Guide to the quality and safety of TISSUES AND CELLS for human application



European Committee
on Organs, Tissues and Cells
(Partial Agreement)
(CD-P-TO)

EDQM

6th Edition

2026

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European Directorate for the Quality of Medicines & HealthCare | Direction européenne de la qualité du médicament & soins de santé

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Guide to the quality and safety of TISSUES AND CELLS for human application

6th Edition

European Directorate for the Quality of Medicines & HealthCare (EDQM)

French edition: *Guide relatif à la qualité et à l'innocuité des cellules et tissus destinés à des applications humaines*
ISBN 978-92-871-9570-8

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Editorial director: Dr P. Doerr
Page layout and cover: EDQM
Cover photo: ©Shutterstock

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Internet: www.edqm.eu

FAQs & EDQM HelpDesk: www.edqm.eu/hd

ISBN 978-92-871-9569-2
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Co-funded by the European Union

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Co-funded and implemente by the Council of Europe

The standards incorporated in the guide were produced with financial assistance of the European Union.

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Foreword

Established in 1949, the Council of Europe is the oldest and largest of all European institutions, currently comprising 46 member states.[1] One of its founding principles is that of increasing co-operation between member states to improve the quality of life for all Europeans. Within this framework of intergovernmental co-operation in the field of health, the Council of Europe has consistently selected ethical problems for study. One of the most important of these ethical issues relates to the prohibition of financial gain from the human body and its parts, including blood, organs, tissues and cells.

Transplantation-related activities at the Council of Europe are co-ordinated by the European Directorate for the Quality of Medicines & HealthCare (EDQM). This directorate is a pivotal European organisation involved in the harmonisation, co-ordination, standardisation, regulation and quality control of medicines, blood transfusion, organ, tissue and cell transplantation, pharmaceuticals, pharmaceutical care, consumer health, cosmetics and food contact materials.

In recent decades, the fields of transplantation and reproductive medicine have undergone transformative advances, many of which were previously inconceivable. Despite these innovations, the demand for certain tissues and cells continues to exceed supply, posing significant clinical and ethical challenges. Human tissues and cells used for therapeutic purposes have the potential to restore vital physiological functions and, in some cases, save lives. However, their use is inherently associated with risks, particularly the transmission of infectious diseases. These risks require rigorous donor selection and testing protocols, as well as the implementation of robust quality management systems throughout the entire process – from donation to clinical application.

This Guide

The *Guide to the quality and safety of tissues and cells for human application*, published first in 2013 and now in its 6th edition, consolidates the most up-to-date information to provide healthcare professionals with a comprehensive overview of recent scientific developments in the field as well as technical guidance on ensuring the quality and safety of human tissues and cells applied to patients. This edition also reflects the updated regulatory framework introduced by implementation of the latest EU legislation governing substances of human origin (SoHO).

To enhance safety for the recipients of human tissues and cells, it is essential that SoHO professionals involved in identifying potential donors, transplant co-ordinators managing the process of donation after death, bone marrow and cord blood and other cell and tissue type collection centres, fertility clinics, SoHO establishments processing and storing tissues and cells, testing laboratories, organisations responsible for human application, inspectors auditing these establishments and Health Authorities responsible for SoHO for human application all have easy access to this information. They play a critical role in ensuring the integrity and safety of the entire process, from donation to clinical application.

This Guide is also the annex to Recommendation CM/Rec(2020)5 of the Committee of Ministers to Council of Europe member states on the quality and safety of tissues and cells for human application, adopted by the Committee of Ministers on 7 October 2020. It contains instructions accepted as harmonised standards for the quality and safety of tissues and cells, which are aligned with the Council of Europe's fundamental principles and the relevant European Union (EU) directives.

1. Albania, Andorra, Armenia, Austria, Azerbaijan, Belgium, Bosnia and Herzegovina, Bulgaria, Croatia, Cyprus, Czech Republic, Denmark, Estonia, Finland, France, Georgia, Germany, Greece, Hungary, Iceland, Ireland, Italy, Latvia, Liechtenstein, Lithuania, Luxembourg, Malta, Republic of Moldova, Monaco, Montenegro, Netherlands, North Macedonia, Norway, Poland, Portugal, Romania, San Marino, Serbia, Slovak Republic, Slovenia, Spain, Sweden, Switzerland, Türkiye, Ukraine, United Kingdom.

The overarching objectives of this Guide are to support professionals in their practical work and to contribute to improving the success rate and safety of clinical applications involving SoHO.

Changes in the 6th edition

SoHO entities and establishments in EU member states are required to comply with EU directives, and after 7 August 2027, the SoHO Regulation. This Guide has a broader scope and is intended to support Council of Europe member states, including those in the EU, through education and the provision of non-binding guidelines to facilitate ongoing improvements in the donation, collection, testing, processing, preservation, storage, release, distribution and import/export of SoHO. It is envisaged that the Guide will have a global reach extending beyond the borders of the Council of Europe.

Implementation of these guidelines among member states and individual SoHO entities and establishments may vary, and alternative procedures, practices and equivalent standards of quality and safety based on a careful risk-based assessment may already be in place.

In this edition, the content of the chapters has been structured into four distinct levels of information to facilitate practical use, and the terms "*must*", "*should*" and "*advised*" have been carefully reviewed to enhance clarity and consistency in their usage. For further details, please refer to the section How to use this Guide.

Unless otherwise stated, the guidelines apply only to SoHO intended for transplantation or clinical use (including medically assisted reproduction, intestinal microbiota and human milk). In this 6th edition, chapters have been updated and extended and some new ones introduced. This responds to an increased interest in the field of SoHO, not only from professionals with regard to the potential applications and quality standards but also from Health Authorities, since the rapid development of novel processing methods and clinical applications requires the establishment of well-defined quality and safety criteria on which to base regulatory requirements. Therefore, in this 6th edition substantial efforts have been made to address these evolving needs. As a result, the Guide has been restructured into three main sections – Parts A to C – where the Good Practice Guidelines (GPG) have been reorganised and integrated into the corresponding chapters to enhance clarity and usability.

Part A

Chapter 1 covers introduction. Part A (Chapters 2-19) covers general requirements applicable to all organisations (entities and establishments) involved in the donation, collection, testing, processing, preservation, storage, distribution and import/export of SoHO, including the introduction of novel processes and clinical applications. Some of the main changes in this part include:

- **Chapter 2** – Quality management outlines the general principles of a quality management system and has been completely revised and updated, the responsibilities of key personnel – including responsible person, physician, releasing officer and quality manager – have been defined and the differences between the various processes within quality management systems have been clarified.
- A new chapter, **Chapter 3** – Qualification, validation and verification provides a clearer overview on how to identify where qualification, validation and verification are required. The chapter covers the general principles of how to perform these activities accurately and provides specific guidance on their use in different areas.
- **Chapter 4** – Risk management provides users with tools to evaluate the risks in their entities or establishments. The EDQM Microbiological Risk of Contamination Assessment tool (MiRCA), assists in identifying potential microbiological risks in novel, existing or changed aseptic processes, and supports management decisions to mitigate risks in aseptic processes. In conjunction with MiRCA, the EuroGTP II tool aims to determine whether a preparation or process has any novelty, to assess the associated risks and to determine the extent of studies or follow-up required to assure its safety and efficacy. Both tools are hosted on a dedicated IT portal, and it is hoped that they will prove valuable for SoHO entities and establishments, inspectors and Health Authorities when evaluating risks.
- **Chapter 6** – Donor evaluation: In addition to general guidance on donor assessment, this chapter now features a dedicated section outlining specific donor eligibility criteria depending on the type of SoHO, as well as detailed guidance on evaluating risk conditions associated with SoHO donations.
- **Chapter 7** – Screening for markers of transmissible infections: Drafted together with the ECDC, this chapter includes new algorithms for the interpretation of results of tests for HIV, HCV, HBV and *Treponema pallidum* infectious markers. It also provides an interpretation and decision-making proposal.
- **Chapter 8** – Collection **and Chapter 10** – Processing: Guidance in both chapters has been fully revised. These two chapters, used in conjunction with the MiRCA tool, facilitate an extensive assessment of individual and aggregated risk-of-contamination factors, which may be used by entities and establishments to decide on and prioritise risk-mitigation actions, to improve collection and processing activities.
- **Chapter 9** – Cleanroom premises: This chapter has been completely redrafted and now provides generic guidance for the design, qualification, monitoring, maintenance and cleaning of

cleanroom premises used for aseptic processing of SoHO.

- **Chapter 12** –Microbiology: The guidance on the correct use of microbiological test methods described in the *European Pharmacopoeia* has been updated. In addition, the list of micro-organisms for which the discarding of grafts is recommended (unless treated with a validated sterilisation process) has been revised.
- **Chapter 14** – Interaction between SoHO establishments and organisations responsible for human application: Emphasises the need for close co-operation between SoHO establishments, organisations responsible for human application and Health Authorities in responding to the introduction of novel processes and clinical applications in the field of SoHO. The chapter has been updated with the new requirements for these entities from the SoHO Regulation.
- **Chapter 19** – Introduction of novel processes and clinical applications: Aims to outline key elements to be considered when developing and authorising novel processes and/or new clinical applications.

Part B

Part B (Chapters 20-34) contains detailed guidelines and requirements for the different SoHO addressed in this Guide. Each chapter has undergone comprehensive revision, with updates and extensions to reflect current practices and scientific advances. Notably, a dedicated section on patient follow-up has been added to all relevant chapters. Some additional changes include:

- **Chapter 20** – Ocular tissue, guidance on indications and contraindications for application by eye condition has been revised and expanded.
- **Chapter 24** – Musculoskeletal tissue now more clearly presents the critical quality attributes that are commonly evaluated by routine quality control.
- **Chapter 25** – Haematopoietic progenitor cells from bone marrow and peripheral blood and **Chapter 26** – Umbilical cord blood progenitors have been updated in alignment with the new SoHO legislation and major international guidelines.
- Dedicated chapters expand the technical guidance and clinical applications for pancreatic islets (**Chapter 27**) and hepatocytes (**Chapter 28**).
- **Chapter 30** – Medically assisted reproduction and **Chapter 31** – Fertility preservation have been revised with new information to provide updated guidance.
- **Chapter 32** – Human milk and **Chapter 33** – Intestinal microbiota contain the most important scientific knowledge required for assessing these types of SoHO.
- **Chapter 34** – SoHO as starting materials: This chapter provides guidance for authorised SoHO establishments on quality and safety aspects, mainly in donation, collection, testing and distribution of the starting material for further processing for the production of novel therapies involving human tissues and cells.

Part C

Finally, Part C of the Guide comprises SoHO monographs, offering detailed information on SoHO preparations and their clinical applications. These monographs describe products that are precisely defined and have demonstrated safety and efficacy in patient use – representing consolidated processes for established clinical indications. In each edition, monographs undergo regular reassessment to ensure their continued relevance. This edition introduces additional monographs, bringing the total to 52, significantly expanding the scope of guidance available for clinical practice.

Expertise involved and acknowledgements

A dedicated working group, composed of well-recognised international experts nominated by member states and relevant professional associations, was convened for the preparation of this Guide. The final composition of the working group was decided after considering the technical and scientific expertise in the field of the nominated experts, the drafting needs of the Guide and the active participation in the elaboration of previous editions of the Guide while ensuring broad and balanced geographic representation. For those areas where additional knowledge was needed, external expertise was engaged.

Chaired by Jacinto Sánchez-Ibáñez (Spain) and Akila Chandrasekar (United Kingdom), this expert group made exceptional contributions by sharing their expertise, reviewing the literature in their respective areas and extracting and distilling knowledge from numerous international guidelines, collaborative projects and websites, with the aim of ensuring that all this up-to-date information is made available and accessible to professionals and regulators. Members of the group co-ordinated preparation of the chapters and ensured access to appropriate outside expertise, where relevant, through the engagement of a number of additional experts from European countries and beyond, who co-authored and contributed to the discussions on various parts of this Guide. All the professionals who participated in the preparation of the Guide are listed in Appendix 38.

The final draft was submitted to a stakeholder consultation where the Council of Europe European Committee on Organs, Tissues and Cells (CD-P-TO), Health Authorities, relevant professional associations and additional experts nominated by them carefully revised the

text and provided comments and suggestions. During the consultation for this 6th edition, 3 717 comments were received; all of them were carefully analysed by the working group and 68% of the comments led to changes in the final text. In some instances (7%), comments were deemed relevant but required extensive research and/or discussion, so their inclusion was postponed to a future edition, and in 25% of cases the comments did not require any changes to the text. Our gratitude is extended to all those individuals who provided extremely useful feedback during this stakeholder consultation.

The elaboration of this Guide was partly funded by the European Commission through a Direct Grant Agreement (contract number 2024/SI2.929092) entitled "Strengthening European SoHO systems". Special thanks are due to Stefaan van der Spiegel and Rita Pitiera, from DG-SANTE, who ensured that the current text remained in alignment with EU legislation and who made available the results from EU-funded projects. Several professional associations – in particular, the European Association of Tissue and Cell Banks (EATCB), the European Eye Bank Association (EEBA), the European Society for Human Reproduction and Embryology (ESHRE), the European Society for Blood and Marrow Transplantation (EBMT), the World Marrow Donor Association (WMDA) and the European Milk Bank Association (EMBA) – are to be thanked for sharing their experience and knowledge.

The drafting and publication of the 6th edition of the Guide was co-ordinated by Mar Lomero (Scientific Officer in charge of the Guide), with the assistance of Muriel Burstin, Carlotta Vizioli, Federica Domini, Cathydja Patel, Đana Džafić and Gerard M.-F. Hill. Marta López Fraga is to be thanked for sharing her expertise and knowledge as co-ordinator of previous editions of the Guide.

The entire project has been an exceptional combined effort of everyone involved, with extensive discussions dedicated to the common goal of improving the safety, efficacy and quality of SoHO for human application. The result is this 6th edition of the Guide, which constitutes a common European standard, based on the long-standing expertise and knowledge of the working group and of the CD-P-TO.

How to use this Guide

New structure of the Guide

Chapter 1 provides general information on the field of substances of human origin (SoHO), covering ethical principles and the legislative framework for the field. The rest of this Guide is structured in three main parts: A, B and C.

Part A

Part A (Chapters 2-19) contains general requirements applicable to all substances of human origin, and to SoHO entities and establishments which have developed activities with SoHO, namely: donation, collection, testing, processing, preservation, storage, release, distribution, import/export, clinical application and the introduction of novel processes, preparations or clinical indications.

The chapters have an introduction section to provide background. The content provided in Part A has been structured in four different levels of guidance for users:

- **Standards**. This first level contains the standards that are derived from the European Union (EU) SoHO Regulation. They represent the requirements associated with the different SoHO activities. The aim is to summarise and transpose the requirements in the appropriate chapters. For the EU Member States (MS), these standards are mandatory. The term "must" is used in these standards, to indicate the necessity of compliance. These standards should not be understood as literal excerpts (verbatim) from the regulatory provisions. At the end of the specific standard, the relevant source in the EU SoHO Regulation (Article) is quoted in brackets. This allows the user of the Guide to establish correspondence with the original legal text, if needed.
- **Good Practice Guidelines** (GPGs). The GPGs are intended to elaborate on the key elements that should be defined, implemented and monitored by SoHO entities and establishments. The GPGs may not always be directly linked to a standard but they provide relevant technical guidance. For the EU MS, any guidance provided as GPGs is understood as mandatory, unless the SoHO entity can demonstrate that deviating from it results in equivalent or better quality and safety. The term used within this level is "should".
- **Recommendations**. This third level contains all the advice and guidance that is relevant to the chapter but is not considered significant enough to be classed as mandatory. The aim of this category is not to impose additional mandatory requirements but to inform and recommend the best practices and knowledge available, to ensure the quality and safety associated with SoHO activities. The terms used are "recommended" or "advised".
- **Supplementary information**. This fourth level includes all the relevant information that is supplementary to the three levels above. It will include, for example, text explaining the rationale for the standards, guidance and advice, or general background information. Unless otherwise specified, the information provided in sections without a heading level should be considered as supplementary information.

Part B

Part B (Chapters 20-34) contains specific technical guidelines and requirements applicable to SoHO

entities or establishments, in addition to and/or in deviating from the general requirements that are specified in Part A. The structure of Part B of the Guide follows the same different levels of guidance defined in Part A. The relevant Part A chapters or sections are cross referenced in Part B. All chapters from Part B should be read in conjunction with the general chapters from Part A.

Part C

Part C (SoHO monographs) – The SoHO monographs give more detail of the technical requirements and specifications related to established SoHO preparations and clinical indications. They reflect the current status of preparation methods and their corresponding safety and effectiveness for known clinical uses (i.e. consolidated processes for consolidated uses). SoHO monographs are to be used as a reference to support, but not as a substitute for, entity-specific validation and authorisation processes.

These monographs set out harmonised standards for the quality, safety and quality control of SoHO preparations across Europe. It should be noted that the SoHO monographs included here do not necessarily cover all SoHO preparations currently prepared and used in Europe. As such, SoHO monographs are provided for those preparations where sufficient data on safety, efficacy or standardisation are available.

In addition, the EU SoHO Regulation refers to these SoHO monographs in the context of preparation authorisation, indicating that they should be taken into account, where available. However, it is important to note that the authorisation of SoHO preparations within the EU is not dependent on the existence of a corresponding SoHO monograph in this Guide.

To ensure that new preparations can be considered for inclusion, the publication of data supporting preparation processes is strongly encouraged. Such contributions are essential for the continued development of the Guide and the harmonisation of practices.

Each SoHO monograph combines two aspects, a consolidated process and a consolidated use, in a single document that is complementary to other sections of the Guide. SoHO monographs complement and link generic requirements in Part A and specific requirements from Part B to define specific critical properties and quality-control requirements for SoHO, prepared in specific ways and to be used for specific clinical indications.

When developing and implementing new SoHO preparations and clinical indications, these monographs should be incorporated into the necessary risk assessment to help users and Health Authorities (HAs) to evaluate the level of novelty and risk associated with the preparations and clinical indications being considered. SoHO monographs will undergo regular reassessment to ensure their relevance.

The field of SoHO is a dynamic one and approaches continually change with advances in the development of new processing methods and the emergence of new indications. It is important that these new improvements are carefully evaluated by SoHO entities and HAs, notably via the process of authorisation. Chapter 19 of this Guide provides generic guidance on good practice for the introduction of new or changed procedures and their subsequent authorisation. By reference to these SoHO monographs, both SoHO entities and HAs will know that products that do not have a matching monograph may need more attention (more risk assessment, validation, possible clinical studies etc.) before they can be authorised and supplied for routine use.

Target audience

The contents and technical standards defined in this Guide are meant to assist the activities of all SoHO stakeholders, such as SoHO professionals, SoHO entities (and establishments) and HAs.

The current edition is structured into 34 chapters; Table 1 provides an overview of how the chapters can correlate with the needs of different SoHO professionals based on their specific activity profiles. Therefore, it is essential for users to have a comprehensive understanding of all relevant chapters to ensure their overall knowledge and compliance with the technical requirements.

Table 1. **Chapters of this Guide relevant for the different stakeholders**

	SoHO entities						
					SoHO establishments		***Health authorities***
	Registries	***Donation centres***	***Testing / Quality control labs***	***ORHAs***	***SoHO establishments / banks***	***Importers / SoHO establishments***	
Chapter 1. Introduction	X	X	X	X	X	X	X
Chapter 2. Quality management	X	X	X	X	X	X	X
Chapter 3. Qualification, validation and verification	X	X	X		X	X	X

	SoHO entities						Health authorities
					SoHO establishments		
	Registries	*Donation centres*	*Testing / Quality control labs*	*ORHAs*	*SoHO establishments / banks*	*Importers / SoHO establishments*	
Chapter 4. Risk management	X	X	X	X	X	X	X
Chapter 5. Recruitment of potential donors, identification and consent	X	X			X	X	X
Chapter 6. Donor evaluation		X			X	X	X
Chapter 7. Screening for markers of transmissible diseases		X	X		X	X	X
Chapter 8. Collection		X			X	X	X
Chapter 9. Clean room premises		X	X		X		X
Chapter 10. Processing					X		X
Chapter 11. Storage					X	X	X
Chapter 12. Microbiology			X		X		X
Chapter 13. Release, distribution, import and export				X	X	X	X
Chapter 14. Interaction between SoHO establishments and ORHAs				X	X	X	X
Chapter 15. Computerised systems	X	X	X	X	X	X	X
Chapter 16. Coding, packaging and labelling	X	X	X	X	X	X	X
Chapter 17. Traceability	X	X	X	X	X	X	X
Chapter 18. Biovigilance	X	X	X	X	X	X	X
Chapter 19. Introduction of novel processes and clinical application				X	X	X	X
Part B (Chapters 20–34)				X	X		X
SoHO Monographs				X	X	X	X

Note. ORHA: organisation responsible for human application.

More information about the *6th edition of the Guide*, its aims, scope and terminology are addressed in Chapter 1 Introduction, §1.1.

Chapter 1. Introduction

1.1 Scope and aim of this Guide

Substances of Human Origin (SoHO)[2] are defined as "any substance collected from the human body whether it contains cells or not and whether those cells are living, or not, including SoHO preparations resulting from the processing of such substances" [1]. Included in the definition of SoHO are organs, blood and blood components, tissues, cells, including replacement and reproductive tissues and cells (including embryos, even if they are not collected from the human body), as well as progenitor cells and other SoHO, such as human milk and intestinal microbiota. A brief history of the preparation of tissues and cells for human application can be found in Appendix 1.

SoHO preparations have a wide variety of therapeutic indications, and their clinical applications can have a significant impact on patients, ranging from life-saving therapies to substantial improvements in quality of life for those who require such treatments.

However, due to their biological nature, SoHO activities involve risks both to living donors and recipients. Therefore, it is paramount to ensure that these activities are carried out according to high-quality standards and stringent safety protocols to minimise those risks and protect public health.

This Guide establishes the good practices and standards of quality and safety for SoHO preparations intended for human application. However, it should be noted that this Guide does not apply to blood and blood components or to organs, because they are addressed in the *Guide to the preparation, use and quality assurance of blood components* and the *Guide to the quality and safety of organs for transplantation*, respectively [2].

This Guide establishes technical guidelines, developed on the basis of scientific knowledge, for the activities related to tissues, cells (including replacement and reproductive tissues and cells, as well as progenitor cells) and other SoHO, such as human milk and intestinal microbiota. For simplicity, all these are referred to as SoHO or 'tissues and cells' in this Guide.

This 6th edition of the Guide has three main objectives:

- To provide sound information and scientifically based guidance – for all professionals involved in donation, collection, testing, processing, storage, preparation and clinical applications of SoHO preparations;
- To optimise the quality of SoHO preparations and minimise the risks for donors and recipients;
- To promote the highest underlying ethical principles that are to be applied when donation of SoHO and its application in humans is considered.

This 6th edition of the *Guide to the quality and safety of tissues and cells for human application* collates the most up-to-date information and provides a comprehensive overview of the most recent advances in the field of SoHO. The requirements outlined in the Guide represent the consensus of experts in the field of SoHO on what they consider the safest and most effective practices, based on documented evidence, scientific principles and their professional knowledge and experience. It also provides technical guidance on ensuring the quality and safety of SoHO for clinical use, including best practices associated with SoHO-specific activities. More information on how to use this Guide is shown in the section 'How to use this Guide'.

Unless otherwise indicated, the term 'member states' signifies member states of the Council of Europe (CoE). For EU Member States (MS) this edition of the

2. In the context of this guide, SoHO means tissues and cells (including replacement and reproductive tissues and cells, and embryos and progenitor cells) and other SoHO, such as human milk and intestinal microbiota.

Guide, together with the guidelines of the European Centre for Disease Prevention and Control (ECDC), serves as an appropriate means to demonstrate compliance with Regulation (EU) 2024/1938 of the European Parliament and of the Council of 13 June 2024 on standards of quality and safety for SoHO intended for human application (hereafter referred to as the EU SoHO Regulation) and which repeals Directives 2002/98/EC and 2004/23/EC.

Moreover, this Guide expands upon the European Union (EU) legal requirements by describing good practice at a technical level and providing additional recommendations and explanatory information, as well as consideration of ethical issues. It is intended to facilitate ongoing improvements in the donation, collection, testing, processing, preservation, storage, release, distribution, import/export, human application and clinical-outcome registration of SoHO, through the provision of non-binding recommendations for all CoE MS, including those in the EU, but also beyond the CoE borders. Therefore, it represents a useful source of practical information for those working within the EU legislative framework and those working within national legal frameworks in all CoE MS and beyond.

Implementation of this guidance among EU MS and individual SoHO entities may vary, and alternative procedures, practices and equivalent standards of quality and safety of SoHO based on a careful risk-based assessment may be in place. When adopting those alternative guidelines or practices, EU organisations and/or EU MS must verify and document whether these alternative standards adequately achieve compliance and are equivalent to the technical guidelines published by the ECDC and the European Directorate for the Quality of Medicines & HealthCare (EDQM) in this Guide, as well as in the EU SoHO Regulation.

It is important to take into account that this Guide does not provide any guidance on how SoHO are or should be regulated or classified, so users of this Guide are advised to carefully consider the national legal requirements that apply to the activities they are undertaking.

This Guide only covers SoHO used for current therapeutic purposes. Similarly, SoHO that are both collected and applied within the same medical procedure are outside the scope of this Guide, as long as they are not removed from the surgical field.

Additionally, it is worth noting that, in the EU, in the case of SoHO collected for the purposes of manufacturing medical devices, regulated by Regulation (EU) 2017/745, medicinal products, regulated by Directive 2001/83/EC, advanced therapy medicinal products, regulated by Regulation (EC) No. 1394/2007, or investigational medicinal products, regulated by Regulation (EU) No. 536/2014 for investigational medicinal products, the provisions of the SoHO Regulation are applicable to the following SoHO activities: donor registration, donor history review and medical examination, and testing of donors or of persons from whom SoHO are collected for autologous use, collection and release. Insofar as storage, import and export are carried out on SoHO up to and including their distribution to a manufacturer regulated by other Union legislation, the SoHO Regulation also applies. It is also worth noting that this Guide does not provide guidance on the regulatory aspects of released SoHO once it has been distributed by the SoHO establishments for manufacturing or further processing under a different framework. In these situations, users are advised to carefully consider the national, and where appropriate, EU legal framework that applies to the activities they are undertaking.

As of the publication date of this Guide (6 October 2026), the EU Directives and current national legislation regarding SoHO remain applicable. However, it is essential to note that the SoHO Regulation, set to come into effect in August 2027, will supersede existing requirements and good practice. Upon its entry into effect, the technical guidance provided in this Guide will become mandatory compliance for all EU MS, ensuring a harmonised approach to the safety and quality of SoHO and SoHO activities.

This Guide is the result of the collective joint effort and expertise gathered by experts nominated by the CoE MS and professional associations in the field (see Appendix 37), as well as by the members and observers of the European Committee on Organs, Tissues and Cells (CD-P-TO) (see Appendix 38).

1.2 Terminology used in the Guide

In addition to the glossary provided in Appendix 3, this Guide includes some terms that differ from those in previous editions.

The previous references to 'tissue' or 'grafts' have been replaced, where relevant, by 'SoHO preparation' to designate a type of SoHO that has been subjected to processing and/or any other SoHO activity with impact for the quality and safety, has a specific clinical indication and is intended for human application to a recipient or is intended for distribution [1].

EU Regulation 2024/1938 differentiates between the terms 'SoHO entity' and 'SoHO establishment', where 'SoHO entity' means an entity legally established in the Union that carries out one or more of the SoHO activities referred to in Article 2(1).c (donor registration, donor history review and medical examination, testing, collection, distribution, quality control, human application and clinical outcome registration). Therefore, this includes 'SoHO establishment', which refers to a 'SoHO entity' that carries out any of the following activities: processing and storage, release, import, export.

In the context of the same EU Regulation, depending on the type of activities performed, it will be up to each Health Authority to decide what classification should be assigned to each entity within the EU MS. Although the scope of this Guide extends beyond the EU and provides guidance to CoE MS, these terms are used in the present Guide, where relevant.

In this Guide, the term 'Health Authority' (HA) is used throughout to refer to a body that has been delegated the responsibility for SoHO activities on a national or regional basis by their government. Other similar terms, such as 'regulatory authority', 'regulatory agency' or, in the EU, 'Competent Authority', are equivalent to it. It should be noted that in some countries, the activities described in this Guide may be controlled by different Health Authorities – e.g. separate authorities may regulate medically assisted reproduction (MAR) and the donation, banking and human application of other SoHO.

1.3 Quality management

The level of safety, efficacy and quality of donated SoHO for human application as SoHO preparations or as SoHO-derived medicinal products of any nature needs to be maintained and continually optimised. This strategy requires implementation of quality systems (see Chapter 2) that include traceability (see Chapter 17) and vigilance (see Chapter 18) on adverse events and reactions that need to be reported both nationally and for imported/exported SoHO preparations. High-quality, safe and efficacious procedures are essential for donors and recipients alike.

The long-term outcomes of SoHO donated for human application should be assessed for the living donor, as well as the recipient, to document benefit and harm. Optimising the outcome of the human application of SoHO entails a rules-based process that encompasses clinical interventions and *ex vivo* procedures from donor selection through to long-term follow-up. Under the general supervision of Health Authorities, all transplant and MAR programmes should monitor donors and recipients, according to agreed long-term follow-up protocols, to ensure that they receive appropriate care, including information about long-term risks and benefits and ways of (self) reporting relevant findings/events/outcomes. Evaluation of information on long-term risks and benefits is essential to the consent process and for adequately balancing the interests of donors and recipients. The benefits to both have to outweigh the risks associated with donation and human application.

Clinical application of SoHO should be ensured through traceability for donor and recipient. Internationally agreed means of coding to identify SoHO used in transplantation are essential for full traceability (see Chapter 17).

In the specific case of MAR, traceability should include the outcome of the pregnancy as well as the health of the donor, recipient and newborn. It is of the utmost importance to put the welfare of donors (especially with respect to third-party donors) in a central position in determining what constitutes acceptable practice. This requirement might entail additional effort in the context of cross-border reproductive care. MAR registries are essential, collecting data on a systematic basis to follow up the long-term health effects of MAR activity, including the health of the donor, recipient and newborn, but also including information on third-party donors [3, 4].

1.4 Ethical principles

SoHO can be derived only from the human body – hence the ethical challenges associated with their donation and application. The wide range of SoHO described in this Guide makes explicit the very different circumstances under which a person can donate. The person providing these may be living or deceased, the SoHO may be used almost immediately or stored for long periods of time, and the SoHO may be used unprocessed or heavily processed. Whatever the case, handling and disposal of SoHO should be carried out in a manner that shows respect for fundamental rights and for the human body.

Ethical standards of all aspects of SoHO donation and human application have to comply with the Oviedo Convention on Human Rights and Biomedicine (1997) [5] and the Additional Protocol on transplantation of organs and tissues of human origin (2002) [6] and must also, in the EU, comply with the Charter of Fundamental Rights of the European Union (2012) [7]. Other important guidelines to observe from an ethical viewpoint are the Council of Europe Committee of Ministers Resolution (1978) 29 on harmonisation of legislation of CoE MS relating to removal, grafting and transplantation of human substances [8], the World Health Organization (WHO) Guiding Principles on human cell, tissue and organ transplantation [9], the Declaration of Istanbul on Organ Trafficking and Transplant Tourism [10] and the Barcelona Principles on the use of human donated tissue for ocular transplantation, research and future technologies from the Global Alliance of Eye Bank Associations (GAEBA) [11].

For tissue donation from deceased individuals, the 'dead-donor rule' (which states that patients have to be declared dead before removal of any vital organs or tissues for transplantation) needs to be strictly respected [12].

1.4.1 Informed consent

The Oviedo Convention states that an intervention in the health field may be carried out only after the person concerned has given free and informed consent to it. This person has to make a free choice in the absence of any undue influence and needs to be given appropriate information beforehand as to the intended use and nature of the intervention as well as its consequences and risks. The person concerned may freely withdraw consent at any time.

Together with the Declaration of Istanbul, a joint initiative of the International Society for Nephrology (ISN) and The Transplantation Society (TTS), the Additional Protocol to the Convention on Human Rights and Biomedicine concerning transplantation of organs and tissues of human origin expands these provisions

further for the specific case of donation and transplantation. These provisions are explained further in detail in Chapter 5. Specific cases related to consent in MAR procedures are outlined in Chapter 30.

SoHO must not be collected from the body of a deceased person unless that person has been certified dead in accordance with the national law and consent or authorisation has been obtained. The collection cannot be carried out if the deceased person had objected to it.

Finally, it is crucial to emphasise the importance of consent in creating and maintaining the trust of the general public in health professionals and the healthcare system as a whole. 'Medical mistrust', or mistrust of the healthcare system, is one of the reasons why people are reluctant to donate bodily substances. This may be associated with concerns about consent in that the terms of the consent might be misused (for example, by using the donated material in a manner which is not in accordance with consent, including its use for non-therapeutic purposes, research and/or profit) and that additional material might be taken without explicit consent. Values such as honesty and trust are central in both the professional and personal relationships when donation of bodily material takes place. Therefore, it is of vital importance that the limits of the consent are clearly established, made explicit and scrupulously respected.

The recipient – and, if appropriate, the person or official body providing authorisation for the human application – needs to be given suitable information beforehand on the purpose and nature of the procedure, its consequences and risks, and the alternatives to the intervention.

In summary, to continue their activity all donation and transplantation programmes are dependent upon the goodwill and voluntary donation of relevant material from donors. It is, therefore, important that public confidence is maintained by standards of good practice. By engaging donor trust and commitment when obtaining consent, healthcare professionals will reduce the risk of nefarious trading and potential physical harm from the clinical use of SoHO.

1.4.2 Absence of conflicts of interest

To avoid any potential conflict of interests, physicians determining the death of a potential donor should not be directly involved in SoHO collection from the donor or subsequent clinical application procedures, and nor should they be responsible for the care of any intended recipient of such SoHO preparations. Health Authorities will set out the legal standards for determining that death has occurred and specify how the criteria and process for determining death will be formulated and applied.

All living donors need to receive complete information before giving their consent. Moreover, this information has to be impartial and, whenever possible, given by a treating physician who is not treating the recipient of the SoHO. This also applies in cases of allogeneic donation or third-party donation in MAR. Scarcity of SoHO collected from living donors should never influence the decision to accept a donor into a programme. Also, financial incentives are not used to encourage donations because they will render living donors more likely to consider repeat donations or to continue donating despite potential risks to their health. Furthermore, financial incentives may lead donors to not disclose all the information necessary for a complete and adequate donor selection, thus compromising the quality and safety of the donated SoHO.

1.4.3 Financial neutrality

Discussions around how best to increase the supply of SoHO often focus on questions of donor motivation: specifically, how individuals may best be encouraged to donate different forms of bodily material. Nevertheless, it is essential to recall the Oviedo Convention which, in Article 21, clearly states that the human body and its parts must not give rise to financial gain. The *Guide for the implementation of the principle of prohibition of financial gain with respect to the human body and its parts from living or deceased donors* [13] provides further guidance on how to interpret Article 21 of the Oviedo Convention in order to facilitate its implementation. This notion is reiterated in the additional protocol to that Convention, which also clearly states in its Article 21 that the human body and its parts must not, as such, give rise to financial gain or comparable advantage. The aforementioned provision does not prevent payments that do not constitute a financial gain or a comparable advantage, in particular:

a. compensation of living donors for loss of earnings and any other justifiable expenses caused by the removal of SoHO or by the related medical examinations;
b. payment of a justifiable fee for legitimate medical or related technical services rendered in connection with transplantation;
c. compensation in cases of undue damage resulting from the removal of SoHO from living persons.

In the donation of any SoHO, removal of barriers to donation must not render a decision to donate non-altruistic. Initiatives that reduce the barriers to donation should only facilitate individuals in carrying out an action that they were already inclined to take by concern for the welfare of the recipient.

In this sense, the Nuffield Council on Bioethics suggests distinguishing between two types of intervention, both of which aim to increase donation by changing its costs and benefits [14]. The first type is 'altruist-focused interventions', which typically involve removal of various disincentives to act and, in doing so, remove countervailing concerns that may hinder potential donors from acting on their altruistic motivations. For the purpose of this Guide, we call these interventions 'compensation'. The second type is 'non-altruist-focused interventions', which are targeted at potential donors who have no strong motivation to help others through donation of their SoHO and who, therefore, if they are to donate, need to be provided with different reasons for action, perhaps in the form of payment or 'incentive' going well

beyond the reimbursement of expenses. These incentives are particularly worrisome in the case of gamete donors, where they may change the donor's perception of the relative risks and benefits of a donation that is not free of potential health hazards and psychological consequences. In addition, gamete donation for treatment purposes presents further ethical implications because it involves the potential conception of a new human being.

In summary, voluntary unpaid donation, long promoted as the only ethical basis for donation of SoHO, should continue to have a central role in the donation process. Compensation to donors should cover only justifiable expenses and loss of income and should not act as a direct or indirect incentive or inducement.

Physicians and other health professionals should not engage in transplantation procedures, and health insurers and other payers should not cover such procedures, if the SoHO concerned have been obtained through exploitation or coercion of, or payment to, the donor or the next of kin of a deceased donor.

Promotion of altruistic unpaid donation of SoHO by means of advertisement or public appeal may be undertaken in accordance with domestic regulations. However, advertising the need for availability of SoHO with a view to offering or seeking financial gain or comparable advantage for the donor, or their next of kin where the individual is deceased, should be prohibited. Brokering that involves payment to such individuals or to third parties should also be prohibited.

SoHO establishments storing and supplying SoHO have developed largely in response to the increasing demand for supplies of SoHO for therapy and research. However, professional bodies should ensure that their guidelines reflect their members' responsibilities in the acquisition and supply of SoHO. SoHO should be supplied on an operational cost basis and no payment should ever exceed the justifiable fee for the services rendered; in other words, SoHO establishments can claim a reasonable amount for certain expenses, but should not quote an unfair amount greater than the actual cost in order to make profits. When calculating the operational costs of a SoHO entity, which may have a variety of funding sources, consideration should be given to the long-term sustainability of the SoHO entity. In order to do this, it is important to analyse the clinical need (for the different types of SoHO that the SoHO establishment will be handling) and to efficiently manage the SoHO entity's 'value chain', which includes the costs of collection, processing, storage, distribution, personnel, transport, infrastructure and administration, and the need to incorporate state-of-the-art processes and equipment, among others.

1.4.4 Equitable access to healthcare

Healthcare in general is a human right because it secures and protects people's access to the normal range of opportunities and because it allows people to thrive. Given the importance of health for the general wellbeing of a person, every person, regardless of his/her income or financial means, should have access to decent healthcare.

Requests (the demand) for SoHO preparations for clinical application may often exceed what is available (the supply). Significant practical and ethical questions of efficiency and fairness arise in deciding how to distribute these limited resources. Article 3 of the Additional Protocol to the Convention on Human Rights and Biomedicine concerning transplantation of organs and tissues of human origin states that transplantation systems must exist to provide equity in access to transplantation services for patients. Except in the case of direct donations, SoHO preparations are to be allocated among patients only in conformity with transparent, objective and duly justified rules according to medical criteria. The persons or official bodies responsible for the allocation decision must be designated within this context.

With regard to access to MAR, infertility treatment covers a broad range of 'causes' (e.g. age-related decline in fertility, male factors, blocked Fallopian tubes) and applications that cannot necessarily all be fitted into the same category. The pivotal point in this scenario seems to be whether the desire for a child should be considered a fundamental need or a personal wish. The debate on ethical and social aspects (including access to MAR for social indications, anonymity of gamete donors, genetic selection of donors, compensation for donation, posthumous reproduction or surrogacy) is ongoing. There is, however, general agreement that reproductive cloning must be forbidden.

Inclusion of infertility treatment in the basic healthcare tier is dependent upon the general level of welfare in society. Cultural, educational and religious backgrounds may also affect the availability of these therapies. Nevertheless, given the rightful claims of other types of healthcare and other fundamental needs in society (as well as limited availability of non-partner gametes and embryos), access cannot be expected to be unlimited. Ultimately, access to MAR should be considered in a structured way to include efficiency, safety and equity to avoid discrimination [15]. Some countries have opted to give public access to a fixed number of cycles/treatments for everyone, even if this means that those who need more treatments have to pay for it themselves. Cross-border reproductive care refers to a widespread phenomenon whereby patients seeking MAR treatment cross international borders to obtain reproductive treatment outside their country of residence. The reasons for travelling vary between countries, but the most common reason is access to certain treatments or techniques not legally allowed in the country of origin (e.g. non-partner donation treatment, pre-implantation genetic testing of embryos) or if a particular group is excluded from treatment (e.g. same-sex couples, single women, women above a certain age). There may be other limitations to access at home (e.g. long waiting lists). Further reasons for travelling may be better quality of care and less expensive treatment [16, 17].

The ideal situation is fair access to fertility treatment in the home country for all patients. This ideal should be promoted at all levels [17]. However, if for some reason treatment is not possible in the country of

residence, cross-border reproductive care may provide a solution. Furthermore, it is in accordance with the principle of freedom of movement of patients within Europe [18]. However, crossing borders may also lead to increased shortage of scarce resources in the visited country and to the detriment of local citizens. Health Authorities may want to introduce a system for fair allocation of scarce resources that considers local needs, such as a maximum number (or percentage) of treatments provided to foreign patients. In addition, cross-border reproductive care should always follow the same strict quality and safety criteria that govern domestic treatments, including appropriate traceability and biovigilance measures that cover both recipients and their children born as a result of the MAR treatment.

1.4.5 Equity in donation

Individual motivation and choice are only one part of the donation picture; the central role of organisations, entities and professionals in facilitating donation should not be underestimated, nor indeed the importance of trust in these systems. An example of such organisational aspects is that, whenever a person dies in circumstances where donation is a possibility, and there was no decision by the deceased in life not to donate, this should be raised with their family.

The role of the state with respect to donation should be understood as one of stewardship: that is, actively promoting measures that will improve general health (thereby reducing the demand for some forms of bodily material) and facilitating donation. Such a stewardship role should extend to taking action to remove inequalities that affect groups or individuals with respect to donation. Equity in donation refers to the absence of systematic disparities in the burden of donation between social groups who have different levels of underlying social advantage or disadvantage (i.e. different positions in a social hierarchy). Inequities in donation would, in a systematic manner, put groups of people who are already socially disadvantaged (e.g. by virtue of being poor, female and/or members of a particular racial, ethnic or religious group) at further disadvantage with respect to their health.

As discussed above, introduction of financial incentives for donation in the field of MAR renders certain social groups particularly susceptible to disparities based on social and economic status.

With respect to cross-border care, safeguards need to be in place to guarantee that all donors, regardless of their origin, receive similar care and follow-up. To prevent abuse of donors coming from abroad, the use of intermediate agencies – which may lead to violations of the rules of good clinical practice and, in the worst-case scenario, to trafficking – should be avoided. Post-donation care needs to be provided to the best possible standards.

1.4.6 Anonymity

The identity of the donor and recipient should (except in the case of donation between persons having a close personal relationship) be maintained in strict confidentiality. Such precautions will prevent abuse and protect the families of donors and recipients from feelings of anxiety associated with emotional involvement, a sense of obligation to return favours, or guilt.

In the specific case of MAR, different regulations are applied in different CoE MS with regard to the anonymity of third-party donors. Debate has focused on the donor's right to anonymity, the welfare of the resulting offspring and his/her right to family life, and the effect of removal of donor anonymity on the supply of gametes for treatment. Presently, some countries require that donors always remain anonymous, whereas other countries require that their identity may be known only in exceptionally urgent medical situations. Some countries allow the possibility of the offspring gaining access to non-identifying information about the donor (e.g. hair colour, ethnicity). Other countries allow the offspring to contact donors after the offspring has reached a certain age. Hybrid models exist in some countries. A common standard seems to be that donors do not have the right to information about children generated from their gametes (unless the child chooses and is legally allowed to obtain information about the donor).

1.4.7 Transparency

The organisation and execution of activities based on donation and human application, as well as their clinical results, need to be transparent and open to scrutiny, while ensuring that the personal anonymity and privacy of donors and recipients are protected (if relevant).

Transparency can be achieved by maintaining public access to regularly updated comprehensive (standardised) datasets on activities, in terms of donation, collection, storage, allocation, distribution (including import/export) and application, as well as treatment outcomes for recipients, living donors and children born as a result of MAR procedures. Any additional data on the needs for SoHO and for organisational funding may be made publicly available. Such transparency is consistent with shielding (from public access) information that could identify individual donors or recipients (anonymity), while still respecting the requirement of traceability. The objective of the system should be not only to help meet the goal of self-sufficiency for countries or regions and to maximise the availability of data for scholarly study and governmental supervision to allow determination of clinical outcomes and efficacy of treatments but also to identify risks (and facilitate their mitigation) to minimise harm to donors and recipients.

1.5 Policies and legislative framework

1.5.1 Council of Europe

The Council of Europe, based in Strasbourg (France), is an international organisation that promotes co-operation between all European countries in the areas of human rights, democracy, rule of law, culture and public health. After the 3rd Conference of European Health Ministers on the Ethical, Organisational and Legislative Aspects of Organ Transplantation [19], held in Paris in 1987, the Council of Europe Committee of Experts on the Organisational Aspects of Co-operation in Organ Transplantation (SP-CTO) was created. This committee consisted of experts in different aspects of transplantation: immunologists, surgeons and physicians, as well as co-ordinators and representatives from organ-sharing and organ-procurement organisations. In 2007, the secretariat responsible for activities related to organs and SoHO was transferred to the EDQM of the Council of Europe [20], and the newly appointed CD-P-TO took over as the steering committee [21].

Today, the CD-P-TO is composed of internationally recognised experts from CoE MS, observer countries, the European Commission and the WHO, as well as representatives from the Steering Committee for Human Rights in the fields of Biomedicine and Health (CDBIO) and several professional societies and non-governmental organisations. It actively promotes the non-commercialisation of organ donation, the fight against organ trafficking, the development of ethical, quality and safety standards in the field of organs and SoHO, and the transfer of knowledge and expertise between CoE MS and organisations.

Within the framework principle of sharing knowledge through international co-operation, the Council of Europe has established widely recognised recommendations and resolutions in the field of transplantation, covering the ethical, social, scientific and training aspects of the donation and transplantation of organs and SoHO [22]. Whereas agreements and conventions are binding on the states that ratify them, resolutions and recommendations are policy statements to governments that propose a common course of action to be followed.

The Council of Europe Convention for the Protection of Human Rights and Fundamental Freedoms (European Treaty Series, No. 5) [23] is an international treaty to protect human rights and fundamental freedoms in Europe. It was drafted in 1950 by the then newly formed Council of Europe and came into force on 3 September 1953.

The European Agreement on the Exchange of Therapeutic Substances of Human Origin (European Treaty Series, No. 26) [24], signed in Paris on 15 December 1958, aims to provide mutual assistance with respect to the supply of therapeutic SoHO.

The European Agreement on the Exchange of Tissue-Typing Reagents (European Treaty Series, No. 84) [25], signed in Strasbourg on 17 September 1974, laid the groundwork for development of mutual assistance in the supply of tissue-typing reagents and the establishment of joint rules between signatory parties. By this agreement the signatory parties undertake to make reagents available to other parties who are in need of them, by the most direct route, subject to the condition that no profit is made on them, that they must be used solely for medical and scientific purposes and are free of import duties. The Additional Protocol (European Treaty Series, No. 89) [26], which was opened for signature on 24 June 1976 and came into force on 23 April 1977, provides for the accession of the European Community to this agreement.

The Oviedo Convention – the Convention for the Protection of Human Rights and Dignity of the Human Being with regard to the Application of Biology and Medicine (European Treaty Series, No. 164) [5], which was opened for signature on 4 April 1997 and came into force on 1 December 1999 – is the first legally binding international text designed to preserve human dignity, fundamental rights and freedoms, through a series of principles against the misuse of biological and medical applications. The Convention is inspired by the principle of the primacy of human beings over the sole interest of science or society. It lays down a series of principles applying to medical practice as well as biomedical research, organ transplantation and genetics. The Convention includes the principle of consent, non-discrimination based on genetic characteristics, and protection of private life and access to information. The Convention specifically prohibits any financial gain from the body and its parts. The *Guide for the implementation of the principle of prohibition of financial gain with respect to the human body and its parts from living or deceased donors* [13] provides further guidance on how to interpret Article 21 of the Oviedo Convention in order to facilitate its implementation.

The Oviedo Convention was extended by an Additional Protocol to the Convention on Human Rights and Biomedicine concerning transplantation of organs and tissues of human origin (European Treaty Series, No. 186) [16], which was opened for signature on 24 January 2002 in Strasbourg and came into force on 1 May 2006. This additional protocol aims to protect the dignity and identity of everyone and to guarantee, without discrimination, respect for his/her integrity and other rights and fundamental freedoms regarding the transplantation of organs and tissues of human origin, thereby establishing principles for the protection of donors and recipients. However, the additional protocol does not apply to gametes and embryos.

The Council of Europe Convention on Action against Trafficking in Human Beings (European Treaty Series, No. 197) [27], which was opened for signature in Warsaw on 16 May 2005 and came into force on 1 February 2008, alongside its Explanatory Report, addresses the trafficking of human beings for the purpose of organ removal.

The Council of Europe/United Nations joint study on Trafficking in Organs, Tissues and Cells and Trafficking

in Human Beings for the Purpose of the Removal of Organs [28], presented at the United Nations headquarters in New York on 13 October 2009, focuses on trafficking in organs, tissues and cells for the purpose of transplantation. The joint study made it evident that existing criminal-law instruments dealing exclusively with trafficking in human beings (including for the purpose of organ removal) left loopholes that allowed several unethical transplant-related activities to persist. This is why the Council of Europe decided to undertake the task of drafting a new international legally binding instrument against trafficking in human organs.

The Council of Europe Convention against Trafficking in Human Organs (European Treaty Series, No. 216), with its Explanatory Report [29], adopted by the Committee of Ministers on 9 July 2014, identifies distinct activities that constitute 'trafficking in human organs'. The central concept is 'the illicit removal of organs', which consists of removal without the free, informed and specific consent of a living donor; removal from a deceased donor other than as authorised under domestic law; removal when, in exchange, a living donor (or a third party) has been offered or received a financial gain or comparable advantage; or removal from a deceased donor when a third party has been offered or received a financial gain or comparable advantage. The CD-P-TO has collected evidence on the different illicit and unethical activities identified in the field of tissues and cells, involving lack of consent, falsification of documents, commercialisation of tissues, processing, transport and storage of tissues in illegal facilities, etc.

A document has been elaborated to raise awareness among Council of Europe decision-making bodies of the necessity to explore the need for an additional protocol to define, prevent and combat illicit activities in the chain of donation to clinical application of human tissue and cells, and to protect donors and recipients [30].

As a result, there is Recommendation 2173 (2020) on 'Combating trafficking in human tissues and cells' from the Council of Europe Assembly [31].

The CD-P-TO has evaluated the risks of commodification of SoHO in the current European setting [32]. The outcome is a position statement setting out a series of proposals aimed at ensuring that the ethical principles of voluntary and unpaid SoHO donation and the non-commercialisation of the human body are respected, that the sustainability of healthcare systems is protected and that SoHO continue to be available (and accessible to patients), even in the context of innovative therapies that may, on occasion, need to be regulated under different regulatory frameworks.

Other major resolutions and recommendations in the field of tissues and cells include:

- Resolution (78) 29 of the Committee of Ministers on Harmonisation of legislation of MS relating to removal, grafting and transplantation of human substances [33];
- Recommendation No. R (94) 1 of the Committee of Ministers to MS on human tissue banks [34];
- Recommendation No. R (98) 2 of the Committee of Ministers to MS on provision of haematopoietic progenitor cells [35];
- Recommendation Rec(2004)8 of the Committee of Ministers to MS on autologous cord blood banks [36];
- Recommendation Rec(2006)4 of the Committee of Ministers to MS on research on biological materials of human origin [37];
- Recommendation CM/Rec(2020)5 on the quality and safety of tissues and cells for human application [38];
- Recommendation CM/Rec(2020)6 on establishing harmonised measures for the protection of haematopoietic progenitor cell donors [39];
- Recommendation CM/Rec(2022)19 of the Committee of Ministers to MS on establishing measures for the harmonised collection of activity data related to the availability and use of tissues and cells of human origin with a view to supporting self-sufficiency and facilitating international data sharing [40];
- Recommendation CM/Rec(2024)8 of the Committee of Ministers to MS on the family approach to discuss deceased organ and tissue donation [41].

Monitoring of practices in CoE MS has become an evident need for the sake of transparency and international benchmarking. Keeping this goal in mind, since 1996 the EDQM/CoE has published *Newsletter Transplant* [42], which is co-ordinated by the Organización Nacional de Trasplantes (ONT) in Spain. This publication summarises comprehensive data (provided by national focal points designated by governments) on organ, tissues and cells donation and transplantation activities, management of waiting lists, organ-donation refusals and authorised centres for transplantation activities. *Newsletter Transplant* provides information from ≈ 70 countries, including CoE MS, observer countries and observer networks (e.g. Iberoamerican Donation and Network Council on Organ Donation and Transplantation, Mediterranean Network). The *Newsletter Transplant* database is connected with other international projects on data collection (e.g. WHO Global Observatory on Organ Donation and Transplantation, Eurocet database) [43] to avoid duplication of efforts. *Newsletter Transplant* has evolved into a unique official source of information that continues to inspire policies and strategic plans worldwide.

Achieving self-sufficiency based on voluntary unremunerated donation and security of supply, as well as timely and equitable access to safe SoHO therapies, are important national and European goals. To ensure rational, fair, timely and equitable access to safe therapies based on the use of SoHO of human origin, governments need to know how many SoHO are available and how many are required for their populations so the transplantation requirements can be consistently met and an appropriate level of funding to support donation programmes is maintained. This information is also essential

in order to avoid over-reliance on a limited number of providers or countries, as well as to prepare for risk scenarios that might affect supply.

Furthermore, having accurate activity data is also essential in order to put into context the figures related to serious adverse events and reactions related to the use of tissues and cells of human origin. In an attempt to streamline and harmonise data-collection exercises in Europe, in the framework of a co-operation Grant Agreement between the European Commission and the EDQM/CoE and with the support of the main professional societies and existing registries in the field, the EDQM co-ordinated the identification of a minimum dataset that would serve the abovementioned objectives [44]. The exercise entailed reaching an agreement on parameters, units and expected quality of the data to be collected, as well as making recommendations on who should be accountable for the collection and validation of these data and ensuring dissemination among all relevant stakeholders.

Besides the *Guide to the quality and safety of tissues and cells for human application*, the Council of Europe also produces the *Guide to the quality and safety of organs for transplantation* and the *Guide to the preparation, use and quality assurance of blood components* [2].

In addition, the CoE has also produced various booklets to provide guidance to the general public on certain aspects of SoHO donation [45]:

- Umbilical cord blood banking (2nd edition, 2016)
- Donation of oocytes, a guide for women to support informed decisions (2018)
- Fertility preservation: A guide for people facing an illness or life events that may affect their fertility (June 2021)
- Tissue donation – Everything you need to know (2022)
- Corneal transplants – All you need to know (2023)
- "Add-ons" in medically assisted reproduction treatments (2025).

1.5.2 European Union

Article 168 (4)(a) of the Treaty on the Functioning of the European Union [46] (previously Article 152 of the Treaty of Amsterdam) gives the EU a mandate to establish high quality and safety standards for SoHO.

The EU has published the regulation on SoHO – Regulation (EU) 2024/1938 of the European Parliament and of the Council of 13 June 2024 on standards of quality and safety for SoHO intended for human application and repealing Directives 2002/98/EC and 2004/23/EC ('new SoHO Regulation') – which applies from 7 August 2027, with an extended one-year period for the implementation of certain provisions [1].

This regulation came after an evaluation of the previous EU Directive 2004/23/EC, which confirmed that while the Tissue and Cells Directives (still in force at the time of publication of this current Guide [47-52]) had improved the safety and quality of tissues and cells used for transplantation or MAR, a number of gaps and shortcomings were identified and revision was required to ensure that the legal framework was able to be kept up-to-date, fit for purpose and future-proof. The new SoHO regulation aims also to strengthen donor protection and oversight by authorities, to facilitate innovation in the field and to support measures in EU MS to achieve sufficiency and sustainability of supply.

This regulation merges the basic acts into a single instrument and updates the legislation in the direction of a more flexible alignment with scientific and technological developments, tackles the (re-)emergence of communicable diseases, including lessons learnt from the Covid-19 pandemic, and removes from legislation many technical provisions, which will allow a faster update of standards.

This regulation also aims to ensure better protection for donors and recipients, as well as for children born following MAR. The text adopted broadens the scope of SoHO to include human milk and intestinal microbiota. It also aims to future-proof the EU's legislation by covering other SoHO that may be applied to humans in the future and by allowing more flexible future updates. It should be mentioned that all SoHO that are intended to be applied to humans fall within the scope of this regulation.

With this legislation, entities applying SoHO to SoHO recipients are subject to provisions concerning traceability, reporting activity data and notifying adverse reactions or events, where relevant, and provisions concerning the monitoring of clinical outcomes when applying SoHO in the context of a plan for SoHO preparation authorisation. There are also obligations not to apply SoHO unnecessarily and to obtain SoHO recipient consent.

For EU/European Economic Area (EEA) countries, SoHO Regulation 2024/1938 also elevates the technical guidelines produced by expert bodies such as EDQM and the ECDC and considers the guidance produced by these bodies as an appropriate means to demonstrate compliance with the regulation and the standards thereof to ensure high levels of quality, safety and effectiveness.

SoHO national authorities are involved in the process of establishing those guidelines through their participation in the governance bodies of both ECDC and EDQM. CoE MS should be able to adopt other guidelines as a reference for SoHO entities located in their territory. When adopting such other guidelines, EU MS should verify and document that those guidelines achieve compliance with the standards set by the EU regulation. In cases of detailed technical issues for which neither Union legislation nor the ECDC and the EDQM, nor other guidelines, have defined a technical guideline or rule, SoHO entities should apply a locally defined rule that is in line with relevant internationally recognised guidelines and available scientific evidence and is appropriate to mitigate any risk identified.

In order to promote a co-ordinated application of this regulation, a SoHO Coordination Board (SCB) has been set up. The Commission and the EU MS are actively participating in its activities and co-chairing it. The SCB

contributes to co-ordinating the application of this regulation throughout the Union, including by helping EU MS to conduct SoHO supervisory activities. The SCB is composed of persons appointed by the EU MS based on their roles and expertise in their SoHO Competent Authorities (CAs) and also involves experts that are not working for SoHO CAs, for specific tasks where access to necessary in-depth technical expertise in the field of SoHO is required.

With the aim to limit the administrative burden on SoHO CAs and the Commission, the 'EU SoHO Platform' has been established to facilitate timely submission of data and reports. The EU SoHO Platform contributes to improving transparency of reporting and SoHO supervisory activities and to the exchange of information between relevant parties, including decisions on the regulatory status of substances, products or activities. Furthermore, this platform also serves as a reliable source of information for the general public regarding the work of the SCB, SoHO national authorities, expert bodies, including EDQM and ECDC, and SoHO entities. The SoHO Platform will be further used for the sharing of best practice as documented and published by the SCB on SoHO supervisory activities.

As from 25 May 2018, the new General Data Protection Regulation 2016/679 (GDPR) entered into force, repealing Directive 95/46/EC [53]. The regulation is directly applicable in all EU MS and regulates the processing of personal data relating to individuals in the EU by an individual, a company or an organisation. This Regulation applies to companies or organisations that process personal data as part of the activities of one of their branches established in the EU; it also applies to a company or organisation established outside the EU, but processing data of data subjects in the EU in the context of offering products or services in the EU or monitoring behaviour of data subjects in the EU. It does not apply, however, to the processing of personal data of deceased persons, anonymised data or legal persons.

In addition to this legislative framework, the European Commission has, throughout the years, supported the different EU MS and its stakeholders in their efforts to implement EU legal requirements related to SoHO activities, providing funding for several initiatives under the Programme of Community Action in the Field of Health. Some of the projects supported include the following.

- EQSTB (European Quality System for Tissue Banking) focused on four main work packages: (i) identification of the key requirements for tissue banking; (ii) development of a registry to support exchange of tissues; (iii) provision of training programmes, both online and face-to-face, to fulfil the needs of tissue and/or cell establishment professionals; and (iv) development of an audit model and audit guide for tissue and/or cell establishments, with recommendations for tissue and/or cell establishments and guidance for auditors.
- EUSTITE (European Standards and Training in the Inspection of Tissue Establishments) [54] developed guidance and training courses for EU CAs on the inspection of tissue and/or cell establishments and on vigilance procedures for tissues and cells used in transplantation and in assisted reproduction. The guidance document served as a basis for the guidelines on implementation of inspection and control measures in the field of human tissues and cells included in Commission Decision 2010/453/EU of 3 August 2010.
- POSEIDON (Promoting Optimisation, Safety, Experience sharing and quality Implementation for Donation Organisation and Networking in unrelated haematopoietic stem cell transplantation in Europe) provided recommendations for improvements in the safety of unrelated haematopoietic progenitor cell transplantation, for the optimisation of human stem-cell donation policy, and for promoting equal access to this therapy throughout the EU.
- EUROCET [55] is a platform that was funded initially by the European Commission but is now maintained by the Italian National Transplant Centre. It collects and publishes annual activity data on donation, processing and human application of tissues and cells. However, the Tissue Establishment Registry has been temporarily suspended in order to avoid confusion with the official EU Tissue Establishment Compendium.
- EuroGTP (European Good Tissue Practices) [56] developed a guide to good tissue practice and personnel training guidelines for tissue establishments on the recovery, processing and preservation of tissues, to help ensure that all tissue establishments can achieve the highest level of quality and safety of tissues for human application. EuroGTP has provided a crucial basis for much of the technical content of this Guide.
- The project SoHO V&S (Vigilance and Surveillance of Substances of Human Origin) [57] addressed the harmonisation of terminology and documentation relating to adverse events and reactions. It aimed to find a consensus on how information should be exchanged between EU MS, the European Commission and third countries to enhance efficient management of incidents involving cross-border distribution of tissues and cells. The project drafted important guidance documents for the EU CAs, on the detection and investigation of suspected illegal and/or fraudulent activity related to tissues and cells, the communication and investigation of serious adverse events and reactions associated with human tissues and cells, and vigilance and surveillance in the field of assisted reproductive technologies. The project also prepared a guidance document for healthcare professionals on vigilance and surveillance of human tissues and cells. It also provided a training model for CAs in the investigation and management of vigilance and surveillance of tissues and cells.

- The joint action ARTHIQS (Good Practice on Donation, Collection, Testing, Processing, Storage and Distribution of Gametes for Assisted Reproductive Technologies and Haematopoietic Stem Cells for Transplantation) [58], launched in 2014, was a 3-year project to build institutional and inspection guidelines for assisted reproductive technologies as well as guidelines related to the set-up and regulation of haematopoietic stem cell donor follow-up registries and banking of cord blood.
- The joint action VISTART (Vigilance and Inspection for the Safety of Transfusion, Assisted Reproduction and Transplantation) [59], launched in 2015, aimed at promoting and facilitating the harmonisation of inspection, authorisation and vigilance systems for blood transfusion and tissues and cells for human application.
- The project EuroGTP I (Good Tissue Practices for demonstrating safety and quality through recipient follow-up) [60] and EuroGTP II (European Good Tissue Practices part II) [61] developed good practice guidance for tissues establishments for evaluation of safety, quality and efficacy of tissue and cellular therapies and products. The key outputs included a systematic and interactive assessment tool [62] to identify and evaluate risks and the methodologies to mitigate these risks when novelties are introduced.
- The joint action European Cornea and Cell Transplant Registry (ECCTR), launched in 2016, aimed to develop a common assessment methodology, based on the three existing European corneal transplant registries in the Netherlands, Sweden and the UK, and establish a web-based European registry to assess and verify the safety, quality and efficacy of ocular tissue transplantation [63].
- The EU-funded project TRANSPOSE (TRANSfusion and transplantation: PrOtection and SElection of donors) [64] was launched in 2017 and aimed at harmonising European donor selection and protection policies, while maintaining adequate health and safety protection of the recipient.
- The joint action GAPP (Facilitating the Authorisation of Preparation Process for blood, tissues and cells) [65] was launched in 2018 and aimed at facilitating the development of a common and optimal approach to assessing and authorising preparation processes in blood and tissue and/or cell establishments, adapting requirements as prescribed by Article 29 of Directive 2002/98/EC and Article 28 of Directive 2004/23/EC.
- SAVDON (2018-2021) – a project to set up infrastructure to report serious events and adverse reactions for donors donating haematopoietic progenitor cells and to analyse a pathway to import haematopoietic progenitor cells to EU MS.
- EuMAR (European monitoring of Medically Assisted Reproduction) [66] was launched on 10 March 2023 and is aimed at building on these national-level registries and developing a pan-European registry of prospective cycle-by-cycle data on the use and outcomes of MAR treatments.
- EGALiTE (European Group for Accreditation and Liaison of Blood-Tissues and Cells Establishments) [67] (2022-2024) – a project to enhance the ability of SoHO organisations to respond during a crisis, to ensure supply of essential SoHO and to define technical assistance programmes.
- EuroTraCTOR (European Transplant and Cellular Therapy Online Registry) [68] (2023-2026) – a project to develop the European Transplant and Cellular Therapy Online Registry. This new European Society for Blood and Marrow Transplantation (EBMT) registry will be built based on the needs identified by haematopoietic stem cell transplant professionals and National Competent Authorities (NCAs).
- GAPP PRO (Piloting GAPP model approach for assessing and authorising novel SoHO preparation PROcess) [69] (2024–2027) – a joint action to promote standard specifications of a product's preparation processes and a common approach for the authorisation procedures, which are still very different among EU MS.
- ReaderSHip (2023-2025) – a project to map out organisational models for administrative and legal support for the use of SoHO in EU hospitals and to develop recommendations and guidance documents on the optimal organisational management of SoHO in hospitals.
- SightSoHO [70] (2022–2026) – a project providing training activities to further encourage and promote: the "formal" recognition of the commonalities among blood, tissues and cells (BTC) oversight processes and the adoption of a common approach for the evaluation and inspection of SoHO; the harmonisation and standardisation of some key minimum competencies/knowledge of SoHO inspectors, assessors and vigilance officers; and the strengthening of the existing network to foster trust between EU inspectorates and CAs to allow mutual recognition of certifications and authorisations of BTC establishments.
- IMAGINE-HMB (Implementation of human Milk hArmonized Guidelines for Infant Nutrition in Europe) [71] (2024–2025) – a project conducting research and producing EU-wide guidelines on human milk and human milk bank practice, the IMAGINE-HMB Project aims to constitute an EU Forum of Experts in human milk.
- HARMONIZE FMT [72] (2025–2026) – a project dedicated to the promotion of expert groups, activities and guidelines that secure the preparation, distribution and application of safe and effective faecal microbiota transfer/transplantation (FMT) preparations in the EU.

These projects have strengthened collaboration among Health Authorities, and between these Health Authorities and the professional associations in the

area of tissues and cells for human application, allowing continuous input from field practice into the regulatory framework.

1.5.3 World Health Organization

In 1987, the 40th World Health Assembly (WHA), concerned about the trade for profit in human organs, initiated preparation of the first WHO Guiding Principles on transplantation, endorsed by the Assembly in 1991 in resolution WHA 44.25 [73]. These Guiding Principles have greatly influenced professional codes and practice as well as legislation around the world for decades. After a consultation that took several years, on 21 May 2010 the 63rd WHA adopted resolution WHA 63.22 [74], which endorsed the updated WHO Guiding Principles on human cell, tissue and organ transplantation [75] and called on WHO MS to implement these Guiding Principles, promote voluntary and unremunerated donation, oppose trafficking and promote transparent and equitable allocation. It also urged its members to strengthen oversight, to collect and publish activity data, including adverse events and reactions, and to implement globally standardised coding. These WHO guidelines are intended to provide an orderly, ethical and acceptable framework for the acquisition and transplantation of human cells, tissues and organs for therapeutic purposes.

The WHA adopted resolution WHA 57.18 [76] in 2004, which urged WHO MS to take measures to protect the poorest and vulnerable groups from transplant tourism and the sale of tissues and organs, including attention to the wider problem of international trafficking in human tissues and organs. Subsequently, the Declaration of Istanbul on Organ Trafficking and Transplant Tourism [10] was adopted in 2008, as an initiative of The Transplantation Society (TTS) and the International Society for Nephrology (ISN). In 2018 it was updated to incorporate revised definitions and briefer and more comprehensive principles to provide up-to-date guidance and practical advice for health professionals, policy makers and law-enforcement authorities. The declaration emphasises that organ trafficking and transplant tourism should be prohibited because they violate the principles of equity, justice and respect for human dignity, targeting impoverished and otherwise vulnerable donors and inexorably leading to inequity and injustice.

United Nations Resolution 71/322, adopted by the WHO General Assembly on 8 September 2017, aimed at strengthening and promoting effective measures and international co-operation on organ donation and transplantation to prevent and combat trafficking in persons for the purpose of organ removal and trafficking in human organs [77].

Robust bi-directional donor–recipient traceability is a prerequisite to achieving effective vigilance and surveillance worldwide. For this reason, Resolution WHA 63.22 [74] also urged WHO MS to collaborate in collecting data (including adverse events and reactions) in addition to implementation of globally consistent coding systems. The Resolution also requested WHO to continue collecting and analysing global data related to the transplantation of 'medical products of human origin' (MPHO), and to facilitate access by WHO MS to appropriate information including severe adverse occurrences. The Notify project was a specific follow-up action that was led by the Italian National Transplant Centre (CNT) and WHO to promote the sharing of information on adverse incidents for improving safety and efficacy [78].

As a result of resolutions WHA 57.18 and WHA 63.22 (which requested that global data on the practice, safety, quality, efficacy and epidemiology of transplantations be collected in the WHO MS that have transplant programmes), an international watchdog on transplantation was set up as a collaborative initiative between the Spanish ONT and WHO and was termed the Global Observatory on Donation and Transplantation [79]. The universal availability of these data is recognised as a prerequisite for global improvements in demonstrating transparency, equity and compliance, and for monitoring systems. In addition, the data provided also help to give an overview of the legal and organisational aspects in very different settings and countries, which enables regulating bodies to monitor transplantation activities.

The WHO has also published two *aides-mémoire* specifically on the donation and transplantation of tissues and cells [75, 80].

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Related material

Appendix 1. A brief history of the preparation of tissues and cells for human application
Appendix 2. Acronyms and other abbreviations
Appendix 3. Glossary
Appendix 37. Active members of the working group for the elaboration of the *Guide to the Quality and Safety of Tissues and Cells for Human Application* (6th edition) and other members and contributors
Appendix 38. Members of the European Committee (Partial Agreement) on Organs, Tissues and Cells (CD-P-TO)

Part A.

General requirements

Chapter 2. Quality management system

2.1 Quality management system

This chapter outlines the general principles of a quality management system (QMS). The quality, safety and effectiveness of Substances of Human Origin (SoHO) and of SoHO preparations are achieved through compliance with requirements at four levels:

a. the legal framework;
b. the QMS, which is a management method to ensure that SoHO consistently comply with standards and Good Practice Guidelines (GPGs);
c. the technical requirements which are specific to each type of SoHO and which ensure quality, safety and effectiveness, as detailed in this Guide; and
d. the authorisations in place for the specific activities, from the relevant Health Authority (HA).

The following non-exhaustive list of relevant standards and legal instruments includes tools to support the construction of a robust and efficient QMS:

- Regulation (EU) 2024/1938 of the European Parliament and of the Council of 13 June 2024 on standards of quality and safety for Substances of Human Origin (SoHO) intended for human application and repealing Directives 2002/98/EC and 2004/23/EC [1] – which sets the standards of quality and safety for SoHO intended for human application; these requirements are legally binding in EU member states (EU MS).
- European Directorate for the Quality of Medicines & HealthCare (EDQM) Guide: the guidelines are based on a QMS approach. They form the basis of good practice and have been developed for use in the implementation of a QMS.
- European Centre for Disease Prevention and Control (ECDC) guidelines on the prevention of transmission of communicable diseases through Substances of Human Origin [2].
- FACT–JACIE International Standards for haematopoietic cellular therapy product collection, processing and administration, published by the Foundation for the Accreditation of Cellular Therapy and the Joint Accreditation Committee of the International Society for Cellular Therapy and the European Society for Blood and Marrow Transplantation [3].
- NetCord-FACT International Standards for cord blood collection, banking and release for administration [4].
- European Society of Human Reproduction and Embryology (ESHRE) Guidelines for good practice in *in vitro* fertilisation (IVF) laboratories [5].
- World Marrow Donor Association (WMDA) International Standards for Unrelated Haematopoietic Stem Cell Donor Registries [6].
- International Organization for Standardization (ISO) requirements, as addressed in the ISO 9001 QMS standards. ISO standards have been developed to assist organisations of all types and sizes to implement and operate effective QMS [7].

2.2 Applying a quality management system

Quality management is a wide-ranging concept that covers activities from donor registration, through donor history review and medical examination, testing, collection, processing, quality control, storage, release, distribution, import, export, human application to the patient and clinical outcome registration, and tasks that individually or collectively influence the quality, safety and effectiveness of SoHO. It is the sum total of the organised arrangements made with the objective of

ensuring that SoHO are of the quality required for their intended use. Quality management incorporates good practices.

Standards

S2.1 SoHO entities must establish, maintain and update a QMS that is appropriate, taking into account their SoHO activities, and that achieves a high level of quality of SoHO (Art. 37.1).

S2.2 SoHO entities must design their QMS in a way that ensures that SoHO activities are carried out in a consistent manner, by personnel that have documented and periodically assessed competence to perform the tasks allocated to them and in facilities that are designed and maintained in a manner that prevents SoHO contamination, or cross-contamination between SoHO, or loss of traceability. In that respect, SoHO entities must take into account the technical guidelines for quality management published by the EDQM, together with the EDQM good practice guidelines, as indicated on the EU SoHO Platform. Alternative approaches to the design of the QMS may be applied where SoHO entities can demonstrate to their SoHO competent authorities that they achieve an equivalent level of quality (Art. 37.2).

Good Practice Guidelines

G2.1 SoHO entities should prepare, document and maintain a QMS that assures compliance with those Standards and GPGs documented in this Guide, with any national (and, for EU MS, any EU) legislation that is/are pertinent to their activities.

G2.1.1 Where an entity is formally associated with a parent registered entity, they may adopt the QMS of that entity. Where this is done, a service level agreement (SLA) should be in place, detailing the responsibilities of each party.

G2.2 The QMS should be documented. A quality manual or equivalent documents should be established and contain a description of the QMS (including management responsibilities).

G2.3 The QMS should be designed to assure the quality, safety and effectiveness of SoHO, as well as to ensure donor and staff safety and robust end-user service. This strategy requires the development of clear policies, objectives and responsibilities.

G2.4 The design of the QMS should incorporate risk management principles, including the use of appropriate tools (see also Chapter 4, Risk management).

G2.5 The QMS should ensure that all critical processes are specified in appropriate procedures or instructions, that they are carried out in accordance with the standards and GPGs of this Guide, and that they comply with appropriate regulations and other guidelines as set out in the chapters in this Guide.

G2.6 All processes, procedures and instructions should be defined clearly and reviewed periodically at defined intervals and also systematically in the light of new knowledge and be shown to be capable of consistently delivering SoHO that meet(s) defined quality and safety specifications. This strategy requires that critical steps and significant changes to the process are identified and verified or validated.

Recommendations

R2.1 It is recommended that the basic concepts of quality management, good practice and quality risk management be interrelated and interconnected. They are described here in order to emphasise their relationships and their fundamental importance to all activities.

R2.1.1 A good QMS may address quality management under the following topics:

a. personnel and organisation;
b. premises;
c. equipment, materials and software;
d. outsourced activities management (contractual/service level agreements);
e. processes and procedures;
f. registrations;
g. quality control;
h. quarantine and release;
i. change management;
j. traceability;
k. complaints;
l. investigation and reporting of deviations, adverse events and reactions;
m. recall;
n. self-assessment, internal and external audit;
o. validation, verification and qualification;
p. quality risk management;
q. continuity planning;
r. tools for continuous quality improvement.

Supplementary information

Entities that perform activities for an establishment only on an occasional basis, such as a collection centre conducting a single SoHO collection, do not need to register as an entity when they are formally associated with a parent registered entity and are covered by that entity's QMS. An SLA documenting roles and responsibilities ensures adequate oversight. Nonetheless, compliance with national legislation is mandatory and takes precedence.

2.3 Personnel and organisation

Standards

S2.3 SoHO entities must put in place procedures and specifications covering, where applicable to their SoHO activities, the documentation of roles, responsibilities of personnel and organisation (Art. 37.3.a).

Good Practice Guidelines

G2.7 Quality should be recognised as the responsibility of all persons involved in SoHO activities, with management ensuring a systematic approach towards quality and the implementation and maintenance of a QMS.

G2.8 Key personnel, including the responsible person, physician, releasing officer and quality manager, are responsible for achieving this quality objective. This requires the participation and commitment of staff, across different departments and at all levels in the organisation, as well as the involvement of the organisation's suppliers and distributors.

G2.9 Key personnel have the ultimate responsibility for ensuring that an effective QMS is in place and resourced adequately, and that roles and responsibilities are defined, communicated and implemented throughout the organisation.

G2.10 There should be an organisation chart in which the relationships between key personnel and other staff are clearly shown in the managerial hierarchy.

G2.11 All personnel should have up-to-date job descriptions, which clearly set out their tasks, roles and responsibilities.

G2.12 Personnel should be available in sufficient numbers to carry out the activities undertaken by their SoHO entity and they should be qualified, trained and competent to perform their tasks.

G2.13 Key personnel should establish a quality policy that describes the overall objectives and vision of the organisation in relation to quality. They should ensure the QMS and governance of good practice through reviews of the performance of the QMS at periodic intervals so as to verify its effectiveness, and they should ensure continual improvement of all processes affecting the quality, safety and effectiveness of SoHO and the QMS itself, and introduce preventive and corrective measures if deemed necessary. Key personnel are also responsible for regulatory supervision to ensure compliance with all legal and regulatory frameworks.

G2.14 Key personnel should have adequate authority to carry out their tasks and responsibilities. Their tasks and responsibilities should be delegated to designated deputies in the event of prolonged absence. There should be no gaps or unexplained overlaps in the responsibilities of key personnel.

G2.15 Key personnel may delegate selected tasks to other personnel who should be qualified and competent by training and experience to perform such tasks. In such cases, this person will carry out these tasks under the responsibility of the key person delegating these tasks. All parties involved should be aware of, and agree to, the delegations made.

Recommendations

R2.2 It is recommended that the delegation of tasks and/or responsibilities of key personnel be documented in procedures, job descriptions, agreements, digital systems or other types of documents.

Supplementary information

It is acknowledged that, in practice, separating responsibilities of key personnel can be challenging in smaller SoHO entities and establishments, where limited staffing may require individuals to fulfil the roles of responsible person, physician, releasing officer and quality manager concurrently, provided that the individual meets all applicable qualification or experience requirements as laid down in those standards and GPGs (G2.9).

2.3.1 Responsible person

Standards

S2.4 Every SoHO entity must appoint a person responsible, within their entity, for ensuring that SoHO activities carried out by the SoHO entity comply with the requirements of the SoHO Regulation applicable to those SoHO activities (Art. 36.1).

S2.5 The responsible person must be in possession of a diploma, certificate or other evidence of formal qualifications in the field of medical, pharmaceutical or life sciences awarded on completion of a university course of study, or a course recognised as equivalent by the MS concerned, and must have at least two years of experience in the relevant field (Art. 36.2).

S2.6 Every SoHO entity must inform their competent authority (CA) of the name and contact details of the responsible person. Where the responsible person is permanently or temporarily replaced, SoHO entities must without undue delay inform their SoHO CA of the name and contact details of the new responsible person and the date on which that person assumed responsibility (Art. 36.3).

Good Practice Guidelines

G2.16 The responsibilities of the responsible person, or their designated replacement, should include (but are not limited to):

G2.16.1 ensuring that all activities carried out, in the SoHO entity or establishment for which that person is responsible, are performed in accordance with the standards and GPGs of this Guide and with national (and, for EU MS, EU) legislation;

G2.16.2 providing information to the HA on any substantial changes to the SoHO entity's activities and its management;

G2.16.3 implementing appropriate control measures to ensure compliance with the standards

and GPGs of this Guide and other guidelines if applicable and with national (and, for EU MS, EU) legislation;

G2.16.4 keeping accurate and comprehensive records of activities of their SoHO entity or establishment;

G2.16.5 ensuring that the HA is notified of any serious adverse event and/or reaction and is provided with a report analysing the cause and the outcome;

G2.16.6 implementing and maintaining a QMS based on the GPGs of this Guide;

G2.16.7 providing training and qualifying personnel directly involved in the SoHO entity's activities.

2.3.2 Physician

Standards

S2.7 Each SoHO establishment must appoint a physician who carries out their tasks in the same MS and who must at least fulfil the following conditions and have the following qualifications:

a. possession of formal qualification as a physician;

b. at least two years' practical experience in the relevant field (Art. 50.1.b).

S2.8 The physician must be responsible for at least the following tasks (Art. 50.2):

a. development, review and approval of procedures for establishing and applying SoHO donor eligibility criteria, procedures for SoHO collection and criteria for the allocation of SoHO;

b. supervision of the implementation of procedures referred to in point (a) when they are carried out by SoHO entities contracted by the SoHO establishment;

c. the clinical aspects of investigation of suspected adverse reactions in SoHO donors, SoHO recipients and offspring from medically assisted reproduction from the perspective of the SoHO establishment;

d. design and supervision, in collaboration with treating physicians, of clinical-outcome monitoring plans to generate evidence required to support applications for SoHO preparations authorisations;

e. other tasks relevant to the health of SoHO donors, SoHO recipients and offspring from medically assisted reproduction in relation to SoHO collected or supplied by the SoHO establishment.

S2.9 The physician may delegate the tasks referred to in S2.8 to other persons who must be qualified by training and experience to perform such tasks. In such cases, those persons must perform those tasks under the responsibility of the physician (Art. 50.3).

S2.10 By way of derogation from S2.9, where SoHO entities are authorised as SoHO establishments by their national HA, the physician must be responsible for those tasks that are relevant to the SoHO activities performed by the SoHO entities and that have a direct influence on the health of SoHO donors, SoHO recipients and, where relevant, offspring from medically assisted reproduction (Art. 50.4).

2.3.3 Releasing officer

Standards

S2.11 In cases where a SoHO establishment releases SoHO, it must appoint one or more releasing officers (Art. 49.1).

S2.12 The releasing officer must be in possession of a diploma, certificate or other evidence of formal qualifications in the field of medical, pharmaceutical or life sciences awarded on completion of a university course of study, or a course recognised as equivalent by the MS concerned and must have at least two years of experience in the relevant field (Art. 49.2).

S2.13 The releasing officer may delegate the task of releasing SoHO as referred to in S2.11 to other persons who must be qualified by training and experience to perform such a task. In such cases, those persons must perform that task under the responsibility of the releasing officer who will always be responsible for the release (Art. 49.3).

S2.13.1. The responsibility for releasing SoHO may be delegated to an alternate in the event of short-term absence of the releasing officer, provided that the alternate meets the requirements set out in S2.12 (Art. 49.3).

Good Practice Guidelines

G2.17 The responsibilities of the releasing officer should include:

G2.17.1 approval of procedures for releasing SoHO (see Chapter 13);

G2.17.2 ensuring that all release criteria are verified and documented prior to release.

2.3.4 Quality Manager

Good Practice Guidelines

G2.18 SoHO entities should designate one or more persons to design, implement and manage a QMS.

G2.19 The quality manager(s) support(s) the responsible person establishing, implementing and maintaining a QMS.

Recommendations

R2.3 It is recommended that processing and quality control activities be independent of each other, to ensure effective and reliable evaluation of pro-

cesses; and these two types of activity are preferably carried out by separate personnel. However, it is recognised that in practice this separation of responsibilities may be difficult to accomplish in smaller SoHO establishments, where due to staffing constraints individuals may have to perform both processing and quality-control activities. It is, however, recommended that individuals do not take responsibility for quality-control checking of activities that they themselves have performed.

2.3.5 Training and qualification of staff

Standards

S2.14 SoHO entities must put in place procedures and specifications covering, where applicable to their SoHO activities, the selection, training and competence assessment of personnel (Art. 37.3.b).

Good Practice Guidelines

G2.20 SoHO entities should implement policies and procedures to describe the approach to selection, training, re-training, qualification and disqualification of personnel.

G2.21 Personnel involved in SoHO activities should be qualified, trained and competent by defined procedures and documented as such.

G2.22 SoHO entities should specify the competence requirements for each function and/or role, including requirements for education, training, re-training, technical knowledge, skills, experience and competence assessment.

G2.23 Personnel should be provided with initial/basic training, re-training as required (when procedures change or when scientific knowledge develops) and adequate opportunities for relevant professional development. The training programme should ensure and document that each individual:

G2.23.1 has demonstrated competence in the performance of their designated tasks and responsibilities;

G2.23.2 has an adequate knowledge and understanding of the scientific/technical processes and principles relevant to their designated tasks and responsibilities;

G2.23.3 understands the organisational framework, QMS and health and safety rules of the entity/establishment in which they work; and

G2.23.4 is adequately informed of the broader ethical, legal and regulatory context of their work.

G2.24 Training, re-training and competence assessment records should be maintained, taking into account the requirements of the General Data Protection Regulation (GDPR).

G2.25 The competence of personnel should be evaluated periodically, and the effectiveness of training programmes should be periodically assessed.

Supplementary information

A training programme may include the following information:

- minimal education requirements for each function;
- training needs; These may include but are not limited to: training in the organisational framework, the quality principles, health and safety rules, ethical principles, legal and regulatory context, handling of personal data, security and data management, knowledge and understanding of the scientific and technical aspects relevant to their designated activities and tasks;
- training objectives and competence requirements for each training and re-training;
- training resources, including trainers, training facilities, training material and equipment;
- responsibilities for the implementation of the training programme, who will deliver the training, who will schedule the training, who will manage the training material and who will assess the competence of the trainee;
- qualification of the operators' aseptic techniques. Media fill tests could be used to assess the aseptic technique working competences;
- the capability of individual operators to meet the desired competences, which could be assessed through observation and tests before they are approved as qualified to carry out tasks unsupervised;
- personnel hygiene and principles of personnel health status. Where appropriate, personnel are typically asked to have relevant knowledge of, and to be constantly aware of, the importance of avoiding microbial contamination of themselves, donors, recipients and SoHO.

Training programmes and the competence of personnel are generally monitored periodically: for example, by the monitoring of quality indicators, proficiency testing, external quality assurance and internal or external audits.

Examples of competence assessment methods that can be used in any combination include:

- direct observations of an activity;
- monitoring the recordings and reporting of SoHO activities;
- review of work records;
- assessment of problem-solving skills;
- inter-personnel comparison;
- media fill tests.

In the EU, training records fall under the general employee records category of the GDPR, which emphasises that personal data could not be kept longer than necessary. A retention period of 10 years could be justified in analogy with other records that are critical to the quality, safety and effectiveness of the SoHO, starting from the moment the personnel leave(s) the SoHO entity.

2.3.6 Personnel hygiene and personnel health condition

Standards

S2.15 SoHO entities must mitigate risks arising from microbial contamination of SoHO from the personnel coming into contact with SoHO during collection, processing, storage or distribution. SoHO entities must mitigate such risks by specifying and verifying the hygiene procedures of the personnel of the SoHO entity in contact with the SoHO throughout the SoHO preparation chain (Art. 58.5.b).

Good Practice Guidelines

G2.26 There should be safety and hygiene instructions in place, as applicable, and these should be adapted to the activities and tasks to be carried out.

G2.27 There should be instructions ensuring that health conditions that can be relevant to the quality of SoHO, e.g. infectious disease or having open lesions on the exposed surface of their body, are reported by personnel. The necessary precautions should be taken to ensure fitness for work and personal health.

G2.28 Visitors or untrained personnel should, preferably, not be taken into the collection, processing and laboratory areas. If this is unavoidable, they should be given information in advance, particularly about personal hygiene and the prescribed protective clothing. They should be closely supervised.

G2.29 A written safety procedure and personal protective equipment (PPE) should be available to minimise the risks to the health of personnel and visitors.

G2.30 Eating, drinking, chewing, smoking or vaping, or the storage of food, drink, smoking materials or personal medication (with the exception of emergency medication e.g. for chronic diseases such as diabetes or epilepsy), in the collection, processing, testing and storage areas should be prohibited. In general, any unhygienic practice, within the processing areas or in any other area where the tissues or cells might be adversely affected, should be forbidden.

G2.31 Documented procedures should be in place describing the actions to be taken if an individual is directly exposed to blood, tissues or cells of a donor or their donation (e.g. needle-stick injury).

G2.31.1 These procedures might include: accelerated and extended testing of the donor, rapid testing of the staff member, and prophylaxis for the transmissible agent(s) where appropriate.

Recommendations

R2.4 It is recommended that SoHO entities and establishments define and monitor rules for personal hygiene and safety and the behaviour of personnel in the collection, processing, testing and storage areas. It is advisable to take the following risks into consideration:

R2.4.1 the use of private cell phones (mobiles) in collection and processing areas;

R2.4.2 health status of personnel and the potential impact of their personal health condition on their suitability for working with SoHOs, because compromised immunity, their vaccination status or infectious disease may increase the risk to themselves, their co-workers or the SoHO they are manipulating;

R2.4.3 poor personal hygiene, particularly regarding hair, beard and hands, and the use of personal protective equipment (PPE) when necessary;

R2.4.4 the potential for accidents that carry risks of disease transmission;

R2.4.5 risks from gas manipulation, anoxia, burns and liquid nitrogen.

Supplementary information

Personnel coming into contact with SoHO are exposed to a risk of transmission of diseases. In some cases, donors will not have been fully tested at the time of collection or initial processing and, even where they have been tested, a residual risk of infection by untested agents or infectious agents that were below the limit of detection at the time the sample was collected, remains. There may also be occasions when a donation is processed following receipt of positive test results, increasing the risk to healthcare personnel, for example when an autologous donation is assessed as being the most appropriate treatment methodology. Standard universal precautions, appropriate procedures and the use of suitable PPE all help to avoid transmission of infections.

2.4 Premises

Standards

S2.16 SoHO entities must put in place procedures and specifications covering, where applicable to their SoHO activities, the procurement, qualification, validation and monitoring of premises (Art. 37.3.c).

S2.17 SoHO entities must mitigate risks arising from microbial contamination of SoHO from the environment coming into contact with SoHO during collection, processing, storage or distribution. SoHO entities must mitigate such risks by specifying and verifying the cleanliness of collection areas, taking into account the degree of exposure of SoHO to the environment during collection, and the cleanliness of storage areas. In cases where SoHO are exposed to the environment during processing, SoHO entities must mitigate such risks by specifying, based on a structured and documented risk assessment for each SoHO preparation, validating and maintaining a defined air quality in processing areas (Art. 58.5.b.c).

Good Practice Guidelines

G2.32 Premises should be designed, located, constructed, adapted and maintained to suit the activities to be undertaken. Their layout and design should aim to minimise the risk of errors and permit activities to proceed in an orderly sequence. Their layout should also allow effective cleaning and disinfection to avoid contamination and cross-contamination, as applicable.
G2.33 SoHO entities should have floor plans or procedures which document rooms and their function.
G2.34 SoHO entities should specify appropriate air cleanliness levels to be maintained in the processing areas, based on a risk assessment and the rationale for the air cleanliness levels applied.
G2.35 When required, environmental conditions such as lighting, temperature, humidity and ventilation should be appropriate and checked to ensure safety and comfort to patients, donors and personnel, and to assure the accurate functioning of equipment.
G2.36 Premises should be secure to prevent the entrance of unauthorised people.
G2.37 A pest control system should be in place, if indicated by a risk assessment performed by the SoHO entity.
G2.38 All waste generated by the process(es) in the premises should be disposed of, in accordance with applicable laws and regulations.

Recommendations

R2.5 The minimal air cleanliness levels maintained in cleanroom areas are specified in Chapter 9 of this Guide. Nevertheless, it is recommended to use MiRCA tool in selecting the appropriate grade of air quality and cleanliness for areas involved in the aseptic processing of SoHO; see Chapter 4. Further guidance on facilities for SoHO collection, processing and storage is given in Chapters 8, 10 and 11 of this Guide.

2.5 Equipment and materials

Standards

S2.18 SoHO entities must put in place procedures and specifications covering, where applicable to their SoHO activities, the procurement, qualification, validation and monitoring of materials and equipment, including IT systems (Art. 37.3.c).
S2.19 Applications for SoHO preparation authorisation must include a description of equipment, reagents and materials coming into direct contact with the SoHO during processing and their certification status in accordance with Regulation (EU) 2017/745, where applicable, and – in the case of the use of in-house developed equipment, reagents or materials – evidence of the validation of their quality (Art. 39.2.b.v).
S2.20 SoHO entities must mitigate risks arising from microbial contamination of SoHO from the equipment and the materials coming into contact with SoHO during collection, processing, storage or distribution. SoHO entities must mitigate such risks by specifying, procuring and decontaminating equipment and materials that come into contact with SoHO during collection, processing, storage or distribution, such that their sterility, where necessary, is ensured (Art. 58.5.d).

2.5.1 Critical equipment, materials and software

Good Practice Guidelines

G2.39 SoHO entities and establishments should identify and document all critical equipment (apparatus and appliances), materials (reagents, instruments, medicines, implants, consumables) and software used for all SoHO activities in which they are involved.
G2.40 Critical equipment, materials and software should meet documented requirements and specifications and, when applicable, comply with national laws and, for EU MS, with Regulation 2017/745 of the European Parliament and of the Council of 5 April 2017 concerning medical devices [8] and Regulation 2017/746 of the European Parliament and of the Council of 5 April 2017 on *in vitro* diagnostic medical devices [9].

Supplementary information

Those equipment or materials that come in direct contact with the SoHO, or influence the quality, safety and effectiveness of the SoHO, either directly (e.g. an additive) or indirectly (e.g. donor testing kits), or provide information by means of *in vitro* examination of specimens derived from the human body, including organ, blood and tissue donations, are considered 'critical'.

In the EU, some of the critical equipment, materials and software will be considered as medical devices as laid down in Regulation 2017/745 of the European Parliament and of the Council of 5 April 2017 concerning medical devices [8] and Regulation 2017/746 of the European Parliament and of the Council of 5 April 2017 on *in vitro* diagnostic medical devices [9].

Further guidance on software is given in Chapter 15 of this Guide.

2.5.2 Equipment

Good Practice Guidelines

G2.41 SoHO entities and establishments should have equipment appropriate to the activities for which they are authorised.
G2.42 All critical equipment should be designed, located, qualified and maintained to suit its intended

purpose and should comply with the general safety requirements of this Guide and the specific requirements relevant to the type(s) of SoHO.

G2.43 Documented systems for purchasing equipment should be available. These should identify the specific requirements for establishing and reviewing contracts for the supply of critical equipment.

G2.44 Equipment should be selected to minimise any hazard to donors, personnel or SoHO.

G2.45 Equipment should be designed or selected so that it can be thoroughly cleaned and, where necessary, decontaminated. This should be performed according to documented procedures.

G2.46 Equipment should be installed in such a way as to mitigate any risk of error or of contamination.

G2.47 Fixed gas pipework should be clearly labelled to indicate the contents and, where applicable, the intended direction of flow.

G2.48 To ensure appropriate performance of equipment, a maintenance plan should include instructions for use, maintenance, cleaning, disinfection and sanitation. The plan should take into account the criticality of the equipment, and should specify monitoring, user-notification and problem-resolution mechanisms. If an unusual event is observed, personnel should follow the standard actions described in the maintenance plan. The standard actions should involve the notification of affected personnel and, where appropriate, initiation of a resolution of, or a response to, the problem. Depending on the severity of the problem and the criticality of equipment, a back-up plan should be in place and be implemented to keep the process or equipment operating.

G2.49 Where equipment affects critical collection, processing, testing or storage parameters (e.g. temperature, pressure, particle counts, microbial contamination levels), they should be identified and should be the subject of appropriate monitoring, alerts, alarms and corrective action, as required, to detect malfunctions and defects and to ensure that the critical parameters are maintained within acceptable limits at all times.

G2.50 Repair and maintenance operations should not present any hazard to the donor or staff, or to the quality, safety and effectiveness of the SoHO.

G2.51 Equipment and washing/cleaning solutions should be chosen and used so that they are not sources of contamination or toxicity.

G2.52 Distilled, deionised and, where appropriate, other water pipes should be sanitised according to a risk assessment that details the action limits for microbiological contamination and the measures to be taken according to the impact on the process.

G2.53 Records of maintenance activities should be clear and comprehensible and should detail the specific activities performed as part of maintenance.

G2.54 Defective equipment should be labelled clearly as such and, if possible, removed from processing areas.

G2.55 Apparent deviations in the quality and/or performance of equipment should be investigated and registered promptly. Outcomes of these investigations should be reported in a timely manner to the responsible person, who should consider the corrective and preventive actions to be implemented.

G2.56 The ability of a supplier to maintain its activities relating to equipment should be requalified on a regular basis, particularly to anticipate weaknesses in services or to manage changes in the system, equipment or supplier. The periodicity and detail of the requalification process should be linked to the level of risk of using the system or equipment and should be planned for each supplier.

G2.57 Serious adverse events caused by the design or use of the equipment should be reported to the manufacturer and the relevant national HA.

G2.58 All modifications, enhancements or additions to qualified equipment should be managed through the change-control procedure of the SoHO entity. The effect of each change to the system or equipment, as well as its impact on quality, safety and effectiveness of SoHO, should be determined to identify the extent of revalidation required.

Recommendations

It is recommended that:

R2.6 the maintenance of equipment be scheduled, performed and registered as defined by the manufacturer. Deviations from the manufacturer's schedules or instructions are typically specified through risk assessment or validation data and documented;

R2.7 the cleaning and disinfection intervals be generally defined through risk assessment, considering reported deviations, while also monitoring results and the validation of the cleaning and disinfection procedure.

Supplementary information

Relocating defective equipment from its environment may be an appropriate solution in certain situations. It prevents the use of defective equipment and eliminates the need for technical staff to enter the controlled environment. However, it is generally important to ensure that relocation does not cause additional damage or increase the risk of cross-contamination.

2.5.3 Calibration of measuring equipment

Good Practice Guidelines

G2.59 Measuring equipment with an appropriate range and accuracy for measuring, weighing, recording and monitoring should be available, and should be calibrated and checked at defined intervals using appropriate methods.

G2.60 A calibration plan should be in place that outlines the criteria for determining which equipment requires calibration, the acceptable tolerance limits and the recalibration frequency.

G2.61 All equipment with a critical measuring function should be calibrated against a traceable standard if available. Adequate records of such calibrations should be maintained, including the values obtained prior to any adjustment. The calibration result of each piece of critical measuring equipment or system should be traceable through a documented and unbroken chain of calibrations back to the International System of Units or to a recognised standard. Calibration reports should include the measurement uncertainty.

G2.62 If the calibration result plus/minus the measurement uncertainty falls outside the tolerance limits of the measuring equipment or system, there should be provisions for remedial action to re-establish conformity with these limits. These remedial actions should be documented. If calibration activities provide evidence that tissues or cells were processed and released for use when critical measurement equipment was not measuring accurately, risk assessment should be applied to decide on appropriate corrective or preventive actions regarding the fate of the SoHO.

Supplementary information

Calibration is an operational qualification method that confirms, under defined conditions, the relationship between values obtained from the measuring equipment or system and those obtained using an appropriate certified standard. Calibration addresses measurement uncertainty (U). 'Measurement uncertainty' refers to the closeness of agreement between a measured quantity value and the true quantity value of what is being measured. Hence, if the measured value is close to the true value, the measuring system has high accuracy [10].

Calibration can be conducted either in-house or by a third-party provider. The organisation responsible for calibration is also responsible for demonstrating traceability to the International System of Units or a recognised standard. This can be done either through accreditation of the calibration method to ISO 17025 [11] or by providing evidence of a validated calibration method, along with copies of ISO 17025 calibration certificates for the equipment used.

2.5.4 Materials, reagents and consumables

Good Practice Guidelines

G2.63 SoHO entities should have materials (reagents, instruments, medicines, implants, consumables) appropriate to the activities for which they are authorised.

G2.64 Documented systems for purchasing materials should be available.

G2.65 A controlled list should be constructed of all critical materials.

G2.66 A policy should be in place to select the use of critical materials, their characteristics, their specifications and their source if that is relevant (e.g. animal source).

G2.67 All critical materials should be designed and qualified to suit their intended purpose and should comply with the GPGs of this Guide and the specific requirements relevant to the type(s) of SoHO.

G2.68 Specifications for the critical materials in contact with SoHO should include, if applicable:

- **G2.68.1** a description of the materials, including the designated name and the internal code reference; the reference, if any, to a pharmacopoeia monograph; the approved suppliers; and, if possible, the original manufacturer of the products;
- **G2.68.2** instructions for acceptance testing or reference to procedures;
- **G2.68.3** qualitative and/or quantitative requirements with acceptance limits;
- **G2.68.4** storage conditions and precautions;
- **G2.68.5** the expiry date.

G2.69 Critical materials used to perform any evaluation of the donor should not have any negative impact on the management of the donor or the reliability of the evaluation results.

G2.70 Critical materials that come into contact with SoHO should not be reactive, additive or absorptive to such an extent that they affect the quality, safety or effectiveness of the SoHO or present any hazard.

G2.71 Instruments and devices used in SoHO activities for aseptic processes should be sterile. Instruments or devices should be of good quality, qualified or specifically certified and regularly maintained.

G2.72 If applicable, critical materials used during the collection, processing and storage of SoHO should be qualified as being:

- **G2.72.1** free of viral contamination;
- **G2.72.2** free of transmissible spongiform encephalopathy (TSE) contamination as much as possible;
- **G2.72.3** fulfilling Medical Device Regulation requirements (if classified as medical device);
- **G2.72.4** suitable for human use, when available;
- **G2.72.5** of defined identity, purity, sterility and quantification of endotoxins;
- **G2.72.6** free of human/animal origin reagents if possible;
- **G2.72.7** free of antibiotics whenever possible;
- **G2.72.8** supplied with risk assessment of potential residues in the final SoHO preparations.

G2.73 All critical incoming materials should be checked to ensure that the consignment corresponds to the order.

G2.74 Only critical materials from qualified suppliers that meet their documented requirements and specifications should be used. Batch acceptance testing or checking of each delivery of critical materials should

be carried out, and recorded, before release for use in tissue or cell activities.

G2.75 Critical materials should be stored under the conditions established by the manufacturer and in an orderly manner that permits segregation by status, batch and lot as well as stock rotation. If necessary, environmental storage conditions should be monitored.

G2.76 When critical reusable instruments need to be used, a validated cleaning and sterilisation procedure for removal of infectious agents should be in place.

G2.77 Each batch of critical materials should be traceable to the activities in which it was used. Product identification, batch number, lot number and expiry date should be recorded.

G2.78 Changes to critical materials should be managed in accordance with the principles of change control.

G2.79 Apparent deviations in the quality and/or performance of critical materials should be investigated and recorded promptly. Outcomes of these investigations should be reported in a timely manner to the responsible person, who should consider and approve the corrective and preventive actions to be implemented. Serious adverse events or reactions caused by poor-quality materials should be notified to the manufacturer and reported to the health authority.

Recommendations

R2.8 To ensure that SoHO entities and establishments use appropriate critical materials, it is recommended that when selecting critical materials, user requirement specifications (URS) be documented, setting out the minimum criteria for the critical materials and their suppliers. These criteria can form the basis for qualification of potential suppliers and acceptance testing for critical materials.

Supplementary information

The qualification of critical materials can be achieved by, for example, checking certificates of analysis, conducting internal quality control tests, sterility testing and toxicity testing. For critical reagents or medicines that are temperature-sensitive, this may be necessary with each delivery due to critical transport conditions.

The qualification of suppliers can be achieved by, for example, checking of certificates of the QMS of the manufacturer applicable to medical device manufacturing, documentary audits or external audits on the manufacturing site. Several management standards could be applicable to medical device manufacturing: for example, ISO 13485 [12] and ISO 9001 [7]. The purpose is to ensure that they can be relied upon to continue supplying the materials that meet the URS.

Based on a risk analysis or validation data, SoHO establishments could deviate from a manufacturer's storage conditions. Good inventory records and appropriate labelling could ensure traceability and prevent the use of materials after their expiry date. Storage and use of materials generally follow the 'first-in first-out' principle (i.e. the materials that entered storage first are used first) taking into account the expiry date of materials.

Further guidance on the traceability of critical materials, reagents and consumables is given in Chapter 17 of this Guide.

Further guidance on reagents and materials used in SoHO processing is provided in Chapters 8 and 10.

2.5.5 In-house medical devices within the EU

Good Practice Guidelines

G2.80 Medical devices manufactured and used within a health institution, and lacking Conformité Européenne (CE) marking, may be used within the institution only if they comply with national regulations and, for EU MS, Regulation (EU) No. 2017/745, Article 5(5) and/or (EU) No. 2017/746, Article 5(5).

G2.81 In the EU, the production and use of an in-house medical device should be notified to the national HA.

Supplementary information

Medical devices and *in vitro* diagnostic medical devices intended for the collection, processing, testing and storing of SoHO for human application is a small market for medical device manufacturers. Many manufacturers consider that the potential revenue from marketing or keeping these medical devices on the EU market no longer justifies the necessary investment to comply with the provisions of Regulations (EU) 2017/745 (medical devices Regulation, MDR) [8] and (EU) 2017/746 (*in vitro* diagnostic medical devices Regulation, IVDR) [9]. If there is no equivalent medical device on the market, SoHO establishments may be required to use equipment and materials for laboratory use, or produced under Good Manufacturing Practice (GMP) conditions, or research-use-only (RUO) devices that were not specifically designed and validated for this purpose. In these cases, the SoHO entity assumes full responsibility for their use, quality and safety.

The following applications fall under the definition of an in-house device [13]:

1. CE-marked medical devices or *in vitro* diagnostic (IVD) tests that are used for an application other than their intended use;
2. CE-marked devices or IVD tests that were substantially modified to create a new medical device;
3. Devices intended for RUO but being used for human application;
4. CE-marked devices combined with another device or another type of product (e.g. pharmaceutical product) to create a new device;

5. Devices manufactured from raw materials, from parts or components of a device or of another type of product, or from an existing device or another type of product.

In-house medical devices can be used within the health institution where they were manufactured and used, including hospitals, as well as institutions such as laboratories and public health institutes that support the healthcare system and/or address patient needs, but which do not treat or care for patients directly.

Combining multiple medical devices and/or medicines for immediate use within the same application is not considered the manufacture of a new in-house medical device because the risks are not different from separate administration or use within the same application, for example a SoHO storage medium. If the combination of multiple medical devices and/or medicines is not used immediately within the same application, the SoHO establishment could assess, based on a risk analysis, whether the risks of application have increased to such an extent that it is considered a new in-house medical device.

The EuroGTP II tool can help SoHO establishments to determine the extent of the clinical performance testing of in-house medical devices [14]. Further guidance on in-house medical devices is given in Guidance document MDCG 2023-1 on the health institution exemption [13].

2.6 Outsourced activities management

Standards

S2.21 SoHO entities must put in place procedures and specifications covering, where applicable to their SoHO activities, the management of contracted third parties (Art. 37. 3.i).

Good Practice Guidelines

G2.82 Outsourced arrangements should be clearly defined, agreed upon and monitored to prevent misunderstandings that could lead to the use of SoHO or activities of unsatisfactory quality. There should be a formal agreement outlining these arrangements, the SoHO or processes involved, and any technical provisions associated with them.

G2.83 All outsourced activities should be done in accordance with a written agreement, with reference to the specification for the tissues or cells concerned.

G2.84 The responsibilities of each party in outsourced activities should be clearly documented to ensure that the standards and GPGs are maintained.

G2.85 All agreements should be dated, reviewed and renewed when necessary.

- **The contract giver**

G2.86 The contract giver should assess the competence of the contract acceptor to successfully carry out the arrangements being outsourced.

G2.87 The contract giver should provide the contract acceptor with all the information necessary to carry out the contracted arrangements correctly and in accordance with the specification and any other legal requirements. The contract giver should ensure that the contract acceptor is fully aware of any problems associated with the equipment, materials, software, samples or contracted processes that might pose a hazard to the premises, equipment, personnel, other materials or other tissues or cells of the contract acceptor.

G2.88 The contract giver should ensure that all tissues or cells, analytical results, equipment, materials and software delivered by the contract acceptor comply with their specifications and that they have been released under a quality system approved by the Responsible Person or other authorised person.

- **The contract acceptor**

G2.89 The contract acceptor should have adequate premises, equipment, materials, software, knowledge, experience and competent personnel to satisfactorily carry out the arrangements requested by the contract giver.

G2.90 The contract acceptor should ensure that all equipment, materials, software or test results delivered by the contract giver are suitable for their intended purpose.

G2.91 The contract acceptor should not pass to a third party any of the arrangements entrusted under the contract without the contract giver's prior evaluation and approval of the arrangements. Arrangements made between the contract acceptor and any third party should ensure that the relevant information is made available in the same way as between the original contract giver and contract acceptor.

G2.92 The contract acceptor should refrain from any arrangements that may adversely affect the quality, safety and effectiveness of the tissues or cells processed and/or analysed for the contract giver.

- **The agreement**

G2.93 An agreement should be drawn up between the contract giver and the contract acceptor that specifies their respective responsibilities relating to the contracted arrangements and these responsibilities and arrangements should be agreed by both parties.

G2.94 The agreement should set out a procedure enabling the responsible person or releasing officer to verify that the contract agreement has been processed and/or distributed in accordance with the applicable GPGs, standards and regulation if applicable.

G2.95 Processing and distribution records, including reference samples if relevant, should be kept by, or be available to, the contract giver. Any records relevant to assessment of the quality of the tissues or

cells in the event of complaints or a suspected defect should be accessible and specified in the defect/recall procedures of the contract giver.

G2.96 The agreement should permit the contract giver to perform audits of the activities undertaken by the contract acceptor, if relevant.

Recommendations

It is recommended:

R2.9 to document agreements for at least the following arrangements:
- R2.9.1 suppliers of testing;
- R2.9.2 SoHO activities;
- R2.9.3 transport companies responsible for the transport of SoHO;
- R2.9.4 meeting records, communications records;
- R2.9.5 suppliers of critical medical devices and *in vitro* medical devices;
- R2.9.6 suppliers of critical services such as cleaning, disinfection or sterilisation;
- R2.9.7 suppliers of critical IT services;
- R2.9.8 suppliers of cleanroom qualification services;
- R2.9.9 suppliers of critical equipment;

R2.10 that the review of outsourced SoHO activities and supporting tasks be achieved through on-site audits, questionnaires, monitoring of quality control results, reported non-conformities, assessment of complaints or a combination of these methods.

Supplementary information

The outsourcing of arrangements to third parties could be documented in written agreements, SLA or shared procedures authorised by both parties, or terms and conditions that form part of application forms or orders.

2.7 Documentation

Standards

S2.22 SoHO entities must put in place procedures and specifications covering, where applicable to their SoHO activities, the other documentation relevant for the QMS put in place (Art 37.3.d).

Good Practice Guidelines

G2.97 Good documentation constitutes an essential part of the QMS and is key to operating in compliance with the standards and GPGs of this Guide and with national (and, for EU MS, EU) legislation. The various types of documentation used should be defined fully in the QMS of the organisation.

G2.98 The main objective of the system of documentation used should be to establish, control, monitor and record all activities and tasks that directly or indirectly impact on all aspects of the quality, safety and effectiveness of SoHO. The QMS should include sufficient instructional detail to facilitate common understanding of the requirements.

G2.99 A document-control system should be established for the periodical review, revision history and archiving of documents and to ensure that only current versions are in use.

G2.100 A periodic review process should be established to ensure that the documentation for any given process, system or equipment is complete, current and accurate.

G2.101 All document changes should be reviewed, dated, approved, documented and promptly implemented by authorised personnel. Document approval should be recorded either on paper or through an electronic system.

G2.102 Documents should be protected from unauthorised changes, deletions or removals. Handwritten or digital entries in authorised and published documents should be made in a clear, legible and indelible way, signed and dated by authorised personnel.

G2.103 Retained or archived documents should be appropriately identified to prevent their inadvertent use.

G2.104 Quality system documentation should be retained for a minimum of 10 years.

Recommendations

R2.11 Documents are recommended to include:
- R2.11.1 unique identifier;
- R2.11.2 title;
- R2.11.3 edition or current revision date or revision number;
- R2.11.4 number of pages;
- R2.11.5 name of the SoHO entity or establishment, author and authoriser.

R2.12 It is recommended that QMS documentation include the following non-exhaustive list of document types:
- R2.12.1 a quality manual, if required by national legislation or other applicable quality standards;
- R2.12.2 policies and Standard Operating Procedures (SOPs) for all activities and tasks that directly or indirectly influence the quality, safety and effectiveness of the tissues or cells, including the management of the quality system itself;
- R2.12.3 specifications for personnel, premises, equipment, materials and software;
- R2.12.4 qualitative and quantitative specifications for SoHO;
- R2.12.5 technical guidelines;
- R2.12.6 agreements;
- R2.12.7 job descriptions;
- R2.12.8 instruction manuals;
- R2.12.9 risk assessments as part of the QMS, notably for critical activities;

R2.12.10 validation or verification dossiers;
R2.12.11 flow charts;
R2.12.12 manufacturers' instructions;
R2.12.13 software documentation;
R2.12.14 floor plans;
R2.12.15 external documents such as laws, guidelines, textbooks.

Supplementary information

Documentation may exist in various forms: paper-based, electronic or photographic. Many documents could also exist in hybrid forms (i.e. some elements are electronic, and others are paper based).

2.7.1 Records

Standards

S2.23 SoHO entities must collect and report data relating to any of the following SoHO activities (Art. 41.1):
a. SoHO donor registration;
b. collection;
c. distribution;
d. import;
e. export;
f. human application.

S2.24 SoHO entities must keep the data necessary to ensure traceability, appropriately safeguarded and accessible to the SoHO CA, for a minimum of 30 years from the SoHO distribution date or, where applicable, from the date of disposal or export. They may store the data in electronic form (Art. 42.6).

Good Practice Guidelines

G2.105 Records should be made, manually and/or by recording instruments, during activities and critical tasks which demonstrate that all the steps required by the defined procedures and instructions were in fact taken and that the quantity and quality, safety and effectiveness of the SoHO met specifications.

G2.106 Records should be legible and may be handwritten, transferred to another medium or recorded in a computerised system.

G2.107 Records should be made or completed at the time each action is taken and in such a way that all significant activities, including aspects relating to quality control and quality assurance of SoHO, are traceable.

G2.108 Records of activities and critical tasks should enable the complete history of the SoHO to be traced, and these records should be retained in a comprehensible and accessible form.

G2.109 For every critical activity, the materials, equipment and personnel involved should be identified and recorded.

G2.110 Each significant step should be recorded in a manner that permits the SoHO or the procedure to be traced, in either direction, from the first step to final use/disposal.

G2.111 Any alteration made to the entry on a record should be signed and dated; the alteration should permit reading of the original information. Where appropriate, the reason for the alteration should be recorded. In the case of electronic records, there should be an audit trail or alternative, so that it is possible to trace what data have been altered, when the record was altered and who altered it.

G2.112 Access to records (registers and data) should be restricted. A process regarding the handling of confidential data and access to registers by personnel, including contractors, personnel of external bodies, or individuals with access to the SoHO entity or establishment, should be implemented and monitored.

G2.113 Data protection and confidentiality measures should be in place. Management of donor, patient and personnel data should include privacy and confidentiality. Except for any potential sharing of data in the interest of donor health monitoring and/or of public health, as necessary and proportionate, all other data should be regarded as confidential.

G2.114 Records (except data necessary to ensure traceability) that are critical to the safety and quality of the SoHO should be kept for at least 10 years after the expiry date, clinical use or disposal.

Recommendations

R2.13 It is recommended that the records provide evidence of various actions taken to demonstrate compliance with instructions, e.g. SoHO activities, events, investigations and a history of all SoHO, including their distribution:
R2.13.1 records are recommended to include the raw data that are used to generate other records;
R2.13.2 for electronic records, it is recommended that designated users define which data are to be used as raw data;
R2.13.3 all data on which quality decisions are based are defined as 'raw data'.

Supplementary information

Records may include the following non-exhaustive list of record types:
a. records of the performance of operations critical to quality, safety and effectiveness of SoHO;
b. records of critical premises, equipment, information systems, materials, consumables and reagents;
c. records of complaints and deviations;
d. records of corrective and preventive actions;
e. records of internal audits, external audits and inspections;
f. training and competency records of personnel;
g. records of internal and external quality control;

h. identification and analysis of risks and a risk-mitigation plan;
i. donor and recipient records;
j. information on the final destination of SoHO;
k. records of quality indicators.

A SoHO entity or establishment that processes and stores confidential records generally follows strict rules on data protection and can guarantee that the information is:

a. used fairly, lawfully and transparently;
b. used for specified, explicit purposes;
c. used in a way that is adequate, relevant and limited to only what is necessary;
d. accurate and, where necessary, kept up to date;
e. kept for no longer than is necessary;
f. handled in a way that ensures appropriate security, including protection against unlawful or unauthorised processing, access, loss, destruction or damage.

Records which are deemed more sensitive may require additional protection. This includes records on:

- race;
- genetics;
- biometrics (where used for identification);
- health.

2.8 Change control

Standards

S2.25 SoHO entities must not make any significant change as to the steps of the processing applied, or the activities performed for an authorised SoHO preparation, without the prior written SoHO preparation authorisation from the CA. Significant changes for which an application for an updated SoHO preparation authorisation is required must be those having an impact on the intended clinical indication or on the quality, safety or effectiveness of the SoHO preparation (Art. 39.6).

Good Practice Guidelines

G2.115 A formal change-control system should be in place to describe the actions to be taken to plan, evaluate and document any planned change which is proposed for the range and/or specifications of activities and tasks or any other change that may affect the reproducibility of a process, the quality, safety or effectiveness of SoHO, or the health of donors or recipients/patients.
G2.116 Change-control procedures should ensure that sufficient supporting data are generated to demonstrate that the revised process results in a SoHO product of the desired quality, consistent with the approved specifications. Supporting data, e.g. copies of documents and/or records, should be reviewed ahead of going live with the planned change to confirm that all risks that have been identified prospectively have been sufficiently mitigated; further, the data generated once the change is active should also be reviewed at a defined time period post-go-live to confirm that the impact of the change has been demonstrated as intended, without any unintended consequences, before final close-out of the change control.
G2.117 The potential impact of a proposed change should be evaluated, and the degree of revalidation or additional testing, qualification, verification and validation needed should be determined, based on the principles of quality and risk management.
G2.118 Changes should be authorised and approved by the Responsible Person(s) or relevant personnel who have the expertise and competence to determine adequacy in accordance with the QMS.
G2.119 After implementation of any change, an evaluation should be undertaken to confirm that the quality objectives were achieved and that there was no unintended deleterious impact.
G2.120 Where temporary and time-limited changes are implemented, provisions should be in place to ensure and verify that the changes are reversed as appropriate.
G2.121 All changes should be evaluated for any requirement of notification to, or approval from, a national HA.

Recommendations

It is recommended that:

R2.14 change control be supported by a comprehensive risk assessment, for example the EuroGTP II tool, for which see Chapter 4. The purpose of this is to ensure that the change is justified, is documented and will not impair the quality, safety and effectiveness of SoHO;
R2.15 as a minimum, the following aspects of any change be evaluated:
R2.15.1 effect on quality, safety and effectiveness of SoHO;
R2.15.2 the need for SOPs to be updated;
R2.15.3 any need to validate or re-validate the process;
R2.15.4 effects on quality-control analyses;
R2.15.5 requirement to inform, and be authorised by, HAs;
R2.15.6 need to train personnel;
R2.15.7 effect on previously conducted risk analyses.

2.9 Complaints

Standards

S2.26 SoHO entities and establishments must put in place procedures and specifications covering, where applicable to their SoHO activities, any complaints (Art. 37.3.k).

Good Practice Guidelines

G2.122 Complaints should be recorded and examined, and appropriate measures should be taken to resolve the complaints.
G2.123 Complaints should be managed within the time limits specified within the policy of the entity or establishment.
G2.124 Based on the potential seriousness and frequency of appearance of complaints, a root cause analysis and possible identification of corrective measures should be performed to prevent recurrence.
G2.125 The systems in place for the management of complaints should be linked as appropriate to the systems in place for the management of deviations, adverse events and adverse reactions.

Recommendations

It is recommended that:
R2.16 a description of the process for handling complaints be available at publicly accessible information points such as the website of the entity, health institutions, brochures and/or consent forms. It is recommended that complaints from any stakeholders are taken into consideration;
R2.17 a mechanism for categorising, tracking and trending complaints generally will facilitate risk-based auditing, preventive and corrective actions, continuity planning and quality review.

2.10 Investigation and reporting of deviations, adverse events and adverse reactions

Standards

S2.27 SoHO entities and establishments must put in place procedures and specifications covering, where applicable to their SoHO activities, the management of cases where procedures have not been followed or specifications have not been met (Art. 37.3.j).

Good Practice Guidelines

G2.126 A process should be in place to report, record, investigate and correct any significant deviations or non-compliance with the QMS. Adequate resources should be made available for the timely resolution of deviations.
G2.127 The investigation of deviations should include an assessment of the impact on the SoHO, including a review and evaluation of relevant operational documentation and an assessment of deviations from specified procedures. Deviations should be categorised depending on how critical they are to the quality, safety and effectiveness of SoHO, and how frequently they occur.
G2.128 If it is determined that deviations could recur and/or there is doubt about compliance with requirements of the QMS, based on the estimated frequency of occurrence and severity (e.g. risk matrix) of the deviations, a root cause analysis should be performed to identify the underlying cause or causes of the deviation. This can be determined using risk-management principles.
G2.129 A process for the implementation of corrective and preventive actions should be in place. Appropriate corrective and/or preventive actions (CAPAs) should be identified and taken in response to investigations, with a view to ensuring that root causes of the non-conformity are identified and corrected, and that recurrence of the root causes is prevented. The need to consider a recall of SoHO or the need to quarantine materials should also be considered. CAPAs should be completed in a timely and effective manner.
G2.130 The effectiveness of CAPAs should be monitored and assessed, in line with quality and risk-management principles.
G2.131 The systems in place for the management of deviations should be linked as appropriate to the systems in place for the management of Serious Adverse Reactions and Events (SAREs) (see Chapter 18).
G2.132 If SoHO are distributed to Advanced Therapy Medical Product (ATMP) or medical device manufacturers, SAREs should also be reported to pharmacovigilance, medical device vigilance and biovigilance systems for donor reactions, or other events and reactions detected before distribution of the SoHO.
G2.133 Data relating to deviations should be routinely analysed to identify quality problems or trends that may require preventive action.

Supplementary information

For detailed guidance on vigilance of SoHO, see Chapter 18.

2.11 Recall

Standards

S2.28 SoHO entities and establishments must put in place procedures and specifications covering, where applicable to their SoHO activities, the withdrawal of SoHO from the inventory of released SoHO and recalls (Art. 37.3.g).

Good Practice Guidelines

G2.134 The SoHO entity or establishment should have personnel authorised to assess the need for recall and to initiate and co-ordinate the necessary actions.
G2.135 A recall procedure should be in place that includes a description of the responsibilities, actions

to be taken within pre-defined periods of time and requirements for notification to the HA.

G2.136 Recall actions should be taken within pre-defined periods of time and should include tracing all relevant SoHO. The purpose of the investigation is to identify any donor who might have contributed to causing the adverse reaction and to retrieve available SoHO from that donor, as well as to notify consignees and recipients of components collected from the same donor in the event that they might have been put at risk.

G2.137 The progress of the recall process should be recorded, and a final report issued, including reconciliation of the delivered and recovered quantities of the SoHO.

G2.138 The effectiveness of the arrangements for recalls should be regularly evaluated.

2.12 Quality review

Standards

S2.29 SoHO entities and establishments must put in place procedures and specifications covering, where applicable to their SoHO activities, the quality control and monitoring of key performance indicators of SoHO activities (Art. 37.3.e) and internal audits (Art. 37.3.h).

S2.30 SoHO entities must mitigate risks arising from microbial contamination of SoHO from the environment, the personnel, the equipment and the materials coming into contact with SoHO during collection, processing, storage or distribution. SoHO entities must mitigate such risks by performing quality control testing of SoHO to detect microbial contamination and using methods of inactivation or elimination of micro-organisms, where feasible and appropriate (Art. 58.5.e).

2.12.1 Quality control

Good Practice Guidelines

G2.139 A quality-control system should be in place to ensure that SoHO are not released for use until their quality, safety and effectiveness has been assessed as satisfactory. Activities such as verification steps, sampling and testing should be carried out to assess that the SoHO, and also materials, equipment, software and processes, comply with established acceptance criteria.

G2.140 Samples for quality control should be collected and recorded in accordance with processes that describe the method of sampling, including the amount of sample to be taken, precautions to be observed and storage conditions.

G2.141 At all stages of quality-control testing, sampling containers should be labelled with relevant information on the type of quality-control sample, origin of the sample and, if applicable, date and time of sampling.

G2.142 There should be a person responsible for quality control who supervises all quality-control procedures, who ensures that the premises and equipment where quality-control operations are carried out are appropriate and maintained under suitable conditions and who also ensures that all personnel working under their responsibility are adequately trained.

G2.143 Documentation should be in place that governs quality control at key stages during processing. The documentation should include at least:

- **G2.143.1** the test method;
- **G2.143.2** the sample size and sampling plan;
- **G2.143.3** the acceptance criteria.

G2.144 Sampling and testing methods should be validated/verified to show the representativeness of the sample and the suitability of the selected methods.

G2.145 The performance of the testing procedures should be regularly assessed.

G2.146 Records related to quality-control testing should be part of the documentation of SoHO. Data to be recorded and maintained (by either the SoHO establishment or the testing laboratories) should include:

- **G2.146.1** identification information and batch number of the material or products used;
- **G2.146.2** reference to the relevant specifications and testing procedures, and to the equipment used;
- **G2.146.3** test results, including observations and calculations, and reference to any certificates of analysis if relevant;
- **G2.146.4** date of testing, and date and time of sample collection;
- **G2.146.5** identification of the person(s) who performed the testing;
- **G2.146.6** identification of the person who verified the testing;
- **G2.146.7** statement of approval or rejection of the test results.

G2.147 Minimum acceptance criteria should be based on a defined specification for each type of SoHO.

Supplementary information

Internal quality control in a testing laboratory includes use of positive, weakly positive or negative control samples as appropriate. External quality assessment (sometimes called 'proficiency testing') involves analysis of unknown samples and evaluation of the results by a third party.

Detailed guidance on microbiological testing is provided in Chapter 12. Guidance on specific quality-control tests for specific types of SoHO is provided in Part B of this Guide.

2.12.2 Self-assessment, internal audit and external audit

Good Practice Guidelines

G2.148 Self-inspection or audit systems should be in place to verify compliance with the GPGs. They should be carried out regularly by trained and competent persons, in an independent way, and according to approved procedures.
G2.149 All non-conformities should be documented, and appropriate CAPAs should be implemented in a timely and effective manner if applicable.

Supplementary information

Auditing is a tool for ensuring compliance with the quality system and for supporting continuous quality improvement. Internal audits may be conducted by the organisation's quality assurance personnel, by internal staff of the SoHO establishment who are not involved in the subject matter of the audit or by an authorised external expert with the required competence.

External audits are undertaken by independent bodies (often designated as approved ISO certifying bodies), including JACIE, ESHRE and WMDA, for certification and accreditation purposes. External audits provide an opportunity for critical review by experts unfamiliar with the systems in place locally. They provide an excellent opportunity for systems improvement.

2.12.3 Quality indicators

Good Practice Guidelines

G2.150 Processes for establishing and monitoring of quality indicators should be established, which include establishing the objectives, methodology, interpretation, limits action plan and duration of monitoring.
G2.151 Quality indicators should be periodically reviewed, to ensure continued appropriateness, unless specific indicators are mandated by other applicable guidelines (e.g. JACIE, ESHRE), in which case additional periodic review may not be required.

Supplementary information

A monitoring plan could be developed and implemented to ensure appropriate performance of activities. The plan could consider the criticality of the premises, system, material or equipment and could outline monitoring, user notification and problem-resolution mechanisms. If an unusual event is observed, personnel will follow the standard response described in the monitoring plan. The standard response will include notification of relevant personnel and, if possible, initiation of a response to resolve the problem and risk assessment of the affected SoHO.

2.12.4 Quality management system review

Recommendations

R2.18 It is advised to conduct a management review with the objective of verifying the consistency of all activities performed within the entity, the appropriateness of the current QMS, and the identification of trends and potential improvements. Such a review could be conducted periodically, taking into account the conclusions of previous reviews; it could document and encompass all processes and activities of the entity, including all SoHO that are imported, exported or intended for use in the manufacture of other products.
R2.19 It is advised that a QMS review include:
- R2.19.1 review of starting materials, including SoHO;
- R2.19.2 review of critical in-process controls;
- R2.19.3 review of results of quality control and quality monitoring;
- R2.19.4 review of all changes;
- R2.19.5 review of the qualification status of equipment;
- R2.19.6 review of technical agreements and contracts;
- R2.19.7 review of all significant deviations and the CAPAs implemented;
- R2.19.8 review of the findings of internal and external audits and inspections;
- R2.19.9 review of complaints and recalls;
- R2.19.10 review of donor-acceptance criteria;
- R2.19.11 review of donor deferrals;
- R2.19.12 review of look-back cases.

2.13 Continuity planning

Standards

S2.31 SoHO entities and establishments must put in place procedures and specifications covering, where applicable to their SoHO activities, continuity planning (Art. 37.3.m).
S2.32 Where a SoHO entity ceases its activity, the traceability data must be transferred to a contracted SoHO entity for the remaining part of the traceability period, after informing the SoHO CA (Art. 42.6).
S2.33 Critical entities must establish appropriate mechanisms for the continuous monitoring of their stocks of critical SoHO and must be able, in the event of shortages or upon request, to communicate such information to their SoHO CA (Art. 62.3).

Good Practice Guidelines

G2.152 SoHO entities and establishments should have a continuity plan in place that details how collection services, donated SoHO and all associated documents will be maintained in the event that activities are temporarily suspended or permanently ceased.

2.14 SoHO supply: contingency and emergency planning

Contingency planning and emergency preparedness are always key elements in safeguarding the supply of SoHO preparations, but particularly during disaster or emergency situations. They ensure that, when faced with such situations, a safe and adequate SoHO preparations supply can be maintained.

Standards

S2.34 Each critical SoHO entity must draw up a SoHO entity emergency plan that implements the national SoHO emergency plan (Art. 67).

Good Practice Guidelines

G2.153 Each entity responsible for critical SoHO should establish, implement, test periodically and maintain a contingency and emergency plan.
G2.154 Key stakeholders within the supply chain of critical SoHO preparations should be identified and their expected responsibilities and actions should be defined according to different key risk scenarios.
G2.155 The roles and actions of each key stakeholder for each key risk scenario should be defined, based on the role of the stakeholder within the supply chain of critical SoHO preparations and on the severity of risk.

Recommendations

R2.20 It is recommended that each SoHO entity identify its critical processes and establish, implement and maintain an emergency plan that ensures the continuity, safety and traceability of those processes and SoHO preparations in situations that may disrupt normal operations.

Supplementary information

One of the main aspects of designing a strategy for emergency preparedness plans is the implementation and communication between all key stakeholders. This aspect of strategy would cover who, what, when, with whom and how to communicate prior to, during and after a response to an emergency situation. The Blood Supply Contingency and Emergency Plan (B-SCEP) Project was launched by the EDQM with a view to providing practical tools to assist European countries in establishing or strengthening contingency and emergency plans for their blood systems [15].

A standardised toolkit for contingency and emergency preparedness for SoHO establishments has been established by the Egalite project [16].

Contingency planning focuses on anticipating and mitigating supply chain disruptions before they occur. It is proactive and scenario based.

Typical elements include:

- identifying vulnerabilities in the supply chain;
- mapping critical processes and dependencies;
- defining alternative suppliers, routes or procedures;
- stock-management strategies;
- scenario planning for shortages or delays.

Emergency preparedness deals with acute, high impact events that require immediate action, such as natural disasters, cyberattacks, major accidents or sudden large scale shortages.

It includes:

- activation of emergency plans;
- crisis communication;
- co-ordination with national authorities;
- rapid decision-making structures;
- maintaining minimum essential SoHO supply under extreme conditions.

In the EU, the SoHO Coordination Board (SCB) is responsible for establishing the criteria that determine which SoHO are considered critical and for maintaining a harmonised EU level list. National CAs will identify the critical SoHO within their jurisdiction on the basis of these SCB-defined criteria [17].

2.15 References

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Chapter 3. Qualification, validation and verification

3.1 Introduction

Qualification and validation (or verification) are critical activities that are essential to maximise the safety and efficacy of substances of human origin (SoHO) and SoHO preparations. They are informed by systematic risk mapping of the potential risks associated with the critical processes conducted by a SoHO entity, encompassing personnel, premises, equipment, materials and the computerised systems used to support these processes. Qualification and validation are integral to a SoHO entity's quality management system (QMS).

This chapter provides guidance for SoHO entities on how to identify where qualification, validation and verification are required, the general principles of how to perform these activities accurately and specific guidance relating to their use in different areas. This chapter does not cover the validation of computerised systems; this is a specialised technical activity and is addressed in Chapter 15. In accordance with the terminology used in Chapter 2, the term 'materials' used in this chapter encompasses all reagents, instruments, medicines, implants and consumables used in critical processes.

3.1.1 General principles of qualification, validation and verification

It is important for SoHO entities to clearly understand the differences between qualification, validation and verification, in order to understand when and where they are appropriate. Brief definitions and examples are given below, and further examples are included in later sections of this chapter. Note that these definitions are specific to how the terms are used in this Guide. Drawing a clear distinction between 'validation' and 'verification' is challenging due to the different definitions used in different standards. For the purposes of this Guide, the definition of 'verification' is derived from that used by ISO9001:2015, rather than the more specific definition used in the field of medical device regulation.

Qualification is applied to confirm the compliance of individual components of a process, e.g. objects or concepts but not procedures, with their predefined requirements; this may include items as diverse as personnel, premises, equipment, materials and computerised systems. These components are proven (qualified) to achieve their defined objectives. For example, personnel working in controlled environments are required to demonstrate that their aseptic technique meets a designated level, or that they can perform a process to a required standard. Qualification of all relevant components is an essential prerequisite for the validation of a process. Certain types of equipment that are required to measure or maintain specific parameters also need to be calibrated against external controls; for example, balances, pH meters or thermometers. Their role is to ensure accuracy.

Validation is required for whole processes; its intention is to demonstrate, through objective evidence, that a process reliably achieves its intended purpose. Validation requires that the acceptable outcome(s) of a process be pre-determined. For example, a validation study may be used to confirm that a process consistently produces SoHO preparations that meet the required critical quality attributes (CQAs).

Verification is used to confirm that a well-established process, that has previously been validated, continues to achieve its specified requirements. Monitoring of key process indicators can be a useful tool for verification.

The term 'validation' may be also used generically to cover all three activities, and this precedent is followed in this chapter to avoid repetition. It will be clear from the context what the intended meaning is.

The factors for which qualification, validation or verification is appropriate are shown in Table 3.1. Please

refer to Chapter 2 (§2.5.3 Supplementary information) for further details on, and the definition of calibration.

The processes of risk management and change control are integral to determining the requirements for qualification and validation. This relationship is shown in Figure 3.1.

Standards

S3.1 SoHO entities and establishments must mitigate any risk that the inherent properties of SoHO preparations, which are essential for their clinical effectiveness, could be significantly adversely altered by any processes involved in their collection, processing, storage or distribution, rendering them less effective to recipients. To achieve this, the following measures must be combined (Art. 58.7):

S3.1.1 conducting process validation and equipment qualification according to the principles of quality risk management, with critical processes being prioritised;

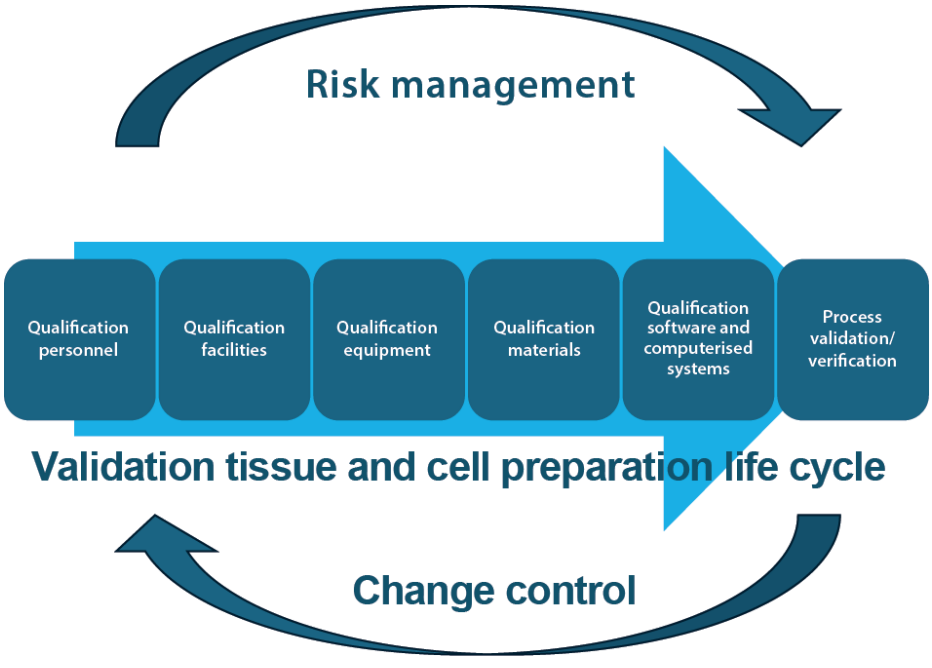
S3.1.2 collecting evidence of effectiveness when needed.

Table 3.1 **Processes or critical resources that are to be qualified and validated or verified or calibrated**

	Qualification	Validation	Verification	Calibration
Premises	×			
Personnel	×			
Equipment	×			×
Computerised systems	×			
Measuring equipment	×			×
Materials	×			
Suppliers	×			
Analytical tests referred or sourced by IVDR, *Ph. Eur.* monographs, etc.			×	
In-house analytical tests[a]		×		
In-house process methods[a]		×		
Established process methods (e.g. guidelines, SoHO monographs, other SoHO entities/establishments)			×	
Cleaning and disinfection methods[a]		×		

[a] After initial validation, periodic re-validation (verification) is required.

Figure 3.1 **Validation of SoHO preparation life**



Good Practice Guidelines

G3.1 SoHO entities should establish a system to document evidence that provides a high degree of assurance that a specific process, piece of equipment or environment will consistently produce processed SoHO that meet pre-determined specifications and quality attributes.

G3.2 All critical personnel, facilities, equipment, materials and software should be identified and qualified.

G3.3 SoHO entities should identify what verification or validation work is needed to prove control of the critical aspects of their particular processes.

G3.4 All critical processing procedures should be verified or validated and should not render the SoHO clinically ineffective or be harmful to the recipient.

G3.5 Any significant changes to the facilities, equipment, materials, computerised systems or processes that might affect the quality of the SoHO should be qualified/verified/validated and authorised by a Health Authority (HA) if applicable.

G3.6 A risk-assessment approach should be used to determine the scope and extent of validation/verification/qualification. This should consider where relevant:

- **G3.6.1** personnel;
- **G3.6.2** equipment (e.g. autoclave, incubator, freeze dryer);
- **G3.6.3** premises (e.g. cleanrooms, laminar flow module);
- **G3.6.4** materials (e.g. reagents and disposable items);
- **G3.6.5** computerised systems (e.g. cleanrooms, environmental monitoring system, tissue processing system);
- **G3.6.6** analytical methods;
- **G3.6.7** processes (any processes that may affect the quality of the SoHO, including those relating to transport, storage and direct processing of the SoHO, as well as more general processes such as facility and equipment maintenance that could also impact on the quality of the SoHO).

G3.7 The results from the risk-assessment study of the scope of validation/verification/qualification activities within a SoHO entity should be covered in a Validation Master Plan.

G3.8 SoHO entities performing very simple, minimal manipulation of a limited range of SoHO, in accordance with published methods or following long-established practices using the same materials and equipment, may rely on ongoing quality control and periodic reviews. Such entities should still document their validation policy, explaining their approach on the basis of risk assessment, and use retrospective data or historical data to conduct the verification of their critical processes to confirm that the method has the intended (clinical) outcome.

G3.9 The status of processes, facilities or materials should be clearly labelled to identify those which have been validated and those undergoing or awaiting validation.

G3.10 Items and processes that are awaiting or undergoing validation should be clearly labelled and identified as such and not used for production of SoHO preparations for clinical use.

G3.11 Qualification, validation and verification activities should be repeated until enough replicates have been performed to prove that the outcomes are consistent. The number of replicates should be informed by analysis of the outcome data and will be determined by the level of variation.

G3.12 Qualification and validation should be completed before a process, or process modification, is implemented for routine use and then be repeated at pre-determined intervals. A risk-assessment approach should be used to determine these intervals.

G3.13 Completed qualification and validation activities should be documented in a formal report. This should include at least:

- **G3.13.1** the scope of the report (what specific factors or processes it covers);
- **G3.13.2** what protocols were used;
- **G3.13.3** details and calibration status of any measuring equipment used;
- **G3.13.4** the pre-defined acceptance criteria;
- **G3.13.5** any deviations from the planned protocol, and the reasons for this;
- **G3.13.6** detailed results;
- **G3.13.7** confirmation of whether or not the pre-defined acceptance criteria were met.

G3.14 SoHO entities should document how and when qualification and validation reports will be reviewed and authorised, and by whom.

G3.15 Routine qualification and validation activities may be formalised within the quality system; in this situation a formal report is not required for each activity.

Recommendations

R3.1 It is recommended that SoHO entities seek expert statistical advice in the design and outcome analysis of qualification, validation and verification activities.

Supplementary information

Not every change to a process requires formal validation. As per G3.5, SoHO entities will evaluate if a proposed change is significant; while there is some subjectivity in this determination, structured tools such as EuroGTP II (see Chapter 4) provide a standardised model to assist with this determination. Where a change is determined to be significant, a risk assessment helps in determining what (if any) validation is required to support the change; this may result in a determination that there is no significant risk, and no validation is indicated. Factors that may influence the determination of validation requirements

for new or modified processes include, but are not limited to:

- whether the method has been used by the SoHO entity, or other SoHO entities, previously;
- whether there is an established monograph for the preparation process;
- the requirements of national legislation;
- the probability and consequences of any identified risks;
- the number of recipients that could receive SoHO preparations prepared from the SoHO;
- the clinical profile of probable recipients;
- the inclusion of any routine quality-control checks to confirm that the SoHO preparations meet specific CQAs.

Process validation can also occur during the processing of SoHO preparations intended for release for human application (concurrent validation), for example in situations where clinical demand for a SoHO preparation exceeds supply and it is not considered acceptable to sacrifice donated material for validation purposes. In cases where processes or methods have been in routine use without change for many years, but are not supported by formal validation, existing data and information can be used as a basis for retrospective verification of critical processes to confirm that the method achieves the intended clinical outcome.

The objective of validation is to rigorously test the critical aspects of items through a series of controlled tests that replicate the conditions under which they are expected to operate, demonstrating that they consistently generate SoHO preparations that meet predefined acceptance criteria for quality and safety. Operational considerations are generally incorporated into the design of validation protocols, and it is good practice for them to encompass a range of critical variables. For example, if a validation protocol requires that a process be carried out at 37°C for 60 minutes, any deviation from these parameters (e.g. if the temperature drops to 36.9°C for part of the process, or the process takes 61 minutes) results in the process not conforming with its validated requirements.

It can be beneficial to design validation protocols to cover a range for each critical variable, so that the routine protocol permits an acceptable range. In the example above, this range might be 35°C to 39°C for 55 to 65 minutes. Depending on the process and variable, it may only be necessary to validate one extreme of the acceptable range. This is the 'worst case' approach and is applicable in situations such as the transport of frozen SoHO preparations from a SoHO entity to an organisation responsible for human application (ORHA). In this case the requirement may be that the SoHO preparation remains below a temperature of –20°C, but there is no requirement for a lower limit. In this situation, the SoHO entity usually establishes what the real-world, worst-case conditions for the procedure could be (commonly for frozen SoHO preparations transport protocols this is the highest ambient temperature and the longest possible transport time) and carry out the validation protocol at these limits. By default, this will validate any process that falls within these parameters.

Parameters commonly specified in validation protocols include operating temperatures, exposure times, air flows, bioburden, location and product characteristics, in addition to any relevant failure detection methods and alarms.

3.2 Validation master plan

The schedule, test methods and acceptance criteria for a SoHO entity's qualification, validation and verification activities are typically summarised in a controlled document approved by the SoHO responsible person. The Responsible Person may delegate this responsibility to another individual (or individuals) whom they deem appropriately qualified and experienced. This document is often referred to as the validation master plan (VMP). The VMP is a high-level strategic document that provides an overview of the processes, personnel, equipment, materials and computerised systems to be validated. It will reference other controlled documents that cover specific validation processes. The VMP is an integral part of a SoHO entity's QMS, and its core purpose can be summarised as providing assurance that all critical aspects of a SoHO entity's operations are adequately controlled and monitored to ensure that the SoHO preparations consistently meet their requisite criteria of safety and efficacy.

Good Practice Guidelines

G3.16 The policy and process for planning, executing and recording validation should be documented in a VMP.

G3.17 The VMP should ensure that all designated CQAs for the SoHO preparations provided by a SoHO entity are adequately addressed by validation protocols.

G3.18 As a minimum, the VMP should consist of:

- **G3.18.1** description of the SoHO entity, or reference to where the description may be found;
- **G3.18.2** list of personnel, premises, equipment, materials, computerised systems and processes that need to be qualified, validated or verified;
- **G3.18.3** current state of validation/verification/qualification of each element within the scope of the plan, unless any other information system is in place to provide this information;
- **G3.18.4** validation/verification/qualification programme;
- **G3.18.5** organisational structure, including roles and responsibilities for validation activities;
- **G3.18.6** procedures related to validation/verification/qualification activities;
- **G3.18.7** criteria for requalification, revalidation or reverification.

G3.19 Specific qualification, validation or verification activities should be described in standard operating protocols (SOPs) containing at least:

G3.19.1 the objective(s);
G3.19.2 the protocol;
G3.19.3 scope of the activities;
G3.19.4 who is responsible;
G3.19.5 any related documents;
G3.19.6 the stages of qualification, validation or verification;
G3.19.7 the acceptance criteria.

Recommendations

It is recommended that:

R3.2 in the development of either a VMP or individual validation protocols, a validation team that includes representation from all relevant stakeholders, for example composed of operational, quality, regulatory and medical experts, be assigned to oversee the process;

R3.3 when determining the validation requirements for activities and facilities that are outside the experience of SoHO entity personnel, external experts be consulted, for example for the qualification of new cleanroom premises.

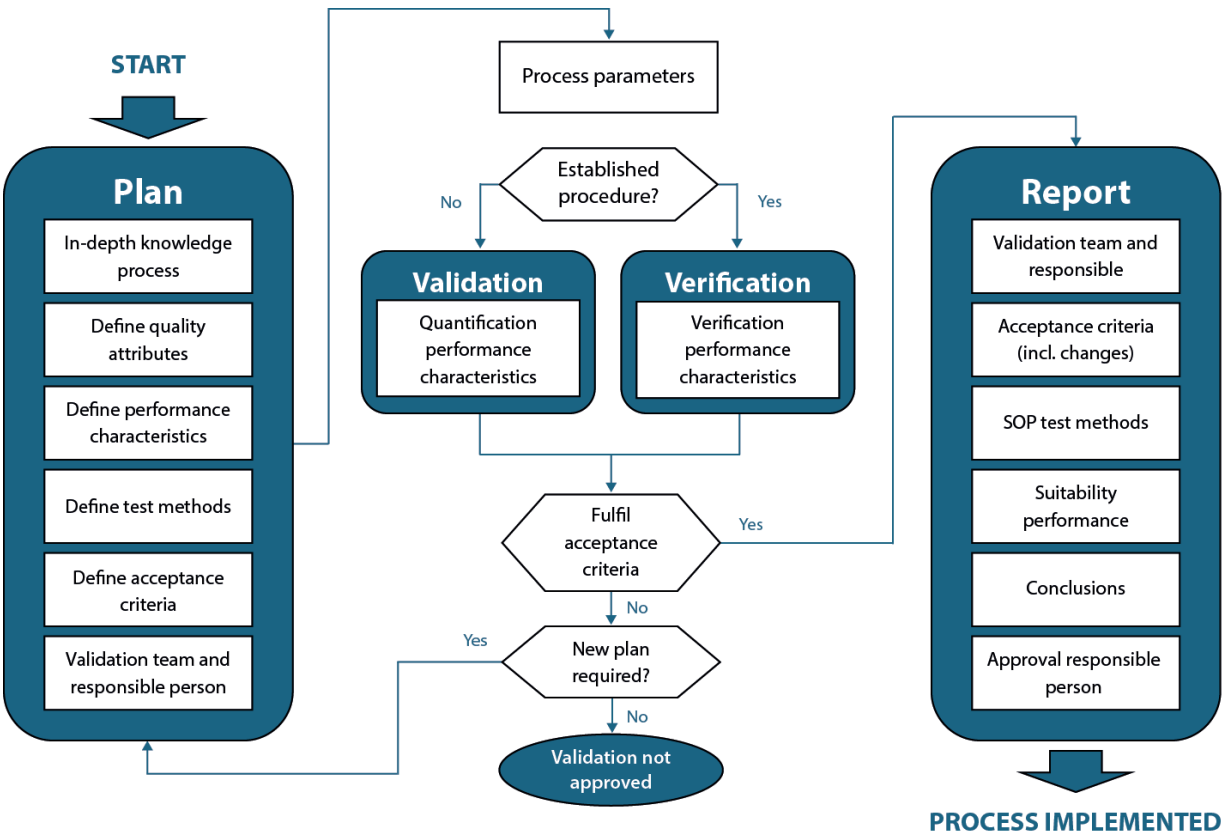
Supplementary information

In existing SoHO entities, the VMP will develop organically according to the activities it performs. When designing a novel facility, the VMP typically commences with a process design phase aimed at gaining in-depth knowledge of the process. During this stage, the CQAs of SoHO preparations that the SoHO entity provides are identified, together with acceptable ranges. Based on these parameters, a process control strategy can then be developed to ensure the required CQAs of the SoHO preparations can be met.

Following completion of the process design phase, the validation phase commences. Here, the process control strategy is implemented, and all related elements – such as personnel, premises, equipment, materials, computerised systems and suppliers – are qualified before moving forward with process validation [1]. This process is shown in Figure 3.2.

All validation activities described in a SoHO entity VMP ultimately derive from the need to ensure that the SoHO preparations provided by the SoHO entity consistently meet their designated specifications and CQAs. When determining the validation requirements for a process, it may be beneficial to employ risk management techniques. These analyses commonly address risks to donors, recipients and product quality, and the requirements of regulatory compliance. They consider possible failure modes and detection methods, and include product specifications for processing stages, test specifications for test methods and user-requirement specifications (URS) for items used in processes. For custom equipment and facilities, the manufacturer or supplier interprets the URS to create a design specification, which guides the engineers in developing detailed technical specifications. For off-the-shelf items, the URS

Figure 3.2 **Development and implementation of a process validation**



Source: adapted from Ned Tijdschr Klin Chem Labgeneesk 2017, vol. 42, no. 1

helps in assessing their suitability and informs purchase decisions.

If procedures cannot be completed exactly as planned, the validation team reviews the situation and decides whether to repeat the validation or allow a deviation for the modified procedure. These decisions are generally documented in the validation records and report.

The process for performing and documenting ongoing validation activities mirrors the process described in G3.18.

3.3 Qualification of materials

The materials used in the composition of SoHO preparations play an important role in their overall safety and quality. To help ensure consistent standards, it is helpful to define clear specifications and to check that the materials align with them.

Standards

S3.2 SoHO entities must put in place procedures and specifications to address the procurement, qualification and validation of materials (Art. 37.3.c).

S3.3 Where materials (including reagents) are prepared in house, applications for SoHO preparations authorisation must include evidence of the validation of their quality (Art. 39.2.b.v).

Good Practice Guidelines

G3.20 If components of animal origin are used in the composition of SoHO preparations, for example in reagents or excipients, a risk assessment should be performed to mitigate the risks of zoonotic infections.

G3.21 The stability of materials should be established under their designated storage conditions, to ensure that they maintain their minimal quality criteria for the duration of the shelf life applied.

G3.22 When extending expiry dates for materials, or changing storage conditions beyond those already validated, additional stability testing should be undertaken to demonstrate that they will remain effective throughout the extended period.

G3.23 Any deviations from validated storage parameters that are not supported by validation data should be risk assessed to determine their impact on the quality of a material.

G3.24 The toxicity of materials should be evaluated to ensure their safety for their intended application. This may be accomplished by review of the extent of toxicological data and/or by conducting in-house *in vitro/in vivo* studies.

G3.25 The risk of materials introducing impurities into SoHO preparations should be evaluated. A risk analysis should evaluate what levels of impurities are acceptable, and these requirements should be detailed in the specification for the material. Documentation provided by suppliers, such as certificates of analysis, should be consulted to confirm compliance.

G3.26 SoHO entities should consider the possibility that different materials that come in contact with each other during processing may cross-react in undesirable ways, potentially impacting the quality and/or safety of the SoHO preparations. Where this is considered a risk, compatibility studies should be performed.

Recommendations

R3.4 It is recommended that SoHO entities source components of animal origin from suppliers that comply with relevant national safety regulations, ensuring traceability and validating the absence of contaminants.

Supplementary information

Components of animal origin present specific risks of the potential transmission of zoonotic infections. Testing and documentation of the safety of these materials confirms their suitability for use in SoHO activities.

Transport and storage conditions play a significant role in maintaining the stability and shelf life of critical materials and reagents. Proper storage conditions, such as controlled temperature, humidity and protection from light, are critical to preserving material integrity. Similarly, transport processes need to prevent exposure to conditions that could lead to material degradation.

Where information regarding the purity of a material is insufficient, the *European Pharmacopoeia* may contain suitable analytical methods to measure purity.

3.4 Qualification of premises and equipment

The qualification of new critical premises or equipment commonly begins with Design Qualification (DQ) and progresses successively through Installation Qualification (IQ), Operational Qualification (OQ) and Performance Qualification (PQ), as per Figure 3.3.

Figure 3.3 **Steps in qualification of premises and equipment**



Protocols for DQ, IQ, OQ and PQ will vary depending on the premises or equipment being qualified. Further details are provided in the supplementary information section.

Standards

S3.4 SoHO entities must put in place procedures and specifications, where applicable to their SoHO activities, that address the sourcing and qualification of premises and equipment (Art. 58.7).

SoHO entities must ensure that any equipment that may have an impact on the safety and efficacy of SoHO preparations is qualified for its intended use (Art. 58.7.a, Art. 39.2.b.viii).

Good Practice Guidelines

G3.27 DQ, IQ, OQ and PQ protocols should be documented and approved prior to being implemented.

G3.28 Assessment of the performance of critical premises and equipment should be undertaken in the following situations:

G3.28.1 at initial commissioning;

G3.28.2 after relocation, repair, modification or adjustment subject to risk assessment to determine impact;

G3.28.3 if there is evidence they may not be functioning appropriately (and this assessment should include OQ and PQ).

Supplementary information

Design Qualification

DQ is the first phase in the qualification of premises and equipment. DQ involves the documented verification that the proposed design of premises and equipment meets the necessary regulatory and user requirement specifications. It ensures that all aspects of the design align with the intended purpose, relevant quality standards and operational needs.

This phase typically includes defining, reviewing and approving performance characteristics, test and verification methods, and predetermined acceptance criteria. The process also involves evaluating whether the design considerations adequately encompass factors such as safety, efficiency and maintainability.

Installation Qualification

IQ is the second phase in the qualification of premises and equipment. IQ involves the documented verification that the premises and equipment have been installed according to the approved design specifications and manufacturer's recommendations, such that consistent quality and compliance can be expected throughout their operational life.

It confirms that the installation is completed correctly and that all components are installed in their proper location, correctly connected and functioning as intended. The process typically includes checking the installation against engineering drawings, equipment manuals and other relevant documentation.

Examples of activities that may be undertaken during IQ include the following:

1. verification of equipment installation – documenting that the equipment is installed according to the manufacturer's instructions, including confirming the correct placement, orientation and assembly of components;
2. utility verification – ensuring that utilities such as electricity, water, gas or compressed air are correctly connected and within the required specifications for the equipment or system to function correctly;
3. calibration of instruments – verifying that all measuring instruments and control devices associated with the equipment are calibrated and functioning within specified tolerances;
4. component and material checks – confirming that all materials used in the installation, such as gaskets, seals and wiring, meet the specified standards and are properly installed;
5. documentation review – ensuring that all relevant documents, including manuals, drawings and certificates of compliance, are available, accurate and complete.

Through these activities, IQ establishes a solid foundation for subsequent qualification stages by ensuring that the installation is robust, compliant and capable of supporting the intended operations.

Common steps of IQ protocols may include:

1. verification of Conformité Européenne (CE) approval, or equivalent, where required;
2. verification that the location and environmental conditions of the premises/equipment are compatible with the manufacturer's recommendations and with any specific additional requirements that the SoHO entity may have for internal performance characteristics;
3. verification that the correct items have been provided by the manufacturer (e.g. checking part numbers against requests);
4. verification that items are installed in accordance with the supplier's recommendations and the SoHO entity's requirements;
5. verification that all parts of the equipment are free from defects;
6. verification that the connections of services, such as electricity, water, steam, pressure, vacuum etc., are functional and that their operating ranges are appropriate to the proper functioning of the installation;
7. identification of the items that require calibration, with checks of appropriate calibration certificates

and documentation of the programme and procedure for periodic calibration;
8. verification of the provision of instructions for performing preventive maintenance.

The execution of IQ may require expertise and measuring equipment that are unavailable to the SoHO entity and can be outsourced to the suppliers or an appropriate third party. However, the ultimate responsibility for the qualification, including IQ, always remains with the SoHO entity managing the SoHO preparations. The SoHO entity may also choose to engage an external expert to undertake this review and oversight on their behalf.

When a supplier or third party is engaged to perform IQ, the SoHO entity remains responsible for ensuring that:

1. the contracted party is demonstrably qualified and possesses the necessary expertise and experience to carry out the IQ tasks appropriately, including adherence to any required quality standards;
2. clear and detailed agreements are established with the contracted party, including a comprehensive description of the IQ procedures, specification of the necessary documentation and details of how to report the results;
3. all IQ documentation, including protocols and reports, is complete and accurate – the SoHO entity, or its designated representative, has the responsibility for approving these documents;
4. the results of IQ activity performed by suppliers or other third parties are reviewed and approved by the SoHO entity, or its nominated expert – this review and approval requires verifying that all specifications and requirements have been met and that the installation is ready for further qualification steps.

Operational Qualification

OQ is the third phase in the qualification of premises and equipment. OQ focuses on verifying that the premises or equipment function according to their specified parameters and function under actual operating conditions. During OQ, the performance of the premises or equipment is tested to ensure it consistently produces outcomes within the established acceptance criteria. This involves executing predefined tests and procedures that simulate the extremes of 'real world' operating conditions.

Through these activities, OQ provides documented evidence that the facility, equipment or computerised system operates as intended in the actual environment, setting the stage for the next phase of qualification validation, PQ. OQ ensures that the system or equipment can consistently deliver the desired outcomes in routine use. The completion of a successful OQ permits the finalisation of calibration, operating and cleaning procedures, operator training and preventive maintenance requirements, effectively permitting a formal 'release' of the premises and/or equipment.

Common steps of OQ protocols may include:

1. tests to evaluate the functionality of premises/equipment, tests which are usually performed under conditions encompassing upper and lower operating limits, sometimes referred to as 'worst case' conditions;
2. identification of performance characteristics, tests performed, alarms, security devices and acceptance criteria;
3. verification that, where relevant, items of premises/equipment have been correctly connected to mains services.

Examples of OQ protocols are provided in Appendices 4 and 5.

Performance Qualification

PQ is the fourth and last phase in the qualification of premises and equipment. PQ focuses on verifying that premises and equipment consistently perform according to the defined process parameters under 'real world' operating conditions, as opposed to the created 'worst case' conditions used for OQ. PQ will normally follow on from successful completion of IQ and OQ, although it may in some cases be appropriate to perform it in conjunction with OQ.

During PQ, the process is tested in the real-world environment where it will be used, often over an extended period. This phase involves using the premises or equipment to prepare SoHO preparations suitable for clinical application under normal operating conditions, to ensure that the desired outcomes are consistently obtained.

The key objective of PQ is to verify that the process not only functions correctly but performs reliably and consistently over multiple runs or cycles, over an extended period, by testing:

- process performance, which ensures that the premises or equipment operate within the specified performance characteristics (e.g. temperature, pressure, processing time) under actual production conditions;
- quality, confirming that the SoHO preparations prepared meet their required quality standards and specifications; for example, as specified in the relevant SoHO monographs (if one exists, see Part C).

The driver for the extent and duration of PQ is the criticality of the SoHO preparation: the potential risks it presents and the potential benefits that it can deliver to patients. This is essentially a risk-based determination. For example, where a SoHO preparation has the potential to cause serious harm to a patient if it does not meet its specifications, and where these specifications cannot be independently tested for each SoHO preparation, extensive PQ may be indicated. The variability of data may also play a role in this determination.

Common steps of PQ protocols may include:

1. simulated processes, using materials, qualified substitutes or model SoHO, that have been

developed from knowledge of the process and the facilities or equipment; these may include processes performed at extremes of the expected operating condition ('worst case' approach);
2. a detailed process description and protocol, or reference to protocol development where relevant;
3. a list of qualified personnel, facilities, equipment, materials and computerised systems involved;
4. the performance characteristics and operating ranges of the premises/equipment being validated;
5. a description of the tests to be performed, or control variables, including sampling methods, times and locations of sampling, and analytical methods.

3.5 Process validation and verification

Process validation and verification ensure that SoHO preparation processing protocols reliably accomplish their intended outcomes. This is generally completed before use of a protocol to prepare SoHO preparations for clinical use (prospective validation). In some circumstances, this may be combined with the preparation of SoHO preparations for clinical use (concurrent validation). In the case of well-established protocols that have been used to produce SoHO preparations for clinical use for a significant period of time, but are not supported by prospective validation, it may be acceptable to justify them on the basis of historical data demonstrating safety and efficacy.

Standards

S3.5 SoHO entities must ensure that any process that may have an impact on the safety and efficacy of SoHO preparations is validated to achieve its desired outcome (Art. 39.2.b.viii and Art. 58.7.a).

S3.6 Applications for SoHO preparations authorisation must include any specific conditions and time limits for storage and transport, including validation of those conditions and limits (Art. 39.2.b.vi).

Good Practice Guidelines

G3.29 Personnel, premises, equipment, materials, reagents and computerised systems to be used should be qualified before use, and analytical *in vitro* testing methods should be verified or validated.

G3.30 Processes should be validated to reliably achieve their documented outcome.

G3.31 All personnel, premises, equipment, materials, reagents and processes should periodically be re-assessed to verify that they are still operating in a valid manner. The periodicity of this re-assessment should be risk based and may take the form of a desk-based review of process data, or physical re-qualification.

G3.32 Process validation/verification should be completed prior to distribution of any novel SoHO preparations for clinical use (prospective validation/verification).

G3.33 A prospective validation or verification protocol should include, but need not be limited to, the following:

G3.33.1 a short description of the process;

G3.33.2 a summary of the critical processing steps to be investigated;

G3.33.3 a list of the personnel, premises, equipment, materials or reagents to be used (including measuring, monitoring and/or recording equipment together with its calibration status);

G3.33.4 quality-control checks for release of the processed SoHO;

G3.33.5 list of analytical *in vitro* test methods, as appropriate;

G3.33.6 proposed in-process controls with acceptance criteria;

G3.33.7 additional testing to be carried out, with acceptance criteria and analytical validation/verification, as appropriate;

G3.33.8 sampling plan;

G3.33.9 methods for recording and evaluating results;

G3.33.10 teams/departments and persons responsible;

G3.33.11 proposed timetable.

G3.34 Using this defined process (including specified components), a series of batches of the final SoHO preparations should be produced under routine conditions.

G3.35 The number of process runs carried out and observations made should be sufficient to allow the normal extent of variation and trends to be established and to provide sufficient data for evaluation.

G3.36 Batches, where applicable, made for process validation or verification should be the same size as the routine scale batches.

G3.37 In exceptional circumstances it may be acceptable not to complete a validation programme before routine processing starts and instead to validate a process during routine processing (concurrent validation) for novel SoHO preparations. The decision to carry out concurrent validation should be based on a documented risk assessment, justified, documented and approved by a person authorised by the Responsible Person (RP).

G3.38 Documentation requirements for concurrent validation are the same as specified for prospective validation.

G3.39 In the case of well-established SoHO preparations that have been provided for routine clinical use over a significant period, retrospective process validation/verification based on historical data may be acceptable. This should only be considered where there have been no recent changes in relation to starting materials, the SoHO preparations, operating

procedures, premises, equipment or critical materials or reagents.

G3.40 Retrospective validation/verification requires preparation of a specific protocol and reporting of the results of the data review, leading to a conclusion and a recommendation.

G3.41 Batches selected for retrospective verification should be representative of all batches made during the review period, including any batches that failed to meet specifications, and should be sufficient in number to demonstrate process consistency.

G3.42 Additional testing of retained samples, where possible, should be considered to obtain the necessary amount or type of data to retrospectively verify the process.

Recommendations

R3.5 It is recommended that the source of data for this retrospective verification (G3.40) may include, but need not be limited to:

- R3.5.1 batch processing and packaging records;
- R3.5.2 process control chart (or key performance indicators);
- R3.5.3 maintenance records;
- R3.5.4 records of personnel changes;
- R3.5.5 process-capability studies;
- R3.5.6 processed SoHO data;
- R3.5.7 storage stability results.

This list is not comprehensive and is recommended to be adapted for the SoHO in question.

Supplementary information

The scope of process validation is very broad, encompassing relatively simple processes such as site-to-site transport under temperature-controlled conditions through to validation of entire *de novo* SoHO preparations processing protocols. Some examples of validation are in Appendices 6 and 7.

3.6 Revalidation or requalification

Good Practice Guidelines

G3.43 Revalidation or requalification should be performed when there is a significant change in the process, premises, equipment, materials or reagents used to prepare a SoHO preparation that may affect the quality of the SoHO.

G3.44 Routine revalidation or requalification in the absence of significant changes is not necessary, provided that routine quality review is in place and confirms that the system or process is consistently producing material meeting its specifications. This should include review of meaningful quality indicators, including but not limited to:

- **G3.44.1** review of quality incidents associated with the process;
- **G3.44.2** review of process key performance indicators;
- **G3.44.3** process audits.

Recommendations

It is recommended that:

R3.6 when implementing changes to personnel, premises, equipment, materials or reagents, the impact on validated processes be assessed and their continued compliance with the performance characteristics be ensured: change management plays a crucial role in maintaining compliance and ensuring the continued quality, safety and effectiveness of SoHO preparations;

R3.7 a systematic approach, including risk assessments (e.g. EuroGTP II), be employed to tailor the validation requirements to the nature of the change and the risks arising from it. This approach ensures that validated systems continue to operate within predefined performance characteristics, maintaining product quality and regulatory compliance.

3.7 Validation and verification of *in vitro* diagnostic tests (IVDs)

The approach to validating or verifying analytical tests varies, depending on whether the test systems and kits being used are certified as compliant with the EU *in vitro* Diagnostic Medical Device Regulation [2] or are developed in house. In all cases it is important that the validation plan considers the variety of sample types (matrices) and analytes to be tested, as certain substances may be present that could cause interference.

Good Practice Guidelines

G3.45 Validation studies should be done to demonstrate that the performance of the IVD kit or test system, as used in the SoHO entity, meets the expected specification. For sterility testing, the methods should be verified in accordance with the details of the corresponding *Ph. Eur.* chapter.

G3.46 In-house developed IVD tests should be validated.

Recommendations

It is recommended that:

R3.8 for the validation of CE-IVD tests, the performance characteristics evaluated include trueness (bias), precision, interferences, linearity, detection limits, stability and the verification of reference values within the relevant population;

R3.9 measurement uncertainty also be determined for quantitative IVD tests [3, 4];

R3.10 for qualitative tests, specificity, sensitivity and interferences be the performance characteristics to be considered;

R3.11 for the validation of in-house IVD tests, the performance characteristics include consideration of trueness (bias), precision, analytical sensitivity, analytical specificity, linearity, stability, diagnostic specificity, diagnostic sensitivity and verification of the reference values of their own population.

Supplementary information

More detailed information concerning the validation or verification of *in vitro* diagnostic tests can be found at https://clsi.org/ [5].

Related material

Appendix 4. Example of cleanroom qualification
Appendix 5. Examples of operational qualification protocols
Appendix 6. Example of process validation – SoHO transportation
Appendix 7. Example of method validation – oocyte vitrification

3.8 References

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Chapter 4. **Risk Management**

4.1 Introduction

The collection and application of substances of human origin (SoHO) may generate risks for both donors and recipients, and a key finding from the evaluation of EU blood legislation in 2019, a finding which informs EU SoHO Regulation 2024/1938, was that these two groups were not fully protected from avoidable risks. This only applies where the donation event potentially places the donor at elevated risk; if the donation is a by-product of routine surgery, the donor will not be placed at elevated risk. Consequently, the Regulation establishes risk management as a fundamental, ubiquitous process that is integral throughout the SoHO preparation pathway, from donor screening through to application to the recipient. When performed accurately and thoroughly, risk management permits the identification, evaluation and (where necessary) mitigation of risks and is an essential tool for ensuring the protection of donors and recipients.

Other chapters of this Guide contain Standards, Good Practice Guidelines (GPGs) and recommendations stating specific situations where risk management is, or may be, required. The format of this chapter differs from that of other chapters in Part A in that it does not contain any specific mandatory Standards, and only a small number of GPGs and recommendations. Instead, it provides guidance on the core principles of performing risk assessment and also outlines specific tools for the performance of risk assessments. These tools may be generic processes that can be used in many different situations in the healthcare environment and beyond, or they may be specifically formulated for use with SoHO preparations.

4.2 Principles of risk management

The fundamental objectives of a risk assessment are to inform decision making, protocol design and policy setting to minimise risks to donors, recipients and staff.

The scope and format of risk assessments is informed by the process they are evaluating. For example, some risk assessments will address the consequences of discrete processing protocols [1] or the use of specific chemicals, whereas others may be more wide ranging, for example addressing risks of contamination of SoHO preparations during collection and processing, or risks arising from aspects of a donor's medical history [2]. There are, however, certain basic concepts that are common to all risk assessments. The core steps of any risk assessment are:

1. The identification of potential risks (or hazards) and determination of potential consequences arising from the risk.
2. Evaluation of the probability (or likelihood) that the risk will occur, and the severity (or impact) of the consequences if the risk occurs.

This is termed a 'two-dimensional' risk assessment, in that it focuses on probability and severity. Some models also incorporate an additional factor, that of detectability; this factor may be applicable to risks where it may not be immediately obvious that an event has occurred, for example transmission of a disease with a long incubation period. This is called a 'three-dimensional' risk assessment. When risks have been identified and their probability and consequences quantified, a determination can be made as to which of them need to be mitigated, and in what order of priority.

This order of priority will generally be based on the overall current risk score, and an acceptable residual risk score will be determined; it will rarely be possible to reduce a risk score to zero. If the targeted score is lower than the currently assessed score, action is indicated to

reduce one or more of the probability, severity or detectability scores for the risk to bring the score down to or below the acceptable level. Note that, for the detectability factor, that the objective is to increase it as much as possible; however, if the mathematical basis of the risk assessment calculation is maintained, increasing the level of detectability results in a lower scoring. The level of acceptable risk is also influenced by the potential benefits of the process under assessment; higher levels of residual risk may be acceptable, depending on factors such as the clinical benefits of the SoHO preparations and the availability of alternative treatment options if the SoHO preparation is not available.

Risk management can be applied prospectively and/or retrospectively. Prospective management of risk has the advantage of detecting and mitigating risks before they actually occur, while retrospective management can make use of historical data from a process to inform assessment of probability and severity, which in turn facilitates prioritisation of the most critical risks.

Retrospective risk management may also be instigated by corrective and preventive action arising from Serious Adverse Reactions and/or Events (SAREs) and quality incidents. SoHO entities are encouraged to make a risk management approach integral to all of their processes, considering that risk assessment is a cyclical process, not a once-only event; see Figure 4.1.

Risk assessment is only effective if it is followed by risk avoidance and reduction (if possible) and continual re-evaluation of residual risk to ensure that new information and data are incorporated. An entity's risk assessments may be considered by Health Authorities (HA), if appropriate, to inform the frequency and content of their inspection activities.

Risk assessments may be performed on a planned basis, to inform policies and protocols and to manage risks associated with novel or amended processes, or on an *ad hoc* basis in response to deviations from standard protocols, for example to determine the impact of the deviation on the suitability of the SoHO preparation for clinical application. Examples of the latter situation include:

- selection of a donor where full compliance with the normal criteria has not been met, but where the donation has a particular clinical value, and the potential risk can be mitigated sufficiently to justify the deviation from standard procedures;
- exceptional release of non-complying SoHO on the basis that the potential benefits for the recipient and the lack of availability of alternatives outweigh the potential risks;
- retention or removal of SoHO in storage that had been historically released according to former criteria, when new, more sensitive procedures or tests have been implemented that imply an additional level of safety or quality and new, more stringent criteria for release.

Specific sources of information that SoHO entities may incorporate in risk assessments include records of incidents recorded in the quality management system (QMS) and data from biovigilance programmes. These are useful to inform determination of probability and severity. Undertaking risk assessment during process development helps to define requirements and alternatives, aids the process of supplier selection and helps to determine the scope and extent of validation.

Figure 4.1 **Cycle of risk assessment**



Good Practice Guidelines

G4.1 All risk assessments should be documented.
G4.2 SoHO entities should maintain up-to-date copies of their risk assessments and be able to provide them to the HA on request.
G4.3 SoHO entities should periodically review their completed risk assessments at time intervals proportionate to the identified level of risk, reviewing those with higher scores more frequently.
G4.4 Where they are known and defined, risks should be related to the critical quality attributes of the SoHO preparation, and any factors that may adversely impact any of these attributes should be considered.

Recommendations

It is advised that:

R4.1 the output of risk assessments is determined by the quality and accuracy of its input and as such it is important that the data and expertise used are of the highest quality. It is also advisable that the risk assessment process includes as much available, relevant data as possible and that individuals with appropriate knowledge and experience take part;

R4.2 SoHO entities or establishments ensure that training in risk management, and the use of risk management tools, is integrated into their QMS, and forms part of the training matrix for personnel who will have responsibility for risk assessment. Where resources permit, it is advisable to organise training from experts in the field;

R4.3 SoHO entities or establishments prioritise the management of risks that score highly either for probability or severity within their QMS, with risks that score highly for both factors given the highest priority.

4.3 Generic risk management tools

Risk management is ubiquitous across many different industries and specialities, and a variety of different tools have been developed to provide standardised approaches to the process. Risk assessment is fundamentally a subjective process that requires educated estimations; however, it can be made more objective through the inclusion of as much data as possible, and the use of standardised methodologies.

A detailed discussion of these tools is beyond the scope of this Guide, but a comprehensive list of well-established tools can be found in ISO 31010: Risk management – Risk assessment techniques [3], together with a comprehensive review of the general concept and principles of risk management. Different tools are more appropriate for specific situations, and it is important that the tool selected is appropriate for the specific analysis.

However, there is rarely a definitive 'right' or 'wrong' approach to risk management in any given situation, and SoHO entities or establishments will select the resource that best meets the needs of the specific situation and is available to perform the assessment. In some cases, different models may be equally valid for carrying out the analysis. Allocation of the necessary resources, in terms of personnel and time, and identification and inclusion of relevant data are equally, if not more, important than choosing the 'best' model.

In addition to the international standard ISO 31010 referenced above, further guidance may be obtained from the EU Good Manufacturing Practice (GMP) rules and guidelines as to how risk management techniques can be applied to the preparation of medicinal products [4]. While these may not be directly applicable to the preparation of SoHO preparations, they do provide useful examples of how generic tools can be adjusted and applied to specific situations. This section (§4.3) outlines the key features of some of the generic risk management tools commonly used by SoHO entities or establishments and provides examples of situations where they might be used. Where SoHO entities have access to dedicated QMS expertise, this may assist them with adapting standard tools to their own needs.

4.3.1 Hazard analysis and critical control points

Hazard analysis and critical control points (HACCP) was developed in the 1950s in the food industry, but is now widely used for many manufacturing processes, including biological control. The objective of HACCP analysis is to identify potential hazards, and control them at specific, key points in a process, rather than through inspection of the end product. HACCP is widely used in process design within the pharmaceutical industry, and there are helpful publications from the World Health Organization [5] and in the *Quality Assurance Journal* [6] providing guidance on its use in this area. These may be adapted and interpreted for use within a SoHO entity or establishment.

4.3.1.1 *Hazard Analysis Critical Control Points Process*

The key steps of HACCP are:

1. Conduct a hazard analysis to identify hazards and related preventive measures.
2. Identify the critical control points (CCP). These are points in the process where the identified hazards can be controlled.
3. Establish the critical limits necessary to control the hazards, for specific parameters.
4. Monitor the critical limits for each identified CCP and establish set time intervals for this monitoring.
5. Define the appropriate corrective actions to be taken if a process falls outside the critical defined limits.

6. Establish verification procedures to ensure that outputs are accurate.
7. Ensure that appropriate record keeping and documentation procedures are in place for all steps of the process.

4.3.1.2 *When is hazard analysis and critical control points applicable?*

The nature of the HACCP process ensures that it links well with quality control protocols, through the generation of documented evidence. It takes a practical approach by which to identify where in a process the risks can be most effectively controlled (not merely monitored), and continual control throughout the process is more effective than relying on quality control of the final product. It is especially suitable for identifying where hazards can result from human actions. However, it requires that hazards can be accurately identified, and the resultant risks and suitable controls defined.

With regard to SoHO preparation, the HACCP process is particularly suited to activities such as the appropriate placement of environmental monitoring locations in cleanroom facilities.

4.3.2 Root Cause Analysis

Root Cause Analysis (RCA) is employed in response to significant quality incidents, which may have caused the loss of donations or resulted in major regulatory non-conformances, or any other type of incident where rapid action is necessary to reduce the risk of its recurrence. The key principle of RCA is to systematically analyse the whole process to identify the root cause of the problem, rather than jump to obvious conclusions. In some models RCA may be performed prospectively where potential fault or failure mechanisms can be identified at the design stage of a process. Examples of RCA can be found in Appendices 8, 9 and 10.

4.3.2.1 *Root Cause Analysis Process*

RCA can be performed according to various defined methodologies; however, these all follow the same core sequence:

1. Establish a group of experts with the requisite knowledge to analyse the process.
2. Document the scope and objective of the RCA.
3. Ensure that all evidence (including data) relating to the incident is collected and available to the RCA team.
4. Undertake a structured analysis to identify the root cause of the incident. There are different methodologies available for this process, two of which, Fault (or failure) Tree Analysis (FTA), and Failure Mode, Effects and Criticality Analysis (FMECA), are discussed in more detail below. FMECA may also be used as a standalone risk assessment tool in its own right.
5. Determine evidence-based solutions and make recommendations as to how these will be implemented.
6. Oversee the implementation of these recommendations.
7. Verify that the recommendations have been successfully implemented, and that they have been successful in reducing the risk of the incident recurring. While the content of any given RCA is specific to the incident in question, there are generally five common factors that will need to be considered: machinery and equipment, personnel, methodology, the environment and the product itself.

4.3.2.2 *Fault (or failure) tree analysis*

Fault (or failure) tree analysis (FTA) is a structured risk analysis technique that commences with the event in question and identifies all the causal factors that led to the event occurring. There may be multiple levels of causal factors which lead on from each other resulting in the index event occurring. These can be shown pictorially in a 'tree' diagram. If sufficient data are available, it may be possible to assign probabilities to these causal factors to determine which are most relevant to controlling the risk, ensuring that resources are used most effectively.

FTA is particularly suited to the analysis of complex events which may result from multiple factors and multiple interactions. The output can be analysed to determine multiple potential failure pathways and identify the most relevant where resource input will result in maximum risk reduction. However, the model relies on simple binary states (pass/fail) and may be less effective in evaluating error modes that are more complex, especially human factors.

4.3.2.3 *When is RCA applicable?*

Typically, RCA is performed by a SoHO entity or establishment reactively in response to incidents, although certain models may be appropriate for use proactively during process design. The simplicity of the process enables it to be adapted to multiple different types of incidents, and there are many types of RCA model that can be employed, in addition to the two described above. Other widely used RCA models include fishbone diagrams and the '5 whys' technique. Examples of how these models can be applied to SoHO preparation are provided in Appendices 8, 9 and 10 of this Guide. However, it bears repetition that the quality of the output of an RCA is dependent on the expertise and data that can be assembled to analyse it, and on the process being allocated sufficient time and resources. It may also not be practical to implement all recommendations where resources are limited.

4.3.3 Failure Modes, Effects and [Criticality] Analysis

Failure Modes, Effects and [Criticality] Analysis (FMECA) is an extension of the Failure Modes and Effects Analysis (FMEA) process. FMEA is a bottom-up process that aims to identify all potential modes of failure in a system, and then assess their likelihood, severity and detectability. FMECA adds a further assessment that permits the failure modes detected to be ranked in terms of importance, or criticality. This enables the identified failure modes to be prioritised and addressed in order of relevance.

4.3.3.1 *Failure Modes, Effects and [Criticality] Analysis Process*

The FMEA and FMECA processes can be summarised as follows:

a. Identify potential failure modes, and their effects.
b. Determine the severity of each failure mode.
c. Determine the likelihood of each failure mode.
d. Determine the detectability of each failure mode.
e. Assess the criticality of each failure mode.

Steps a. to d. are the FMEA process; for FMECA a fifth step, a criticality analysis (e.), is performed when FMEA has been completed; see Figure 4.2.

4.3.3.2 *When is FMEA/FMECA applicable?*

The FMEA process is commonly applied at the process design stage, where it can be used prospectively to identify potential causes of failures in novel systems. When used at this point, it can help define the process itself to ensure greater reliability and to inform routine maintenance testing and quality control protocols for the process. FMECA outputs rely on accurate data to inform estimations of probability and severity, and the criticality component of the analysis may be carried out following the implementation of a process when such data are available.

4.4 SoHO preparation-specific tools

The generic risk management tools discussed in the previous section are based on tried and tested principles that have been utilised in multiple sectors for many years. However, they may be adapted for use in specific situations; there is very rarely a one-size-fits-all approach and, where possible, tools which have been specifically designed to be utilised when assessing SoHO related activities are to be used. The final section of this chapter covers two such SoHO preparation-specific tools.

4.4.1 Euro Good Tissue Practices II

Whenever a novel process is implemented, or an existing process revised, it is essential for the SoHO entity to assess the risk resulting from this change. The Euro Good Tissue Practices II (EuroGTP II) project was funded by the European Union to develop a process to enable SoHO entities or establishments to do this in a systematic and consistent manner [7, 8]. The project brought together SoHO entities, Competent Authorities, professional societies and independent experts, and produced a number of key deliverables, including an interactive assessment tool (IAT) to perform risk assessment and a detailed Guide to application of the IAT. Both of these deliverables can be accessed online [9].

4.4.1.1 *Euro Good Tissue Practices II Process*

There are three steps to the EuroGTP II methodology (see Figure 4.3):

1. Identification of novelty

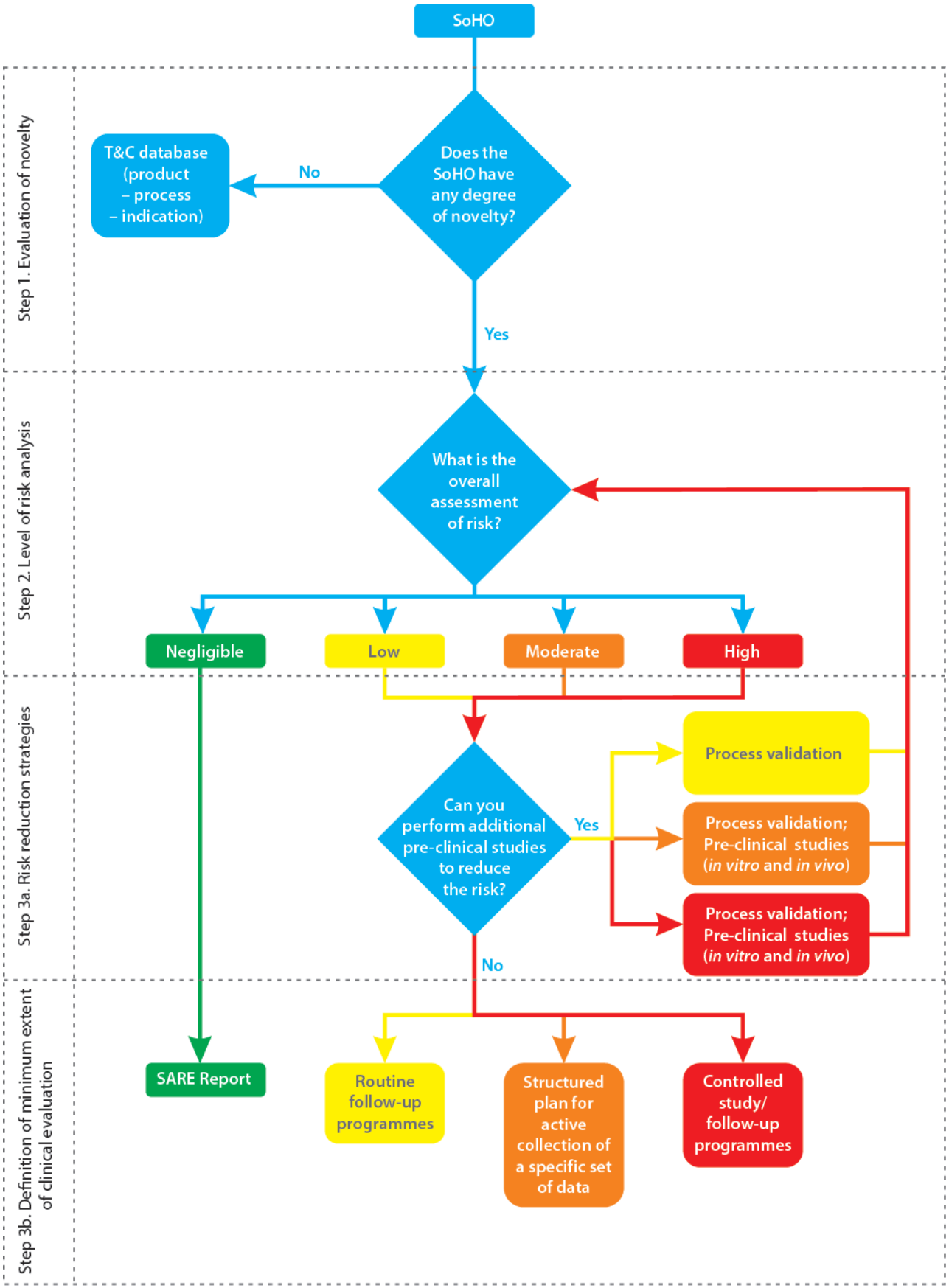
With any novel process, it is very likely that significant changes, which could impact on the safety and/or clinical efficacy of the product, will need to be implemented. This is also possible when a change is made to an existing process. The first step of the process requires that the user considers each step of the supply chain, from donor selection to transplantation, to determine if any such significant change has been made. If any is identified, the

Figure 4.2 **Failure mode, effects and criticality analysis (FMECA)**



user proceeds to step 2. If not, no further action is necessary, and the IAT can generate a documented record that the evaluation was undertaken and the rationale for the decision.

Figure 4.3 **Overview of the EuroGTP II process: assessing SoHO preparations**



2. Identification and evaluation of specific risks

If significant change is identified, the SoHO entity then determines the specific risks and risk consequences that the change raises, and the likelihood, severity, detectability and evidence that can be used to mitigate each risk. Based on the scores allocated, the IAT calculates an overall risk score for the change, ranging from negligible to high.

3. Risk mitigation and extent of clinical evaluation

Depending on the outcome of step 2, the user may need to take steps to reduce the risk resulting from the change. The EuroGTP II tool Guide provides guidance as to how this can be done, listing *in vitro* and *in vivo* assays that can be used to further investigate specific risks. The Guide also suggests clinical follow-up methodologies that are appropriate to designing clinical evaluations or registries for the products in question, either to collate additional data to inform the risk analysis or to implement routine follow-up methods and registries following implementation of lower-risk products. Where higher levels of risk are identified, clinical application may only be appropriate in the context of an ethically approved, controlled clinical investigation. It is worth noting that the EU SoHO regulation applies a very similar approach in this respect to the EuroGTP II methodology. Please see Chapter 19 for further details.

4.4.1.2 *When is the EuroGTP II process applicable?*

As discussed previously, risk management is a continual, cyclical process. The EuroGTP II methodology exemplifies this principle in that it can be performed at any stage of the product design cycle, from evaluating potential risk in the initial concept to quantifying the impact of minor changes in established processes. The design of the tool allows existing risk assessments to be amended to accommodate subsequent process changes or incorporate new data without the need to re-analyse the entire process. Furthermore, the online IAT can be used to document and record the decisions made and their rationale at all stages of the risk-assessment process, and the outcome can be printed and shown to an HA as evidence that an appropriate risk-assessment process has been undertaken with respect to novel and changed processes.

As with all types of risk assessment, the quality of the output depends on the quality of the input, and a key requirement of the process is that sufficient resource and expertise be committed to the risk assessment to ensure an accurate outcome. The EuroGTP II tool has been used successfully to evaluate risks from different types of SoHO, and an expanding list of publications is maintained on the EDQM website [8]. This provides a useful library of examples for any SoHO entity planning this type of risk assessment.

4.4.2 EDQM Microbiological Risk of Contamination Assessment tool (MiRCA)

The aseptic collection and processing of SoHO are some of the more difficult processes conducted by SoHO entities and entail some degree of risk. Failure in some of these process steps may lead to microbiological contamination and loss of SoHO preparations or may even pose a potential health risk if the contamination is not detected before implantation or use. Microbial safety cannot be assured only by finished-product testing because the available microbiological test methods are not always fully conclusive, with the possibility of false negative results, and sometimes test results are not yet available at the time of implantation or use. Furthermore, for some types of SoHO preparation, such as those used in medically assisted reproduction (MAR), options for microbiological testing are limited due to the nature of the SoHO. In addition, the environmental monitoring that is often performed during aseptic processing of SoHO cannot be considered as a reliable quantitative measurement of the level of cleanliness of the aseptic environment owing to the limitations of classic microbiological air-sampling methods.

However, prior to the development of the MiRCA tool, there was no specific risk-management process available to assess the risk of SoHO preparations being contaminated during collection and processing. This risk is influenced by a large number of variables, including cleanliness of the local infrastructure, type of garment, SoHO type, number and competence of personnel, degree of qualification of infrastructure, equipment and materials used, collection and processing methods, decontamination processes, environmental monitoring processes and local expertise, among others. This gap was recognised during the revision of the 5th edition of this Guide, and EDQM assembled an expert group to develop the MiRCA tool to meet this need [9].

4.4.2.1 *The MiRCA Process*

The core objectives of the MiRCA process are to:

a. identify potential risks in novel, existing or modified aseptic processes;
b. alert the SoHO entity or establishment management to the degree of risk of introducing microbiological contamination during the collection or processing of SoHO;
c. support management decisions to mitigate risks in aseptic processes.

The fundamental objective of MiRCA is to help SoHO entities to prevent the loss of SoHO preparations due to microbial contamination during aseptic collection and processing and to reduce the risk of infection being transmitted to recipients. MiRCA starts from the assumption that the primary source of microbial contamination during aseptic processing is human, more specifically personnel and donors/patients. This assumption is based on the knowledge that a gowned operator may

release continuously as many as 10 000 colony-forming units per hour [10].

However, the MiRCA tool utilises a much broader approach, focusing on all sources of contamination affecting the deposition of micro-organisms during aseptic processing. In many aseptic processes, operators are using inanimate objects, such as equipment, materials and reagents, that could disperse their load of microbial contamination to the environment or directly to SoHO preparations. Also, the training of personnel and the qualification and monitoring of environments and equipment has an impact on the deposition of micro-organisms. Special relevance is also given to the presence and effectiveness of decontamination processes, quality control and monitoring processes. The implementation or non-implementation of these processes is included in MiRCA and each process is assessed as an individual risk factor.

The MiRCA process identifies in total 55 individual risk factors: 22 individual risk factors that affect the deposition of micro-organisms during aseptic collection of SoHO preparation and 33 individual risk factors that affect the deposition of micro-organisms during aseptic processing and distribution of the SoHO preparation. All standard risk-assessment tools consider two dimensions: probability of occurrence and impact (severity) of occurrence on the objective. Normally, the duration of occurrence is part of the probability of occurrence. In MiRCA it was not always possible to incorporate the duration of exposure into the probability of occurrence. For that reason, a third dimension was included: temporal distribution.

The semi-quantification of risk, expressed as an individual risk value (IRV), is calculated by multiplying the probability of occurrence (P), the impact of the occurrence on the objective (I) and the temporal distribution (T) of each risk factor:

$$IRV = P \times I \times T$$

4.4.2.1.1 Probability of occurrence

The probability of occurrence (P) estimates how likely is the deposition of micro-organisms to occur due to individual risk factors. The probability spectrum is expressed on a scale of 0 to 5:

0 = negligible or not applicable
1 = very low
2 = low
3 = moderate
4 = high
5 = very high

For example, for the risk factor 'air filtration system of the collection environment' the probability of deposition of micro-organisms is:

0 = (negligible) if either not applicable or a closed system;
1 = (very low) if the air filtration technology is temperature-controlled high-efficiency particulate air (HEPA)-filtered air flow;
2 = (low) if the air filtration technology is vertical or horizontal laminar HEPA-filtered air flow;
3 = (moderate) if the air filtration technology is turbulent mixed HEPA-filtered air flow;
4 = (high) if the air filtration technology is turbulent mixed non-HEPA-filtered air flow;
5 = (very high) if there is no ventilation because no air filtration technology is available.

4.4.2.1.2 Impact of occurrence on the objective

Different risk factors have different impacts on the risk of loss of SoHO preparations. In addition, different risk factors have a different impact on the loss of SoHO due to microbial contamination. In order to reflect this in the tool, MiRCA applies an impact factor (I) of 1 to 5 for each risk factor, based on the assumption that the main mechanism for microbial dispersion during aseptic processing is airborne deposition:

1. The risk factor indirectly affects the deposition of micro-organisms in the background of the environment that SoHO preparations are exposed to. For example, periodical qualification of the background at rest has no direct influence on the deposition of micro-organisms in the background, but it tells us whether the microbiological load of the background is within the specifications.
2. The risk factor directly affects the deposition of micro-organisms in the background of the environment that SoHO preparations are exposed to. For example, the number of personnel present in the background of the processing environment has a direct influence because these operators continuously release micro-organisms in the background of the environment that SoHO preparations are exposed to.
3. The risk factor indirectly affects the deposition of micro-organisms in the environment that SoHO preparations are exposed to. For example, the frequency of sanitisation of the collection environment affects the microbiological load of furniture, equipment, floors and walls, which has in turn an impact on the microbiological load of the collection environment that SoHO preparations are exposed to.
4. The risk factor directly affects the deposition of micro-organisms in the environment that SoHO preparations are exposed to. For example, the number of personnel present during collection is a risk factor because they continually release micro-organisms into the collection environment that SoHO preparations are exposed to.
5. The risk factor directly affects the deposition of micro-organisms onto SoHO preparations; for example, the storage of SoHO preparations in non-sterile reagents could directly contaminate SoHO.

4.4.2.1.3 Temporal distribution

The temporal distribution (T) of risk factors that have an impact on the airborne deposition of microbial contamination is estimated by the time during which the SoHO preparations are exposed to their direct environment, expressed in hours – for example, the time that an open container containing SoHO preparations is exposed to the process environment, or the time that musculoskeletal tissues are exposed to the air of the collection environment during collection. Other risk factors, mostly intervention-dependent, are assessed as present/applicable or not present/not applicable – for example, the use of reagents, or personnel competence.

At the end, all IRVs are added up to calculate a final risk score for the collection of SoHO. The outcome of the collection of SoHO preparations is added as an individual risk factor in the assessment of the processing and distribution of SoHO preparations. The outcome of the global process is calculated as the sum of all individual risk values of processing, multiplied by the individual risk value of the terminal sterilisation or decontamination method, expressed as a percentage, with the sterility assurance level (SAL) shown in brackets:

- Validated sterilisation process (SAL $\leq 10^{-6}$), e.g. an appropriate dose of gamma irradiation: 99% reduction of the final risk score.
- Any validated method that achieves (SAL $\leq 10^{-5}$ or $\leq 10^{-4}$), for example a chemical treatment: 90% reduction of the final risk score.
- Any validated method that achieves (SAL $\leq 10^{-3}$), for example a validated antibiotic cocktail at 22° to 37°C: 50% reduction of the final risk score.
- Any validation method that achieves (SAL $\leq 10^{-3}$ and $\leq 10^{-2}$); for example, a validated antibiotic cocktail at 2° to 8°C: 20% reduction of the final risk score.
- No decontamination or non-validated decontamination method: no reduction of the final risk score.

4.4.2.1.4 Calculation of final risk score

The final risk scores, expressed as a percentage of the maximal achievable risk score, are then translated to a risk profile by an exponential algorithm, which is proportional to the overall risk of introducing microbial contamination during procurement or processing and distribution of SoHO:

- 0 to 3.12% Extremely rare
- 3.13 to 6.24% Rare
- 6.25 to 12.4% Unlikely
- 12.5 to 24.99% Possible
- 25.00 to 49.99% Likely
- 50.00 to 100% Almost certain

4.4.2.2 *When is the MiRCA process applicable?*

MiRCA provides a structured and systematic methodology to enable SoHO entities to identify, quantify and mitigate aseptic risks of established and novel aseptic processes. Furthermore, it is a continual process, which can be repeated to inform and support management decisions, or as a step within the Corrective and Preventive Action (CAPA) process or the change-control process. The aseptic risk outcome can be used during the EuroGTP II exercise when assessing the probability of a specific aseptic risk consequence.

MiRCA can help SoHO entities to define the minimal requirement of their collection and processing facilities with respect to all applicable aseptic risk factors and any subsequent decontamination step taken after collection or processing (see also Chapters 8 and 10). Within the EU, however, MiRCA cannot be used to justify deviation from the minimal air quality requirements set by the EU Tissue and Cell Directives and/or national laws. EU SoHO Regulation 2024/1938 will permit SoHO entities to establish their own minimal air-quality requirements based on a risk-assessment approach. It is anticipated that MiRCA will be an extremely valuable tool for this purpose.

As with the EuroGTP II process, the online tool [9] can be used to document and record the decisions made and the rationales used in different stages of the risk-assessment process, and the outcome can be printed and shown to an HA as evidence that an appropriate risk-assessment process has been undertaken with respect to novel and changed aseptic processes.

4.5 References

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Related material

Appendix 8. Example of root cause analysis: 5 whys – receipt of tissue
Appendix 9. Example of root cause analysis: fishbone diagram – receipt of SoHO
Appendix 10. Example of root cause analysis: fishbone diagram – medically assisted reproduction

Chapter 5. Recruitment of potential donors, identification and consent

5.1 Introduction

Substances of human origin (SoHO) can be obtained from living or deceased donors. However, some SoHO can only be obtained from living donors. In order to ensure availability of SoHO, and the safety and success of any transplantation or human application programme, potential donors need to be identified and recruited. In every case, donor evaluation is the first, crucial step to ensure the safety of the SoHO donated, to exclude any contraindications to donation and, in the case of a living donor, to exclude any medical situation that could potentially harm the donor themselves. This chapter describes the process, requirements and key elements at the beginning of the donation pathway for both living and deceased donors.

5.2 Donor protection

Standards

S5.1 A SoHO entity must ensure respect for the dignity and integrity of donors (Art. 52.1).

S5.2 A SoHO entity must ensure high levels of safety and protect the health of living donors from risks related to the donation (Art. 52.2) (see Chapters 6 and 8).

S5.3 A SoHO entity must (Art. 53.1):

a. meet all applicable consent or authorisation requirements in force in their country;

b. provide donors or, where applicable, any persons granting consent on their behalf, in accordance with national legislation, with all appropriate information relating to the donation process, in accordance with national legislation. This information must be provided in a way that is adequate in view of their capacity to understand it, alongside the contact details of the SoHO entity responsible for collection from which they can request further information, if needed;

c. safeguard the rights of the living donor to physical and mental integrity, to non-discrimination, to privacy and to the protection of their personal data, including health data concerning them; Regulation (EU) 2016/679 applies in EU countries;

d. ensure that any SoHO donation is voluntary and unpaid. This also applies when the SoHO are donated exclusively for use in research without any human application (Art. 54.6); see section 5.3;

e. identify and minimise risks to health of living donors (see Chapters 6 and 7);

f. in cases where SoHO can be donated repeatedly, and frequent donation might negatively influence the living donor's health: verify, by means of registries, that living donors are not donating more frequently than is indicated as safe in technical guidelines and monitor relevant health indicators to evaluate whether their health is not compromised;

g. in cases where donation implies a significant risk to a living donor, develop and implement a plan for monitoring the SoHO donor's health after donation;

h. in the case of an unrelated donation, refrain from revealing the donor's identity to the recipient or to the offspring from medically assisted reproduction, apart from in circumstances where such information exchange is permitted in the Member State concerned.

S5.4 SoHO entities must collate data relating to their SoHO activities and submit the data to the EU SoHO platform as part of their annual report (Art. 41.1).

Additional standards for donor protection: local and national registries are useful tools for medium- and long-term follow-up of SoHO donors. More information on registries and relevant standards is given later in this chapter (section 5.6).

Recommendations

It is recommended that:

R5.1 for living donors, there be a donor follow-up protocol for each type of SoHO donation, except where SoHO donation is incidental to a separate surgical treatment procedure, and each protocol [1, 2] includes a list of physical examinations and tests to be performed at recommended intervals;

R5.2 donor protection include the measures prior to donation (e.g. age restrictions, defined number of maximum donations) and post-donation (e.g. collection of donor outcome data and long-term follow-up of donors);

R5.3 measures for the protection of haematopoietic progenitor cell (HPC) donors be harmonised, including guidance for the medical suitability assessment and eligibility criteria of HPC donors and for the collection of a minimum dataset on all HPC donors and donations [3, 4]. The European Committee on Organs, Tissues and Cells (CD-P-TO) recommendation on establishing harmonised measures for the protection of HPC donors is a helpful tool for all Health Authorities and facilities involved in HPC donor clearance and clinical follow-up of donors after the HPC donation process;

R5.4 procedures include prospective collection of donor outcome data and long-term follow-up of all donors through data collection and analysis in large registries, including measures to ensure the protection of donors [5].

5.3 Donor recruitment

Standards

S5.5 A SoHO entity must not provide financial incentives or inducements to donors or any persons granting consent on their behalf (Art. 54.1).

S5.6 In cases of compensation of living donors, this must be in accordance with the principle of voluntary and unpaid donation and based on transparent criteria, including through fixed allowances or non-financial forms of compensation. Any SoHO entity must follow conditions of compensation established within the national legislation of their country including the upper limit set to guarantee financial neutrality. SoHO donors may choose not to be compensated (Art. 54.2).

S5.7 In the case of compensating living donors, at the request of their Health Authority, the SoHO entity must provide information in a transparent manner on the details of how they have implemented the conditions laid down in national legislation (Art. 54.5).

S5.8 Promotion and publicity activities in support of the donation of SoHO must not refer to compensation (Art. 54.4).

Good Practice Guidelines

G5.1 Public campaigns should aim to diversify and increase the pool of available donors, the number of donations and registration in living donor registries.

G5.2 All necessary measures should be taken to ensure that any promotion and publicity activities in support of the donation of human SoHO comply with guidelines or legislative provisions laid down by the member states (MS) and the associated restrictions or prohibitions on advertising the need for, or availability of, human SoHO with a view to offering or seeking financial gain or comparable advantage.

G5.3 Procedures should be implemented and maintained in accordance with the applicable legislation for recruiting living donors in an ethical manner, for ensuring their safety and wellbeing, and for the identification and referral of all potential deceased donors.

G5.4 Professionals involved in the recruitment of living donors, and in the identification and referral of deceased donors, should be trained and appropriately qualified.

G5.5 The method of verifying the donor's identity should be described in an identification procedure. The verification should be performed based on at least two independent factors, like date of birth and name, or name and hospital patient number.

G5.6 Identification and referral of deceased tissue donors should be in compliance with the national deceased donation programme.

G5.7 A system should be established between the SoHO entity responsible for the collection and the corresponding SoHO establishment to ensure that any deceased individual who is a potential donor can be detected within an adequate period of time to allow an effective donation.

Supplementary information

Donor recruitment is a continuous process. It begins with increasing the awareness of the general public, by education at local and national levels, about the benefits of the clinical applications for different SoHO.

Success stories describing donors' and patients' experiences and testimonials of family members may encourage people to consider whether SoHO donation is right for them. The most common instances of donation from living donors where donor recruitment plays

an essential role are the donation of HPC and of gametes and embryos for medically assisted reproduction (MAR) procedures as well as donation of human milk or intestinal microbiota.

5.3.1 Recruitment of haematopoietic progenitor cell donors

Successful HPC transplantation depends on matching the donor and recipient for human leukocyte antigens (HLA). If a patient needs an HPC transplant, their siblings and close relatives are screened to determine if their HLA types are compatible [6]. Information on specific considerations when the donor is under the age of legal consent can be found in Chapter 25.

For patients without a matching family donor, there is a possibility that an HLA-compatible voluntary donor can be found [6]. Therefore, volunteer donors are generally recruited and registered on donor registries around the world, particularly from diverse ethnic communities. Donor registries and cord blood banks are linked internationally, in a global database operated by the World Marrow Donor Association, using tissue-typing to establish rapid identification of potential donors [7].

When a person volunteers to be an HPC donor, an initial evaluation is performed. Suitable donors are HLA-typed and their details are placed on a donor registry. Potential donors are requested to inform the registry if there is any change to their health status that could contraindicate donation or the possible use of the cells.

Potential HPC donors will only be requested to donate HPC if they are identified as the best possible match for a patient. In this case they will be requested to attend a donation centre for a full explanation of the procedures by the clinical staff and a thorough medical examination with blood tests to detect any medical and/or infectious contraindication to donation. Further information on donor registries and recruitment of HPC donors can be found in Chapter 25.

5.3.2 Recruitment of cord blood donors

Umbilical cord blood (UCB) donor recruitment usually starts during pregnancy, but it may also occur as late as at admission into the maternity unit, provided the mother is fit to provide an informed consent. The UCB donation can be made available for public or private uses. Public banks provide UCB units stored for unrelated transplantation, in order to increase access to UCBs through worldwide donor registries, whereas in private banks, which are usually managed by for-profit organisations, the donor keeps ownership of the product. The latter offer the processing and storage services for future potential medical applications for donors and their family.

Information leaflets or brochures about UCB banking are an important part of the recruitment plan, and any claims made in recruitment materials are supported by scientific evidence [8] (see Chapter 26). In this regard, the Council of Europe CD-P-TO, composed of internationally recognised experts, has created a guide for parents to provide clear, accurate and balanced information about the use of cord blood in medical treatment and to guide parents through their cord blood storage options [9].

5.3.3 Recruitment of donors in medically assisted reproduction

This section focuses on the recruitment of third-party donors.

Gamete and embryo donation is a complex practice in which multiple ethical considerations, legal restrictions, medical facts, and social and psychological consequences intermingle. Things are further complicated by such factors as:

a. the reasons why a person donates;
b. what kind of reproductive cells are donated (donation of oocytes or sperm or embryos commits the donor to a different investment in the medical interventions required and the risk of harm);
c. the level of anonymity, whether a known donor, identifiable or anonymous;
d. how often a donor can donate and how many offspring may be derived from a single donor.

As with any living donors, gamete donors are entered into registries, not as a database of potential donors, such as for HPC, but to guarantee traceability and transparency of practice (see section 5.5 and Chapter 17). In addition, registries allow adequate follow-up of the donors, including the collection of data on serious adverse events or adverse reactions in donors, in recipients and in the resulting offspring (see Chapter 18).

Further information on recruitment of gamete and embryo donors can be found in Chapter 30.

5.3.4 Identification and referral of deceased SoHO donors

SoHO donation depends on professional training, organisational commitment and the development of strong working relationships between various professionals in different organisations for identifying and referring possible tissue donors to a SoHO entity. The referral may originate from SoHO entities, hospitals and other centres, such as medical examiners' offices, mortuaries, coroners' offices, forensic institutes, funeral homes, emergency medical services and nursing or retirement homes [10]. For a successful deceased donation programme, identifying all of the parties who may be involved allows the process to be co-ordinated and facilitated and maximises the opportunities for successful collection. The key parties are summarised in Table 5.1.

Alongside the WHO Critical Pathway for deceased donation [11], which was conceived as a useful clinical tool applicable in every country, region or hospital for assessing the potential of deceased organ donation, evaluating performance in the deceased donation process and identifying areas for improvement, a Clinical Pathway for deceased tissue donation has been designed by the CD-P-TO with the same goal [12]. The value

of this tool is that it creates uniformity in the description and assessment of the deceased donation process and defines types of donors based on the different phases of the donation process: possible, potential, eligible, actual and utilised tissue donors (see Figure 5.1).

Recommendations

It is recommended that:

R5.5 systems be in place to ensure that:

- R5.5.1 any potential donors considered for organ donation are also referred for potential tissue donation [13];
- R5.5.2 all deaths (typically hospital but also community deaths) are routinely referred to a donor co-ordinator, collection entity or SoHO establishment, regardless of the age of the patient or the cause of death;

R5.6 donation professionals learn to obtain a decision about donation while showing empathy to loved ones and also taking the wishes of the deceased individual into account and with understanding of their cultural and religious beliefs;

R5.7 every SoHO entity perform an analysis of the potential donors, actual donors, causes to reject a potential donor and all the statistics considered helpful to improve the detection system and increase donation efficacy.

Supplementary information

Routine referral of all deceased individuals with no known medical contraindications for SoHO donation gives every individual the opportunity to be a donor. It is beneficial that donation professionals bear in mind that grief and mourning are essential processes that individuals need to endure when coping with the death of a loved one. When approaching a bereaved family to discuss donation, donation professionals typically take a sensitive approach, understanding the nature of grief and the different ways in which it may manifest in individuals. They can then use their skills and experience to support donor families and provide help as they judge appropriate.

5.3.5 Donor consent

Standards

S5.9 A SoHO entity or establishment must provide living donors or, where applicable, any persons granting consent on behalf of a donor, with all appropriate information relating to the donation, in accordance with national legislation (Art. 55.1).

S5.10 A SoHO entity or establishment must provide the relevant information before the consent to donate is granted. The information must be provided in an accurate and clear manner, using terms that are easily understood by the donors or, if applicable, any persons granting consent on their behalf. The information must not be misleading, in particular as to the benefits of the donation for future recipients of the SoHO concerned (Art. 55.2).

S5.11 In the case of living donors or, where applicable, persons granting consent on their behalf, the SoHO entity or establishment must provide information regarding (Art. 55.3):

a. the purpose and nature of the donation;

b. i. the intended use of the donated material, specifically covering proven benefits for future recipients;
ii. any possible research or commercial uses, including any use to manufacture products under a different regulatory framework , to which specific consent must be granted;

c. the consequences and risks of the donation;

d. the obligation for consent, in accordance with national legislation, in order for the collection to be carried out;

e. the right to revoke consent and any restrictions on that right after the collection;

f. the purpose of the tests that will be performed in the course of the donor health evaluation;

g. the right of the donor or, where applicable, the person granting consent on their behalf, to receive the confirmed results of the test when relevant for their health, in accordance with national legislation;

h. the recording and protection of the donor's personal data, including health data, and medical confidentiality, including any potential sharing of data in the interests of monitoring the donor's health and of public health, as necessary and proportionate, in accordance with data protection regulation;

i. the possibility that the donor identity may be revealed to the offspring from medically assisted reproduction that is born as a result of their donation in cases where national legislation grants that right to such offspring;

j. other applicable safeguards to protect the donor.

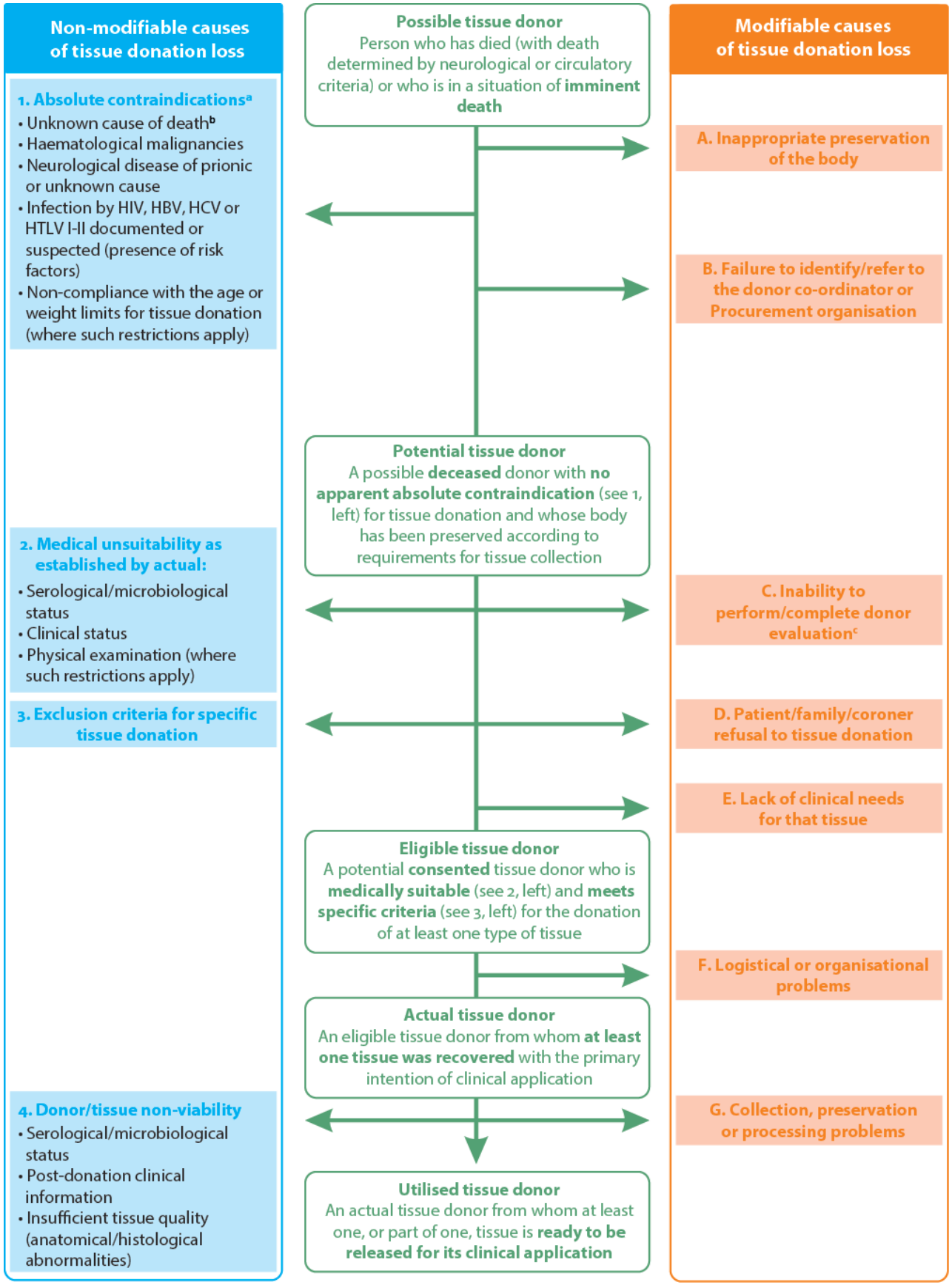
S5.12 In the case of a deceased donor, SoHO entities concerned must provide any persons granting consent to collection on their behalf, in accordance with national legislation, with relevant information as below (Art. 55.4):

a. the purpose and nature of the donation:
i. the intended use of the donated material, specifically covering proven benefits for future recipients;
ii. any possible research or commercial uses, including any use to manufacture products under a different regulatory framework , to which specific consent must be granted;

b. the obligation for consent, in accordance with national legislation, in order for the collection to be carried out;

c. the right to revoke consent and any restrictions on that right after the collection.

Figure 5.1 **CD-P-TO Critical Pathway for deceased tissue donation [adapted from [12]]**



[a] See European Guidelines, https://eur-lex.europa.eu/legal-content/EN/TXT/?uri =OJ:L_202401938.

[b] Unless an autopsy is performed to clarify the cause of death after tissue collection.

[c] Including feasibility to perform validated serological/microbiological blood testing. See corresponding non-modifiable causes of tissue loss.

Good Practice Guidelines

G5.8 Procedures or protocols for expressing consent to donation (depending on the type of donor, the specific circumstances and the different legal systems for consent) should be implemented and maintained.

G5.9 There should be an authorised person who confirms and records that consent for the collection has been obtained in accordance with the legislation in place in their country.

G5.10 The discussion about consent should be conducted in a suitable environment.

G5.11 The person who requests the consent of the donor should have received specific training for this purpose.

G5.12 Consent should be recorded and/or documented in the donor/patient's record.

G5.13 Informed consent should be obtained from all donors, or any persons granting consent to collection on behalf on deceased donors, for the use of their tissue SoHO for specific purposes for infectious disease marker screening, and for other testing when appropriate (e.g. HLA typing).

G5.14 In the case of living donors, the scope and duration of the consent should be stated explicitly.

G5.15 In the case of autologous donation, the patient needs to be informed about options and the balance of risks and benefits of the procedure. The consent needs to specify what would happen if the clinical application could not be performed for any reason (e.g. contamination of the transplant or if the patient's condition contraindicated application).

G5.16 Disposal of cellular therapy preparations should include a pre-collection written agreement between the storage facility and the designated recipient or the donor and the transplant centre, defining the length of storage and the circumstances for disposal of cellular therapy products. In case of an unrelated donor, this agreement should be signed between the donor and the registry/donor centre.

G5.17 In the case of a donor who is a minor or a donor with no legal capacity, the consent should be obtained from parents or the legal representative, provided the following conditions are met [14]:

- **G5.17.1** there is no compatible donor available who has the capacity to consent;
- **G5.17.2** the recipient is a brother or sister of the donor;
- **G5.17.3** the donation has the potential to be life-saving for the recipient;
- **G5.17.4** the authorisation of the donor's representative, or of an authority or a person or body provided for by law, has been given specifically and in writing and with the approval of the competent body;
- **G5.17.5** the potential donor concerned does not object.

G5.18 In the case of discarded tissue or surgical by-product, the donor should be made aware that he or she can express any intention as to how he or she desires such surgical residues to be dealt with, or else the surgical residues should be discarded by the healthcare institution as per documented SOP while ensuring that any human tissue is handled with due care and attention.

G5.19 For deceased donors, in cases where a legal process applies, judicial consent should be obtained according to local regulations before starting any collection activities. Collection should only be carried out if it does not affect the judicial autopsy.

Recommendations

R5.8 If translation is needed for proper information, the use of an unrelated translator, i.e. with no personal relation to the donor, is recommended.

Supplementary information

In the case of allogeneic HPC donors, consent can be obtained at several stages: before HLA typing, before donor testing and before the conditioning regimen on the recipient has begun [15]. The interview includes information about donation procedures, their risks and side-effects, the collection method and the need to administer growth factors when peripheral HPC is being collected. HPC donors are made aware of the possible consequences for the recipient if they withdraw their consent once the conditioning regimen on the recipient has already begun. However, this situation cannot be used to coerce the donor or influence their decision.

For HPC and autologous SoHO it is beneficial to document that the conditions for disposal defined in the consent prior to collection have been met, including (where applicable) the option to transfer the SoHO to another facility if the designated recipient is still alive after the agreed storage period; see Chapter 11 – Storage.

Some examples of forms to obtain consent for MAR can be found in Appendices 11, 12 and 13.

5.4 Deceased donors

5.4.1 Legal consent systems

Consent for the donation of organs and SoHO from deceased donors is subject to national legislation and regulation in each country.

There are two legal consent systems for expressing consent to donation:

a. Opting-in is a system in which consent to donation has to be obtained explicitly from the donor during their lifetime or from an authorised individual (usually the next of kin).

b. Opting-out is a system in which consent to donation is presumed, no objection to donation has been registered by an individual during their lifetime or is known to have existed, or consent is

inferred by the donor's family who could testify to the donor's position.

In practice, variations exist within both systems, and the relatives may play a prominent role in the decision.

According to opt-in legislation, consent for collection of tissue from deceased donors is given by the person when they were alive or, after their death, by their legally authorised representative or a 'person in a qualifying relationship to the deceased'. A hierarchy of qualifying relationships may exist (e.g. ranked from highest to lowest: spouse or partner, parent or child, brother or sister, grandparent or grandchild, niece or nephew, etc.)

Table 5.1 **Key parties in deceased SoHO donation programmes and the challenges and opportunities they may pose**

	Opportunities	**Weaknesses/Obstacles**
Public	• Learn about donation and the benefit of transplantation to recipients • Promote solidarity and altruism • Express wishes to family and friends in relation to donation • Increase awareness of tissue donation at every opportunity – media, schools etc.	• Lack of knowledge about donation and tissue needs • Unwillingness to be confronted with death • Fear that an expressed desire to become a donor may interfere with medical care • Lack of trust in the fairness of the donation and transplantation system
Donor family	• Honour the wishes of the loved one • Make some sense out of the death of a loved one • Find comfort in knowing that donated SoHO of the loved one helped save or improve lives • Gain some control after the death of a loved one • Know that a part of the family member 'lives on' • Recognition and gratitude from society and recipients	• Grief • Relatives are emotionally exhausted • Lack of understanding or knowledge about donation • Fear of disfigurement • Not knowing the wishes of the loved one • Lack of trust in the medical profession or fairness of the donation system • Dissatisfaction with healthcare staff and process
Hospital staff	• Support wishes of the donor and their family • Increase availability of SoHO for patients in need • Contribute to public awareness of donation and transplantation • Take part in the chain of donation, contribute to saving lives	• Discomfort or lack of knowledge about donation • Lack of trust in the donation and transplantation system • Not acknowledging the value of SoHO • Increased workload • Lack of acknowledgement from those involved in collection • Lack of resources
Transplant co-ordinator	• Support wishes of donor and their family • Maximise donation opportunities – tissue donation may benefit as many as 100 patients per donor • Increase availability of tissue for patients in need • Contribute to public and professional awareness of donation and transplantation	• Concern that tissue consent will have an impact on consent for some organ donation [16, 17] • Lack of knowledge about tissue donation and its benefits • Increased workload
Coroner	• Mutually beneficial for coroner and collection team/SoHO establishment, who may share findings, tissue/blood samples and test results to help investigations • Support wishes of the donor and their family • Increase availability of SoHO for patients in need	• Lack of knowledge about donation • Concern over potential impact on death investigation
Funeral homes	• Extend support to bereaved families • Raise awareness by including donation information in funeral homes and obituaries • Allow collection of certain SoHO to occur in funeral homes • Contribute to the preparation of the body • Increase availability of SoHO for patients in need	• Lack of knowledge about donation • Fear of difficulty in preparing body for funeral services • Increased time and costs • Delay in funeral services
Retirement homes, nursing homes and hospices	• Support wishes of the donor and their family • Raise awareness by including donation information • Allow collection of certain SoHO to occur on their premises • Increase availability of SoHO for patients in need	• Lack of knowledge about donation • Lack of trust in the medical profession and in the donation and transplantation system • Many of the residents may be affected with conditions that contraindicate donation

and consent is ideally obtained, where possible, from the person ranked highest in the hierarchy.

The opt-out system was introduced to help meet the shortfall in organs and SoHO available for transplantation. However, in most programmes, if the family is against the idea of the donation proceeding, tissue collection will not proceed; this is called a 'soft' opt-out system.

Even if the legislation is based on a presumed consent or opt-out system, the family and/or other persons who knew the donor well have to be engaged in the donation process to provide information on medical and behavioural risks about the potential donor to ensure the safety of donated SoHO.

Table 5.2 gives an overview of national consent systems in Europe. The information is up to date as of August 2024. Of the 44 answering countries, it appears that the majority (28 countries) have an 'opt-out' system, and 12 countries have an 'opt-in' system. One country is in the process of changing systems, and the remaining three countries have other systems.

Among the means to express wishes regarding organ and SoHO donation after death are donor cards and donor registries. Individuals who have donor cards are also often simultaneously recorded in the national donor registry. Consent to donation recorded on a donor card or in a registry may contain detailed information, e.g. consent or not to various types of donations (donations of specific organs or SoHO). In some countries, those who apply for a passport or driving licence have to state whether they are willing to donate organs or SoHO after death. Advance directives ('living wills') may also enable individuals to state prospectively under which medical conditions they do not want to receive life-sustaining therapy and allow documentation of people's wishes related to donating organs and SoHO after death.

National systems generally enable individuals to modify or withdraw their consent or objection at any time. This ensures that the most recent information about an individual's wishes is recorded in some way and is available at all times should an enquiry be received from a physician or a donor co-ordinator involved in the donation process.

In countries with no legal framework for consent to donation, or where a potential donor is not able to express their donation preference, for example a minor, the decision is, as a rule, left to the family members of the potential donor, based on the assumption that the family would respect and represent the potential donor's wishes. Alternatively, consent may pass to those who are the nominated legal representatives of the potential donor, according to the laws of the country.

5.4.2 Conversations with the family

Conversations with a family about SoHO donation do not generally differ from conversations related to organ donation. Therefore, where applicable, it is best to perform interviews about the donation of organs and SoHO in a single interview session with the family, allowing them time to reach a decision with which they are comfortable. It is, therefore, important that the physician or donor co-ordinator conducting the interview is fully prepared and is familiar with the context, the history of the hospitalisation and the situation of the relatives, and has gathered as much information as possible prior to starting the conversation.

It is frequently impractical to discuss donation with many family members, and it is necessary to limit participating family members to the lawful next of kin and/or those who are key to making the decision, considering the legal framework in place and cultural or religious practices. If this is well explained to the other family members, they will not feel excluded and will support the donation.

When there are social, cultural or language barriers or difficulties, the support of friends of the potential donor who have a greater level of integration or similar religious sympathies may be beneficial for the family. These persons are generally informed previously about the donation and about the processes involved so that they can support the family and ensure that the potential donor's wishes about donation are supported as much as possible; they are typically not limited to making a simple translation [18-21]. It is beneficial to provide the family with an environment that helps facilitate the conversation, perhaps located close to the place where their loved one died, to give family members the opportunity to say goodbye. The conversation is sensitive and generally happens in a quiet room with resources that meet the minimum needs (e.g. telephone, handkerchiefs, water, food) so that they can feel comfortable and have confidence to speak freely.

In circumstances where a face-to-face interview is not feasible, the interview can be conducted by telephone or live video conversation. The same principles of empathy, compassion and professionalism apply in all interviews. Consent for donation can be recorded in the video or sound recording of the conversation, or alternatively, registered in a document completed by a suitably trained professional.

5.4.3 Family objections

It is difficult to proceed with donation when a family is strongly against it, even if there is evidence that their deceased family member wished to be a donor. The family has the right to express their opinion about donation, and clinicians will either take a balanced decision to continue with the collection without the support of the family and risk damaging the emotional health of the relatives, incurring possible bad publicity and a loss of public confidence in the donation programme, or accept that it is not feasible to follow the wishes of the deceased and therefore abandon the donation process. Details of the conversation may help to understand their reasons for objections and to address concerns of the family so that donation can be facilitated where possible.

Table 5.2 **Legal provisions in European countries for consent to/authorisation of organ donation from deceased persons**

Country	National consent system	Donor registry	Non-donor registry
1 Andorra	opting-out		
2 Armenia	opting-out		
3 Austria	opting-out		×
4 Belarus	opting-out		×
5 Belgium	opting-out	×	×
6 Bosnia Herzegovina	opting-out		
7 Bulgaria	opting-out		×
8 Croatia	opting-out		×
9 Cyprus	other	×	×
10 Czech Republic	opting-out		×
11 Denmark	opting-in	×	×
12 Estonia	opting-out	×	×
13 Finland	opting-out		
14 France	opting-out		×
15 Georgia	opting-in	×	
16 Germany	opting-in		
17 Greece	opting-in	×	×
18 Hungary	opting-out		×
19 Iceland	opting-out		×
20 Ireland	opting-in		
21 Israel	opting-in	×	
22 Italy	other	×	×
23 Latvia	opting-out	×	×
24 Lithuania	opting-in	×	
25 Luxembourg	opting-out		
26 Malta	opting-in		×
27 Moldova	other	×	×
28 Montenegro	opting-in		
29 Netherlands	opting-out	×	×
30 North Macedonia	opting-out	×	×
31 Norway	opting-out		
32 Poland	opting-out		×
33 Portugal	opting-out		×
34 Romania	opting-in	×	
35 Russian Federation	opting-out	×	×
36 San Marino	opting-out	NA	NA
37 Serbia	opting-out		×
38 Slovak Republic	opting-out		×
39 Slovenia	other	×	×
40 Spain[a]	opting-out	×	×
41 Sweden	opting-out	×	×
42 Switzerland	currently opting-in, but an opting-out system is to be implemented not before 2026	×	×
43 Türkiye	opting-in	×	
44 United Kingdom	opting-out	×	×

[a] Some countries do not have opting-in or opting-out registries, but this requirement is fulfilled by advance decisions (living wills) and/or registries that allow individuals to record their wishes about donation.

Source: Adapted from a survey by the European Committee on Organs, Tissues and Cells (CD-P-TO) of the Council of Europe in 2021.

5.5 Registries

Standards

S5.13 SoHO entities that collect SoHO from living donors, in cases where SoHO can be donated repeatedly, must register these donors in the SoHO entity registry or, where available, in national or recognised international registries, to verify donation frequency. Those registries must have the possibility of interconnectivity with other such registries (Art. 53.3).

S5.14 Where the circumstances point to a risk that a donor is donating too frequently in more than one SoHO establishment located in one or more member states, the SoHO entity must verify donation frequency by consulting with interconnected donor registries on a case-by-case basis. SoHO entities must be in a position to demonstrate to their Health Authorities, on request, that an appropriate procedure that mitigates such risk is in place (Art. 53.3).

S5.15 SoHO entities or establishments that collect SoHO from living donors that are subjected to a surgical procedure in order to donate, or that are treated with prescribed medication to facilitate donation, must ensure that the plan for monitoring the donor's health after donation is proportionate to the risks associated with the donation. SoHO entities must include in the plan the time period during which the monitoring must continue (Art. 53.4).

S5.16 Any SoHO entity that distributes reproductive SoHO from third-party donation must comply with rules established in national legislation regarding the limits to the number of offspring from medically assisted reproduction or of human applications with reproductive SoHO from a single donor, where applicable. In such a case, the SoHO entity must monitor compliance with such rules via registries for reproductive donors, in accordance with the national legislation applicable in the country where SoHO is applied (Art. 58.10) (see Chapter 30).

S5.17 In the EU, registries must comply with data protection regulations EU 2016/679 and EU 2018/1725 (Art. 76).

Good Practice Guidelines

G5.20 Recruitment of persons that are not able to consent should never be done through public registries. In addition, in some countries, specific regulations restrict donation in these circumstances (e.g. some countries do not allow collection of peripheral blood progenitor cells from minors and/or administration of growth factors).

G5.21 Registries should minimise the risk that information could be used to identify the donors and should limit the information included to that necessary for carrying out their designated tasks and obligations.

G5.22 Donor privacy should be protected against unauthorised access to personal and medical data.

Supplementary information

Registries allow clinicians to gain the knowledge required to ensure the highest possible protection of donors and the timely identification of donation-associated risk factors so that adverse occurrences can be addressed adequately and prevented in the future. They are valuable at a national level to evaluate the quality and safety of healthcare related to donation and transplantation, to develop preventive strategies and health policies, and to provide good quality and cost-effective healthcare.

Combining data from various national registries would allow knowledge and experience to be shared at the international level, enable a faster accumulation of data to identify the absolute risk and the risk factors, facilitate further improvement of donation across Europe, promote research into donation that is based on high-quality data and support the alignment of healthcare throughout Council of Europe member states and beyond.

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Related material

Appendix 11. Example of consent form: female (HFEA, UK)
Appendix 12. Example of consent form: female (CNPMA, Portugal)
Appendix 13. Example of consent form: male (HFEA, UK)

Chapter 6. **Donor evaluation**

6.1 Introduction

Donor evaluation is critical in ensuring the safety and quality of substances of human origin (SoHO), SoHO preparations and living donor safety [1, 2].

This chapter considers the principles of evaluation of three types of donor:

a. deceased allogeneic tissue donors;
b. medically assisted reproduction (MAR), including third-party donation; and
c. living autologous and allogeneic SoHO donors, including donors of haematopoietic progenitor cells (HPC), human milk and intestinal microbiota.

The main objectives of donor evaluation are:

1. to identify risk conditions by careful assessment of information in the medical and social history and thus, to identify conditions that might adversely affect the quality and safety of SoHO and influence efficacy and clinical outcome;
2. to minimise the risk of transmitting a disease from a donor to recipient; and
3. to ensure that the donation will not cause harm to a healthy living donor.

In the EU, the designated SoHO establishment physician is responsible for the development, review and approval of procedures for establishing and applying donor eligibility criteria, including the donor health evaluation (see Chapter 2, section 2.3.2; Art. 50.2.a).

6.2 Assessment of potential allogeneic donors

Allogeneic donors can be living donors related to the intended recipient, unrelated voluntary living donors or deceased donors. This section includes assessment of:

a. living tissue donors – for example, femoral heads surgically removed during primary hip replacement, amniotic membrane donated by mothers at the time of delivery and heart-valve donation from the discarded heart of a heart transplant recipient (known as a 'domino donor');
b. HPC donors: related or unrelated, and cord blood – for example, bone marrow collected in theatre under general anaesthesia or peripheral blood stem cells collected by apheresis techniques;
c. deceased tissue donors – for example, musculoskeletal, cardiovascular, ophthalmologic or skin tissue donated after death;
d. MAR donors – in the case of third-party donation (sperm/oocyte donors and embryos);
e. other SoHO such as human milk and intestinal microbiota.

Standards

S6.1 SoHO entities must have donor selection policies and procedures in place to provide a high level of assurance that pathogens, toxins or genetic conditions that are potentially life-threatening, disabling or incapacitating and originate from a third-party donor are not transmitted to SoHO recipients or offspring from medically assisted reproduction (Art. 58.1).

S6.2 The review and evaluation of donors must include at least the following (Art. 58.2):

- Current and past medical history;
- Personal and social history (behavioural history);
- Travel history;
- Relevant family history ;
- Evaluation of test results for markers of transmissible communicable disease ;
- Evaluation of the risk of transmitting (Art. 58.3.a):
  - cancer cells;
  - serious genetic conditions;

- other non-communicable diseases.

S6.3 Where the transmission of serious genetic conditions is an identified risk, and in particular in the case of MAR with third-party donation, and insofar as national legislation allows, for any of the following testing:

i. routinely testing SoHO donors for potentially life-threatening, disabling or incapacitating genetic conditions with a significant prevalence in the SoHO donor population; OR
ii. testing SoHO recipients to identify genetic risk for potentially life-threatening, disabling or incapacitating conditions, according to family history, combined with testing third party SoHO donors for such identified serious genetic conditions to ensure matching that will prevent such conditions occurring in the offspring from MAR (Art. 58.3.b).

S6.4 SoHO entities must apply temporary or permanent deferrals when risks cannot be minimised by donor testing (Art. 58.2 and 58.3):

a. Donor eligibility must be assessed by conducting an interview with (Art. 58):
  i. living donors or, where applicable, with any persons granting consent on their behalf (Art. 53.2); or
  ii. in the case of deceased donors, an interview with a relevant person who is informed about the donor's health and lifestyle history (Art. 58.13.b).

b. Donor evaluation of living donors must identify and minimise any risk that the collection procedure might pose to the donor's health (Art. 53.1.e).

c. For living donors who donate repeatedly, the interviews may be limited to aspects that might have changed and interviews may be replaced with questionnaires. Interviews must be added in cases where responses provided in questionnaires indicate changes in relevant information (Art. 58.13).

d. SoHO entities must conduct interviews with the donors and gather information on the donors' present and recent state of physical and, where appropriate, mental health and their health histories to assure the safety of the donation process for those donors. SoHO establishments may perform additional tests as part of the donor health evaluations (Art. 53.2).

e. SoHO entities must document the results of SoHO donor eligibility verification and must communicate and clearly explain the results of SoHO donor eligibility verification to SoHO donors or, where relevant, any persons granting consent on their behalf, in accordance with national legislation (Art. 53.1.g & Art. 58.14).

f. Where tissues are collected from deceased donors, SoHO entities must communicate and explain the results of the donor eligibility assessment, in particular any condition identified in the deceased donor that might imply a risk for the health of the deceased donors' relatives or close contacts, to the relevant persons, in accordance with national legislation (Art. 58.14).

Good Practice Guidelines

General

G6.1 Selection criteria for allogeneic donors should be established and documented by SoHO entities/establishments, considering the SoHO-specific donor criteria shown in Table 6.2 (see Chapter 2):

a. Eligibility for donation should be evaluated on an individual basis, taking into consideration the possible risks and benefits, and should be based on all available information, including the relevant medical history, travel and relevant behavioural histories, results of laboratory tests and physical examination results.

b. Donor age criteria and any other events with risk of exposure should be established, documented and recorded.

c. Procedures for donor selection and evaluation should be implemented and maintained. They should take place before each collection, except in the case of repeated donations (see S6.4.c, Art. 58.13).

G6.2 The following actions for donor selection and evaluation should be conducted:

- examine donor/donor family consent details;
- examine donor's medical history (including genetic disease, a family history of disease, exclusion criteria, additional exclusion criteria for deceased child donors);
- examine donor's social history (including personal, travel and other events with risk of exposure, behavioural, risk assessment);
- donor's physical examination (including exclusion signs);
- evaluation of test results for markers of communicable transmissible disease (see Chapter 7);
- final decision making about eligibility.

G6.3 An authorised person should collect and record the donor's relevant medical and behavioural information.

G6.4 Donor's identity should be confirmed with at least two forms of identifiable information, such as given name and family name, date of birth or medical history number, and documented.

G6.5 An interview should be conducted, during which a standardised questionnaire should be completed. The interviews should be done, documented and signed by a trained and appropriately qualified person.

G6.6 In the case of both living and deceased donors, screening should be performed to exclude any contraindications to donation and, in the case of a living donor, to exclude any medical situation that could potentially harm the donors themselves.

G6.7 In the case of a deceased donor, and in the case of a living donor when justified, a physical examination of the donor should be performed to detect any sign that may be sufficient on its own to exclude the donor or which should be assessed together with the donor's medical and personal history.

G6.8 If a physical examination is not performed, this should be justified and documented.

G6.9 In order to acquire the appropriate information, different relevant sources should be used, including the following when appropriate:

- **G6.9.1** the medical records of the donor;
- **G6.9.2** an interview with a person who knew the donor well (relative or close person), for deceased donors;
- **G6.9.3** an interview with the treating physician;
- **G6.9.4** an interview with the general practitioner;
- **G6.9.5** the autopsy report, for deceased donors.

G6.10 The complete donor records should be reviewed and assessed for the donor's suitability and signed by a qualified health professional.

Living donors

G6.11 In the case of living donors:

- **G6.11.1** The clinician caring for the donor should make the decision on donation according to clinical guidelines and relevant scientific data from the perspective of whether the donation is safe for the potential donor;
- **G6.11.2** The potential benefits and risks should be analysed on an individual basis and a decision made by the clinician in charge, subject to full informed consent from the patient. This process should be clearly documented.

G6.12 The health professional responsible for obtaining the health and social history should ensure that the donor has:

- **G6.12.1** understood the information provided (see Chapter 5);
- **G6.12.2** had an opportunity to ask questions and been provided with satisfactory responses;
- **G6.12.3** confirmed that all the information provided is true to the best of his/her knowledge.

G6.13 Depending on the tissue or cell to be donated, other specific exclusion criteria should be added, such as the potential for transmission of genetic diseases (e.g. HPC or donor of reproductive cells).

G6.14 In the case of MAR

- **G6.14.1** If reproductive cells and tissues are processed and/or stored, and will be cryopreserved, they should meet the following criteria:
  - **G6.14.1.1** The clinician responsible for the donor should determine and document, based on the patient's medical history and therapeutic indications, the justification for the donation and its safety for the recipient and any child(ren) that might result;
  - **G6.14.1.2** Biological tests should be carried out to assess the risk of cross-contamination;
  - **G6.14.1.3** Positive results will not necessarily prevent within-relationship use in accordance with national rules.

Deceased donors

G6.15 The cause, time and circumstances of death should be assessed and recorded. The sources of information used for deceased donors should be reviewed.

G6.16 The donor information analysis should preferably be performed before collection but, if this is not possible, the collected SoHO should be quarantined until a final decision is made by the physician of the SoHO establishment.

Recommendations

It is recommended that:

R6.1 the donor´s haemoglobin be measured before the donation if large volumes of blood are collected in HPC donations;

R6.2 potential donors with low haemoglobin levels be adequately prepared (e.g. through iron supplementation in cases of iron deficiency) or excluded from donating;

R6.3 if bone marrow is aspirated to obtain bone marrow stromal cells or a skin biopsy is performed, or wherever a procedure may lead to a risk of bleeding, the donor's platelet count and coagulation parameters meet predefined criteria to ensure they are not at increased risk of bleeding [1, 2, 3];

R6.4 for living allogeneic donation, where applicable (e.g. umbilical cord blood or gamete donation) attention be paid to cases of family adoption or conception by donated gametes/embryos, as it may not be possible to trace the genetic family history.

Supplementary information

Sources of information

An example of a standardised medical and social questionnaire for interviews can be found in Appendix 14.

Collection of SoHO from deceased donors takes place after circulatory arrest. Thus, the time available for full donor evaluation is normally limited. During the interview with the person giving information regarding the potential donor's medical and social history, they may be under emotional stress, and some details might be forgotten. Even when donor relatives trust the interviewer, they may be reluctant to disclose sensitive information, or they may not know the entire truth. More detail of sources of information can be found in Table 6.1.

Careful review of all the collected donor information will help ensure an accurate donor evaluation and assessment of the risks, including the identification of any potential contraindications for donation, either absolute or tissue-specific (see Part B for specific chapters and Table 6.2).

Table 6.1 **Deceased donors: sources of information and types of record for donor evaluation**

Sources of information	Types of record
1. interview with family and friends/close acquaintances 2. interview with attending clinician and nurse, as well as the healthcare provider 3. detailed review of the medical notes (see *Types of record*, to the right) 4. general practitioner notes 5. physical examination findings 6. autopsy findings (for deceased donors), which need to be communicated as soon as possible after collection 7. tests for infectious markers (see Chapters 7 and 12) and other relevant test results (see *Types of record*, to the right)	1. emergency room and emergency medical transport (ambulance) records 2. admission records, progress notes, clinician's orders/notes, nursing observations 3. surgical records 4. records of consultations (e.g. psychiatry, infectious disease, neurological, orthopaedic, oncology, rheumatology, counselling) 5. discharge summary or death certificate (for deceased donors to confirm cause of death or to determine whether an autopsy is planned) 6. results of laboratory tests (microbiology, chemistry, haematology, virology, toxicology, genetic screening, pathology) 7. physical evaluation form (see Appendix 15) 8. information relating to transfusions and infusions (to be used for evaluation of haemodilution) 9. radiography/magnetic resonance imaging/computed tomography
For deceased donors whose death occurred outside a healthcare facility, the record types 10 to 12 listed to the right may also be available and, if so, they need to be reviewed	10. police records 11. records from the medical examiner or coroner 12. records from the extended-care facility

Table 6.2 **SoHO-specific donor criteria**

SoHO	Age	Specific criteria
Cornea	No age restrictions	Absolute contraindications: • Retinoblastoma, uveal malignant melanomas and malignant tumours of the anterior segment are absolute contraindications • Some haematological neoplasms can be accepted after a risk assessment • Individuals with acute localised ocular infection (bacterial, viral, fungal, protozoal, parasitic) are excluded from donation of ocular tissues. This exclusion includes those with a known history of past ocular herpes infection · Individuals suffering from bacterial septicaemia may be considered for cornea donation, provided that the corneas are stored by organ culture and the medium tested for microbial contamination before transplantation
Amniotic membrane	No age restrictions	Absolute contraindications: • significant local bacterial, viral, parasitic or mycotic infection of the genital tract, especially amniotic infection syndrome • (known) malformation of the unborn/newborn • pathological premature rupturing of membranes • endometritis • meconium ileus • IgM positive for toxoplasmosis (if available)

SoHO	Age	Specific criteria
Skin	No age restrictions	Absolute contraindications: • autoimmune diseases and systemic connective tissue diseases affecting skin (e.g. scleroderma) • diseases affecting the dermis (e.g. dermal mucinosis, nephrogenic fibrosing dermopathy, porphyria, lupus erythematosus) • toxicity of the skin as a result of the presence of toxic agents or poisons • systemic use of corticosteroids or Cushing disease, inducing severe skin atrophy Relative contraindications: • extensive lacerations, haematoma or scars • skin diseases with extensive involvement (e.g. psoriasis, eczema) • relevant skin ulcers, pressure ulcers, stoma, pyoderma or mycoses • skin disorders interfering with procurement or aesthetically not acceptable for patients (e.g. extensive tattoos, jaundice) • diabetes mellitus with severe skin complications (e.g. ulcers, amputation, neuropathy) • pre-malignant conditions such as multiple actinic keratoses and Bowen's disease • mechanical, thermal or microbial damage where skin is to be collected • extreme peripheral oedema • high body mass index (BMI ≥ 40) or malnutrition (BMI < 18.5) affecting collection or body reconstruction
Cardiovascular tissue	Arteries • male aged 17-55 • female aged 17-60 Veins • aged up to 70 Aortic valves • newborn (3 kg) to age 70 Pulmonary valves • newborn (3 kg) to age 70 Note: The advice given on age limits is based on experience of the quality of tissues collected from donors within these age limits. Aortic valves could also be collected from donors up to 70 years of age, but in most cases these valves may not be suitable for banking. Collection of pulmonary valves from the same heart, however, would show a much higher yield, explaining the acceptance of these hearts for donation.	Absolute contraindications: • valvulopathy of both the aortic and pulmonary valves, with moderate-to-severe stenosis or incompetence (the vessels can still be acceptable) • aortic dissection (detachment of the intima and adventitia) • Marfan's syndrome and related diseases (e.g. Loeys–Dietz syndrome, Ehlers–Danlos syndrome) • bacterial or fungal endocarditis, myocarditis, ankylosing spondylitis (if cardiac involvement), haemachromatosis (if cardiac involvement), muscular dystrophy, rheumatic fever Relative contraindications: • dilatation of ventricles and valve alteration such as dilated roots, wall alteration of aorta or pulmonary trunk • untreated pneumonia without evidence of resolution, due to highly pathogenic bacteria or fungi, such as *Staphylococcus aureus*, pneumococci or *Candida* spp. • previous surgical cardiovascular interventions on the tissue to be collected
Musculoskeletal	No age restrictions	Absolute contraindications: • iatrogenic or degenerative tears or lesions detected during collection of cartilage, menisci, tendons and osteoarticular grafts • presence of articular prosthesis, implant or other medical devices
Haematopoietic progenitor cells	Allogeneic: • any decision to collect from young (< 18 years) and elderly donors (> 60 years) shall include an accurate risk assessment and appropriate informed consent of the donor stating that risk	Absolute contraindications: Allogeneic • Donors with a history of malignant disease (excluding haematological malignancies) after a minimum disease-free period of 5 years can be considered as suitable for sibling donation after careful assessment of the risk of malignant disease transmission

SoHO	Age	Specific criteria
Cord blood	No age restrictions	Absolute contraindications: • autoimmune diseases if the mother received treatment in the last 12 months • malignancy (except basal cell carcinoma and *in situ* cancer treated and cured) • inflammatory bowel disease (e.g. Crohn's disease and ulcerative colitis) • evidence of active or chronic infection • live immunisation (vaccination) during this pregnancy • haematological disorders that can be transmitted thorough transplantation • organ recipient
Pancreatic islets	No age restrictions	BMI restrictions are not absolute contraindications. Absolute contraindications: • donor with Type 1 diabetes mellitus
Hepatocytes	No age restrictions	Absolute contraindications: • steatotic livers • liver-originated disease of the donor that could be transferred to the recipient and might cause disease, e.g. hyperoxalosis, familial amyloid polyneuropathy; • alterations to the liver extracellular matrix or vessels that could complicate perfusion and prevent efficient isolation of hepatocytes; • donor liver characteristics that might affect hepatocyte quality, such as the size of liver tissue, the degree of steatosis, the length of both warm and cold ischaemia, and hypoxia
Adipose tissue	No age restrictions	Absolute contraindications: Donors do not have any major systemic diseases or lipid disorders, and they are not underweight.
Medically assisted reproduction	Oocyte donors: > 18 years or < 36 years	Absolute contraindications: • diminished ovarian reserve based on criteria defined within the SoHO establishment • family history of dominant genetic disease • abnormal karyotype • any risk factor associated with the donor's health • unsuitability for donation based on interview • cases in which the donor family history cannot be documented, such as adoption or loss of contacts with her biological parents
	Sperm donors > 18 years and < 45 years	Absolute contraindications: • family history of dominant genetic disease • poor sperm quality • abnormal karyotype • poor sperm survival rate after thawing • unsuitability for donation based on interview • cases in which the donor family history cannot be documented, such as adoption or loss of contacts with his biological parents or lack of relevant information; • relevant testicular pathology · cryptorchid or hypermobile testes (operated or not) · testicular tumours · history of orchitis, epididymitis and grade III varicocele

SoHO	Age	Specific criteria
Human milk	No age restrictions	Absolute contraindications: • any tobacco exposure (active or passive) or use of nicotine-containing products • illegal substance abuse (including the use of recreational drugs) or use of methadone • any consumption of alcohol (occasional consumption of not more than one standard drink – a glass or a pint/570 mL of beer – may be accepted if milk collection for donation purposes is avoided for 12 hours) • consumption of high quantities (> 300 mg) of substances containing caffeine (coffee, tea, cola or cacao); occasional consumption may be accepted • vegan donors without vitamin B12 supplementation • using prescription or over the counter medication or other pharmacologically active substances (including herbal products) that are not compatible with milk donation (more information can be found at www.ncbi.nlm.nih.gov/books/NBK501922/ and www.e-lactancia.org/) • women immunised with attenuated live virus (contraindication of donating milk for 4 weeks after the immunisation) • women who have recently been in contact with infectious patients (e.g. chickenpox, mumps, measles) unless they have been immunised; if they have not been immunised, they are excluded for a period equivalent to the incubation period or, if not known, for 4 weeks • women with mastitis or fungal infections of the nipple or areola until all symptoms have disappeared and any course of medication completed • women with reactivation of *Herpes simplex* (HSV) or varicella–zoster infections in the mammary or thoracic region • women with a history of malignancy can be considered if an individual risk assessment is performed
Intestinal microbiota	≥ 18 and ≤ 65 years	Absolute contraindications: • Cold sores, anal ulcers, persistent pruritus ani within the last three months • BMI (accepted if ≥ 18 and ≤ 30 kg/m^2) • Previous or scheduled gastrointestinal surgery • Gastrointestinal symptoms within the last 3 months (e.g. diarrhoea, constipation, haematochesia, vomiting, abdominal pain) • Any other relevant clinical sign or symptom within the last 3 months (e.g. fever or rash) • Family history (first-degree relations) of large bowel cancer • Allergies or atopy (e.g. food allergy, medication allergy or any kind of anaphylactic allergy, asthma) • Hospitalisation within the last 4 months • Current pregnancy • Antibiotic treatments, scheduled or received within the last 3 months • Regular medication or nutritional supplements (except oral contraception) unless accepted following review by e.g. physician. • Information on nutritional supplements is to be collected, but not lead to exclusion • Chronic diseases

When dealing with living donors, an important part of the evaluation is to assess whether the collection process itself could harm the donor, and whether it is necessary to implement long-term follow-up of living donors after collection; see Chapter 5.

Pre-screening of HPC and oocyte donors is helpful to identify factors to assess if the donation process would place them at increased risk of adverse incidents or harm to their health. Pre-screening may include general health status, medical history questions, physical exam, blood tests and other relevant medical evaluation.

More aspects of living donation are detailed in Chapter 5 – Recruitment of potential donors, identification and consent; Chapter 25 – Haematopoietic progenitor cells from bone marrow and peripheral blood; Chapter 30 – Medically assisted reproduction; and Chapter 31 – Fertility preservation.

Physical examination

The purpose of physical examination is to detect any sign that may be sufficient to exclude the donor, either on its own or when taken together with the donor's medical and personal history.

For living donors, the extent of physical examination to ensure the safety of donors and recipients will vary according to the specific requirements of the tissue or cell donated.

The physical examination may include taking a photograph of suspicious lesions that may indicate a risk and/or taking a sample for histology (see Table 6.3 and Appendix 16). Any findings that may indicate the risk of transmissible disease or unsatisfactory quality of the tissues can result in exclusion of the donor [4]. In all cases of abnormal findings, further investigations may be necessary, according to specific standard operating procedures (SOPs), taking into account in the risk assessment the limited sensitivity and specificity of physical examination for discovering pathologies. Appendix 15 contains an example of a tissue-donor physical assessment form.

Donor medical and social history

Assess carefully donors with trauma, intra-operative blood loss or ruptured aneurysms, including bleeding from oesophageal varices, spleen rupture etc., as the donors may also have received blood products with the possibility of haemodilution. When haemodilution is suspected or confirmed (> 50%), special actions may need to take place; please refer to Chapter 7 for more information.

Social history, evaluation of behavioural and personal risk and other events for risk of exposure

Make an assessment of behavioural and personal risk (including travel history) for history that may completely exclude a donor or can indicate that certain SoHO could be compromised or may suggest an increased risk of infectious diseases [5]. Asking lifestyle questions, including sexual history and habitual drug use, allows the clinician to determine behavioural risk as detailed in section 6.2.1.

Travel history helps to identify risks related to places/countries with less rigorous regulatory standards or incidence of certain communicable diseases.

Obtaining information about hobbies (e.g. home, garden, animals, woodlands) and seeking information about contact with fauna, especially bites from pets, domestic or wild animals (bats and birds), will help to evaluate the risk of infections.

6.2.1 Risk conditions for SoHO donation

6.2.1.1 *Transmissible infections*

Good practice guidelines

G6.17 SoHO establishment physicians should regularly review and update donor selection policies and guidelines in line with emerging evidence and with recommendations from the European Centre for Disease Prevention and Control (ECDC) [4]. SoHO-specific considerations for donor selection are summarised in Table 6.2.

Table 6.3 **Deceased donors' physical examination prior to donation**

Look for signs of	
Possible systemic disease	• malignancy (suspicious skin or subcutaneous lesions; see Appendix 16) • malnutrition, multiple deformities
Bacterial or viral infection	• recent receipt of a live vaccination (vaccination site infection, scabs, vaccinia) • recent receipt of a tattoo, body piercing or acupuncture (shaved area, redness, swelling or scabbing may require further investigation to assess risk) where there is a risk that non-sterile instruments may have been used • skin lesions such as a rash, petechiae, skin ulcers, blue/purple or grey/black lesions, shingles, scabs • oral lesions such as ulcers or thrush (not always possible to examine due to rigor mortis) • visible/palpable enlarged lymph node(s) • icterus (jaundice)
High-risk behaviour	• possible injection of non-prescription drugs (non-medical injection sites) • inspection of tattoos for hidden injection sites or for any additional information (e.g. some tattoos may suggest imprisonment, which is linked with a high communicable disease risk or high-risk sexual behaviours) • genital or peri-anal skin lesions indicative of a sexually transmitted disease
Trauma	• fractures, avulsions, lacerations or abrasions that may affect (contaminate or compromise integrity of) the tissue to be collected • suspected internal trauma that can cause cross-contamination between cavities (e.g. injury to the bowel, penetrating or crushing injuries) • cleanliness of the body, the condition in which the body was found (this can also relate to increased risk for contamination/cross-contamination) • scars (surgical or other); if findings do not match the donor's history, further investigation may be required

G6.18 When assessing individuals who are considered high risk for transmissible infections due to events with risks of exposure to such infections, or if they have had sexual contact with persons who have high risk of infection, SoHO entities should consider those events due to the risk of window period transmission (see Table 6.4 for a list of events considered relevant; see also G6.19 and Chapter 7).

G6.19 The donor deferral period since last at-risk event should be at least more than three times the validated window period of the test used (see Table 6.4 and Chapter 7). If the benefit of transplant outweighs the potential transmission, a documented rationale for decision making should be in place before accepting the donor on a case-by-case basis [4].

G6.20 If the aetiology of an active infection cannot be established, the donor should not be a suitable candidate for donation.

Recommendations

R6.5 It is advised that SoHO establishments and entities consider available evidence from the epidemiological data on transmissible blood-borne infections such as HIV, HCV and HBV in the population, the performance (sensitivity and specificity) of screening tests used for detecting these infections and the residual risk of undetected infection that could be potentially transmitted to the recipient.

R6.6 When SoHO establishments do not have access to data to perform risk assessment, it is recommended to exclude the donor.

Supplementary information

All SoHO have the potential to transmit infections to a recipient. Evidence-based donor selection is the first safeguard in minimising the risk of transmission while not compromising sufficiency of valuable grafts for clinical use.

The incidence and prevalence of these SoHO-related infections varies, depending on different risk factors [6, 7], and the causes of *de novo* infection vary between European regions [4, 8]. This residual risk may be the result of one or all of several factors such as: error in the process, poor assay sensitivity or a donation collected from a donor in the infection window period, etc.

When a donor is considered as high-risk, a risk-assessment analysis, such as the Alliance of Blood Operators model [9], may be used systematically to analyse the information and document the decision, based on the acceptable level of risk tolerance with the potential measures that reduce or minimise the risk (e.g. nucleic acid testing (NAT), processing method). The outcome of this systematic approach provides the basis for evidence-based donor deferral and acceptance policies for donors with high-risk behaviours or who have sexual partners with high-risk behaviours [10].

Donors with bacterial septicaemia may be evaluated and considered for cornea donation, provided that the corneas will be stored by organ culture (see Chapter 20).

In the case of an active infection with unknown origin, communication with the physician or medical staff caring for the potential donor is beneficial if there is any doubt. These healthcare providers may know if there was a suspicion of sepsis or another infectious disease at the time of death, which may not have been well documented in the records.

6.2.1.2 *Children under 18 months or breastfed children*

Good Practice Guidelines

G6.21 Newborns, and infants aged 18 months or less, who were born to a mother with confirmed HIV, HBV, HCV or HTLV infection (HTLV 1 and 2), should not be considered as donors, regardless of the results of analytical tests of their sample (see Chapter 7, section 7.2.1.3) with the following exceptions:

G6.21.1 If the birth mother has a known or suspected HBV infection and the child has received appropriate immunoprophylaxis for HBV according to national recommendations, donations from children aged 9 months or above with a negative NAT and negative HBsAg can be accepted if the child's anti-HBs concentration is 10 IU/L or above.

G6.21.2 In case of a documented previous resolved hepatitis C infection in the birth mother, the child can be tested with a NAT detecting HCV RNA.

G6.22 Children who have been breastfed by infected mothers or other individuals, if relevant, within the 6 months before death should not be accepted.

6.2.1.3 *Organ-transplant recipients*

Good Practice Guidelines

G6.23 All donor medication should be always interpreted by a risk assessment for impact on the tissue.

Recommendations

It is recommended to:

R6.7 verify the traceability of the organ donor that donated the organ by checking if the donor had a disease that potentially could be transmitted to the organ recipient. If so, a risk assessment is advised before accepting tissue donation from the organ recipient;

R6.8 evaluate the risk on a case-by-case basis, considering the feasibility of performing NAT and the level of immunosuppression in combination with the records relating to the quality, safety and traceability of the organ.

Table 6.4 **Risk conditions for SoHO donation**

	Risk factors	Explanation	Action
1	***Exposure events***		
1a	Acquire/vertical transmission	People from a high-risk region for endemic disease, as well as the risk of vertical transmission, e.g. HIV1, human T-cell lymphotropic virus (HTLV1), *Trypanosoma cruzi* or Zika virus [4].	Consider risk of transmission with specific tissues
1b	Animal bites	Evaluated to rule out the possibility of transmissible diseases such as rabies since the incubation period for this disease can span several months.	Consider risk of transmission
2	***Events with risks of exposure to transmissible communicable diseases***		
2a	Tattoos, ear piercings, body piercings and/or acupuncture	Usually, they are applied by sterile methods in approved settings and, in many countries, there are specific approvals for those establishments. If tattoos, piercings or acupuncture treatments were done in approved settings, the donor can be accepted without temporary deferral, but in case of doubt the associated risk is generally considered to be the same as that of non-medical injections (see 2.b).	Consider risk of transmission
2b	Risks of exposure specific to HIV, HBV, HCV and *Treponema pallidum*	Active sexually transmitted infection (STI); Sex in exchange for money, drugs or other payments; Use of antibiotics as post-exposure prophylaxis (PEP) for the prevention of a bacterial STI; Use of injectable or oral pre-exposure prophylaxis (PrEP) or PEP for the prevention of HIV infection; Needle sharing and/or injection of non-prescription drugs; Non-occupational needlestick injuries, tattoos/piercing in unhygienic conditions; Condomless* anal sex with a new partner or one-off partner (casual partner); Condomless* anal sex with more than one partner; Condomless* sex in exchange for money, drugs or other payments; Condomless* sex with a partner using HIV PrEP or PEP; Condomless* sex with a partner who injects non-prescription drugs; Condomless* sex with a partner with an active STI; Condomless* sex with a person living with HIV; Condomless* sex with an individual with an ongoing acute or chronic HBV infection; Close household contact with an individual with acute or chronic HBV infection; Exposure to known or suspected HIV, HBV or HCV infection following an occupational incident (e.g. needlestick injury); Recent healthcare encounter: receipt of SoHO, haemodialysis, intravenous fluid/contrast infusion. * Condomless should be understood as a situation where no condom was used or situations where it was used incorrectly (e.g. condom failure).	Consider risk of transmission Risk exposure within 16 weeks should be considered. This can be reduced to 8 weeks if a highly sensitive DNA NAT (HBV) is used (see Table 7.1). - HIV: exceptions: 12 weeks for oral HIV PrEP and PEP use, and 24 months for injectable HIV PrEP use. - *T. pallidum* exception: 16 weeks when antibiotic use as PEP.
3	***Infections***		
3a	Active systemic infection	Active systemic infection that is not controlled at the time of donation (including bacterial diseases, viral, fungal, protozoan or parasitic infections, or significant local infection) in the SoHO to be donated. Donors with bacterial septicaemia may be evaluated and considered for eye donation but only where the corneas are to be stored by organ culture to allow detection of any bacterial contamination of the tissue.	Exclusion
3b	Active transmissible viral infections/past infections	Clinical or laboratory evidence of active transmissible infections: HIV, HBV, HCV* and human HTLV I/II. *For HCV, provided a negative NAT and a documented sustained virologic response (12/24 months) after treatment, the donor can be accepted (see Table 7.2).	Exclusion

	Risk factors	Explanation	Action
3c	Active infection or recently treated sexually transmitted diseases	e.g. syphilis, gonorrhoea, *Chlamydia* or genital ulcers.	Consider risk of transmission
3d	Chronic persistent infection	History of bacterial and protozoic diseases that can lead to chronic persistent infections, including tuberculosis, brucellosis, leprosy, Q fever, chlamydiosis and salmonellosis. Also tick/arthropod-borne diseases such as borreliosis, rickettsiosis, trypanosomiasis, leishmaniasis, babesiosis and ehrlichiosis.	Consider risk of transmission with specific tissues
4	***Recent vaccination***	Received any of these live vaccines during the **previous 4 weeks**: Viral: Live influenza vaccine given by intra nasal spray (age 2-18), measles, mumps, rubella (MMR), oral polio, yellow fever, varicella (chickenpox), shingles (both live & non-live available for shingles prevention, chikungunya, Japanese encephalitis and oral rotavirus; treat as live if unknown type). Bacterial: tuberculosis (BCG), oral *Salmonella typhi.* Received live **smallpox** vaccines during the **previous 8 weeks.** **Non-live***: Administration of hepatitis B vaccine can lead to low-level reactivity in HBsAg screening assays. For this reason, donors may have to be excluded until at least 14 days after receiving a dose of vaccine, even if they have not been exposed to HBV. Hepatitis B vaccine is sometimes given in a combined vaccine with hepatitis A vaccine.	Non-live vaccinations can be accepted*
5	***Risk of prion disease***	Prion-related diseases: Creutzfeldt–Jakob disease (CJD), Gerstmann-Stäussler-Scheinker (GSS), Kuru and fatal familial insomnia (FFI) are contraindications to tissue or cell donation. To rule out transmissible central nervous system (CNS) infections as a cause in following circumstances: a. any suspicion of prion-associated disease, such as rapid progressive dementia; b. a diagnosis of dementia without a confirmed primary cause (unless prion-associated disease has been ruled out by microscopic examination); if dementia has a primary cause (e.g. dementia of vascular origin), donation may be accepted; c. degenerative or demyelinising disease or a disorder of unknown aetiology involving the CNS; d. persons treated with hormones derived from human pituitary gland, such as growth hormone; e. recipients of *dura mater*; f. individuals who have been told that they may be at increased risk because a recipient of blood or tissues that they have donated has developed a prion-related disorder; g. recipient of blood transfusion from a donor who had subsequently developed prion disease.	Exclude Evaluate for transmissible cause
6	***Malignancy***		
6a	Haematological malignancies	Myeloid and lymphoid neoplasia and leukaemia.	Exclude
		Other myeloproliferative diseases (e.g. polycythaemia vera, essential thrombocythaemia and mastocytosis, myelodysplastic syndromes).	Consider risk of transmission with specific tissues
6b	Non-haematological malignancies	Premalignant condition.	Evaluate follow-up
		CNS tumours.	Consider risk of transmission with specific tissues
		Non-CNS tumours.	Consider risk of transmission with specific tissues
		Carcinoma *in situ* not successfully treated.	Consider risk of transmission with specific tissues

	Risk factors	Explanation	Action
7	***Toxic substances***	e.g. cyanides, lead, mercury, gold, arsenic, pesticides.	Consider risk of transmission with specific tissues
8	***Xenotransplantation***	With living cells, tissues or organs.	Consider risk of transmission
9	***Genetic diseases***	Family history.	Risk of transmission for MAR, HPC and cord blood
10	***Additional risk***	Unknown cause of death even after an autopsy.	Exclusion

Supplementary information

Organ recipients receive immunosuppressive drugs to prevent rejection, but this could make the serology testing unreliable; moreover, organ donor-selection criteria are less stringent than for SoHO.

Treatment with immunosuppressive agents can weaken the immune system and thus influence the reliability of serological tests (Chapter 7). Steroid therapy in high doses causes immunosuppression and may mask infective and inflammatory conditions that would otherwise prevent donation. Evaluating the effect of the immunosuppressive agents on the haematological parameters (erythrocytes, leukocytes and thrombocytes) can be indicative for immunosuppression. Treatments such as chronic use of corticosteroids can affect the quality of skin (see Table 6.2) and also increase the possibility of a secondary malignancy.

6.2.1.4 *History of travel and new and emerging diseases*

Good Practice Guidelines

G6.24 Professionals responsible for donor selection should be vigilant in monitoring global infectious-disease risks.

G6.25 The risk of transmission of an infectious agent through collection of tissues or cells from a donor who may have visited an affected area needs to be balanced against the likelihood of transmission occurring.

G6.26 If the donor was infected, they should only be accepted after full recovery and when the donation is no longer infectious.

G6.27 In cases of community transmission, further risk assessment should be performed taking into account all available scientific and clinical information, mitigation factors and any other relevant external factors.

G6.28 A multidisciplinary approach with involvement of different stakeholders should be performed to respond to the threat posed by new and emerging infections.

Recommendations

It is recommended that:

R6.9 in Europe, donor selection be based on regular monitoring of the Rapid Communication Reports originating from the Eurosurveillance website as well as actively seeking information to assess the epidemiological status of diseases in the areas where a donor has lived or travelled [11];

R6.10 the donor be deferred for at least twice the length of the incubation period in cases of recent travel or known contact with someone infected, provided the donor remains well [4];

R6.11 for donors with history of travel to, who originate from, or who have visiting relatives in malaria-endemic areas, the minimum deferral period for blood donors in European Directorate for the Quality of Medicines & HealthCare (EDQM) guidance [8] for all groups of potential donors (visitor, origin/previous resident, travel-related illness, history of malaria) be four months if the result of a validated malaria antibody assay, performed at least four months after last exposure incident or resolution of symptoms, is negative with a complete risk assessment. The same guidance applies to SoHO donors.

Supplementary information

The evaluation of recent or pertinent travel history or residence abroad/overseas allows to rule out the risk (including vertical transmission) of specific communicable diseases in endemic countries. It is important to note that regional risks within an affected country can vary, such as between rural and urban areas. Examples of relevant communicable disease risks in travellers could include: Chagas disease, chikungunya virus infection, dengue, Ebola virus disease, Middle East respiratory syndrome (MERS), mpox, plague, Q fever, rabies, trypanosomiasis, tuberculosis, multidrug-resistant organisms (MDRO), West Nile virus infection, yellow fever and Zika virus infection [11, 12].

Specific information about the global geographic distribution of infectious diseases and ongoing outbreaks can be obtained from the websites of the ECDC (www.ecdc.europa.eu/en/publications-and-data/monitoring/weekly-threats-reports), the World Health Organization (WHO) (www.who.int/emergencies/disease-outbreak-news) and the Centers for Disease Control and Prevention in Atlanta (the Yellow Book at wwwnc.cdc.gov/travel/page/yellowbook-home/).

It is important to note that emerging vector-borne diseases are also reported in some European regions, e.g. West Nile virus fever, chikungunya virus disease, dengue. Relevant surveillance information for the European

Union can be obtained from the ECDC (www.ecdc.europa.eu/en/substances-human-origin/surveillance). The *Guide to the quality and safety of organs for transplantation* (Chapter 8 – Risk of transmission of infectious diseases) [13] provides useful guidance on assessing the risk of infection transmission through organ transplantation, based on published evidence in the literature and national transplant registries.

6.2.1.5 *Malignancies*

6.2.1.5.1 Haematological malignancies

Good Practice Guidelines

G6.29 Any information indicating haematological alterations that would be suggestive of any of these malignancies should be evaluated further.

Recommendations

R6.12 In the case of cornea donors, it is recommended to do a risk assessment because some of the haematological malignancies can be accepted.

R6.13 Before accepting donors with a history of haematological malignancy, it is recommended that:

- R6.13.1 the potential donor be confirmed as 'disease-free' status either by the haematological clinician(s) if the donor is still followed up or else based on the most recent medical records for the donor by the physician in the SoHO establishment;
- R6.13.2 a minimum of five years have elapsed since completion of treatment for diffuse large B-cell lymphomas, Hodgkin lymphoma and leukaemias;
- R6.13.3 donors with a history of myeloma, follicular lymphomas or small lymphocytic lymphoma/chronic lymphocytic leukaemia be avoided due to prolonged time to cure;
- R6.13.4 for donors over 55 years old, the disease-free period generally be extended to 10 years. This is based on epidemiological studies indicating a longer time-to-cure in this age group.

R6.14 Additionally, when assessing these donors, as with any malignancy, review of other factors affecting corneal quality is recommended. These factors may include toxicity from antineoplastic therapies, ocular graft-*versus*-host disease following haematopoietic stem cell transplantation or a clinical history of keratitis due to opportunistic infections.

Supplementary information

Ocular manifestations in patients with blood malignancies can be caused by primary lymphomas or be secondary to systemic haematological cancers in the form of leukaemic retinopathy [14].

The normal central cornea lacks blood and lymphatic vessels. In the very periphery of the cornea, vessels are limited to the transition between the cornea and sclera, known as the limbal area.

Generally, an active haematological malignancy is a contraindication for any tissue donation. However, due to the cornea's immunological privilege, certain circumstances allow for consideration of corneal donation eligibility of donors with a history of haematological cancers after thorough risk assessment by the designated physician of the SoHO establishment [15, 16].

The disease-free status may also be a verbal (oral) confirmation, provided by a named person answering the relevant medical and social history questions about the donor, or by a physician with access to records of the donor's treatment.

The negligible risk of transmission can be reduced even further if the amount of transplanted corneal donor tissue is minimised. Compared with a full-thickness corneal graft (25 mg weight), endothelial grafts to be used for DSAEK (5 mg weight) or DMEK (1 mg weight) based surgery reduce the volume of grafted donor material by around 80% and > 95%, respectively.

The WHO has published classifications of myeloid neoplasms and acute leukaemia [17], and of lymphoid neoplasms [18]. A recent blood test carried out before death, if available, may offer valuable information indicative of these conditions. Although an experienced haematologist will be able to provide a differential diagnosis, certain results can be individually evaluated [18], such as the examples shown in Table 6.5.

Table 6.5 **Haematological parameters**

Haematological parameters	Range	Reason for assessment
Altered haemoglobin	men > 18.5 g/dL women > 16.5 g/dL	aims to assess for potential contraindications, such as polycythaemia vera.
Altered haematocrit	men > 55.5% women > 49.5%	
Platelet count	< 50 × 10^9/L	is highly indicative of a haematological disorder.
	50-100 × 10^9/L	aims to assess for contraindications due to a possible haematological problem.
	> 450 × 10^9/L	aims to assess for contraindications such as essential thrombocytosis.
Altered white blood cells	> 50 × 10^9/L	aims to assess for contraindications such as chronic myeloid leukaemia.

6.2.1.5.2 Non-haematological malignancies

Good Practice Guidelines

G6.30 Detailed history of type, duration, course/recurrence and treatment history should be documented and assessed.

Supplementary information

Availability of screening programmes has improved early detection, which increases the treatment options and can lead to cure. For donors who had been diagnosed with a pre-malignant condition (e.g. Bowen's disease, polyposis coli or Barrett's oesophagus), further information for the follow-up can be obtained to exclude malignancy (see Appendix 17).

Some international bodies provide assessments on risk of transmission of malignancies through organ transplant for CNS and non-CNS tumours [19], which can be used as a basis for determination of the risks of transmission through tissue transplant. The role of processing steps applied to SoHO in reducing the risk of transmission of malignancy for SoHO following transplantation can be considered. The effect of high-dose terminal sterilisation (25-40 KGy) and the decellularisation process removing viable cells are examples of tissue-processing steps that reduce the potential for transmission of malignant cells. Also, tissue recipients usually do not require immunosuppression.

The *Guide to the quality and safety of organs for transplantation* (Chapter 9: Risk of transmission of cancer) [13] provides useful guidance on assessing the risk of malignancy transmission through organ transplantation, based on published evidence in the literature and national transplant registries. Other than absolute contraindications (unacceptable risk), the risk of transmission is classed as minimal (< 0.1%), low (0.1 to 1 or < 2%), intermediate (1 or 2% to 10%) or high (> 10%) for CNS and non-CNS tumours. A similar approach could be applied for SoHO transplantation based on a literature review.

6.2.1.6 *CNS tumours*

Good Practice Guidelines

G6.31 In CNS tumours, cerebral lymphoma and secondary intracranial lymphomas should be considered absolute contraindications.

G6.32 Malignancy gradation in the CNS should be thoroughly evaluated, including a complete histological exam rather than a simple biopsy, due to possible heterogeneity of the mass.

G6.33 Individual risk assessment should be performed for decisions about whether to accept donors with malignancies that have low or intermediate transmission risk for tissue donation.

Supplementary information

In 2021 the WHO updated their 2016 classification [20] of selected CNS tumours, which is shown in Appendix 17 (Table A17.1 compiles a combination of 2016 and 2021 classifications). In addition, following UK Advisory Committee on the Safety of Blood, Tissues and Organs (SaBTO) guidelines, WHO Grade 1 and 2 CNS tumours have a minimal or low risk of metastasis and would not contraindicate tissue donation. The Grade 3 and 4 primary CNS tumours have either low (WHO grade 3: < 2% transmission risk) or intermediate (WHO grade 4: 2.2% with an upper 95% CI of 6.4% transmission risk) risk of transmission through organ transplantation [13] (Appendix 17, Table A17.2). There are no described cardiac metastases with any CNS tumours in the literature. There are published reports of extracranial bone metastasis of glioblastoma multiforme (Grade 4) [21-27].

Appendix 17 provides a generic risk assessment for accepting a donor with CNS tumours (Grade 3 & 4) for heart valves.

6.2.1.7 *Non-CNS tumours*

Good Practice Guidelines

G6.34 The scarcity of certain types of SoHO should be taken into consideration where the risk of transmission is negligible or very low, and when there are no metastases described in the relevant tissue (see Appendix 17).

Supplementary information

The assessment of transmission risk for non-CNS tumours in an organ transplant setting [19] is summarised in Appendix 17, Table A17.3. Malignant neoplasms have been transmitted to immunosuppressed recipients through transplantation organs from donors with known or unknown malignancies [13].

The risk–benefit analysis is very different in a tissue-transplant setting. SoHO preparations are mostly used in elective settings and are life-enhancing (rather than life-saving).

The processing steps and terminal sterilisation may vastly reduce the potential for transmission of tumour cells, and SoHO recipients, unlike organ recipients, usually do not require immunosuppression. Thus, the risk of transmission is generally much lower compared with organ transplant. Guidance for undertaking these assessments is provided in Figure 6.1 (Decision tree scheme).

Transmission of malignancy via SoHO preparations is rare; there have been two case reports, in 2017, of donor-derived malignancy in viable keratolimbal allograft recipients [10, 28, 29].

Malignant melanoma with known metastatic disease is a contraindication, but in the case of cornea a detailed slit-lamp examination of the cornea helps to determine their suitability. Any vascularised ocular tissues,

such as sclera, limbal tissue or cells derived from limbal tissue, are not covered by this exclusion and may be evaluated as discussed above.

6.2.1.8 *Carcinoma* in situ

Carcinoma *in situ* (CIS) is an early form of cancer that is defined by the absence of invasion of tumour cells into the surrounding tissue, usually before penetration through the basement membrane with no potential for metastasis unless it progresses into an invasive carcinoma. There may be differences in terminology used to describe CIS depending on the anatomical location where it occurs, but all share the same characteristics. A list of all types of malignancy considered as CIS is provided in Appendix 17.

Its removal eliminates the risk of subsequent progression into a life-threatening condition. As most forms of CIS have a high probability of progression into invasive carcinoma [13], it is usually completely removed. If a donor had been successfully treated and cured after a CIS (e.g. uterine cervical or vulval carcinoma *in situ*, some intraductile carcinoma of the breast, intra-epithelial cancer of the prostate, etc.) the donation could be suitable because CIS do not adversely affect the safety or quality of other types of tissues.

6.2.1.9 *Exposure to toxic substances*

Recommendations

R6.15 In cases of ingestion of or exposure to a toxic substance (e.g. cyanides, lead, mercury, gold, arsenic, pesticides), it is recommended to assess the timing of exposure to the toxin in relation to when donation will take place. Exposure to asbestos in the past is a risk for developing a mesothelioma. In this case a thorough risk assessment is advised; see the *Guide to the quality and safety of organs for transplantation* (Chapter 10: Risks related to the use of organs from donors with other conditions and diseases) [13].

6.2.1.10 *Xenotransplantation*

Recommendations

R6.16 It is recommended that precautionary donor exclusion be applied if a potential donor had previous xenotransplantation with living SoHO and organs.

Supplementary information

Xenotransplantation is any procedure that involves the transplantation, implantation or infusion into a human recipient of either (a) live cells, tissues or organs from a non-human animal source, or (b) human body fluids, cells, tissues or organs that have had *ex vivo* contact with live non-human animal cells, tissues or organs [30-34].

Biological products, drugs or medical devices sourced from non-human animals that do not contain living cells, tissues or organs, including (but not limited to) porcine insulin, porcine heart valves, porcine skin and acellular porcine corneal stroma, and collagen matrices derived from acellular porcine, bovine or any other xenogeneic source [34-35], are not considered as xenotransplantation.

The main concern with the use of xenotransplantation is the potential infection of recipients with both recognised and unrecognised infectious agents and the possible subsequent transmission to their close contacts and into the general human population. Moreover, new infectious agents may not be readily identifiable with current methods/tests used for screening.

Exposure to non-human live animal material has the potential for cross-species infection by retroviruses, which may be latent and may lead to disease years after infection. Infections that would not normally affect humans such as porcine endogenous retroviral viruses (PERVs) that are integrated into the pig genome could potentially integrate into the host genome [36-38] and be transmitted through donated material. *In vitro* studies have demonstrated transmission of a PERV to a human cell line lacking the intracellular machinery that protects against retroviral infection [39, 40]. However, available data from clinical studies, with a limited number of patients, did not report any transmission of pathogens in the recipients who received porcine cell-based bioartificial liver transplant or porcine islets [40-44].

Although the published studies have not demonstrated any transmission of retroviruses via clinical xenotransplantation, the risk is difficult to quantify given the small number of recipients to date [35, 37], and long-term monitoring may help to demonstrate its safety.

Those donors who have received biological SoHO preparations can be accepted as SoHO donors.

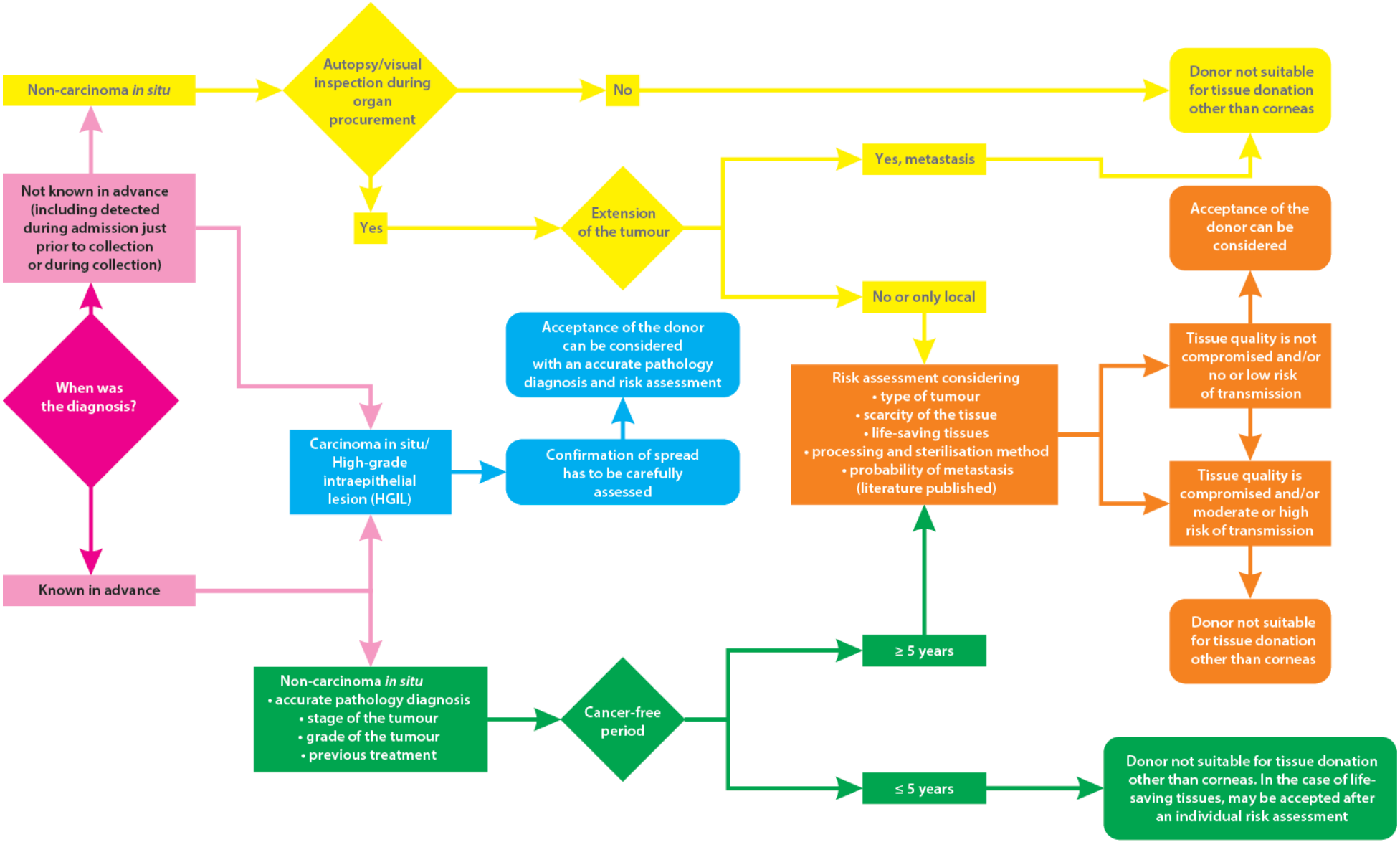
6.2.1.11 *Deferred for blood donation for known reason*

If it is known that the potential donor was excluded or deferred from donating blood by a blood-collection establishment, it is important to know the specific reason for the deferral, and the eligibility of the donor is then evaluated on an individual basis. If the reason is not known, it may be safer to exclude the donor for tissue donation.

6.3 Assessment of potential autologous donors

There are no absolute contraindications for autologous donors. In those cases, donor protection remains the main objective and the potential benefit.

Figure 6.1 **Decision tree algorithm for solid tumour approach**



6.4 References

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Related material

Appendix 14. Medical and social history questionnaire (NHS, UK)
Appendix 15. Physical assessment form (NHS, UK)
Appendix 16. Evaluation of pigmented skin lesions
Appendix 17. Evaluation of malignancies for risk assessment in SoHO donors

Chapter 7. Screening for markers of transmissible infections

7.1 Introduction

Screening donors for markers of transmissible infections, in the context of the donor evaluation, is critical to prevent the transmission of pathogens or disease through substances of human origin (SoHO) to recipients and to offspring of medically assisted reproduction (MAR).

In addition to allogeneic donors, this chapter is relevant to persons from whom SoHO are collected:

- for application to themselves;
- as part of their own current or future MAR treatment;
- as part of MAR treatment in the context of within-relationship use.

This chapter is also relevant to donors from whom SoHO are provided as starting material for the purpose of manufacturing medical devices, medicinal products, advanced therapy medicinal products or investigational medicinal products.

The donor screening requirements provided in this chapter do not apply to SoHO collected and applied during bedside and in-surgery procedures in the autologous context.

Standards

S7.1 SoHO entities must establish procedures to achieve a high level of assurance that pathogens that are potentially life-threatening, disabling or incapacitating are not transmitted from a third-party donor to recipients or to offspring from MAR (Art. 58.1).

S7.2 SoHO entities must screen donors for markers of communicable transmissible infections, in addition to conducting donor evaluation including current and past health, travel and relevant behavioural histories (Art. 58.2).

S7.3 SoHO entities in the EU or EEA must consider the requirements for donor screening set out in the most recent guidelines published on the EU SoHO platform by the European Centre for Disease Prevention and Control (ECDC) (Art. 59.4.a.i) [1].

Good Practice Guidelines

G7.1 Mandatory screening should include markers for human immunodeficiency virus (HIV), hepatitis B virus (HBV), hepatitis C virus (HCV) and *Treponema pallidum* as a minimum. In the EU/EEA, in addition to HIV, HBV and HCV, any other pathogens determined necessary by available guidelines published by the ECDC on the SoHO platform should also be included. See Table 7.1.

7.2 Sample collection and time of sampling

All personnel involved in ensuring the quality of the donor sample throughout the screening process, from the collection of the sample to the laboratory setting, have an impact on the screening results, and have a role in ensuring that SoHO are safe to use.

Good Practice Guidelines

G7.2 The screening should be carried out on the donor's serum or plasma. Screening should not be performed on other fluids or secretions unless specifically justified clinically using a validated or verified test for such a fluid.

Table 7.1 **Guidance on screening on SoHO donors**

		HIV[a]	HBV[a]	HCV[a]	*Treponema pallidum*[b]	HTLV-I/II[b]
Window period[c]		**6-10 days** (NAT HIV-1 RNA, 95% limit of detection [LOD] 100 IU/mL)	**15-20 days** (NAT HBV DNA, 95% LOD 20 IU/mL)	**<7 days** (NAT HCV RNA, 95% LOD 50 IU/mL)	**3-4 weeks** for treponemal-specific tests [e.g. enzyme imunoassays (EIA), *T. pallidum* passive particle agglutination (TPPA)]	**8-12 weeks**
Deceased donors	**Required**	All donors should be screened for: • Anti-HIV-1/2 • HIV-1 using RNA NAT: 95% LOD for HIV-1 RNA ≤100 IU/mL[d,e].	All donors should be screened for: • HBsAg • Anti-HBc • HBV using DNA NAT. If a highly sensitive NAT is used, anti-HBc is not required.	All donors should be screened for: • Anti-HCV • HCV using RNA NAT: 95% LOD for HCV RNA ≤50 IU/mL[d,e].	All donors should be screened with a treponemal-specific test such as: • *T. pallidum* haemagglutination (TPHA) test (or) • *T. pallidum* particle agglutination (TPPA) test (or) • treponemal EIA (or) CLIA.	High-risk donors (see section 7.7 and Chapter 6, Table 6.4) should be screened for: • Anti HTLV-I/II .
	Advice and practical considerations	• HIV-1/2 RNA NAT is advised. • Antigen-antibody combination tests can be used instead of antibody only.	HBV DNA NAT: 20 IU/mL or lower could be considered as highly sensitive.		It is advised to further test samples reactive on the initial and confirmatory treponemal test with a non-treponemal test.	It is advised to test all donors for HTLV I/II where risk factors for HTLV-I/II are unknown.

[a] These requirements and advice are based on the ECDC technical guidelines on the prevention of communicable disease transmission through SoHO that will apply from 7 August 2027. Guidance is available at www.ecdc.europa.eu/en/infectious-disease-topics/related-public-health-topics/substances-human-origin/technical-guidelines.

[b] These recommendations are based on EDQM guidance and discussions with ECDC while awaiting ECDC's technical guidelines on the prevention of communicable disease transmission through SoHO in the European Union and European Economic Area (EU/EEA) which are ongoing. Once published, the information will be updated accordingly.

[c] These are the conservative assumptions considered by ECDC. Shorter window periods might be reported in the literature.

[d] If a dilution of the sample is needed, and the LOD threshold cannot be achieved, the dilution factor should be documented.

[e] A higher LOD can be considered if justified by a documented risk assessment including the endemicity of the disease. An update of the risk assessment should be performed if there are significant changes to the epidemiology of the disease or if there is a transmission event from donor to recipient.

[f] Human milk and intestinal microbiota donors are based on EDQM guidance and discussions with ECDC while awaiting ECDC's technical guidelines on the prevention of donor-derived communicable disease transmission through SoHO to recipients in the EU/EEA which are ongoing. Once published, the information will be updated accordingly.

		HIV[a]	HBV[a]	HCV[a]	*Treponema pallidum*[b]	HTLV-I/II[b]
Window period[c]		**6-10 days** (NAT HIV-1 RNA, 95% limit of detection [LOD] 100 IU/mL)	**15-20 days** (NAT HBV DNA, 95% LOD 20 IU/mL)	**<7 days** (NAT HCV RNA, 95% LOD 50 IU/mL)	**3-4 weeks** for treponemal-specific tests [e.g. enzyme imunoassays (EIA), *T. pallidum* passive particle agglutination (TPPA)]	**8-12 weeks**
Living non-reproductive SoHO donors[f]	**Required**	All donors should be screened for: • Anti-HIV-1/2 • HIV-1 using RNA NAT: 95% LOD for HIV-1 RNA ≤100 IU/mL[e].	All donors should be screened for: • HBsAg • Anti-HBc • HBV using DNA NAT: If a highly sensitive NAT is used, anti-HBc is not required.	All donors should be screened for: • Anti-HCV • HCV using RNA NAT: 95% LOD for HCV RNA ≤50 IU/mL[e].	All donors should be screened with a specific treponemal test such as: • *T. pallidum* haemagglutination (TPHA) test, (or) • *T. pallidum* particle agglutination (TPPA) test (or) • treponemal EIA (or) CLIA.	High-risk donors (see section 7.7 and Chapter 6, Table 6.4) should be screened for: • Anti HTLV-I/II .
	Advice and practical considerations	• HIV-1/2 RNA NAT is advised. • Antigen-antibody combination tests can be used instead of antibody only.	HBV DNA NAT: 20 IU/mL or lower could be considered as highly sensitive.		It is advised to further test samples reactive in the initial and confirmatory treponemal test with a non-treponemal test.	It is advised to test all donors of haematopoietic progenitor cells for HTLV- I/II. It is advised to test all other living SoHO donors where risk factors for HTLV-I/II are unknown.

[a] These requirements and advice are based on the ECDC technical guidelines on the prevention of communicable disease transmission through SoHO that will apply from 7 August 2027. Guidance is available at www.ecdc.europa.eu/en/infectious-disease-topics/related-public-health-topics/substances-human-origin/technical-guidelines.

[b] These recommendations are based on EDQM guidance and discussions with ECDC while awaiting ECDC's technical guidelines on the prevention of communicable disease transmission through SoHO in the European Union and European Economic Area (EU/EEA) which are ongoing. Once published, the information will be updated accordingly.

[c] These are the conservative assumptions considered by ECDC. Shorter window periods might be reported in the literature.

[d] If a dilution of the sample is needed, and the LOD threshold cannot be achieved, the dilution factor should be documented.

[e] A higher LOD can be considered if justified by a documented risk assessment including the endemicity of the disease. An update of the risk assessment should be performed if there are significant changes to the epidemiology of the disease or if there is a transmission event from donor to recipient.

[f] Human milk and intestinal microbiota donors are based on EDQM guidance and discussions with ECDC while awaiting ECDC's technical guidelines on the prevention of donor-derived communicable disease transmission through SoHO to recipients in the EU/EEA which are ongoing. Once published, the information will be updated accordingly.

		HIV[a]	HBV[a]	HCV[a]	*Treponema pallidum*[b]	HTLV-I/II[b]
Window period[c]		**6-10 days** (NAT HIV-1 RNA, 95% limit of detection [LOD] 100 IU/mL)	**15-20 days** (NAT HBV DNA, 95% LOD 20 IU/mL)	**<7 days** (NAT HCV RNA, 95% LOD 50 IU/mL)	**3-4 weeks** for treponemal-specific tests [e.g. enzyme imunoassays (EIA), *T. pallidum* passive particle agglutination (TPPA)]	**8-12 weeks**
Reproductive cells third-party donors	**Required**	All donors should be screened for: • Anti-HIV-1/2 • HIV-1 using RNA NAT: 95% LOD for HIV-1 RNA ≤100 IU/mL[e].	All donors should be screened for: • HBsAg • Anti-HBc • HBV using DNA NAT. If a highly sensitive NAT is used, anti-HBc is not required.	All donors should be screened for: • Anti-HCV • HCV using RNA NAT: 95% LOD for HCV RNA ≤50 IU/mL[e].	All donors should be tested with a specific treponemal test such as: • *T. pallidum* haemagglutination (TPHA) test, (or) • *T. pallidum* particle agglutination (TPPA) test (or) • treponemal EIA (or) CLIA.	High-risk donors (see section 7.7 and Chapter 6, Table 6.4) should be screened for: • Anti HTLV-I/II.
	Advice and practical considerations	• HIV-1/2 RNA NAT is advised. • Antigen-antibody combination tests can be used instead of antibody only.	HBV DNA NAT: 20 IU/mL or lower could be considered as highly sensitive.		It is advised to further test samples reactive on the initial and confirmatory treponemal test with a non-treponemal test.	It is advised to test all reproductive SoHO donors for HTLV I/II where risk factors for HTLV-I/II are unknown.

[a] These requirements and advice are based on the ECDC technical guidelines on the prevention of communicable disease transmission through SoHO that will apply from 7 August 2027. Guidance is available at www.ecdc.europa.eu/en/infectious-disease-topics/related-public-health-topics/substances-human-origin/technical-guidelines.

[b] These recommendations are based on EDQM guidance and discussions with ECDC while awaiting ECDC's technical guidelines on the prevention of communicable disease transmission through SoHO in the European Union and European Economic Area (EU/EEA) which are ongoing. Once published, the information will be updated accordingly.

[c] These are the conservative assumptions considered by ECDC. Shorter window periods might be reported in the literature.

[d] If a dilution of the sample is needed, and the LOD threshold cannot be achieved, the dilution factor should be documented.

[e] A higher LOD can be considered if justified by a documented risk assessment including the endemicity of the disease. An update of the risk assessment should be performed if there are significant changes to the epidemiology of the disease or if there is a transmission event from donor to recipient.

[f] Human milk and intestinal microbiota donors are based on EDQM guidance and discussions with ECDC while awaiting ECDC's technical guidelines on the prevention of donor-derived communicable disease transmission through SoHO to recipients in the EU/EEA which are ongoing. Once published, the information will be updated accordingly.

		HIV[a]	HBV[a]	HCV[a]	*Treponema pallidum*[b]	HTLV-I/II[b]
Window period[c]		**6-10 days** (NAT HIV-1 RNA, 95% limit of detection [LOD] 100 IU/mL)	**15-20 days** (NAT HBV DNA, 95% LOD 20 IU/mL)	**<7 days** (NAT HCV RNA, 95% LOD 50 IU/mL)	**3-4 weeks** for treponemal-specific tests [e.g. enzyme imunoassays (EIA), *T. pallidum* passive particle agglutination (TPPA)]	**8-12 weeks**
Reproductive SoHO – within relationship use	**Required**	All partners should be screened with: • antigen-antibody combination assays detecting anti-HIV-1/2.	All partners should be screened for: • HBsAg or HBV using DNA NAT.	All partners should be screened for anti-HCV.		High-risk partners (see section 7.7 and Chapter 6, Table 6.4) should be screened for anti-HTLV-1/II.
	Advice	HIV-1/2 RNA NAT is advised in addition to screening with antigen-antibody combination assays.		HCV RNA NAT is advised: • In case of a positive confirmatory test result following a reactive anti-HCV. • In case a partner reports recent events with a risk of exposure to HCV.	It is advised that all partners are screened with a specific treponemal test such as: • *T. pallidum* haemagglutination (TPHA) test, (or) • *T. pallidum* particle agglutination (TPPA) test (or) • treponemal EIA (or) CLIA.	It is advised to test all tissue donors for HTLV I/II where risk factors for HTLV-I/II are unknown.

[a] These requirements and advice are based on the ECDC technical guidelines on the prevention of communicable disease transmission through SoHO that will apply from 7 August 2027. Guidance is available at www.ecdc.europa.eu/en/infectious-disease-topics/related-public-health-topics/substances-human-origin/technical-guidelines.

[b] These recommendations are based on EDQM guidance and discussions with ECDC while awaiting ECDC's technical guidelines on the prevention of communicable disease transmission through SoHO in the European Union and European Economic Area (EU/EEA) which are ongoing. Once published, the information will be updated accordingly.

[c] These are the conservative assumptions considered by ECDC. Shorter window periods might be reported in the literature.

[d] If a dilution of the sample is needed, and the LOD threshold cannot be achieved, the dilution factor should be documented.

[e] A higher LOD can be considered if justified by a documented risk assessment including the endemicity of the disease. An update of the risk assessment should be performed if there are significant changes to the epidemiology of the disease or if there is a transmission event from donor to recipient.

[f] Human milk and intestinal microbiota donors are based on EDQM guidance and discussions with ECDC while awaiting ECDC's technical guidelines on the prevention of donor-derived communicable disease transmission through SoHO to recipients in the EU/EEA which are ongoing. Once published, the information will be updated accordingly.

Abbreviations: DNA, deoxyribonucleic acid; ECDC, European Centre for Disease Prevention and Control; EDQM, European Directorate for the Quality of Medicines & HealthCare; HBc, hepatitis B core antigen; HBsAg, hepatitis B surface antigen; HBV, hepatitis B virus; HCV, hepatitis C virus; HIV, human immunodeficiency virus; HTLV, human T-lymphotrophic virus; LOD, limit of detection; NAT, nucleic acid amplification test; RNA, ribonucleic acid.

G7.3 The donor samples should be obtained and handled according to instructions provided by the screening laboratory following the specifications laid out by the manufacturer of the relevant pathogen screening assay (e.g. timing, collection tube, sample type, size and handling, transport and storage conditions) to ensure optimal screening assay performance.
G7.4 The donor sample collection and labelling procedures should be clearly designed to ensure accurate sample identification, linking the sample with the corresponding donor and the SoHO donation for traceability. At least two donor identifiers, such as the donor's full birth name, date of birth, medical record number or alternative unique electronic identifiers should be used.
G7.5 The label or embedded documentation should include:
G7.5.1 The date and time when the donor sample was collected.
G7.5.2 A notification in case the donor sample was collected *post mortem* from a deceased donor.

Recommendations

R7.1 As regards the information that needs to be included in the label or embedded documentation (see G7.5), it is recommended to include, if relevant, a description of the site on the donor's body that the sample was collected from (e.g. cephalic vein, femoral artery, subclavian artery, superior vena cava).

7.2.1 Time of sampling

7.2.1.1 *Deceased donors*

Good Practice Guideline

G7.6 In the case of a deceased donor, blood samples should have been obtained just prior to death or, if not available, as soon as possible after death (within 24 hours after death). If the period is extended beyond 24 hours, additional validation of each infectious disease screening assay using such specimens is necessary under the conditions accepted by the national regulations.

Supplementary information

Delays in blood sampling of deceased donors have previously been shown to increase the incidence of haemolysis (see section 7.3.2) and contamination, due to growth of micro-organisms and release of enzymes (including by-products of tissue and cell death). This can lead to poor quality blood samples from deceased donors and may cause non-specific screening results or lead to rejection of samples as they may be unsuitable for screening. For this reason, blood samples are normally obtained within 24 h after death [2].

There is some evidence that blood samples obtained more than 24 h *post mortem* can yield valid results after validation of screening assays [3-5].

Appendix 19 contains information on validation of screening for infectious disease assays for use with blood from deceased donors.

7.2.1.2 *Living donors*

7.2.1.2.1 Haematopoietic progenitor cell (HPC) donors

Good Practice Guidelines

G7.7 In the case of autologous and allogeneic HPC and mononuclear cell (e.g. donor lymphocyte infusions) donors, blood samples should be obtained within 30 days prior to the collection of cells or at the time of collection. Test results should be available before the conditioning regimen of HPC recipients.
G7.8 In the case of cord blood HPC donors, maternal blood samples should be obtained at the time of collection, or within 7 days before or after the collection of cells.

7.2.1.2.2 Non-reproductive SoHO other than HPC donors

Good Practice Guidelines

G7.9 In the case of non-reproductive living SoHO donors other than HPC donors, blood samples should be obtained at the time of donation, or within 7 days before or after collection of SoHO.
G7.10 In the case of human milk, mandatory tests should be performed on blood samples at the beginning of donation within 7 days before the first collection, as outlined in Chapter 32.
G7.10.1 Donor testing should be performed at first donation and:
G7.10.1.1 if the milk will be pasteurised, should be repeated at least every 3 months or when the last donation is taking place;
G7.10.1.2 in the case of raw milk, should be repeated at least every 2 months or when the last donation is taking place; and,
G7.10.1.3 if risk factors for transmissible communicable disease are identified during health interviews during regular donor follow-up, repeat testing should be done.
G7.10.2 If there is a suspicion risk factor for infection such as *T. cruzi* during the health interview, additional testing (for relevant infection) should be done, especially when the milk is not pasteurised.
G7.11 Intestinal microbiota: In addition to mandatory tests on donor blood samples described in this chapter, blood and faeces tests specific to intestinal microbiota should be done as outlined in Chapter 33. Complete donor screening including blood and

faeces tests should be done at the beginning and after the ending of a donation period (not exceeding 3 months).

7.2.1.2.3 Reproductive SoHO donors

7.2.1.2.3.1 *Within relationship use and own MAR treatment*

Good Practice Guideline

G7.12 In the case of oocytes/sperm and ovarian/testicular tissues, within relationship use or own MAR treatment, blood samples should be obtained within 3 months before the first collection of oocytes or sperm. For further collection of oocytes or sperm, additional blood samples should be obtained according to national legislation no later than maximum 24 months or when a risk is identified.

7.2.1.2.3.2 *Third-party donors*

Good Practice Guidelines

G7.13 In the case of reproductive third-party oocyte and sperm donors, samples should be obtained at the time of donation.

G7.14 In the case of serial sperm cell donations, the donors should be sampled and screened at the first donation and prior to the release of a donation, at least 30 days after the last donation in the series. If the period of serial donations extends beyond 90 days, the donor should be re-screened every 90 days for as long as the serial donations are ongoing.

Supplementary Information

In case of third-party oocyte donation, the donation of oocytes could be considered as the starting point of stimulation, and the screening could be performed at that point in time.

7.2.1.3 *Paediatric donors*

A child may acquire an infection through vertical transmission from an infected birth mother or from a person breastfeeding the child.

The following Good Practice Guidelines (GPGs) include blood sample screening of the birth mother and the person breastfeeding a possibly infected child donor, following the pathway for non-HPC/non-reproductive living donors, though they are not donors in this context.

Good Practice Guidelines

G7.15 If the child donor **is 18 months old or less**:

G7.15.1 The screening should be carried out on a blood sample from the birth mother.

G7.15.2 If the child has been breastfed within 6 months prior to donation, a blood sample from the person breastfeeding the child, if not the birth mother, should also be screened in addition to the maternal sample.

G7.15.3 If it is not possible to test the mother or the person breastfeeding, but the risk related to transmissible communicable diseases can be ruled out through other means in the mother or in the person breastfeeding, a blood sample from the child, if 3 months of age or above, should be tested with NAT and serology (see Table 7.2).

G7.16 If the child donor is **more than 18 months old**:

G7.16.1 If the cessation of breastfeeding **is more than 6 months prior to donation**, a blood sample from the child should be obtained at the time of donation;

G7.16.2 If the cessation of breastfeeding **is within the 6 months prior to donation**:

G7.16.2.1 the child should be screened. Interpretation of test results should consider testing limitations in case of potential exposure to HBV and HCV through breastfeeding;

G7.16.2.2 a blood sample from the breastfeeding mother or from any other person breastfeeding should also be tested if the infectious diseases of concern cannot be ruled out through other means.

Supplementary Information

Screening of the birth mother is to identify the risk of vertical transmission because a newborn's immune systems are not fully developed to mount an immune challenge.

If the human milk has been obtained from an authorised milk bank (SoHO establishment), a separate sample from the milk donor is not required, if the milk bank follows the donor selection and screening procedures described in this Guide.

If it is not possible to test the person breastfeeding in the past 6 months, but an infection can be ruled out through other means, e.g. with recent negative test, performed for other reasons than the donor screening, the child alone can be tested (see G7.16.2.2).

7.3 Quality of samples

Some donor-related conditions, such as haemodilution and haemolysis, may affect the quality of the donor samples and thereby have the potential to affect the accuracy of the screening results.

7.3.1 Haemodilution

Good Practice Guidelines

G7.17 Sampling in close proximity to an intravenous line should be avoided to prevent the collection of a diluted blood sample.

G7.18 If a potential donor has lost blood, and has received blood, blood components, colloids or crystalloids, an assessment of the extent of haemodilution should be carried out (see Appendix 18).
G7.19 In case of haemodilution, a blood sample collected before administration of any infusion or transfusion should be sought.
G7.20 If a pre-infusion sample is not available, post-infusion or transfusion samples should only be accepted if testing procedures have been validated for such samples.

Supplementary Information

Assessment of the extent of any haemodilution includes the use of a formula (see Appendix 18 for an algorithm to calculate the potential haemodilution) to calculate dilution of the donor's original circulating blood volume (and circulating levels of antigen and/or antibody, if present). Current practice in a number of countries is to consider 50% calculated haemodilution to be the maximum allowable with minimal risk of a false-negative screening result arising because of the haemodilution [2].

In cases of acute or untreated chronic infections, viral loads are usually in a range where haemodilution may not cause false-negative results. If initial screening results are suggestive of low viraemia, a highly sensitive NAT should be performed. SoHO from a donor with suspected occult HBV infection [6, 7] and haemodilution might not be considered to be safe.

7.3.2 Haemolysis

Haemolysis is a destruction of erythrocytes. The lysis of the blood cells is generally visually detectable after centrifugation of blood samples. Depending on the degree of haemolysis, the colour of the serum/plasma may be more or less "reddish". The darker colour may affect the accuracy of the screening assays and promote a higher density reading by the optical component of the screening equipment and may lead to a false-positive screening result. Haemolysis may be due to an incorrect blood withdrawal technique, such as rapid collection of blood through a small-bore needle, or inappropriate sample handling (e.g. extreme temperatures, delayed processing or prolonged storage). It can also be donor-derived due to intrinsic factors [8].

7.4 Screening laboratories

Screening laboratories with dedicated expertise have a crucial role in providing timely and accurate screening results.

Standards

S7.4 The testing laboratories, screening donor samples for transmissible communicable diseases, must be accredited, certified or authorised. The laboratories must use certified and validated testing methods for screening or, when not feasible, use other methods validated by those laboratories (Art. 58.2.b).

Good Practice Guidelines

G7.21 Testing laboratories, screening donor samples for transmissible communicable diseases, should be accredited, certified or authorised by the designated body.
G7.22 The laboratories should participate in relevant external quality assessment schemes (proficiency screening), if available, and be subject to regular internal and external audits to ensure consistent levels of competence and performance. Audits should be undertaken periodically by the SoHO entities or by qualified external experts as part of the SoHO entity's quality system.
G7.23 If the screening is performed by a contracted laboratory, the activities should be covered by a written mutual third-party agreement between the SoHO establishment and the laboratory; see Chapter 2 for outsourced activities.

7.5 Screening of donor samples

Standards

S7.5 In the EU, *in vitro* diagnostic medical devices as defined by Regulation (EU) 2017/746 must be used for screening of donor samples [9].

Good Practice Guidelines

G7.24 Upon receipt of donor samples at the laboratory, positive identification of the samples received against those expected should be carried out.
G7.25 The type of test used should be validated or verified for the purpose, in accordance with current scientific knowledge.
G7.26 There should be data confirming the suitability of any laboratory reagents used in testing of donor samples.
G7.27 Laboratories should have Standard Operating Procedures (SOP) with defined criteria for acceptance or rejection of donor samples, based on pre-screening circumstances that could affect the screening results, such as sampling procedure, storage and transport conditions, and should share the information in a timely manner with the SoHO establishments concerned.
G7.28 There should be a reliable process in place for transcribing, collating and interpreting results.
G7.29 Screening algorithms should be defined precisely in writing (by SOPs) for dealing with initially reactive specimens and for resolving discrepancies in results after re-testing.

G7.30 There should be clearly defined procedures to resolve discrepant results. Appropriate confirmatory testing should take place.

7.6 Documentation and reporting of screening results

Good Practice Guidelines

G7.31 The written agreement between the SoHO establishment and the screening laboratory should include requirements for reporting of screening results. The procedures should ensure accurate, rapid and verifiable reporting of results.

G7.32 There should be a process in place at the screening laboratory to ensure prompt alerts using an immediate notification system when an indicative screening result for an infectious disease occurs.

G7.33 In the case of confirmed positive results, the SoHO entity/establishment responsible for release of SoHO, as well as those persons responsible for donor management, should be notified without delay.

G7.34 Data-security measures should be implemented, as well as safeguards against any unauthorised additions, deletions or modifications to donor screening results.

Recommendations

R7.2 In the reporting of screening results, it is recommended to consider other precautionary measures such as the following:

- R7.2.1 using computerised procedures for the transfer of screening results from laboratory equipment to the laboratory data-processing management system (e.g. medical records) to eliminate the need for manual transcription of data;
- R7.2.2 using clearly interpretable, computerised graphic symbols to highlight positive screening results;
- R7.2.3 recording (semi)quantitative values (e.g. titre, IU/mL) of antibodies and/or the related positivity threshold next to the viral negative/positive result;
- R7.2.4 recording the number of copies/mL, if applicable (or, preferably, IU/mL), of nucleic acid measurement as well as (for qPCR systems) the linear range of the assay (if qualitative PCRs are used, then semiquantitative values should be recorded, e.g. Ct-Values, Cp-Values);
- R7.2.5 documenting the name of the screening assay being used (or making available on demand the data of the diagnostic assays, e.g. name, manufacturer).

7.7 Mandatory tests and interpretation of results

Good Practice Guidelines

G7.35 SOPs that define the criteria for acceptance or rejection of SoHO based on test results should be in place and should include special considerations for acceptance or rejection of SoHO from infected donors, including donations in an autologous setting or within relationship use.

G7.36 In exceptional circumstances, where test results from an allogeneic donor do not meet defined acceptance criteria, the SoHO preparation should only be accepted when the benefits outweigh risks to recipients. See Chapter 13, section 13.3.

G7.37 A donor management policy should be in place, including the provision of information to the donor and follow-up procedures, in the case of confirmed positive results, and the fate of the SoHO preparations (see Table 7.2).

Deceased donors

G7.38 In the case of reactive screening test(s) and absence of negative confirmatory test results, the transplant co-ordination team(s) should be informed. A risk assessment should be performed to determine if any previous donations are potentially infectious and if look-back procedures should be initiated. Recipients who received potentially infectious donations (e.g. organs and any other SoHO) should be tested.

Living donors

G7.39 In the case of reactive screening test(s) and absence of negative confirmatory test results, the donor should be notified through any means available and referred to relevant clinical care; look-back procedures of previous, potentially infectious donations should be initiated for repeated donations.

Algorithms to support decision making in non-reproductive deceased or living donors for HIV, HBV, HCV and *Treponema pallidum* tests results are set out in Figures 7.1, 7.2, 7.3 and 7.4 respectively.

Approaches to confirm screening test results should rely on nationally established algorithms.

Supplementary information

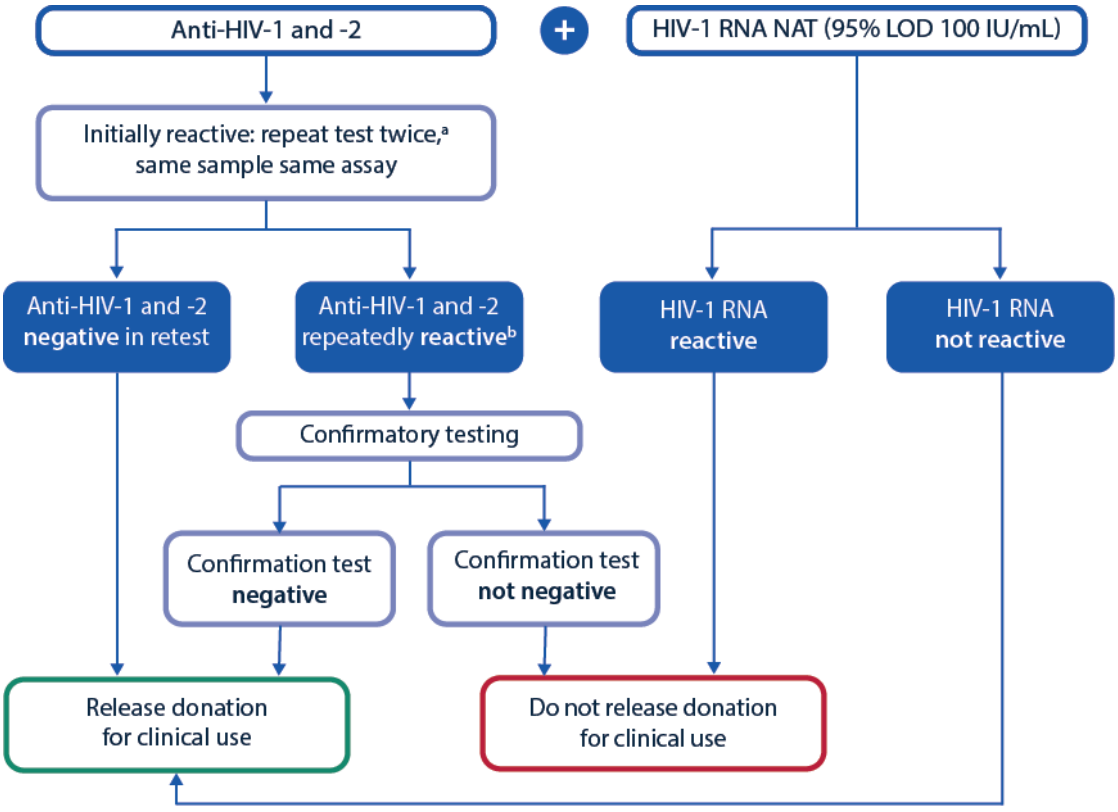
It is well recognised that NAT assays for HIV, HBV and HCV reduce the risk of inadvertent disease transmission due to the substantial decreases in window periods when compared with routine serological screenings [10, 11].

Table 7.2 **Interpretation and decision-making framework**

	Deceased and living non-reproductive SoHO donors	Reproductive third-party donors	Paediatric donors
HIV-1 and HIV-2[a]	Donations from donors with a reactive NAT on screening should not be released for clinical use. Donations from donors with a reactive serological screening test (anti-HIV-1/2) should only be released with a negative confirmatory test result,[b] provided there is a negative HIV RNA NAT in screening (see Figure 7.1).	Donations from donors with a reactive anti-HIV-1/2 serological test and/or HIV RNA NAT should not be released for clinical use. Confirmatory test results[b] are used to inform donor follow-up or re-entry procedures.	Donations from children should not be released for clinical use if either the child, the birth mother or the person who has breastfed the child in the past six months, has a reactive NAT, a reactive serological test in the absence of a negative confirmatory test result, or a known HIV infection. Donations should be released for clinical use if the test result requirement is met by all tested individuals (as given in this table).
HBV[c]	Donations from donors with a confirmed reactive HBsAg serological test and/or reactive HBV DNA NAT should not be released for clinical use. In the case of a reactive HBsAg screening, donations should only be released with a negative confirmatory test result[b] for HBsAg and provided there is a negative HBV DNA NAT in screening. If anti-HBc is reactive, donations should be released only if highly sensitive HBV DNA NAT (95% LOD for HBV DNA ≤ 20 IU/mL) and HBsAg are negative (see Figure 7.2).		If the birth mother has a known HBV infection and if the child has received appropriate immunoprophylaxis for HBV according to national recommendations, donations should be released for clinical use only if the anti-HBs concentration for the child is above 10 IU/L. Donations should be released for clinical use if the test result requirement is met by all tested individuals (as given in this table).
HCV[d]	Donations from donors with a reactive HCV RNA NAT should not be released for clinical use. In the case of negative HCV RNA NAT and reactive anti-HCV in screening, the donation should be released only with a negative confirmatory test result[b] for anti-HCV (OR) where there is documented SVR12/24[e] evidence available (see Figure 7.3).	Donations from donors with a reactive anti-HCV serological test and/or HCV RNA NAT should not be released for clinical use. Confirmatory test results are used to inform donor follow-up or re-entry procedures.	Donations from children should not be released for clinical use if either the child, the birth mother or the person who has breastfed the child in the past six months has • reactive NAT, • a reactive serological test in the absence of a negative confirmatory test result, or • a documented SVR12/24 for the mother, and the child is HCV RNA NAT negative. Donations should not be released if the mother has a known active HCV infection.
Treponema pallidum	Donations from donors with reactive screening results should only be released with negative confirmed results[b] (see Figure 7.4).	Donations from donors with a reactive screening test should not be released for clinical use.	
HTLV- I/II	If anti-HTLV-1/2 is reactive in screening, donations should only be released after a negative confirmatory test, on the basis of a risk assessment taking into account the type of SoHO donated and the type of confirmatory test(s) used.	Donations from donors with a reactive screening test should not be released for clinical use. Confirmatory test results are used to inform donor follow-up or re-entry procedures.	Donations from children should not be released for clinical use if the child has a reactive anti-HTLV-1/2 test result, or if the birth mother, or the person who has breastfed the child, has a known or suspected HTLV-1/2 infection.

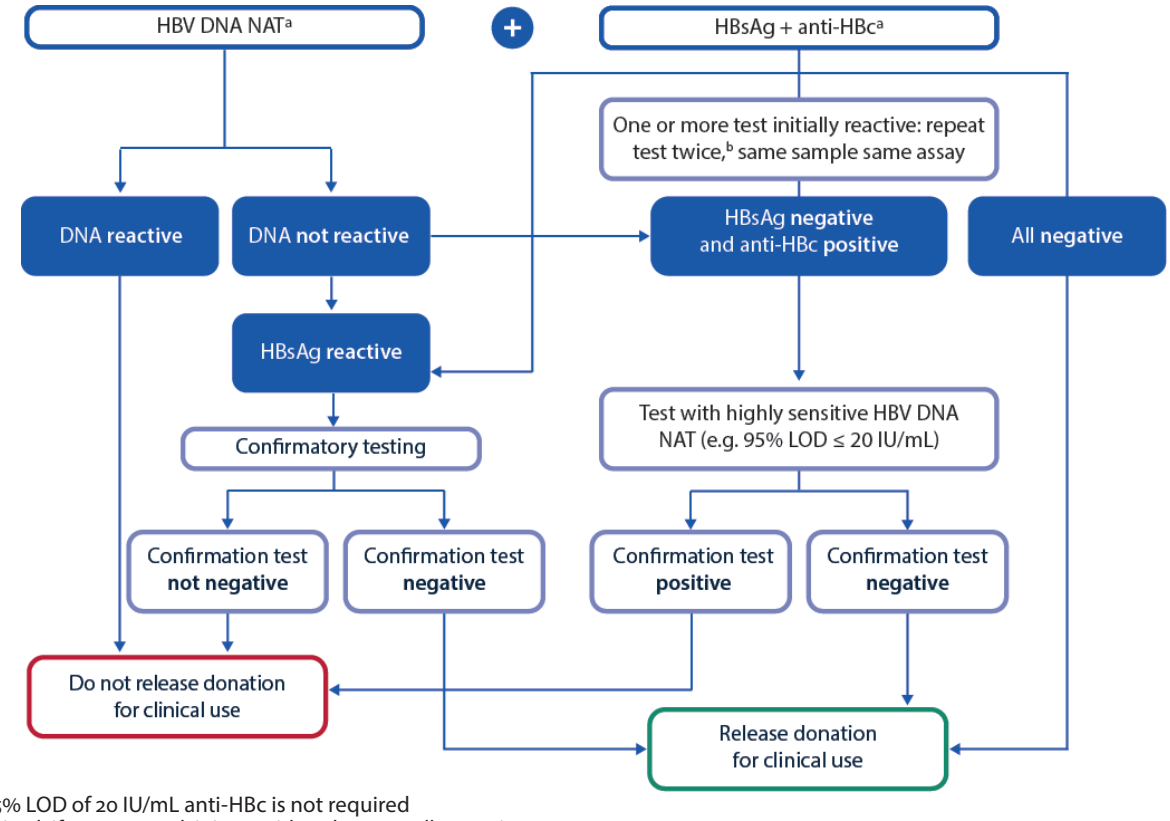
[a] Adapted from ECDC HIV guidelines available at www.ecdc.europa.eu/en/publications-data/guidelines-prevention-hiv-transmission-through-substances-human-origin
[b] Approaches to confirm screening test results should rely on nationally established algorithms.
[c] Adapted from ECDC HBV guidelines available at www.ecdc.europa.eu/en/publications-data/guidelines-prevention-hepatitis-b-virus-transmission-through-substances-human
[d] Adapted from ECDC HCV guidelines available at www.ecdc.europa.eu/en/publications-data/guidelines-prevention-hepatitis-c-virus-transmission-through-substances-human
[e] Sustained virological response (SVR) defined by undetectable HCV RNA in blood at 12 weeks (SVR12) and 24 weeks (SVR24) after the end of therapy, assessed by HCV RNA nucleic acid test (NAT) with a limit of detection (LOD) ≤ 15 international units (IU) per mL (EASL, https://doi.org/10.1016/j.jhep.2020.08.018)

Figure 7.1 **Algorithm/scheme of HIV screening**



[a] Advised, if not retested, it is considered repeatedly reactive
[b] At least one retest is reactive

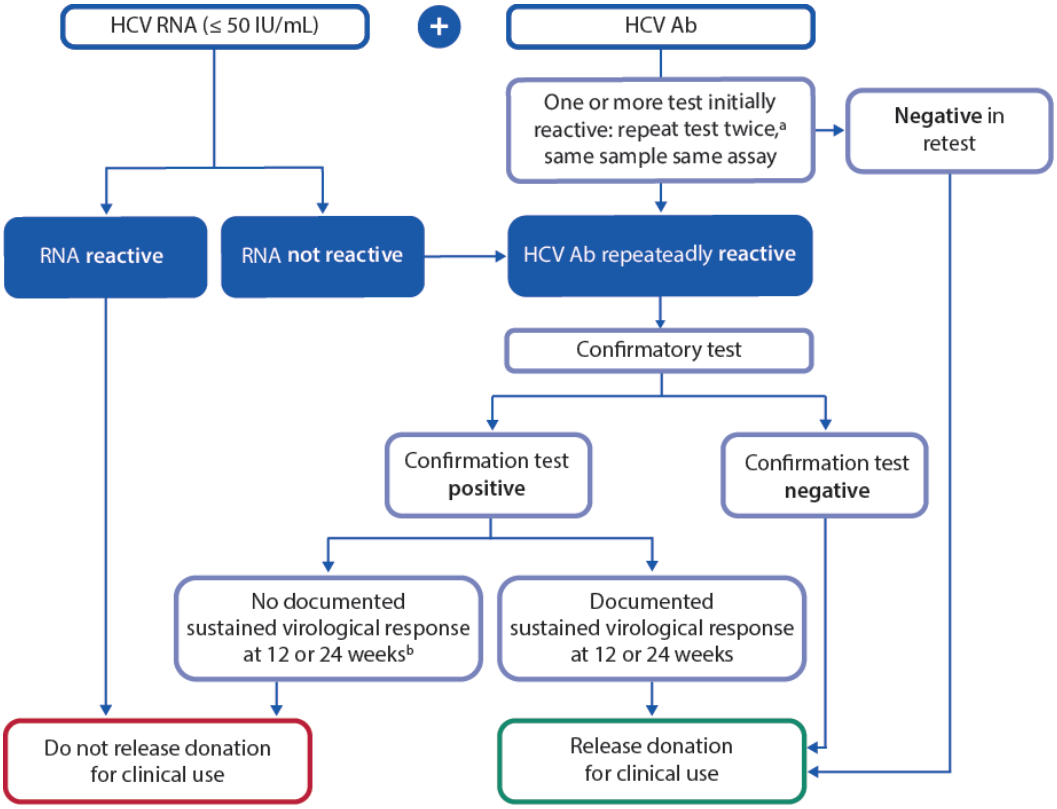
Figure 7.2 **Algorithm/scheme of HBV screening**



[a] If 95% LOD of 20 IU/mL anti-HBc is not required
[b] Advised, if not retested, it is considered repeatedly reactive

Approaches to confirm screening test results should rely on nationally established algorithms.

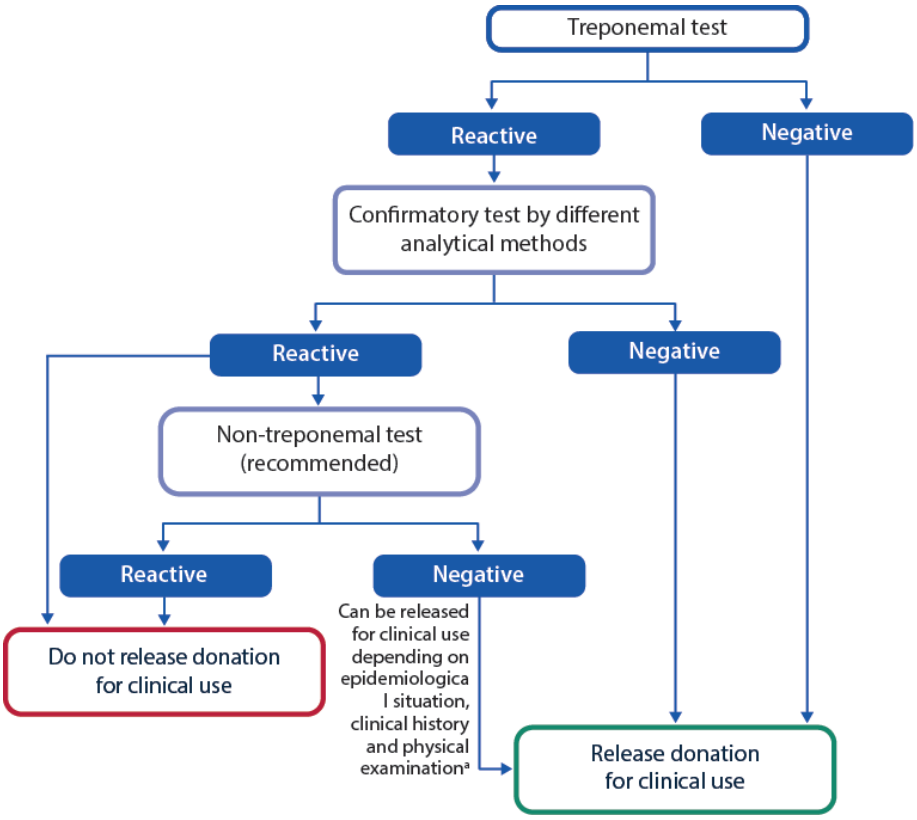
Figure 7.3 **Algorithm/scheme for HCV screening**



[a] Advised, if not retested, it is considered repeatedly reactive
[b] SVR after documented treatment

Approaches to confirm screening test results should rely on nationally established algorithms.

Figure 7.4 **Scheme for *Treponema pallidum***



[a] documentation of treatment and cure, and absence of recent risk factors for the donor, as well as refrigeration or freezing of the donated tissue or cell which could lead to pathogen inactivation.

Supplementary information

Treponemal tests are serological tests with specific antibodies that recognise and bind to species of the genus *Treponema*. No distinction can be made between the different treponematoses (spirochetes within the group of *Treponema*) due to immunological cross-reactivity. These tests usually remain indefinitely positive after the initial infection, which means that they cannot be used to monitor the response to treatment or diagnose reinfections.

Non-treponemal screening tests are tests that detect IgG and IgM directed against the lipids that are released from the damaged human cells during an early stage of the infection. The goal of these screening tests is to search for antibodies to antigens that are not specific to an infection with species of the genus *Treponema*, as reflected in the term reaginic antibodies. The non-specific nature of this category of serological screening tests is reflected in the fact that many other causes, such as advanced age, pregnancy, various types of malignant tumours, autoimmune diseases and other unrelated infections may result in the formation of anti-lipoid antibodies, thus generating false-positive results. Moreover, non-treponemal screening tests usually display low sensitivity in the detection of early syphilis, and the first positive results are not obtained until some 4-8 weeks after infection. The screening tests belonging to this category have mainly a diagnostic purpose as part of the therapeutic follow-up of patients with syphilis. Thus, a declining titre over a certain period of time is indicative of a favourable response to treatment. As a rule, successful treatment leads to negative results.

Organ donors undergo routine screening for markers in addition to the screening required for SoHO donation (e.g. *Cytomegalovirus*, Epstein–Barr virus, toxoplasma). If the results indicate presence of such active infection in the donor, SoHO can only be released after risk assessment and expert advice.

7.8 Additional screening in selected circumstances

Good Practice Guidelines

G7.40 Additional donor testing should be performed where indicated for other markers and agents, taking into account the epidemiological profile of any given region or country along with travel and personal exposure risks.

G7.41 In EU/EEA, any epidemiological alert from ECDC or in the SoHO platform should be taken into account.

Recommendations

R7.3 It is recommended that:

- R7.3.1 risk factors for infections as well as incidence and prevalence data compiled by ECDC [1] be considered when assessing donors on a case-by-case basis, for risk factors to those specific infections/diseases associated with high-prevalence areas;
- R7.3.2 in the case of paediatric donors, such infections be reviewed for their impact in mother-to-child transmission.

Supplementary information

Screening for new or emerging infections would be feasible, if and when a suitable screening test is available.

Additional screening of donor samples could include vector-borne infections such as malaria, trypanosomiasis, strongyloidiasis, schistosomiasis, *T. cruzi*, West Nile virus, dengue, chikungunya and Zika virus if risk is identified in donor evaluation (see Chapter 6).

Hepatitis E is an example of zoonotic infection that can be transmitted to humans by contaminated food and water. Testing HEV RNA (by NAT) in donor samples, where necessary, could help to protect recipients by identifying infection in donors.

Additional tests can also be used for donor-recipient risk stratification and to determine recipient follow-up protocols where relevant: for example, antibodies to *Cytomegalovirus*, EBV, *Coxiella burnetti* and *Toxoplasma gondii*.

Bacteriological tests are excluded from this chapter; nevertheless, results of blood culture testing can be very useful tools to aid in the determination of bacteraemia in a donor of tissues and/or cells (see Chapter 12, section 12.9).

7.9 Long-term storage of samples

Archived samples may be used for several purposes such as look-back testing or if investigating a report of a serious adverse reaction in a recipient of SoHO due to a potentially transmitted infection.

Good Practice Guideline

G7.42 If there are no national requirements, a risk-analysis-based decision based on the category of "donor" (autologous, allogeneic, living, deceased or multi-donor) should be performed to decide if, and for how long, archived samples should be retained.

Recommendations

R7.4 It is recommended that samples have a storage time of at least 1 year after the distribution of the last SoHO preparation from the donor.

7.10 References

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Related material

Appendix 18. Sample haemodilution algorithm

Appendix 19. Example of validation screening: infectious disease assays of blood from deceased donors

Chapter 8. **Collection**

8.1 Introduction

Collection refers to the process by which substances of human origin (SoHO) are obtained from an individual, including pre-treatment steps, such as administration of hormones, growth factors or other medicinal products needed to facilitate the process at, or under the supervision of, a SoHO entity.

The focus of this chapter is to set out requirements and guidance for the procedures for collection of SoHO from living and deceased donors. In some cases (e.g. human milk, intestinal microbiota, sperm) the collection is done by the donor themself.

This chapter is to be read in conjunction with Chapter 2, which sets out the generic requirements about the personnel, equipment, materials and documentation that are applicable to all chapters in this Guide. The requirements for donor evaluation can be found in Chapter 6; consent and authorisation requirements are detailed in Chapter 5.

The main objectives of collection can be summarised as follows:

- To protect the living donor: ensure all necessary measures are taken to minimise risks during the collection process.
- To maximise donation from deceased persons: develop organ and SoHO donation from deceased individuals to its fullest therapeutic potential [1].
- To diminish the risk of contamination: collect SoHO with the least possible manipulation and in the shortest possible time in an environment appropriate for the type of SoHO.
- To ensure quality and safety during SoHO collection: maintain appropriate conditions throughout the collection process to preserve integrity and prevent adverse events.

Standards

S8.1 SoHO entities must ensure high levels of safety and protect the health of living donors and recipients (Art. 58.6) before, during and after the collection procedure (Art. 52.2).

S8.1.1 This includes identifying and minimising any risks during the SoHO collection procedure, risks such as exposure to reagents or solutions that might be harmful to the health of living SoHO donors (Art. 53.1.h).

S8.2 SoHO entities must mitigate the risks that any reagents or solutions added to SoHO or coming into contact with SoHO during collection might be transferred to SoHO recipients and might have harmful effects on their health (Art. 58.6). See Chapter 2.

S8.3 SoHO entities in charge of collection must be registered before commencing any SoHO collection activities (Art. 35). See Chapter 2.

S8.4 SoHO entities must collect data relating to their collection activities and submit such data to the EU SoHO platform as part of their annual report (Art. 41.1).

Good Practice Guidelines

G8.1 Collection of SoHO should take place only after donor consent requirements have been satisfied and donor evaluation has been carried out.

G8.2 The collection process should not interfere with or compromise the health or care of the donor. In the case of cord blood, amniotic membrane and human milk, this applies to both mother and baby.

8.2 Personnel

Since collection activity can impact the safety of both the donor and the recipient, as well as the quality and safety of the SoHO, the designated SoHO establishment physician holds overall responsibility for developing, reviewing and approving collection procedures. This ensures the protection of the donor and the recipient, as outlined in Chapter 2, section 2.3.2 (Art. 50.2). For information on the need for persons undertaking collection to have appropriate qualifications, training, expertise and experience, see Chapter 2.

Recommendations

R8.1 Information on recommended components of a training programme for SoHO collection and the necessary requirements for outsourced activities, particularly third-party collections, are described in Chapter 2 (sections 2.3.2 and 2.6, respectively).

8.3 Facilities, equipment and materials

8.3.1 Facilities

General guidance about facilities is shown in Chapter 2, section 2.4.

Good Practice Guidelines

Collection areas

G8.3 The facility should be of adequate size to allow proper operations and ensure donor privacy and anonymity.

G8.4 Prior to undertaking any collection activity, a risk assessment should be performed to ensure the collection venue is fit for purpose (see Appendix 20).

G8.5 Facilities in which SoHO are collected should meet appropriate grades of air quality and cleanliness where applicable. The appropriate standard of cleanliness will depend on the type of SoHO being collected, the degree of exposure of the SoHO during the collection process, and the decontamination or sterilisation processes that will subsequently be applied to the SoHO during processing.

G8.6 The collection facility should be divided into different areas of adequate size to prevent improper labelling and packaging, mix-ups or cross-contamination of SoHO.

G8.7 In cases of SoHO donation from living donors, if required, SoHO collection should be carried out in an area appropriately equipped for the initial treatment of donors experiencing adverse reactions associated with the donation, if such reactions are possible. Access to an intensive care unit and/or emergency service should also be available, where applicable.

Supplementary information

Collection of SoHO may take place in various facilities, ranging from a hospital operating room, dedicated facility, hospital clinic, mortuary, funeral home or care home to a donor's own home [2, 3]. Risks of contamination during collection are often multi-factorial so, to help quantify and map them, a dedicated chapter (Chapter 4) has been developed. The Microbiological Risk of Contamination Assessment tool (MiRCA) can be used in the risk assessment (https://soho-guides.edqm.eu/home/).

8.3.2 Equipment and materials

Guidance relating to equipment and materials to be used during collection, processing and storage can be found in Chapter 2 (section 2.5).

8.3.3 Identification, packaging, containers and labelling

After collection, SoHO are identified, packaged and labelled correctly following guidance given in Chapter 16 and Chapter 17 and then transported to the SoHO entity/establishment for further processing, or to the clinical team for direct use, in accordance with established requirements (see Chapter 16).

8.4 Procedures

Good Practice Guidelines

Pre-collection identification

G8.8 Before the collection of SoHO can proceed, an authorised person of the donation team should confirm and record how the donor has been reliably identified beyond any doubt and by whom. The verification should be based on at least two independent factors, such as date of birth and birth name, or birth name and hospital patient number. An example of the type of form that can be used for this purpose can be found in Appendix 21.

G8.9 The source of the donor's identity should be documented. For living donors this should include officially recognised means of identification such as identity cards, passports, etc. For deceased donors, the presence of toe tags, wrist bands or other confirmation of the deceased's identity should be noted.

G8.10 To facilitate and ensure the traceability of the whole process, a unique identifier should be allocated to the donor.

Collection procedures

G8.11 Procedures should be authorised by the physician of the SoHO establishment or delegated person, be appropriate for the type of donor and the type of SoHO collected, and they should be standardised. There should be procedures in place to protect the safety of the living donor.

G8.12 The collection procedures should protect those properties of the SoHO that are required for their ultimate clinical use, and at the same time minimise the risk of microbiological contamination during the process, particularly when SoHO cannot subsequently be sterilised.

G8.13 Policies and procedures should be in place to minimise the risk of SoHO contamination by staff who might be infected with transmissible diseases.

G8.14 Where feasible, initial microbiology testing of SoHO should be performed before any processing that may impact the bioburden. Activities that may be classified as processing include, but are not limited to, washing, shaping, separation, decontamination and sterilisation. If pre-process testing is not feasible or not required due to the nature of the SoHO, the rationale should be documented in a risk assessment. No further microbiological testing is required for SoHO preparations that are used immediately as part of a surgical intervention in an autologous setting. A validated or verified test method should be performed (see Chapter 12).

Collection procedures for deceased donors

G8.15 The sequence in which the various SoHO are collected should be well defined to assure the quality of each type of SoHO.

G8.16 If deceased donation occurs after organ donation, all surgical approaches to obtain the organs should have been sutured to reduce as much as possible the risk of contamination of thoracic and abdominal SoHO before their collection.

G8.17 SoHO should be collected as soon as possible after death, within time limits defined as per policy of the SoHO entity/establishments or national regulation.

G8.18 Once the SoHO have been collected, the donor body should be reconstructed so that it is as similar as possible to its original anatomical appearance.

G8.19 SoHO entities should ensure that appropriate arrangements are in place for the effective body reconstruction of deceased donors, including the availability of suitably trained staff and appropriate equipment.

Recommendations

It is recommended that:

R8.2 written Standard Operating Procedures (SOPs) for collection procedures include details of required steps for collection, including how to register deviations from the approved collection procedure. In addition, SoHO entities are expected to have procedures in place to notify of any Serious Adverse Reaction (SAR) or Serious Adverse Event (SAE) that occurs during SoHO collection (See Chapter 18);

R8.3 the personnel involved understand the risks and potential consequences if policies and procedures on collection are not followed as directed in the written SOP and according to relevant legislation (see section 2.3 in Chapter 2);

R8.4 additional procedures and policies intended to minimise the risk of microbiological contamination during collection, and related to the specific SoHO that are collected (see also Part B), incorporate the information listed here:

- R8.4.1 the number of personnel allowed to be present during collection and the importance of following such restrictions;
- R8.4.2 requirements for the preparation of the donor anatomical sites, following the recommended standards of practice used for surgical patients, prior to commencing collection using an appropriate antimicrobial agent designed for this purpose;
- R8.4.3 the procedure for the disinfection of the collection site [4], taking into account the reduction or elimination of microbial spores as well as vegetative micro-organisms, and listing the types of suitable disinfectants, their concentrations and durations of exposure;
- R8.4.4 prior to use, all materials and equipment should be visually inspected by collection personnel to verify compliance with specifications (e.g. sterile, seals not broken, equipment functioning as expected, expiration date);

R8.5 For deceased donors, it is recommended that:

- R8.5.1 the body be cooled or placed in a cold environment with a temperature range of 0-10°C within the first six hours *post mortem*, in order to reduce microbial metabolism and thus slow down the degradation processes. *Post mortem* time limits [3, 5-7] could be defined in collection procedures for deceased donors;
- R8.5.2 SoHO collection be performed as soon as possible within the first 24 hours. Depending on the SoHO to be collected, if a risk analysis has been performed and the process validated, the time can be extended as far as the risk analysis makes it advisable. When the body cannot be refrigerated in the first six hours, it is recommended to shorten the removal times to 12 hours *post mortem*;
- R8.5.3 the materials and sutures used for body reconstruction be suitable for cremation (G8.19);
- R8.5.4 SoHO be collected before the autopsy takes place. If this is not possible, provision of detailed written procedures to address the increased potential for contamination when collection takes place after autopsy is recommended.

Supplementary information

Collection procedures generally include steps to protect those properties of SoHO that are required for their ultimate clinical use (described in more detail in the specific chapters of Part B of this Guide), including requirements related to temperature during storage and transport, and definition of maximum periods for different procedures (e.g. to avoid unnecessary delays in transport). During collection, important anatomical structures and other relevant SoHO characteristics usually need to be preserved in order for the SoHO to be suitable for their intended use.

Instead of specialised collection teams collecting different SoHO from a deceased donor, a multi-tissue collection team may be used. This team, consisting of a group of individuals who are trained and have defined roles, can collect all SoHO for which there is consent. The main advantages of a multi-tissue collection model are:

- better co-ordination, because all SoHO are collected by the same team;
- less time taken to collect all SoHO, thereby decreasing the risk of microbial contamination caused by long warm ischaemia times, as well as the number of technical errors that could affect the quality, safety or suitability of the SoHO [3, 8-13] and avoiding delays in funeral rites.

SoHO entities can set out the sequence in which the various SoHO are collected to assure the quality of each type of SoHO. Based on expert consensus the collection sequence, whether carried out by separate teams or by a multi-tissue team, could be: skin (first back and lower limbs), eyes/corneas, cardiovascular and musculoskeletal. Justification for this collection process flow includes the following reasons:

- Skin is the first collected SoHO because the donor is placed in a prone position to obtain skin from the back and lower limbs, and the support provided by the presence of musculoskeletal SoHO (in particular, bones) facilitates the procedure. Reconstruction after skin collection is suggested to be performed at the end of donor collection to avoid any interference in subsequently procured SoHO (e.g. musculoskeletal).
- It may be preferable to collect eyes after skin to avoid eye bleeding from the sockets if the donor is placed in a prone position following enucleation of the eyes (see Chapter 20).
- Cardiovascular and musculoskeletal SoHO are usually collected last because the same donor draping may be used. Some cardiovascular SoHO (e.g. femoral arteries) may be collected simultaneously with the musculoskeletal SoHO.
- If the collection is performed simultaneously with organ collection, the sequence varies: starting with the SoHO from the cavities open for organ recovery, thorax and abdomen (arteries, heart for heart valves or vertebral bodies), then the suggested sequence of skin, eyes, cardiovascular and musculoskeletal can be followed. All collection teams involved are aware that SoHO will be collected after organs, usually first preparing the body before starting surgery; second, they guarantee sterile conditions during the whole procedure to minimise the risk of cross-contamination; finally, in the event of a situation arising during organ collection that may have compromised factors such as the sterility of the surgical field or the collection of a specific SoHO due to iatrogenic conditions in the field, they usually promptly notify the collection team. This notification allows for a thorough assessment of the incident's significance and facilitates appropriate actions to address any potential issues.

8.5 Processing at the collection site

Good Practice Guidelines

G8.20 Excluding closed systems, where processing that includes activities such as decontamination, shaping, cleaning, sizing and final packaging is unavoidably carried out at the collection site, a risk assessment should be performed to ensure that the required quality and safety of the SoHO are achieved. This risk assessment should take into account the criteria applicable to the processing area, as well as appropriate coding, graft traceability and release criteria.

G8.21 Processing duration and extent should be limited to the minimum and fulfil the same requirements as any other processing area for the open processing steps.

G8.22 Records supporting the qualification and monitoring of the processing site should be available for inspection.

G8.23 Selection of the use of suboptimal conditions has to be supported by written justification and should be authorised by the relevant Health Authority (HA).

Recommendations

R8.6 Simultaneous undertaking of processing steps during the collection phase, or in the collection area, is not recommended, as control of contamination and cross-contamination at the collection site is typically less stringent than the controls applied in a SoHO establishment (see Chapters 4, 12 and Part B for more information).

8.6 Tissue packaging, coding and temporary storage prior to transportation

Good Practice Guidelines

G8.24 Packaging of the collected SoHO should be appropriate and conform to the standards for the type of SoHO. Double or triple wrapping should be necessary, depending on the tissue-specific requirements. See Part B for additional information.
G8.25 Following collection, all SoHO should be packaged in a manner which minimises the risk of contamination and should be stored at temperatures that preserve the required characteristics and biological function of the SoHO.
G8.26 The packaging should prevent contamination by those responsible for packaging and transportation of the SoHO.
G8.27 Once the SoHO is collected and until it arrives at the SoHO entity, critical variables related to maintaining the quality of the SoHO (e.g. temperature, sterile packaging) should be monitored and documented in accordance with each type of SoHO.
G8.28 Records to demonstrate compliance with specified storage conditions should be completed and maintained.

8.7 Documentation

Good Practice Guidelines

Collection report

G8.29 The SoHO entity performing the collection should produce a collection report, which is passed on to the SoHO entity/establishment. Records of collection should be retained. This collection report should contain at least:

- **G8.29.1** donation unique number, which will be either the donation identification sequence of the Single European Code (SEC) for EU countries, or a code generated by an HA or by use of an internationally recognised coding system such as ISBT-128 or Eurocode; in the case of sperm from the partner (within-relationship use) in the EU, for medically assisted reproduction (MAR) there is no need for the SEC (see Chapter 30);
- **G8.29.2** information about the type of donation;
- **G8.29.3** the identity and address of the SoHO establishment that is to receive the SoHO;
- **G8.29.4** donor identification data (including how and by whom the donor was identified; see Appendix 21).

The collection report should also contain:

G8.30 A list of observations made during the physical examination of the donor's body – but, for a living donor, only when such an examination is justified (see Appendix 15).
G8.31 Description and identification of collected SoHO, including samples (taken before or during collection) for testing of infectious diseases.
G8.32 When applicable, a description of other SoHO from the same donor sent to different SoHO establishments, including their identification.
G8.33 If collection from a living donor involves a directed donation, the recipient's identification, which needs to be documented to ensure traceability (see Chapter 17).
G8.34 Identification of the person who is responsible for the collection session.
G8.35 Date, time (where relevant, start and end) and location of collection, the procedure (SOP) used and any incidents that occurred; also, where relevant, environmental conditions at the collection facility (description of the physical area where collection took place); see also Appendix 21.
G8.36 ID/batch numbers of reagents and transport solutions used.
G8.37 For deceased donors, the report should also contain the date and time of death where possible, conditions under which the body is kept, i.e. refrigerated or cooled (or not), and the start and end times of refrigeration.
G8.38 Where SoHO is collected at home (such as sperm for within-relationship use, donor human milk), the collection report should state this and contain only the following:

- **G8.38.1** name and address of the SoHO entity/establishment to receive SoHO;
- **G8.38.2** the donor identification;
- **G8.38.3** the date and time of collection.

G8.39 Following collection, the collection report should include confirmation that all collected SoHO were packaged and labelled in accordance with the SOP.

Recommendations

R8.7 Where SoHO are sent across national borders, it is important to assess and mitigate any risks that may arise, for example due to differences in language. In this context, it may be appropriate to agree on a common language for all documentation related to donors and/or SoHO.

Supplementary Information

If the time of death is not known (G8.37), evidence of the last time the donor was seen alive could be taken into account in determining the time of death.

Collection records are maintained to facilitate robust and reliable traceability. Usually, collection records are sufficiently detailed to provide a complete history of the work undertaken and will offer links to the records of the donor of the collected SoHO (see Chapter 17).

In addition, the maintenance of records of materials, equipment and identification personnel is included in traceability. More information on unique identifiers (e.g. a donation number for a donation event and/or a donor identification number) and coding are given in Chapter 16.

8.8 References

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Related material

Appendix 15. Physical assessment form (NHS, UK)
Appendix 20. Sample form to assess working environment (NHS, UK)
Appendix 21. Sample donor-identification form (NHS, UK)

Chapter 9. **Cleanroom premises**

9.1 Introduction

This chapter provides generic guidance for the design, qualification, monitoring, maintenance and cleaning of cleanroom premises used for aseptic processing of substances of human origin (SoHO). Aseptic processing of SoHO preparations, while exposed to the environment, takes place in cleanroom premises with specified air quality and cleanliness in order to minimise the risk of microbiological, particulate and endotoxin contamination, including cross-contamination between SoHO of different donors.

For processing steps at the collection site see Chapter 8 (section 8.5).

General guidance on facilities (i.e. design, documentation, access, equipment and hygiene) can be found in Chapter 2. Requirements for collection facilities are in Chapter 8; for storage facilities which are outside of cleanrooms, see Chapter 11.

Standards

S9.1 SoHO entities must mitigate risks arising from microbial contamination of SoHO from the environment, the personnel, the equipment and the materials coming into contact with SoHO during collection, processing, storage or distribution. SoHO entities must mitigate such risks by specifying and verifying the hygiene procedures of the personnel of the SoHO entity in contact with the SoHO throughout the SoHO preparation chain (Art. 58.5.a).

S9.2 SoHO entities must mitigate risks, in cases where SoHO are exposed to the environment during processing, by specifying, validating and maintaining a defined air quality in processing areas, based on a structured and documented risk assessment for each SoHO preparation. See Chapter 10 (Art. 58.5.c).

S9.3 SoHO entities must put in place procedures and specifications covering, when applicable to their activities, validation and monitoring of premises (Art. 37.3.c).

9.2 Design

Good Practice Guidelines

G9.1 The aseptic processing of SoHO preparations should be carried out in appropriate cleanroom premises. Cleanroom premises should be maintained to an appropriate air cleanliness class and supplied with air that has passed through filters of an appropriate efficiency.

G9.2 In cleanrooms all exposed surfaces should be smooth, impervious and unbroken in order to minimise the shedding or accumulation of particles or micro-organisms and to permit the repeated application of cleaning agents and disinfectants.

G9.3 The premises should be designed, equipped and maintained to prevent, as far as possible, the entry of insects and other animals.

G9.4 Sinks and drains should not be installed in cleanrooms with specified air cleanliness classes equivalent to those of grade A or B; see Table 9.2. In other cleanrooms, air breaks should be fitted between the machine or sink and the drains.

G9.5 Hand washing facilities should be provided for personnel before entry to the cleanroom premises.

G9.6 Cleanrooms should be supplied with a filtered air supply that maintains a positive pressure cascade and/or an airflow to the adjacent cleanrooms of a lower air cleanliness class under all operational conditions and should flush the area effectively. For cleanroom premises, these pressure cascades should be clearly defined between different cleanrooms.

G9.7 Stringent biosafety requirements should be followed if processing SoHO from patients having known transmissible pathogenic viral infections, e.g. hepatitis B virus (HBV), hepatitis C virus (HCV) or human immunodeficiency virus (HIV). These should be determined by a documented risk analysis, considering the pathogenicity of the virus involved and with respect for personnel safety, according to Directive 2000/54/EC on the protection of workers from risks related to exposure to biological agents at work [1].

G9.8 When handling SoHO of potential transmissible diseases, negative pressure may be required in specific cleanrooms for containment reasons (e.g. handling of SoHO of transmissible pathogenic viral positive donors). In such cases, the negative pressure cleanrooms or areas (e.g. Class II biosafety laminar air flow cabinets or isolators) should be surrounded by a positive pressure cleanroom of appropriate grade. The entrance doors of these cleanroom premises should be clearly labelled with a biosafety sign.

G9.9 SoHO of transmissible pathogenic viral-positive donors should be processed in class II biosafety cabinets or isolators. For exceptions for medically assisted reproduction (MAR), see G30.61.

G9.10 Aseptic processing of SoHO that are exposed to the environment and are not subjected to a subsequent sterilisation process should take place in an environment with a specified air cleanliness class equivalent to those of grade A in terms of particle concentration and microbial counts in air volume, unless one of the conditions specified in G9.12 applies.

G9.11 The air cleanliness class of the background environment of a grade A environment should be determined through a risk assessment, but it should be at least equivalent to grade D in terms of particle concentration and microbial counts in air volume. The risk assessment should consider factors associated with the processing, testing and implantation of the types of SoHO concerned.

G9.12 An aseptic processing environment with a specified air cleanliness class less stringent than grade A should only be used if at least one of the following conditions is met:

G9.12.1 where a validated terminal sterilisation process is applied, or

G9.12.2 where it is demonstrated that exposure to a grade A environment has a detrimental effect on the required properties of the SoHO, or

G9.12.3 where it is demonstrated that the route of administration has a significantly lower risk of transmitting bacterial or fungal infection to the recipient than transfusion or implantation, or

G9.12.4 where it is not technically possible to carry out the required aseptic process in a grade A environment, e.g. due to requirements for specific equipment in the processing area that is not fully compatible with grade A.

G9.13 The risk assessment for determination of a less stringent aseptic processing environment than grade A should consider potentially significant risk factors, including:

G9.13.1 SoHO microbiological contamination during open *v.* closed processing;

G9.13.2 effectiveness of the processing method to remove pathogenic contaminants;

G9.13.3 suboptimal detection of contaminants can occur due to limitations in both the sampling method and the detection techniques used. Both the sampling approach and the detection methods may have inherent restrictions that affect the accuracy and sensitivity of contamination identification.

Recommendations

It is recommended that:

R9.1 adjacent cleanrooms of different air cleanliness classes have a minimum pressure differential in the range of 7.5 Pa to 15 Pa [2];

R9.2 the environmental temperature and humidity of cleanrooms be maintained to assure the comfort of personnel and to reduce the risk of proliferation of micro-organisms. The influence of temperature and humidity is assessed with regard to the process and, where necessary, monitoring is introduced;

R9.3 projecting ledges, shelves, cupboards and equipment be kept to a minimum and are positioned in a logical way in the cleanrooms to minimise the risk of cross-contamination, errors, omission or wrong application of any processing or control step;

R9.4 a structured risk assessment tool be used to determine the minimum air cleanliness classes for aseptic processing of specific SoHO preparations and their adjacent cleanrooms. The MiRCA (Microbiological Risk of Contamination Assessment) tool (see Chapter 4) has been designed for this purpose. Further advice can also be found in the specific chapters in Part B of this Guide;

R9.5 cleanrooms be entered through change rooms for personnel, and airlocks or pass-through hatches for equipment and materials.

Supplementary information

Cleanrooms are typically surrounded or enclosed by other cleanrooms with lower air cleanliness classes, following the principles of the 'box in box' concept. The movement of personnel and materials between adjacent cleanrooms increases the risk of contamination transfer. Segregation between adjacent cleanrooms is achieved through a combination of physical barriers, such as airlocks, transfer hatches or doors, and aerodynamic precautions like air pressure differentials and controlled airflow. See Figure 9.1.

Airlocks are commonly employed to facilitate the movement of materials and personnel between cleanrooms with lower air cleanliness classes and the

background room of aseptic processing environments, within which the aseptic processing environment, such as laminar airflow hoods, isolators and restricted access barrier systems (RABS) is located. To reduce contamination risk, airlocks designated for personnel movement are typically kept separate from those for material movement. If separation is not feasible, time-based procedures may be implemented to distinguish between personnel and material movement.

Additionally, airlocks can be used to transition materials and personnel between surrounding rooms of lower air cleanliness classes, such as from grade D to grade C or from an unclassified area to grade D. It is common practice for these areas that two doors cannot be opened at the same time. An interlocking system or a visual and/or audible warning system needs to be operated to prevent the opening of more than one door at a time. When cleanrooms with lower air cleanliness classes are separated only by a door, a risk assessment and mitigation measures may be applied and documented. These measures could include specific disinfection and monitoring programmes.

It may be possible to temporarily generate a containment cleanroom in standard cleanroom premises by increasing the air pressure in the change rooms to result in the processing room having reduced air pressure with respect to the last change room. This is not a simple process and raises many potential risks. Detailed guidance on how to manage and control this process can be found in Chapter 2.

9.3 Qualification of cleanrooms

Qualification of cleanroom premises ensures that they meet the required GPGs for air cleanliness classes as defined in this Guide for environmental conditions and contamination control. Proper qualification helps maintain the integrity of processing methods, where the quality, safety and effectiveness of SoHO preparations depend on a controlled, aseptic environment. Qualification ensures that cleanrooms consistently operate (at rest and in operation) within defined parameters, minimising the risk of cross-contamination and ensuring compliance with regulatory requirements.

Good Practice Guidelines

G9.14 Cleanrooms and clean zones (e.g. laminar air flow cabinets, isolators and RABS) should be qualified in compliance with the guidelines in EN ISO 14644-1 [3] and EN ISO 14644-3 [4].

G9.15 The validation master plan (see also Chapter 4) should be built following a risk-assessment exercise aiming to define the selection, extent and time interval of the qualification tests as defined in Table 9.1 in a proportional manner to the risks identified.

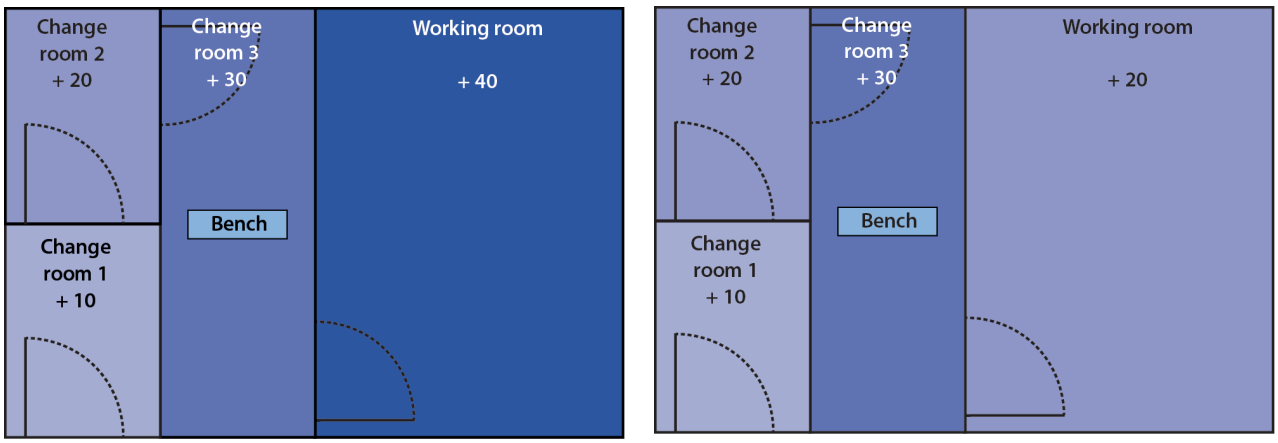
G9.16 The validation plan should consider the initial and subsequent qualification, as well as the at-rest and simulated-operation classification of all cleanrooms. Depending on the risk assessment and the design of the cleanroom premises, supporting tests that should be considered include:

- **G9.16.1** airflow tests (e.g. air changes, laminar flow velocities in laminar airflow areas);
- **G9.16.2** airflow direction test and visualisation;
- **G9.16.3** installed filter system leakage test;
- **G9.16.4** temperature and humidity tests;
- **G9.16.5** air pressure difference test;
- **G9.16.6** recovery test.

G9.17 The cleanroom qualification and requalification strategies should be documented and justified. Where routine processing sessions are subject to a continuous air quality monitoring programme, the frequency of requalification activities may be adapted accordingly to a less stringent programme.

G9.18 For cleanroom classification, the total of particles equal to or greater than 0.5 and 5 µm should be measured. This measurement should be performed both at rest and in operation in accordance with the limits specified in Table 9.2. The minimum air-sample volume per sampling location is determined in compliance with EN ISO 14644-1 Annex A [3].

Figure 9.1 **Schematic plans indicating air pressure differentials between adjacent cleanrooms**



Design on the left: typical processing facility design; design on the right: biohazard processing facility design.

G9.19 The selected supporting tests should be conducted in accordance with EN ISO 14644-3 and the time intervals specified in Table 9.1, as part of the validation master plan, by qualified professionals, at least during at-rest condition. Any deviations from the predetermined frequencies should be based on a formal risk assessment.

G9.20 The microbial contamination level of the cleanrooms should be determined as part of the cleanroom qualification. The number of sampling locations should be based on a documented risk assessment, considering the results obtained from room classification, and knowledge of the process and operations to be performed in the cleanroom. The maximum limits for microbial contamination during qualification for each grade are given in Table 9.3. Qualification should include both "at rest" and "in operation (or simulated operation)" states.

Recommendations

It is recommended that:

R9.6 ISO 21501-4 [5] be considered for calibration of the particle counter used for the classification of cleanrooms;

R9.7 the choice of qualification tests be based on factors such as the design of the installation, its operational states and the required level of air cleanliness;

R9.8 for RABS and isolators, further critical factors be considered for qualification, such as sanitisation and decontamination method and the transfer process of materials and equipment.

Supplementary information

Qualification tests enable cleanrooms to be classified into their appropriate air cleanliness class. Classification is a method of assessing the level of air cleanliness against an air cleanliness class for a cleanroom or clean-zone device by measuring the airborne particle concentration. The classification is part of the qualification of cleanrooms and clean zones and will be clearly differentiated from monitoring operational processes.

For particle counts, the minimum number of sampling locations related to the area of each cleanroom or clean zone to be classified is provided in EN ISO 14644-1. It divides the whole cleanroom or clean zone into sections of equal area and selects in each section a sampling location considered to be representative of the characteristics of the section. The position of the particle probe may be located at a critical point as defined by risk assessment.

Classification in operation may be carried out during routine (or simulated) operations, with a specified number of personnel present.

The cleanroom or clean zone has met the specified air cleanliness class if the particle concentration measured at each of the sampling locations does not exceed the concentration limits as defined in Table 9.2.

The maximum permitted microbial levels are defined in Table 9.3. as colony forming units (CFU). If different or new technologies are used that present results in a manner different from CFU, the SoHO entity could scientifically justify the limits applied and where possible correlate them to CFU.

Table 9.1 specifies supporting qualifications test methods that characterise the performance of cleanrooms and clean zones.

Table 9.1 **Supporting qualification tests for cleanrooms and clean zones**

Supporting tests	Principle	Recommended time interval
Total airborne particle count (classification test)	The total count of airborne particles (viable and non-viable) performed at rest and in operation, to determine air cleanliness class	• Initially • Yearly
Airflow test	Average airflow velocity *or* air changes per hour at rest	• Initially • Time interval as defined by risk assessment
Airflow direction test and visualisation	To demonstrate the airflow pattern type, i.e. unidirectional, non-unidirectional or mixed at rest	• Time interval as defined by risk assessment • After significant changes in the design
Installed filter system leakage test	Detection of leaks in the filter, and integrity testing of seals between filter and mounting arrangements at rest	• Initial • Time interval as defined by risk assessment
Microbial contamination level	The total count of viable particles performed at rest and in operation	• Initially • Time interval as defined by risk assessment
Temperature and relative humidity	The purpose of these tests is to verify that the temperature and relative humidity levels are within control limits	• Initially • Time interval as defined by risk assessment
Air pressure difference	Differential pressure between adjacent cleanrooms at rest	• Initially • Time interval as defined by risk assessment
Recovery test	The time required for a cleanroom to recover after a particle-generation event – normally tested for cleanrooms classified as grade A or B	• Time interval as defined by risk assessment

Table 9.2 **Maximum permitted total particle concentration for classification of the air cleanliness class**

Classification		*Maximum number of particles/m³*			
ISO 14644-1	**EU GMP**	**EU GMP**			
		at rest		*in operation*	
class	*grade*	*≥ 0.5 µm*	*≥ 5.0 µm*	*≥ 0.5 µm*	*≥ 5.0 µm*
ISO 5 at rest and in operation	A	3 520	not specified[a]	3 520	not specified[a]
ISO 5 at rest ISO 7 in operation	B	3 520	not specified[a]	352 000	2 930
ISO 7 at rest ISO 8 in operation	C	352 000	2 930	3 520 000	29 300
ISO 8 at rest	D	3 520 000	29 300	not predetermined[b]	not predetermined[b]

[a] Classification including 5 µm particles may be considered where indicated by risk assessment or historical trends.

[b] For grade D, in-operation limits are not predetermined. The SoHO entity should establish in-operation limits based on a risk assessment and routine data.

Table 9.3 **Maximum permitted microbial contamination level during qualification**

Grade	**Air sample CFU/m³**	**Settle plates (diam. 90 mm) CFU/4 hours)[a]**	**Contact plates (diam. 55 mm) CFU/plate**
A	No growth	No growth	No growth
B	10	5	5
C	100	50	25
D	200	100	50

[a] Settle plates should be exposed for duration of operation and changed as required after a maximum of 4 hours. Exposure time should be based on recovery studies and should not allow desiccation of the media used. CFU = colony forming unit.

9.4 Environmental monitoring

Monitoring provides evidence of continuous compliance with the specified air cleanliness class as well as evidence of (cross)-contamination control of aseptic processes and cleaning and sanitation methods. EN ISO 14644-2 [6] and EN 17141:2020 [7] offer guidance for establishing a monitoring plan for cleanrooms, focusing on air cleanliness through particle concentration and microbiological monitoring.

Good Practice Guidelines

G9.21 SoHO entities should establish a monitoring plan for cleanrooms and clean zones (e.g. laminar airflow cabinets, isolators, RABS), based on a risk assessment, the results obtained during qualification and the selected air cleanliness class, that specifies:

- measuring locations required after identifying the critical care points of each aseptic process performed;
- sampling methods and equipment;
- sampling plan and frequencies (continuous, sequential or periodical);
- measuring methods and equipment;
- acceptance limits per air cleanliness class and process (action and alert levels);
- analysis and evaluation of results (including trend analysis);
- handling of out-of-specification results.

G9.22 The selection of sampling methods and frequencies should involve consideration of risks generated by the sampling method during aseptic processing, the design of the cleanroom, number of personnel, materials and equipment used, and the nature of the SoHO. Based on the outcome of the risk assessment the SoHO entity should select one or more of the following environmental monitoring methods (see Table 9.4):

- total airborne particle concentration monitoring;
- volumetric air sampling;
- settle plates;
- glove, clothing and surface sampling (contact plates or swabs).

G9.23 Critical facility parameters identified to be a risk to the SoHO, such as air-supply conditions, pressure differentials, particle numbers and microbial contamination – and if applicable, humidity and temperature – should be monitored and recorded.

G9.24 The frequency (continuous, sequential or periodic) and duration of monitoring should be based on the criticality of the aseptic process and should permit the detection of changes in levels of contamination. Monitoring does not need to cover the entire duration of the aseptic process.

G9.25 The monitoring plan for the background and surrounding cleanrooms should be set having regard to the risks and in particular the nature of the aseptic processes conducted.

G9.26 An appropriate culture medium should be selected that permits the growth of the expected micro-organisms most commonly identified in the relevant area. Where applicable, it should be verified that residues of biocides or cleaning agents do not interfere with the detection and evaluation of these micro-organisms.

G9.27 Rapid microbiological monitoring methods should be considered if the SoHO preparations are intended to be used in human application shortly after processing (e.g. hours up to a couple of days) without terminal sterilisation.

G9.28 In grade A and B cleanrooms, any detected CFU should be identified by the genus and species, and for other cleanliness classes according to the microbial monitoring programme of the SoHO entity.

G9.29 The monitoring plan should define appropriate alert and action limits based on Tables 9.5 and 9.6. With a view to identifying potential changes that may be detrimental to the aseptic process, the alert limits for grades B, C and D should be lower than those specified as action limits and should be based on risk assessment and on routine qualification and monitoring data.

G9.30 The monitoring plan should ensure that, when defined alert limits are exceeded, the event is quickly identified and recorded. If action limits are exceeded, the event should be promptly identified, recorded and registered as a non-conformity, with appropriate corrective actions taken when needed.

G9.31 Results of monitoring should be considered when making the decision whether SoHO can be released. If action limits are exceeded, the SoHO entity should evaluate whether the finding will affect the risk that tissues or cells could have been contaminated during processing.

Table 9.4 **Environmental monitoring methods**

Method	Air or surface, qualitative or quantitative	Notes
Total airborne particle concentration	Air quantitative	Can be used to quantify viable and non-viable particles suspended in the air during aseptic processes. The position of the sampling probe of the light-scattering airborne particle counter is selected based on the design, set-up and air cleanliness class of the measuring environment, as well as the critical care points of the aseptic process performed.
Volumetric air sampling	Air quantitative	Can be used to quantify micro-organisms suspended in the air but it is not a reliable measure of the sporadic contamination that occurs during aseptic processes. In general, the manufacturer of the sampling instrument will recommend sample sizes, and this recommendation may be taken into account in the design of the sampling plan. Sample sizes of 1 m^3 of air are typically used for measurement in grade A areas. If this sample size leads to an unreadable number of colonies, smaller volumes may be used to monitor e.g. Class, B, C and D areas, provided this approach is justified. The sampling volume and time, as well as the transport time of the culture plates to the microbiology lab, could lead to capture-plate drying, which might have a detrimental effect on the growth promotion of micro-organisms.
Settle plates	Air qualitative	This is a method that can provide continuous monitoring of micro-organisms. It can be used to detect bacteria and fungi that descend in a column of air over the plate. Sampling time and the laminar airflow speed of the test environment, as well as the transport time of the culture plates to the microbiology lab, could lead to capture-plate drying, which has a detrimental effect on the growth promotion of micro-organisms. Usually settled plates can be open for 2-4 hours depending on the agar volume and composition. Exposed plates may be replaced by unused ones so that the total time of the process is reached.
Contact plates[a]	Surface qualitative	Contact plates can be used to detect contamination by micro-organisms of surfaces. The pressure and duration of contact have a significant influence on microbial yield (recommendation: uniform pressure for 10 s). Tested surfaces are cleaned after sampling.
Swabs[a]	Surface qualitative	Used for wiping surfaces that cannot be sampled with contact plates. A swab dampened with sterile physiological (0.9%) NaCl solution is used to test dry surfaces. A dry swab is used to test damp surfaces. The sample material collected is then wiped onto an agar plate. The sample area should be ≈ 25 cm^2, if possible. Tested surfaces are cleaned after sampling.
Glove prints	Glove or fingertips qualitative	Fingertips are the most likely area to come into contact with microbial contamination during aseptic processes, on materials or arising from the operator and then transferred onto SoHO preparations. Glove prints are taken from both hands by pressing all five fingers for 5 to 10 s on the agar surface of a settle plate. Samples are taken after aseptic processing or before changing gloves.

[a] Sampling should be performed at the end of processing session.

G9.32 The performance of the monitoring plan should be periodically reviewed.

Recommendations

R9.9 It is recommended that any fungi detected in grade C and grade D cleanrooms during qualification or routine monitoring be identified to the genus and species level to inform appropriate corrective actions.

Supplementary information

Critical care points for sampling are chosen after identifying areas representing particular risk to the contamination of the SoHO and the maintenance of the required grading of the area in question (G9.21).

Although measurement of total airborne particle concentration of ≥ 5.0 µm particles is not required for qualification purposes in grade A and grade B at rest, it might be considered for routine monitoring as it serves as a valuable diagnostic tool for early detection of potential failures. While occasional spikes in ≥ 5.0 µm particle counts may be false positives, consistent or recurring low-level counts can indicate possible contamination. Such events may signal early failure of the heating, ventilation and air conditioning (HVAC) system or filling equipment, or they may reflect poor practices during machine setup and routine operation.

The sample volume taken for monitoring purposes is determined, preferably based on a risk assessment, and does not need to be the same as the sample volume used for qualification purposes. When using automated systems, the sample volume will usually be a function of the sampling rate of the system used. If using sequential systems, consider particle losses because of the length of the tubes and kinks in the tubing.

The nature of some processes like bone cutting may generate numerous particles or aerosols which can have a detrimental effect on the light-scattering airborne particle counters. Such particles or aerosols will also generate false positive total particle concentration results.

Table 9.7 can provide support for determining the minimum frequencies for environmental monitoring, considering the nature of the collected SoHO and their bioburden level, the expected bioburden level after processing, the sterilisation methods applied and the location where the SoHO preparation is implanted or transfused [8].

Additives inhibiting residual biocides and cleaning agents are a component of the culture medium for environmental monitoring. At present no commercial

Table 9.5 **Recommended limits for total particle concentration monitoring**

Classification		Maximum number of particles/m³	
ISO 14644-1	*EU GMP*	*in operation*	
class	grade	≥ 0.5 µm	≥ 5.0 µm
ISO 5	A	3 520[a]	29[a]
ISO 7	B	352 000	2 930
ISO 8	C	3 520 000	29 300
ISO 9	D	not predetermined[b]	not predetermined[b]

[a] Sometimes it is technically not possible to carry out the required aseptic process within the specified grade A particle concentration limits due to requirements for specific equipment in the processing area (e.g. microscopes) or processing activities (e.g. cutting of bones). The SoHO entity could in these circumstances define deviations based on a risk assessment.

[b] For grade D, in-operation limits are not predetermined. The SoHO entity should establish in-operation limits based on a risk assessment and routine data where applicable.

Table 9.6 **Recommended limits for microbiological monitoring**

Grade	Air sample CFU/m³	Settle plates (diam. 90 mm) CFU/4 hours)[a]	Contact plates (diam. 55 mm) CFU/plate	Glove/clothing print[c] CFU/plate
A	No growth[b]	No growth[b]	No growth[b]	No growth[b]
B	10	5	5	5
C	100	50	25	25
D	200	100	50	50

[a] Settle plates should be exposed for the duration of the aseptic process and changed as required after a maximum of 4 hours. It is recommended to define the maximum exposure time that is possible without having any effect on the suitability of the media used.

[b] Sometimes it is technically not possible to carry out the required aseptic process within the specified grade A microbiological limits due to requirements for specific equipment in the processing area (e.g. microscopes) or the initial bioburden level of the collected tissue and cells. The SoHO entity could in these circumstances define deviations based on a risk assessment.

[c] Depending on the function of the cleanroom (e.g. processing or background environment) it could be recommended to take microbiological monitoring samples of gloves and cleanroom clothes.

neutraliser is able to inactivate all biocides. The concentration of residue left on the surfaces after cleaning depends on the type of biocide and the sanitation programme. Additionally, it is common practice to ensure that any residues from the sanitation process do not affect the ability to recover micro-organisms.

Environmental monitoring samples are incubated at a minimum of two different temperatures for 3-5 days at 20-25°C followed by incubation at 30-35°C for an additional 2-3 days; this method has been shown to be sufficient to detect most micro-organisms. Incubation conditions for environmental monitoring samples are set out in Table 9.8. Alternative methods are acceptable if high recoveries (> 50%) of micro-organisms of interest can be demonstrated consistently [9].

9.5 Cleanroom clothing and aseptic precautions

Good Practice Guidelines

G9.33 There should be a policy outlining the requirements for wearing cleanroom clothing, based on a risk assessment and the recommendations listed in Table 9.9. The clothing and its quality should be appropriate for the aseptic process and the air cleanliness class of the cleanroom.

G9.34 Personnel should be trained in the gowning requirements appropriate to various air cleanliness classes. Cleanroom clothing should be worn in such a way as to protect the operator and SoHO from the risk of contamination.

G9.35 Non-permanent cosmetics (make-up) should not be worn in cleanrooms.

G9.36 Jewellery should not be worn in cleanrooms unless justified by risk assessment.

G9.37 Cleanroom clothing should be cleaned and handled in such a way that it does not gather additional contaminants which can later be shed. When working in a contained area, single-use cleanroom clothing should be discarded before leaving the contained area.

G9.38 Personnel working in cleanrooms should be given specific training on aseptic techniques, including the basic aspects of microbiology.

G9.39 Only the minimum number of personnel required for efficient processing should enter the processing areas. The need for additional persons to be present in processing areas should be risk assessed when the procedure is being designed, taking into account cleanroom qualification data.

Table 9.7 **Recommended minimum frequencies of environmental monitoring**

	SoHO preparations where the terminal bioburden level before infusion or implantation is expected to be < 1 CFU		*SoHO preparations where the terminal bioburden level before infusion or implantation is expected to be ≥ 1 CFU or where processing is followed by terminal sterilisation or where the implantation occurs in a non-sterile environment*	
Method	**Direct working environment**	**Background environment**	**Direct working environment**	**Background environment**
Total airborne particle concentration	to be defined by risk assessment	quarterly	to be defined by risk assessment	quarterly
Volumetric air sampling	to be defined by risk assessment	quarterly	to be defined by risk assessment	quarterly
Settle plates	every working session	weekly	monthly	quarterly
Gloves prints	every working session	quarterly	monthly	quarterly
Contact plates	weekly	quarterly	monthly	quarterly
Clothing prints	quarterly	quarterly	not applicable	not applicable

Table 9.8 **Recommended incubation conditions for microbiological monitoring**

Aerobic	Trypticase soy agar irradiated	20-25°C + 30-35°C	3-5 days + 2-3 days
Fungi			
Alternative incubation conditions			
Aerobic	Trypticase soy agar irradiated	30-35°C	2-3 days
Fungi	Sabouraud agar	20-25°C	5-7 days

Recommendations

It is recommended that:

R9.10 gloves be changed regularly during aseptic processing. If sterile cleanroom clothing is used, it is recommended to change the suits, caps, shoes and arm covering each work session (on exit from the cleanrooms). The same clothing may be worn for more than one session if monitoring results justify;

R9.11 sterile and non-sterile clothing be changed at least daily and that non-sterile shoes be disinfected for each entry;

R9.12 facemasks be changed regularly during aseptic processing;

R9.13 samples be taken from the surface periodically at several fixed sites on clothing (see Table 9.7);

R9.14 microbiological monitoring results be used for the qualification or re-qualification of personnel;

R9.15 if an operator is disqualified, they be retrained and re-qualified before they can be involved in aseptic operations;

R9.16 the retraining/re-qualification of a disqualified operator include participation in a successful process-simulation test. The usual approach is to conduct simulated defined-critical process steps using culture medium in place of, or added to, SoHO;

R9.17 while it is best practice not to wear jewellery in cleanrooms, give sympathetic consideration to religious, cultural and emotional aspects associated with items of jewellery when consequently it may cause distress if staff are asked to remove them. It may be possible to mitigate the risks arising from the wearing of certain types of jewellery through actions such as covering them with sticking plasters, for example. It is recommended that case-specific risk assessment be performed in these situations to determine if exemptions can be justified.

Supplementary information

If disinfection of gloves is performed, consider whether the efficacy of disinfection depends on the disinfectant/type of gloves combination. Disinfection of some gloves has an influence on the tightness of the gloves [10].

Many cosmetics are powder based and can shed particles, and thus increase particle counts in clean environments. For this reason, their use in clean environments is not permitted.

Table 9.9 **Recommended clothing requirements**

Classification	Clothing	Description
Grade D	Facemask	Non-sterile, single-use. Beard and moustache are covered
	Cap	Non-sterile, single-use
	Suit	Non-sterile, general protective suit
	Shoes	Non-sterile, disinfected shoes dedicated to the cleanroom or non-sterile overshoes, or single-use overshoes
	Gloves	Non-sterile, non-powdered, single-use rubber or plastic gloves
Grade C	Facemask	Non-sterile, single-use. Beard and moustache are covered
	Eye protection	Non-sterile (the use of eye protection depends on the risks of the process and the use of protective equipment such as laminar airflow hoods, RABS or isolators)
	Cap	Non-sterile, single-use
	Suit	Non-sterile, high particle retention, single- or two-piece trouser suit gathered at the wrists and with high neck
	Shoes	Non-sterile, disinfected shoes dedicated to the cleanroom, or non-sterile or single-use, high particle retention overshoes
	Gloves	Directly handling SoHO or sterile materials: sterile, non-powdered, single-use rubber or plastic gloves Otherwise: non-sterile, non-powdered, single-use rubber or plastic gloves
Grade A/B	Facemask	Sterile, single-use. Beard and moustache are covered
	Eye protection	Sterile (the use of eye protection depends on the risks of the process and the use of protective equipment such as laminar airflow hoods, RABS or isolators)
	Cap	Sterile, high particle retention, tucked into the neck of the suit
	Suit	Sterile, high particle retention
	Arm covering	If applicable: sterile, high particle retention
	Shoes	Sterile, high particle retention, boot-like structure to enable the trouser legs to be tucked inside the footwear
	Gloves	Sterile, non-powdered, single-use rubber or plastic gloves

9.6 Cleaning

Good Practice Guidelines

G9.40 The cleaning and disinfection process should be validated or verified and monitored to achieve the required air cleanliness class and to confirm the effectiveness of the cleaning and disinfection process.
G9.41 Appropriate disinfectants should be selected, based on the types of micro-organism found in the environment. More than one type of disinfectant should be employed to ensure that, where they have different modes of action, their combined usage is effective against bacteria, fungi and viruses. Disinfection should include the periodic use of a sporicidal agent.
G9.42 Cleaning programmes should effectively remove product and disinfectant residues.
G9.43 Where fumigation or vapour disinfection (e.g. vapour-phase hydrogen peroxide) of cleanroom and clean zones is used, the effectiveness of any fumigation agent and dispersion system should be validated.
G9.44 Inactivation of prions should be considered if risk of prion contamination has occurred, e.g. if tissues or cells from a Creutzfeldt–Jakob disease-positive donor have been processed or stored.

Recommendations

It is recommended:

R9.18 to rotate between different types of disinfectant to avoid any resistance effect due to biocides [11] and to cover a wider range of micro-organisms;

R9.19 to determine cleaning and disinfection intervals and methods by validation, referring to historic data and trends, where available;

R9.20 that disinfectants and detergents used in grade A and B cleanrooms or clean zones be sterile prior to use;

R9.21 to define how long the cleaning and disinfection process can be delayed after processing and how long the disinfection process remains effective;

R9.22 to replace containers of disinfectants and detergents within a defined period after opening as, once opened, they may become contaminated with micro-organisms which can then proliferate;

R9.23 to take care in choosing the appropriate cleaning products and disinfectants based on validation data [11] in MAR centres, where volatile organic compounds released by biocides (e.g. alcohols) might be detrimental for gametes and embryos.

9.7 References

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Chapter 10. **Processing**

10.1 Introduction

This chapter provides generic guidance on the processing of substances of human origin (SoHO) by SoHO establishments/entities. It does not cover process validation, storage, release, distribution, import/export, coding, packaging and labelling, or traceability, which are covered elsewhere in the Guide.

The EU SoHO Regulation defines processing as:

"Any operation involved in the handling of SoHO, including, but not limited to, washing, shaping, separation, decontamination, sterilisation, preservation and packaging, except for the preparatory handling of SoHO for immediate human application during a surgical intervention, without the SoHO being removed from the surgical field before they are applied" (Art. 3.23).

In addition to the procedures specified in the Regulation definition, processing is also considered to include more specific activities including cutting, grinding, centrifugation, soaking in antibiotic or antimicrobial solutions, decellularisation, concentration/purification of cells, freeze drying, cryopreservation and thawing. Processing is considered to commence when any procedure intended to affect the microbiological status, physical structure or biological/mechanical properties of the tissue/cell is used, in addition to the activities defined in the Regulation. Details of which specific established process methods are applied to different SoHO can be found in the SoHO monographs (Part C of this Guide).

The objectives of processing SoHO may include:

i. promoting the intended clinical outcome by removing those elements that may contribute to a reduction in the performance of the SoHO – for example, decellularisation of a SoHO, or removing seminal plasma by density gradient centrifugation before intra-uterine insemination;
ii. reducing the risk of transmission of infections by the inactivation of micro-organisms by disinfection, or sterilisation in circumstances where cell viability is not required;
iii. preservation of the required properties of SoHO, making extended storage for future use possible;
iv. facilitating and optimising clinical use by dividing a donation into multiple, ready-to-use units of SoHO.

Although it can deliver significant improvements in safety and effectiveness, processing can also introduce risks. These include:

- microbial contamination from the environment or the operator, or cross-contamination from other SoHO;
- errors in identification or labelling, leading to loss of traceability;
- incorrect characterisation of, or damage to, the SoHO, that reduces their clinical effectiveness.

For these reasons, processing methodologies for SoHO undergo validation to demonstrate that:

- the quality and effectiveness of the final product have not been unacceptably compromised,
- the risk of introducing exogenous microbial contamination is minimised, and
- unintended cross-contamination with SoHO from other donors cannot occur.

It is important to consider that most processing protocols will have some impact on the native properties of SoHO, including those pertinent to their effectiveness and/or safety. However, the impact of these changes, when considered in relation to the clinical benefits that the SoHO provide, can be used in determining the level of change that can be tolerated.

10.2 Receipt of SoHO at the SoHO establishment/entity for processing

Following collection, SoHO are ideally transferred to the SoHO establishment/entity as soon as practicable, where they can be processed and stored under controlled conditions. There are occasions when the initial steps of processing are performed at the collection facility for operational reasons. This may result in elevated risks of microbial contamination and cross-contamination, and these are typically reflected in process risk assessments.

Refer to Chapter 13 for guidance relating to receipt of SoHO preparations imported from other countries. Information on outsourced activities can be found in Chapter 2, section 2.6.

Standards

S10.1 Each SoHO entity must put in place procedures and specifications covering, where applicable to their activities, quarantine of SoHO (Art. 37.3.f).

Good Practice Guidelines

G10.1 Each SoHO establishment/entity should ensure that the SoHO received are quarantined until they, along with the associated documentation, have been inspected or otherwise verified as conforming to documented requirements.

G10.2 The review of relevant donor and collection information and thus acceptance of the donation should be carried out by specified and authorised persons.

G10.3 If the SoHO and testing samples are not being transported by their own personnel, the SoHO entity should ensure that they have an agreement in place with the third party undertaking the transport to the SoHO entity (e.g. general terms and conditions). This agreement should address how the safety and security of the SoHO are ensured during transit, and how the required temperature is maintained.

G10.4 The acceptance or rejection of received SoHO should be documented.

G10.5 When the collected SoHO arrive at the SoHO establishment/entity, there should be documented verification that the consignment, including the transport conditions, packaging, labelling and associated documentation and samples, meets the specifications of the receiving establishment/entity. The following aspects should be checked and recorded:

- **G10.5.1** (no) evidence of unauthorised opening or manipulation;
- **G10.5.2** (no) signs of damage that might result in the deterioration of SoHO and (no) signs of incidents relating to storage or transport;
- **G10.5.3** transport conditions, including (where relevant) transit time, and temperature during transport (unless a validated transport method has been used, or a validated surrogate method of confirmation, such as the presence of solid refrigerant in the container, can be employed);
- **G10.5.4** unique identification of the donor;
- **G10.5.5** description of the SoHO (including number of units per ampoule);
- **G10.5.6** collection report including collection date and time;
- **G10.5.7** if applicable, details of any associated samples (including blood).

G10.6 Received SoHO, if awaiting final test results or if subject to additional testing or confirmatory medical assessment, should be held in quarantine until such test results or medical data become available, except where it is essential to process the SoHO immediately to retain the critical qualities or attributes of the grafts prepared.

G10.7 The SoHO establishment/entity should have documented procedures for the management and segregation of non-conforming consignments, or those with incomplete test results, to ensure that there is no risk of contamination of other SoHO being processed, preserved or stored.

G10.8 The collection report and shipping record (if the donation was transported by a third party) should be cross-checked with the contents of the package.

G10.9 The data registered at the SoHO establishment/entity (except reproductive cells intended for within-relationship use) should include, where relevant:

- **G10.9.1** confirmation that the consent/authorisation has been obtained, including the purpose(s) for which the SoHO may be used (i.e. therapeutic or research, or both therapeutic use and research) and any specific instructions for disposal if the SoHO are not used for the purpose for which consent was obtained;
- **G10.9.2** all required records relating to the collection of SoHO and the taking of the donor history, as described in the donor documentation section;
- **G10.9.3** results of physical examination (if performed), of laboratory tests and of any other relevant tests or examinations (such as the autopsy report, if one was performed);
- **G10.9.4** for allogeneic donors, a documented review of the complete donor evaluation against the selection criteria by an authorised, trained and qualified person;
- **G10.9.5** documentation of any residual processing reagents (such as antibiotics) that may provoke an allergic response in the recipient.

G10.10 In the case of reproductive cells intended for within-relationship use, the data to be registered at the SoHO establishment/entity should include:

- **G10.10.1** consent, including the purpose(s) for which the SoHO may be used (such as reproductive only and/or for research) and any specific instructions for disposal if the SoHO are not used for the purpose for which consent was obtained;

G10.10.2 partner identification;
G10.10.3 place of collection;
G10.10.4 SoHO obtained and relevant characteristics.

10.3 Processing methods

Standards

S10.2 SoHO entities must take measures to mitigate the risks of disease transmission to SoHO recipients resulting from cross-contamination between SoHO during processing. These measures must ensure that physical contact between SoHO from different donors, as well as SoHO collected from different persons for future autologous or within-relationship use, is avoided (Art. 58.4).

S10.3 In cases where pooling of SoHO is necessary for the effectiveness or feasibility of the SoHO preparation, contact between SoHO from different donors must be minimised and justified by a documented risk–benefit assessment (Art. 58.4).

S10.4 SoHO entities must mitigate risks arising from microbial contamination of SoHO from the environment, personnel, equipment and consumables that come into contact with SoHO during processing. This mitigation must include the following elements for each SoHO (Art. 58.5):

S10.4.1 Specify the hygiene procedures that their personnel who come into contact with the SoHO must follow and verify that these are complied with.

S10.4.2 Where SoHO are exposed to the environment during processing, specify a defined air quality in the processing areas based on a documented risk assessment, and verify that these conditions are attained and maintained.

S10.4.3 Ensure that equipment and materials that come into contact with the SoHO during processing are adequately decontaminated or sterilised.

S10.4.4 Perform quality control testing of SoHO to detect microbial contamination.

S10.4.5 Where feasible and appropriate, treat the SoHO to inactivate or eliminate microorganisms.

S10.5 SoHO entities must mitigate the risks that the properties of SoHO they process, that are deemed necessary for clinical effectiveness, might be adversely impacted by any processing activity performed. Such mitigation must include conducting comprehensive process validation and equipment qualification, and, when needed, gathering evidence of clinical effectiveness (Art. 58.7, Art. 39.2.b.viii, Art. 39.4).

S10.6 Where feasible, SoHO entities must mitigate the risks that SoHO cause an unexpected immune reaction in recipients by implementing processing protocols to remove or reduce those elements of the SoHO that could stimulate an unintended immune response (Art. 58.8.b).

Good Practice Guidelines

G10.11 SoHO establishments/entities should document all processes that could affect quality and safety of the SoHO and should ensure that they are carried out under controlled conditions.

G10.12 All processing procedures and protocols should be documented and should conform to the validated or verified methodologies.

G10.13 Where validation/verification is based on retrospective evaluation of the clinical outcome and/or vigilance data supplied by the establishment/entity, data should be collected and analysed that include the number of SoHO implanted following processing by the method under consideration, and the time period (start and end dates/times) during which these implantations occurred.

G10.14 SoHO establishments/entities should ensure that all processes are conducted in accordance with the approved Standard Operating Procedures (SOPs).

G10.15 Each distinct process should be carried out under defined environmental conditions to minimise any risk to the quality and safety of SoHO and to protect SoHO establishment/entity personnel.

G10.16 The selection of the appropriate processing environment should be based on a documented risk assessment, see Chapter 9.

G10.17 Facilities for aseptic and clean, non-sterile processing should be dedicated to this activity, and should be designed, qualified and monitored to ensure that the air quality is appropriate for the process being carried out.

G10.18 SoHO establishments/entities should ensure that the equipment used, the environment where processing takes place, the process design, validation and control conditions are compliant with the requirements referred to in this chapter and elsewhere in the Guide, or according to equivalent national measures.

G10.19 It should be demonstrated that all validated or verified processes can be carried out consistently and effectively in the SoHO entity environment by the staff available.

G10.20 All processing procedures should undergo regular review to ensure that they continue to achieve the intended results.

G10.21 Pooling of SoHO from two or more donors during processing should only be done where it has been demonstrated to be the only way of providing sufficient clinically effective SoHO.

G10.22 In the case of medically assisted reproduction (MAR), only mixing of SoHO derived from one male and one female donor should take place.

G10.23 Pooled SoHO should be treated as a single batch, and it should be documented which original donations have been pooled together.

G10.24 A separate set of clean, sterile instruments should be used for each batch.

G10.25 The procedures used to prevent or reduce contamination during processing should be validated/verified depending on the type of tissue and how it is processed.
G10.26 Maximum times from collection until processing and storage should be defined.
G10.27 All critical processing parameters (e.g. times and temperatures) should be documented in the records for SoHO preparations.
G10.28 The acceptance, rejection or disposal of SoHO, including those to be discarded or used for non-clinical purposes, should be properly documented and traceability maintained.
G10.29 If any parts of the SoHO preparation processing protocols are outsourced to third parties, the SoHO establishments/entities should ensure an agreement is in place clearly documenting the responsibilities of both parties. (For further guidance on outsourced activities, see Chapter 2, section 2.7.)
G10.30 In EU member states (MS), reagents used during processing, decontamination and preservation of SoHO should be CE (Conformité Européenne) marked. Elsewhere, equivalent national conformity standards may be used as an alternative to CE marking.
G10.31 Where no suitable CE-marked or equivalent conformity-assessed reagent is available, SoHO entities may use alternative reagents subject to a documented risk assessment.

Recommendations

It is recommended that:

R10.1 to ensure graft safety, risks of contamination be minimised during processing activities;

R10.2 those concerned in determining the appropriate category of processing environment recognise that risks are often multi-factorial and, to help quantify and map them, it is helpful to use a structured process;

R10.3 in this respect Chapter 4 be consulted, in particular the tailored Microbiological Risk of Contamination Assessment tool (MiRCA) that has been developed specifically for this purpose since, by using this tool, SoHO establishments/entities can better understand the overall risk of their protocols, how risk factors are distributed along the SoHO supply chain, from collection to distribution, and how to implement the most efficient mitigation or risk-reducing measures;

R10.4 those involved consider that some types of processing applied to a SoHO may result in the final product falling into another regulatory category, namely: medical devices, regulated by Regulation (EU) 2017/745 of the European Parliament and of the Council; medicinal products, regulated by Directive 2001/83/EC of the European Parliament and of the Council; advanced therapy medicinal products, regulated by Directive 2001/83/EC and Regulation (EC) No. 1394/2007 of the European Parliament and of the Council; and investigational medicinal products, regulated by Regulation (EU) No. 536/2014 of the European Parliament and of the Council. In case of doubt, SoHO entities are advised to consult the SoHO Compendium for any relevant precedents, and if still unclear to contact their Health Authority for advice;

R10.5 those involved refer to Chapter 9 for detailed guidance regarding cleanroom facilities used for processing.

Supplementary information

Note that the same process may have very different impacts on different types of SoHO. It is important that processes are optimised and adapted where necessary when applied to different types of SoHO.

Common processing activities include, but are not limited to:

- *Cleansing* of collected material by removal of extraneous tissues and bodily fluids is a common initial processing step. Typically, scalpels, scissors and gauze wipes are used in this process.
- *Separation* is used to partition the specific type of SoHO to be processed from another type, for example to divide dermis and epidermis, or amniotic membrane and chorion. It may involve the use of enzymes to separate and isolate the specific tissue.
- *Cutting and shaping* allows initial preparation of collected SoHO into the shapes and forms required for transplantation. Different types of cutting device can be used, depending on type of SoHO. For cutting bone, different types of saws may be used, such as oscillating saws, bandsaws or rotary saws, whereas for soft tissues such as skin and tendon, scissors or scalpels may be used.
- *Grinding*, by different types of mills, is used to pulverise bone tissue into smaller pieces. Depending on the type of bone being ground, actively cooled grinding mills that dissipate heat may be used, to prevent the bone being damaged by excessive temperatures caused by grinding friction.
- *Washing* may be performed (using any of several types of washing solution, e.g. distilled water, 0.9% NaCl, balanced salt solution, phosphate-buffered saline or alcohols, depending on the requirements of the washing process and the critical quality attributes of the SoHO preparations) for one or more of the following purposes:
  - as an initial step in processing, e.g. to remove surface blood and lipids;
  - as an integral part of a process, e.g. to remove bone–marrow components from musculoskeletal allografts and/or to remove traces of chemical compounds used during processing, for example antibiotics used for decontamination;
  - to decrease the bioburden of SoHO.
- *Centrifugation* may be used to concentrate and separate cells from a suspension or different fractions of suspensions, or to remove marrow, blood

and lipid components from musculoskeletal allografts.

- *Supercritical CO_2* treatment involves the use of carbon dioxide under high pressure. Under these conditions, CO_2 behaves as a supercritical fluid solvent, which is very effective at removing fluids, lipids and cells from solid tissues, and reduces bioburden.
- *Disinfection* by soaking or rinsing in antibiotic or antimicrobial solutions is commonly used for decontamination of viable SoHOs that cannot be terminally sterilised, or as a stage in the processing of subsequently sterilised SoHO to reduce the bioburden.
- *Cell concentration and selection* is used as an initial step for HPC processing or for *in vitro* cell cultures. This may also include the isolation of particular cell types, e.g. mononuclear cells from peripheral blood.
- *Filtering* is often used to separate SoHO from extraneous material, for example after HPC collection from bone marrow, to remove particulate matter.
- *Decellularisation* is a technique that aims to remove most of the cellular content of a SoHO preparation, leaving behind just the extracellular matrix (ECM). These ECMs may be implanted directly or used as a scaffold for the manufacture of tissue engineered products. See Chapters 22, 23 and Appendix 30 for a more detailed discussion of specific applications of decellularisation to skin allografts and a general overview of the different protocols that can be used.
- *Devitalisation* is a term used to describe a process which will intentionally kill donor cells in a tissue but not necessarily remove the dead cells from the tissue, such as freeze thawing or glycerolisation.
- *Demineralisation* is a process of chemical removal of the bone mineral, resulting in exposure of biologically active bone morphogenetic proteins present in bone tissue. Demineralisation is usually performed using a dilute hydrochloric acid solution.
- *Freeze-thawing* of tissues can be used as a processing step, for lysis of cells prior to other procedures.
- *Pasteurisation* is a method using controlled heating, to a defined temperature for a specified period of time, to inactivate micro-organisms while maintaining the critical quality attributes of the SoHO.

10.4 Processing: reagents and consumables

Standards

S10.7 SoHO entities must mitigate the risks that any reagents and solutions used during the processing of SoHO, and that may have a harmful impact on a recipient's health, are retained within the SoHO and transferred to recipients (Art. 58.6). Such mitigation must include as a minimum the following measures:

S10.7.1 specifying such reagents and solutions prior to their purchase and use;

S10.7.2 verifying that such reagents and solutions have any required certifications;

S10.7.3 demonstrating the removal of such reagents and solutions, when necessary, prior to distribution.

Good Practice Guidelines

G10.32 The use of all reagents (including antibiotics) during processing should be recorded and, if there is any risk that residual quantities of these reagents may remain in the SoHO preparations when issued for clinical use, the end user should be made aware of this.

G10.33 The use of any reagents that may provoke a toxic or immunological response in recipients should be eliminated where possible, and minimised where not.

G10.34 The reagents used in processing should be of an appropriate grade for their intended use and be sterile (if applicable).

G10.35 Reagents not approved for human use should not be used if an equivalent reagent of appropriate grade is available. Their use should be supported by risk–benefit assessment.

G10.36 Consumables and reagents used in processing should undergo risk assessment and validation to confirm that they are suitable for their intended purpose. Following this initial evaluation, it is not necessary to continually validate consumables and reagents obtained from third parties where the supplier confirms compliance with the required specification. Any batches of consumables and reagents prepared in house should be evaluated to ensure they meet specification.

G10.37 The origin, characteristics and conditions for storage (physical, chemical, microbiological) and expiry dates of consumables and reagents should be monitored and recorded.

G10.38 Consumables and reagents should be used in a manner consistent with the instructions provided by the manufacturer.

G10.39 Critical consumables and reagents should have documented specifications describing, if applicable:

G10.39.1 the designated name and the internal code reference;

G10.39.2 the reference (if any) to a pharmacopoeia;
G10.39.3 their approved suppliers and, if possible, their original manufacturer;
G10.39.4 directions for sampling and testing, or reference to procedures;
G10.39.5 critical quality attributes, with acceptance limits;
G10.39.6 shelf life, storage conditions and any necessary precautions to be taken when handling and using them.

G10.40 All critical consumables and reagents obtained from third parties should have a certificate of compliance/analysis from the manufacturer.

10.5 Preservation methods

Preservation of SoHO for long-term banking is part of the operation of a SoHO establishment/entity. Selecting a preservation technique that aligns with the specific characteristics of the SoHO preparations allows a SoHO establishment/entity to achieve reliable results.

Good Practice Guidelines

G10.41 The selected preservation technique should be validated to be capable of retaining the critical properties of the SoHO preparations (e.g. viability, structural integrity) for the duration of the maximum permissible storage period.

Supplementary information

Types of preservation

SoHO preparations can be preserved by different techniques including, but not limited to:

- *Refrigeration*, or hypothermic storage, is used for short-term storage of SoHO preparations following collection, or during processing. It may also be used for the short-term preservation of SoHO between processing and transplantation, where other types of preservation are not feasible, for example the storage of corneas, or fresh osteochondral SoHO preparations where donor cell viability is essential. Refrigeration involves storing SoHO above freezing point, normally at temperatures between 0 and 10 °C or a tighter range within these overall limits.
- *Freezing* (uncontrolled) is used for pre-processing storage of collected SoHO, for in-processing storage between different processing steps of non-viable SoHO and for storage of processed SoHO awaiting release for transplantation. Uncontrolled freezing can disrupt cells [1] and will usually result in SoHO being non-viable on thawing, so is not used for cellular SoHO preparations, or where viability of SoHO preparations is a critical quality attribute.
- *Cryopreservation and vitrification* are processes where the biological and structural functions of SoHO preparations are preserved by cooling to sub-zero temperatures in a cryoprotectant. This is used where retention of donor cell viability is a desired objective of the preservation process. Controlling the rate of cooling to prevent formation of intracellular ice crystals within cells, which can result in damage and loss of viability and integrity, is crucial. Once cryoprotectants are added, SoHO preparations may be placed in a freezing device (such as a controlled-rate freezer) that gradually reduces the temperature of the SoHO preparations and ensures that a slow, constant cooling rate is achieved, including over the critical period when the SoHO preparations freeze, by rapidly dissipating the latent heat of crystallisation while ensuring no sudden changes in temperature. When the cooling is complete, cryopreserved SoHOs are stored below their glass transition temperature; this will differ according to the nature of the SoHO and any storage reagents, but a temperature of below −140°C is generally used.
- *Lyophilisation* (or freeze-drying) achieves dehydration of SoHO by freezing and then reducing the surrounding pressure to allow the frozen water in SoHO to sublimate directly from the solid phase to the gas phase. Lyophilisation prevents autolysis and microbial proliferation during the drying process and allows storage at room temperature.
- *Dehydration* accomplishes the removal of water from SoHO by passive evaporation, or with the use of solvents. This avoids freezing and negative pressure treatments required for lyophilisation. Passive evaporation is generally used for the preservation of thin tissues (such as amniotic membrane), which can dry quickly at room temperature) and can be accelerated by placing the tissue under a continuous air flow, by raising the ambient temperature, or by placing desiccants within the environment, and/or by the use of hygroscopic consumables in contact with, or adjacent to, the tissue. Solvent dehydration is used for certain types of bone allograft and involves displacement of water through the use of volatile solvents.
- *Organ culture* involves storing SoHO preparations at normothermic temperatures, for a short period between completion of processing and transplantation. It is generally used where preservation of donor cell viability and phenotype is essential and requires the use of nutrient-rich storage media.
- *Glycerolisation* involves impregnating SoHO in a concentrated glycerol solution (85% or higher). The glycerol sequesters water in SoHO and thus slows the rate at which degradative processes occur. It is primarily used for the preservation of skin grafts, and it permits long-term storage at refrigerated temperatures. It does not preserve cell viability.

The preservation methodology is a key determinant of how long any preserved SoHO preparations can be stored prior to implantation (their 'shelf life'). It is incumbent on SoHO entities to justify their selected preservation methodologies and shelf lives for different SoHO preparations. This may be based on specific validation studies, relevant data from the literature or records of historical practice.

10.6 Decontamination methods

Eliminating the possibility of viable micro-organisms being present in transplanted SoHO or, where this is not feasible, reducing the overall bioburden prior to application, is crucial to ensure the highest possible level of safety. The microbiological safety of SoHO is influenced by donor selection protocols and minimisation of initial contamination, supported by the use of protocols to control and monitor contamination during the entire collection process. Chapter 12 describes methods of microbiological control.

Additionally, processing protocols that are not specifically designed for decontamination may also be effective in reducing bioburden, for example through the physical removal of micro-organisms by washing, or the use of reagents with antimicrobial activity. Even if the specific decontamination method employed is not sufficient to ensure sterility, this threshold may be attained by considering the cumulative bioburden reduction contributions of other aspects of the process.

In many cases, it is highly probable that collected SoHO sent to a SoHO entity for processing will harbour micro-organisms, because:

- the SoHO preparation originates from parts of the body which contain microbiota, and/or
- the SoHO preparation is contaminated during the collection process.

Where the donor is deceased, *post-mortem* translocation of micro-organisms from the alimentary tract may also occur. Pre-processing disinfection is unlikely to be 100% effective in removing this contamination.

Good Practice Guidelines

G10.42 If the process includes a sterilisation or viral-inactivation step, process-specific validation/verification studies should be completed to demonstrate the log reduction achieved by the process.

G10.43 Where a microbial inactivation procedure is applied to SoHO preparations, the effectiveness of the procedure should be shown for micro-organisms that are commonly found in the SoHO preparations themselves.

G10.44 SoHO establishments/entities should employ the most robust decontamination methodologies possible, with the provision that they do not unacceptably impact critical properties of the SoHO preparations.

G10.45 Sterilisation processes used for SoHO preparations should be validated. National and international standards should be considered when designing validation protocols. Where it is not possible to ensure that the final SoHO preparation is sterile, a risk–benefit assessment should be undertaken to determine what level of residual risk arising from the possibility of contamination is acceptable.

G10.46 SoHO establishments/entities should undertake studies to evaluate the bioburden present on allografts prior to sterilisation and, based on these studies, determine appropriate parameters to ensure the killing of all micro-organisms (in the case of sterilisation processes) or to ensure the required level of bioburden reduction (in the case of decontamination processes).

G10.47 When used specifically for the inactivation of micro-organisms, the disinfection processes should be validated.

G10.48 When using chemical-based sterilisation or disinfection protocols, the risk of toxic chemical residues remaining in the tissue may be a concern and, if applicable, this risk should be included in the information provided to SoHO preparations users.

G10.49 Likewise, where a SoHO preparation processing or decontamination protocol utilises antibiotics, the possible presence of residual antibiotics should be checked for and the validation process should determine whether these may compromise post-disinfection sterility assessment. If this is the case, this uncertainty should be reflected in the SoHO establishment's microbiology policy.

Recommendations

It is recommended that:

R10.6 when validating disinfection and sterilisation protocols, a panel including micro-organisms such as Gram-positive and Gram-negative bacteria, spores and fungi, and including known, relevant 'resistant' species, be used (see Chapter 12 for further guidance);

R10.7 in the case of viruses or an established model virus, relevant species of enveloped and non-enveloped viruses covering a range of virus particle sizes be used [2].

Supplementary information

Types of decontamination

Disinfection

Disinfection is a term used for non-sterilising processes that kill bacteria, and/or fungi and/or spores, and/or inactivate viruses via a known, direct and quantifiable physical or chemical mode of action. In the preparation of SoHO preparations, disinfection processes are either precursors to sterilisation processes or in some cases, where sterilisation is either not required or not possible,

are themselves the primary mechanism for ensuring microbiological SoHO preparations safety.

Generally, when used as a precursor to sterilisation, a disinfection process is intended to reduce the bioburden on the SoHO prior to sterilisation such that it does not exceed a level that can be reliably and completely eliminated by the sterilisation process.

Even where a disinfection method is validated, it is still beneficial to perform post-disinfection sterility tests on SoHO preparations.

The type of disinfection that can be applied to SoHO preparations depends on the nature of the SoHO preparation, how vulnerable it is to different methodologies and the critical quality attributes that have to be retained. For example, some entities utilise a thermodisinfection process for the attainment of microbiological transplant safety [3], although the impact of elevated temperatures on their critical quality attributes restricts the types of SoHO preparation that this process can be applied to. Conversely, for SoHO preparations where maintenance of cell viability is essential, elevated temperature and chemical disinfectants may not be feasible. In these cases, disinfectants that specifically target micro-organisms such as antibiotic-based solutions are the preferred option. However, with these techniques the risk that antibiotic residues could remain in the SoHO preparations post-disinfection and compromise post-disinfection sterility testing [4] is a consideration when defining acceptance criteria.

Peracetic acid-ethanol treatment

This method is typical of the 'panel' approach discussed above. In one example of a peracetic acid (1%) ethanol decontamination process, the method was tested in allogeneic avital bone transplants with a thickness of 15 mm. The process led to a reduction of virus titres of more than 4 $\log_{10}$ $TCID_{50}$/mL. For viable bacteria, fungi and spores, a titre reduction below the detection level (5 $\log_{10}$ CFU/mL) was achieved after an incubation time of two hours [5].

Sterilisation

Sterilisation is defined as a process that results in the complete absence of all cell-based micro-organisms capable of replication. In the preparation of SoHO preparations a sterilisation process will usually also have to meet the requirements for virus inactivation.

Sterilisation processes can be based on moist or dry heat, chemical agents, irradiation or high pressure. However, for SoHO preparations the only true sterilisation method in general use is irradiation. Individual validation methods for processes used to sterilise SoHO transplants are feasible, but the following established, standardised approaches are available:

a. Sterility Assurance Level (SAL). For sterilisation processes with a well-defined dose/kill relationship, a very high degree of sterility assurance can be achieved, and quantified with a SAL. The SAL is expressed as an experimentally-derived number defining the likelihood of a contaminant surviving the process. The smaller the number, the higher the likelihood of sterility. For some classes of medical device and for some medicinal products, there is a European requirement that a sterilisation process is validated to give a value of SAL $\leq 10^{-6}$ for the product to be labelled 'sterile'. This SAL means that the likelihood of non-sterility is 1 in 1 million. This is often interpreted as meaning that in a theoretical batch with 1 million 'units', at least 999 999 units will be sterile following application of the sterilisation process. More relevant for SoHO transplants is the interpretation that the process results in all units being sterile in 999 999 from 1 million cycles.

b. Potency against a panel of bacteria, spores and viruses. Validation of potency can be achieved by application of the EN 1040 standard and/or Committee for Proprietary Medicinal Products (CPMP) (now the Commitee for Medicinal Products for Human Use) guidelines [2, 6]. This type of validation requires that the maximum anticipated level of bacterial, fungal and viral contamination can be eliminated, by establishing the elimination capacity in terms of the number of log-scale reductions in the concentration of samples spiked with a panel of bacteria, fungi and viruses.

Sterilisation methodologies applicable to SoHO preparations

Currently, the only commonly applied process applied to SoHO allografts that is recognised as true sterilisation is high-dose irradiation. Historically, ethylene oxide gas sterilisation was applied to lyophilised SoHO preparations, but this has been discontinued due to concerns of carcinogenicity arising from residuals remaining in the tissue.

Irradiation with gamma particles or accelerated electron beams can be used for the sterilisation and viral inactivation of tissue transplants. For the sterilisation component, such processes are well suited to validation according to SAL [7]. Gamma radiation is effective in killing bacteria, fungi, spores and, to a more variable degree, viruses. Irradiation can have negative effects on the properties of SoHO preparations, depending on the dose applied [8] and the conditions under which they are irradiated. Applying appropriate, validated and verified irradiation conditions can substantially reduce these negative effects and protect the SoHO preparations, integrity. The use of low temperatures and radioprotective chemicals can mitigate some of the damage caused by gamma irradiation [9]. The dose of irradiation required for sterilisation is determined by the number and identity of potential contaminants. SoHO entities may undertake studies to evaluate the bioburden present on allografts prior to irradiation and based on these, calculate an appropriate dose of irradiation to reliably accomplish sterility [8, 10].

High doses of irradiation, in the absence of any coolant, may generate high temperatures (≥ 60°C) in the SoHO preparations, which may have effects on SoHO transplant properties. These effects, while statistically significant, may not always impact the clinical performance of SoHO preparations. Some of the adverse effects of irradiation can be ameliorated by reducing the temperature during the irradiation process, and/or the addition of radioprotectant chemicals to the SoHO preparations [9, 11]. Irradiating musculoskeletal SoHO preparations in the frozen state retains the primary effects of gamma irradiation sterilisation (the breaking of covalent bonds by high-energy gamma rays) while minimising the secondary effects of the process (generation of free radicals). Overall, this can reduce the damage done to allografts by the irradiation process but may also provide some protection to micro-organisms.

10.7 Preparation authorisation

Demonstrating the safety and efficacy of the processes used to prepare SoHO preparations contributes to ensuring their appropriate use. In the EU, it will be obligatory under the SoHO Regulation that all combinations of SoHO preparations and processing protocols provided by a SoHO entity have been granted a Preparation Authorisation (PA) from their national competent authority (CA) before they are provided for clinical use. Any SoHO preparation that was authorised, accredited or licensed under Directive 2004/23/EC prior to the SoHO Regulation coming into force will be deemed to have been granted a PA under the Regulation (Art. 82.1).

If a PA indicates significant potential residual risk from the SoHO preparations, a SoHO entity may need to include a clinical outcome monitoring plan with their application. Where this is required, detailed guidance on how to prepare one, proportionate to the level of risk identified, can be found in Chapter 19.

Standards

S10.8 SoHO entities must submit applications for PAs to their HA (Health Authority) (Art. 39.1).

S10.9 PA applications must include the following data and information, where applicable (Art. 39.2. a, b):

- **S10.9.1** the name and contact details of the SoHO entity applying for the PA;
- **S10.9.2** a description of the source SoHO used to prepare the SoHO preparations;
- **S10.9.3** a list of selection criteria and specific tests applied to the donors of the source SoHO (including generic criteria applied to all SoHO, and any specific criteria applied to the SoHO in question);
- **S10.9.4** a description of the collection procedures for the source SoHO;
- **S10.9.5** any quality control tests and checks performed on the source SoHO prior to it being used to make a SoHO preparation;
- **S10.9.6** a description of the processing protocol(s) applied, including consumables and equipment used in the processing protocols, and relevant parameters and controls;
- **S10.9.7** a description of the equipment, reagents and consumables that come into contact with the SoHO preparations, and any relevant certification, in accordance with the EU Medical Devices Regulation (EU 2017/745), where applicable;
- **S10.9.8** if equipment, reagents or consumables are prepared in house, evidence of validation of their quality;
- **S10.9.9** acceptable transport and storage conditions and times, and evidence that these have been validated;
- **S10.9.10** the specification of the SoHO preparations, including quality control tests performed and parameters required for release;
- **S10.9.11** data resulting from process validation and equipment qualification;
- **S10.9.12** where relevant, details of any aspects of the processing protocol contracted out to third parties, including other SoHO entities;
- **S10.9.13** the clinical indication(s) for which the SoHO preparations are intended to be provided, and the clinical data justifying this;
- **S10.9.14** any non-clinical data (*in vitro* and/or *in vivo*) relevant to the effectiveness and toxicity of the SoHO preparations.

S10.10 PA applications must include a documented risk–benefit assessment that considers the processes applied to the SoHO preparations and the specific clinical indication(s) for which the application has been submitted. These must take into account the following (Art. 39.2.c):

- **S10.10.1** whether the SoHO preparation process is aligned with an existing European Directorate for the Quality of Medicines & HealthCare (EDQM) SoHO preparations monograph, equivalent national standards or other relevant Guidelines or Technical methods (Art. 59.4, Art. 27.6.b, c);
- **S10.10.2** whether the SoHO preparation meets the quality criteria defined in an existing monograph or specification;
- **S10.10.3** whether the intended clinical application of the SoHO preparations is covered by an existing monograph or specification;
- **S10.10.4** any information regarding previous authorisation or use of the SoHO preparation, or a SoHO preparation which is comparable to that addressed in the PA application, by other SoHO entities; EU MS must include any relevant data present on the EU SoHO platform;
- **S10.10.5** evidence of clinical functionality, generated as part of conformity assessment procedures according to EU/2017/745 for certified medical devices used in the preparation process where:
  - they are critical to the preparation process and
  - the applicant has access to this data;

S10.10.6 evidence that a standardised process has been performed to identify, quantify and evaluate any potential risks to SoHO preparations donors, SoHO preparations recipients or offspring from MAR arising from the protocols used to prepare the SoHO preparations, considering technical guidelines published by the EDQM relating to protection of SoHO preparations donors and offspring from MAR;

S10.10.7 where indicated risk is greater than negligible or potential clinical efficacy is unknown, a proposed plan for clinical outcome monitoring, which must be included;

S10.10.8 an indication if any of the data contained in the PA are proprietary, accompanied by a verifiable justification if so.

S10.11 If the PA application includes a proposal to record clinical outcome data in an existing Registry, the application must include details of the Registry, and evidence that approval to use the Registry for the SoHO preparations in question has been requested (Art. 39.3).

S10.12 Where the risk–benefit assessment for the SoHO preparations does not clearly demonstrate that the benefit of using the SoHO preparations outweighs the risks, or that the risks are greater than negligible, the SoHO entity must only distribute the SoHO preparations under a clinical outcome monitoring plan that has been approved by the HA (Art. 39.4).

S10.13 Any clinical outcome monitoring plan submitted in relation to a PA must be proportionate to the projected risk and benefit of the SoHO preparations (Art. 20.4.d & 21).

S10.14 The results of any clinical outcome plan authorised by the HA must be submitted within the time limit specified by the HA (Art. 19.2.d).

S10.15 The SoHO entity submitting a PA application has responsibility for collecting the indicated clinical data and must make such data available to the HA on request (Art. 39.5).

S10.16 SoHO entities must not make significant changes to a protocol described in a PA without written authorisation from their HA. In this context, 'significant' means any changes to the approved clinical indication, or any changes that may affect the quality, safety or efficacy of the SoHO preparations (Art. 39.6).

S10.17 The SoHO entities that prepare a SoHO preparation authorised by a PA must be based in the EU MS where the PA is submitted (Art. 39.7).

Good Practice Guidelines

G10.50 Risk–benefit assessments submitted in support of PA applications should include, where available, validation, verification and/or clinical studies performed by the SoHO entity itself and performed by other SoHO entities, in addition to relevant data present in the EU SoHO compendium.

G10.51 When submitting clinical data in support of PA applications, SoHO entities should indicate the level of clinical evidence these data provide.

Recommendations

It is advised:

R10.8 that when preparing risk–benefit assessments in support of PAs, SoHO entities use a structured risk analysis tool that has been developed for SoHO preparations, such as the EuroGTP-II process discussed in Chapter 4;

R10.9 in cases where a SoHO preparation is not aligned with an existing SoHO monograph, to refer to monographs for similar SoHO preparations for guidance on what critical properties of the SoHO preparations are generally expected to be retained. SoHO monographs for SoHO preparations can be found in Part C of this Guide.

Supplementary information

Different types of clinical study generate different levels of clinical evidence. Studies that generate higher levels of evidence may be accorded proportionate levels of significance in the risk–benefit assessment accompanying a PA. The standard hierarchy for clinical evidence is:

Level 1 - Systematic review or meta-analysis of all relevant randomised controlled trials.

Level 2 - Controlled trials with true randomisation.

Level 3a - Controlled trials without true randomisation.

Level 3b - Case-control and cohort studies.

Level 3c - Systematic reviews of descriptive and qualitative studies.

Level 4 - Single descriptive or qualitative studies.

Level 5 - Expert opinion (not a clinical study but may provide some relevant evidence).

10.8 Quality control

Good Practice Guidelines

G10.52 Tests and procedures should be carried out to measure, assay or monitor processing, preservation and storage methods, equipment and reagents to ensure compliance with established acceptance criteria.

G10.53 Written procedures should be in place that govern quality control at key stages during processing. As a minimum, these should include:

G10.53.1 the test method,

G10.53.2 the sample size,

G10.53.3 acceptance criteria.

G10.54 The results of all tests or procedures should become part of the processing record.

G10.55 If in-process quality controls are undertaken in the processing area, they should be carried out in

a manner that does not pose a risk to the processing steps that need to be carried out.

G10.56 Non-conforming products should be identified and separated from conforming products. The fate of non-conforming products should be decided by the Responsible Person (RP) in charge of the SoHO establishment/entity, in consultation with the releasing officer where issue for clinical use is considered.

G10.57 Sampling and testing methods should be validated to demonstrate that the sampling method as much as possible represents the SoHO and that the testing methods are suitable and fit for purpose.

Supplementary information

The minimum quality control requirements for evaluation of each type of SoHO are described in SoHO chapters, and the SoHO monographs.

10.9 References

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Related material
Appendix 30. Decellularisation

Chapter 11. **Storage**

11.1 Introduction

Storage is the maintenance of substances of human origin (SoHO) preparations for clinical application under appropriate controlled conditions to ensure that the quality of the SoHO preparations will be maintained. SoHO preparations are normally stored in a SoHO entity or establishment prior to their release. For the remit of this chapter, storage covers the stages from collection to distribution as well as storage at organisations responsible for human application (ORHAs) (SoHO entities).

The opportunity to store SoHO is also usual during processing and brings great advantages:

a. preservation of the required properties of the biological material, making extended storage for future use possible;

b. facilitating and optimising clinical use by dividing a donation into multiple, discrete units of SoHOs;

c. reducing the risk of disease transmission by allowing SoHO entities to await test results of infectious disease testing and microbial contamination prior to release and clinical use, though this is only possible if the SoHO can be stored under defined conditions for an adequate time while awaiting the test results;

d. performing tests and awaiting other quality-control results before release, such as cell counts, clonogenic assays for haematopoietic progenitor cells (HPC), and residual moisture in lyophilised or dehydrated grafts.

Several different types of storage can be distinguished:

- Short-term storage
  Because of processing methods and the lifespan of the SoHO preparations, some viable SoHO preparations can only be stored for a short period of time (e.g. corneas stored in organ culture for about 4-5 weeks).
- Long-term storage
  If SoHO preparations need to be stored for a longer period, other strategies (e.g. cryopreservation, deep-freezing or lyophilisation) will be necessary (see also Chapter 10, section 10.5 on preservation methods) for maintaining the critical properties of the SoHO preparations.
- Quarantine storage
  All SoHO preparations that are stored before having determined their suitability (including known non-conforming ones, e.g. accepted for autologous use) are kept under quarantine with provisions in place to avoid any cross-contamination or mix-up with other products.
- Storage at an ORHA
  ORHAs have short- and long-term storage for SoHO preparations awaiting application, surplus or unused SoHO and SoHO preparations recalled by SoHO establishments. Therefore, a system for identification of SoHO preparations throughout any phase of the clinical application at the ORHA will clearly distinguish SoHO preparations suitable for clinical use and/or discarded ones (see Chapter 14).

This chapter provides generic guidance on the storage of SoHO preparations. Further SoHO-specific guidance is provided in Part B of this Guide.

11.2 General considerations

Commonly used temperature ranges for storage of SoHO are shown in Table 11.1. Storage facilities for materials or SoHO are present in most SoHO establishments and SoHO entities, including collection sites, and in ORHAs. The time limits between collection, processing and storage as well as the shelf life and storage

temperatures of SoHO preparations are described in the SoHO-specific sections of this Guide (see Part B).

Standards

S11.1 SoHO entities must take measures to prevent the risks of cross-contamination between SoHO, starting or interim material during storage and distribution. Such measures must ensure that physical contact or transfer of material by other means between SoHO from different donors, as well as SoHO collected from different persons for future autologous or within-relationship use, is avoided (Art. 58.4).

S11.2 If a SoHO establishment or SoHO entity partially or totally ceases its activities it must (Art. 35.7, Art. 42.6):

S11.2.1 Communicate this change to their Health Authority (HA), or in EU member states to the register of SoHO activities, without delay, according to national procedures.

S11.2.2 Where relevant, inform their HA to which entity or entities that any SoHO preparations that it has in storage, and any traceability data relating to those SoHO preparations, will be transferred.

S11.2.3 Inform persons from whom SoHO were collected and stored, if the SoHO were intended for autologous or within-relationship use, or if they are a highly matched SoHO preparation for a specific recipient, and provide them with information about the new SoHO entity or establishment that will store the SoHO preparations (Art. 35.8).

Good Practice Guidelines

G11.1 Maximum storage time and storage conditions should be defined and validated for each type of SoHO preparation.

G11.2 There should be a system in place to separate and distinguish SoHO preparations in quarantine from those that are released and from those that are rejected, in order to prevent mix-ups and cross-contamination.

G11.2.1 Quarantined SoHO preparations should be physically separated or visibly different (by labelling and/or packaging whenever possible) or separated by any other means, e.g. computerised systems, from released SoHO preparations.

G11.2.2 To maintain physical separation, specific areas or storage devices (or secured segregation within the device) should be defined for SoHO preparations in quarantine, for SoHO preparations that should comply with special criteria and for SoHO preparations that have been released; a separate area should be dedicated to the storage of media and other materials used during the process of preparation.

G11.3 A documented risk assessment approved by the SoHO establishment physician should be in place to determine the fate of all stored SoHO preparations following the introduction of any new donor selection or testing criterion that enhances safety or quality.

G11.4 The seals and the material used in the packaging and labelling of SoHO employed should be qualified for their use at the designated storage temperature and for the conditions of use, to demonstrate that the packaging and labelling can retain their integrity under such conditions.

G11.5 A written agreement should be in place each time that a SoHO establishment (or SoHO entity that carries out storage) entrusts storage of SoHO to a third party. (See Chapter 2, section 2.6 on outsourced activities.) They should evaluate and select third parties based on their ability to meet the established standards of quality set out in this Guide or equivalent.

G11.6 An inventory audit of SoHO preparations stored should be performed regularly. The frequency of such audits should be determined by risk assessment.

G11.7 Policies and procedures should be developed for the emergency transfer of tissue to designated alternative storage facilities, and for alternative monitoring methods, e.g. in the event of mechanical failure, loss of coolant, lack of access or loss of the whole facility. These should include specification of tolerance limits or temperatures and time limits after which the initi-

Table 11.1 **Temperature range for storage of SoHO**

Storage method	[a]°C minimum	[a]°C maximum
Cryopreservation	−196	−140
Deep frozen[b]	−80	−60
Frozen[c]		< −15
Refrigerated[c]	+2	+8
Cold or cooled[c]	+8	+15
Room temperature[c]	+15	+25
Dehydrated and/or lyophilised	+4	+30
Organ culture	+28	+37

[a] SoHO-specific temperatures are indicated in the relevant SoHO chapters as well as the associated SoHO monographs.

[b] Based on general practice.

[c] Based on the *European Pharmacopoeia* [1].

ation of the emergency transfer is required. Actions to be taken when limits have been exceeded should also be specified in the relevant standard operating procedure (SOP). Further information on emergency planning can be found in Chapter 2, section 2.14.

Recommendations

R11.1 It is recommended that, when applicable, reference samples of SoHO for quality control be stored under the same conditions as the SoHO preparations themselves (e.g. HPC and cord blood reference samples, for which see Chapters 25 and 26 respectively).

Supplementary information

In certain specific cases, it may be possible to prolong the expiry date, especially in the case of cryopreserved HPC (e.g. cord blood) (see see Chapters 25 and 26), reproductive SoHO for within-relationship use or in cases of fertility preservation (see Chapters 30 and 31). Further details on SoHO-specific shelf life can be found in the tissue-specific chapters of Part B.

Obtaining and analysing the clinical outcome data where available can provide evidence to be used to verify the safety and efficacy of the storage procedures.

If there is no direct contact between the donor/patient and the SoHO entity in which storage takes place, and the SoHO entity would need to inform the donor/patient about ceasing of the entity's activities (see S11.2.3), the treating clinician in charge of the application can contact the donor/patient and inform them.

For transitional provisions in the EU, concerning SoHO already in storage before 7 August 2024, please refer to Article 84 of the SoHO Regulation.

11.3 Requirements for storage facilities

Standards

S11.3 SoHO entities must mitigate microbial contamination risks arising from environment, personnel, equipment and materials coming into contact with SoHO also during storage. This must be avoided by the following applicable measures:

S11.3.1 specifying and verifying the hygiene procedures of the personnel of the entity in contact with the SoHO throughout the preparation chain;

S11.3.2 specifying and verifying the cleanliness of collection areas, taking into account the degree of exposure of SoHO to the environment during collection, and of storage areas;

S11.3.3 specifying, procuring and decontaminating equipment and materials that come into contact with SoHO during collection, processing, storage or distribution, such that their sterility is ensured and can be verified, where necessary (Art. 58.5).

Good Practice Guidelines

G11.8 Storage rooms should be clean and located in a secure and dedicated area, and access should be limited to authorised personnel.

G11.9 A documented cleaning plan for the storage area should be implemented.

G11.10 Storage areas should be of appropriate size to allow orderly storage of materials and reagents, and of SoHO preparations.

G11.11 Storage areas for SoHO preparations should be maintained within defined temperature limits. Where special storage conditions are required (e.g. humidity) these should be checked, monitored and recorded.

G11.12 Storage facilities should ensure that the equipment, working environment, process design and storage conditions in use comply with the standards of quality and safety set out in this Guide or equivalent (see Chapter 2).

G11.13 An alarm system should be in place to alert users in a timely manner to any deviation from the pre-defined storage conditions. Alarm systems placed in storage devices should be continuously active and able to alert personnel on a 24-hour basis. The functionality of the alarm systems should be checked regularly.

G11.14 Provision should be in place in the event of equipment or power failure.

G11.15 A backup unit should be available in case of cleaning, repair or defect to provide the same safe conditions as the storage equipment. Additionally, based on risk assessment, a backup energy system should be implemented.

G11.16 Oxygen sensors should be appropriately placed, and personal protection equipment should be available in areas where liquid nitrogen is present.

Recommendations

It is recommended that:

R11.2 a secure, clean and stable environment be maintained for storage. This can be achieved by ensuring that the storage facility is:
- *a.* large enough for its intended purpose and allows easy access and exit for personnel and equipment; and
- *b.* located in a dry, cool, well-ventilated place, away from external heat sources;

R11.3 where mechanical refrigerators and freezers are utilised, suitable air conditioning systems be installed, to dissipate excess heat;

R11.4 in case of loss of electrical power, the storage facility have a backup system to guarantee continuous conditions. Furthermore, it is advised to document it in emergency plans;

R11.5 in order to minimise the risk of environmental microbial contamination, storage areas be separated from corridors or the outdoors by doors;
R11.6 as with the processing rooms, monitoring systems be put in place for storage rooms.

Supplementary information

The necessary air-conditioning capacity for the storage area can be calculated on the basis of the actual heat load of the equipment and the environmental factors. Storage rooms can be equipped with an HVAC (heating, ventilation and air conditioning) system for maintaining the temperature and humidity by adequate air exchange.

The frequency of monitoring systems for storage rooms, as well as the implementation of alarm limits, depends on the degree of stringency needed and the results of risk analysis.

11.3.1 Special safety measures for liquid nitrogen facilities

Good Practice Guidelines

G11.17 SoHO should be stored in the vapour phase of liquid nitrogen, rather than by immersion, if no alternative is available.
G11.18 SoHO immersed in liquid nitrogen should be double-wrapped during storage or stored in a high-security primary container especially designed for liquid nitrogen (depending on the storage system, type of sample and after risk assessment).

Recommendations

Operational –

It is advised that:
R11.7 within the organisation of the storage room there is space that allows circulation and manipulation around the cryogenic tanks, and that the room is clearly identified with pictograms indicating the dangers and the location of personal protection equipment;
R11.8 tank-filling systems are designed to minimise evaporation of nitrogen and be equipped with safety valves and rupture discs, and external de-gassing valves for long lines;
R11.9 condensation on the tanks and the formation of ice inside them are controlled;
R11.10 the floors are covered with a material resistant to low temperatures and high loads, allowing easy movement of the tanks without shock.

Safety –

It is recommended that:
R11.11 before entering the room, staff members ensure that ventilation is functioning correctly and are able to check the oxygen level. An adapted mechanical ventilation (extraction and fresh-air intake) system, working continuously, is mandatory, ensuring that under normal operating conditions the oxygen concentration is kept above a minimal level. Continuous monitoring of the oxygen level is ensured by detectors, checked and maintained to the supplier's recommendations;
R11.12 detectors for oxygen levels be placed in lower areas of the room in order to monitor any accumulation of nitrogen at floor level;
R11.13 the oxygen concentration or alarm status be visible and audible outside the storage room;
R11.14 the entrance door of the room be equipped with an oculus allowing surveillance from outside. Where the entirety of the room cannot be seen from the oculus, alternative surveillance systems, such as closed-circuit television, may be used;
R11.15 the door to the liquid nitrogen room open outwards;
R11.16 the staff have access to gloves with long sleeves able to protect against cold, cryoprotective aprons and shoes, as well as safety glasses (EN166) or visors protecting the face;
R11.17 the room have in place an alarm (visual and audible) warning of nitrogen evaporation that leads to an oxygen level equal to or lower than 19%;
R11.18 when the alarm is activated, staff leave the room and await normalisation of the oxygen level;
R11.19 the alarm be connected to a continuous monitoring station allowing alerts for the staff working in the room and the rescue or assistance of staff, if necessary;
R11.20 likewise, there be a procedure in place to ensure that, when the oxygen level in the facility is lower than 19%, the facility can only be entered by suitably trained and qualified personnel equipped with an independent air supply [2].

Supplementary information

In the case of G11.17 and G11.18, this is highly important for storage with liquid nitrogen owing to the accumulation of microbial contaminants in liquid nitrogen storage vessels, which could lead to cross-contamination of samples unless appropriate precautions are taken [3-5].

11.4 Documentation

Good Practice Guidelines

G11.19 Records of the storage of SoHO preparations should be retained, and reports should be available that detail:
G11.19.1 storage location (and transfer record if location is changed);
G11.19.2 date placed in storage and date removed from storage;
G11.19.3 storage temperature;

G11.19.4 any incidents that occurred during storage.

G11.20 When a SoHO preparation is stored in liquid nitrogen and shipped in dry ice or stored in a SoHO entity (ORHA) at a higher temperature than its designated storage temperature within the SoHO establishment, considerations should be put in place regarding the new expiry date. The new expiry date should be incorporated in the accompanying documentation.

11.5 Expiry date

Recommendations

R11.21 When determining the maximum storage period, it is recommended that the following factors be considered, as a minimum:

- R11.21.1 possible deterioration of the required properties of SoHO;
- R11.21.2 risks related to microbiology;
- R11.21.3 package integrity over time;
- R11.21.4 expiry of storage solutions;
- R11.21.5 stability at the storage temperature;
- R11.21.6 quality criteria (viability, functionality after thawing);
- R11.21.7 the time of collection (if relevant for the particular SoHO type).

11.6 Disposal of SoHO

Good Practice Guidelines

G11.21 SoHO entities should include in their SOPs special provisions for the handling of SoHO preparations to be discarded, in order to prevent the contamination of other SoHO preparations, the processing environment or personnel. If applicable, these procedures should comply with national regulations.

G11.22 Disposal of SoHO should be carried out in a manner that shows respect for fundamental rights and for the human body.

G11.23 Disposal records should include details of date, involved personnel and reasons for disposal.

G11.24 There should be documentation to show that the conditions for disposal, as defined prior to collection, have been met, including (where applicable) the option to transfer the SoHO R11.21.6 to another facility if the designated recipient is still alive after the agreed storage period.

11.7 References

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Chapter 12. **Microbiology**

12.1 Introduction

This chapter sets out a comprehensive, risk-based strategy for the assessment of the microbiological quality and safety of substances of human origin (SoHO) intended for human application. It outlines the relevant microbiological test methods described in the European Pharmacopoeia (*Ph. Eur.*) [1], while explicitly taking into account the specific characteristics and inherent limitations of SoHO such as the initial microbial load (bioburden) after collection, the nature and availability of the test sample in view of the scarcity of SoHO, the intrinsic microbiological composition of the product, the feasibility of sampling, the possibility of decontaminating or sterilising the final SoHO preparations, and the intended mode and route of clinical application (*Ph. Eur.* 2.6.39).

The principles described in this chapter provide a general framework for microbiological assessment; however, their practical application is to be adapted to the specific characteristics of each SoHO, including its intended clinical use.

For certain SoHO – such as those with very limited material, products containing excipients that may interfere with microbiological assays, or SoHO where the presence of commensal micro-organisms is intrinsic to the intended function – not all approaches described in this chapter may be applicable. In such cases, an alternative microbiological control strategy is to be defined using a justified, SoHO-specific and risk-based approach. Additional guidance on microbiological testing for specific SoHO processes is provided in the SoHO-specific chapters in Part B of this Guide.

Standards

S12.1 SoHO entities must mitigate risks arising from microbial contamination of SoHO from the environment, the personnel, the equipment and the materials coming into contact with SoHO during collection, processing, storage or distribution. SoHO entities must mitigate such risks by performing quality control testing of SoHO to detect microbial contamination and by using methods of inactivation or elimination of micro-organisms, where feasible and appropriate; see Chapter 2 (Art. 58.5.e).

Good Practice Guidelines

G12.1 All entities that process SoHO should have access to the services of a microbiology laboratory, licensed or accredited to relevant national or international standards, and should have access to the advice of a suitably qualified expert microbiologist.

12.2 Testing

12.2.1 Sampling

Good Practice Guidelines

G12.2 SoHO entities should perform a risk assessment to determine whether microbiological testing should be carried out on representative samples collected prior to the commencement of processing, at any point from the end of the collection process until the commencement of any processing protocols. The risk assessment should take into consideration the nature of the SoHO, their origin and collection method, the critical steps during collection and packaging, the use of a closed system, the initial bioburden after collection, the prophylactic use of antibiotics, the time between collection and application, the storage conditions, the route of administration and their intended application.

G12.3 Microbiological testing should be carried out as a minimum on representative samples collected at the end of the processing protocols, immediately before final packaging of the SoHO preparation. For SoHO that do not undergo processing, there should be at least one sampling point prior to placing the SoHO preparation in its final package.

G12.4 If an appropriately validated terminal sterilisation process is applied (sterility assurance level [SAL] of $\geq 10^{-6}$ [2]), parametric release allows SoHO entities to replace post-processing microbiological testing as a release criterion with acceptance criteria for the control of identified process parameters (Ph. Eur. 5.1.1).

G12.5 Despite the limited availability of SoHO, samples for testing should as much as possible be representative of the whole SoHO preparation and suitable to ensure that the chosen analytical method can be performed with acceptable sensitivity and specificity to prevent false-negative or false-positive test results.

G12.6 In cases where the nature, scarcity or size of the collected SoHO prevents direct sampling, an alternative sampling approach should be implemented which may include sampling adjacent SoHO, sampling liquids in contact with the SoHO (e.g. storage media, transport media or rinsing solutions) or using swabs.

Recommendations

R12.1 It is recommended to conduct a risk assessment to determine whether it is necessary to perform in-process microbiological testing at relevant steps of the SoHO preparation process.

Supplementary information

Microbiological contaminants may be present within the SoHO, as well as on the surface of the SoHO or in solutions in contact with the SoHO (G12.3). Therefore, the processing of representative SoHO samples is relevant for microbiological testing [3, 4]. Most SoHO are exposed to the direct environment at certain processing stages between collection and packaging. Avoiding open processing unless it is absolutely necessary minimises the risk of contamination during open processing. This will help to ensure that aseptic conditions are maintained throughout processing. The requirements for microbiological sampling and testing are expected to be most stringent in these situations.

By testing at relevant steps of the aseptic process, one can verify that the SoHO meet predefined standards for microbiological quality set by the SoHO entity throughout the whole preparation process. This helps in maintaining consistency and ensures that the SoHO preparations will be safe for clinical application. In-process testing also enables the verification that decontamination, sterilisation and other processing steps have been effective in reducing microbial bioburden.

When a closed system is employed during processing, it may not be necessary to carry out in-process testing because it does not provide any additional information regarding the microbiological status of SoHO preparations. However, if the starting material is not sterile and the process involves prolonged incubations under conditions conducive to microbial growth, a risk assessment may indicate the need for in-process testing. Given the special nature of human bodily material and the desire to keep as much material as possible available for transplantation, it is acceptable to base the release of terminally sterilised SoHO in their final container only on process and validation data (parametric release) and not on microbiological testing of the final SoHO preparations. This approach requires the validation of SoHO collection, transport, washing, antibiotic treatment and other processing steps, packaging and storage. In some countries, such an approach may require permission from the Health Authority (see also section 12.2.6 below).

12.2.2 Microbiological testing for bacteria and fungi

Testing for aerobic bacteria, anaerobic bacteria and fungi in SoHO samples under appropriate conditions is the most accurate qualitative measure of microbiological contamination.

Good Practice Guidelines

G12.7 Considering the nature of the collected SoHO and any subsequent processing step, the microbiological testing approach should follow the procedures outlined in *Ph. Eur.*, specifically chapters 2.6.1, 2.6.13 or 2.6.27. Deviations from such standards should be justified, and alternative test methods should be documented and justified in accordance with *Ph. Eur.* 5.1.6.

G12.8 Microbiological test assays for bacteria and fungi should be inspected visually or by an automated system, at pre-determined intervals both during and at the end of the testing period.

G12.9 Samples for subculture should be collected from positive test results as soon as possible to identify the bacteria and fungi, and an antibiotic susceptibility profile should be conducted if required.

G12.10 Alternative rapid microbiological testing methods should be considered, especially for preparations of SoHO with a short shelf life. Independent of the applied method, the fitness for purpose of rapid microbiological testing methods should be justified and validated according to *Ph. Eur.* 2.6.27 or 5.1.6.

Recommendations

R12.2 It is recommended that further processing after the decontamination step be conducted without the use of antimicrobial agents, if possible. If this is not feasible, it is advised that these agents are removed from the sample matrix before microbiological testing or inactivated by suitable techniques (e.g. resins, penase) [5].

12.2.3 Testing for *Mycoplasma*

Mycoplasma contamination poses a potential risk of infection to patients, particularly to patients who are immunocompromised. Additionally, *Mycoplasma* contamination can critically affect the SoHO preparations itself. *Mycoplasma* are known to disrupt cell function, causing changes in gene expression, cellular signalling and metabolic activity.

Good Practice Guidelines

G12.11 Testing for specific infectious agents such as *mycoplasma* should be carried out if they are known to constitute a relevant risk for the SoHO preparation.
G12.12 Considering the nature of the collected SoHO and any subsequent processing step, the testing approach should follow the procedures outlined in *Ph. Eur.*, Chapter 2.6.7. Deviations from these standards should be documented and justified. Nucleic acid testing (NAT) methods should be validated in accordance with *Ph. Eur.* 2.6.7 and other alternative methods should be validated in accordance with *Ph. Eur.* 5.1.6.
G12.13 As mycoplasmas are cell-associated microorganisms that may adhere to the cells or locate in the cells, testing should always include the SoHO, if *Mycoplasma* testing is applicable.

Supplementary information

Possible sources for *Mycoplasma* contamination include the cellular starting material itself or its collection (e.g. improper aseptic techniques, poor laboratory practice), animal- or human-derived raw materials such as untreated sera, personnel in the clinic or manufacturing site, and the entire processing method.

Mycoplasma can penetrate sterilising-grade filter membranes with a nominal pore size of ≤ 0.2 µm. They also lack a rigid cell wall, which makes them unsusceptible to antimicrobial agents that target the cell wall. Furthermore, many broad-spectrum antibiotics inhibit the proliferation of *Mycoplasma* but do not kill them. Hence, elimination with antibiotics is difficult. These characteristics are relevant when evaluating approaches for *Mycoplasma* testing.

12.2.4 Testing for mycobacteria

The risk of mycobacteria in SoHO transplantation is a significant concern due to the potential for serious infections and complications. Mycobacteria can be transmitted through contaminated donor SoHO, posing various risks to transplant recipients such as infections, especially to those who are immunocompromised. These infections can lead to severe complications such as pneumonia, sepsis or even organ failure, depending on the type of *Mycobacterium* and the site of infection. Mycobacterial infections often have a slow onset and can be difficult to detect early, making diagnosis challenging. Symptoms may not appear until weeks, months or even years after the transplant, complicating treatment and increasing the risk of severe outcomes.

Good Practice Guidelines

G12.14 Testing for mycobacteria should be carried out if they are known to constitute a relevant risk.
G12.15 Considering the nature of the collected SoHO and any subsequent processing step, the testing approach should follow the procedures outlined in *Ph. Eur.*, Chapter 2.6.2. Deviations from such standards should be justified, and alternative test methods should be validated in accordance with *Ph. Eur.* 5.1.6.

12.2.5 Testing for pyrogens

Pyrogens are microbial components that lead to fever if applied parenterally (see *Ph. Eur.* 5.1.13). In some specific situations, pyrogen contamination may be responsible for unexplained SoHO preparation failure, e.g. pancreatic islet transplantation [6]. The major pyrogen of concern is lipopolysaccharide (LPS) derived from the outer membrane of Gram-negative bacteria, the so-called bacterial endotoxins, but also other non-endotoxin pyrogen contaminants are relevant, e.g. bacterial flagellin. Pyrogen contaminations can originate from the donor material itself or from raw materials, solutions and instruments coming into contact with the SoHO during processing.

Good Practice Guidelines

G12.16 Testing for pyrogens should be carried out if they are known to constitute a relevant risk. If risk assessment reveals the necessity of bacterial endotoxin control, method *Ph. Eur.* 2.6.14 (Bacterial endotoxins) should be used. Depending on the risk assessment, testing of non-endotoxin pyrogens in addition to endotoxin or mixtures of both should be performed with the monocyte activation test (MAT; *Ph. Eur.* 2.6.30).
G12.17 Because raw materials are potential sources of pyrogens, raw materials declared by their manufacturers to be free of pyrogens or with low content of endotoxin (such as some medical devices) should preferably be used for the culture of SoHO, if available.

Recommendations

R12.3 It is recommended to test for pyrogens following the elimination of viable microbes through decontamination or sterilisation steps, particularly in cases where the starting material has a high bioburden;
R12.4 For certain SoHO preparations (e.g. pancreatic islets) that are administered immediately to the patient and that cannot be cryopreserved without damaging the viability and quality of the cells, it is recommended to use a rapid method for pyrogen testing.

Supplementary information

Guidance for selection of a suitable test strategy for pyrogens is provided by *Ph. Eur.* 5.1.13. In *Ph. Eur.* 5.1.10 the limit for bacterial endotoxin is specified as 5 IU per kg of patient weight per bolus or hour for intravenous drugs (*Ph. Eur.* monograph 5.1.10 Table 5.1.10.-1), which is usually applied for all parenteral preparations.

12.2.6 Microbial enumeration testing

Microbial enumeration testing is the process of determining the number of viable micro-organisms (bacteria, fungi and mycobacteria) present in a SoHO sample or alternative sample such as liquids which came in contact with the SoHO preparations. Microbial enumeration testing helps assess the effectiveness of the decontamination and sterilisation processes and can demonstrate that the microbial enumeration is reduced to acceptable levels. Microbial enumeration test results are usually expressed as the total number and/or identity of micro-organisms associated with a specific SoHO sample determined by testing.

Good Practice Guidelines

G12.18 The microbial enumeration status of starting material (pre-processing) should be measured as part of the validation and monitoring of the efficacy of decontamination procedures and/or terminal sterilisation [7] according to *Ph. Eur.* test methods 2.6.12 or 2.6.13. If applicable, the microbial enumeration test results should be used by SoHO entities to define acceptance/rejection criteria for SoHO starting material for further decontamination or sterilisation.

G12.19 Regular microbial enumeration testing should be carried out, based on a risk assessment if post-processing microbiological testing is replaced by parametric release or if post-processing microbiological test results are not available at the time of release.

Supplementary information

Microbial enumeration testing generally involves filtering of a liquid sample through a membrane filter. The filter is then cultured to count the viable micro-organisms. SoHO can be tested by using a representative cell sample suspended in a broth medium, or less commonly by employing a culture swab or suspension from an initial wash of the specimen. Although swab-based methods are less efficient in recovering micro-organisms, with recovery rates typically below 20% [8, 9], they still provide a rough estimate of microbial contamination levels – categorised as heavy, moderate or light. Identifying specific species aids in assessing the potential risk posed by the contaminants. To improve recovery efficiency, more rigorous methods like sonication or mechanical shaking can be used, where the SoHO preparation sample is exposed to an extraction fluid containing a surfactant [8]. (See also §12.3.)

Microbial enumeration testing is the quantitative measure of the microbial load (bioburden) of SoHO. Although microbial infection post-transplantation is rarely reported, some virulent microbes when present in high numbers on a SoHO surface may form cytotoxic exo-products such as proteases and toxins, which can have a deleterious effect on the cellular integrity or structural properties of the SoHO preparation.

12.3 Conditions and methods of microbiological testing

Good Practice Guidelines

G12.20 Each batch of the microbial culture medium used for microbiological testing should be tested for its growth-promoting capabilities and, where relevant, used to test for the microbial strains listed in the relevant *Ph. Eur.* chapters.

Supplementary information

Frequently, growth-promotion testing has been performed by the vendor of microbial media using a defining group of suitable micro-organisms as documented by the certificate for each particular medium batch. In this case, growth promotion need not be repeated by the end user, provided growth-promoting testing, transport and storage conditions are properly documented and are in accordance with the requirements of the vendor and the relevant chapter of *Ph. Eur.*

12.4 Sterility testing (*Ph. Eur.* 2.6.1)

Incubation conditions for sterility testing are detailed in Table 12.1.

It is of the upmost importance to take precautions against microbial cross-contamination during a sterility

Table 12.1 **Incubation conditions for sterility testing**

	Culture medium	Incubation temperature °C	Testing period
Aerobic	Soya bean casein digest medium	20-25	14 days
Anaerobic	Fluid thioglycolate medium	30-35	14 days
Fungi	Soya bean casein digest medium	20-25	14 days

Note: Fluid thioglycolate medium will also detect aerobic bacteria.

test (*Ph. Eur.* 2.6.1 and 5.1.9). At the least, sub-cultivation is carried out in a biohazard laminar airflow cabinet, properly disinfected before the test, and no other activity is to be conducted at the same time. The preferred microbiological control procedure is dependent on the sample material.

12.4.1 Membrane-filtration method

This method uses membrane filters having a nominal pore size ≤ 0.45 µm whose effectiveness in retaining micro-organisms has been established.

12.4.2 Direct inoculation method

The direct inoculation method is suitable for solutions and SoHO samples (i.e. solid substances). Sample quantities are listed in *Ph. Eur.* 2.6.1.

Microbial growth can be assessed macroscopically for signs of contamination in both methods. During incubation, an intermediate reading and a final reading are made. In the event of turbidity in the culture medium following testing of the sample material, sub-cultivation is performed (refer to *Ph. Eur.* 2.6.1).

Fungi are detected along with aerobic bacteria in soya bean casein digest medium (see *Ph. Eur.* 2.6.1). However, other media and/or incubation temperatures may be used, provided that the method is validated according to *Ph. Eur.* 5.1.6.

12.5 Microbiological testing using automated culture systems (*Ph. Eur.* 2.6.27)

Testing is performed as described in *Ph. Eur.* 2.6.27 supplemented with the following additional instructions for samples containing cellular material.

12.5.1 Incubation conditions

Samples containing cellular material may result in turbidity of the culture media immediately after inoculation. Use of validated automated culture systems, which do not rely on turbidity for pathogen detection, may be advantageous in this case.

Incubation in automated culture systems is carried out over at least 7 days. The testing time can be adapted to specific requirements arising from the characteristics of the preparation. For example, if risk assessment identifies potentially slow-growing micro-organisms such as *Cutibacterium acnes* the testing time could be extended up to 14 days.

The temperature of incubation usually established for clinical microbiology (at 37°C) might be too limited to sufficiently account for a broad range of contaminating micro-organisms found in the environment or in SoHO. Hence, the incubation conditions detailed in Table 12.2 are commonly used as alternatives based on risk assessment, considering the expected micro-organisms and environmental conditions.

12.5.2 Sample volume

For automated culture systems, recommended sample volumes from 100 µL up to 10 mL can be inoculated per culture bottle. Usage for testing of a small percentage part of total volume bears the risk of an increased sampling error, leading to false-negative results, especially if bioburden is low (see also §12.2.6).

12.5.3 Samples with antimicrobiological additives

The type, amount and mixture of antimicrobial growth agents used during collection and processing or used for patient treatment (and therefore present in samples for microbiological testing) is highly variable. Culture media bottles containing neutralising or adsorbing substances are established for the testing of patients' blood with therapeutic doses of a limited number of antibiotics or antimycotics. This ensures that any residual antimicrobial activity is effectively eliminated (see also §12.4). Therefore, such samples should be validated thoroughly for residual antimicrobial activity to prove the suitability of the chosen method.

Table 12.2 **Conditions for microbiological control testing according to *Ph. Eur.* 2.6.27**[a]

	Aerobic incubation	**Anaerobic incubation**
Option 1	20-25°C normally (automated system) 30-35°C if necessary (automated system)	30-35°C (automated system)
Option 2	35-37°C (automated system) and, where relevant, additional incubation at a lower temperature (manual method)[b]	35-37°C (automated system)
Option 3	30-32°C (automated system)	30-32°C (automated system)
Option 4	30-32°C (automated system)	35-37°C (automated system)

[a] According to *Ph. Eur.* 2.6.27, testing period not less than 7 days with an automated growth-based method and may be extended up to 14 days.

[b] Where relevant, incubate in addition at 20-30°C. Incubation can be done using commercially available microbiological media, either aerobic bottles intended for automated systems or tryptic soy broth (TSB).

12.5.4 Period between inoculation of culture bottles and incubation in an automatic culturing system

Inoculated culture media bottles are placed into the automated culture system as soon as possible. In the event of a delay, the temperature is maintained between 15 and 20°C [10]. If the delay exceeds 12 hours, the results from the automated culture system can be verified by subculturing, unless otherwise validated. For certain automated systems and delayed kinetic culture media bottles, manufacturer specifications may allow a delay of up to 48 hours at room temperature. Due to variability in delay times, pre-incubation temperatures and types of micro-organisms, any delay exceeding 12 hours requires validation.

12.6 Microbial enumerations tests (*Ph. Eur.* 2.6.12 and 2.6.13)

The SoHO-associated bioburden of aerobic mesophilic bacteria and fungi can be quantitatively enumerated either by membrane filtration or plate-count methods according to *Ph. Eur.* 2.6.12. The preferred microbiological recovery procedure is dependent on the type of sample. Different mechanical techniques can be used, alone or in combination, to extract micro-organisms from the product before filtration or plate-count method (e.g. sonication, crushing, blending, shaking) if validated. The addition of a surfactant, such as polysorbate 80, can improve the extraction. A correction factor can be calculated from the validation data. The SoHO-containing jar can further be sonicated for 5 minutes (at 47 kHz) followed by mechanical shaking for 30 minutes (at 200 strokes/minute) on a linear reciprocal shaker [8].

12.6.1 Membrane-filtration method

An appropriate volume of the extraction fluid is filtered through at least two appropriate filters (e.g. 0.45 µm pore size nitrocellulose filter). One filter is transferred to the surface of a casein soya bean digest agar plate for determination of the total aerobic microbial count (TAMC) and one filter to the surface of a Sabouraud dextrose agar plate for determination of the total combined yeasts/moulds count (TYMC). The casein soya bean digest agar is incubated at 30-35°C for 3-5 days and the plate of Sabouraud dextrose agar at 20-25°C for 5-7 days. In addition, anaerobic bacteria can be enumerated by transfer of a filter to an appropriate medium plate (e.g. thioglycolate agar) and anaerobic incubation at 30-35°C for 3-5 days. The microbial count can be calculated as colony-forming units (CFU) per unit weight or volume (gram or millilitre) or surface area of SoHO.

12.6.2 Plate-count methods

At least two Petri dishes for each level of sample dilution for each medium are prepared, either by the pour-plate method or by the surface-spread method. Plates of casein soya bean digest agar, Sabouraud dextrose agar and anaerobic medium agar are incubated as stated above. The plates exhibiting the highest colony count below 250 for TAMC and 50 for TYMC, in accordance with a specific dilution, are counted. The arithmetic mean per culture medium of the counts is used to calculate the number of CFUs per unit weight or volume (gram or millilitre) or surface area of SoHO.

For determination of the presence or absence of specified micro-organisms, test conditions are described in *Ph. Eur.* 2.6.13, in which the growth-promoting, inhibitory and indicative properties of specific media are described.

12.7 Alternative methods for detecting microbiological contamination (*Ph. Eur.* 5.1.6)

Alternative microbiological testing methods based on novel technologies that provide automated, rapid and more sensitive microbiological results, as compared with classic or conventional testing methods, may be used to control microbiological quality of pre-processing samples, in-process samples or final SoHO preparations samples, in particular for preparations with a short shelf life. Validation of such alternative microbiological testing methods (examples in Table 12.3) is conducted in accordance with requirements of *Ph. Eur.* 5.1.6.

Table 12.3 **Alternative methods for control of microbiological quality**

Growth-based methods	Electrochemical
	Gas consumption/production
	ATP bioluminescence
	Turbidimetry
Direct methods	Solid-phase cytometry
	Flow cytometry
Cell components methods	Phenotypic
	Genotypic
	NAT
	Genetic fingerprinting
	Sequencing

12.8 Validation or verification of microbiological testing methods

Good Practice Guidelines

G12.21 Whatever the applied method, the method suitability should be verified with respect to specificity, sensitivity and robustness (method suitability test).

G12.22 The microbiological test methods employed for microbiological testing should be verified in the presence of the intended sample material and volume. The method suitability test should be carried out using the bacterial and fungal species indicated in the chapters applicable from *Ph. Eur.*

G12.23 In addition to the prescribed test strains, method suitability should also be demonstrated for SoHO-associated contaminants, when not adequately covered by the test strains prescribed in the *Ph. Eur.* method (e.g. *Cutibacterium acnes* and *Micrococcus* spp., which are typical skin contaminants).

G12.24 For microbiological test samples obtained after decontamination steps, such as treatment with antibiotics and antifungal agents, methods for microbiological testing should be evaluated carefully to assess possible inhibition of microbial growth due to decontaminating agents or their residues.

G12.25 If inhibition of microbiological growth by the sample material is identified at the method suitability test, the method should be adapted in an appropriate way, for instance by using a higher volume of the culturing media or by the addition of binding or enzymatic substances that inhibit anti-microbiological agents.

G12.26 The SoHO entity should determine, based on a risk assessment, whether the suitability test needs to be repeated following significant changes in the collection, processing or storage of SoHO preparations.

G12.27 Interpretation of verification results should include at least the following factors:

- **G12.27.1** assessment of the growth of micro-organisms in the presence of the SoHO to be tested;
- **G12.27.2** specification of the microbial count for evaluation of the method;
- **G12.27.3** period of time until a positive result has been detected for test assays and positive controls;
- **G12.27.4** proof of identity of inoculated micro-organisms.

G12.28 For negative controls or test assays without detection of micro-organisms, the total testing period and results of the subculture (including the methods used) should be specified.

Recommendations

It is recommended that:

R12.5 when validating testing methodologies, consideration be given to the potential impact of sample size and the presence of residual processing and/or decontamination reagents (for example, antibiotics) on the accuracy of the results, since false-negative results are a significant concern in these circumstances;

R12.6 the efficacy of the decontamination procedure for each SoHO type be validated. Additionally, any changes to the decontamination procedure be accompanied by a risk assessment and validation to ensure the new procedure maintains similar (non-inferior) efficacy.

Supplementary information

Some micro-organisms can survive as facultative intracellular parasites, allowing them to reside within host cells where they are shielded from many external decontamination measures. In addition, certain species are capable of entering a dormant, non-growing metabolic state. In this state, they remain viable but inactive, and normal growth resumes once antimicrobial pressure or environmental stress is removed. Because these organisms can persist in protected intracellular niches and can temporarily halt metabolic activity, they are less affected by treatments that rely on active growth or extracellular exposure. These characteristics are essential considerations when assessing the effectiveness and limitations of decontamination and testing strategies, and they are also taken into account during product-specific microbiological risk assessments and when selecting sampling strategies and test methods.

Cutibacterium acnes is not readily accessible to skin disinfection due to its prevalence in the sebaceous glands, and detection of this species in SoHO preparations is not unusual. *Cutibacterium acnes* grows under anaerobic or micro-aerophilic conditions as a 'slow-grower' and is associated with a particularly long detection time, so it may be included in method validation and additionally verification.

12.9 Microbiological examination of donors

Although they do not forecast what the microbiological status of the SoHO will be, microbiological blood cultures can be a useful tool to give a picture of the microbiological status of the donor, especially for the diagnosis of infections in deceased donors [11], in addition to the required serological examinations. In the case of living donors, see Chapter 25 – Haematopoietic progenitor cells from bone marrow and peripheral blood – for more information.

If available, the results might also be helpful information when assessing potential contamination from the collection team and environment, as well as cross-contamination between SoHO preparations, or to

evaluate the efficacy of antimicrobial treatment, if applicable [12].

Recommendations

It is recommended that:

R12.7 action be taken to ensure blood samples are of sufficient quantity, collected properly within a suitable time frame, and prepared/stored appropriately for testing;

R12.8 a clear protocol be followed that addresses skin disinfection, blood volume and the number of blood cultures;

R12.9 skin disinfection be prioritised to avoid secondary contamination;

R12.10 at least a two-step alcoholic disinfection be performed (preferably with sporicidal disinfection);

R12.11 blood cultures (both aerobic and anaerobic) be taken, ideally in different vessels, bearing in mind that larger blood quantities and multiple independent blood cultures improve the probability of detection of an infection in the donor;

R12.12 blood cultures be incubated for ≥ 5 days because such a period generally gives time for the potential micro-organisms to grow;

R12.13 blood samples for culture be obtained:

- R12.13.1 before circulatory arrest, since the results of blood cultures using samples collected before circulatory arrest provide useful information about the clinical status of the donor (e.g. infections with pathogenic organisms) and can be a useful supplementary tool for evaluation of donor suitability and the quality and safety of specific SoHO, especially if the SoHO are not terminally sterilised [6, 11-17];
- R12.13.2 after cardio-circulatory arrest (in which case the organs and SoHO may be at a higher risk of endogenous microbiological contamination), bearing in mind that the information provided by blood cultures may be questionable due to agonal spread and *post mortem* bacterial translocation;

R12.14 in the case of locally acquired contamination or a local infection, the microbial result apply only to the SoHO where the contamination was detected and to SoHO that could have been cross-contaminated. If bloodstream infection (anamnestic or blood culture) or any other distribution of the pathogenic micro-organisms (during collection, storage, transport or manufacturing) cannot be excluded, it is recommended that the SoHO be rejected.

Supplementary information

Manipulation of the deceased donor can lead to dissemination of micro-organisms from the lung and visceral organs to the heart. However, published data suggest that neither agonal nor *post mortem* spread can be expected to produce false-positive cultures if the body is appropriately cooled, the SoHO collection is performed within 24 hours after death and the samples for microbiological culture are collected early during SoHO collection, with minimum possible manipulation of the deceased donor [14, 18].

Nevertheless, a positive culture, in the particular context of organ or SoHO collection, may signify contamination or cross-contamination during procedures, indicating problems with *post mortem* microbial spread, collection techniques and/or the environment.

12.10 Interpretation of results and actions to be taken

Good Practice Guidelines

G12.29 In general, source material that demonstrates contamination should be rejected unless the SoHO preparation undergoes decontamination and/or terminal sterilisation, and the detected bioburden can be reliably inactivated or removed by the intended procedure, or if it is justified by clinical circumstances (refer to Part B for specific examples) [18-21]. In the event that there is no alternative to using the SoHO, measures should include full identification of the contaminating micro-organisms and their antibiotic susceptibility profile, as well as adequate prophylaxis of the donor/recipient, except for SoHO preparations where the natural microbiota is essential for their function and viability (e.g. intestinal microbiota, human milk).

G12.30 Contamination with specific pathogenic micro-organisms should result in the discard of the SoHO preparation unless treated with a sterilisation process validated to eliminate the infectivity of such organisms [18-22]. If applicable, a non-exhaustive list of pathogens whose presence necessarily excludes the collected SoHO from further processing should be established for each type of SoHO preparation based on Table 12.4 and the SoHO establishment/entity's own experience. A risk assessment based on the urgency of the application, judgement of infection risk and treatment options should be conducted.

G12.31 If release of the SoHO preparation is necessary before the end of the officially verified/required incubation period, a 'negative-to-date' reading of the results should be carried out. In this case, intermediate results of the final testing in combination with final results of in-process controls should be used for SoHO release.

G12.32 Before release of the SoHO preparation a risk-assessment analysis of the microbiological results should be conducted to assess the suitability of the SoHO preparation, considering the microbiological results of other SoHO from the same donor and the immune status of the recipient.

G12.33 If micro-organisms are detected after the release of SoHO preparations, pre-defined measures such as identification and antibiotic susceptibility profile of the species should be carried out, and information should be provided to clinicians caring for the patient.

Recommendations

It is recommended that:

R12.15 contaminated source materials be assessed, using an exclusion list for pathogenic micro-organisms, and their bioburden status evaluated;

R12.16 particular attention be paid to multidrug-resistant micro-organisms, potential toxin-producing micro-organisms, yeasts and filamentous fungi, and the SoHO preparations are rejected if that is appropriate (see Table 12.4).

Table 12.4 **Micro-organisms[a] that, if identified in pre-processing SoHO samples, will trigger Recommendation R12.16 to discard the SoHO[b] unless treated with a validated decontamination or sterilisation process to eliminate infectivity of such organisms**

Staphylococcus aureus, *Staphylococcus lugdunensis*
Streptococcus spp., *Enterococcus* spp.
Clostridioides (Clostridium) spp.
Enterobacteriaceae (e.g. *Escherichia coli, Enterobacter* spp., *Salmonella* spp., *Shigella* spp., *Klebsiella* spp.)
Yeast and filamentous fungi (moulds)

[a] Except in specific cases where a documented risk assessment determines that the clinical benefit justifies the risk (e.g. haematopoietic progenitor cell transplantation), where the decision is life-saving and where antibiotics can be applied to the recipient. In those cases, performance of a case-specific risk assessment is recommended.

[b] Intestinal microbiota is excluded from this table; please refer to Chapter 33 for guidance on intestinal microbiota.

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Chapter 13. **Release, distribution and import/export**

13.1 Introduction

This chapter sets out the procedures for the release and distribution, import and export of substances of human origin (SoHO) and SoHO preparations that are deemed suitable for clinical application; it also describes the requirements for distribution of SoHO preparations and defines recommended controls for their import and export.

For the purposes of this chapter, the definitions of terms are as given below:

- Release: a process through which it is verified that a SoHO preparation meets defined quality and safety criteria and fulfils the conditions of any applicable authorisation, before distribution or export.
- Distribution: the process of transporting and delivering released SoHO preparations intended for human application, either in general or for a specific recipient, whether within the same SoHO entity responsible for human application (ORHA) or in another ORHA, or intended for the manufacture of products regulated by other legislation to a manufacturer of such products.
- Transport: the act of transferring a SoHO preparation between facilities under the control of suitably trained, designated and authorised staff at the distributing and receiving facilities. 'Shipment' is a type of transport where the transfer of the SoHO from the distributing to the receiving facility is carried out by means of a contract with a third party, usually a specialised logistics company.
- Import: activities carried out to bring SoHO preparations into one country from another country before their release.
- Export: activities carried out to send SoHO preparations from one country to another country.

In the European Union (EU) 'import' and 'export' refer to movements of SoHO preparations from and to a third country outside the EU. A third-country supplier is an organisation located outside the EU that is contracted to supply SoHO preparations or to perform activities that might influence the quality and safety of the SoHO preparations imported.

13.2 Release

Standards

S13.1 SoHO establishments must have a procedure in place to ensure that, prior to release, the relevant authorisations are complied with, and the quality and safety of SoHO preparations released have been verified and documented by the release officer (RO) (Art. 60).

S13.2 SoHO preparations for autologous use or within-relationship use, without storage, do not require release before human application but the SoHO preparation authorisation must include a specification of the quality control parameters to be monitored during processing (Art. 60).

Good Practice Guidelines

G13.1 Prior to release, a comprehensive record review should ensure that all elements of collection, donor assessment and testing, processing, quality control and temporary storage have met the established quality criteria, including the identity of the SoHO.

G13.2 The RO, or personnel designated by the RO, should register (in writing or digital format) the review and fulfilment of all legal requirements, SoHO specifications and quality-release criteria, thereby releasing

the SoHO for storage in an inventory of SoHO that are available for human application.

G13.2.1 If release cannot be approved, the SoHO should be discarded or can be made available for non-clinical use (research/educational use), if a specific consent for research/educational use was given.

G13.3 Packaged SoHO should be examined visually for appropriate labelling and container integrity.

Recommendations

R13.1 It is recommended, before release, that the following documentation be reviewed where relevant:

- R13.1.1 approval of donor eligibility;
- R13.1.2 consideration and approval of the processing and storage records;
- R13.1.3 final evaluation of the label and container to ensure traceability, accuracy and integrity;
- R13.1.4 results of screening tests on incoming SoHO preparations;
- R13.1.5 in-process controls;
- R13.1.6 specifications for final release of SoHO preparations based on testing results used to determine final release (e.g. quality controls such as viability or cells count, microbiology test results, biological activity);
- R13.1.7 evaluation of any non-conformity registered from collection until release.

13.3 Exceptional release

Standards

S13.3 Under exceptional circumstances, SoHO preparations can be released to an intended recipient even in cases where the preparation does not meet all the relevant standards or its process authorisation, or in cases of authorised import of preparations for immediate human application to a specific recipient (Art. 61, Art. 26.6).

S13.4 Exceptional release must be authorised on the basis of a documented request from the physician treating the intended recipient, where such a request includes a confirmation of full knowledge of, and agreement to, any deviation or reason for exceptional release. The intended recipient, or person granting consent on their behalf, must be informed of the exceptional release and required to give consent in accordance with national legislation prior to the human application (Art. 61.2).

S13.5 The SoHO establishment physician must document the benefit–risk assessment and the agreement with the treating physician (Art. 61.2) and authorise exceptional release by the RO (Art. 61.1 & Art. 61.2).

S13.6 The exceptional release condition must be explicitly indicated on the label or in the documentation accompanying the released SoHO preparations (Art. 61).

S13.7 Exceptional release for export must be authorised on the basis of a documented request from a treating physician, or regulatory authority in another country, including confirmation of full knowledge of, and agreement to, any deviation from the EU SoHO Regulation (Art. 61.3).

S13.8 Exceptional release for the manufacture of a product intended for a specific recipient must take place on the basis of a documented request from the manufacturer, including confirmation of full knowledge of, and agreement to, any deviation from the EU SoHO Regulation (Art. 61.4).

Good Practice Guidelines

G13.4 In the case of microbiological contamination of autologous SoHO, or SoHO received from a specific allogeneic donor whereby a repeated collection cannot be conducted or involves a high degree of risk, the risk assessment and risk–benefit analysis should be based on the nature and extent of the contamination and should specifically consider the risk based on identification of the contaminating micro-organisms and the potential for adequate prophylaxis of the intended recipient.

G13.5 If release of the SoHO is necessary before the end of the verified or officially required incubation period, a 'negative-to-date' reading of the results may be carried out. In this case, intermediate results of the final testing in combination with final results of in-process controls should be used for SoHO release.

G13.6 If micro-organisms are detected after SoHO release, pre-defined measures such as identification and antibiotic sensitivity of the species should be carried out, and information should be provided to clinicians caring for the patient.

13.4 Distribution

Standards

S13.9 SoHO establishments must collect data relating to their distribution activities and submit an annual report of the data collected to their Health Authority (HA) or, in the EU, to the EU SoHO Platform as instructed by their HA (Art. 41.1, Art. 41.4 & 41.5).

Good Practice Guidelines

G13.7 SoHO for clinical application should not be distributed without an order from a clinician or other authorised person at the ORHA.

G13.8 Distribution for clinical application should be restricted to authorised ORHAs or to authorised health professionals and should comply with all applicable national laws and regulations. In cases where SoHO require additional procedures (such as thawing) to

be undertaken by the ORHA, the associated specific instructions should be provided to the ORHA by the SoHO establishment.

G13.9 A validation or verification of the distribution process should be documented and used as reference to any procedure resulting from that distribution process.

G13.10 Written agreements for the shipment of SoHO preparations should be in place between the shipping SoHO entity and the SoHO establishment or advanced therapy medicinal product (ATMP) manufacturer.

Supplementary information

For transfers of SoHO preparations between countries that are within the EU, such transfers usually being referred to as 'distribution', the EU legislation [1] provides for equivalence of requirements between EU member states. SoHO preparations coming from a duly authorised SoHO establishment under duly authorised processes by the HA might not require additional control. However, some EU member states may opt to apply more stringent requirements (e.g. no direct distribution to an ORHA, additional testing requirements, additional labels and/or product inserts in national language(s), etc.).

13.5 Transport

Good Practice Guidelines

G13.11 The container/package should be secure, and transport or shipment conditions such as temperature and duration should be defined to ensure maintenance of the required properties of the SoHO preparations.

G13.12 When it is necessary to maintain temperature within certain limits during transport or shipment of SoHO, temperature loggers or temperature indicators should be used, or the transport or shipment of SoHO should be validated under the worst-case scenario.

G13.13 The mode of transport or shipment should comply with the applicable laws and regulations on transportation of biological substances. Appropriate, legible labelling should be included; see Chapter 16.

Recommendations

R13.2 It is recommended that an alternative plan of transport or shipping be made available in case of emergency situations, to prevent possible clinical complications for the recipient.

13.6 Documentation

Good Practice Guidelines

G13.14 The SoHO establishment should demand from the courier physical records or access to digital registries of pick-up and delivery. In this way the SoHO establishment can ensure complete traceability of the SoHO.

G13.15 Records of the distribution of SoHO should be retained, and reports should be available that detail:

- **G13.15.1** name of party responsible for distribution;
- **G13.15.2** identification of establishment, courier or individual who transported SoHO at any stage between collection and end use;
- **G13.15.3** packaging;
- **G13.15.4** date and, if critical for quality and safety of the SoHO, time of distribution and delivery;
- **G13.15.5** identification of receiving entity, clinician or ORHA;
- **G13.15.6** any incidents that occurred during distribution;
- **G13.15.7** identification code (in the EU, the Single European Code [SEC]).

13.7 Recall and return procedures

Good Practice Guidelines

G13.16 The SoHO establishment should have personnel authorised to assess the need for recall and to initiate and co-ordinate the necessary actions.

G13.17 A recall procedure should be in place that includes a description of the responsibilities, actions to be taken within pre-defined periods of time and requirements for notification to the HA(s).

G13.18 Recall actions should be taken within pre-defined periods of time and should include tracing all relevant SoHO and, where applicable, should include trace-back.

G13.19 The progress of the recall process should be recorded and a final report issued, including reconciliation of the delivered and recovered quantities of the SoHO preparations.

G13.20 The effectiveness of the arrangements for recalls should be periodically evaluated.

Supplementary information

In the case of a recall because of an adverse reaction in the recipient, the purpose of the investigation is to identify whether any other SoHO prepared from the same donor might have contributed to causing the adverse reaction. If so, and if concerns exist about the safety of those SoHO, the OHRAs and consignees are notified, to retrieve any of those unused SoHO. Recall may also be applicable in case of an adverse event but may also be

related to any step prior to the human application of the SoHO where the event in question has the potential to affect the quality and/or safety of the SoHO.

13.8 Import and export

13.8.1 Import

Standards

S13.10 SoHO entities must collect data relating to their import and export activities and submit an annual report of the data collected to their HA or, in the EU, to the EU SoHO Platform as instructed by their HA (Art. 41.1, Art. 41.4 & 41.5).
S13.11 Prior to importing SoHO preparations, SoHO establishments must have authorisation from their HA to import (Art. 47).
S13.12 SoHO establishments must put in place written agreements with their third-country suppliers (Art. 48.2). These written agreements must include at least (Art. 48.3):

- Details of the third-country supplier contracted.
- The requirements to be met to ensure quality, safety and effectiveness of the SoHO preparations imported.
- In the EU, the right of the Competent Authority to inspect activities, including facilities of any third-country supplier or entity sub-contracted by that supplier.

S13.13 The SoHO establishment must be responsible for the physical reception and visual examination and verification of imported SoHO preparations prior to their release. The SoHO establishment must verify coherence between the SoHO preparations received and the associated documentation, and must conduct an examination of the integrity of packaging, labelling and transport conditions (Art. 48.4).
S13.14 The RO of the importing SoHO establishment must release imported SoHO preparations for distribution after verifying their compliance with the quality and safety and effectiveness requirements and when the physical and documentation controls are satisfactory (Art. 48.5). SoHO establishments may delegate the physical reception, visual examination and verification to the SoHO establishment that will apply the SoHO preparations to the recipient in cases where the import is organised for a specific recipient (Art. 48.6) and the release may be completed remotely.
S13.15 Importing SoHO establishments must ensure an equivalent level of traceability with regard to the imported SoHO (Art. 42.1).

Good Practice Guidelines

G13.21 The importing SoHO establishment should require that any changes to authorisation status be immediately communicated by the exporting facility.
G13.22 Acceptance at the SoHO establishment should include a documented procedure to verify compliance with the written agreement in place with the exporter.
G13.23 Containers should be examined for any evidence of tampering or damage during transport.
G13.24 SoHO should be stored in quarantine in an appropriate secure location under defined conditions until they, along with the accompanying documentation, have been verified as conforming to requirements.
G13.25 The importing SoHO establishment should identify and code the imported SoHO with the appropriate identification code (in the EU, the SEC) and in accordance with applicable regulations; see Chapter 16.
G13.26 A written agreement between the exporting SoHO establishment and importing SoHO establishment should clearly define the relevant roles and responsibilities, including procedures for transport, packaging and required shipment conditions.
G13.27 The agreement should specify how SoHO preparations will be identified, and a unique identifying code should allow unambiguous identification and traceability.

Recommendations

It is recommended that:
R13.3 potential language barriers be considered and a common language agreed for all donor and SoHO-related documentation;
R13.4 transport (preferably direct) from exporting SoHO establishment to importing SoHO establishment be arranged, without intermediate stops when possible, using an approved courier;
R13.5 any non-compliance be reported to the exporting SoHO establishment;
R13.6 any third-country supplier be audited to verify that the requirements ensuring the quality, safety and effectiveness of the SoHO being imported have been met.

Supplementary information

Except for emergency situations, as referred to in S14.4, SoHO establishments intending to import SoHO will (ideally) provide a clear justification – either demonstrating that the need cannot be adequately met by comparable material from domestic sources or presenting another valid reason for the import. This may include the justification of the import in terms of accessibility, quality, speed of supply, risk of infection, quality of service, cost-effectiveness, or scientific or research needs. Companies that import and/or export SoHO have responsibilities equivalent to those of SoHO establishments for ensuring the equivalent requirements for safety and quality, for maintaining traceability and for having adequate vigilance systems in place.

13.8.2 Customs and security clearance

Recommendations

R13.7 It is advised that all SoHO supplied from abroad have a clear description of the content of the consignment and its destination. This is to ensure that frozen SoHO, which are usually packed in dry ice or stored in a dry-shipper, as well as fresh SoHO for urgent medical need, are not delayed at border crossings for customs clearing.

Supplementary information

For information about labelling, please see Chapter 16.

In daily practice there is a consensus that viable SoHO preparations, including stem cells, gametes and embryos, are not exposed to irradiation.

A study published in 2002 concluded that even 10 passages through the hand-luggage control system resulted in no harm to haematopoietic progenitor cells (HPC) and lymphocytes in terms of viability and potency. Interestingly, the radiation dosage of passage through the hand-luggage control system is of 1.5 + 0.6 µSv compared to a radiation dose of 60 µSv received by the HPC during a 10 h flight [2]. Based on current knowledge, the low radiation exposure from the hand-luggage control system can be assumed to have no effect on cell viability and/or potency. If damage does occur, it is more likely to be caused by radiation during the flight.

It may be expedient for the importing SoHO establishment to inform Customs in advance of a prospective consignment and that any enquiries by Customs be answered promptly. The agreement with the exporter could include the responsibility for meeting the cost of transport, refrigeration and/or storage at a Customs facility for any items that may be detained pending Customs enquiries.

13.8.3 Export

Standards

S13.16 Within the EU, SoHO establishments must ensure that SoHO preparations released for export comply with the requirements of the EU Regulation (Art. 51.1).

S13.17 SoHO preparations may be released for export in the case of exceptional release, but in those cases SoHO establishments must ensure that they comply with requirements of donor protection, vigilance and traceability (Art. 51.2).

Good Practice Guidelines

G13.28 Exported SoHO should be collected, handled, stored, transported, used and discarded in accordance with the consent that has been given by the donor, when applicable.

G13.29 SoHO preparations should be exported only to countries that have proper controls on the use of donated material and only for the purposes for which they can lawfully be used in the country of destination.

G13.30 Documentation obtained from the exporting SoHO establishment should be archived for the time period required by national regulations (e.g. 30 years in EU member states).

Recommendations

It is advised that:

R13.8 a SoHO establishment carefully checks the entry conditions for each country if it wants to export SoHO and that it enters into written agreements to clarify the rights and responsibilities of the parties involved;

R13.9 SoHO preparations are not to be exported if there is an unmet clinical need for the material in the country of origin.

13.9 International co-operation

For many patients waiting to be treated with HPC it is difficult to find a matching donor in their own country. To facilitate a global search for a matching donor, the World Marrow Donor Association (WMDA) operates a search and facilitation platform that links national registries; see Appendix 32.

To harmonise the quality of SoHO preparations worldwide, professional societies (FACT, JACIE, WMDA, EFI, ASHI, NetCord-FACT) have established accreditation and certification programmes [3-5].

13.10 Health emergency situations

Standards

S13.18 Distribution of SoHO without preparation authorisation can take place when justified by a health emergency situation (Art. 65), but the SoHO establishment must prove that:

S13.18.1 the human application of the SoHO preparation is in the interest of public health;

S13.18.2 the SoHO preparation has a level of quality and safety that is acceptable considering the available data indicating a positive benefit–risk assessment;

S13.18.3 the SoHO preparation is for immediate human application to a defined group of SoHO recipients who have no other therapeutic alternative, treatment cannot be postponed, the prognosis is life-threatening and the expected benefit outweighs the risk;

S13.18.4 the intended recipients or, where applicable, persons granting consent on their behalf, must be informed about the intended application of SoHO without preparation authorisation and

must give their consent to the immediate human application, in accordance with national legislation, prior to human application.

13.11 References

1. Regulation (EU) 2024/1938 of the European Parliament and of the Council of 13 June 2024 on standards of quality and safety for substances of human origin intended for human application and repealing Directives 2002/98/EC and 2004/23/EC (Text with EEA relevance), available at https://eur-lex.europa.eu/legal-content/EN/TXT/?uri=OJ:L_202401938, accessed 26 July 2026.
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3. Netcord–Fact International Standards for Cord Blood collection, banking, and release for administration, 8th edn, v.8.2, 11 February 2025, available at www.factglobal.org/standards/cbb-standards/, accessed 10 September 2026.
4. WMDA, available at https://wmda.info/ensuring-quality/, accessed 27 February 2026.
5. WMDA, Courier Company Certification Programme, available at https://share.wmda.info/x/vgATIQ, accessed 27 February 2026.

Related material
Appendix 32. Donor search through registries for haematopoietic progenitor cell transplantation

Chapter 14. Interaction between SoHO establishments and organisations responsible for human application

14.1 Introduction

An organisation responsible for human application (ORHA), a type of SoHO entity in the EU, is a healthcare facility that carries out human application of substances of human origin (SoHO). ORHAs play a key role in protecting the health of recipients and have a responsibility to get informed consent for clinical application of SoHO and/or SoHO preparations, to maintain traceability, to identify and report any serious adverse reactions related to clinical application of those SoHO preparations, to participate in the investigation of reported incidents and (where required) to implement corrective or preventive actions [1-3]. The type and frequency of interactions between a SoHO establishment and/or SoHO entity and an ORHA may vary, depending on the type of SoHO, geographical location and clinical environment. Having a collaborative approach with well-defined roles and responsibilities is beneficial to all stakeholders.

The remit of this chapter is human application of SoHO preparations in ORHAs. If additional activities are carried out in ORHAs such as donor recruitment, collection, storage, distribution, import or export, please see relevant chapters for applicable standards and guidance, and Chapter 2 for Quality Management.

Standards

S14.1 ORHAs are considered as SoHO entities in the EU and must register their activity with the Health Authority (HA) or relevant register before commencing any activity (Art. 35.1). The registered activities must be carried out by persons who operate within a registered entity (Art. 35.2). ORHAs must maintain and update the required Quality Management System (Art. 37.1) for registered activities.

S14.2 ORHAs must collect data relating to their SoHO human application activities and submit an annual report of the data collected to their HA or, in the EU, to the EU SoHO Platform as instructed by their HA (Art. 41.1, Art. 41.4 & Art. 41.5).

14.2 Core principles for organisations responsible for human application on ordering and applying SoHO preparations

Standards

S14.3 ORHAs must not (Art. 58.12):

S14.3.1 apply SoHO preparations to recipients unnecessarily without clinical justification, although they must make optimal use of SoHO, taking into account therapeutic alternatives and following the most up-to-date guidelines;

S14.3.2 advertise or promote particular SoHO to potential recipients, or to any persons granting consent on their behalf or to healthcare professionals, and neither must they use information that is misleading, especially about the potential use and benefit to recipients, or information that minimises the associated risks of the SoHO concerned.

S14.3.3 apply allogeneic SoHO for purposes other than the prevention or treatment of a medical condition, including through reconstructive surgery, or for medically assisted reproduction (MAR).

Good Practice Guidelines

G14.1 SoHO preparations should be requested by a clinician or other authorised person at the ORHA.
G14.2 Clinicians intending to use SoHO preparations for human application should be aware of the risks associated with the application of SoHO preparations and consider the risks and benefits of the procedure to the intended recipient.

14.3 Choosing a supplier of SoHO

Good Practice Guidelines

G14.3 ORHAs should obtain SoHO preparations from an authorised SoHO establishment or through a registered SoHO entity.

Supplementary information

In most cases, collected SoHO require processing and storage at the SoHO establishment before their distribution to ORHAs for human application. Sometimes, the ORHA may have to use an organisation that mediates distribution between SoHO establishments and ORHAs. In certain cases, collection entities may send collected SoHO directly to the ORHA for immediate transplantation without any intermediate steps at a SoHO establishment, such as processing or storage. The ORHA has the responsibility to make sure that the SoHO preparations are obtained from a SoHO establishment, or the distributor/ collection entity authorised or registered as a SoHO entity appropriately by an HA (G14.1). This will provide assurance that the donors of SoHO have been selected and tested in compliance with the legal and technical standards on quality and safety, and that all quality system requirements are in place for the collection, processing, storage and distribution of SoHO in the SoHO establishment/entity.

In the case of collection of haematopoietic progenitor cells (HPC) or lymphocytes for unrelated allogeneic use, there is no direct interaction between the collection entity and the ORHA before cell collection. This means that the ORHA does not directly select the collection facility that will collect the HPC or lymphocytes. Suitable donors are identified through donor registries, which in the EU are considered as SoHO entities. The registry is then responsible for ascertaining whether the collecting facility complies with appropriate quality and safety standards, including traceability of the collected cells.

14.4 Importing SoHO from other countries

Standards

S14.4 In emergency situations, if an ORHA wishes to import SoHO directly for immediate clinical application for a specific recipient, they must request from their HA a derogation of the requirement for an importing establishment, setting out justification based on clinical circumstances on a case-by-case basis (Art. 47.3). The HA may also authorise imports in emergency situations for immediate human application to SoHO recipients whose health would be seriously endangered without such an import of SoHO (Art. 26.6).

Supplementary information

If an ORHA intends to import SoHO preparations from another country (for the EU, that means from countries outside the EU) on a regular basis and takes responsibility for release, HA authorisation for import as a SoHO establishment is required as it is a regulatory requirement in EU countries. Please see Chapter 13, section 13.8 for standards and Good Practice Guidelines (GPGs) for importing SoHO establishments.

The importing SoHO establishment takes responsibility for the verification that the quality and safety standards are met, and that the SoHO preparation is suitable for clinical application.

In certain cases, and particularly in the case of HPC, national and international donor registries play a key role in the organisation of the import of matching stem cells for an individual recipient. These registries can take responsibility for verifying compliance with required quality and safety standards, provided in the EU they have authorisation as importing establishments. They may delegate the steps of physical checks on imported SoHO preparations and their documentation to the ORHA receiving and applying the SoHO preparations. Similarly, an authorised SoHO establishment may import SoHO preparations without physically receiving them and arrange for the SoHO to be sent directly to an OHRA for application to a specific recipient; see Chapter 13.

14.5 Accepting SoHO preparations on exceptional release

Standards

S14.5 The physician or surgeon treating the intended recipient must confirm full knowledge of, and agreement to, any deviation from quality standards as the basis of exceptional release and confirm acceptance of SoHO preparations for the intended recipient based on a documented risk–benefit assessment shared by the SoHO establishment physician.

The intended recipient, or person granting consent on their behalf, must be informed of the exceptional release and must give consent in accordance with national legislation prior to the human application (Art. 61.2).

S14.6 The SoHO establishment and the ORHA must establish a plan, where applicable, for monitoring the recipient's health after clinical application in relation to risks associated with the SoHO preparations applied after exceptional release, for a specified time period (Art. 61.2).

Supplementary information

The responsibilities and relevant standards applicable for a SoHO establishment approving exceptional release are detailed in Chapter 13, section 13.3.

The clinician responsible for the care of the recipient may agree to accept SoHO preparations that do not meet the normal release criteria for clinical application for a specific individual after discussion with the SoHO establishment physician responsible for authorising this exceptional release; see section 13.3. This decision is on the basis of a risk–benefit analysis, taking into consideration the alternative options for the individual and the consequences of not providing the SoHO preparations. The risk assessment giving the reason for the exceptional release of the SoHO is documented by the SoHO establishment physician and is shared with the treating clinician. The treating clinician takes responsibility to inform the recipient as part of the normal consenting procedure of the intention to use SoHO under exceptional release and agree a clinical monitoring plan with the SoHO establishment physician where applicable (see §14.6).

14.6 Recipient consent

Standards

S14.7 An ORHA applying SoHO preparations to recipients must obtain their consent or, where relevant, that of any person granting consent on their behalf, in accordance with national legislation, for the human application of SoHO preparations (Art. 58.15).

S14.8 ORHAs must inform the recipient(s) or any person granting consent on their behalf of, at least, the following:

S14.8.1 the safeguards intended to protect the personal data, including health data, of the recipient(s) and, where relevant, of the offspring from MAR;

S14.8.2 the need for the recipient(s) to report back any unintended reactions following the human application of SoHO preparations or any serious genetic conditions in offspring from MAR with third-party donation (Art. 58.15).

S14.9 SoHO recipients taking part in a clinical study, or the persons granting consent on their behalf, must be informed that the SoHO has not yet been authorised for routine clinical use and it is applied in the context of a clinical study that forms part of the authorisation process for that SoHO preparation (Art. 40.3).

Supplementary information

The information given to a prospective recipient to obtain an informed consent would include information on the reason for use of the SoHO preparation, its potential benefits and risks, and available alternatives (see §14.2 above). The risks can be explained with a description of any adverse outcomes that have been reported for the given type of SoHO preparation and estimated frequency, whether the treatment is well established or if it involves novel methods of processing/clinical application.

Where the collection of clinical follow-up data is proposed, including use of national or international registries (see §14.13), recipients may need to provide consent for the sharing and secondary use of their data in accordance with national legislation and guidance.

In the EU, the General Data Protection Regulations (GDPR, Regulation EU 2016/679) provide certain exemptions from the need for consent for the collection and use of such data under Articles 6.1.e and 9.2.h. However, recipients still will be informed about the collection, registration and use of their clinical data and, although they do not need to give consent, they do have the right to refuse to allow their data to be collected and used. However, their therapeutic care will remain unchanged. Moreover, they can request that their data be withdrawn at any time even though they may not initially have refused their permission.

14.7 Checks by ORHAs on accepting delivery of SoHO preparations

Good Practice Guidelines

G14.4 When SoHO are received by an ORHA, authorised personnel should verify and record whether:

G14.4.1 the SoHO preparations received correspond to what was ordered and to the information in the accompanying documentation, and that this is complete and legible;

G14.4.2 the shipping containers and primary containers are labelled with the required information – including, where appropriate, the Single European Code (SEC) – and that labels are affixed and legible (see Chapter 16), and also that separate accompanying documents provide any other relevant information that is not on the primary container label;

G14.4.3 the shipping container and primary container are intact;

G14.4.4 the specified expiry dates of SoHO have not been exceeded;
G14.4.5 the transport temperature range was monitored when necessary or maintained adequately.
G14.5 Where the delivered SoHO do not comply with the stated requirements, the ORHA should liaise with the SoHO establishment to decide the correct course of action, which could include disposal of the SoHO preparations or their return to the SoHO establishment.

Supplementary information

For SoHO preparations that are transported at low temperatures, maintenance of the required transport temperature can be confirmed by data readout from a temperature logger placed in the shipping container (depending on type of SoHO preparation) or by a residual coolant in the container (e.g. water ice for refrigerated SOHO and dry ice or vapour/ liquid nitrogen for frozen SoHO). An ORHA can request a supplying SoHO establishment to provide a validation report to show that the required temperature can be maintained in the shipping container for a period of time that exceeds the maximum duration of transport.

14.8 Package insert/instructions and temporary storage before use

Good Practice Guidelines

G14.6 ORHAs should follow the instructions for storage and handling of SoHO preparations according to the guidance of the supplying SoHO establishment.
G14.7 Where a SoHO preparation is stored from receipt at an ORHA until clinical application in a temperature-controlled environment, the storage device (e.g. refrigerator, freezer, liquid nitrogen storage tank, incubator) should meet the following requirements:

- be regularly maintained, be qualified and have functional alarms to alert for temperature excursions;
- be dedicated to the storage of healthcare products and cleaned according to a defined protocol and frequency;
- be stored in a secure location with restricted access.

Recommendations

It is recommended that:
R14.1 the ORHA contact the SoHO establishment for advice regarding actions to be taken if the temperature is outside defined limits or in the event of equipment/power failure;
R14.2 during the storage of SoHO preparations at the ORHA before clinical application, the SoHO preparations be kept together with their associated documentation or that steps are taken to ensure that the documentation is reliably linked to the SoHO preparations and easily accessible.

Supplementary information

Once SoHO have been distributed by a SoHO establishment for clinical use, the appropriate storage and handling become the responsibility of the ORHA. In the EU, storage in the ORHA is also a SoHO activity that needs appropriate registration. SoHO preparations are stored under various temperature conditions, depending on their type, method of preservation and packaging. Failure to monitor and maintain controlled temperatures can result in waste of a precious resource and, if the SoHO are used, serious adverse outcomes due to deterioration in their quality.

14.9 Inspection of the container, documentation and SoHO prior to application

Good Practice Guidelines

G14.8 Before human application, the container/packaging should be inspected, and the accompanying documentation reviewed and confirmed to be complete and legible.
G14.9 The label should be checked and compared with the description on the package insert to confirm that the material is indeed what was ordered for the patient and is what is shown on the label.
G14.10 The packaging and the contents should be inspected for any signs of damage during transport or after local storage.
G14.11 Where temperature during transport and storage at the ORHA is critical, there should be confirmation that the required temperature has been maintained.

14.10 Preparation of SoHO before use

Good Practice Guidelines

ORHAs should follow:
G14.12 instructions for opening the container or package;
G14.13 instructions for any required manipulation or reconstitution (e.g. thawing, washing, rehydration);
G14.14 information on expiry time limit after opening/manipulation.

Supplementary information

Any deviation from the instructions provided by the SoHO establishment is at the discretion of the clinical user, who takes full responsibility, based on benefit–risk analysis, for any adverse outcome resulting from not adhering to the instructions provided by the SoHO establishment.

Routine steps applied to SoHO preparations at ORHA, to get them ready just before their clinical application, are not considered as a processing activity. Examples include thawing SoHO preparations following instructions provided by the SoHO establishment, shaping of tendons or bone grinding for impaction grafting.

14.11 Surplus or unused SoHO

Good Practice Guidelines

G14.15 Any SoHO preparation issued for use in a named patient should not be redirected to another recipient without informing the SoHO establishment that distributed the SoHO preparation and maintaining traceability to the new recipient.

G14.16 Any unused surplus material should not be used for quality or non-clinical use without checking with the SoHO establishment that supplied the SoHO that a valid donor consent for non-clinical use is in place.

G14.17 Any unused surplus material of a SoHO preparation remaining after application in a clinical procedure in one patient should not be used in another recipient.

G14.18 Any part or whole SoHO preparation that is not used clinically should be discarded as clinical or anatomical waste, in accordance with national rules, or returned to the supplying SoHO establishment for appropriate and lawful disposal.

G14.19 SoHO provided to one ORHA should not be transferred to another ORHA for clinical application without agreement of the SoHO establishment.

G14.20 SoHO establishments that do accept the return of unused and unopened SoHO should be able to confirm that the required storage conditions have been maintained, that the packaging has not been tampered with and that the quality and safety of the SoHO have not been compromised.

14.12 Traceability

Coding and traceability are addressed fully in Chapters 16 and 17.

Good Practice Guidelines

G14.21 The ORHA should keep an electronic or paper log of all SoHO preparations received, stored, transplanted or discarded.

G14.22 Details of the SoHO preparations applied should be in the recipient's record and in the record of the treatment room or operating theatre where they have been applied.

G14.23 ORHAs should have a system for rapid identification of SoHO preparations and an audit trail to identify the SoHO preparations and to link them to the recipient(s) of SoHO preparations in the event of recall or serious adverse reactions/events (SAR/SAE) related to the SoHO preparations.

G14.24 ORHAs should have a process in place for archiving records that are necessary to maintain traceability.

Recommendations

R14.3 It is recommended that when individuals who have been treated with SoHO preparations are discharged from an ORHA, their discharge documentation specifically mention this fact.

Supplementary information

Some SoHO establishments require the ORHA to return a traceability form or card providing details that will be sufficient to unambiguously identify the recipient and the applied SoHO preparations. In addition, the ORHA can retain a copy of the documentation in the recipient's medical record because returning the card does not release the ORHA from its responsibility to maintain the above-mentioned traceability records for 30 years after clinical use or final disposal. Where cards or forms are returned to the SoHO establishment, the national data-protection regulations still apply to ensure confidential information is stored in secure systems and that the recipient's privacy is not compromised in any way.

When a discharge summary mentions the use of SoHO preparations in a recipient, general practitioners looking after the patient in the longer term will be able to associate unexpected symptoms with possible transmission of disease or other reactions from the SoHO preparations applied and contact the ORHA for follow-up as required.

14.13 Recipient follow-up and clinical outcome registries

Good Practice Guidelines

G14.25 ORHAs should ensure before submission of data to any registry that the clinical registry has data quality management procedures in place that ensure adequate accuracy and completeness of data.

Supplementary information

Depending on the healthcare system, routine clinical follow-up of the SoHO preparations recipient is performed either by the ORHA or by another healthcare organisation. Clinical follow-up data are kept at the ORHA in the recipient's records and may be submitted to national/international clinical outcome registries with appropriate consent. Some registries are maintained by scientific or professional associations whereas others are held by HAs. In some countries there may be legal obligations to collect clinical follow-up data. Registries have been established for several SoHO preparations, including HPC, MAR and corneal transplantation.

Clinical outcome registries provide real-world data that may give a more realistic overview of outcomes compared with single-centre studies. Registries fulfil an important role in allowing studies on large datasets that can be used to determine recipient outcomes for a wide range of conditions, evaluating factors that influence clinical outcome or that may increase the risk of adverse events, and for validation of SoHO establishment protocols and practices [4].

The level of clinical follow-up to support an application for preparation authorisation of a new or novel SoHO preparation is determined by risk assessment using standard methodology such as the EuroGTP II tool. See Chapter 19 for more information on how an ORHA and SoHO establishment could work together in developing and getting approval for new applications with robust clinical monitoring plans.

14.14 Adverse events and reactions

General aspects on biovigilance are fully addressed in Chapter 17; responsibilities specific to the ORHA are given below.

Standards

S14.10 ORHAs must maintain a system for detecting, investigating and recording information concerning adverse reactions and adverse events (Art. 44.1).
S14.11 All reasonable efforts must be taken to encourage prospective parents of children born from third-party donation to communicate information concerning serious genetic conditions, as soon as they emerge in the children, to the ORHA where they were treated. The ORHA must communicate, without undue delay, that information to the SoHO establishment that released the reproductive SoHO for human application, in accordance with national legislation on the storage and use of reproductive SoHO (Art. 44.2). See S18.4.
S14.12 The ORHA must communicate adverse reactions or adverse events to the SoHO establishment/SoHO entity that distributed the SoHO preparations to them (Art. 44.4).

Good Practice Guidelines

G14.26 The ORHA should follow instructions provided by the SoHO establishment that supplies the SoHO preparations or the HA on how to report SARs and SAEs using standardised documentation.
G14.27 ORHAs should provide training and awareness to their healthcare professionals on identifying and reporting SAR/Es without undue delay within time limits defined by the distributing SoHO establishment/HA.

Supplementary information

As SARs in recipients might result from various diverse factors, associated with the clinical procedure or with the recipient's underlying condition, clinicians might not consider the applied SoHO to be the cause of or a contributory factor to the SAR. However, there is an obligation, legal in the EU, for ORHAs to report known or suspected SARs to the supplying SoHO establishment and to the Competent Authority according to national regulations. ORHAs are key players in the identification of relevant factors in SARs and in implementation of the vigilance investigation. Examples of SARs are documented in the World Health Organization's Notify Library [5].

14.15 Response to recalls and reviews

Good Practice Guidelines

G14.28 ORHAs should have a system in place to identify and quarantine SoHO preparations if instructed by a SoHO establishment/entity.
G14.29 If a recalled SoHO preparation is in storage, the ORHA should discard it or return the SoHO preparation to the SoHO establishment as instructed.
G14.30 If a recalled SoHO preparation is already transplanted, the SoHO establishment and ORHA should agree recipient follow-up procedures, where applicable.

Supplementary information

There are various reasons why a SoHO establishment may recall SoHO that were distributed to an ORHA. A recall may be related to the receipt of new information on the donor's medical or behavioural history that implies a risk of disease transmission, or it may be related to the discovery of an error in processing or a fault or contaminant in a reagent or solution used in processing. It may be instigated by the SoHO establishment or required by the HA.

When a SoHO establishment issues a recall, it generally traces very quickly all the recipients of the particular batch (or donation) of SoHO that is implicated. The existence of a centralised logbook or electronic database that

maintains a two-way audit trail of SoHO preparations received, with dates of use or disposal and identification of recipients, will greatly facilitate conducting a recall, so that appropriate actions may be taken.

A review may also be required as part of an investigation into the safety of particular SoHO that have been applied to patients in the past. It may require recalling patients for additional testing or other investigations. Again, maintenance of a two-way audit trail allows effective identification of potentially affected patients. More information can be found in Chapter 18.

14.16 References

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Chapter 15. **Computerised systems**

15.1 Introduction

Computerised systems are playing an ever-increasing part in the management of business operations, including those related to healthcare. Substances of human origin (SoHO) entities or establishments may use a wide range of computerised systems. These can range from simple stand-alone computer systems that use a software package to track and trend data through to those fully integrated systems that control a range of SoHO processing steps and present data that will allow release of SoHO preparations for clinical applications. In some cases, those systems are relied upon to record consent and donor identity. Computerised systems may also have a role in managing the quality system documentation and controlling the facility (premises) or ensuring that the required environmental conditions, such as air-pressure differentials or particle counts, are maintained (e.g. a building-management system).

Errors and malfunctions of computer systems can go unnoticed and may have serious consequences [1]. The controlled introduction of changes (i.e. change control) in software is an important aspect of maintaining data integrity. A review conducted in 2010 reported the discovery of a systematic error in documentation of the wishes of organ donors that had probably occurred in 1999, and which potentially affected the records of more than 900 000 individuals.

Computerised systems that capture, generate, record, process, retain and archive critical data impacting the donation, processing and release of SoHO preparations are selected and qualified according to the procedures for critical equipment (see Chapter 3) [2].

Standards

S15.1 When carrying out SoHO activities, SoHO entities must, to the extent possible, make use of technologies that reduce the risk of human error (Art. 58.11). SoHO entities may store data in electronic form to ensure traceability (Art. 42.6).

15.2 Interconnectivity

Infrastructure guarantees the correct handling of data between workstations hosting the computer system and the relevant server(s). Infrastructure includes, but is not limited to, communication by physical lines (e.g. ethernet), switches and routers. Correct design of a computer system generally includes the use of suitable tools (e.g. test suites, servers, version- and configuration-control systems, modelling and architecture tools, communication tools, traceability and behavioural-modelling tools). With increasing availability of flexible working solutions, some infrastructure limitations may ensue from the use of personal workstations.

SoHO entities may gain access to resources such as data storage, servers, databases, networking and software on the internet through cloud service providers, rather than owning and having on their premises their own computing infrastructure or data platforms. While some of the basic functionality remains the same, there are a number of benefits, including increased speed, scalability and flexibility. However, information security may be a major challenge.

15.3 Planning the implementation of a computerised system

Good Practice Guidelines

G15.1 Hardware and software should be protected against unauthorised use or unauthorised changes.

The backup procedure should prevent loss of, or damage to, data from both expected and unexpected downtimes or function failures.

G15.2 There should be a role-based access to read, extract, print, enter, authorise and amend data.

G15.3 Computerised systems used in inventory management and release should be designed to prevent the automatic release of SoHO until all necessary release criteria are available and compliant. Computerised systems should also allow entities to identify and, where applicable, prevent the release of currently available or future SoHO donations from a deferred donor.

Recommendations

It is recommended that:

R15.1 the user have close contact with their information technology (IT) department, or an IT consultant independent of any supplier of computerised systems, before implementation of a computerised system at a SoHO entity;

R15.2 the SoHO entity or establishment define the system by generating a written description of the functions that it is designed to carry out and all the expected human interactions, i.e. functional and non-functional requirements, that define the user requirements specification (URS). For bespoke software, custom-designed software that is specifically tailored to meet the unique needs and requirements of a particular organisation or user, the URS serves as a basis for software system engineers to develop a detailed technical specification, which guides programmers in writing new or revised code for the system. In the case of off-the-shelf software, the URS is used to evaluate potential software candidates and to guide local IT staff on configuring the software. The URS will be the basis for subsequent testing and verification of the developed/supplied system;

R15.3 the list of minimal requirements for the computerised system include, (but not be limited to):

- R15.3.1 the need to manage calculations and printouts (e.g. reports and labels);
- R15.3.2 the need for data protection, e.g. personal access to the system or parts of the system (see §15.11);
- R15.3.3 the duration of and options for record storage (in general in the EU, a minimum of 10 years is recommended for quality system-related data and a minimum of 30 years for traceability-related data) (see Chapters 2 and 17);
- R15.3.4 backup conditions ensuring data restoration;
- R15.3.5 the need to interface with other computerised systems/registries (e.g. social security registries, administrative systems, financial systems), as well as system availability over time;
- R15.3.6 the need for encryption in case information is transferred over an open network;
- R15.3.7 fulfilment of the applicable legal requirements if the computerised system is intended to be used for a purpose specified in the definition of a 'medical device' (in the EU, this is defined in the medical devices regulation EU 2017/745);
- R15.3.8 the need for audit trails (documentation of data changes or data deletion, including identification of the person making the changes);
- R15.3.9 read-only access, including audit trails by the Quality Assurance team to the computerised system to review data, metadata and audit trail(s);
- R15.3.10 handling of data originating/accumulating from measurements;
- R15.3.11 e-archives for long-term storage and access in a readable form for the duration of the records, retention period;
- R15.3.12 the need to handle archive migration to other SoHO entities in compliance with data protection and national regulatory authority directives;

R15.4 the SoHO entity or establishment evaluate the different systems available and chooses one that meets the established requirements (including maintenance). The degree of user friendliness may also be taken into consideration, provided that established requirements are met;

R15.5 there is an audit of the developer/manufacturer to ensure that they can provide a product that meets regulatory requirements on the basis of a risk assessment (see Chapter 2).

Supplementary information

Figure 15.1 shows the different steps in implementing a computer system. It illustrates the design flow and documents related to specific phases (life-cycle documentation), together with the division of responsibilities between supplier/vendor and user with regard to testing, user instructions, maintenance, system improvements and access to source code. In Figure 15.1, diagram A reports the models for system software categories 4-5 and diagram B reports the simplified model for system software category 3 (as in Table 15.1).

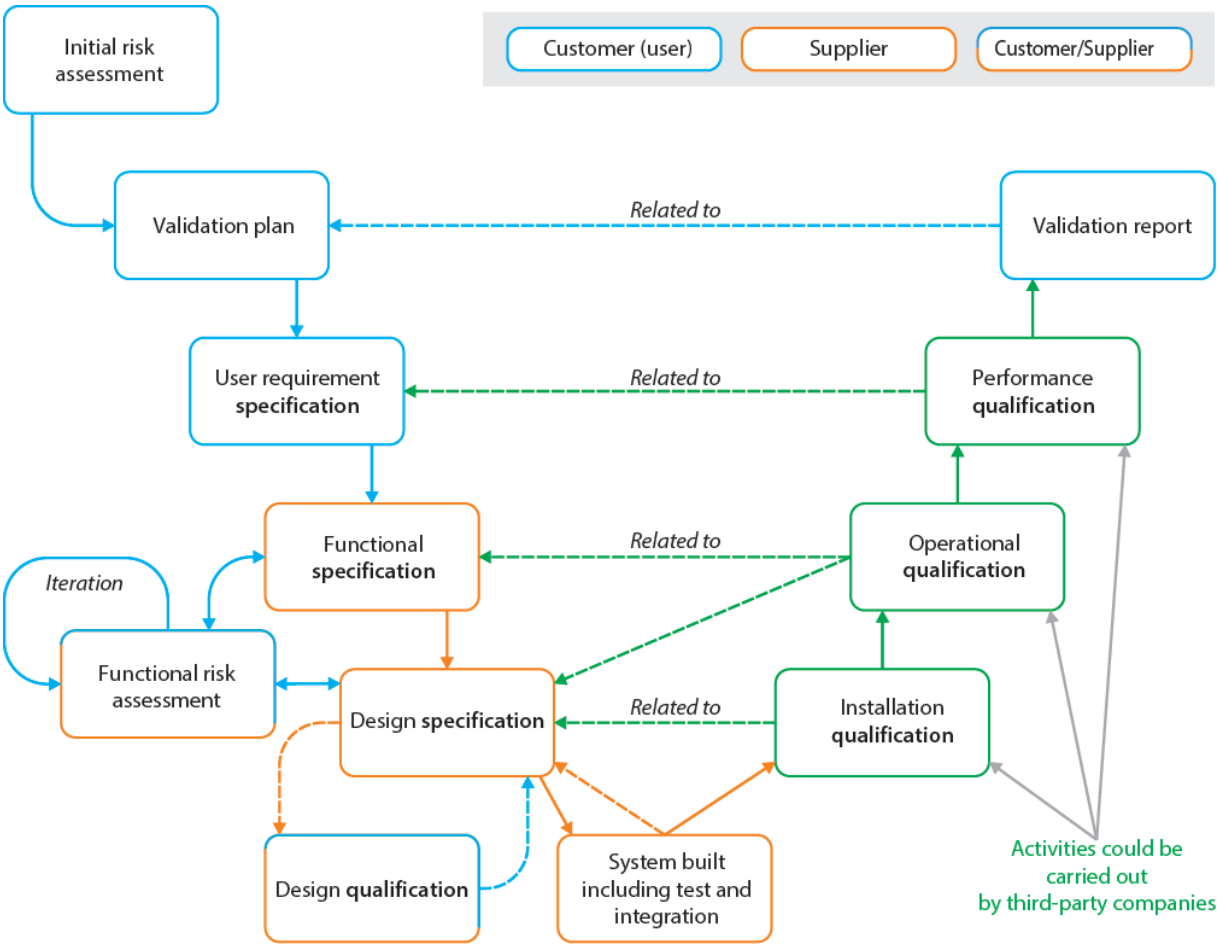
These steps will ensure that the user has all the necessary information about the system to be purchased and that the IT department or IT consultant has received the relevant technical information. The developer/supplier of the computerised system typically receives accurate and complete information about integrated systems linked to the system to be purchased. This course of action also minimises the need for 'work around' by the user (which can be a source of error).

The computer system that manages the activities of a SoHO entity or establishment usually includes hardware, software, peripheral devices and documentation such as manuals and standard operating procedures (SOPs). For further information, refer to the International Organization for Standardization ISO/IEEE 12207:2017, ISO/IEC/IEEE 29148:2018, ISO/IEC 27001:2013 and ISO/IEC 27007:2011 [3-6].

Dashed lines indicate relationships between testing and specification documents. Section A (top) describes system software categories 4-5; section B describes category 3 (categories as in Table 15.1).

Figure 15.1 **'V' model diagrams for computer system implementation**

A. Models for system software categories 4-5



B. Simplified model for system software category 3

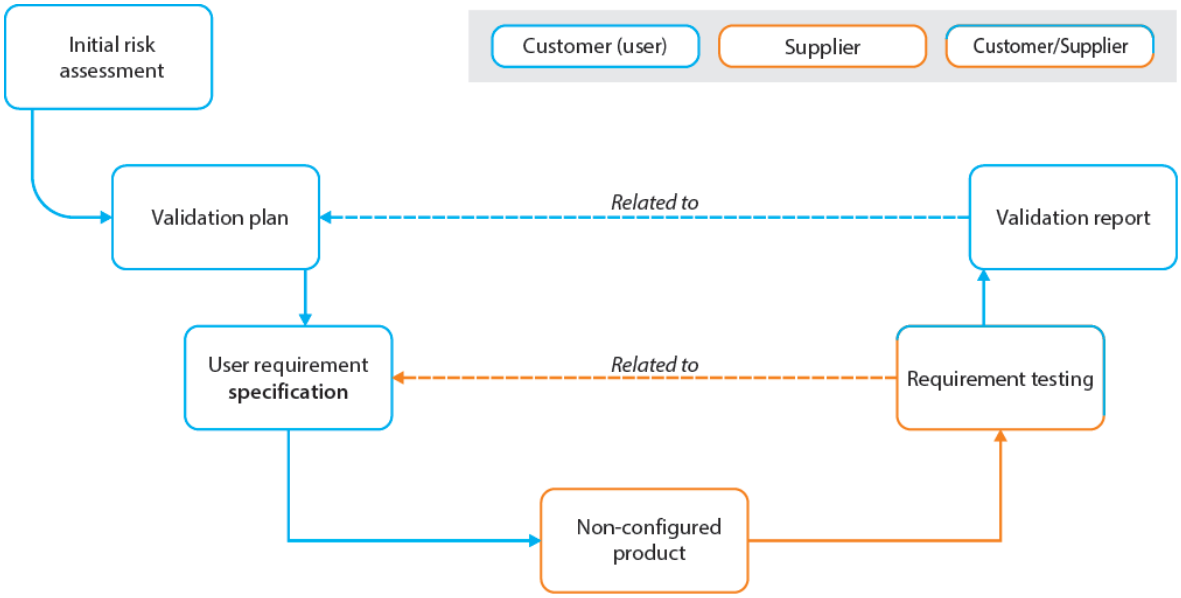


Table 15.1 **An approach to qualification and control of computerised systems by system category**

Category	Description	Typical examples	Typical approaches
Infrastructure software (Cat.1)	• Software on which applications are built • Software used to manage the operating environment	• Operating systems • Database engines • Statistical packages • Spreadsheets • Network monitoring tools • Scheduling tools • Document version control tools	• Record version number and verify correct installation by following approved installation procedures
Non-configured (Cat.3)[a]	• Software that cannot be configured to suit the specific process, although working parameters can be set to suit the intended use	• Firmware-based application • Commercial off-the-shelf software packages (spreadsheets without macros) • Instrument software (e.g. software associated with machines used for testing bacteriology or serology, cell counters)	• Specify user requirements before selection • Risk-based approach to supplier assessment • Record version number and verify correct installation • Risk-based tests against requirements as dictated by use (for simple systems, regular calibration may substitute for testing) • Procedures in place for maintaining compliance and fitness for intended use
Configured (Cat.4)	• Software, often very complex, that can be configured by the user to meet the specific needs of the user's business process; software code is not altered	• Management system for donation, processing, storage and distribution of SoHO, building-management systems (monitoring air pressures in rooms, temperature and/or particles, temperatures of fridges, freezers and incubators) • Clinical trial • Monitoring • Note: specific examples of the above system types may contain substantially customised elements	• Risk-based approach to supplier assessment • Demonstrate supplier has adequate quality management system • Some life-cycle documentation retained only by supplier (e.g. design specifications) • Configuration documentation supplied to the customer • Record version number and verify correct installation • Risk-based testing to demonstrate that the application works as designed in a test environment • Risk-based testing to demonstrate that the application works as designed within the routine environment • Procedures in place for maintaining compliance and fitness for intended use • Procedures in place for managing data
Custom (Cat.5)	• Software custom-designed to suit business process	Varies, but may include: • Internally or externally developed management systems for donation, processing, storage and distribution of SoHO • Internally or externally developed process control applications • Spreadsheet macro (i.e. database spreadsheet for clinical trial monitoring)	Same as for 'Configured' above, but also: • More rigorous supplier assessment, with possible supplier audits • Possession of full life-cycle documentation (as indicated in Figure 15.1)

[a] In GAMP4, firmware applications represented category 2, which is now integrated into category 3.
Source: International Society for Pharmaceutical Engineering. Good Automated Manufacturing Practice (GAMP) 5 [7].

15.4 Qualification and testing

Standards

S15.2 SoHO entities must put in place procedures and specifications, where applicable to their SoHO activities, that address the procurement, qualification and validation of IT systems (Art. 37.3.c).

Good Practice Guidelines

G15.4 If computerised systems are used, the software, hardware and backup procedures should be validated/qualified before use. Re-validation/qualification is not required in all cases and should be based on risk.

G15.4.1 Periodic evaluations of system performance and data integrity should be conducted.

These evaluations should follow predetermined plans that incorporate random checks. Additionally, any quality incidents should be reviewed as part of these evaluations.

Recommendations

It is recommended that:

R15.6 effective version control be maintained over software in use and that new software is qualified and existing software be re-qualified when changes are made, while

- R15.6.1 the qualification of computerised systems in a SoHO entity is incorporated in the general validation plan of the centre, and such qualification includes:
  - i. the identity of the computerised systems and interfaces that are subject to qualification;
  - ii. a brief description of the qualification strategies for different categories of computerised systems, as well as other qualification activities;
  - iii. an outline of the protocols and related test procedures for all qualification activities of the computer system, including defining the reporting requirements for documenting the qualification exercises and related results;
  - iv. the identity of the process owner and system owner, and their responsibilities as part of the qualification programme.

It is also recommended that:

R15.7 the level of qualification for computerised systems be dependent on the criticality of the systems to the quality and safety of the SoHO preparations. Therefore, it is recommended that a criticality rating based on a risk assessment, taking into consideration also the complexity and novelty of the system, be applied to all computerised systems in place. The method of qualification of these critical systems depends on the type/category of software used. Table 15.1 gives some examples with suggested approaches to qualification;

R15.8 qualification be commensurate with the level of risk, intended use and potential implications of malfunction to quality and safety;

R15.9 a full set of documentation be made available that is as detailed as necessary to ensure appropriate operation of the system, before qualification of a newly installed computerised system can be carried out, and the required documentation can include, as a minimum, the following:

- R15.9.1 a detailed specification (inventory) of the hardware, software and peripheral devices, including their environmental requirements and limitations;
- R15.9.2 diagrams or flowcharts of the system's operations that describe all component interfaces, a network diagram and all database structures (e.g. file sizes, input and output formats) – if applicable: i.e. for system software categories 4 and 5;
- R15.9.3 SOPs that describe how the system is used. The user can develop the SOPs, based on the instructions for use provided by the software developer and the internal procedures of the establishment. In particular, SOPs may address all manual and automated interactions with the system, including:
  - i. routine backup, maintenance and diagnostic procedures, including assignment of responsibilities, restoration, path management and archiving of electronic data;
  - ii. proactive and preventive measures to identify and address potential safety risks (safety leading indicators) [5-6, 8];
  - iii. 'work-arounds' for system limitations,
  - iv. procedures for handling errors, incidents and problems, including assignment of responsibilities;
  - v. procedures for handling disasters and contingency planning, including assignment of responsibilities;
  - vi. procedures for database amendments by formal request by authorised persons;
  - vii. procedures for change control;
  - viii. a training system that includes manuals, documentation and procedures for training.

Supplementary information

Guidance is provided in Chapter 3 on the qualification of new equipment. Computerised systems controlled by bespoke and off-the-shelf software are extensively used by SoHO entities or establishments to help manage SoHO activities and data. The design of, and changes to, software can have a significant impact on the quality, safety and effectiveness of SoHO and the integrity of critical data. Before new or revised versions of software are put into service, they undergo operational qualification and performance qualification by users through User Acceptance Testing (UAT). This testing process involves verifying critical settings encoded in the software, such as during a version update for software used in running an apheresis machine or testing system. Typically, software is tested in its operational state through process-simulation tests or parallel running as part of the overall system (equipment) qualification. UAT also includes regression testing to ensure that existing functionality continues to operate as expected while testing new functionalities.

Qualification documents and the results of tests undertaken are expected to be part of the documentation supplied to the user, who can then carry out tests according to a predefined and documented test plan [9]. Types of risk to consider include inadequate design of a system, errors that may occur in use (errors of use or

system defects) and loss or distortion of data [10]. Testing will involve the system, and the way it is expected to perform routinely in the entity.

Testing may be done by a third party, in which case the test team will also include personnel from the SoHO entity and will be generally approved by the SoHO entity. The organisation of ownership, system management, maintenance and support, and the plan for regular internal revisions, are commonly included in the quality-management system (see Chapter 2). If the operating system and/or firmware platform are subject to changes, including but not limited to patching or updating for security reasons, the performance of a risk analysis will help evaluate whether those changes could affect the system, and a revision of the quality system assures the procedures (see §15.4).

The following types of basic testing are examples of what could be conducted initially and when new versions of the software are installed:

a. Functional testing of components

Initial qualifications are usually carried out by the supplier/vendor, who will provide the user with documentation related to the tests performed. At minimum, those documents can include:

i. details of the methods employed to conduct verifications and testing of requisites stated in the URS document;
ii. qualification documents with results of tests (test scripts) for each functionality, including test procedure, expected result, test result, acceptance criteria;
iii. conformity statement with relevant signatures;
iv. any supporting documentation (e.g. print screens) to verify the specific test case, for traceability and to facilitate quality assurance review and follow-up.

b. Data migration

The process for data migration will be defined, documented and tested appropriately. This will ensure full maintenance of traceability, including archiving of data (if necessary).

c. Environmental testing (installation and verification instructions, or IVI)

In the actual operating environment, functional tests are carried out to demonstrate that:

i. the software systems work appropriately with the hardware;
ii. all applications of the software perform appropriately with the software operating system;
iii. appropriate information passes correctly through system interfaces, including appropriate data transfer to or from another laboratory, and through automated (e.g. serology testing, cell counting) systems (if applicable);
iv. accessories such as barcode scanners perform as expected with the barcodes in use (if applicable);
v. printed reports are formatted appropriately and completed correctly;
vi. personnel are trained and use the system correctly;
vii. the system performs appropriately at peak production times and with the maximum number of concurrent users;
viii. backups restore data in a correct way;
ix. data security, confidentiality, audit trails and access levels, where applicable, are operating as required.

Monitoring of personnel usage training can be performed periodically as part of the quality system.

15.5 Change control

Good Practice Guidelines

G15.5 Changes in computerised systems should be qualified; applicable documentation should be revised, and relevant personnel trained appropriately, before any critical change is introduced into routine use. Computerised systems should be maintained in a qualified state. This should include user testing to demonstrate that the system is correctly performing all specified functions at initial installation. If re-qualification analysis is needed, it should be based on risk assessment and conducted not only for qualification of the individual change, but also to determine the extent and impact of that change on the entire computerised system.

Supplementary information

Changes might be handled following the V diagram, such as the example shown in Figure 15.2. Depending on the system, it may be desirable to have a test version of the computerised system containing the same data (mirrored).

15.6 Maintenance and scheduled operations

Good Practice Guidelines

G15.6 Systems should be properly maintained at all times. Documented maintenance plans should be developed and implemented.

Recommendations

It is recommended that:

R15.10 data be checked periodically and systematically by qualified IT personnel to identify and remove unwanted data (e.g. duplicate records) and to

ensure that data entries are stored accurately and appropriately. Please refer to ISO/IEC 14764 for further guidance on maintenance [7, 10]; and

R15.11 security provision cover at least:

R15.11.1 an adequate change history of the system, for both software and hardware (when necessary);

R15.11.2 periodic review of user access, such as periodic alteration of passwords and removal of unnecessary or outdated access;

R15.11.3 creation of records of all data changes (i.e. an audit trail), including a retained record of original data and the reason for the change;

R15.11.4 appropriate use of malware protection (e.g. computer virus) software;

R15.11.5 control of administrative security access to ensure that only authorised personnel can make changes to the software, system configuration and data;

R15.11.6 regular testing to verify the appropriate integrity and accuracy of backed-up data;

R15.11.7 consideration of whether permanent storage (e-archive) of specific data is necessary.

15.7 Quality assurance

Recommendations

R15.12 It is recommended that the data-processing systems that affect the quality of SoHO preparations be considered as critical equipment within the quality assurance programme, which as a minimum will:

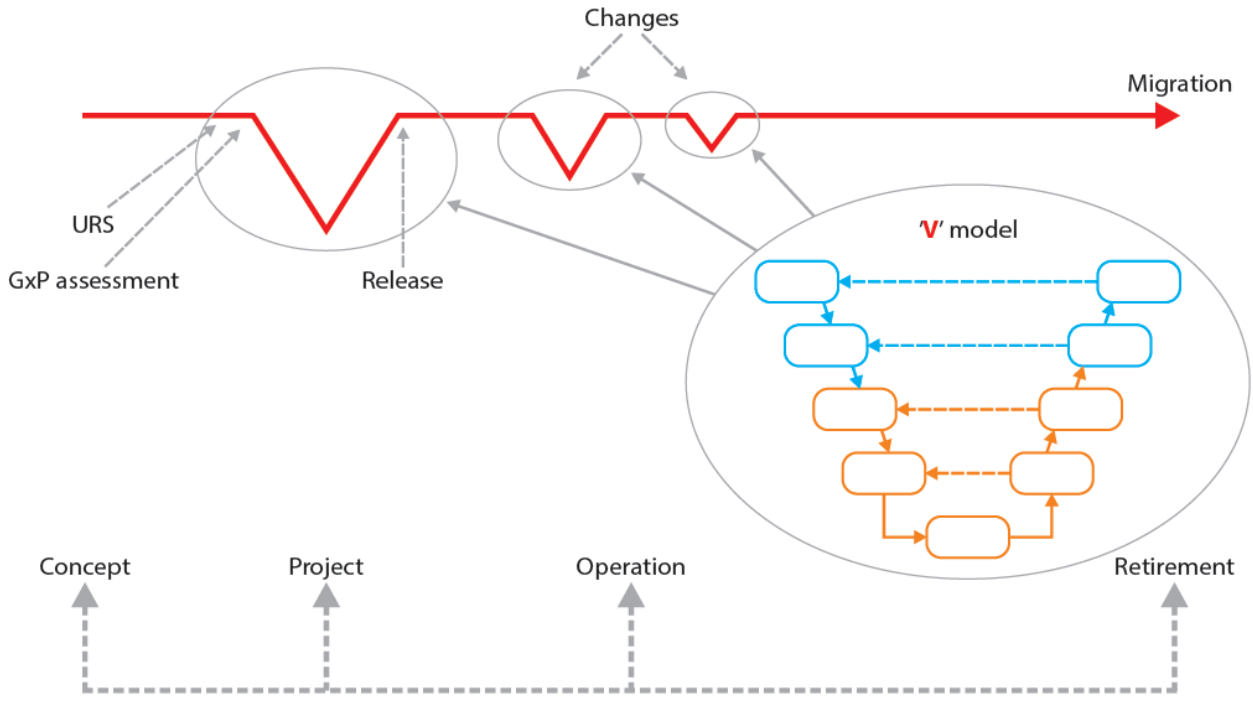
R15.12.1 ensure the ongoing accuracy and completeness of all documentation on equipment, software maintenance and operator training;

R15.12.2 ensure that periodic evaluations are undertaken to verify appropriate accomplishment of all performance tests, routine maintenance, change procedures, data-integrity checks (including audit-trail checks), error investigations and operator-competence evaluations.

15.8 Regulations and official guidance on qualification of computerised systems

Regulatory documents can be useful sources of reference for SoHO entities or establishments. The most common industry guide used for qualification of computerised systems is that from the International Society for Pharmaceutical Engineering (ISPE) [7]. More specific guidance related to blood and tissues is available from the British Committee for Standards in Haematology [11]. A guideline on computerised systems and electronic data in clinical trials published by the European Medicines Agency (EMA) could also provide useful information [12].

Figure 15.2 **Handling of software life-cycle activities**



Supplier may provide knowledge, experience, documentation and services throughout life-cycle.

URS: user requirements specifications. GxP: good [specialism] practice. Orange: user. Blue: supplier.

Source: modified from ISPE Good Automated Manufacturing Practice (GAMP) 5 [7]

Regulation of computerised systems is well established in the pharmaceutical industry, with EU Good Manufacturing Practices (GMP) [13] acting as the regulatory reference in the EU. The advice of the OECD Working Group on Good Laboratory Practice on applying these principles to computerised systems is also recommended [14]. Inspectors in the EU also use the Pharmaceutical Inspection Co-operation Scheme Guidance (PIC/S) [15]. The pharmaceutical industry operates on a global scale, so many European companies maintain compliance with the US Food and Drug Administration (FDA) [16].

Appendix 22 contains an example of a checklist that can be used for internal or external audits, adapted from ISO/IEC 27007:2020 and the guidance document of the Swedish Board for Accreditation and Conformity Assessment (SWEDAC).

15.9 Failure of the system

Recommendations

It is recommended that:

R15.13 the time taken to enact alternative arrangements be based on risk assessment and be appropriate for the particular system and the business process it supports;

R15.14 these arrangements be documented and tested adequately [17]. Testing of these alternative systems and their ability to retrieve data can be assessed periodically (based on risk assessment).

Supplementary information

For computerised systems that support critical processes, that there is provision (e.g. disaster recovery or business contingency plan/procedure) to ensure continuity of support for those processes in the event of a system breakdown (e.g. alternative manual procedures or degraded modes, alternative system). See G2.5 in Chapter 2.

15.10 Electronic signatures

Records may be signed electronically. In the EU, the eIDAS Regulation (910/2014) sets the legal framework for advanced electronic (digital) signatures [17].

Recommendations

R15.15 It is recommended that electronic signatures:

- R15.15.1 have the same impact as handwritten signatures within the boundaries of the organisation;
- R15.15.2 be permanently linked to their respective record;
- R15.15.3 include the reason, the time and the date that they were applied.

Supplementary information

When electronic signature is not supported by the platform, records or documents may be approved electronically by a user account access process that ensures only authorised users can access the system. This may be achieved by various authentication methods such as passwords, biometrics and two-factor authentication to verify a user's identity and may be tracked by audit trail.

15.11 Data protection

Standards

S15.3 The processing of personal data, including health data, must be carried out in accordance with applicable national data protection legislation and, in the EU, as per requirements defined in EU 2016/679 and 2018/1725 (Art. 76).

Good Practice Guidelines

G15.7 Methods of preventing unauthorised entry should be in place, such as personal identity codes or passwords that are changed regularly.

G15.8 All necessary measures should be taken to ensure protection of data. These measures should ensure that safeguards against unauthorised additions, deletions or modifications of data and transfer of information are in place and are used to resolve data discrepancies and to prevent unauthorised access/disclosure of such information.

G15.9 Procedures for personal data protection should comply with national legal requirements.

Recommendations

R15.16 It is recommended that appropriate technical and organisational measures be taken to guarantee a level of security appropriate to the risk, measured against the context and purposes of the processing. The factors which may be analysed to determine the appropriateness of the measures include the degree of data sensitivity, the risks to data subjects in the event of a breach and the costs involved in implementing specific types of security measures. The latter may include:

- R15.16.1 encryption or pseudonymisation;
- R15.16.2 measures to ensure the confidentiality, integrity and resilience of processing systems;
- R15.16.3 methods which enable the timely access to, restoration of or availability of personal data in the event of an incident;
- R15.16.4 regular tests and evaluation to ensure that the measures implemented meet their desired objective of maintaining the security of data processing;
- R15.16.5 periodical verification of integrity and availability of the backed-up data;

R15.16.6 implementation of a scheme to ensure that the computer system enacts the desired authority structure for those applications in which all users generally do not have identical authority.

15.12 Cybersecurity management

Good Practice Guidelines

G15.10 Organisations should have protocols in place to assure cybersecurity according to the criticality level of the system and of the data.

Recommendations

R15.17 It is recommended that cybersecurity risk assessments and controls be reviewed at regular intervals and immediately when something significant changes, such as a change in the threat or a significant change in the way a system or service is used.

Supplementary information

Cybersecurity is increasingly becoming a prominent concern among healthcare providers in adopting digital technologies for improving the quality of care delivered to patients. Increasingly frequent cyber threats with global reach, ranging from ransomware to distributed denial of service attacks, have recently exposed the vulnerabilities of IT systems and caused extended downtimes that have disrupted clinical operations and negatively impacted patient care [18, 19].

Given escalating cybersecurity risks and threats, cybersecurity programs are expected to evolve from reacting to intrusions after they occur to proactively preventing and deterring threats before they happen. A combination of both reactive and proactive controls forms the cybersecurity foundation of information assurance strategies [20, 21].

Among the reactive controls, the organisation may implement measures to:

i. detect incidents, such as intrusion detection systems, e-mail spam filters and scheduled malware scans;
ii. mitigate the impact of a cyberattack, such as network segmentation and restriction of administration privileges;
iii. reverse the effects of an attack in order to resume operations, such as off-site backups, incident response plans, continuity of operation plans and disaster recovery plans.

Managing cybersecurity includes the pre-emptive identification of security weaknesses through an appropriate risk assessment, based on probability of occurrence, impact on the organisation, prioritisation of the risk [6, 21, 22] and the development of a mitigation strategy based on an assurance plan, built through definition of policies, procedures, training and active processes to identify threats to and vulnerabilities of the IT system.

15.13 Archiving

Recommendations

It is recommended that:

R15.18 critical data be archived in a long-term stable medium and placed 'off site' physically or as an e-archive backup at a location remote from the hardware, to ensure secure storage;
R15.19 archived critical data be checked for accessibility, readability and integrity;
R15.20 the ability to retrieve archived data be ensured and tested if changes are made to the system (e.g. new computer equipment or software is installed) [11];
R15.21 files be stored in databases, if possible, in a time-durable format.

Supplementary information

Backup is essential for disaster recovery, and a common approach, one that keeps data safe in almost any failure scenario and is easy to remember, is the 3-2-1 backup rule, i.e. at least three different versions of your data over different periods of time stored on two different pieces of media, one of which is placed off-site [23].

Archiving may be conducted using secure software methods such as databases compliant with ACID (atomicity, consistency, isolation and durability) recommendations, that guarantee data integrity.

Among formats more commonly used are software-encrypted files and CRC (cyclic redundancy check)-secured files, which may require dedicated software to manage them. As this characteristic may condition future retrieval, an ISO-standardised version of the Portable Document Format (PDF), called PDF/A [24], has been implemented to ensure document reproduction using any device in years to come, as the format is independent of hardware and software platforms.

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Related material
Appendix 22. Checklist for system audit of computerised systems

Chapter 16. **Coding, packaging and labelling**

16.1 Introduction

The quality and safety of substances of human origin (SoHO) and SoHO preparations are dependent not only on the way they are collected or processed, but also on the way they are coded, packaged and labelled before being sent to an organisation responsible for human application (ORHA) [1-3]. The World Health Organization (WHO) has published an aide-mémoire on the key safety requirements (including storage, packaging and labelling) for essential minimally processed human SoHO preparations for transplantation, and some countries have adopted legal requirements to ensure that human SoHO preparations are appropriately coded, packaged and labelled [4, 5]. These steps are also addressed in EU Regulation 2024/1938 [6].

Although this chapter establishes specific guidance for coding, packaging and labelling and for the collection, processing and distribution phases, this guidance applies equally to intermediate phases, such as in-process steps, in which all materials, containers, equipment and SoHO are adequately identified at all times.

With increasing movement of SoHO preparations across borders, the capacity to uniquely identify them is of huge importance to maintain traceability. This can be achieved by coding that facilitates tracing the SoHO from donor to recipient and vice versa while respecting data protection and confidentiality rules.

16.2 Coding

Standards

S16.1 SoHO entities distributing SoHO preparations must apply a code that contains the information required by the traceability system. SoHO entities must ensure that the code generated (Art. 42.3):

S16.1.1 is unique (not applicable in the context of autologous or within-relationship use of SoHO preparations collected in the same organisation where they are applied); see S16.2;

S16.1.2 is machine-readable (unless the size or storage conditions mean that a machine-readable code cannot be applied; in which case refer to S16.2);

S16.1.3 does not reveal the identity of the SoHO donor or the person from whom SoHO preparations are collected in the case of autologous use;

S16.1.4 in the EU, complies with technical rules for the Single European Code (SEC). (See below for standards relating to the SEC.)

S16.2 SoHO entities must include these codes on the labels applied to the SoHO preparation, prior to its distribution, or on the documents accompanying the distributed SoHO preparation, where it can be guaranteed that such documents will not be separated from the SoHO preparation or will be kept digitally linked to the SoHO preparation concerned (Art. 42.4).

16.3 Single European Code

Standards

S16.3 In the EU, SoHO entities must apply an SEC to SoHO preparations distributed for human application (Art. 43.1).

S16.4 In cases where SoHO are transferred for further processing in another SoHO entity or released for manufacture of products under a different regulation, or exported to a country outside the EU, SoHO entities must, at least, apply the elements of the SEC

that allow for the identification of the donation (Donation Identification Sequence) (Art. 43.1).

S16.5 The SEC or the relevant elements of the SEC, as applicable, must appear on the primary packaging of the SoHO preparation or on a label attached to the primary package, or on the documents referring to that SoHO preparation that accompany it (Art. 43.1).

S16.6 SEC labelling is not a requirement in the following circumstances (Art. 43.2):

S16.6.1 reproductive SoHO preparations for within-relationship use;

S16.6.2 SoHO preparations applied to a recipient without being stored;

S16.6.3 SoHO preparations imported for immediate human application to a specific recipient, authorised directly by the Health Authority;

S16.6.4 SoHO preparations that are imported to or collected in the same SoHO entity where they are applied.

Good Practice Guidelines

G16.1 SoHO establishments should implement a coding system (such as EUTC, ISBT 128 or Eurocode) for the identification of SoHO, in order to ensure the traceability of all SoHO preparations, or more than one code in parallel.

G16.2 The coding system should identify and link each donation event to the donor, because donors can potentially donate SoHO on several occasions at different time points. A donation code should be applied to all SoHO preparations collected. The donation code should be traceable to the donor code.

G16.3 The unique codes should be physically attached and recorded in documents and/or data, to guarantee traceability and biovigilance at all stages from donor identification until clinical application or disposal of the SoHO preparations.

G16.4 The code should incorporate at least:

G16.4.1 identification of the SoHO establishment(s);

G16.4.2 unique donation number;

G16.4.3 SoHO code (basic nomenclature);

G16.4.4 split number (if applicable);

G16.4.5 expiry date.

Recommendations

It is recommended that:

R16.1 the use of an SEC not preclude the additional application of other codes, in accordance with national requirements;

R16.2 countries already using existing coding systems compatible with SEC requirements (i.e. ISBT 128, Eurocode), which are standards for barcodes and other forms of machine readability, continue using these systems and incorporate the new legal requirements;

R16.3 a machine-readable code be used, unless the size or storage conditions prevent its application for any of the following reasons:

i. to guarantee the accuracy of the records;
ii. to avoid manual transcription errors;
iii. for easy transfer of machine outputs into electronic databases;

R16.4 traceability mapping be retained in the importing SoHO establishment between the identifier assigned and the original identification of the imported SoHO, and between these and the supplier;

R16.5 split numbers carried by imported SoHO that do not exceed three alphanumeric characters be used directly in the SEC; if the imported SoHO carry a longer split number, or if no split number is provided, the importing SoHO establishment assign a new split number with a maximum of three alphanumeric characters to ensure uniqueness of the SEC.

Supplementary information

Coding started with the development of local coding systems applied in individual SoHO establishments, but in the last two decades there has been significant movement towards the use of national and international coding standards, building on the longer and more consolidated experience in blood transfusion.

At EU level, the permitted SoHO coding systems are ISBT 128, Eurocode and EUTC (Figures 16.1 and 16.2).

EUTC provides only the basic nomenclature, but ISBT 128 and Eurocode include more detailed product information (e.g. EUTC may represent a product type such as a tendon, whereas the other two systems may specify whether the tendon is whole, shaped or irradiated).

The ISBT 128 and Eurocode product descriptions are mapped to the high-level product description codes provided by the EUTC.

SoHO in the three product coding systems are mapped to each other to ensure that a SoHO code in the SEC can be 'translated' irrespective of the system used.

ISBT 128 is managed by ICCBBA (the International Council for Commonality in Blood Banking Automation) [7-9], which is the most widely used information standard for SoHO. ICCBBA is a not-for-profit, non-governmental organisation that develops and maintains the ISBT 128 health information data standard. ICCBBA relies on input from more than 200 volunteer experts in the fields of transfusion and transplantation from around the world and supports a structured product terminology with more than 5 600 SoHO codes, including reproductive and ocular tissues [9-13]. Currently, only ISBT 128 has a secure two-dimensional symbol that allows SEC to be machine-readable and to encode all the electronically readable information.

The Eurocode International Blood Labelling Systems e.V. (Eurocode IBLS) [14] is a not-for-profit association under German law. Eurocode IBLS manages the coding standard Eurocode, which is an

ISO/IEC 15418-listed information standard providing worldwide unique identifiers for labelling blood products and SoHO to enhance security in blood transfusion and SoHO transplantation. Today it is used in various countries, such as Germany and Austria.

The EUTC preparation coding system was developed by the European Commission (EC) for tissue establishments not using the other two coding systems. The EUTC covers all types of SoHO along with high-level terminology and their corresponding product codes. A mapping of the more detailed ISBT 128 and Eurocode product codes to the generic EUTC codes is also provided on the EU Coding Platform.

The EU Coding Platform (https://webgate.ec.europa.eu/eucoding) was integrated in the EU SoHO Platform. It is hosted by the EC and is the major tool for implementing coding requirements. The platform contains [15-17]:

a. The EU Tissue Establishment Compendium, which is the register of all SoHO establishments that are authorised, licensed, designated or accredited by each EU member state's competent authority or authorities; it contains information about these SoHO establishments along with their corresponding SoHO establishment codes. The EU Tissue Establishment Compendium is hosted by the EC and maintained by the member states' competent authorities. Each competent authority is responsible for the accuracy of the entries for the SoHO establishments that they have licensed or authorised and for keeping these entries up to date.

b. The EU Tissues and Cells Product Compendium, which is the register of all types of SoHO circulating in the EU and the respective product codes under the three permitted coding systems (EUTC, ISBT 128 and Eurocode IBLS) [18-21].

c. The Code translator, which is an application for decoding the EU SoHO establishment code and each SoHO preparation code.

These three tools are publicly available and free of charge. Therefore, the EUTC used by EU member states may also be used by other countries.

Figure 16.1 **Label with SEC derived from ISBT 128**

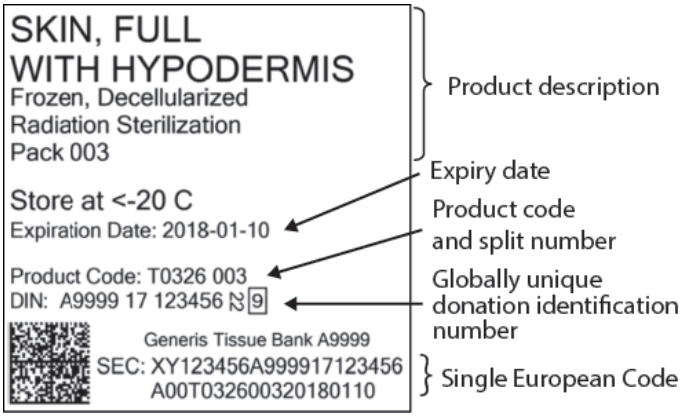


Figure 16.2 **Label with SEC derived from Eurocode**



The SEC is a unique identifier within the EU that consists of two elements (see Table 16.1): the Donation Identification Sequence (DIS) and the Product Identification Sequence (PIS).

1. Donation Identification Sequence (DIS)

The DIS indicates the origin of the SoHO, without revealing the identity of the donor, and consists of the SoHO establishment's code and the unique donation number. The SoHO establishment code is a combination of the SoHO establishment number allocated in the EU Tissue Establishment Compendium with the International Organization for Standardization (ISO) country code. The unique donation number may be created locally by the SoHO establishment, centrally (by a Health Authority) or globally as a unique number provided by an international organisation (e.g. ICCBBA or Eurocode IBLS). Donation numbers with fewer than 13 characters will be padded with leading zeros in the SEC. Taken together, these codes will ensure that each donation event has a unique DIS that can be used to label each SoHO preparation. In the case of pooling, a new DIS is allocated to the final SoHO preparation.

2. Product Identification Sequence (PIS)

The PIS describes the type of SoHO and consists of the assigned product code, the split number (if applicable) and the SoHO preparation expiry date (if applicable) in ISO standard format (yyyy/mm/dd). For SoHO preparations without a defined expiry date, the expiry date is oooooooo. The product code includes an identifier of the coding system used ('E' for EUTC, 'A' for ISBT 128 and 'B' for Eurocode) followed by the appropriate product number corresponding to the SoHO preparation type.

The DIS and PIS are separated by a single space, electronically, on the label and on the accompanying documentation; if the label dimensions do not allow this space, the SEC is generally set on two successive lines.

Further information on the SEC and its application can be found on the relevant webpages of the EC [15-17].

SoHO establishments have appropriate procedures in place to ensure the traceability and coding of SoHO preparations (Article 32 of the Regulation 2024/1938); see Chapter 17.

16.4 Packaging

SoHO preparations packaging has an important role during all procedures, starting from collection, through the processing and storage steps, to distribution and human application. Adequate packaging minimises the risk of SoHO preparations being contaminated, protects the persons involved in transport and aids retention of required characteristics and biological functions.

Good Practice Guidelines

G16.5 Storage packaging should be appropriate for the type of SoHO, the temperature (ambient temperature, refrigeration, freezing, cryopreservation), the method of storage and the intended application. The packaging should withstand sterilisation (where this is to be applied), not produce toxic residues during storage and be adequately robust to remain intact when handled during transport. Selection of packaging, or a combination of packaging systems, should result in a sealed environment that prevents leaks. The selected materials should not leach harmful chemicals, and should be sterile or should be sterilised by a safe method (if required).

G16.6 The packaged SoHO preparations should be shipped in a container which is suitable for the transport of biological materials and maintains the safety and quality of the SoHO. Temperature conditions should be appropriate for the type of SoHO to preserve the required characteristics and biological functions (i.e. temperature and duration of transport to the SoHO establishments where the processing will take place). The container should be completely closed and, if not hand-carried by a courier, be fitted with a tamper-evident seal and not opened until the SoHO preparation has been received.

G16.7 SoHO preparations immersed in liquid nitrogen should be double-bagged or stored in validated high-security packaging (specifically designed for liquid nitrogen) in order to prevent cross-contamination during storage [22]. See G11.17 and G11.18.

G16.8 Special consideration should be given to the primary packaging that will be in direct contact with SoHO preparations: materials should be suitable for use with SoHO preparations and submitted to visual

Table 16.1 **Single European Code for SoHO**

Donation Identification Sequence			**Product Identification Sequence**			
Tissue establishment code		Unique donation number	Product code		Split number	Expiry date (yyyy/mm/dd)
ISO country code	TE establishment number		Product Coding System identifier	Product number		
2 alphabetic characters	6 alpha-numeric characters	13 alpha-numeric characters	1 alphabetic character	7 alpha-numeric characters	3 alpha-numeric characters	8 numeric characters

Source: Annex VII of EU Directive 2015/565.

inspection before use; single-use packaging should be used whenever feasible. Where this is not possible, an adequate cleaning process should be assessed and applied along with suitable sterilisation methods such as irradiation or autoclaving of materials and containers.

Recommendations

It is recommended that:

R16.6 the selected materials be sealable, leakproof and traceable; closure of the packaging may ensure integrity during storage and transport;

R16.7 the type of SoHO preparation and its intended use determine the requirements needed to carry out a packaging operation in a safe manner;

R16.8 processing facilities establish and document validated packaging protocols to demonstrate that SoHO preparations maintain the necessary properties and integrity.

Supplementary information

Storage of packaging materials is performed in a clean area, under specified conditions and carefully specified in written procedures. Cleaning transport containers before use ensures that they are suitable for their intended use.

Current information on quality and good practices is provided in Chapter 2 and on risk management in Chapter 4.

16.5 Labelling

Good Practice Guidelines

G16.9 Labels should be designed to adhere firmly to the container under all storage and transport conditions and under the processing techniques used.

G16.10 The type of label to be used, as well as the labelling methodology, should be defined and established in written procedures; each labelling phase for all SoHO preparations should be documented.

G16.11 Labels applied to containers, equipment or premises should be clear, legible, indelible, unique, unambiguous and in the agreed format of the SoHO establishments concerned.

G16.12 All excess labels should be destroyed or maintained in a secure manner, when necessary, to prevent cross-contamination or mix-ups.

G16.13 Obsolete labels should be destroyed according to written procedures.

G16.14 Printed labels should be carefully examined to ensure that the information on the label conforms to the corresponding SoHO preparation; the results of this examination should be documented.

G16.15 The label applied should not be removed, altered or obscured.

G16.16 A sufficient area of the primary packaging should remain uncovered to permit inspection of the contents, whenever possible.

G16.17 A printed label, representative of those used, should be included in the SoHO records, either on paper or in electronic format.

G16.18 Any accompanying tissue or blood samples for testing should be accurately labelled to ensure identification with the donor and include a record of the date, time and place where the sample was drawn.

Recommendations

It is recommended that:

R16.9 labels be machine-printed for clarity, using ink that does not run or otherwise become unreadable when exposed to water or other liquids;

R16.10 labels maintain integrity and remain attached to primary packaging and transport containers under all anticipated storage and transport conditions;

R16.11 all printed labels for primary packaging, for secondary packaging and for documentation intended to accompany the SoHO be stored in access-controlled areas;

R16.12 the production of labels be controlled; when applicable, reconciliation of labels that have been edited, used or returned/rejected be undertaken according to written procedures.

Supplementary information

Further guidance on traceability is provided in Chapter 17 and on handling instructions and type of use in Chapter 13.

16.6 Packaging and labelling of SoHO

Good Practice Guidelines

Primary packaging and labelling after collection

G16.19 Every package containing SoHO preparations should be labelled at the time of collection. The label of the primary packaging should indicate the information described in Table 16.2; if there is insufficient space on the primary container label, the remaining information should be included in a separate document accompanying the primary packaging.

G16.20 Accompanying documentation should include the unique donation number or code specified on the primary packaging.

G16.21 Each unit of SoHO should be packed separately in sterile packaging as soon as possible after collection; double or triple wrapping may be necessary, depending on the SoHO-specific requirements.

G16.22 If labels with all the required information are not attached to the primary packaging, they should

be attached to the secondary packaging, which should be closed, ensuring that any unique identification number on the primary label is present on the label for the secondary packaging and on the accompanying documentation.

Table 16.2 **Labelling of SoHO at the end of collection**

Information shown below in bold should be included on the label; information in normal font can be on the label, if size permits, or in the accompanying documentation
• **unique donation number**
• **product code**
• **type of SoHO**
• **nature and volume of any additives/transport medium** (if used)
• **storage conditions (temperature range)**
• **warning BIOLOGICAL HAZARD** when SoHO are from a donor known to be positive for a relevant infectious disease marker
• dimensions/volume (even if it is nominal)
• date and, where possible, collection end time
• identification of the collection centre
• statement for autologous use only for autologous collections
• identification of the intended recipient for directed donations
• identification of the donor (code or name if applicable)
• SoHO attributes

Final labelling for distribution

G16.23 The label on the primary packaging for distribution should include the information in Table 16.3.
G16.24 SoHO entities should adopt labels that contain the minimum information required and, if possible, linear bar codes.
G16.25 The accompanying document should be packaged with the primary packaging in a manner that ensures they remain together.
G16.26 SoHO preparations collected or processed for research purposes should be clearly identified as such on their packages and labels (e.g. 'FOR RESEARCH USE ONLY' or 'NOT FOR CLINICAL USE'). If the container is too small to include the DIS on the label, the DIS should be included in the accompanying documentation.
G16.27 The division code, represented by the split number that is part of the SEC, should be used on the label in the case of a tissue released for circulation (e.g. pack number).
G16.28 The accompanying documentation should contain a statement that it is the ORHA's responsibility to maintain the SoHO in accordance with the specified storage conditions and to follow the instructions for opening the container, package and, where relevant, any required manipulation/reconstruction. The same applies to a statement regarding incomplete determination of donor eligibility and instructions for reporting serious adverse occurrences to the originating SoHO establishment.

Table 16.3 **Labelling of SoHO released for distribution to an organisation responsible for human application or for circulation to another operator for further processing (other SoHO establishments, third parties, manufacturers of advanced therapy medicinal products)**

Information shown below in bold should be included on the label; information in normal font can be on the label, if size permits, or in the accompanying documentation
• **unique donation number**[a]
• **product code**[a]
• **SEC** (for SoHO entities in the EU and if intended for human application) or **DIS** (as applicable to SoHO released for circulation, other than distributed for human application)
• **types of SoHO and lot or batch number** where applicable
• **expiry date of the SoHO preparations**[a] **and, where relevant, time** (in UTC if the SoHO are to be shipped to another time zone)
• **nature and volume of additives** (if used)
• **SoHO attributes**
• **warning BIOLOGICAL HAZARD** when SoHO are from a donor known to be positive for a relevant infectious disease marker
• **description (definition) and, if relevant, dimensions/volume**
• **storage conditions (temperature range)**
• **identification of the donor (code or name if applicable)**
• **statement FOR AUTOLOGOUS USE ONLY** for autologous collections
• identification of the intended recipient for directed donations
• identification of the originating organisation (collection centre (SoHO entity) or SoHO establishment), including the country[a]
• date of collection (and, where possible, collection end time)
• biological tests/assessments carried out on the donor and results
• presence of potential harmful residues (e.g. antibiotics, ethylene oxide)
• morphological and functional data, where relevant

[a] unless included in the SEC

External labelling of the shipping container

G16.29 For transport, the primary packaging should be placed in a suitable shipping container that should be labelled with at least the information described in Table 16.4.

Recommendations

It is recommended that:

R16.13 labelling of SoHO preparations be performed in a continuous process [23] during all phases of collection, processing, storage and distribution to guarantee identification;

R16.14 before labelling a donated or processed SoHO preparation, some verification procedures be carried out: to inspect the primary packaging for evidence of impurities, defects, broken seals or contamination that could compromise the quality, integrity or safety of the product; to check the facility to remove all previous materials, namely misleading labels; and to verify the correct match between container label and primary packaging label, namely when additional labels are applied to packaging;

R16.15 collected SoHO preparations be mostly packaged in disposable bags; these bags can be also double wrapped before the SoHO preparation is transported;

R16.16 the package insert include the accompanying documentation for the SoHO collected, indicating (where applicable) that they are in a state of 'quarantine';

R16.17 standard nomenclature be used in the attached labels to identify and describe the contents (SoHO preparations characteristics, maintenance and use);

R16.18 premises and procedures for the packaging and labelling of SoHO preparations be designed to prevent cross-contamination or mix-ups (e.g. avoid simultaneous operations and, if they are unavoidable, to put in place adequate additional safeguards).

Supplementary information

Management of packaging and labelling materials covers the following elements:

- written specifications, including suitability for the intended purpose;
- documented procedures describing the receipt, identification, quarantine, sampling, examination, testing, release and handling of both packaging and labelling materials;
- records of each shipment of labels and packaging materials, including their acceptance or rejection;
- a label version-control system to ensure that the current version is the one in use;
- the SoHO preparation expiry date, to be determined not only by the properties of the SoHO but also by the integrity and stability of the packaging and labelling materials, among other factors.

Details of transport conditions are described in Chapter 13.

16.7 Customs clearance

Recommendations

It is recommended that:

R16.19 SoHO establishments ensure that all required documentation is included with SoHO preparation shipments to avoid undue delays whenever SoHO are subject to customs clearance when crossing international borders;

R16.20 transport of frozen or cryopreserved SoHO preparations packed in dry ice or stored in a dry-shipper not be delayed at border crossings;

R16.21 if SoHO preparations are being transported by air, containers be labelled with the appropriate International Air Transport Association (IATA) codes: UN1845 for dry ice or UN1977 for liquid nitrogen in a dry-shipper, and UN3373 for shipment of biological substances by air [24, 25];

Table 16.4 **External labelling of the shipping container**

Information shown below in bold should be included on the label; information in normal font can be on the label, if size permits, or in the accompanying documentation
• **identification of the originating organisation** (collection centre (SoHO entity) or SoHO establishment), including name, address and phone number of a contact person
• **identification of the destination organisation** (SoHO establishment, ORHA (SoHO entity), other operator), including name, address and phone number of a contact person
• **statement that the package contains HUMAN TISSUES/CELLS and the warning HANDLE WITH CARE**
• **warning DO NOT IRRADIATE** where living cells are for human application
• **shipping conditions** (e.g. do not delay, keep cool, keep in upright position, do not freeze)
• **warning BIOLOGICAL HAZARD** when SoHO are from a donor known to be positive for a relevant infectious disease marker
• date and time (in UTC if the SoHO are to be shipped to another time zone) of distribution/circulation
• instructions for opening the container, packaging and, where relevant, any required manipulation/reconstitution
• expiry date after opening/manipulation
• for human application

R16.22 when SoHO preparations are shipped by air, an IATA Time- and Temperature-sensitive label be attached to the outside of the shipping container (the permitted external temperature range in degrees Celsius is in the lower half of the label);

R16.23 responsibilities be defined between the shipping SoHO establishment and the receiving facility for meeting the cost of transport and storage under appropriate conditions.

Supplementary information

It may be expedient for the importer to inform customs of a prospective consignment, and any enquiries by customs need to be answered promptly (see Chapter 13).

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Chapter 17. **Traceability**

17.1 Introduction

Traceability means the ability to locate and identify substances of human origin (SoHO) and SoHO preparations from collection to human application, disposal, distribution or use as starting material for the manufacture of products regulated by other legislation. The system of traceability is dependent on the coding system (see Chapter 16). The increased transportation of SoHO preparations across national boundaries has made traceability challenging [1]. Effective traceability and biovigilance in the global context depend on the use of globally unique identification for all donated biological products [2]. It is essential to facilitate rapid action to prevent harm when links in the safety and quality chain are found to have been compromised. Apart from quality and safety, traceability is also crucial for ethical reasons, as it allows legitimate donation with proper consent to be verified for every SoHO preparation.

Standards

S17.1 SoHO entities must implement a traceability system, to unambiguously link each SoHO donor or the person from whom SoHO are collected for autologous or within-relationship use, to their collected SoHO preparation and to all the documents, samples and SoHO entities that are associated with the SoHO at any point. Importing SoHO establishments must ensure an equivalent level of traceability with regard to imported SoHO preparations (Art. 42.1).

S17.2 The traceability system implemented by a SoHO entity must be able to (Art. 42.2):

S17.2.1 identify the SoHO donor or the person from whom SoHO are collected for autologous, or within-relationship use and the SoHO establishment releasing SoHO;

S17.2.2 identify the SoHO recipient at the SoHO entity or organisation responsible for human application (ORHA) applying the SoHO to the recipient, or receiving it as the starting material for the manufacturer of products regulated under other legislation;

S17.2.3 locate and identify all relevant data relating to the quality and safety of the SoHO preparation and any materials or equipment that have come into contact with those SoHO that might pose a risk to their quality or safety.

S17.3 SoHO entities must keep the data necessary to ensure traceability, appropriately safeguarded and accessible to the Health Authority (HA), for a minimum of 30 years from the SoHO distribution date or, where applicable, from the date of disposal or export. They may store the data in electronic form. Where a SoHO entity ceases its activity, the traceability data must be transferred to a contracted SoHO entity for the remaining part of the traceability period, after informing the HA (Art. 42.6).

Supplementary information

SoHO entities and establishments play a special role in assuring traceability, collecting the data needed to locate and recall SoHO, or to inform clinical users in situations where concerns about quality and safety arise. SoHO entities are responsible for communication with other entities, such as organ transplant units, and with other SoHO entities involved in the collection or processing.

The time interval between detecting a risk to the quality and safety of SoHO preparations and preventing the SoHO preparation from being used in patients has been referred to as the 'traceability window period' [3]. Recalls can be due to inappropriate evaluation of donors, positive serology tests in the donor, contamination of SoHO, infection in recipients of other tissues donated by

an individual donor and other risks introduced during the processing or storage (see Chapter 12). Specifically for medically assisted reproduction (MAR), restriction of donor use is practised due to a suspected or confirmed disease risk of transmitted disorders for future children [4]. The increasing global circulation of SoHO for clinical use, the fact that several SoHO products can originate from one donor who may also donate organs, or the fact that many children may be born from one gamete or embryo donor, and the existence of international markets for equipment, consumables and additives all add to the need for robust systems of traceability in the interests of recipient/offspring safety.

Records and procedures required to maintain traceability are kept long after the clinical use of products, allowing SoHO entities to track and trace all SoHO long after their clinical application, making adequate biovigilance and follow-up procedures possible. Traceability underpins biovigilance (see Chapter 18). Investigation of adverse events and adverse reactions, and of deviations from standard procedures, can be carried out only if a system of traceability is in place.

17.2 How traceability works

Traceability is the 'thread' that joins all the pieces of critical information together, from the moment that a potential donor is identified until the moment when SoHO are applied to the recipient or are discarded. This means that traceability is a concept that allows:

- the tracing of procedures and documents relating to materials coming into contact with SoHO and relating to critical equipment used when donors and/or recipients show any adverse reaction that could be linked to these items;
- tracing the SoHO available to be re-tested if new risks become evident after release, e.g. from collection, processing, testing, handling, storage or transport;
- tracking the fate of recipients;
- tracking follow-up of the health and the number of MAR children; and
- tracking those units associated with incidents detected after distribution or clinical application.

Accurate transcription of critical identification and traceability data is therefore of the utmost importance.

Standards

S17.4 SoHO entities must communicate information concerning serious adverse reactions and/or events (SAREs) to other SoHO entities engaged in the collection, processing, testing, storage and distribution of SoHO collected from the same donor, or otherwise possibly affected. They must only communicate information necessary and appropriate in order to facilitate traceability and ensure quality and safety of SoHO and must limit the information to details necessary to take risk mitigating actions (Art. 44.8).

Good Practice Guidelines

G17.1 Responsibilities for traceability among the different SoHO entities involved in collection, processing, testing, storage and distribution should be clearly defined and documented in a written and technical or legal agreement as required.

G17.2 Audits of traceability from donor to recipient (or offspring from MAR) and vice versa should be included in the quality-management plan.

G17.3 Traceability should be ensured to identify personnel performing critical processing tasks or approving critical results or documents.

Supplementary information

The following could be a robust and effective traceability system:

I. Unique identification

At each stage in the pathway, from donor to recipient or offspring from MAR treatments, each SoHO entity has records of the donor, the donation and the donation samples, and ensures that they are identified and labelled uniquely within their own organisation (see Chapter 16 on coding).

II. Double witnessing protocols

Manual witnessing could be associated with human errors due to the occurrence of conscious automaticity, involuntary automaticity, ambiguous accountability and stress. To mitigate these errors, different electronic models for witnessing the identification of samples and patients, using barcoding or radiofrequency, may be considered for verifying their identity [5, 6]. Critical witnessing points can be identified through a risk-assessment exercise [7] (see Chapter 2 on risk management).

III. Safe transfer of critical information

The traceability trail depends on the accurate transcription of critical identification information. Manual transcription errors can cause disruption of the traceability trail. A qualified electronic transfer system is generally used for critical information (barcodes or other machine-readable codes). If manual transcription of critical information is used, double checking of data is implemented. Electronic storage of data – in well-protected databases for easy and quick access by authorised personnel – is preferred to paper-based information collections.

IV. Timeliness

If a risk is identified, it is possible to rapidly trace all implicated products or all potentially affected recipients and, more specifically for MAR, children conceived. A delay could result in harm to patients or children conceived through MAR. Systems are ideally quickly accessible,

with efficient links between organisations to reduce the 'traceability window period'.

V. Responsibilities between organisations

To support traceability, ensure that each SoHO entity or third-party organisation in the chain clearly understands its responsibilities to allow for effective tracking. This is important because, in several well-known cases of disease transmission during SoHO application, SoHO entities often were unable to trace all recipients [8].

VI. Long-term storage of secure records

For effective reviews, traceability data are usually maintained for long periods of time; therefore, secure premises equipped with adequate protection systems are necessary. If the location of the storage of traceability records changes due to merger or closure of the facilities, the transfer of records to the new location and notifying the HA of the move allows them to maintain effective traceability.

17.3 Traceable records

17.3.1 Records of identification, donor tests and clinical evaluation of the donor

Recommendations

R17.1 It is advised to include the following donor information in traceability records of SoHO activities:
- R17.1.1 donor code or identity,
- R17.1.2 age, sex (if relevant), medical and behavioural history of the donor,
- R17.1.3 outcome of clinical and physical examinations for the donors,
- R17.1.4 completed haemodilution algorithm (where applicable),
- R17.1.5 consent/authorisation form,
- R17.1.6 relevant clinical data, laboratory test results and the results of any other tests carried out,
- R17.1.7 for deceased donors, if an autopsy was performed, the autopsy report. Where the report is not available before the designated expiry date of specific SoHO preparations prepared from the donated material, a verbal confirmation from the pathologist performing the autopsy that 'no findings were reported that could contraindicate transplantation of those SoHO preparations' can be accepted in lieu of the report; other SoHO preparations collected from the donor with longer expiry dates are generally held in quarantine until the full report is available.

R17.2 Specifically, for SoHO entities responsible for testing activities, it is advised to include the following donor information in traceability records of SoHO activities:
- R17.2.1 date and time when donor blood samples were taken,
- R17.2.2 date of receipt of the blood sample at the testing facility,
- R17.2.3 each test kit used to test donor blood sample (i.e. manufacturer, lot number, expiry date),
- R17.2.4 results of donor testing, including repeat testing (if applicable).

17.3.2 Records of collection

Recommendations

R17.3 It is advised to include the following collection information in traceability records of SoHO activities:
- R17.3.1 identification of the entity executing the SoHO collection,
- R17.3.2 donor identification and consent data (including how and by whom the donor was identified), including records of testing of the donor,
- R17.3.3 description or type(s) of collected SoHO (including a description of the samples for testing),
- R17.3.4 identification of the person who was responsible for the collection,
- R17.3.5 reference to the procedure(s) describing the collection process,
- R17.3.6 date, time (start and end, if relevant) and location of the collection,
- R17.3.7 for deceased donors, storage conditions of the deceased donor, i.e. refrigerated (or not) and time of start and end of refrigeration,
- R17.3.8 lot numbers (and if applicable expiry dates) of reagents and transport solutions used,
- R17.3.9 any incidents that occurred during collection,
- R17.3.10 critical equipment used during collection,
- R17.3.11 the identification of the entity receiving the collected SoHO.

17.3.3 Records of processing of SoHO

Recommendations

R17.4 It is advised to include the following processing information in traceability records of SoHO activities:
- R17.4.1 type(s) of SoHO received and evaluation of its/their suitability,
- R17.4.2 reference to the procedure(s) describing the process(es),
- R17.4.3 personnel executing critical processing steps and, if applicable, witnessing steps,
- R17.4.4 critical equipment used during processing with traceable periodic maintenance records,
- R17.4.5 records of critical consumables used during processing (manufacturer, product reference, lot number – if appropriate – and expiry date),
- R17.4.6 records of sterilisation or decontamination, if applicable,

R17.4.7 records of cryopreservation and freezing procedures, if applicable,
R17.4.8 records of environmental monitoring (temperature monitoring, microbial monitoring and particle counts as appropriate),
R17.4.9 records of product testing, including microbial testing, if applicable,
R17.4.10 any incidents that occurred during processing.

17.3.4 Records of storage and distribution

Recommendations

R17.5 It is advised to include the following information on storage and distribution in traceability records of SoHO activities:
R17.5.1 storage location and a transfer record if storage locations change,
R17.5.2 date placed in storage,
R17.5.3 date removed from storage,
R17.5.4 records of storage temperature, including details of nitrogen storage (e.g. vapour phase above liquid nitrogen),
R17.5.5 any incidents that occurred during distribution and/or storage.
R17.6 In addition, when the SoHO are distributed to another SoHO entity for application, the following records could be traceable:
R17.6.1 name of SoHO entity responsible for distribution,
R17.6.2 identification of the SoHO entity, courier or individual who is transporting the SoHO at any stage between the distributing and the receiving entity,
R17.6.3 packaging records (e.g. records of the dry-shipper used, applicable temperature records during transportation),
R17.6.4 time and date of SoHO preparation distribution,
R17.6.5 time and date of SoHO preparation delivery at the receiving entity,
R17.6.6 identification of the receiving SoHO entity or hospital or clinician,
R17.6.7 any incidents that occurred during the transportation.

17.3.5 Records of clinical application of SoHO preparations

Recommendations

R17.7 It is advised to include the following information on clinical application in traceability records of SoHO activities:
R17.7.1 identification of the supplying and distributing SoHO entity,
R17.7.2 identification of the clinician performing the clinical application,
R17.7.3 type(s) of SoHO preparation and the end indication(s) of application,
R17.7.4 identification and consent of the recipient,
R17.7.5 date of clinical application,
R17.7.6 any incidents that occurred during clinical application,
R17.7.7 records of clinical outcome monitoring of the recipient,
R17.7.8 any adverse reactions or adverse events in the recipient,
R17.7.9 applicable to MAR: records of the health outcome of children conceived through MAR treatment.

17.4 References

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Chapter 18. **Biovigilance**

18.1 Introduction

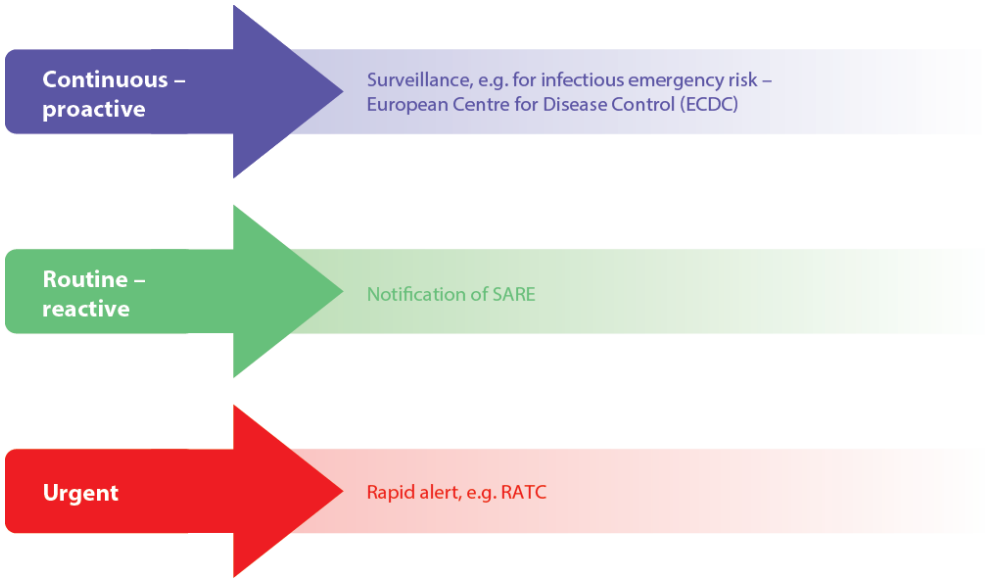
This chapter provides general guidance on the implementation of good vigilance and surveillance (V&S) practices to all those involved in the handling of substances of human origin (SoHO) for human applications, from donation, through banking to clinical use and follow-up.

Systematic monitoring of adverse reactions and events – from the selection of the donor to the follow-up of the donor and the recipient, with the objective of making the application of different SoHO safer and more effective, in conjunction with a programme of V&S – is essential for ensuring the quality and safety of SoHO preparations for human application, as part of a quality management system (QMS) [1, 2].

This chapter is also applicable to autologous patients, couples and medically assisted reproduction (MAR) patients and their offspring in their therapeutic treatment.

Three categories of biovigilance scenario can be described, depending on the type of measures and the speediness of the actions that are recommended for consideration: urgent communication, routine notification and proactive monitoring of possible serious adverse reactions/events (SAREs). Identifications of these categories are based on either a passive surveillance system, where the system waits for events to be submitted (green and red arrows in Figure 18.1) or an active surveillance system, which actively searches for events (purple arrow in Figure 18.1).

Figure 18.1 **Categories and biovigilance scenarios**



RATC: Rapid Alert for Tissues and Cells

The chapters in Part B provide additional guidance on vigilance specific to each type of SoHO.

Standards

S18.1 SoHO entities must maintain a system for detecting, investigating and recording information of adverse reactions and adverse events (AREs) as part of the SoHO preparation authorisation application, including those detected during clinical outcome monitoring (Art. 44.1). See Chapters 10 and 19.

S18.2 In cases where SoHO entities detect or suspect an SARE, they must submit a notification to their Health Authority (HA) without undue delay and must include at least the following information (Art. 44.3):

a. a description of the suspected serious adverse reaction or event;

b. a preliminary assessment of the level of imputability, where applicable;

c. details of any immediate steps taken to limit harm, where applicable;

d. a preliminary assessment of the seriousness of the consequences.

S18.3 SoHO entities must communicate AREs to the SoHO establishments for which they carry out activities, including distribution to them, on the basis of an agreement. In such cases, the SoHO establishment must be responsible for the investigation and must report to their HA when the ARE concerned is deemed to be an SARE. Other SoHO entities that do not have agreements with SoHO establishments must investigate and report SAREs directly to their HA (Art. 44.4).

S18.4 SoHO entities must have systems in place to receive and review communications from organisations responsible for human application (ORHAs) relating to adverse incidents, including serious genetic conditions reported by parents of children born from third party donation. SoHO entities must investigate the suspected SARE relating to released reproductive SoHO preparations as soon as it is identified, to prevent further distribution of SoHO preparations from the implicated donor (Art. 44.2). See S14.11.

S18.5 The physician of the SoHO establishment must be responsible for the clinical aspects of investigating suspected adverse reactions in donors, recipients and offspring from MAR from the perspective of the SoHO establishment (Art. 50.2.c).

S18.6 SoHO entities that collect SoHO must ensure, in cases where SoHO are collected from a living donor by surgical procedure or where a living donor is treated with medication to facilitate the donation, that a plan to monitor the donor's health is designed and implemented according to the risk associated with the donor's health (Art. 53.4) (see also Chapter 14, ORHA).

S18.7 SoHO entities must mitigate the risks that a SoHO preparation causes an unexpected immune reaction in recipients by combining at least the following measures (Art. 58.8):

S18.7.1 adequately typing and matching recipients to donors, when such matching is necessary;

S18.7.2 putting in place procedures to reduce, when feasible, those elements of SoHO preparations that stimulate an unintended immune response, as applicable;

S18.7.3 correctly distributing and applying SoHO to the correct SoHO recipients.

S18.8 SoHO entities must have in place a procedure to accurately, efficiently and verifiably withdraw from distribution or use those SoHO preparations affected, or suspected to be affected, by SAREs. In the case of reproductive SoHO preparations, the procedure must be in accordance with national legislation (Art. 44.6).

S18.9 SoHO entities must communicate information concerning any SARE to other SoHO establishments engaged in the collection, processing, testing, storage and distribution of SoHO collected from the same donor, or otherwise possibly affected (Art. 44.8).

S18.9.1 They must only communicate information necessary and appropriate in order to facilitate traceability and ensure the quality and safety of SoHO in such cases, and must, in particular, limit the information to details necessary to take risk-mitigating actions (Art. 44.8).

S18.9.2 A risk assessment of the seriousness and likelihood of recurrence must be included in the communication. SoHO establishments must, where relevant, also communicate such information to organ procurement organisations, in cases where the donor who is implicated in the SARE has also donated organs, or to manufacturers, in cases where SoHO collected from that donor have been distributed to manufacturers of products regulated by other EU or national legislation (Art. 44.8).

S18.10 SoHO entities must investigate each SARE detected by, or communicated to, them. On completion of the investigation, they must provide an investigation report to their HA. The report must include (Art. 44.7):

S18.10.1 a full description of the investigation of the SARE and the final assessment of the imputability of the SARE to the collection process or to the human application of the SoHO preparations, where applicable;

S18.10.2 a final assessment of the seriousness of harm to a donor, a recipient or the offspring from MAR, or for public health in general, including a risk assessment of the likelihood of recurrence, where relevant;

S18.10.3 a description of the corrective or preventive actions that have been taken to limit any harm or to prevent recurrence.

S18.11 When a SoHO entity that uses medical devices or *in vitro* diagnostic medical devices receives information regarding a serious incident and field safety corrective action, they must communicate with their HA. In the EU, this alert is in the context of Regulation (EU) 2017/745 or (EU) 2017/746 (Art. 44.5).

Good Practice Guidelines

G18.1 There should be systems in place to ensure that adverse events, adverse reactions and all deviations from standard procedures discovered in advance (near misses) are documented, carefully investigated and, where necessary, followed up by the implementation of corrective actions to prevent recurrence.

G18.2 There should be procedures in place for reporting SAREs in a timely manner to the Responsible Person at the SoHO establishment and to the HA. Adequate resources should be made available for their immediate investigation, alerts and resolution, and for the implementation of any corrective and preventive actions.

Recommendations

It is recommended that:

R18.1 SoHO entities have a clearly identified biovigilance co-ordinator (officer at local, hospital, regional level etc.) who has the responsibility for vigilance and surveillance specified in their job description in order to co-ordinate investigations, gather experts from different fields and establish with the experts and the SoHO establishment the measures to be implemented;

R18.2 SoHO entities report SAREs with online webforms because it eases data analysis and follow-up of trends. The implementation of computerised and integrated systems for recording and managing SARE data is recommended to facilitate the assessment and synthesis of data, as well as preparation and dissemination of reports.

18.2 Surveillance

The term 'surveillance' denotes the follow-up of organ/SoHO recipients or living donors, with or without SARE, to provide indicators and information on stratification of risks. An active surveillance system monitors all follow-up, not only for some specific and expected serious reactions or events.

We can consider follow-up as an example of the continuous-proactive category of biovigilance (see Figure 18.1).

Good Practice Guidelines

G18.3 Surveillance systems should be in place with written Standard Operating Procedures (SOPs) to assure the follow-up of living donor, tissue and cell recipients and offspring after MAR treatment.

Recommendations

R18.3 Routine monitoring of clinical outcomes is part of the surveillance system. It is recommended that:

R18.3.1 clinical teams set up a system (e.g. registries) with post-transplant follow-up of SoHO preparations and recipients, as well as living donor outcomes and, in the case of MAR, on pregnancy and obstetric outcomes, to monitor the results and to identify currently unknown risk factors;

R18.3.2 professionals use adapted and robust registries for the follow-up of donors of SoHO for the early detection and reporting of SAREs;

R18.3.3 the recipient be specifically monitored for the presence of disease, if possible including preventive steps, because the donor can develop conditions such as infectious disease or malignancy, detected subsequent to donation, which may potentially have been transmitted to the recipient but not known at the time of donation;

R18.3.4 a complete donor follow-up include physical, phone or laboratory contact at a relevant point in time post-donation;

R18.3.5 periodic analyses be performed to detect and monitor any shift in systematic trend in SAREs or AREs, following surveillance implementation;

R18.3.6 any 'known SARs' be examine further to prevent them from being caused by a systematic error and then recurring.

Supplementary information

In order to facilitate the analysis, for the same kind of SARE, trends can be compared to local trends (data registered in the QMS of the clinical teams/SoHO entities or establishments) or national trends (data registered in national registries where they exist).

Out-of-trend results warrant an investigation to identify the cause(s) of the deviation (negative trend) and corrective measures to be implemented.

Routine monitoring can be complemented by an active surveillance system for well-known adverse reactions. Unfortunately, in the case of SoHO, there are very few transplant registries at European level. One of those registries is the ECCTR (European Cornea and Cell Transplantation Registry), which aims to build a common assessment methodology and establish an EU web-based registry to assess and verify the quality and efficacy of corneal transplantation in ophthalmic surgery [3]. Importantly, registries can help to identify both known and previously unidentified long-term effects of cell or tissue transplantation. These registries may assist with analysis and follow-up of SARE at a national level and also provide trending data [4, 5].

In the field of MAR, the European Society of Human Reproduction and Embryology (ESHRE) has set up a European IVF [*in vitro* fertilisation] Monitoring Programme (EIM) that, among other things, collects data on perinatal outcomes, risks and complications of MAR treatment.

The expected frequency of 'known SARs' can be defined by the relevant experts or professional bodies, based on local or national experience from registry analyses or evidence based on literature data (e.g. expected rate of arterial thrombosis after the use of cryopreserved arteries). When the frequency of these complications increases in one centre beyond a documented threshold of pre-defined rates, a notification of 'suspected SAR' is made to the HA. The surveillance and analyses of the data in these registries are especially important following the implementation and progression of new transplant techniques, such as the advances seen in lamellar corneal transplant since 2005. The root cause analysis (see also Chapter 4 §4.3.2) used by the investigation team makes it possible to determine the reasons for this deviation from the acceptable rate. This investigation falls within the framework of the vigilance system and can trigger corrective measures and improve the quality and safety of care to patients. Such surveillance helps also to determine whether the institution's risk-avoiding behaviour is causing good results or whether they are utilising the most effective clinical practices.

In the case of living donors, short-term follow-up provides assurance of recovery from the donation procedure. Long-term follow-up is desirable to enable any long-term effects of the donation to be identified. The nature and duration of this follow-up will depend on the type of donation (e.g. surgical residues like placenta *versus* specific living donations like haematopoietic progenitor cells after granulocyte colony-stimulating factor [GCSF] mobilisation), the nature of the intervention and its potential impact on the individual's health [6]. Adapted and robust registries benefit from tracing and assessing the data collected from long-term follow-up. The length of follow-up is based on guidance from professional bodies. This follow-up ensures that, if a new condition occurs that may have an impact on the recipient, proper action can be taken; it also ensures that, if donors have been affected by SARs, the collection processes can be improved.

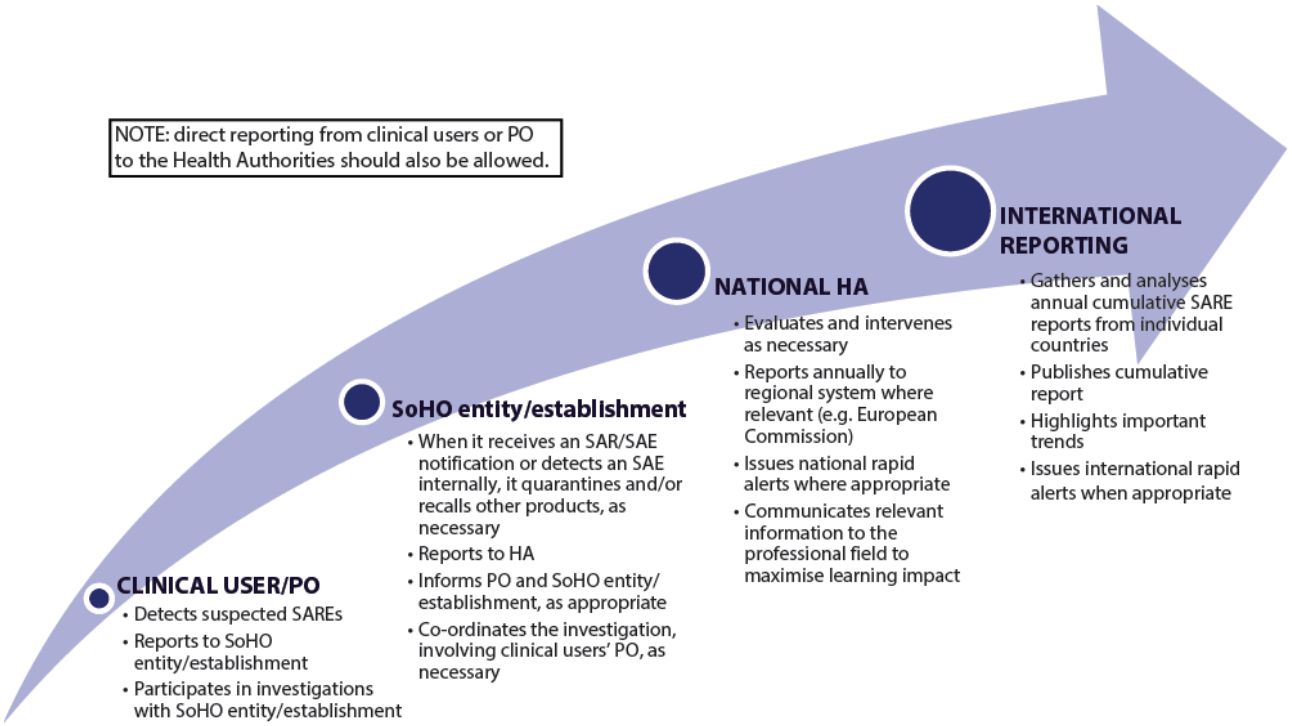
18.3 Vigilance

The term 'vigilance' includes a set of organised surveillance and reporting procedures relating to AREs. HAs play an important role in not only receiving and verifying notifications, but also in supervising them by providing guidance and advice during investigations while also contacting other national authorities (e.g. for organs, medical devices), exchanging information in the case of SoHO rapid alerts, analysing data collection and organising inspections when required. See Figure 18.2. All concerned are reminded that the notification to the HA should be made as soon as possible without waiting until the end of the investigation.

Good Practice Guidelines

G18.4 The organisation of the vigilance system, as well as the roles of the various parties involved, should be defined and broadly communicated within the SoHO establishment involved.

Figure 18.2 **Reporting flow for serious adverse events/reactions**



HA: Health Authority; EU: European Union; PO: Procurement organisation; SAE: Serious Adverse Event; SAR: Serious Adverse Reaction; SAREs: Serious Adverse Reactions and Events; SoHO: Substance(s) of Human Origin.

G18.5 Co-ordination between various systems of vigilance (organ and haemovigilance, material/device vigilance, pharmacovigilance) should be in place at the local level (SoHO establishment) and at the HA level [7].

G18.6 The results of vigilance systems should be communicated effectively and regularly to health-care professionals to ensure that the benefits of these programmes are realised in practice to support continued notification of AREs, with lessons learned and practice improvements.

Recommendations

R18.4 It is recommended that vigilance systems be connected and capable of exchanging information in both directions [8]. Each country decides how to connect all vigilance systems at national and international levels.

Supplementary information

Although some initiatives aim to integrate organisations and technical committees into a single system (R18.4), not all professionals agree to such integration because of their differences in approach to risk assessment [9].

A lack of information or a failure to exchange information can put more patients or the same recipients at risk. Sometimes it is someone within the system who discovers a problem and needs to inform the HA. For example, if there was a problem with a bag used for cryopreserved SoHO and that problem altered the characteristics of the product, or if such a bag was stored at −196°C and after some time this bag was broken, then the medical device vigilance system would need to be informed.

The same caution applies when faced with an SAR or serious adverse event (SAE) with organs if it involves SoHO that were retrieved, or vice versa; in either case, all the corresponding vigilance systems would be informed. Sharing information between vigilance systems and health professionals is beneficial, notably because some materials or devices used in the preparation, shipment or other steps of the process from collection are manufactured by only one supplier and a quality defect or a problem can have huge consequences for SoHO activities.

18.4 Definitions

According to the World Health Organization (WHO) Notify Library for V&S of medical products of human origin (MPHO) [10], adverse occurrences are categorised as follows:

Serious adverse reactions include:

1. harm to recipient;
2. harm to donor, or
3. harm to fetus or offspring.

Serious adverse events involve:

4. risk of harm.

These definitions are also in accordance with the incidents described in EU Regulation 2024/1938 of the European Parliament and of the Council of 13 June 2024 on standards of quality and safety for SoHO intended for human application, and repealing Directives 2002/98/EC and 2004/23/EC as given below.

18.4.1 Adverse reactions

'Adverse reaction' (AR) means any incident which could be reasonably associated with the quality or safety of SoHO, or their collection from a donor or human application to a recipient, that caused harm to a living donor, to a recipient or to offspring from MAR.

'Serious adverse reaction' (SAR) means an adverse reaction that results in any of the following:

i. death;
ii. life-threatening, disabling or incapacitating condition, including transmission of a pathogen or of a toxic substance that might cause such a condition;
iii. transmission of a genetic disorder that:
   - in the case of MAR with third-party donation, resulted in pregnancy loss or might result in a life-threatening, disabling or incapacitating condition in the offspring from MAR; or
   - in the case of MAR in the context of within-relationship use, resulted in pregnancy loss or might result in a life-threatening, disabling or incapacitating condition in the offspring from MAR, due to a pre-implantation genetic test error;
iv. hospitalisation or prolongation of hospitalisation;
v. the need for a major clinical intervention to prevent or reduce the effects of any of the results referred to in points (i) to (iv);
vi. prolonged sub-optimal health of a SoHO donor following single or multiple SoHO donations.

18.4.2 Adverse events

'Adverse event' (AE) means any incident or error associated with SoHO activities that can affect the quality or safety of SoHO in a way that implies a risk of harm to a living donor, to a recipient or to offspring from MAR.

'Serious adverse event' (SAE) means an adverse event that poses a risk of any of the following:

i. inappropriate SoHO distribution;
ii. a defect posing a risk to recipients or donors is detected in one SoHO establishment that would have implications for other recipients or donors because of shared practices, services, supplies or critical equipment;
iii. loss of a quantity of SoHO that causes human applications to be postponed or cancelled;
iv. loss of highly matched SoHO or SoHO for autologous use;
v. a mix-up of reproductive SoHO in such a way that an oocyte is fertilised with sperm from a person other than the intended person, or reproductive

SoHO are applied to a SoHO recipient other than the intended SoHO recipient;

vi. loss of traceability of SoHO.

18.5 Biovigilance steps

For all AREs that may occur, from the time of selection of the donor until clinical application and follow-up, the biovigilance process is generally organised in several steps: detection and recording; evaluation; reporting/notification; investigation; and finally reporting and closing the notification. See recommendations below.

Specific examples of SARs for non-reproductive SoHO and reproductive SoHO can be found in Appendices 24 and 25 respectively. Specific examples of SAEs for non-reproductive SoHO and reproductive SoHO can be found in Appendices 26 and 27, respectively.

Recommendations

R18.5 To fulfil the requirements of the standards, it is recommended that management of the biovigilance process be organised in several specific steps:

R18.5.1 **Detection and recording**. It is recommended that AREs, serious or not, be recorded in the dedicated biovigilance system in accordance with national legislation, so that immediate action can be taken when necessary. Also, near misses are collected within the internal QMS.

R18.5.2 **Evaluation**. It is recommended that every SAR/E be evaluated by the SoHO establishment with the collaboration of all parties involved, and also where necessary a group of relevant multidisciplinary professionals with experience in infectious and malignant or genetic diseases, quality control and quality management, as well as professionals with experience in the use of the SoHO involved in the case. A risk-based approach could be employed, e.g. a risk matrix that would consider severity, frequency of occurrence, likelihood of recurrence and detectability. (See Appendix 23.)

R18.5.3 **Reporting/notification**. It is recommended that the notification include a (preliminary) root cause analysis and proposed mitigation strategies.

R18.5.4 **Investigation**. It is recommended that the actions proposed following the investigation be proportionate to the level of risk and the type of the incident (near misses, AREs and SAREs). Once the investigation is finished, it is recommended that a second evaluation be performed to decide how its findings are to be managed, depending upon what kinds of action have been decided on.

R18.5.5 **Reporting and closing the notification**. It is recommended that the final report considers the information from all previous steps in order to close the notification.

18.6 Serious adverse reactions

Good Practice Guidelines

G18.7 Efficient systems for rapid quarantine and recall of unsafe SoHO preparations should be in place, along with procedures for look-back where donors or recipients are found to have been exposed to a risk.

G18.8 Important outcomes from each adverse reaction should be disseminated appropriately.

G18.9 SoHO establishments that supply SoHO preparations, including MAR, should provide organisations representing clinical users with clear instructions on how to report adverse reactions, preferably using standardised documentation in accordance with national legislation.

G18.10 If SARs are detected in relation to SoHO preparations that have entered international distribution channels, appropriate international collaboration should ensure that all those involved (clinicians, SoHO entities and establishments, and HAs) in each of the countries concerned are informed and participate, if necessary, in the investigation and follow-up.

Recommendations

R18.6 It is recommended that any suspicions of a possible adverse reaction in a donor or recipient be reported to the SoHO entity or establishment in accordance with national legislation.

Supplementary information

In certain circumstances (e.g. emergency case, with no medical alternative), a decision may be taken, based on a risk–benefit analysis, to administer/transplant a non-conforming donation (e.g. *Cytomegalovirus*-positive bone marrow). In that situation if, during the follow-up, an adverse occurrence develops, it will be outside the scope of vigilance.

Below are examples of groups of reportable ARs, although the list is not exhaustive. (For further examples of reportable ARs in each specific tissue, see the chapters in Part B of this Guide):

- Donor harm: suspected harm in living donor related to collection;
- Infection from donor: unexpected primary infections possibly transferred from donor to recipient (e.g. viral, bacterial, parasitic, fungal, prion);
- Suspected transmitted infection not related to the donor (viral, bacterial, parasitic, fungal, prion): possibly due to contamination or cross-contamination by an infectious agent in the collected SoHO or associated materials, between collection and their

clinical application – this could be during processing (e.g. if the previous donor processed was infected and the cleanroom was not disinfected) or during storage (e.g. if the SoHO preparations were stored in a liquid nitrogen package, only one layer of packaging failed and this was not detected before the tissue left the SoHO establishment). Otherwise, it is mixing up deviations and AEs;

- Immunology: unexpected and serious immunological reactions suspected to be related to the SoHO preparations, including allergic reactions, rejection and unexpected haemolytic reactions;
- Malignancy: malignant disease possibly transferred by the SoHO preparations (donor-derived, process-associated or other);
- Failure: unexpectedly delayed or absent engraftment suspected to be related to poor quality of the SoHO preparation, or SoHO preparation failure (including mechanical failure);
- Toxicity: toxic effects due to SoHO preparations or associated materials;
- Mismatch: unexpected immunological reactions due to accidental SoHO mismatch;
- Undue risk: aborted procedure involving unnecessary exposure to risk, e.g. wrong tissue supplied, discovered after patient is anaesthetised and the surgical procedure has begun;
- Genetic abnormality: suspected transmission of genetic disease by transplantation or gamete/embryo donation;
- Other transmission: suspected transmission of other (non-infectious) illness;
- Volume overload: transfusion-associated circulatory overload in haematopoietic progenitor cell (HPC) transplantation;
- Insult: neurological reaction;
- Fever: severe febrile reaction.

18.6.1 Serious adverse reactions: detection and recording

Recommendation

R18.7 It is recommended that all health professionals involved, from donor selection to clinical application and follow-up, be mindful of the possibility of ARs and are aware of the need to report any suspicions of such, in line with local procedures. Such health professionals include:

R18.7.1 medical staff (including surgeons) involved in SoHO collection activities who might become aware or informed of additional safety information on donors during their follow-up;

R18.7.2 staff and personnel carrying out collection of SoHO;

R18.7.3 clinical users paying attention to adverse outcomes that might be associated with the clinical use of SoHO preparations;

R18.7.4 physicians caring for children born after non-partner MAR treatment who may detect a genetic abnormality and, by reporting it, prevent further distribution of gametes/embryos from that donor;

R18.7.5 any other SoHO entity staff involved in any collection, processing, SoHO preparations distribution or follow-up information step.

Supplementary information

Vigilance systems include the detection of donation complications (also considered to be ARs) in living donors that might be associated with the donation process, especially when novel SoHO processes of collection have been applied or new types of SoHO are being collected.

Adverse reactions may be detected after stimulation treatment in living donors and recipients (see Chapter 25 – Haematopoietic progenitor cells from bone marrow and peripheral blood; Chapters 30 and 31 – MAR and fertility preservation; and Chapter 19 Novel processes).

18.6.2 Serious adverse reactions: evaluation

Recommendation

It is recommended that:

R18.8 as a first precautionary measure, any other remaining SoHO preparations from the same donor be quarantined. If SoHO preparations have already been distributed, it is advised to determine where or to whom they are allocated and consider the possibility of recall if that is still an option. These measures are intended to minimise the number of recipients exposed to the same reaction;

R18.9 a 'severity scale' be used to decide whether a particular adverse reaction is an SAR that needs to be reported to the HA;

R18.10 when SARs occur that can have an impact on a lot of patients (e.g. contamination of the medium used to prepare cells) or on public health (e.g. risk of outbreak), then the HA in that country be involved too. Efficient co-ordination of the investigation allows rapid implementation of effective corrective action. If relevant, it is advised that experts in particular fields (e.g. viral transmission) be invited to participate in the investigation of the adverse reaction.

Supplementary information

The scale shown in Table 18.1 is used in the EU. It was proposed by the project European Union Standards and Training for the Inspection of Tissue Establishments (Eustite) [11] for vigilance for SoHO and is based on the scale used for haemovigilance.

18.6.3 Serious adverse reactions: reporting/ notification

Recommendations

R18.11 It is recommended that national V&S programmes allow direct reporting from clinical users to HAs.

Supplementary information

The report of an SAR may involve SoHO quarantine, recall and look-back in patients who have already had implicated SoHO preparation applied. These actions may be taken by organisations other than the one that received the original notification. For example, the organ procurement organisation would play a central part when the deceased donor was an organ and SoHO donor.

Figure 18.3 shows a series of actions that might need to be taken in a report of suspected transmission from a deceased donor of organs and SoHO. Obviously, communication with other organisations that might need to quarantine implicated SoHO, or conduct recalls or look-backs, would be quick and effective.

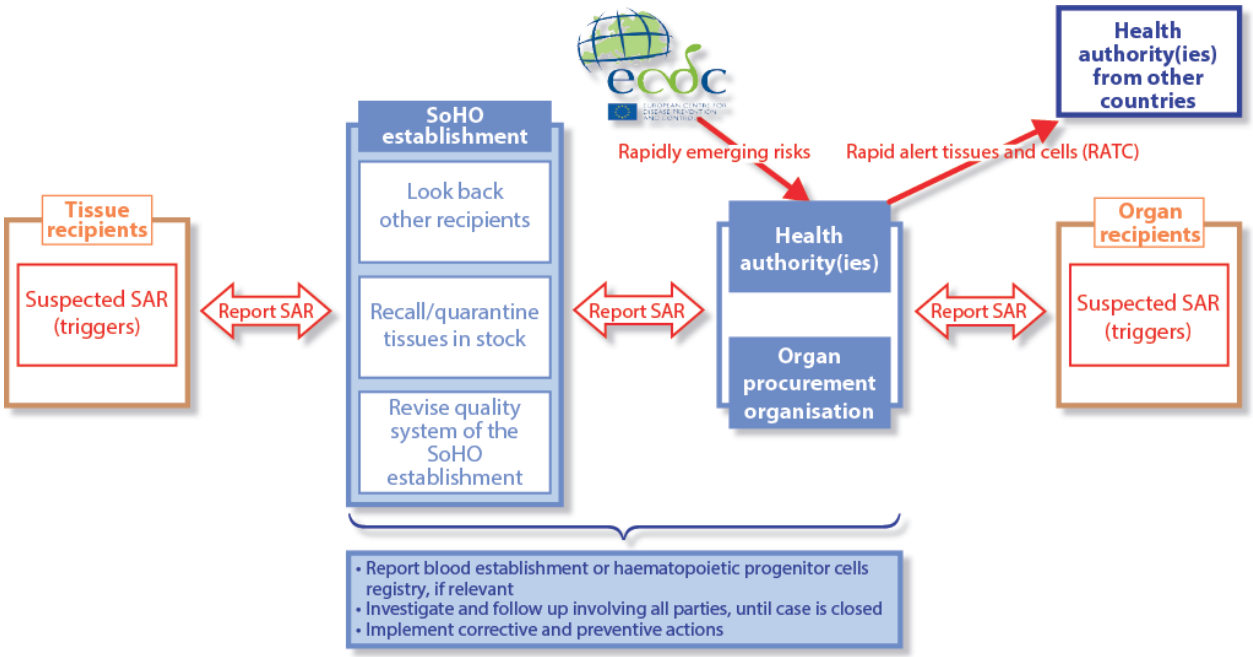
Not all diseases in offspring from MAR are directly related to the donor. Therefore, a careful risk assessment would consider the type of disease (chromosomal, multifactorial, single-gene or mitochondrial disorders, communicable and non-communicable diseases) and the possibility of (genetic) testing of the child, biological parent and/or gametes donor, as well as global prevalence and genetic predisposition. A similar approach applies to HPC transplantation.

Direct reporting by patients to HAs could be facilitated. There are some examples in pharmacovigilance of patients directly reporting outcomes of AEs. The idea

Table 18.1 **Severity scale for adverse reactions**

Not reportable	Insignificant	No harm to the recipient or living donor, and considered to be reportable as an event rather than a reaction, according to EU directives.
	Non-serious	Mild clinical consequences that do not necessitate hospitalisation and/or do not result in long-term disability or consequences for the recipient or living donor.
To be reported	Serious	Adverse reaction resulted in: • hospitalisation or prolongation of hospitalisation and/or • persistent or significant disability or incapacity and/or • medical or surgical intervention to preclude permanent damage or impairment of a body function and/or evidence of a serious transmissible infection and/or • birth of a child with a serious genetic disease after MAR with third party gametes or donated embryos.
	Life-threatening	The living donor or recipient required major intervention after procurement or application of SoHO (vasopressors, intubation, transfer to the intensive care unit) to prevent death and/or there is evidence of a life-threatening transmissible infection.
	Fatal	Death in a living donor or a recipient of SoHO

Figure 18.3 **Example of an adverse reaction involving multiple parties**



with this initiative is to have a full range of self-reporting rather than patients' reports only being collected by clinicians. The project is called PROSPER (Patient - Reported Outcome Safety Event Reporting) [12].

18.6.4 Serious adverse reactions: investigation

Recommendation

It is recommended that:

R18.12 in the case of an SAR, following the assessment of severity and notification to the HA, the next step be an investigation that focuses on establishing the level of imputability and a root cause analysis (i.e. the extent to which the SoHO preparation used clinically can be considered to have caused the adverse reaction);

R18.13 the SAR investigation include a review of all activities from the beginning (donor assessment) to the moment when the SAR was identified, including:

- R18.13.1 all the reagents, substances etc. that were in contact with the SoHO during collection and processing, checking the expiry date and the sterility;
- R18.13.2 the SoHO collected or processed in the same room on the same day, the day before and the day after;
- R18.13.3 the donor's medical and social history: e.g. genetic disease, genetic pathogenic variant carrier analysis, transfusion history, family history, personal and behavioural information, travel history, etc. (see Chapter 5);
- R18.13.4 all the microbiological results of the donor, at each specific step of the process, or the SoHO preparations;
- R18.13.5 the microbiological checks of the processing rooms;
- R18.13.6 the professionals involved: their experience, stress due to unforeseen circumstances, busy days etc.;
- R18.13.7 any shortage of the usual reagents;
- R18.13.8 and the ORHA that uses the SoHO preparation needs to determine whether other patients were operated on or treated in the same operating room, and those patients' clinical and microbiological conditions. This approach is not advised for recipients of gametes or embryos (see Chapter 30).

R18.14 The final step of the investigation is the assessment of the probability or likelihood of recurrence of the AR, considering the consequences to the individual, the system and the supply, selecting the worst-case scenario in each category. Using the impact matrix allows determination of the level of corrective and preventive action to be taken accordingly (see the impact assessment tool given in Appendix 23).

Supplementary information

The scale developed by Eustite [13], shown in Table 18.2, can be applied to describe the outcome of an imputability investigation. It proposes that all ARs be graded in terms of imputability. Table 18.2 also recommends specific approaches to the establishment of imputability for suspected infectious or malignant transmissions, as proposed by Garzoni and Ison in the context of transplantation [14]. Imputability grades might change during an investigation and are, in general, assigned at the point of initial notification and again at the completion of the AR investigation. The evaluation of imputability is evidence-based.

The European Centre for Disease Prevention and Control (ECDC), the WHO or other sources of epidemiological and risk information may be useful to support the process. Consideration can also be given to the practice of archiving pre-transplant samples for transplant recipients to support imputability investigations (including the possibility of genetic material from donors).

18.6.5 Serious adverse reactions: report and closing notification

Based on the results of the investigation and recommended actions, an action plan would be created to implement those measures that are considered adequate, feasible and cost-effective. The last step to close the quality loop is effectiveness verification.

A final report summarising all information gathered during the investigation is generally done before closing.

18.7 Serious adverse events

Adverse events can occur at any moment from donor selection to clinical application.

Some examples of SAEs are (for more information, check the specific SoHO chapters in Part B):

I. Final result of a negative-to-date release with a cultured cornea was reported as positive; no adverse reaction detected in the cornea recipient.
II. *Aspergillus* was detected in an incubator used for storing SoHO preparations that had been distributed. This could be attributed to a processing step.
III. Loss of all embryos during handling; the patient requires a new cycle of MAR.
IV. HPC donor not tested for malaria although donor resided in a malaria-endemic area.
V. Lack of liquid nitrogen, in a liquid nitrogen vessel containing several SoHO preparations, resulting in thawing of the SoHO preparations. This example could have been related to storage.
VI. A frozen bone, specifically selected for this patient, arrives at the hospital and the bag is broken, this being discovered before patient is anaesthetised and before the surgical procedure has begun.

18.7.1 Serious adverse event: detection and recording

Recommendation

R18.15 It is recommended that near misses be assessed within the QMS, in order to prevent recurrence of the same incident.

Supplementary information

Occasionally, an SAE (e.g. discovery of an infectious diseases marker as positive, or a tumour found in the donor after SoHO were used) does not inflict immediate harm on the recipient but requires follow-up during a specified period of time to detect. It is common practice for a SoHO entity and clinicians to jointly establish a relevant period of time, considering the type of SAE (tumoral, infectious etc.) and the potential signs or symptoms if transmission occurs. The notification is closed when the period finishes. Some SAEs, defined at the beginning as SAEs, may be converted later to SARs (serious adverse reactions). Depending on the duration of the SAR occurrence, they can be notified in the biovigilance system or in the registry put in place for the routine monitoring of the recipients. It is notably the case for an SAR that occurs in the long term (e.g. tumoral expression of an SAE).

Following the SoHO V&S project in the EU, some HAs now promote the reporting of what are called near misses, meaning those errors that have real potential to provoke an SAR, which either does not take place (by chance or preventive remedial action) or does not have consequences for the patient, the system or the staff. According to Serious Hazards of Transfusion (SHOT) 2022 [17], near misses are the most prevalent category of events.

18.7.2 Serious adverse event evaluation and reporting/notification

Recommendation

It is recommended that:

R18.16 any deviation from SOP in a SoHO establishment (or any other AE) [18] that has implications for the quality and safety of SoHO in accordance with

Table 18.2 **Scale describing possible outcomes of an imputability investigation**

	Criteria adapted from Eustite-SoHO V&S [11, 15]	**Criteria for infectious and malignant transmissions, adapted from DTAC (Disease Transmission Advisory Committee) [16]**
Not assessable	Insufficient data for imputability assessment.	Insufficient data for imputability assessment.
0. Excluded	Conclusive evidence beyond reasonable doubt for attributing an adverse reaction to alternative cause(s).	Suspected transmission and fulfilment of at least one of the following conditions: • clear evidence of an alternative cause • the appropriate diagnostic tests carried out have failed to document infection by the same pathogen in any recipient from the same donor • laboratory evidence that the recipient was infected with the same pathogen or had a tumour before the application of organs/SoHO.
1. Possible	The evidence is indeterminate for attributing an adverse reaction to the quality/safety of SoHO, to the donation process or to alternative causes.	Either suspected transmission and laboratory evidence of the pathogen or tumour in a single recipient or data suggest transmission but are not sufficient to confirm it.
2. Probable	The evidence is clearly in favour of attributing the adverse reaction to the quality/safety of SoHO (for recipients) or to the donation process (for donors).	Not only are the following two conditions met: suspected transmission and laboratory evidence of the pathogen or tumour in a recipient but also at least one of the following conditions is met: • laboratory evidence of the same pathogen or tumour in other recipients (if multiple recipients) • laboratory evidence of the same pathogen or tumour in the donor If there is pre-transplant laboratory evidence, such evidence indicates if the same recipient was negative for the pathogen involved before transplantation.
3. Definite; certain	The evidence is conclusive beyond reasonable doubt for attributing the adverse reaction to the quality/safety of SoHO (for recipients) or to the donation process (for donors).	All the following conditions are met: • suspected transmission • laboratory evidence of the pathogen or the tumour in a recipient • laboratory evidence of the same pathogen or tumour in other recipients (if multiple recipients) • laboratory evidence of the same pathogen or tumour in the donor If there is pre-transplant laboratory evidence, note that the same recipient was negative for the pathogen before transplantation.

national legislation (see also Figure 18.1) be reported as an SAE;

R18.17 in cases of MAR, any of the following events be considered as an SAE:

- R18.17.1 inappropriate gametes, embryos or gonadic tissues have been released for clinical use, even if not used;
- R18.17.2 any event that could have implications for other patients or donors because of shared practices, services, supplies, critical equipment or donors;
- R18.17.3 any type of gamete or embryo misidentification or mix-up of gametes or embryos;
- R18.17.4 an event that resulted in a loss of traceability of gametes or embryos;
- R18.17.5 contamination or cross-contamination;
- R18.17.6 accidental loss of gametes, embryos, gonadic tissues (e.g. breakdown of incubators, accidental discard, manipulation errors) resulting in a total loss of chance of pregnancy for one cycle.

18.7.3 Serious adverse event: investigation

Recommendation

R18.18 It is recommended that investigation of an AE, and especially of an SAE, be focused on establishing root cause(s), notably which steps of the process can be considered causative of the AE.

Supplementary information

Appendices 24, 25, 26 and 27 provide examples that may serve as guidance. Despite the fact that SAEs, by definition, have not (or not yet) involved harm to recipients or donors, the impact of a SAE can be significant if considered in a broader way. The impact assessment tool given in Appendix 23 can also be applied to SAEs to help reach a decision on the response required.

18.7.4 Serious adverse events: report and closing notification

Recommendation

R18.19 It is recommended that an action plan be created, based on the results of the investigation and the recommended actions, to implement those measures that are considered adequate, feasible and cost-effective. The last step to close the quality loop is effectiveness verification.

18.8 Surveillance for new risks

Good Practice Guidelines

G18.11 Vigilance programmes should include an activity of scanning for new risks that have not been recognised previously or have not yet occurred. Such new risks may be related to donors, emerging infectious diseases, new techniques, new medical devices (including new ancillary products) or new reagents to which SoHO can be exposed during processing, and new inheritable pathogenic DNA variants, that MAR donors have not been tested for.

Supplementary information

Newly emerging infectious diseases, for which targeted testing can be carried out or which might imply the need to exclude certain donors, represent an example of one type of new risk. The ECDC monitors the epidemiology of diseases in Europe and publishes a weekly communicable disease threat report that provides useful data to follow these new risks. See https://www.ecdc.europa.eu/en/publications-and-data/monitoring/weekly-threats-reports [19].

18.8.1 Rapid alerts

In some circumstances, a particular event or reaction requires rapid communication nationally or internationally. The aim is to facilitate urgent action, such as a recall of products or critical materials or the quarantine of SoHO (see Figure 18.1).

The following examples have been identified in the SoHO V&S project [20] as triggers for rapid alerts within or between EU member states:

a. an ARE of a serious or potentially serious nature;
b. potential risk to other individuals or other SoHO establishments;
c. wider public health implications;
d. rapid intervention needed (preventive or corrective measures, urgent communication).

Within the EU, a system for rapid alerts – called Rapid Alerts for Tissues and Cells (RATC) – is hosted by the European Commission and enables the Competent Authorities of EU member states to rapidly share urgent information on risks to patients where that information has consequences in more than one EU member state [21].

18.8.2 International reporting

The European Commission provides annual reports on the notification of SARE, compiled by the European Competent Authorities, which are publicly available [22, 23]. Such international reporting allows for trend analyses on the basis of consolidated data and for sharing of the lessons learned.

In the case of HPC, the World Marrow Donor Association (WMDA) offers a global reporting system to support HA that can be used to facilitate the reporting of HPC-related serious events and adverse reactions [24], and national HPC registries have implemented a global reporting system because HPC products have specific characteristics (for more information, see Chapter 25).

18.9 Vigilance communication: education and training

Good Practice Guidelines

G18.12 Programmes of training and awareness should be organised by HAs to encourage reporting by SoHO entities.
G18.13 Professionals should be trained about what to communicate, when and to whom.
G18.14 The reporting system should be non-punitive and confidential.
G18.15 HAs and professional societies should publish the results of their programmes without identifying individual centres, hospitals or individuals. Those SoHO establishments directly involved in specific incidents should also consider publishing their experience to alert others to the means by which they detected and confirmed the event or reaction.

Supplementary information

The Notify Library is an initiative launched by the WHO and supported by the Italian National Transplant Centre (CNT) that has gathered information on documented adverse occurrences in transplantation and MAR. It has reviewed cases to identify general principles supporting detection and investigation. The database has been constructed from the information gathered and is accessible on a dedicated website [10, 25]. The database is maintained and updated on this platform and is intended as a communication hub for institutions and organisations worldwide collaborating in the facilitation of access to V&S information to improve safety and efficacy.

It is worth noting that learning is an important benefit derived from biovigilance, including feedback to all involved parties. In this context, both health professionals and HAs can report and publish information to the Notify Library on unexpected adverse incidents, observations and experience for teaching purposes.

18.9.1 Educational training and workshops

Education on biovigilance for healthcare professionals can be done with a high-quality educational programme and with well-organised workshops, disseminating the message in meetings, publishing reports with anonymised data, etc.

The purpose is to stimulate reporting in an appropriate manner while avoiding over-reporting, which can collapse the system. Reporting and further analysis are very useful tools for learning how to avoid mistakes and other errors; in the end, the resulting analysis is beneficial for the safety of donor and patients.

18.10 References

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Related material

Appendix 23. Serious adverse reaction or event: impact assessment tool
Appendix 24. Example of serious adverse reaction (SAR) for non-reproductive SoHO
Appendix 25. Example of serious adverse reaction (SAR) for reproductive SoHO
Appendix 26. Example of serious adverse event (SAE) for non-reproductive SoHO
Appendix 27. Example of serious adverse event (SAE) for reproductive SoHO

Chapter 19. Introduction of novel processes and clinical applications

19.1 Introduction

Advances in basic science, technology and medicine create opportunities for the development of novel substances of human origin (SoHO) processes (including changes to donor selection, collection, processing, storage and distribution methodologies) or clinical applications. The key elements that ensure the quality, safety and effectiveness of novel processes and applications, and thus the safety of donors, SoHO preparations and recipients, include:

- focusing on clinical need as the predominant driver for the development of novel processes and applications for SoHO;
- the active involvement and close co-operation among three stakeholders – SoHO entities and establishments, clinicians representing organisations responsible for human application (ORHAs) and Health Authorities (HAs);
- comprehensive risk–benefit analysis that underpins the development and evaluation of novel processes and applications.

Some processes and applications, while new to a specific SoHO entity or establishment, are not novel *per se* as they have been well established by others. For those processes, Part C of this Guide includes SoHO monographs appropriate to different processes and applications for SoHO. These SoHO monographs are useful tools for SoHO entities and establishments and HAs, providing the minimum criteria to ensure the quality and safety of different types of SoHO; they are tools that can be used by SoHO entities and establishments to design appropriate validation studies for new processes.

19.2 Regulatory considerations

When a SoHO entity is developing a novel preparation process, or if it plans to provide SoHO for a new clinical application, one key consideration is whether the process or clinical application might lead to a regulatory classification of the SoHO as medicinal products, or as advanced therapy medicinal products (ATMPs) or as medical devices. If there is a possibility that the change in preparation or application methodologies for the SoHO may result in it falling into a different regulatory classification, the regulatory framework for authorisation of the relevant product type will be applicable in the EU (for further information, see Chapter 34). In these cases, the regulatory requirements of the SoHO legislation for donation, collection and testing still apply. It may be helpful for the SoHO entities to engage with their HA at an early stage of the product development cycle to establish in advance which is the applicable legal framework in their country.

19.3 Preparation authorisation application

In EU member states, it is a requirement that SoHO preparations are authorised by their Competent Authority before they are made available for clinical use. SoHO entities may request guidance or opinion from their Competent Authorities on the authorisation requirements prior to submitting a preparation authorisation (PA) application. Please see Chapter 10 (§10.7) for more detailed guidance on preparing a PA application.

The standards given below, which are based on articles in the EU regulation [1], may benefit not only EU countries but also other countries as a model for HAs to ensure that new and changed SoHO preparations are only made available for clinical use after the benefits and risks of their use have been systematically evaluated.

Standards

S19.1 The PA application must include the results of a risk–benefit analysis conducted in respect of the combination of SoHO activities performed for the SoHO preparation together with the intended clinical indication for which the application for authorisation is submitted (Art. 39.2.c). See Chapter 10 (§10.7).

S19.2 SoHO entities must carry out a risk–benefit assessment with available scientific evidence and clinical data (Art. 20.4.c). In cases where the indicated risk is greater than negligible, or the expected clinical effectiveness is unknown, a proposed plan for clinical-outcome monitoring must be included for the purpose of providing further evidence, where necessary, for the SoHO preparation authorisation (Art. 39.2.d).

S19.3 SoHO entities must request an exemption from their HA if they wish to provide SoHO preparations without authorised PA for a specific recipient when justified by the clinical circumstances as given below (Art. 38.3 and Art. 19.11) or in health emergency situations (Art. 65):

- **S19.3.1** the specific recipient has no therapeutic alternative, the treatment cannot be postponed, or the specific recipient's prognosis is life-threatening and the benefit outweighs the risks;
- **S19.3.2** the safety and effectiveness of the SoHO preparation can reasonably be assumed, based on the available clinical and biovigilance data;
- **S19.3.3** the recipient is informed that the SoHO preparation concerned has not been authorised for routine clinical use.

Supplementary information

The standards derived from the EU regulation are based on the principles and guidance developed by three EU-funded projects: VISTART, EuroGTP II and GAPP.

VISTART (Vigilance and Inspection for the Safety of Transfusion, Assisted Reproduction and Transplantation), an EU Joint Action which concluded in 2019, proposed regulatory principles for HAs for the appraisal and approval of clinical evaluation protocols for blood or other SoHO prepared with new processing methodologies [2]. These VISTART principles provided guidance for stakeholders in the development and implementation of novel preparation processes or clinical applications, ensuring compliance with regulatory and technical requirements, and they proposed an approach whereby the degree of risk associated with the novelty is linked to an appropriate clinical follow-up plan.

EuroGTP II (Good Tissue Practices for demonstrating safety and quality through recipient follow-up) developed good-practice guidance for SoHO establishments for evaluation of the safety, quality and efficacy of SoHO therapies and products. The key outputs from EuroGTP II include a systematic mechanism and interactive assessment tool to identify and evaluate risk, and defined methodologies that can be used to mitigate risk. These outputs are hosted on the European Directorate for the Quality of Medicines & HealthCare (EDQM) SoHO platform [3, 4] (see also Chapter 4).

GAPP Joint Action (facilitatinG the Authorisation of Preparation Process for blood, tissues and cells) focused on the development of a common and optimal approach for Competent Authorities to assess and authorise preparation processes in blood and SoHO establishments [5]. This project developed Good Practice Guidelines for Competent Authorities for PA for blood, tissues and cells. It also included three annexes: 1. Authorisation changes in donation, procurement and collection, processing, preservation, storage and distribution; 2. Assessing the quality and safety of donor testing, microbial inactivation/reduction and sterilisation steps as part of the PA; and 3. Assessing clinical data as part of the PA. The EuroGTP II tool and methodologies are recognised by the GAPP project as a suitable mechanism for performing this type of evaluation based on VISTART principles.

19.4 Clinical-outcome monitoring

Standards

S19.4 In cases where the scientific evidence and clinical data (evaluated as part of the benefit–risk assessment) are insufficient or point to the risk as being more than negligible, the SoHO entity must prepare a clinical-outcome monitoring plan to support the PA application (Art. 21.1).

S19.5 The content of the clinical-outcome monitoring plan must be determined by the level of risk and based on an expected positive benefit–risk assessment. Risks may be classified as low, moderate or high, in which case the following requirements for clinical outcome monitoring apply (Art. 21.3) - see Figure 19.1:

- **S19.5.1** low risk – a proactive clinical follow-up of a pre-defined number of SoHO recipients (clinical follow-up);
- **S19.5.2** moderate risk – a SoHO clinical study of a pre-defined number of SoHO recipients to assess pre-defined clinical endpoints (clinical investigation follow-up);
- **S19.5.3** high risk – and this includes cases where the risk or the benefit cannot be accurately evaluated due to a lack of scientific and/or clinical data or knowledge – a SoHO clinical study of a pre-defined number of SoHO recipients to assess

pre-defined clinical endpoints with a comparison to standard therapy (comparative clinical study).

S19.6 Before commencing a SoHO clinical study, SoHO entities must (Art. 40.2):

S19.6.1 apply for a favourable opinion from a relevant ethics committee to address the ethical, legal and methodological aspects of the clinical study to determine the capacity of the study, as designed, to draw robust conclusions;

S19.6.2 communicate the favourable opinion of the ethical committee to their HA;

S19.6.3 obtain approval from the HA of the clinical-outcome monitoring plan.

S19.7 SoHO recipients taking part in a clinical study, or the persons granting consent on their behalf, must be informed that the SoHO preparation has not yet been authorised for routine clinical use and it is applied in the context of a clinical study that forms part of the authorisation process for that SoHO preparation (Art. 40.3).

S19.8 The person responsible for the clinical study must be adequately qualified and trained (Art. 40.4). See G19.1.

S19.9 The SoHO entities must comply with vigilance and reporting requirements to report incidents to HAs identified in the course of the clinical study as per study protocol (Art. 40.5).

S19.10 If the proposal for PA includes clinical outcome registration, the applicant must provide details of the clinical registry and request approval for its use (for this purpose) from the HA (Art. 39.3).

Good Practice Guidelines

G19.1 The person responsible for the clinical study should be aware of the principles of good clinical practice and study protocol.

G19.2 SoHO entities should collaborate with clinical users to develop plans for clinical follow-up and outcome monitoring or a clinical study, as required, depending on benefit–risk analysis for the PA proposal.

G19.3 The clinical follow-up plan should include:

G19.3.1 the number of SoHO preparation applications/recipients for follow-up;

G19.3.2 the type and duration of clinical follow-up, including information on follow-up procedures (such as samples, imaging);

G19.3.3 the methodology for clinical follow-up data collection;

G19.3.4 the parameters identified to prove safety and/or efficacy of the specific SoHO preparation;

G19.3.5 data consistency assessment and/or data analysis including biometrics and statistics.

G19.4 The clinical investigation follow-up plan and the comparative clinical studies should define as a minimum:

G19.4.1 objectives and purpose of the clinical investigation;

G19.4.2 recipient inclusion and exclusion criteria;

G19.4.3 number of SoHO recipients planned to be included in the clinical investigation;

G19.4.4 alternative therapies or SoHO preparations, if any;

G19.4.5 control treatment (comparative clinical studies);

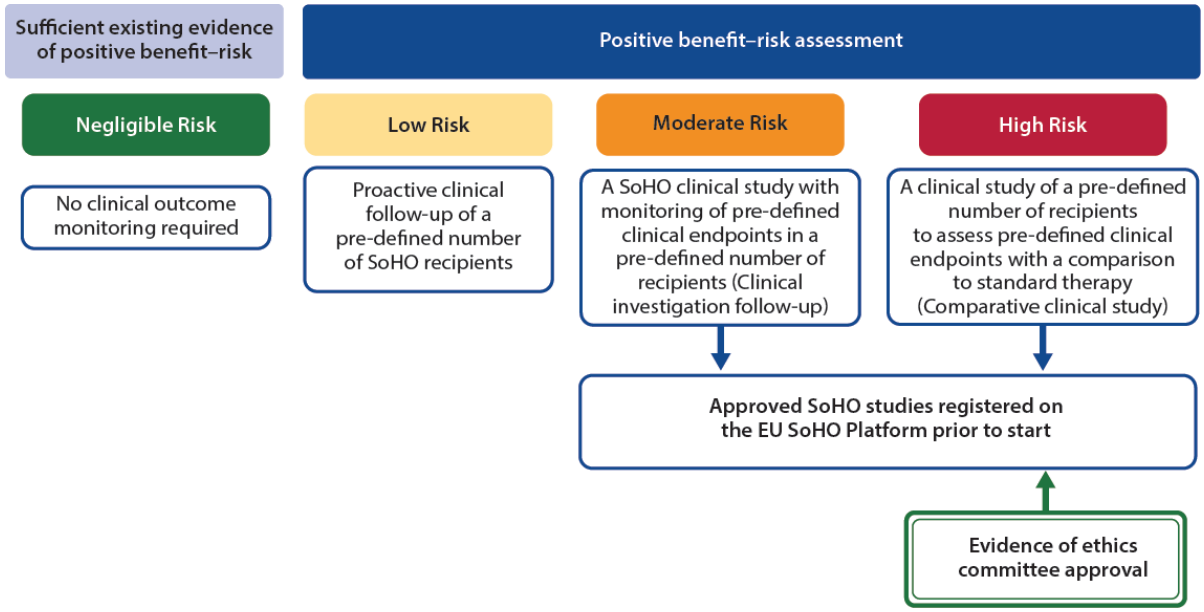
G19.4.6 recruitment procedures and informed consent protocol for the recipients;

G19.4.7 planned follow-up visits and procedures (tests, samples, imaging etc.) and duration of the clinical investigation;

G19.4.8 data collection methodology;

G19.4.9 safety and effectiveness parameters;

Figure 19.1 **Clinical outcome monitoring plans to gather further evidence of safety and effectiveness in recipients**



G19.4.10 endpoints defined by the applicant and end users to assess safety and effectiveness;
G19.4.11 statistical protocols, data handling, record keeping and methodology for data analysis.

Supplementary information

The GPGs above align with outputs of GAPP [6, 7]. The purpose of a PA is for the HA to verify that an adequate level of quality, safety and effectiveness is achieved consistently by SoHO preparations when prepared following the methods detailed in the application. It is recognised that the benefit–risk assessment will differ greatly among different SoHO preparations and for different clinical indications. Close collaboration between the SoHO entities, establishments and clinical end users is crucial to provide sufficient evidence to demonstrate safety and effectiveness for PA authorisation by the HA (see section 19.6).

19.5 Clinical-outcome monitoring plans

Standards

S19.11 SoHO entities must use the guidelines and templates for clinical outcome monitoring plans provided by their HA (Art. 19.1).
S19.12 The applicant SoHO entity remains responsible for collecting the clinical-outcome monitoring data and must make those data available upon request from the HA (Art. 39.5).

Supplementary information

SoHO entities are expected to report incidents identified in the course of the clinical study to HAs, in line with study protocols and the vigilance and reporting requirements set out in Chapter 18 of this Guide (S18.1). Those persons submitting the PA application can choose to record the clinical data generated during the implementation of the clinical-outcome monitoring plans themselves, or they can also use existing clinical registries.

The existence of a registry of approved clinical studies at national, European and international level is critical to facilitate patient participation in such clinical studies, to boost multi-centre studies and to foster collaboration to generate more robust results and conclusions and to make the knowledge generated available to other researchers, healthcare professionals, participants themselves and the general public. Some examples of existing European databases which are assessing and verifying the safety, quality and efficacy of specific treatments include: the European Cornea and Cell Transplantation Registry (ECCTR), the European Society for Blood and Marrow Transplant (EBMT) Patient Registry, and European IVF Monitoring (EIM) [8-10].

19.6 Interaction between key stakeholders in clinical studies and follow-up

When introducing novelties in the field of SoHO, close interaction between the three main stakeholders is vital. These are the SoHO establishments, the clinicians in the ORHA and the HAs. They each contribute their specific expertise to establish a structured, safe and efficient approach to the introduction of new SoHO preparations and clinical applications.

19.6.1 Responsibilities of the SoHO establishment

The SoHO establishment is responsible for ensuring that the quality, safety and effectiveness of SoHO preparations meet the regulatory requirements and is also responsible for defining critical quality attributes necessary for release for clinical application (see also Chapter 4 for risk analysis). In this context, quality and safety result from the donor selection, the collection of SoHO, the testing and the preparation processes. Risk analysis to support a PA application would consider current evidence derived from known preparation processes, processing steps for authorised SoHO preparations that are comparable to the SoHO preparations obtained with novel preparation methods and/or new clinical applications. See Chapter 10 (§10.7) for more information on PA.

In circumstances where evidence is lacking, due to the grade of novelty and uncertainty, an analysis to estimate the risks is necessary. The EuroGTP II tool [3, 4] and methodologies are recognised as a suitable method for performing this type of evaluation; see Chapter 4.

19.6.2 Responsibilities of the clinicians/ organisations responsible for human application

The roles of the clinician(s) are:

- ensuring safety and effectiveness in the context of clinical application of the SoHO preparations;
- explaining to the patient the relevant information on the expected effectiveness and potential risks;
- obtaining appropriate patient consent to participate in the study, for application of the SoHO preparations and for gathering clinical outcome and surveillance data from patients.

Although it is the responsibility of the SoHO entity to submit a PA for authorisation, the details of the clinical follow-up and clinical investigations (number of patients, duration, end points etc.) are developed in collaboration with the end users, who have the clinical knowledge to determine such criteria.

19.6.3 Responsibilities of the Health Authorities

Regulation of SoHO by independent bodies, such as HAs, ensures the quality, safety and efficacy or effectiveness of SoHO preparations [1].

Regulation focuses on two key elements:

- data-driven, risk–benefit assessment of SoHO preparations, based on well-established specifications;
- risk-based decision making on the approval of preparations or clinical applications by the HAs.

19.7 References

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Part B.

Specific requirements for substances of human origin

Chapter 20. **Ocular tissue**

20.1 Introduction

Ocular tissues collected from deceased donors are used for treating loss of vision caused by irreversible corneal disease or trauma, as well as for reconstructive and glaucoma surgery.

The cornea is the principal refractive component of the eye. Good vision depends on its transparency and a smooth, spherical corneal surface. The cornea is also part of the ocular surface and is strong enough to withstand the intra-ocular pressure and to help protect the delicate inner structures of the eye.

20.1.1 Corneal transplantation

A corneal transplant (keratoplasty) is an operation to remove all or part of a diseased or damaged cornea and replace it with healthy donor tissue. In Europe, the main indications for corneal transplantation include:

- Fuchs endothelial corneal dystrophy (FECD) – failure of the corneal endothelium, the monolayer (of non-dividing cells lining the inner surface of the cornea) that controls stromal hydration and thus maintains corneal transparency;
- keratoconus – a connective tissue disorder resulting in thinning and degeneration of the corneal stroma, which normally accounts for 90% of corneal thickness, and also resulting in distortion of corneal shape;
- pseudophakic bullous keratopathy (PBK) – failure of the corneal endothelium as a consequence of previous cataract or other intra-ocular surgery;
- infection, such as *Herpes simplex* keratitis (HSK), or other kinds of infection or inflammation or trauma – mainly treatment of post-infectious scars, but acute infection may require a therapeutic keratoplasty;
- regraft for a failed corneal transplant.

These conditions may all be treated with a full-thickness corneal transplant (penetrating keratoplasty, PK); however, current practice is to replace, where possible, only the dysfunctional part of the cornea [1-3] as illustrated in Figure 20.1.

Endothelial keratoplasty (EK) is the method of choice for treatment of endothelial dysfunction, such as FECD or PBK. The lamellar graft, which consists of endothelium on its basement membrane (Descemet membrane), with or without a thin supporting layer of stroma, is inserted through a small incision into the anterior chamber of the eye and attaches to the posterior surface of the patient's cornea. The initial attachment is ensured by air or sulphur hexafluoride gas tamponade in the anterior chamber. The affected patients are mostly elderly and the advantages of EK over PK include much faster visual rehabilitation, lower rejection rates and, since there are no sutures required to hold the graft in place, negligible surgically induced astigmatism.

There are currently four techniques for preparing tissue for EK:

i. Descemet stripping endothelial keratoplasty (DSEK) – endothelium on Descemet membrane with a thin layer of stroma, prepared by manual dissection.
ii. Descemet stripping automated endothelial keratoplasty (DSAEK) – endothelium on Descemet membrane with a thin layer of stroma, prepared by using a microkeratome.
iii. Descemet membrane endothelial keratoplasty (DMEK) – endothelium on Descemet membrane without any stroma, prepared by manually separating Descemet membrane from the stroma.
iv. Pre-Descemet membrane endothelial keratoplasty (PDEK) – endothelium on Descemet membrane with a thin layer of pre-Descemet stroma, prepared by injecting air to create a 'big bubble', which separates the graft from the bulk of the stroma.

For keratoconus, which typically affects younger patients, deep anterior lamellar keratoplasty (DALK) is an alternative to PK that replaces the full thickness of the stroma, leaving the recipient's endothelium intact. In PK, the immunological rejection against the endothelium is one of the main reasons for failure, and the advantage of DALK is that the patient's endothelium is maintained. Superficial stromal defects and scars can also be treated by anterior lamellar keratoplasty (ALK), without the need to replace the full thickness of the stroma.

Whereas a tissue for PK or EK requires a donor corneoscleral disc with a viable endothelium, a tissue for DALK or ALK does not; however, corneoscleral discs with an endothelium suitable for PK may be requested for DALK owing to the occasional need to switch procedure in the operating room (OR) from DALK to PK. Nowadays tissue for EK is mostly prepared in a substances of human origin (SoHO) establishment, which saves time for the surgeon and avoids the risk of damage to the tissue when prepared in the OR. In this way risks of damage to the tissue are transferred to the SoHO establishment, but the advantage is that this does not happen in the OR and a backup tissue can be arranged. Furthermore, SoHO establishments are increasingly trained to prepare high-quality tissues for EK due to the sheer volume of their preparations.

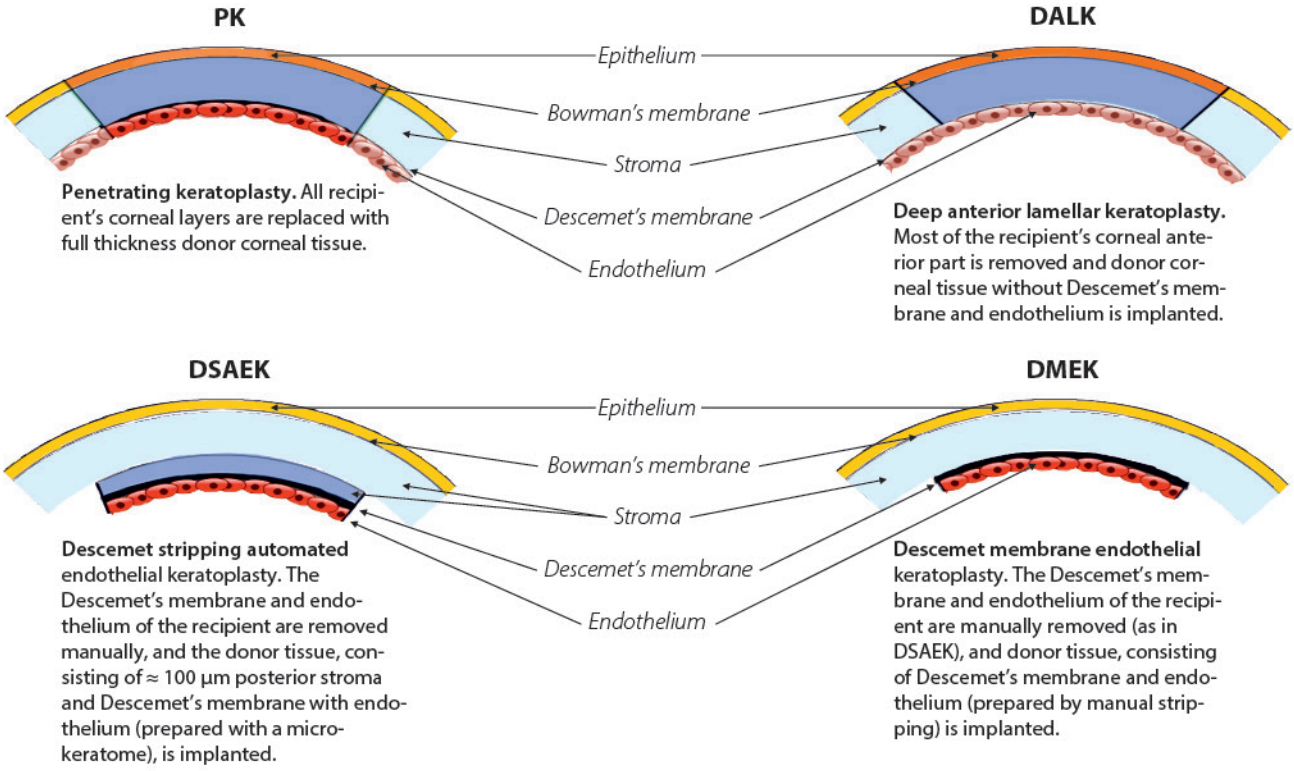
Corneal transplant outcome – in terms of both graft survival and visual acuity – depends on the recipient disease, the presence of pre-operative risk factors, such as vascularised cornea, glaucoma and inflammation, and post-operative complications such as allograft rejection. In the absence of other risk factors, keratoconus and FECD are considered to be low-risk graft indications with 4-year survival rates of 95% and 88%, respectively [4]. Regraft and PBK are more likely to undergo allograft rejection and/or failure and are therefore considered high-risk graft indications with 4-year survival rates of 63% and 63% respectively [4].

A major cause of graft failure is allograft rejection. Most rejection episodes can be successfully treated with topical (sometimes intra-ocular, subconjunctival, peribulbar or systemic) corticosteroids. In some cases, use of systemic immunosuppression (e.g. cyclosporine, tacrolimus, mycophenolate) is considered necessary to reduce the risk of immune reactions. The results from studies of human leukocyte antigen (HLA) matching to reduce the risk of rejection are not as conclusive as for organ transplantation, but matching may be beneficial for high-risk corneal grafts [5].

20.1.2 Further use of ocular tissue

If, for example, a patient has suffered multiple failed corneal grafts, a keratoprosthesis may be an appropriate alternative to a corneal graft [6]. Keratoprostheses are attached to a ring of corneal tissue from a donor, which is then sutured to the recipient's cornea after removal of the failed graft. Sclera and cornea can also be used in glaucoma surgery and for reconstructive surgery of the ocular surface. Limbal tissue, which contains a population of corneal epithelial progenitor cells, may be transplanted as a keratolimbal allograft (KLAL) to treat ocular surface disease caused by failure of the corneal limbus (limbal stem cell deficiency) [7].

Figure 20.1 **Different types of keratoplasty**



There are other SoHO that are used in ocular surgery, but which are addressed in other chapters or fall outside the scope of the SoHO legislation, such as the following:

- Limbal progenitor cells may be isolated from a corneoscleral disc and expanded *ex vivo* for treating ocular surface disease (Chapter 34) [8];
- Corneal endothelial cells may be isolated from a corneoscleral disc and expanded *ex vivo* for injection as a suspension into the anterior chamber for treatment of corneal endothelial disease [9];
- Amnion is used for treating ocular surface conditions and as a support for limbal progenitor cells (see Chapter 21) [10];
- Autologous and allogeneic serum eye drops may be used for treating dry eye and/or persistent epithelial defects [11]. Guidelines are provided in the Guide to the preparation and quality assurance of blood components [12].

However, activities such as donor registration, history review and medical examination, testing, collection and release are still subject to the SoHO Regulation (see Chapters 19 and 34).

20.2 Donor evaluation

20.2.1 Tissue-specific exclusion criteria for ocular tissue donation

Acceptance and exclusion criteria for cornea donation that differ from the criteria for other tissues are based on the avascularity of the cornea and ocular-specific conditions that may affect the cornea.

The general criteria for donor selection are outlined in Chapter 6. The specific criteria for exclusion of ocular tissue are given in the same chapter, in Table 6.2. The section below provides additional information to explain the tissue-specific criteria.

Table 6.4 details the general risk conditions for SoHO donors, mainly relating to communicable infectious diseases requiring careful evaluation. Donor screening is provided in Chapter 7.

20.2.1.1 *Selection criteria for ocular tissue other than cornea*

Good Practice Guidelines

G20.1 For any tissue or cells that are not derived from the avascular cornea, such as sclera, limbal tissue and limbal cells, the common exclusion criteria for non-ocular tissues should be followed (see Chapter 6).

20.2.1.2 *Donor age*

Provided that corneas are examined to exclude those unsuitable for transplantation based on endothelial cell density and/or stromal abnormalities (see §20.7), the upper age limit for eye donors may be determined by the SoHO establishment. The likelihood that corneas will be suitable for PK or EK does decline with increasing donor age but, where the endothelial cell density is considered sufficient, graft survival is little affected by donor age [13, 14].

The minimum donor age is more uncertain and can be determined by the SoHO establishment because corneas from young children lack rigidity and have a high radius of curvature. As most corneal diseases are age-dependent, there is little demand for corneas from young donors for transplantation; however, corneas from these donors may be important as a source of limbal grafts or limbal progenitor cells.

Older donors (over 65 years) are often preferred for DMEK surgery, as grafts from older donors are easier to prepare and to unfold during surgery [15], although a clinical study does not suggest any clinical disadvantage of using donors younger than 55 years [16].

20.2.1.3 *Malignancies*

Good Practice Guidelines

G20.2 In the case of donors with malignant diseases and a potential risk of metastasis formation in the anterior ocular segment, a thorough biomicroscopy (e.g. slit-lamp examination) of the globe or the corneoscleral disc focused on possible metastasis should be undertaken in the SoHO establishment.

G20.3 Corneas should be excluded where there is suspicion of anterior segment metastases of the eye or the corneoscleral disc.

G20.4 The utmost care should be taken to correctly identify the affected eye/cornea to avoid the risk of collecting/processing the affected eye/cornea.

Recommendations

R20.1 The incidence of metastases from non-ocular tumours to the anterior segment of the eye is extremely low [17]. It is recommended to accept the contralateral non-affected eye.

Supplementary information

While discussing the incidence of metastases from non-ocular tumours, a report of metastatic cholangiocarcinoma cells found in the sclera and sclerocornea interface, but not avascular cornea, supports this [18]; there has been a report of malignant cutaneous melanoma (MCM) metastases in peripheral, but not central, avascular cornea [19]. The Medical Standards of the Eye Bank Association of America recommend the exclusion of donors with a history of melanoma with known metastatic disease [20]. More information about malignancies is provided in Chapter 6 (Table 6.2 and Recommendations R6.12 to R6.14).

20.2.1.4 *Infections*

Good Practice Guidelines

G20.5 Donors colonised with multidrug-resistant micro-organisms should undergo a thorough risk assessment before they may be accepted as donors.
G20.6 Individuals with active localised ocular infection (bacterial, viral, fungal, protozoal, parasitic) should be excluded from donation of ocular tissues.
G20.7 An eye with a known history of past ocular *Herpes* infection should be excluded [21]. In this case, the utmost care should be taken to correctly identify the affected eye/cornea to avoid the risk of collecting/processing the affected eye/cornea.

20.2.1.5 *Diabetes mellitus*

Diabetes mellitus increases the risk of unsuccessful DMEK graft preparation [22]. However, this risk may be related to the severity of the diabetes, and an algorithm has been proposed to allow grafts to be prepared from corneas from donors with mild disease [23].

20.2.1.6 *Eye diseases*

Good Practice Guidelines

G20.8 The following should exclude cornea donation:
- **G20.8.1** autoimmune disease (e.g. sarcoidosis, rheumatoid arthritis) if there is ocular involvement, or previous ocular surgery that would prejudice graft outcome.

G20.9 The eye conditions that should be excluded or accepted depending on the planned use of the tissue are indicated in Table 20.1.

20.3 Collection

General information on collection is provided in Chapter 7.

20.3.1 *Post mortem* time

Though it is normal practice in many SoHO entities and establishments to collect corneas within 24 hours after death, some Health Authorities or local practices may allow collection up to 72 h after cardiac arrest for organ-cultured corneas. A recent study has documented the safety of this practice in corneal tissue collection [24].

20.3.2 Collection procedures

The ocular surface is exposed to the environment, and after death, there is no blinking and no tear film. Therefore, it is likely to be contaminated by environmental micro-organisms. For these reasons, a classified area with a specified air quality is not required for ocular tissue collection.

Good Practice Guidelines

G20.10 The donor's eyelids and surrounding skin area should be cleaned with an antiseptic solution and a sterile drape (eye sheet) placed over the face leaving the eyes exposed.
G20.11 The donor's eyes should be flushed with an appropriate sterile solution to remove debris, mucus and foreign matter from the cornea and conjunctival sac.
G20.12 An antiseptic solution suitable for cleaning the ocular surface prior to intra-ocular surgery, or a broad-spectrum antibiotic solution, should be applied.

Recommendations

R20.2 After insertion of a lid speculum, peritomy is performed, preferably leaving a frill of conjunctiva at the limbus to avoid damage to the limbal progenitor cell niche.

Supplementary information

Advantages of *in situ* excision of corneoscleral discs include reduced death-to-preservation time since the corneoscleral disc is placed in storage medium

Table 20.1 **Eye conditions to be excluded or accepted depending on the planned use for PK, DALK or EK**

Eye condition	PK	DALK	EK
Corneal disorders including keratoconus, keratoglobus, and epithelial and stromal dystrophies. As these diseases typically are bilateral, both eyes should be excluded for PK and DALK.	X	X	✓
Corneal opacity, scarring, pterygium or other superficial disorders of the conjunctiva or corneal surface that involve the central area of the cornea.	X	X	✓
Refractive corneal surgical procedures, including radial keratotomy, synthetic lamellar inserts and laser refractive surgery.	X	X	✓

immediately following collection; potentially improved cosmetic reconstruction of the donor; and it may be more acceptable to some relatives or next of kin than enucleation. However, collection by enucleation is simpler, with less risk of harm to the corneal endothelium; and enucleated eyes provide sclera, for glaucoma or reconstructive surgery, and retina, optic nerve, lens and iris for research. Furthermore, a whole eye can be rinsed with physiological salt solution and disinfected easily with, for example, povidone-iodine. Also, trephining the corneoscleral disc is better controlled in the cleanroom environment inside the SoHO establishment. There is no reported evidence of a difference in corneal quality or clinical outcome between these two collection methods.

20.3.2.1 *Collection of the whole eye*

Good Practice Guidelines

G20.13 After enucleation, the whole eye should be placed, cornea uppermost, in a fixed position in a moist chamber and transported to the SoHO establishment.

Supplementary information

The lateral extraocular muscle is located and lifted with a muscle hook and clamped with artery forceps close to its point of attachment to the sclera. The muscle is divided distally, leaving the artery forceps in place to stabilise and steady the eye. The remaining rectus muscles are then lifted in turn with the muscle hook and divided close to the sclera. It is not necessary to divide the oblique muscles. The eye is then gently lifted and the optic nerve severed using curved enucleation scissors.

20.3.2.2 *Collection of corneoscleral discs*

Good Practice Guidelines

G20.14 After excision, the corneoscleral disc should be immersed, endothelium uppermost to avoid the risk of damage, in an appropriate corneal storage solution that may contain antibiotics and antimycotics.

Recommendations

R20.3 Unless the cornea is to be transferred directly to organ culture at the SoHO establishment, it is recommended that, when possible, the container be a corneal viewing chamber or have a flat bottom and adequate optical properties to facilitate subsequent assessment by biomicroscopy and specular microscopy.

Supplementary information

After peritomy, sclerotomy is performed, maintaining a wide scleral rim (about 2 mm) around the cornea. The corneoscleral disc is then gently lifted away from the eye without folding, to avoid damage to the endothelium.

For light microscopy evaluation, other relevant containers and evaluation methods may be used.

20.3.3 Reconstruction of the donor

Good Practice Guidelines

G20.15 The aim should be to mimic as closely as possible the original profile of the donor's closed eyes.
G20.16 After enucleation, the orbit should be filled with an appropriate prosthesis or other suitable material.
G20.17 The eye lids should be then closed to restore the appearance of the donor.

20.4 Temporary storage and transportation to the SoHO establishment

Good Practice Guidelines

G20.18 Whole eyes should be stored and transported in a moist chamber at 1 to 10°C. The time from cardiovascular arrest to processing at the SoHO establishment should not exceed 72 h.
G20.19 The manufacturer's recommendations for storage temperature should be followed or the temperature conditions be validated.

Recommendations

R20.4 It is recommended to place corneoscleral discs collected by in situ excision in a hypothermic storage solution or in a room-temperature storage medium or solution (designated by the manufacturer).

20.5 Processing, preservation and storage

Depending on which ocular tissue arrives at the SoHO establishment, different factors are evaluated.

20.5.1 Cornea processing methods

Good Practice Guidelines

G20.20 When whole eyes are received by a SoHO establishment, they should be subjected to a cleaning protocol to reduce the bioburden on the ocular surface before excision of the corneoscleral disc.
G20.21 Gross examination of the globe or corneoscleral disc at the arrival to the SoHO establishment should be performed to check:
G20.21.1 Presence of abnormalities of the external eye structures;

G20.21.2 Presence of excessive orbital tissue or laceration of the eye globe.

G20.22 Biomicroscopy examination (see above: section 20.2.1.3, G20.2) of the cornea, limbus and surrounding sclera should be performed upon arrival at the SoHO establishment to observe for:

G20.22.1 signs of previous corneal refractive surgery or anterior segment surgery;

G20.22.2 signs of abnormal corneal shape (keratoconus, keratoglobus, micro- or megalocornea);

G20.22.3 presence of central corneal opacities (pterygium, stromal opacities);

G20.22.4 presence of abnormal tissue changes in the corneal periphery, anterior chamber or iris.

G20.23 Microscopic evaluation of corneal endothelium should be done at least once at the SoHO establishment to:

G20.23.1 estimate the endothelial cell density;

G20.23.2 observe for endothelial abnormalities (scars, guttae).

Supplementary information

Cleaning protocol could be, for example, rinsing in sterile saline and immersion in a disinfectant such as povidone-iodine or chlorhexidine [25].

When corneoscleral discs have been collected by *in situ* excision, they will already be in a storage medium on arrival at a SoHO establishment and may not require further processing unless they are transferred to organ culture. Further processing of corneoscleral discs to prepare grafts for EK may be undertaken in the SoHO establishment [26, 27].

Biomicroscopy methods are used for exclusion of pathological changes to the stroma or endothelium, such as scars, oedema, significant arcus, striae, endothelial guttae or disease, infiltrates or foreign bodies, and anterior segment tumours or metastases.

For corneas stored by hypothermia, the microscopic evaluation of corneal endothelium is typically performed at the start of storage. If the corneoscleral disc is not in a corneal viewing chamber, it is turned over so that the endothelium is facing downwards to allow observation by specular microscopy through the base of the container. It is then returned to the endothelium-uppermost position to avoid the risk of subsequent damage.

There are two accepted methods used for endothelial evaluation by microscopy:

1. Specular microscopy. This method allows direct examination of the endothelium without staining; however, the appearance of the endothelial cells varies with temperature, type and time of preservation and the storage medium used. It is general practice that cold-stored corneas are warmed to room temperature to enhance the quality of the endothelial image.
2. Transmitted light microscopy (bright field or phase contrast). If necessary, to enable cell counting, brief exposure to hypotonic sucrose solution (1.8% w/v) or short exposure (few seconds, max. 4 minutes) to either balanced hypotonic salt solution (BSS) or 0.9% (w/v) NaCl is possible to make endothelial cell borders visible. The exposure time to these solutions needs to be limited. Prior use of a stain such as trypan blue (0.06-0.4%) will help to identify dead cells and areas of denuded Descemet membrane.

20.5.2 Cornea storage methods

i. Hypothermic storage at 1 to 10°C

Good Practice Guidelines

G20.24 For corneoscleral discs in storage medium, the manufacturer's recommendations should be followed for storage temperature and for maximum storage time, which can vary up to 21 days [28-30].

G20.25 Corneas prepared in a SoHO establishment for DSAEK or for DMEK should be shipped to SoHO entities in hypothermic storage media.

ii. Organ culture at 28 to 38°C

Good Practice Guidelines

G20.26 Storage time should be up to 4-5 weeks from the start of organ culture.

G20.27 An inspection of the endothelium is mandatory at the end of the storage period and then the transplant should be assigned to the proper kind of surgery based on the cell density.

G20.28 To reverse the stromal oedema that occurs during organ culture, corneas to be used for PK or DALK or DSAEK should be transferred to a medium, the transport or 'deswelling' medium, containing a macromolecule to increase osmotic pressure and induce an efflux of water from the stroma. The cornea should be kept at 10 to 38°C for up to 4-7 days, at the discretion of the Responsible Person (RP) and depending on the medium used [31, 32].

Recommendations

It is advisable to:

R20.5 prepare DMEK grafts by manual dissection, pneumatic dissection or hydrodissection [33];

R20.6 when using DSAEK grafts prepared by a SoHO establishment, mount the corneoscleral disc in a pressurised artificial anterior chamber and then cut away the anterior stroma using a microkeratome [34];

R20.7 supply DMEK grafts rolled in the final diameter direct into medium or attached, either in the centre or at the periphery, and laid back on the stroma and stored in normal medium with or without deswelling properties [35], although research suggests medium without deswelling properties may be superior [36];

R20.8 prepare organ-cultured corneas in the SoHO establishment for DSAEK after pre-thinning in deswelling medium, or for DMEK with or without pre-thinning [35];

R20.9 renew the storage medium using aseptic procedures during the storage period at the discretion of the RP, though this may depend on the manufacturer's recommendations;

R20.10 lay back the anterior stroma onto DSAEK preparation to provide additional support during transport in deswelling medium [37];

R20.11 cut DSAEK grafts as thin as possible because visual acuity is better after grafting with a thin graft compared with a thick graft [38].

Supplementary information

Despite storage times up to 4-5 weeks for organ culture (G20.26), successful transplants after seven weeks have been reported [39].

Pre-loaded grafts for both DSAEK and DMEK can also be provided in order to minimise the time and effort needed for tissue preparation in the theatre (G20.28) [40, 41].

A cornea released to the surgeon for a DMEK procedure to be prepared by the surgeon in the OR may also be sent without pre-thinning.

Clinical results after graft preparation using a femtosecond laser have been found inferior when compared with microkeratome-cut DSAEK grafts [42, 43]. Clinical quality-control studies comparing DSAEK grafts prepared in a SoHO establishment with DSAEK grafts prepared by the surgeon immediately before surgery have not identified differences in early complications (graft detachment), primary graft failure or endothelial cell density two years after surgery [34].

A no-touch technique, without direct physical tissue manipulation during tissue preparation, may be an ideal approach to minimise graft damage [44]. Clinical results after eye bank- and surgeon-prepared DMEK grafts seem similar [45, 46].

Although not yet implemented in SoHO establishment practice, corneal storage under controlled positive pressure (bioreactor) and continuous medium renewal, thereby avoiding corneal swelling, might improve endothelial survival during culture. This technique may become a future significant technical advancement of the organ culture technique [47].

iii. Storage of non-viable corneal tissue

Corneoscleral discs or pieces of cornea for glaucoma or reconstructive surgery are stored in ethanol (≥ 70% v/v) or glycerol for extended periods. Corneal tissues are typically irradiated and stored in albumin, or cryopreserved, frozen [48] or freeze-dried [49].

20.5.3 Sclera processing and storage

Good Practice Guidelines

G20.29 After excision of the corneoscleral disc from the eye, sclera should be prepared using aseptic techniques by removing the intra-ocular contents (vitreous, lens, iris, choroidal and retinal tissue) and adnexa (remnants of muscles, conjunctiva).

G20.30 Sclera stored in saline with antibiotics in a refrigerator should only be kept for short periods (≤ 7 days).

Supplementary information

Sclera can be stored – whole, or divided into smaller, individually packaged pieces – in ethanol (≥ 70% v/v) or glycerol [50], or fixed in formalin, freeze-dried, frozen or kept in physiological solution or medium with antibiotics.

The shelf life for sclera stored by ethanol or glycerol, or fixed in formalin, freeze-dried or deep-frozen is generally accepted as up to two years.

20.5.4 Transport of the cornea to the Organisation Responsible for Human Application (ORHA)

Good Practice Guidelines

G20.31 The organ-cultured cornea as well as prepared grafts for DSAEK and DMEK should be transported to the ORHA at temperatures between 10 and 38°C.

G20.32 The total duration of storage/transport in deswelling medium should not exceed seven days.

G20.33 Hypothermic stored corneas should be transported at 1 to 10°C.

Supplementary information

A more limited temperature interval may be provided by the manufacturer of commercial media.

20.6 Microbiological testing

At the discretion of the transplanting surgeon, any corneoscleral tissue and storage medium remaining after preparation of the graft may be sent for microbiological testing; although there appears to be little predictive value from this [51], it can be helpful for the investigation of post-operative endophthalmitis. For further information, refer to Chapter 12 on the principles of microbiological testing.

Testing may be carried out before processing by swabbing the eye before excision of the corneoscleral disc; however, the predictive value of this procedure is very small and therefore not recommended for ocular tissues [52].

20.6.1 Hypothermic storage of corneas

Supplementary information

Taking a sample of medium for microbiological testing during hypothermic storage of corneas is not standard practice but may be required by national guidelines. Due to low temperature and limited time of storage, sampling of storage medium as a surrogate for direct microbiological testing of tissue may be made immediately upon arrival at the SoHO establishment.

20.6.2 Organ-culture storage of corneas

Good Practice Guidelines

G20.34 Since corneoscleral discs intended for transplants requiring viable cells cannot be sterilised, microbiological testing of samples of organ-culture medium taken during corneal storage should be undertaken to test for microbial contamination.
G20.35 A minimum initial corneal storage period of three days is required before taking samples for microbiological testing.
G20.36 In addition to microbiological testing, the culture medium should be inspected regularly for turbidity and change in pH (e.g. change in colour of phenol red in the medium), which may indicate microbial contamination.
G20.37 If growth is detected after release and transport to the ORHA, the surgeon should be informed immediately to prevent transplantation of the tissue.
G20.38 If the transplantation has taken place, the identification and sensitivities of the contaminating micro-organisms should be established as soon as possible in order to help the surgeon's post-operative management of the recipient.
G20.39 The fellow cornea should be re-evaluated and the donor risk should be re-assessed, whereafter the fellow cornea may be discarded, e.g. in case of contamination with *Candida* spp.
G20.40 If the fellow cornea has already been transplanted, the transplanting surgeon should be informed, and the patient monitored.

Recommendations

It is advised that:
R20.12 further microbiological testing be carried out, if possible, whenever a cornea is re-exposed to the environment, for example after endothelial assessment and transfer of the cornea into the transport medium or after preparation of corneas for DSAEK or DMEK in a SoHO establishment;
R20.13 the organ-culture medium be kept for at least a week after transfer of the corneoscleral disc to transport medium to allow additional monitoring for signs of contamination.

Supplementary information

However, given the restricted time a cornea may remain in transport medium, it is possible that growth of micro-organisms may not be detected before the cornea is transplanted. A negative-to-date release is possible, as described in Chapter 12.

20.6.3 Sclera and non-viable corneal tissue

Good Practice Guidelines

G20.41 Depending on the method of storage, microbiological testing should be carried out after processing.

Supplementary information

Storage in ethanol (≥ 70% v/v), glycerol (≥ 85% v/v). Irradiation of the tissue may render microbiological testing unnecessary (see Chapter 12).

20.7 Quality control and cornea evaluation

Good Practice Guidelines

G20.42 Quality-control tests on corneal grafts should consider at least the following minimum quality criteria:
G20.42.1 no evidence of microbiological growth (aerobic or anaerobic bacteria, yeast or fungi);
G20.42.2 sufficient endothelial quality if the graft is used for PK or EK;
G20.42.3 normal morphology and integrity of the corneal stroma if the graft is used for PK or DALK.
G20.43 If the graft is used for PK or EK, microscopic evaluation of corneal endothelium should be performed before release for transplantation to:
G20.43.1 estimate the endothelial cell density;
G20.43.2 observe for endothelial abnormalities (scars, guttae).

Recommendations

R20.14 Advised indications and contraindications to use of corneas for PK, DALK or EK are given in Table 20.2.

20.8 Corneal transplant registries

Corneal transplant registries, such as those in Australia, the Netherlands, Sweden and the UK, provide an invaluable resource to validate the quality and safety of transplanted corneas. Registries also allow investigation of donor and recipient factors influencing graft survival, post-operative complications (including

immunological rejection and serious adverse reactions) and visual outcome [4, 13, 53-56]. Although randomised clinical trials (RCT) are considered to provide the highest level of evidence, they are costly and complicated to set up, they can be undermined by changes in clinical and surgical practice during the course of the study, and it is not always straightforward to generalise beyond the specific inclusion/exclusion criteria of an RCT, especially when corneal transplantation outcomes and the risk of post-operative complications are influenced by many factors.

Registries, while not without pitfalls, rely on large datasets to reduce selection bias. They provide a broad overview across multiple transplant units and an evidence base that does not always reflect the optimism generated by the excellent results from single-centre studies [53, 54]. In addition to evaluating the outcome of established techniques and monitoring the uptake and success of new processing and surgical techniques, such as endothelial keratoplasty, registry data can also be used for validating eye-bank processes and storage methods in terms of clinical outcome measures rather than simply relying on *in vitro* laboratory measures of quality and safety [13, 57]. A recent project, part funded by the EU and led by the European Society for Cataract and Refractive Surgery, has established a European Cornea and Cell Transplant Registry (ECCTR), building on the existing registries in the Netherlands, Sweden and the UK. The registry is based on donor and recipient factors registered at the time of surgery and a follow-up two years after surgery. The registry is open for all surgeons and SoHO entities and establishments (see https://www.escrs.org/about-escrs/registries/ecctr).

20.9 Biovigilance

Some of the examples below are from the Notify Library, which includes some well-documented cases of adverse reactions and adverse events in transplantation of ocular tissue.

The database is publicly accessible and can be searched by substance type, by adverse occurrence type and by record number. For further evaluated cases of adverse outcomes associated with ocular banking, search the Notify Library at www.notifylibrary.org/.

Serious adverse reactions (SARs) for corneal transplants include:

i. Transmission
   a. Infection
   - local infection (endophthalmitis or other serious ophthalmic infection);
   - transmission of systemic infection (possibly attributable to the transplanted tissue);
   - donor-to-recipient transmission of the *Herpes* simplex virus (HSV) by cornea transplantation was confirmed by polymerase chain reaction-based DNA fingerprinting of donor and recipient HSV strains (Record Number 429);
   - transplant-acquired diagnosis of rabies supported by temporal association of the recipient's illness, lack of other exposure to rabies and the retro-orbital pain of the recipient of the corneal transplant (Record Number 20);
   - cases with suspicion of fungal infection (*Candida* spp.) have been reported post-DSAEK (Record Number 1820);
   - a case of multidrug-resistant *Pseudomonas aeruginosa* infection after positive culture in the contralateral donor cornea (Record Number 2008).

   b. Malignancy
   - transmission of malignancy (possibly attributable to the transplanted tissue);
   - transmission of T-cell lymphoma is described, whereby molecular analyses were used to detect the same alleles in HLA-DQα testing of the recipient and donor of the graft (Record Number 338);
   - metastases from a cholangiocarcinoma in the vascularised limbal region of a corneoscleral disc. There was no evidence of transmission to the recipient of the avascular corneal graft. The authors recommended that tissue from donors

Table 20.2 **Advised indications and contraindications to use of corneas for PK, DALK or EK**

Graft condition	PK	DALK	EK
Insufficient endothelial cell density (typically lower than 2 000 cells/mm^2)	X	✓	X
Moderate to severe signs of polymegathism and pleomorphism	X	✓	X
Significant (> 25%) endothelial cell loss during organ culture	X	✓	X
Abnormalities such as several scattered or confluent guttae in the centre of the cornea	X	✓	X
Presence of dead endothelial cells (corneas with scattered, isolated dead cells are acceptable, whereas corneas with larger areas of dead or missing cells are not).	X	✓	X
Central stromal scars, signs of previous corneal refractive surgery or opacities within the central 7.5 mm zone. Note that for DSAEK endothelial keratoplasty, scars or opacities within the central 7.5 mm zone are contraindicated.	X	X	✓

with a history of malignancy not be used for limbal allografting (Record Number 1663) [16]. The implications for donor-selection criteria have been considered by the Eye Bank Association of America Medical Advisory Board, resulting in the exclusion of donors with a history of melanoma with known metastatic disease [18].

ii. Graft failure
   a. primary graft failure (corneal transplant never cleared);
   b. graft failure due to a defect in the donor tissue, which was out of date, scarred or marked by incisions from previous surgery.
iii. Undue exposure to risk intervention
   a. during surgery the cornea for transplant showed signs of keratoconus. Procedure aborted. Patient already under anaesthesia (Record Number 957).

Serious adverse events (SAEs) include:

i. Tissue or cell defect
   a. infection detected in organ-culture medium after the cornea was supplied to the surgeon;
   b. tissue supplied was damaged or showed signs of unacceptable previous surgery.
ii. System failure
   a. wrong procedure used for cornea allocation.
iii. Materials
   a. contamination of cornea preservation solution.
iv. Equipment failure
   a. malfunctioning of an incubator used to maintain corneas between 28 and 38°C.
v. Human error
   a. wrong tissue supplied for the intended surgical procedure;
   b. tissue supplied beyond its expiry date;
   c. a case of donor ocular tissue being examined and then shipped to the SoHO establishment with a contact lens on the cornea, thereby highlighting the importance of *in situ* inspection before recovery (Record Number 720).

Other types of notification

Partial or complete graft detachment after EK is not uncommon; although the reported incidences vary, they are often in the range of 5-10% for DSAEK procedures and 10-30% for DMEK procedures [58]. Fortunately, graft detachment is most often successfully treated by re-bubbling (repeated air or SF6 tamponade of the anterior chamber of the recipient). It has not been possible to ascribe this SAE to donor factors such as donor endothelial cell density, donor age or method of preservation [59, 60]. Consequently, graft detachment is mainly considered a surgical complication.

Examples of two different SAR notification reports for ocular tissues used in France and in the UK are included as Appendices 28 and 29.

20.10 Patient follow-up

Patients undergoing corneal transplantation typically need systematic and regular clinical follow-up in order to comply with international standards for patient care. Follow-up may take place at the corneal transplant centre exclusively or as shared care between the transplant centre and primary or secondary eye clinics or general ophthalmology practices. Regular routine follow-up allows clinicians to recognise complications, such as suture-loosening, rejection episodes or an increase in intra-ocular pressure. If unnoted and untreated these events ultimately will result in graft failure and/or loss of vision.

The frequency of follow-up visits is individualised according to the type of corneal transplant procedure, the indication for surgery and the presence of possible risk factors in the recipient. Low-risk procedures include, for example, EK for FECD, while high-risk procedures include re-grafting of patients with ongoing ocular inflammation. High-risk procedures generally need closer follow-up.

Systematic longer-term follow-up at one and two years after corneal transplantation is typically performed in many transplant centres, either as part of an internal quality programme and/or as part of participation in a national or international corneal transplant registry. After two years, lifelong annual or biannual clinical follow-up visits at an ophthalmologist are often recommended for all corneal transplant recipients.

Systematic follow-up includes registration of some basic clinical and quality criteria. Most registries record the main clinical indication to surgery, which typically is performed to 1. improve visual acuity, 2. relieve the patient from pain or 3. preserve the integrity of the eye (tectonic keratoplasty). At the follow-up it is, first of all, registered whether the graft is functional (clear). Reasons for non-clarity include primary graft failure, failure due to immunological graft rejection, late endothelial failure or recurrence of disease (for example, if the graft was performed for tectonic reasons in an eye with uncontrolled keratitis). Registries such as most national registries and the ECCTR provide definitions of clinical and quality criteria to improve agreement of the registration in different corneal transplant centres.

Primary graft failure is typically defined as lacking clearance of the graft and cornea after surgery. Although this may be caused by a real failure of the graft itself, for example caused by errors in the SoHO establishment related to endothelial cell evaluation and counting or wrong handling during transport, it is most often caused by surgical complications or recurrence of disease in the recipient. For example, primary graft failure is extremely rare after PK for uncomplicated cases such as keratoconus and FECD, while it is more common in EK performed by surgeons in their learning phase.

Visual acuity is typically registered at follow-up, but this measure may be confounded by other

co-existing eye diseases such as age-related macular degeneration and pre-existing glaucoma damage but also of the refractive optical quality of the graft, which in PK and DALK is determined by the specific performance of the operating surgeon.

Systematic patient follow-up and participation in registries is important for corneal transplant centres, not only for providing the best treatment of individual patients at the level of international standards, but also for comparing their own results to international standards.

Although it is obligatory to report SARs to the national Competent Authority, it is often international personal contacts in corneal societies or groups that actually realise and address rare SARs. Recently, several cases of fungal infection were recognised after EK and also reported as SARs to the relevant Health Authorities but it was not until these cases were presented at a small group meeting of European corneal surgeons that actions were taken. Investigations were initiated and appropriate initiatives to prevent further reactions considered [61]. Therefore, it would be beneficial to encourage international collaboration and the exchange of individual case reports, in addition to the mandatory SAR reports, among clinicians involved in corneal transplant procedures.

20.11 Developing applications for patient treatment

The Bowman layer lies between the epithelial basement membrane and the stroma. It can be dissected from donor corneas and inserted into the mid stroma of corneas with advanced keratoconus to help strengthen and flatten the patient's cornea [62].

Decellularised stroma can be used as a scaffold or for transplantation for corneal scars/ulcers. Decellularised porcine stroma is being used clinically, and development of human decellularised stroma is in progress [63]. Retinal pigment epithelial cells derived from human embryonic stem cells, induced pluripotent stem cells, umbilical cord, fetal brain or bone marrow are being investigated for the treatment of age-related macular degeneration [64].

Corneal endothelial cells may be isolated from a corneoscleral disc and expanded *ex vivo* for injection as a suspension into the anterior chamber for treatment of corneal endothelial disease [9].

Stromal corneal lenticules, obtained as a surgical by-product of keratorefractive lenticule extraction (KLEx) for refractive errors, or potentially prepared in the SoHO establishment, may be used in the future for tectonic or refractive purposes. Intrastromal corneal lenticule implantation (additive keratoplasty), allowed by the minimally invasive femtosecond laser technology or manual dissection of stromal segments, may offer new therapeutic options and expand the surgeon's armamentarium for treating corneal diseases [65-67].

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Related material

Appendix 28. Serious adverse reaction: notification form for ocular tissues (Agence de la Biomédecine, France)
Appendix 29. Serious adverse reaction: notification form for ocular tissues (NHS, UK)

Chapter 21. **Amniotic membrane**

21.1 Introduction

Amniotic membrane (AM) allografts have been used in different medical specialities since the early 20th century. The placental membranes are composed of amnion and chorion. This chapter is dedicated to the amniotic membrane. The scientific evidence to support the use of the chorion membrane or the amnion-chorion membrane is limited and touched upon in section 21.8.2 – Amnion-chorion membrane. Histologically, AM is the inner, semi-transparent layer of the fetal membranes (amnion and chorion), formed by a single layer of cuboidal epithelial cells (epidermis-like cells) that is attached to a thick basement membrane and an avascular stromal matrix consisting of scattered mesenchymal stromal cells in a collagen scaffold. It has some unique properties: in particular, the amnion contains no blood vessels, lymphatic vessels or nerves. Several mechanisms have been suggested to explain the beneficial effects of AM, on the basis of its biological composition.

Clinical and experimental data have shown [1-5] that AM provides a compatible substrate for cell growth, facilitating migration and differentiation of epithelial cells, supporting maintenance of the original epithelial phenotype and having low or no antigenicity.

In this chapter, when discussing collection, the term 'placenta' is used to mean 'not processed tissues', whereas 'AM' here means 'tissues processed in a substance of human origin (SoHO) entity or establishment'.

21.1.1 Properties of amniotic membrane

Overall, AM is mainly formed of three types of components: structural collagen and extracellular matrix, biologically active cells and a large number of important regenerative molecules. Collagen (types I, III, IV, V and VII) and specialised proteins (including laminin and fibronectin) have been identified in the amniotic basement membrane and stroma. Laminin and fibronectin are particularly effective in facilitating epithelial cell adhesion. The presence of a rich extracellular matrix and collagen endows the stroma with anti-inflammatory properties, which arise from the entrapment of inflammatory cells, the presence of various growth factors, the inhibition of protease activity and decreased lipid peroxidation. In addition, AM has other biological properties that include anti-adhesive, antibacterial and antifibrotic effects, along with the ability to decrease scarring and neovascularisation, enhance wound healing and reduce pain [6-8].

Moreover, AM contains various growth factors (e.g. epidermal growth factor, basic fibroblast growth factor, hepatocyte growth factor, keratinocyte growth factor and transforming growth factors) and cytokines (e.g. interleukin 6 and 8) [9-11].

In addition, the special structure and biological properties of AM make it an important potential source for scaffold material [12]. Stem cells derived from AM have been demonstrated to display multilineage potential and immunomodulatory properties [13-14].

21.1.2 Indications of amniotic membrane

These characteristics have led to the use of AM for a wide range of ophthalmic indications (e.g. corneal ulcerations, persistent epithelial defects, conjunctival defects, limbal stem cell deficiency, traumatic macular hole, chemical or thermal burns) and in the treatment of a broad variety of pathological conditions, including management of burns (as a temporary or permanent wound dressing), repair of skin lesions of different aetiologies (e.g. vascular ulcers, epidermolysis bullosa, radiation burns), in surgery (e.g. orthopaedic, nerves, genito-urinary tract, head) and in intra-abdominal and reconstructive surgery as well as oral and maxillofacial surgery [15-29].

Amniotic membrane application is performed for many indications. Some indications are fairly

standardised, such as in pterygium surgery where the membrane is used to cover the bared sclera [30-33], but most indications are for a variety of more complex ocular surface diseases, such as non-healing epithelial defects, deeper corneal ulcers and small perforations, ocular surface burns caused by chemical agents and fireworks, and as an adjunctive in simple limbal epithelial transplantation procedures [34-37].

AM is widely used in extra-ocular applications with encouraging results. Human amniotic membrane has been used for centuries as a biological wound dressing. The first reported use of amniotic membrane in burn wounds was by Sabella in 1913 [38], shortly after Davis reported on the use of the material in skin transplantations in 1910 [39]. Many advantages of AM as a wound dressing have been reported, most notably prevention of infection, alleviation of pain, acceleration of wound healing and good handling properties [16, 40-44].

In recent years, there has been an increasing body of literature addressing the use of AM in chronic wounds and burns, with important outcomes and demonstrations of AM safety and efficacy [45-46], including for paediatric patients [47]. The beneficial effects of AM have been demonstrated for pain relief, fluid loss and scar reduction and, especially in developing countries, for its low costs. The key role for the revival of AM in reconstructive surgery has been the development of long-term preservation techniques to overcome limitations of immunogenicity and infectiosity [48].

AM is widely used also for wound management, including chronic ulcers, venous stasis ulcers, post-operative or post-traumatic chronic wounds, and post-surgical wound dehiscence. AM assists in the healing process through several physical, biochemical and molecular biological pathways to promote regenerative healing while simultaneously reducing scar formation [49]. Serena *et al.* demonstrated that venous leg ulcers treated with allograft had a significant improvement in healing at four weeks compared with multilayer compression therapy alone [50]. Haugh *et al.* performed a meta-analysis examining randomised controlled trials comparing amniotic tissue products with standard of care in nonhealing diabetic foot ulcers; the conclusion indicates that the treatment of diabetic foot ulcers with AM improves healing rates. Further studies are needed to determine whether these products also decrease the incidence of subsequent complications, such as amputation or death, in diabetic patients [51].

For newer applications, such as treatment of maxillofacial and oral pathology [52-55], orthopaedic diseases [56-57], plastic surgery [58], general surgery (e.g. urology, recto-vaginal, cardiovascular) [59] and other innovative uses, there are many reported cases in humans. AM gives promising results, and the scientific literature supports the use of AM to reduce inflammation, pain and scarring.

21.2 Donor recruitment, evaluation and collection

Prior to full-term delivery, potential living donors are approached to ascertain whether they would be willing to donate their placenta, see Chapter 5. The general criteria for donor selection are outlined in Chapter 6. The specific criteria for exclusion of AM are given in the same chapter, in Table 6.2.

Table 6.4 details the general risk conditions for SoHO donors, mainly related to communicable infectious diseases requiring careful evaluation. Donor screening is provided in Chapter 7.

Good Practice Guidelines

G21.1 The collection should take place only if the delivery is without any complications.
G21.2 Placenta should not be collected from a premature birth.

Recommendations

It is recommended that:
R21.1 AM be obtained from elective caesarean delivery. Placenta could be contaminated by normal vaginal flora during vaginal delivery;
R21.2 when obtaining placenta from vaginal or emergency caesarean deliveries, a risk assessment be carried out due to the possibility of cross-contamination, taking into consideration whether a decontamination procedure or terminal sterilisation is needed, and also the indications that the AM will be used to treat and the significance of product contamination in that context [60, 61].

Supplementary information

The placenta is not obtained in cases of premature births (G21.2) because those circumstances affect the properties of the AM.

21.2.1 Temporary storage and transport to the SoHO establishment

Good Practice Guidelines

G21.3 Collected placenta should be placed in a sterile, pre-labelled container.

Recommendations

It is recommended that:
R21.3 placenta be temporarily stored and transported at refrigerated temperature (with or without transport medium) and processing begins within 24 hours of delivery to the SoHO establishment;
R21.4 if transport at refrigerated temperature is not possible and transport is instead at room

temperature, a transport medium be used that ensures effectiveness and stability for 24 hours after delivery to the SoHO establishment;

R21.5 in the absence of a transport medium that ensures effectiveness and stability at room temperature in transport, processing should start within two hours of delivery to the SoHO establishment.

21.3 Processing and storage

General guidance on processing and storage is provided in Chapters 10 and 11 respectively.

21.3.1 Processing methods

Good Practice Guidelines

G21.4 Detached membrane should be washed to remove blood residues.

G21.5 AM should be decontaminated using a validated antibiotic cocktail at a temperature and duration of decontamination that is detailed in standard operating procedures (SOPs) as per the validation, if terminal sterilisation is not applied.

Recommendations

It is advised that:

R21.6 following the decontamination step and rinsing procedure, AM be spread on a suitable carrier membrane, or fine mesh gauze for easier handling for side orientation and if it needs to be cut into multiple pieces. Depending on the intended clinical use, both sides of amnion (epithelial and stromal) can be placed directly on the selected carrier.

Supplementary information

Processing of AM generally includes the mechanical detachment of membranes, according to a documented SOP. After separation from the placenta, the membranes easily split into an amnion and a chorion leaflet, separated by a jelly-like intermediate layer. The chorion is discarded or kept for further processing and then the amnion is typically rinsed several times in sterile saline until blood residues are removed completely.

Several decontamination procedures are described in literature and are mainly based on antibiotic incubation. Further guidance on sterilisation is defined by the International Organization for Standardization (ISO) [60, 61]. However, in literature, ultrastructural alterations of AM were demonstrated following irradiation [62].

General information on microbiology is provided in Chapter 12.

21.3.2 Preservation methods and storage

General information on preservation methods is shown in Chapter 10, section 10.5. There are several methods of AM preparation and preservation, as shown below [63-68].

21.3.2.1 *Cryopreserved amniotic membrane*

Supplementary information

The more frequently used cryoprotectants are glycerol or dimethyl sulphoxide (DMSO). Currently the generally accepted maximum storage period at –196°/–140°C is five years.

21.3.2.2 *Deep-frozen amniotic membrane*

Good Practice Guidelines

G21.6 Deep frozen AM should be preserved by freezing at between –60°C and –80°C.

Supplementary information

The generally accepted storage period of deep-frozen AM is 1-2 years. Longer shelf life of deep-frozen AM is accepted in some European Union (EU) countries. Storage at –60/–80°C can be carried out with a cryoprotectant (e.g. glycerol or DMSO) or in dry conditions. Storage of amniotic membrane at –28°C in dry conditions, without affecting tissue properties, has been reported in literature [69].

21.3.2.3 *Air-dried amniotic membrane*

Good Practice Guidelines

G21.7 The exposure time and critical properties for processed AM if air-dried in a laminar-flow hood should be validated by the SoHO establishment.

G21.8 A maximum limit for residual moisture (water) content should be established and measured.

Supplementary information

Air-dried AM is stored at room temperature [70].

Some Health Authorities or local practice may allow a residual moisture content up to 15%.

21.3.2.4 *Lyophilised (freeze-dried) amniotic membrane*

Good Practice Guidelines

G21.9 A maximum limit for residual moisture (water) content should be established and measured.

G21.10 Lyophilised amniotic membrane should be stored at room temperature.

G21.11 The shelf life should be validated, taking into account the packaging used and residual water.

Recommendations

R21.7 The recommended range for residual moisture (water) content is 5-10%.

Supplementary information

This preservation method induces minimal changes in the properties of the AM, and the product can be stored at room temperature [71] for a long period of time without deterioration.

21.3.2.5 *Glycerolised amniotic membrane*

Good Practice Guidelines

G21.12 AM intended to be glycerolised should be decontaminated by soaking in antimicrobial solution or sterilised by irradiation unless the glycerolisation procedure has been validated as a decontamination.

Supplementary information

Glycerolisation is a preservation method combined with the antimicrobial properties of high concentrations of glycerol. Typically, 85% (v/v) glycerol is used to preserved AM, which can then be stored at +2-8°C for up to two years, although it does lose some of its biological properties like the lack of wound-healing modulating factors and changes in the basement membrane, leading to a reduced ability to support corneal cell migration [72, 73].

21.3.2.6 *Amniotic homogenate*

The amniotic suspension is used for different applications, especially for ophthalmological and orthopaedic surgery [20, 74-78].

For some clinical applications where a liquid form of AM (injectable or drops) is required, the AM is normally sliced into small pieces through pulverisation, micronisation, homogenisation or morselisation. The small pieces obtained are immersed in a suitable certified liquid for the clinical application or freeze dried [79-89].

AM homogenate can be deep frozen or preserved at room temperature after freeze-drying [77].

21.3.2.7 *Amniotic membrane extract*

One of the main applications of AM extract is for ophthalmic use [90-95].

There are various methods for the preparation of AM extract. The steps are washing isolated human AM, slicing into small pieces and submerging in liquid nitrogen. The mixture is homogenised and centrifuged, and then the supernatant is collected.

21.4 Quality control

Good Practice Guidelines

G21.13 During collection and processing of AM, reliable macroscopic examination of the membranes should be undertaken to exclude visible pathological anomalies (e.g. visible holes, coloured membrane or abnormal thickness) and ensure structural integrity of the tissue.
G21.14 Residual moisture or available water should be quantified in lyophilised and air-dried AM preparations.
G21.15 The exposure time and critical properties for processing air-dried AM in a laminar-flow hood should be validated by the SoHO establishment.
G21.16 Quality controls should be performed according to Chapter 12.
G21.17 Freezing and high temperatures to lyophilised/freeze-dried AM (> 30°C) should be avoided.

21.5 Storage and distribution

Good practice guidelines

G21.18 Deep-frozen AM (with or without cryoprotectant) should be stored between –60° and –80°C and transported at a similar temperature as was used during storage.
G21.19 Transport temperatures of cryoprotected AM above –60°C should be avoided to ensure the stability of the product and maximum safety for the recipient.
G21.20 Lyophilised/freeze-dried AM derivates should be stored and transported at room temperature (15-25°C).
G21.21 Cryopreserved SoHO preparations should be transported below –60°C.
G21.22 Cryopreserved SoHO preparations should be stored below –140°C.
G21.23 If transported on dry ice at –60°C to –80°C cryopreserved SoHO preparations should not be returned to liquid or vapour-phase nitrogen tanks unless validated or supported by a documented scientific rationale.
G21.24 Once the released SoHO preparation has been thawed, it should not be re-frozen.

21.6 Biovigilance

Some of the examples below are from the Notify Library, which includes some well-documented cases of adverse reactions and adverse events in transplantation of AM tissue. The database is publicly accessible and can be searched by substance type, by adverse occurrence type and by record number. For further evaluated cases of adverse outcomes associated with AM banking, search the Notify Library at www.notifylibrary.org/.

Serious adverse reactions (SARs) for amniotic membrane transplants:

The 2022 Biovigilance European Commission report contained 11 cases of corneal pannus related to the application of AM.

Serious adverse events (SAEs) included:

i. Tissues and cells defect
   a. Positive culture detected in a sample of the cryopreserved AM medium at the transplant centre during AM application.
ii. System failure
   a. LN_2 ran out from an LN_2 tank containing AM (as a result, all tissues thawed) (Record number 129).
iii. Materials
   a. Collapse of the dispensed AM homogenate vial, blocking its application.
iv. Equipment failure
   a. Loss of significant quantity of AM preparations due to storage at inadequate temperature (e.g. in a case of equipment failure) (Record number 130).
v. Human error
   a. High level of microbial contamination of collected AM (reflecting the hygiene conditions of the delivery room) (Record number 1125).

21.7 Patient follow-up

General information on patient follow-up is described in Chapter 14, section 14.13.

Although there is a patient follow-up related to the clinical application, this information is not included in any registry that links the patient and SoHO. This situation makes it difficult to record the level of risk of an SAR and the level of success of the different methods of preservation.

Systematic follow-up includes registration of some basic clinical and quality criteria. Most physicians record the main clinical indications to surgery and patient-reported outcomes.

21.8 Developing applications for patient treatment

21.8.1 Decellularised amniotic membrane

For many applications, the extracellular matrix (ECM) structure of the AM is particularly suitable for use as a scaffold. In this case, AM is applied after decellularisation. Various methods which are mainly based on enzyme-, detergent- or mechanical procedures can be used for this purpose [96, 97].

21.8.2 Amnion-chorion membrane

So far, literature hardly differentiates between amnion and chorion preparations. However, based on the finding that amnion and chorion present different content of growth factors and cytokines [96, 98], different effects from amniotic and chorionic preparations can be expected [99].

Amnion-chorion membrane, that includes the amnion and chorion layers inclusive of the spongy layer, has been used as a wound covering for different aetiologies. The chorionic membrane is the outermost layer of placental tissue, consisting of the chorionic mesenchyme and trophoblast layer that encapsulates the amniotic tissue and embeds into the mother's endometrium during development. As previously described for AM, amnion-chorion preparations also contain a wide variety of growth factors and cytokines relevant to tissue repair [100].

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Chapter 22. **Skin**

22.1 Introduction

Autologous skin is considered the gold standard for wound treatment and final wound closure. For large wounds, autologous thin split-thickness skin can be widely expanded (meshed) or transplanted as island grafts (Meek wall technique). The autologous skin will grow out to close the wound. In cases of shortage of autologous skin sources (especially in burns), donor skin is used to treat patients with extensive skin loss, for wounds with either split-thickness or full-thickness depth. Deep injuries lead to dermal damage, impairing the ability of the skin to heal and regenerate.

Many countries maintain a 'strategic stock' of human allogeneic skin grafts to meet the need in case of emergencies or mass casualty events (e.g. mass burn casualty disaster) [1, 2].

22.1.1 Indications for skin/dermal allografts

Donor skin basically acts as a temporary coverage, preparing the wound bed, reducing scarring, controlling pain, preventing infection and maintaining patient homeostasis by reducing loss of fluids, proteins and heat through the burn wound. For these reasons, donor skin can be vital in the treatment of severely burned patients, after escharectomy.

In addition, allogeneic skin is considered to be an excellent biological dressing for the treatment of other types of skin defect such as ulcers (venous, decubitus); diabetic foot; pyoderma gangrenosum [3, 4]; surgical wounds; exfoliative skin disorders such as toxic epidermal necrolysis (Lyell's syndrome); severe graft-*v.*-host disease [5] and congenital epidermolytic skin disease; and acantholytic dermatoses (Darier disease) [6]. In these cases, skin allografts promote re-epithelialisation and formation of granulation tissue, shorten healing time, control pain and protect important structures (e.g. tendons, bones, cartilage, nerves) [7]; they are also successfully used as skin substitutes that incorporate the dermal component into the wound bed, guiding a more physiological healing process, having the ability to be integrated into the wound bed of full-thickness burns, wounds or leg ulcers.

Allogeneic skin can also be placed on top of the autologous skin (the 'sandwich technique') to protect it from mechanical damage, dehydration and infection. After 7-10 days the allogeneic skin can be removed from the wound [8-10].

These factors explain the consistent demand for skin allografts by burn centres and reconstructive surgery units, where their capacity to 'take' and integrate into the wound bed is exploited. In the past, allogeneic skin was used sometimes to replace the lost dermis ('Cuono technique') [11], but the donor cells and hairs still present may cause inflammatory reactions with a negative effect on the final scar formation. Nowadays acellular dermis is available. Several substances of human origin (SoHO) establishments have developed types of skin graft that are more suitable as a dermal equivalent and will be integrated onto the wound bed.

The shortage of allogeneic skin grafts has promoted the development of skin-replacement products, and many research teams have focused on biomaterials for skin substitution in wound healing. Since the 1990s, a huge number of biological, semisynthetic and synthetic skin/dermal substitutes have been developed with the aim of producing an artificial skin that is able to replace human skin completely, but an ideal skin substitute has not yet been realised. A further logical development of this research involves the use of stem cells to re-populate the dermal matrix and reproduce 'physiological' skin, but to date there is no ideal skin-replacement product available based on stem cells.

Clinical indications of acellular dermal matrices are in the field of regenerative medicine and surgery and depend on wound thickness. Among them are:

- dermatology, plastic and reconstructive, general surgery and vascular surgery used for the treatment of acute (e.g. burns) and chronic (e.g. skin ulcers in various aetiology) skin wounds with extensive loss of substance: acellular dermal matrices with a thickness of 0.2-0.4 mm can be used in combination with a thin autologous split-skin graft to improve the scar quality of the wound; thicker acellular dermis is used for other indications such as reconstruction of the breast after mastectomy or hernia repair [12-22];
- orthopaedics for the repair of the rotator cuff of the shoulders as well as for the treatment of skin surgical wounds [23];
- maxillo-facial surgery, ear, nose and throat surgery, dentistry for the sinus lift, implant dentistry for augmentation in gum reconstructions [24];
- reconstruction of ocular region (fornix, eyelids) [25];
- urological surgery (treatment of stress incontinence);
- neurosurgery: duraplasty assuring hermetic dural closure in posterior fossa surgery avoiding cerebrospinal fluid leakage [26].

22.2 Skin-specific donor evaluation

The general criteria for donor selection are outlined in Chapter 6. The specific criteria for exclusion of skin are given in the same chapter, in Table 6.2. Especially relevant is the information on how to perform a general physical examination, which is shown in section 6.2.

Table 6.4 details the general risk conditions for SoHO donors, mainly relating to communicable infectious diseases requiring careful evaluation. Donor screening is provided in Chapter 7.

Good Practice Guidelines

G22.1 Physical examination (PE) protocols for skin should include, if not checked as part of the routine PE: screening of dysplastic naevi (see Appendix 16), dermatitis and ectoparasites.

Supplementary information

It is common practice not to collect skin from younger donors, due to low yield and technical difficulties. SoHO entities and establishments generally establish their own limits, which are commonly determined by the physician of the SoHO entity/establishment, according to characteristics and quality of tissues (see Table 6.2 Specific donor criteria, in Chapter 6).

22.3 Skin collection

Skin is generally obtained from deceased donors declared dead by neurological (DBD) or cardio-circulatory criteria (DCD). For general information on collection from deceased donors, please refer to Chapter 8.

In the absence of sufficient *post mortem* donors, skin can also be obtained from living donors. Skin is obtained from patients having abdominoplasty or mammoplasty procedures who have consented to tissue donation. Such tissue is considered to be a surgical residue.

Recommendations

R22.1 It is recommended that preparation protocols include hair removal, where possible.

22.3.1 Collection teams and sequence

Good Practice Guidelines

G22.2 Skin-collection teams should consist of at least two people operating under aseptic conditions and scrubbed, gowned in sterile clothing as appropriate for the type of collection.
G22.3 Where internal tissues donation is taking place, skin should be retrieved first.

Recommendations

R22.2 It is recommended to collect skin before ocular tissue to avoid eye bleeding from the sockets, if the donor has to be placed in a prone position following enucleation of the eyes. Some SoHO establishments place the head at a higher level than the donor's back to minimise bleeding.

Supplementary information

Studies show that, whether the skin collection is done before or after bone collection, the contamination rate of skin is not significantly different if the collection process is controlled and standardised [27, 28]. However, to obtain grafts of consistent quality, SoHO establishments generally collect skin grafts prior to removal of musculoskeletal grafts.

22.3.2 Skin collection procedure

Good Practice Guidelines

G22.4 Skin should be collected under aseptic conditions. Pre-operative scrubbing and disinfection of the donor skin should be performed to reduce the resident microbiota and commonly found pathogenic micro-organisms.
G22.5 Suitable disinfectants should be chosen. Their concentrations and the durations of exposure

should also be evaluated against commonly found pathogenic micro-organisms on skin grafts.

G22.6 A local sterile field using sterile drapes should be used prior to collection to effectively prevent microbial contamination.

G22.7 SoHO establishments should define the key dimensions (length, width and thickness) for the different types of skin allograft they provide and should ensure their collection protocols can achieve them.

Recommendations

R22.3 It is recommended that SoHO establishments evaluate and utilise skin decontamination protocols that significantly reduce the native bioburden prior to collection of skin grafts.

Supplementary information

The procedure of skin disinfection aims to reduce the bioburden, which can significantly decrease the microbial positivity rate of processed skin samples. Thorough cleaning of the skin with a surgical detergent prior to application of disinfectants will enhance their efficacy. The commonly used disinfectants include povidone-iodine or chlorhexidine (see G22.5).

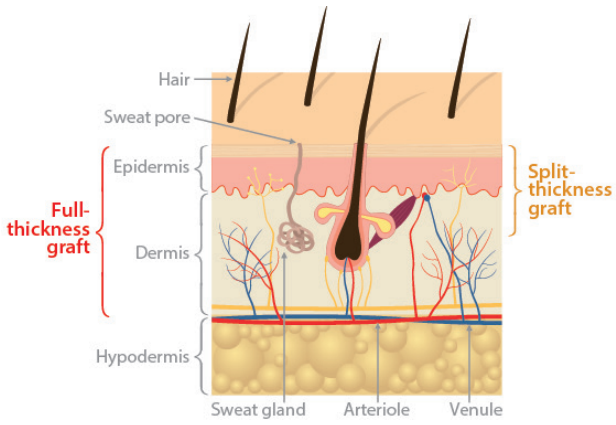
Skin can be collected by manually, electrically, compressed-air or battery-operated dermatomes from areas of the body that are typically not exposed, particularly from the posterior trunk and the lower limbs.

According to graft thickness, skin grafts can be divided into split-thickness and full-thickness grafts. They consist of the entire epidermis and a dermal component of variable thickness. If the entire thickness of the dermis and skin structures is included, the appropriate term is full-thickness skin graft (FTSG). This type of skin graft can be collected using a scalpel instead of a dermatome. If less than the entire thickness of the dermis is included, this graft is referred to as a split-thickness skin graft (STSG). STSGs are categorised further as thin (< 0.3 mm), medium (0.3-0.45 mm) or thick (> 0.45 mm). The choice whether to collect STSG or FTSG can also be determined by the intended clinical use and downstream processing of the collected tissue (e.g. FTSG may be preferred in the production of acellular dermis to be used as a dermal-equivalent). The clinical use of full- or split-thickness grafting (see Figure 22.1) depends on wound conditions, location, thickness, size and aesthetic concerns [29, 30].

In the case of living donors, the tissue obtained will be FTSGs, which can be further dissected into STSGs or used for the preparation of dermal allografts.

Staged separate collection from particular body areas, with placement of collected material from each area into separate containers, effectively as distinct batches, is sometimes preferred in order to reduce eventual cross-contamination of the collected tissue. This approach enables some skin to be banked from a donor where other skin is not suitable for clinical use.

Figure 22.1 **Differences between split -and full-thickness grafts**



22.3.3 Reconstruction of the skin donor

Good Practice Guidelines

G22.8 For aesthetic reasons and with a view to a respectful reconstruction of the donor, skin should not be taken from the neck, face and other typically exposed areas of body that might be visible when people pay their last respects to the donor, unless specific authorisations are obtained.

G22.9 Conditions which may affect body reconstruction after collection such as extreme oedema or a high BMI (≥ 40) should be evaluated prior to collection.

G22.10 Once the SoHO has been collected, or at the end of recovery in the case of multi-tissue donors, appropriate dressing of the donation areas should be carried out to prevent leaking and oozing. This should include the use of sealing agents, padding and/or appropriate garments.

Recommendations

R22.4 In the case of multi-tissue collection teams, it is recommended that effective communication be maintained with all parties to avoid delays in collection time and to meet expectations regarding aesthetic considerations in case of unexpected leakage.

22.3.4 Temporary storage and transport to the SoHO establishment

Good Practice Guidelines

G22.11 All grafts should be stored in sterile, prelabelled containers.

G22.12 Where the retention of tissue viability is required, the grafts should be transported in isotonic transport solution at refrigerated temperatures.

G22.13 After collection, skin grafts should be held at refrigerated temperatures, be transferred to the SoHO

establishment and be processed, or stored under bacteriostatic conditions, as soon as possible.
G22.14 The containers should be sealed securely, refrigerated to +2-8°C and transported to the SoHO establishment.
G22.15 Maximum transport times and transport conditions should be established and documented for each type of graft.
G22.16 Where the transport solution contains antibiotics, pre-decontamination tissue samples should be taken before the tissue is immersed in the solution.

Recommendations

R22.5 For cryopreserved skin grafts it is recommended that:
- R22.5.1 the cell nutrient medium used for viable grafts be changed shortly after receipt of skin grafts, or that the medium be validated for the storage time (i.e. adequate buffering capacity);
- R22.5.2 if antibiotics are added to the transport medium, action be taken to ensure they are ones that are stable and remain active at the designated transport temperature;
- R22.5.3 the antibiotic exposure time be minimised to avoid the risk of damaging the viability of the tissue;
- R22.5.4 when decontaminating tissue, the process validation address the loss of tissue viability where higher antibiotic decontamination temperatures are used;
- R22.5.5 when decontaminating tissue with antibiotics, the optimal activity temperature of antibiotics be considered when selecting the decontamination temperature.

Supplementary information

Transport at low temperatures retards proliferation of most micro-organisms and maintains skin viability (if viable grafts are required). Antibiotics are more effective when used at normothermic temperatures. Many antibiotics are only effective *versus* actively dividing micro-organisms, and pathogenic micro-organisms are more active (and therefore vulnerable) in this temperature range. However, higher temperatures may also result in faster loss of graft viability, and SoHO establishments generally select incubation temperatures and times to minimise loss of viability and maximise antimicrobial potential [31, 32].

If skin grafts are to be glycerolised or decellularised (i.e. they are non-viable grafts), transport protocols do not need to be optimised to retain tissue viability. For example, glycerolised skin grafts are commonly transported at room temperature in 50% glycerol solution. Transport conditions are still to be validated to ensure that any critical quality attributes of the tissue (other than viability) are maintained (i.e. normal morphological structure).

22.3.5 Collection documentation

Good Practice Guidelines

G22.17 The report should contain a description and identification of the collected skin (including samples for testing) as well as deviations from standard operating procedure (SOP) which may impact on the quality and safety of the collected grafts.

22.4 Skin processing

The recovered skin is processed to reduce microbial contamination and allow longer storage periods until transplantation. Skin pieces are trimmed, cut into defined pieces, and any hair remnants are removed.

Different SoHO establishments apply specific preparation methods according to their own SOPs and any applicable local authorisations.

22.4.1 Skin processing methods

Good Practice Guidelines

G22.18 Processing should commence within validated timelines that preserve the quality and safety of the SoHO preparation.
G22.19 To consider a graft as viable, a minimum of 20% of residual cell viability should be achieved.
G22.20 The incubation temperature and the composition of decontamination cocktails should be defined in written procedures by each SoHO establishment, considering the initial tissue microbial contamination.
G22.21 If grafts are meshed by the SoHO establishment, the appropriate mesh ratio should be agreed with clinical users.
G22.22 The graft's irregular edges should be trimmed and, typically, a rectangular shape should be obtained.
G22.23 The dimensions and area of each graft should be measured, recorded and displayed on the label or in the accompanying documentation.

Recommendations

R22.6 It is recommended that SoHO establishments put in place quality control checks for skin grafts based on risk assessment principles, taking into account how they are prepared and their intended clinical application. Ideally, these are batch-specific checks; however, where this is not feasible this can be addressed by process validation. Quality control requirements for evaluation of each type of skin graft are described in Chapter 10 (section 10.8) and the specific SoHO monographs.

Supplementary information

Depending on the intended clinical use and the quality requirements, skin grafts can be processed and preserved according to various methods (deep freezing, cryopreservation, glycerol preservation, lyophilisation, refrigeration) and potentially terminally sterilised with gamma-irradiation. These methods produce different grades of skin viability, integrity and immunogenicity. Skin allografts can also be processed into de-epidermised skin and acellular dermal matrix (ADM).

The usual width of the grafts collected depends on the width of the dermatome blades (usually 8 or 10 cm). Their length varies according to the size of the donor site and the final storage containers' capacity. Collected skin allografts can be cut into specific smaller sizes according to requirements of the end-user clinicians. The skin grafts may be provided as sheets or meshed prior to cryopreservation, to increase the surface area that may be covered by the graft, and to facilitate wound fluid drainage.

Processing of skin grafts generally includes soaking in antibiotic and antimycotic cocktails. Where maintenance of donor cell viability is desired, soaking in antibiotic cocktails selectively inactivates microorganisms while affording some protection to cells.

When tissue viability is not required or when skin tests positive for microbiological contaminants, it can be sterilised by gamma or electron beam irradiation. Ionising radiation (in relation to its dose) can cause structural changes in the irradiated skin allografts, especially in the epidermis [33, 34]. Research has shown that a maximum dose of 25 kGy irradiation of deep-frozen skin in radioprotective solutions sterilises tissues without relevant histomorphological or physical alterations (such as pliability) compared with normal cryopreserved skin [31, 35].

Sterilisation with irradiation will destroy viable donor cells, but if performed appropriately does not compromise the structure and mechanical properties of the graft, meaning it can serve as a biological dressing. This type of graft is not expected to engraft to the wound bed, as cryopreserved skin grafts can. This can, however, result in the graft giving a better cosmetic outcome when applied to facial or hand burns, for example.

22.4.2 Glycerol-preserved skin allografts

Glycerol-preserved skin allografts (GPA) were developed [33] to maintain skin allograft at +2-8°C using an increasing series of glycerol concentrations (generally 50%, 70%, 85%) for preservation and storage without freezing. Glycerol preservation is a method to obtain de-vitalised skin grafts characterised by reduced immunogenicity and low antibacterial/antiviral properties, though it cannot be considered a sterilising agent [36-41]. If there are positive microbiology results from cryopreserved skin, it may be possible, as a collection procedure, to re-process it in 85% glycerol, which has antimicrobial properties unless contaminated by defined pathogens, as specified in Chapter 12, Table 12.4 and/or SoHO establishments' microbiological policy [36, 37, 41]. Most skin establishments validate an expiry date of five years for GPA stored at +2-8°C.

Sterile glycerol solutions meeting pharmacopoeial specification, i.e. *European Pharmacopoeia* monograph 0497 – glycerol 85% – are commonly used for preparations of GPA.

22.4.3 Unprocessed skin allografts

Good Practice Guidelines

G22.24 Unprocessed allografts should only be used where there are no other alternatives and where they are supported by a documented risk assessment.

Supplementary information

The use of unprocessed skin allografts ('fresh skin allografts') is not the preferred option because it may not allow for autopsy reports and/or extensive microbiological testing, which need to comply with the testing period, according to Chapter 12.

However, some SoHO establishments still use unprocessed skin allografts as it is possible to maintain their structural integrity and cell viability for short periods of time (maximum 7-8 days at +2-8°C). These allografts were initially preferred in burn centres due to their high cell viability [41].

22.4.4 Cryopreserved and deep-frozen skin allografts

Good Practice Guidelines

G22.25 When cell viability is required for clinical use, the viability should be assessed using an appropriate test.

G22.26 After cryopreservation, skin allografts should be stored at a temperature below –140°C in liquid nitrogen tanks (vapour phase) or ultralow temperature mechanical freezers.

G22.27 The maximum storage time below –140°C should be determined by the SoHO establishment.

Recommendations

R22.7 A controlled-rate freezing procedure is recommended to preserve cell viability.

Supplementary information

The maximum storage time below –140°C is commonly applied up to five years; however, there are no empirical data supporting longer storage times.

These preservation methods aim to maintain cell viability and structural integrity of skin allografts for extended periods of time. Biological and structural functions of skin tissue are preserved by cooling to subzero temperatures in a freezing medium with cryoprotectants,

such as dimethylsulphoxide (DMSO) or glycerol [42, 43], to protect cells against freezing injury. Cryoprotectants can, however, adversely affect cell viability and graft efficacy.

Cell viability is maintained in cryopreserved and, to a lesser degree, deep-frozen skin grafts. It facilitates the tissue engraftment to the wound bed, as a substrate for revascularisation and recolonisation by host cells [36, 44, 45]. Skin-cell viability, referred to as the mean percentage of cell viability after 10-20 days of storage, is reported to be between 40 and 50% that of the fresh skin [45].

Cell viability can be assessed by various methods including vital dye staining, oxygen consumption, and enzymatic and metabolic assays (described in §22.5.1).

Cryopreservation is considered to be the best method that allows the long-term preservation of viable skin [36]. Storage at higher temperatures (–60°C to –80°C) is a method applied for medium-term (maximum of two years) preservation of viable skin allografts [44-46]. Inappropriate storage compromises the retention of the graft viability and, thus, physiological functioning after transplantation.

22.4.5 Lyophilised skin allografts

Good Practice Guidelines

G22.28 A maximum limit for residual water content should be established and measured.

Recommendations

R22.8 The recommended limit for residual water content is ideally < 5%.

Supplementary information

Processing of grafts by freeze-drying devitalises the grafts while maintaining their structure. Lyophilised skin grafts can be stored at ambient temperature for 3-5 years.

Residual moisture (water) content is expressed as the 'percentage residual moisture' and is equated to the weight (g) of residual water per unit weight (g) of original sample. It is generally agreed that biological materials dried by vacuum sublimation of ice show increased stabilisation when dried to optimum contents of residual moisture [43, 44]. Materials which have been under or overdried, i.e. to moisture contents that are above or below the optimum, will show increased deterioration.

Although the optimal residual moisture content will vary depending on the particular dried sample, certain stability problems can be expected when the levels of moisture are suboptimal. Overdrying a sample, i.e. residual moisture contents less than 1-2% without using a dry stabiliser, generally results in removal of nearly all structured water, allowing modification or blocking of exposed hydrophilic sites of proteins by oxidation. This oxidation causes degradation with a corresponding decrease in the biological activity. On the other hand, residual moisture contents of greater than 5% generally are indicative of underdrying where sufficient amounts of 'free water' remain in the sample which could contribute to transconformation of the protein. The resulting rearrangements of the polypeptide chains shift from the typical ordered arrangement of the native protein to a more disordered arrangement. These protein perturbations can result in poor long-term stability of the dried product.

22.4.6 De-epidermised skin

De-epidermising skin (frequently associated with decellularisation) is a method to lower the antigenicity of the skin graft. Thicker skin obtained from deceased donors is processed aseptically to remove the epidermis. Various methods for separating the epidermis from dermis are reported, such as chemical (sodium chloride, phosphate buffered saline, dispase), physical (heat, freezing and thawing) or mechanical (dermatome). These methods are frequently used in association to obtain optimal de-epidermisation. Full-thickness skin can be obtained from living donors undergoing abdominoplasty or body-contouring procedures and is processed in a similar manner to produce thicker dermal allografts to be used in full-thickness skin loss if primary closure or donor-site availability of autografts is limited or suboptimal [45, 46]. The result is an intact dermal matrix (with fully or partially maintained basement membrane) that can be cryopreserved, frozen, preserved in glycerol or lyophilised.

22.4.7 Skin tissue decellularisation

Tissue decellularisation is a technique that aims to remove all cell remnants from a tissue, maintaining an intact extracellular matrix (ECM). In recent years, several innovative biological products based on decellularisation of donor-derived skin tissue have been developed. Resulting tissues are acellular dermal allografts, as the decellularisation processes invariably remove the epidermis, with the possible exception of the basement membrane. The clinical applications of these grafts extend beyond the traditional role of skin allografts, including tendon repair, abdominal patches and breast reconstruction.

Three methods are actually used to achieve tissue decellularisation: chemical, physical and biological (enzymatic). Each of these methods has a different mode of action and effect on the ECM. A combination of methods helps ensure effectiveness [46]. Chemical methods comprise alkaline/acid solutions, alcohols, hyperosmotic/hyposmotic solutions, ionic detergents and non-ionic, zwitterionic detergents, chelating agents; physical techniques are based on temperature (freeze–thaw cycles), hydrostatic pressure, mechanical agitation and sonication; and biological methods include trypsin and endo/exonucleases (see Appendix 30).

Decellularisation protocols generally start with cell lysis by physical/chemical treatments, detachment

and solubilisation of the cell components using enzymatic treatments and detergents, and removal of the cell remnants from tissues. These processes may be associated with mechanical agitation/sonication to increase the treatment effectiveness. At the completion of the decellularisation process, it is common practice to thoroughly rinse the tissue matrix to minimise the residual amounts of any processing chemicals in the final graft, as these may be toxic or immunogenic, and impact its clinical efficacy.

Sterilisation techniques may be applied to provide a higher level of safety. There are significant advantages in combining decellularisation and sterilisation processes to ensure a clinically safe ECM, minimising the effect on its ultrastructure.

The common goal of all these methods is to obtain an acellular dermal matrix (ADM) characterised by an intact fibrous and collagenous architecture, able to be repopulated by autologous cells of the patient after its engraftment. The absence of immune response and graft rejection in patients is ensured by removing the cellular components (fibroblasts and endothelial cells) as well as the donor DNA and hair remnants. From a functional point of view, these dermal matrices act as cell-free scaffolds able to permanently reconstruct and regenerate damaged and/or pathological skin tissue.

Good Practice Guidelines

G22.29 Quality control checks for the application of ADM should include:

- **G22.29.1** biocompatibility (the ability to take after engraftment and the absence of rejection/inflammatory reaction due to induction of pro-inflammatory cytokines);
- **G22.29.2** integrity of the matrix (the maintenance of integrity of elastic fibres and collagen physiologically identified in the tissue);
- **G22.29.3** pre-decontamination, no pathogenic micro-organisms detectable on the graft;
- **G22.29.4** post-decontamination, no viable micro-organisms detectable on the graft;
- **G22.29.5** malleability and suturability (handling; mechanical resistance with or without stitches);
- **G22.29.6** determine donor cells (and genetic material).

Recommendations

It is recommended that:

R22.9 SoHO establishments put in place quality control checks for ADM grafts based on risk assessment principles, taking into account how they are prepared and their intended clinical application. Ideally these are batch-specific checks; however, where this is not feasible this can be addressed by process validation.

Supplementary information

Decellularisation enables preservation and long-term storage options that are not feasible for cellular grafts, such as lyophilisation, dehydration or refrigeration, facilitating local storage at the site of application and enabling cheaper transport protocols.

See Chapter 34 and Appendix 30 of this Guide for information on decellularisation of tissues as natural ECMs.

22.4.8 Sterilisation of skin allografts

When tissue viability is not required or when skin tests positive for microbiological contaminants, it can be sterilised by gamma or electron beam irradiation. Ionising radiation (in relation to its dose) can cause structural changes in the irradiated skin allografts, especially in the epidermis [47]. Research has shown that a maximum dose of 25 kGy irradiation of deep-frozen skin in radioprotective solutions sterilises tissue without relevant histomorphological or physical alterations (such as pliability) compared with normal cryopreserved skin [48].

Sterilisation with irradiation will destroy viable donor cells, but if performed appropriately does not compromise the structure and mechanical properties of the graft, meaning it can serve as a biological dressing. This type of graft is not expected to engraft to the wound bed, as cryopreserved skin grafts can. This can, however, result in the graft giving a better cosmetic outcome when applied to facial or hand burns, for example.

Good Practice Guidelines

G22.30 Sterilisation methods should be validated for the initial estimated level of bioburden prior to application of the sterilisation method to skin allografts.

22.5 Quality control

General information on microbiological control can be found in Chapter 12.

22.5.1 Microbiological control

Good Practice Guidelines

G22.31 The SoHO establishment's policies for assessing the suitability of skin grafts for clinical use from a SoHO microbiological standpoint should consider:

- **G22.31.1** any contaminants identified during pre-process testing;
- **G22.31.2** any contaminants identified during in-process testing;
- **G22.31.3** any contaminants identified on the finished product;
- **G22.31.4** whether or not different criteria should be applied to skin tissues that may be grafted onto immunocompromised patients.

Supplementary information

The presence of micro-organisms in finished product samples of non-terminally sterilised grafts generally results in a rejection of the donor tissue for clinical use (see Figure 22.2). In exceptional cases, it might be permitted to release grafts with low bioburdens of low-risk organisms such as skin commensals, 'on board' [49], after appropriate risk assessment and consultation with surgeons (e.g. in urgent cases).

22.5.2 Skin allograft performance and quality issues

Good Practice Guidelines

G22.32 In cryopreserved skin allografts, the viability of skin (if required, depending on the intended application) should be tested routinely (specifically testing each batch) or confirming compliance with a validated process (parametric validation):

G22.32.1 No evidence of microbial growth.

G22.32.2 Preservation of structural integrity (normal epidermal/dermal structure).

G22.32.3 Mechanical resistance (if required).

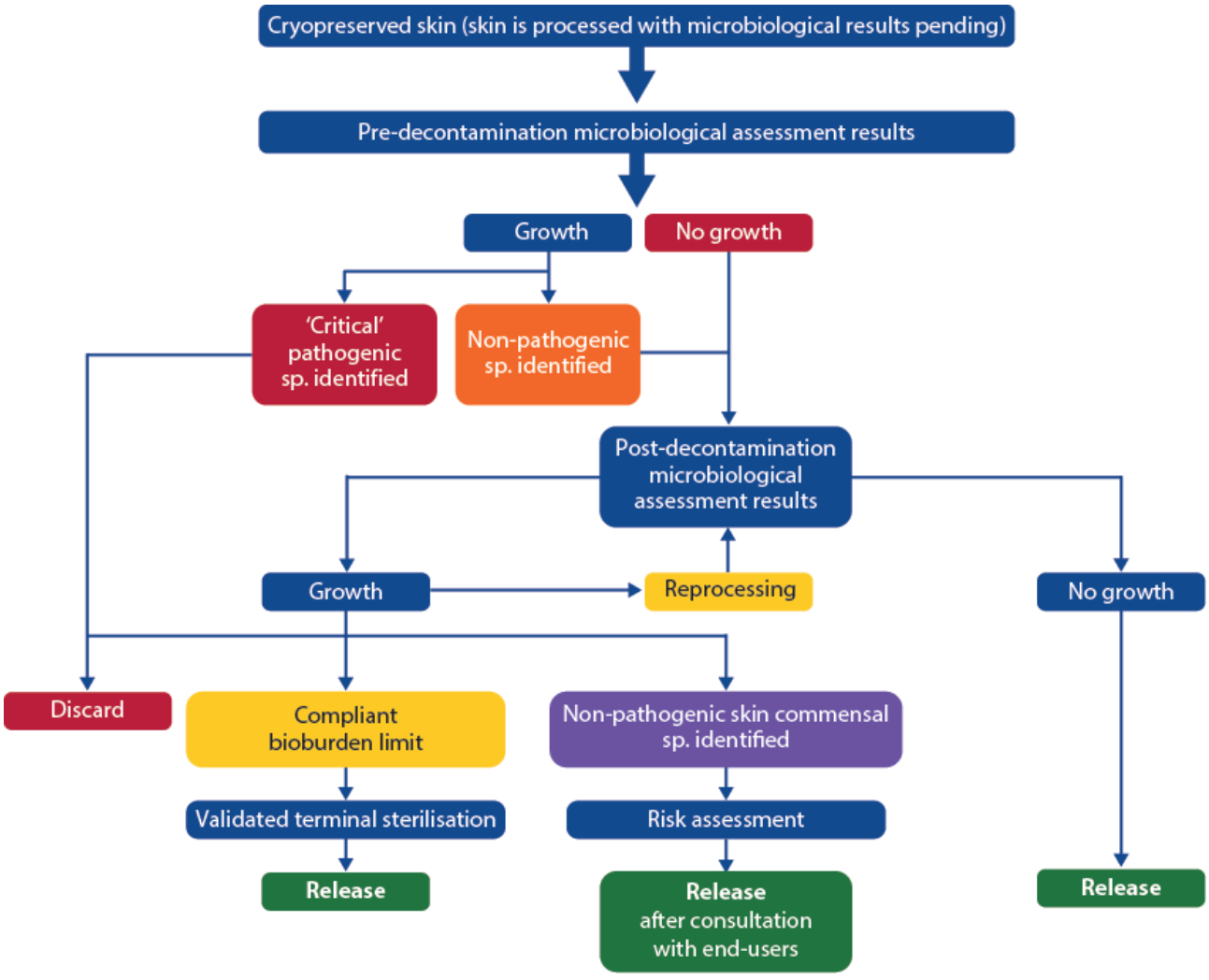
Supplementary information

Viable donor skin is still considered the gold standard for the temporary covering of burns. Different methodologies are used to assess cell viability and tissue morphology in skin grafts before and after thawing:

- quantitative, e.g. tetrazolium salt assay (such as MTT), neutral red test (NRT), resazurin test, oxygen or lactate consumption assay [41, 42];
- and /or qualitative, by histological staining, e.g. orcein, Masson, haematoxylin-eosin.

Controversy exists in the literature data regarding the importance of cell viability in graft performance and quality. It is widely considered that viable skin allografts are superior to all other dressing materials, and the majority of physicians agree that higher viability is usually associated with better wound-bed preparation and graft take [41, 50-52]. Comparison of unprocessed, cryopreserved (viable) and glycerolised skin allografts by the use of animal studies (immuno-competent Balb/c mice) revealed a better performance (evaluated by histology) of unprocessed skin and, to a lesser degree, of deep-frozen (–80°C) and cryopreserved (in liquid nitrogen) skin [53]. These data demonstrated that the performance of cryopreserved skin decreased with time.

Figure 22.2 **Flowchart for acceptance or rejection of skin after bacteriological assessment**



However, non-viable skin allografts can be successfully employed when viable cryopreserved skin allografts are not available or where cell viability is not required for wound treatment [10]. Literature studies [21] indicate that there is no evidence that viability influences healing outcomes. Thus, instead of viability, other aspects, such as structural integrity, clinical outcome and intrinsic antimicrobial safety of the preservation method and cost should be the primary criteria for the choice of preservation method to be used for allografts.

It is demonstrated that allografts' dermal component can easily be integrated [51], whereas homologous epidermis is normally rejected within two weeks [54]. To obtain re-epithelialisation of full-thickness wounds/burns it is usually necessary to follow a two-step surgical procedure: reconstruction of the dermal compartment by skin or dermal allografts and then re-epithelialisation by autologous thin grafting. Recently, some authors [55] have reported a successful one-step procedure in the treatment of severe burns, by using mixed auto- and cryopreserved allograft skin grafting. This technique seems to shorten the wound healing time significantly, thus greatly raising the utilisation rate of autologous skin.

22.6 Packaging and labelling

General information about packaging and labelling can be found in Chapter 16.

22.7 Storage

Good Practice Guidelines

G22.33 Processed skin grafts should be stored under conditions appropriate to the manner of their preservation and maintaining their critical quality attributes (CQA). See Table 22.1 for commonly used storage temperatures used for different types of skin allografts.

22.7.1 Expiry date

If the reagents are used in the storage of tissues at low temperatures (especially frozen or preserved skin), the relevance of their original expiry dates, which assume storage at ambient temperature, is questionable. For further details please see Chapter 11, section 11.5.

Table 22.1 **Storage conditions for processed skin grafts**

Type of skin grafts	Storage conditions
Glycerolised skin	Refrigerators (+2 to +8°C)
Lyophilised (freeze-dried) skin/dermis	Room temperature (15 to 25°C)
Cryopreserved skin	Vapour phase of liquid nitrogen or mechanical freezer (below −140°C)
Deep-frozen skin	Mechanical freezers (−80 to −60°C)

22.8 Skin allograft distribution

Good Practice Guidelines

G22.34 SoHO establishments should have a policy to ensure priority of graft allocation based on clinical severity, in case of shortage.

G22.35 Distribution protocols of cutaneous tissue preparations should account for the fact that they can be distributed at various temperature conditions depending on the processing and storage methods. For storage and transport temperature, please see Table 11.1 and associated specific SoHO monographs.

22.9 Acceptance criteria and exceptional release

General guidance can be found in Chapter 13.

22.10 Biovigilance

Some of the examples below are from the Notify Library that includes some well-documented cases of adverse reactions and adverse events in transplantation of skin. The database is publicly accessible and can be searched by substance type, by adverse occurrence type and by record number. For further evaluated cases of adverse outcomes associated with skin banking, search the Notify Library at www.notifylibrary.org.

Non-exhaustive list of reportable serious adverse reactions (SARs):

i. Transmission
   a. transmission of infectious disease:
      • contaminated skin graft that caused serious infection of a burn wound with *Acinetobacter* (Record Number 428);
   b. transmission of malignant disease.
ii. Graft failure
   a. engraftment failure/delayed engraftment (European Commission SARE report 2022).
iii. Undue exposure to risk intervention
   a. aborted procedure involving unnecessary exposure to risk (e.g. wrong tissue supplied or delayed transport, discovered after patient is anaesthetised and the surgical procedure has begun).

iv. Other
   a. unexpected immunological reactions due to tissue;
   b. allergic reaction (e.g. to antibiotics used for processing media);
   c. bleeding (wound bed preparation).

Non-exhaustive list of reportable serious adverse events (SAEs) described by activity step:

i. Collection
   a. collection without consent.
ii. Processing and labelling
   a. use of non-sterile/expired materials for tissue processing;
   b. mistaken processing media (errors in media preparation);
   c. incorrect labelling;
   d. graft not measured appropriately, resulting in delay in patient treatment and graft loss (Record Number 127).
iii. Product selection
   a. distribution of cryopreserved skin without review of the results of bacterial tests. Several allograft recovery cultures showed virulent pathogens ordinarily not accepted for use that prompted recall of > 100 skin allografts, fortunately without any case of disease transmission (Record Number 128).
iv. Storage
   a. storage at inadequate temperature (e.g. equipment failure; unattended alarm).
v. Transport/distribution
   a. loss of irreplaceable autologous tissues; delayed transport of cryopreserved skin (resulting in tissue discard); incorrect tissue type, i.e. a different type of tissue is supplied than was intended or requested.
vi. Testing
   a. bacterial/viral/fungal contamination of tissues distributed for transplantation; loss of cell viability in cryopreserved viable tissue.
vii. Others
   a. in one case, skin was torn upon thawing and implanting so the graft was lost (Record Number 126).

22.11 Patient follow-up

General information on patient follow up is described in Chapter 14, section 14.13.

Systematic longer-term follow-up (i.e. at 1-2 years after skin transplantation) is performed in many transplant centres.

Systematic follow-up includes registration of some basic clinical and quality criteria, such as the main clinical indications for surgery, the (peri-)operative outcome or complications, and patient-reported outcome.

The possibility of using skin grafts in different presentations (lyophilised, glycerolised etc.) for different clinical indications and the absence of specific national or international registries on skin transplantation, make it difficult to record the level of risk of an SAR and the level of success of the different methods of preservation.

22.12 Developing applications

Wound healing is a major target in tissue-engineering research. Since the 1980s, a large number of biological and synthetic skin/dermal substitutes have been produced. Products such as bioengineered skin equivalents and synthetic/biosynthetic materials, integrated with cultured epidermal cells, have been developed for this purpose, and most of them would be classified in the EU as advanced therapy medicinal products (ATMP) (Chapter 34). However, the ideal skin substitute has not yet been established, and human skin allografts remain a gold standard in the therapy of major burns and skin loss treatment when the lack of available donor sites does not allow autografting and necessitates the use of skin substitutes [48, 51]. Thus, researchers in the field of tissue engineering are still working on the production of an ideal artificial skin able to act as a physiological skin.

22.12.1 Epidermal cell suspensions

Epidermal cell suspensions (non-cultured autologous epidermal cellular grafting) have been used in the surgical management of vitiligo since 1992, when Gauthier and Surlève-Bazeille developed a non-cultured cellular grafting technique [56, 57]. With this technique an epidermal cell suspension is used without cell expansion to treat larger areas (8- to 10-fold size of donor skin) on an outpatient basis with simple laboratory procedures. Epidermal cell suspensions can be useful in a variety of epidermal defects, involving both keratinocytes and melanocytes, and several approaches to delivering autologous keratinocytes/epidermal cells to restore epithelialisation have been developed [58].

A recent technique based on aerosol spraying of non-cultured epidermal cell suspensions represents an efficacious and rapid way to obtain re-epithelialisation. To prepare the epidermal suspension, a cutaneous biopsy is trypsinised and epidermal cells are obtained by scraping off the dermal side of the epidermis. A suspension of autologous keratinocytes, melanocytes and fibroblasts can be delivered onto the wound bed by a spray apparatus. Therefore, epidermal cell sprays can be considered as aerosolised skin grafts that can potentially treat a variety of epidermal defects for burns and traumatic injuries, but also in scar reconstruction, donor-site repair and in skin resurfacing techniques. In consideration of the presence of melanocytes, this technique is also effective in pigmentation defects, including vitiligo and post-burn leukoderma.

Special commercial devices or prefabricated cellular preparation kits have also been developed to isolate and apply non-cultured epidermal cells, dermal cells or adipocytes to wounds in a one-step surgical procedure.

22.12.2 Epidermal 3D cell cultures

It was in 1975 that Rheinwald and Green [59] first described the serial cultivation of human keratinocytes in monolayer culture obtained from primary keratinocytes seeded onto lethally irradiated murine fibroblast feeder layers. Since then, numerous advances have been made in the cultivation of human keratinocytes, in both two-dimensional monolayer and three-dimensional organotypic culture. Cultured epidermis was originally used to re-epithelialise severe burns, but, because of the presence of melanocytes, it was also used in vitiligo and other skin pigmentation disorders and to treat scars, ulcers and skin-graft donor sites.

Three-dimensional (3D) bioprinting, a flexible automated platform for the fabrication of complex living architectures, is a novel approach to the design and engineering of human organs and tissues [60]. Natural decellularised ECM represent emerging biomaterials to be printed and used in tissue engineering and regenerative medicine. These matrices provide a physiological environment acting as a bioscaffold which supports cell proliferation and differentiation. The 3D bioprinting process involves direct printing of the bio-ink (biomaterials + cells) by inkjet, micro-extrusion and laser-assisted bioprinters. The most relevant challenges in skin bioprinting deal with the necessity of skin structures, such as epidermis–dermis interface, vascularisation and adnexal structures, and are still to be addressed.

All cell-culture methods are relevant in the field of tissue engineering, and they have to comply, when considered for clinical applications, with the ATMP regulations. These cell culture constructs can be used to test pharmaceutical products, as well as cosmetics and consumer chemical products, *in vitro* where current regulations require testing that does not use animals.

22.12.3 Skin composite grafts – nipple preservation

Patients with loss of the nipple and areola from cancer can undergo nipple-sparing mastectomy in specific cases (after histological examination of the tissue surrounding the nipple and areola to eliminate the possibility of eventual cancer invasion) and delayed autologous nipple–areola complex (NAC) transfer. It can be cryopreserved as a composite graft to be autografted for reconstruction of the breast after mastectomy. According to published literature and skin-bank protocols [61], a slow cooling procedure for cryopreservation is used by incubating the NAC in a cryoprotectant solution with 10% DMSO or 15% glycerol.

The timing of transfer usually ranges from six months to one year after breast reconstruction. At the time of transfer, the package containing the cryopreserved NAC is thawed in a 37°C water bath and the tissue is grafted on a projection made by a denuded dermal flap on the reconstructed breast.

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Related material

Appendix 16. Evaluation of pigmented skin lesions
Appendix 30. Decellularisation

Chapter 23. **Cardiovascular tissue**

23.1 Introduction

Cardiovascular tissues, i.e. cardiac and vascular (arterial or venous) tissues, can be collected from deceased donors after either neurological death or cardiocirculatory death. Hearts may also be donated by living donors undergoing a heart transplant, where the explanted heart can still yield valvular tissues suitable for transplantation.

Cardiac valvular tissues, obtained from donated hearts, comprise aortic, pulmonary and sometimes mitral valves, and may include patch grafts or conduits from the ascending aorta, with or without arch, and the pulmonary trunk with or without bifurcation. Arterial and venous tissues banked include descending thoracic and abdominal aorta, aorto-iliac bifurcation, iliac and femoral arteries, vena cava, and saphenous and iliac veins. Pericardium is also classed as a cardiovascular tissue, due to its function and anatomical position, and can be recovered together with the heart.

Clinical indications for cardiac valvular tissues include the following:

- Paediatric cardiac surgery for treatment of congenital malformations, such as tetralogy of Fallot [1], valve atresia, bicuspid or mono-cuspid aortic valve, or transposition of great arteries. The most important reason for clinical use of heart-valve allografts in these indications is the similarity between the biomechanical properties and morphology of the allografts and the native tissue of the patient, which enables optimal reconstruction of the left/right ventricular outflow tracts (RVOT/LVOT).
- Reconstruction of the RVOT in the Ross operation, which is often used for the treatment of young female patients of child-bearing age and for athletes. This entails replacement of the diseased aortic valve with the autologous pulmonary valve (autograft) and reconstruction of the patient's RVOT with a pulmonary (or aortic) allograft.
- Endocarditis of either prosthetic or native valves, as the allografts are more resistant to infection. In recent years, allogeneic heart valves have also often been used to repair failed or infected biological valves implanted by endovascular procedure (TAVI, trans-arterial valve implantation).

An important advantage to all patients, but especially children and neonates, is that they do not need long-term anticoagulation due to the non-thrombogenicity of the allograft, thereby avoiding the side-effects of anticoagulation therapy. Long-term follow-up studies suggest that implanted cardiac valvular allografts often undergo premature deterioration, mainly in very young patients and children, possibly due to immune-mediated mechanisms [2, 3]. Therefore, methods for the reduction of immunogenicity in cardiac valvular allografts have been the subject of much research since 1993 [4]. This has involved the development of new procedures to decellularise cardiac valvular tissue (see §23.6).

Clinical indications for vascular allografts include:

- infection of prosthetic and/or native vascular tissue,
- peripheral re-vascularisation,
- vascular reconstruction in cases of malignant infiltration of the vascular wall (arterial, venous) [5],
- reconstruction of the thoracic and/or abdominal aorta,
- coronary artery bypass grafting (CABG),
- arteriovenous access,
- reconstruction of vascular tree in liver transplantation.

Iliac vessels are sometimes collected from deceased donors at the time of organ donation and are stored refrigerated for a short period in case they are required to support transplantation of organs from the same donor including consent and tissue donation

criteria (see Chapter 6). They may be sent to the substances of human origin (SoHO) establishment for further processing as vascular tissue allografts.

Arterial grafts are more widely used than venous allografts; there is a large body of literature demonstrating safe and successful use. Despite the first clinical report of their use being in 1976 [6], clinical use of venous allografts has been more sporadic, perhaps reflecting conflicting reports of their clinical efficacy [7]. However, in recent years there has been an increasing number of clinical reports of their use, especially for saphenous veins [8-10].

23.2 Donor evaluation

The general criteria for donor selection are outlined in Chapter 6. The specific criteria for exclusion of cardiovascular tissues are given in the same chapter, in Table 6.2.

Table 6.4 details the general risk conditions for SoHO donors, mainly relating to communicable infectious diseases requiring careful evaluation. Donor screening is provided in Chapter 7.

As outlined in Table 6.2, donor age limits vary between centres, based on risk assessments or validations related to specific evaluation of the quality of the tissue. In general, the quality of cardiovascular tissue deteriorates with increasing age, and maximum age limits are a simple way to apply donor-selection criteria. However, other factors such as diet, lifestyle and history of smoking also impact on the quality of cardiovascular tissue, though these are more difficult to assess quantitatively. Additionally, risk to patients arises from both the safety and the efficacy of the grafts, as well as from their unavailability, which may result in patients being treated with clinically substandard graft materials. Where clinical demand for particular types or sizes of cardiovascular graft is not being met, SoHO establishments may choose to set higher age limits to increase the donor pool, in the knowledge that a significant proportion of the grafts donated may not be suitable for clinical application and would be discarded instead of banked.

23.3 Collection

Generic requirements for collection are defined in Chapter 8.

Good Practice Guidelines

G23.1 The collection team should work using aseptic techniques, and be scrubbed, gowned in sterile clothing, wearing sterile gloves and protective masks.

G23.2 The ascending aorta and the pulmonary trunk with bifurcation (wherever possible) should be collected together with the heart.

G23.3 All efforts should be made to collect as much length of pulmonary artery distal to the pulmonary bifurcation as possible.

G23.4 For vessel donation, the maximum possible length of the recovered vessel should be maintained, avoiding iatrogenic lesions during manipulation, and collateral branches should be cut 2-3 mm from the arterial wall to allow the surgeon to ligate or suture them during the surgical procedure to avoid unnecessary bleeding in the patient.

Recommendations

It is recommended that:

R23.1 a cardiovascular collection team consist of at least two people;

R23.2 face shields be used while collecting the cardiovascular tissues;

R23.3 SoHO establishments work closely with organ collection teams to ensure that blood vessels collected from the body cavities (e.g. abdominal blood vessels) are collected at the same time as donated organs;

R23.4 cardiovascular tissues be rinsed, immediately after collection, with an isotonic solution such as physiological saline solution.

Supplementary information

Rinsing the tissues after collection can remove surface contamination from the grafts, to decrease the risk of microbial growth in the recovered material before processing commences.

23.3.1 Tissue transport to the SoHO establishment

Good Practice Guidelines

G23.5 The heart and the vascular tissues from each body compartment (thoracic aorta, abdominal vessels or femoral vessels) should all be packaged separately, unless they are to be processed together at the SoHO establishment.

G23.6 Collected grafts should be double packed into at least two sterile layers of packaging and placed in a transport container that ensures a temperature of 1-10°C during transport to the SoHO establishment and protects the collected tissues from mechanical damage during transit.

Recommendations

R23.5 It is recommended that collected cardiovascular tissues be transported immersed in a preferably buffered crystalloid transport solution (e.g. physiological saline, Ringer's solution, Hanks balanced salt solution).

Supplementary information

Separate packaging of tissues from different body compartments reduces the risk of cross-contamination. Some heart valve banks use buffered transport solutions including nutritional/osmotic components in the transport fluid, and some also include antibiotics.

23.4 Processing

Processing of cardiovascular tissues always involves dissection of the graft, evaluation of morphology, and the measurement and recording of key measurements (see §23.6). For valve grafts, their functionality (competence) is also assessed. This information is essential to assure the quality of the grafts and to provide information to implanting surgeons that is necessary for them to determine if a specific graft is suitable for their patient.

The requirements specified in section 23.4.1 apply to all cardiovascular tissues, regardless of how they are processed unless specifically stated otherwise.

23.4.1 Processing requirements applicable to all cardiovascular grafts

Good Practice Guidelines

G23.7 The annular diameter of valves and vessels should be measured using calibrated instruments such as obturators.

G23.8 The length of valve grafts (with or without aortic arch or pulmonary bifurcation) should be measured from the outside of the conduit (myocardium to edge) and, where relevant, from the inside (valve commissure to edge of the conduit).

G23.9 Special care should be taken to avoid overstretching the annulus during measurement of valves; this is particularly critical for the pulmonary valve.

G23.10 The length of vessels, and of valved and non-valved conduits, should be recorded, as should the position and dimensions of any branching vessels. Measurements should be taken with calibrated rulers, if relevant.

G23.11 There should be a rim of myocardium of at least 2 mm depth surrounding the root of the aortic or pulmonary valve.

G23.12 The associated aortic or pulmonary artery should not be cut below the level of 1-2 mm above the commissure.

Recommendations

It is advised that:

R23.6 measurements be taken after processing, prior to preservation, as they may alter following collection and dissection [11];

R23.7 a 24 h time period not be exceeded, from collection of the tissue to the start of processing;

R23.8 72 h of ischaemia time (cardiac arrest) not be exceeded before the start of graft preservation.

Supplementary information

To maximise cardiovascular tissue quality, it is important that the time between circulatory arrest and preservation is as short as possible. There is evidence that some measurements may change following collection and processing. For this reason, they are taken as close as possible to the start of preservation [11]. The anatomical appearance, quality and other attributes noted during processing are documented to assist with the allocation of a suitable allograft for patient need. Appendix 31 provides an example of how these details can be recorded.

It is common practice for any branching vessels to be ligated following dissection.

23.4.1.1 *Processing requirements applicable to antibiotic-disinfected, cryopreserved grafts*

Good Practice Guidelines

G23.13 The duration and temperature [12] of antibiotic treatment and the composition of antibiotic cocktails should be defined by each SoHO establishment, and should take into account:

G23.13.1 initial bioburden of the tissue (i.e. before the tissue comes into contact with an antibiotic solution);

G23.13.2 the efficacy of the antibiotic cocktail against the most common microbes likely to contaminate the tissues.

G23.14 Post-processing microbiological sampling should be performed immediately before the grafts are sealed in their final packaging, prior to cryopreservation and after final measurements have been made (see section 23.6).

Supplementary information

Following decontamination, antibiotic-disinfected cardiovascular grafts that are to be cryopreserved are immersed in a medium containing a cryoprotectant, frozen at a slow, regular cooling rate and stored at a temperature below –140°C (see §23.5).

23.4.1.2 *Processing requirements applicable to decellularised cardiovascular grafts*

Heart valves and large vessels can be decellularised by employing different methods to eliminate cellular components [12-22]. Decellularisation protocols can employ physical methods (freezing, sonication), chemical methods (hyperosmotic solutions, ionic detergents, non-ionic detergents) and enzymatic methods (trypsin, endonucleases). They generally contain multiple steps carried out over several days.

Supplementary information

The most robust and effective decellularisation protocols include a combination of the three methods; please see Chapter 10 and Appendix 30.

The nature of decellularisation protocols, which may involve the use of bacterial-sourced enzymes and/or prolonged incubation periods in the processing protocol, may increase the risk of pyrogen, such as endotoxin, contamination of the finished graft. Endotoxins and other pyrogens, if present in the extracellular matrix (ECM), may induce strong biological responses in the patient, fever being the main manifestation (see Chapter 12, §12.2.5).

23.5 Storage

23.5.1 Cryopreservation

Cryopreservation aims to maintain the biological and structural properties of cardiovascular grafts. It is the most commonly used preservation method for cardiovascular allografts, and cryopreserved grafts have been shown to perform reliably and consistently for long periods of time after implantation; up to 30 years in some cases. Depending on the protocol applied, it may be possible for decellularised cardiovascular tissues to be stored frozen or even refrigerated. As this is an evolving technology, specific guidance cannot be given regarding storage protocols.

Good Practice Guidelines

G23.15 During the cryopreservation process, the parameters of the freezing cycle should be recorded, as well as any deviations that might have occurred during the operation.

G23.16 After cryopreservation, the frozen tissues should be transferred to a temperature-monitored storage tank (cooled by vapour of liquid nitrogen to a temperature below –140°C, or a mechanical freezer that can maintain a temperature below –140°C).

Supplementary information

Cardiovascular tissues are commonly cryopreserved by using a controlled-rate freezer, according to protocols designed to assure a slow and constant rate of cooling. It has been demonstrated that heart valves preserved and stored under these conditions maintain their critical properties for at least 10 years [23]. Currently the generally applied storage period is 5-10 years, on the basis that donor selection and testing criteria will evolve gradually over time.

23.6 Process validation & quality control

Due to their nature, the consequences of cardiovascular allografts failing following implantation, especially if failure is sudden, can be very serious for recipients. This risk is generally factored into process validation studies and reflected in routine quality control checks. This is especially pertinent to the longer and more complex processing protocols used for decellularisation, which create additional potential risks.

Good Practice Guidelines

G23.17 Process validation protocols for **all cardiovascular allografts** should consider and, where indicated by risk assessment, incorporate:

- **G23.17.1** maintenance of the structure of the graft;
- **G23.17.2** maintenance of native biomechanical properties;
- **G23.17.3** confirmation that the processing applied has not adversely affected their biocompatibility;
- **G23.17.4** for valves and vessels, maintenance of native haemodynamic properties;
- **G23.17.5** evaluation of the functionality of grafts prepared according to the protocol in a suitable large animal model;
- **G23.17.6** confirmation that residual quantities of processing reagents present in the grafts do not render the grafts cytotoxic.
- **G23.17.7** This list is not exhaustive; other types of validation may be indicated by process risk assessment.

G23.18 In addition to the requirements specified above, process validation protocols for **decellularised grafts** should incorporate:

- **G23.18.1** assurance that the process does not unacceptably compromise the ability of recipient cells to infiltrate and remodel the grafts;
- **G23.18.2** assurance that the protocol adequately decellularises the grafts, incorporating parameters including, but not limited to:
  - **G23.18.2.1** effective removal of donor cells and cellular components;
  - **G23.18.2.2** elimination of genetic material;
  - **G23.18.2.3** quantification of ECM protein content;
- **G23.18.3** evaluation of the risk of pyrogen introduction.

G23.19 SoHO establishments should establish specific acceptance criteria relating to the following graft properties, and incorporate these into routine quality control tests.

- **G23.19.1** Graft morphology: fissures, cuts, holes and any other structural damage or anatomical abnormalities should be recorded and evaluated

to determine if they impact the clinical utility of the graft;

G23.19.2 Functional competence (cardiac valvular grafts only): the graft should be held in its normal anatomical configuration and filled with physiological fluid; any leakage greater than minimal should constitute a rejection criterion;

G23.19.3 Graft pathology, including but not limited to:

G23.19.3.1 the level and extent of atheroma;

G23.19.3.2 presence of tangible calcification;

G23.19.3.3 fenestrations in leaflets (valves only);

G23.19.3.4 fibrotic thickening of leaflets (valves only);

G23.19.3.5 stenosis or aneurysm (vessels only).

G23.20 Any unusual morphology, and any pathology, should be recorded and provided to potential users of grafts.

G23.21 Unless terminally sterilised, cardiovascular grafts should be microbiologically sampled and cultured for aerobic and anaerobic bacteria, and fungi, according to *European Pharmacopoeia* criteria (see Chapter 12), before and after disinfection prior to final packaging (see section 23.4.1.2).

G23.22 Pre-disinfection testing should include testing of tissue samples.

G23.23 Post-disinfection testing should also include testing of tissue samples, as above. If preservation solution is prepared at the point of use, this should also be tested.

G23.24 Where there is a risk that post-disinfection tests may be compromised by residual disinfection or processing reagents, this should be reflected in the microbiology policy.

Recommendations

It is advised that:

R23.9 any tangible calcifications, with the exception of small calcifications in the distal wall of the aorta or around the coronary ostia where they are most likely not to interfere with graft functioning, constitute a rejection criterion for valves and vessels;

R23.10 histopathological analysis be incorporated as a routine quality-control check; samples of vessel may be analysed to check for evidence of connective tissue disease and evaluate the extent of atherosclerosis; myocardial tissue from valvular grafts can be analysed for evidence of endocarditis and myocarditis;

R23.11 testing of the transport medium be performed pre-disinfection.

Supplementary information

General information on microbiology is provided in Chapter 12.

When assessing functional competence for valves, note that fenestrations within the margins of the lunulae are commonly found and do not constitute an abnormal pathological finding. Provided the coaptation of the graft is ensured by adequate sizing, marginal fenestrations will not permit valve regurgitation.

Complete decellularisation (the elimination of 100% of donor cell material) is not practical. Commonly, SoHO establishments evaluate the effectiveness of the decellularisation protocols by measuring the residual amount of donor cellular material, or nucleic acids, against a pre-established limit. A limit of 50ng/mg of dry tissue is reported in literature [24-29].

23.7 Cardiovascular allograft distribution

Good Practice Guidelines

G23.25 Cryopreserved cardiovascular allografts should be transported at a temperature below –60°C.

G23.26 If the transport temperature for cryopreserved cardiovascular allografts is above –140°C, they should not be returned to storage below –140°C unless such action is supported by validation data or a documented rationale based on maintenance of the critical properties of the graft.

G23.27 The receiving SoHO entity should check package integrity immediately after taking receipt to ascertain that packaging is undamaged and that the requisite transport conditions have been maintained [30].

Recommendations

R23.12 It is recommended that transport of cardiovascular tissues be carried out using dry-shipping containers (vapour phase nitrogen below –140°C).

Supplementary information

Transport temperatures above –60°C for cryopreserved cardiovascular tissues may adversely impact critical properties of cryopreserved grafts. When such preparations are stored at temperatures of above –140°C, ice recrystallisation gradually damages tissue at a rate proportional to the temperature. Transport protocols for cardiovascular tissues that are not cryopreserved are dependent on their storage temperature.

23.8 Cardiovascular tissue thawing

Good Practice Guidelines

G23.28 The documentation that accompanies cryopreserved cardiovascular grafts should contain the instructions for how they should be thawed and the

cryoprotectant eluted, together with a list of the materials required.

G23.29 Once cryopreserved cardiovascular tissues have been thawed, they cannot be re-frozen and should be implanted as soon as possible. The SoHO establishment should define the maximum permissible period between thawing and transplantation, based on validation data or a documented rationale.

G23.30 Where cryopreserved cardiovascular grafts are thawed directly from temperatures below −140°C, for example if they have been transported in a dry-shipper, instructions to users should ensure that thawing to above −140°C is allowed to proceed at a gradual rate.

Supplementary information

Rapid thawing of cryopreserved grafts from below −140°C (for example, plunging directly into a normothermic water bath) can result in micro cracks [fractures] in the grafts) [31, 32]. Controlled, slow thawing and gradual elution of cryoprotective medium to re-establish the isotonic state of the cardiovascular allograft help to maintain the integrity of the cryopreserved tissue.

23.9 Biovigilance

Some of the examples are from the Notify Library, which includes well-documented cases of adverse reactions and adverse events in transplantation of cardiovascular tissue. The database is publicly accessible and can be searched by substance type, by adverse occurrence type and by record number. For further evaluated cases of adverse outcomes associated with cardiovascular banking, search the Notify Library at www.notifylibrary.org.

Serious adverse reactions (SARs) for cardiovascular transplants include:

i. Transmission of pathogens
   a. Bacterial infection (*Staphylococcus epidermis*) transmitted via heart valve (European Commission SARE report 2022).
   b. Hepatitis C virus (HCV) by transplantation of a saphenous vein after confirmation of transmission to a tendon recipient from the same donor. Imputability was confirmed by detection of identical HCV genotype 1a and phylogenetic nucleic acid arrangement (Record Number 564).
   c. Hepatitis B virus by aortic valve allograft resulting in asymptomatic seroconversion in the recipient (Record Number 424).
   d. Rabies virus by a donor with a subarachnoid haemorrhage. Four recipients (kidneys, liver and arterial segment (record number 455; reference ID 1436) developed encephalitis within 30 days after transplantation.
   e. *Mycoplasma hominis* infection transmission from donor to vascular allograft recipient. The allograft was positive before having any contact with the recipient (Record Number 189).
ii. Graft failure
   a. Any factors suggesting rapid (e.g. within 12 months of implantation) unexpected degeneration/deterioration/failure of the graft, e.g. regurgitation with heart valves, or stenosis/claudication with vessels.
iii. Undue exposure to risk intervention
   a. Patient under anaesthesia without a suitable graft available and surgery postponed or rescheduled, incorrect graft size).
iv. Other
   a. Acute arterial allograft rejection [33].

Serious adverse events (SAEs) for cardiovascular transplants include:

i. Tissues and cells defect
   a. Rupture of the allograft after implantation, possibly as a result of inappropriate thawing.
   b. Heart valve determined to be unusable due to excess tissue attachments during transplant (Record Number 123).
ii. System failure
   a. Positive test for *Cutibacterium acnes* in smear of pulmonary heart valve during implantation (European Commission SARE report 2022).
iii. Materials
   a. Improper sealing of packaging impacting sterility of internal package.
iv. Equipment failure
   a. During the cryopreservation process, the parameters of the freezing cycle are out of specifications due to a disruption of the controlled-rate freeze.
v. Human error
   a. Incorrectly sized heart-valve package opened and thawed at transplant centre before discovering wrong size and resulting in discard (Record Number 122).

23.10 Patient follow-up

Generic guidance on patient follow-up is provided in Chapter 14, section 14.13.

Systematic longer-term follow-up at one and two years after transplantation of cardiovascular tissue is typically performed in many transplant centres. Systematic (long-term or longitudinal) follow-up includes registration of basic clinical and quality criteria. Most physicians record the main clinical indications to surgery, the (peri-)operative outcome or complications and patient-reported outcomes.

There are currently no national or international registries monitoring the performance of cardiovascular allografts, although there are some hospital-to-hospital initiatives to record (longitudinal) follow-up. This makes it difficult to evaluate the clinical performance of cardiovascular allografts and compare the efficacy of different

processing and preservation methodologies. It also raises the risk that graft-related SAR/Es are not identified and investigated appropriately.

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Related material

Appendix 30. Decellularisation

Appendix 31. Sample forms for the evaluation of heart valves (NHS, UK)

Chapter 24. **Musculoskeletal tissue**

24.1 Introduction

Human bone and soft-tissue grafts are used widely in surgery to rebuild and replace musculoskeletal (MSK) structures, either for structural or filling purposes. Bone is the most banked and transplanted MSK tissue.

The main indications for bone grafting are:

I. to initiate osteoinduction – the process by which osteogenesis is induced, by stimulating primitive, undifferentiated and pluripotent cells to develop into the bone-forming cell lineage;
II. to facilitate osteoconduction – by providing a scaffold that facilitates the growth of new bone on a surface or into pores or channels.

Fresh autologous bone is considered to be the 'gold standard' in bone-grafting material because it combines all the properties advantageous for a bone graft material. However, use of autografts is limited by the amount that can be collected and the risk of donor-site morbidity; so, in most cases, allografts are used.

Allografting of bone and MSK soft tissues allows, in most cases, adequate and predictable restoration of structure and functionality, including mechanical properties, analogous to the original healthy tissue at the recipient site. In addition, bone allografts offer the benefit of osteoconductive properties and even, depending on the processing applied, different degrees of osteoinduction through growth factors originally present and preserved in the graft [1, 2]. Compared with synthetic materials, allografts offer a better capacity for osseointegration: i.e. direct anchorage of an implant by the formation of bony tissue around the implant, without the growth of fibrous tissue at the bone–implant interface.

MSK tissues can be collected from deceased donors after brain death or after circulatory death and from living donors. MSK tissues include bones, ligaments, tendons, cartilage and other types of fascia, like fascia lata.

The current indications for the transplantation of MSK tissues include, but are not limited to, tumour surgery, prosthesis replacement, filling where there is bone loss, fracture, malunion or bone fusion (spine and limbs), and ligament and cartilage replacement.

MSK tissues can be preserved *ad integrum* or shaped in many different ways, and stored at different temperatures depending on clinicians' needs or their logistical capacities, and on the critical properties of the graft. In some cases, bone grafts are demineralised to enhance their osteoinductivity; the result is demineralised bone matrix (DBM). The appropriately processed bone preparation will provide 'osteoconduction' (so they act as a scaffold and 'guide rail' for osteoclasts and osteoblasts) and will therefore facilitate the incorporation of the substances of human origin (SoHO) preparation and its remodelling [3].

Large osteochondral grafts and whole-bone segments are also provided, matched to the requirements of the recipient site [4].

This chapter defines the specific requirements for MSK tissue donor evaluation and the tissue processing and preservation procedures that meet adequate quality and safety standards, and also addresses biovigilance and new developments.

24.2 Donor evaluation

The general criteria for donor selection are outlined in Chapter 6. The specific criteria for MSK-specific donor criteria are given in the same chapter, in Table 6.2.

Table 6.4 details the general risk conditions for SoHO donors, mainly relating to communicable infectious diseases requiring careful evaluation. Donor screening is provided in Chapter 7.

24.3 Collection

General information on collection is shown in Chapter 8.

Good Practice Guidelines

G24.1 Team members, after surgical hand disinfection, should be gowned in sterile clothing and wear sterile gloves, caps and protective masks (beard protection if applicable).

G24.2 The steps for MSK collection from deceased and living donors should be:

G24.2.1 Donor preparation: it should include washing and pre-operative disinfection of skin to reduce transient and resident microbiota;

G24.2.2 Donor draping: a local sterile field using disposable sterile drapes should be established before collection to effectively reduce risk of microbial contamination;

G24.2.3 Collection of SoHO: all MSK tissues should be collected using an aseptic technique to decrease the risk of contamination. The following best practices should be applied:

G24.2.3.1 personnel located at one side of the donor should not change to the other side until they have finished collecting all tissues from their own side;

G24.2.3.2 the packaging area should be isolated and separate from the operating field;

G24.2.3.3 a fixed collection sequence should be established, from 'cleanest' to 'dirtiest' areas;

G24.2.3.4 gloves and surgical blade should be changed after the collection of tissues in a specific area of the body (e.g. left leg).

G24.2.4 Tissue packaging: collected tissue should be inspected and identified appropriately before packaging and labelling to avoid mix-ups.

G24.2.5 SoHO establishments should ensure that their microbiology policies and protocols reflect how different types of MSK tissues will be collected and processed considering different bioburden and different anatomic locations, and whether different tissues will be batched for processing. See Chapter 12.

Supplementary information

Changing gloves and surgical blades when collecting tissues avoids microbiological cross-contamination between tissue fragments of different zones.

The most frequently collected MSK tissues from deceased donors are:

- long bones (femur, tibia, fibula, humerus, radius, ulna, rib);
- irregular and flat bones (iliac crest, hemipelvis, vertebrae, sternum, clavicle, scapula, mandible);
- soft tissues:
  - tendon: Achilles, anterior and posterior tibialis, peroneus longus, gracilis, semitendinosus, quadriceps; and patellar ligament;
  - cartilage: meniscus, acetabular labrum, costal cartilage, osteoarticular tissues;
  - fascia lata.

MSK tissues can also be collected from living donors, as follows:

- allograft
  - femoral head from hip replacement or bone fragments from knee arthroplasty can be collected as surgical residues;
  - costal cartilage pieces can be obtained from patients undergoing corrective thoracic surgery in cases of pectus carinatum or pectus excavatum (Ravitch procedure).
- autograft
  - skull bone flaps can be removed during neurosurgical procedures in case of brain oedema to avoid cerebral damage due to compression.

Information on donor reconstruction is provided in Chapter 8 (see G8.18 and G8.19).

In most cases of allogeneic MSK tissue donation from living donors, the main purpose of the operation is to treat the patient's bone damage or perform a reconstruction (e.g. hip endoprothesis).

Regarding collection from living donors, the facility (hospital operating room) and equipment (surgical instruments) are provided under the control of the hospital clinic and will by default be suitable for collection of tissue allografts. Lower limbs are considered as the cleanest area and the abdominal cavity as the dirtiest area (G24.2.3.3).

24.4 Temporary storage and transport to the SoHO establishment

Good Practice Guidelines

G24.3 Where maintenance of cell viability **is crucial** (e.g. osteochondreal grafts), SoHO preparations should be refrigerated between +2°C and +8°C within 24 hours of collection in an appropriate storage medium to preserve viability.

G24.4 Where maintenance of cell viability **is not required**, SoHO preparations should be refrigerated between +2°C and +8°C, and subsequently frozen within 24 hours after collection unless additional preservation protocols are implemented.

Recommendations

R24.1 If it is necessary to extend the temporary storage and transport time for viable SoHO beyond 24 hours, it is recommended that a storage solution be used that retards microbial growth and autolysis without unacceptably compromising viability (G24.3).

R24.2 Extended pre-process/preservation storage of non-viable tissues at +2°C/+8°C (beyond 24 hours) may be facilitated in the same way; however, storage solutions that would eliminate donor cell viability, such as hypertonic salt solutions, can be considered in this case (G24.4).

24.5 Processing

24.5.1 Processing methods

Good Practice Guidelines

G24.5 SoHO establishments should determine which critical quality attributes (CQAs) are relevant to their specific processing protocols based on at least the attributes shown on Table 24.1.

G24.6 The safety of excipients (e.g. carriers for DBMs) and their effects on the essential properties of the SoHO should be established.

Recommendations

It is recommended that:

R24.3 to obtain DBM, the bone be soaked in a dilute hydrochloric acid solution to remove the majority (95%) of the mineral content;

R24.4 for osteochondral allografts, the donor and recipient be matched, either by graft dimensions or by donor–recipient height/weight;

R24.5 if DBM is used because of its osteoinductive potential as CQA, the osteoinductive capability be determined.

Supplementary information

The types of SoHO preparation that can be obtained include (but are not limited to):

- whole femoral heads;
- cancellous and corticocancellous (e.g. chips or cubes, blocks, rings and wedges) often obtained from epiphyses of the long bones, vertebral bodies, or ilium, calcaneus or talus;
- cortical SoHO preparations (e.g. chips, struts, cylinders) often obtained from diaphysis of long bone;
- whole bones (with or without tendon insertions);
- structural bone segments (e.g. whole or halved diaphyses, rings, struts or condyles);
- tendons and ligaments (with or without bone blocks);
- menisci, either whole (with or without bone blocks) or sections;
- osteoarticular cartilage SoHO preparations;
- fascia lata patches;
- costal cartilage segments.

Table 24.1 **The critical quality attributes (CQAs) that are commonly pertinent for different types of MSK allograft during processing**

Graft type	Depletion of donor marrow, blood and cell residues[a]	Maintenance of native biomechanical properties[b]	Over 70% donor cell viability at the point of transplant	No microbial growth after processing[c]	Acceptability of excipients
Cancellous/cortico-cancellous bone	✓	✓		✓	
Cortical bone	✓	✓		✓	
Structural bone		✓		✓	
Demineralised bone matrix (DBM)	✓			✓	✓[d]
MSK grafts with excipient(s)	✓			✓	✓
Osteochondral allografts		✓	✓	✓	
Tendons and ligaments	✓	✓		✓	
Fascia lata		✓		✓	
Cartilage	✓	✓		✓	
Meniscus	✓	✓		✓	
Autologous bone flap		✓		✓	

Depending on the nature of the processing, bioburden reduction and preservation protocols applied, some CQAs may not be required, or others may be pertinent. The list is not comprehensive, and SoHO establishments may need to define and include other CQAs depending on their process risk assessments for specific grafts.

[a] SoHO establishments should define relevant residual components and acceptable limits for each; see §24.6 – Quality control.

[b] This attribute applies only to grafts which are indicated to support load constraints.

[c] Not relevant to grafts that are (a) terminally sterilised and (b) where parametric release is feasible.

[d] Tick in orange: this attribute only relevant for DBM putty.

It is common to apply a terminal sterilisation step for non-viable bone tissue in the final package. In these cases, proof of sterility by parametric release, based on confirmed application of a minimum level of sterilisation, is acceptable (for example, achieving a certain core temperature for a defined period, or a defined dose of irradiation).

Any claim of inactivation or elimination of micro-organisms (virus, bacteria, spores, fungi) may be validated using organisms typically found in the tissue, by model organisms that are representative of their type or ideally by a 'worst case' organism known to be highly resistant to the method in question.

The initial processing of bone and other MSK tissue generally involves mechanical steps that remove extraneous tissue. In the case of bone, residual muscle tissue and periosteum are resected, and cartilage may be debrided.

After collection, MSK tissue allografts may undergo no processing prior to packaging and storage for transplantation or may be subjected to various types of physical and/or chemical downstream processing.

Residual bone marrow, lipids and blood components in and/or on MSK tissue preparations may increase the viable microbiological load, which may have a negative effect on sterilisation processes and might be a cause of immunogenic reactions or delayed incorporation in graft recipients. They may also contain infectious agents derived from the donor.

For this reason, various processing steps may be used to break up lipids and cells, including chemical treatments such as organic solvents (e.g. acetone, chloroform or ethanol) or hypotonic aqueous solutions. Blood and bone marrow are usually removed or depleted. This is generally achieved with physical treatments including centrifugation, ultrasound or high-pressure waterjet [5-10]. Elevating the temperature can enhance the efficacy of chemical and physical blood- and marrow-depletion processes. Treatment with supercritical carbon dioxide is an extraction method allowing dissolution and removal of lipid components in a single step due to its low viscosity and high solvent power [4].

For osteochondral allografts, maintenance of donor-cell viability is suggested, so options for disinfection are limited to gentle surface decontamination. It also needs to be considered that many of the routine processing techniques applied to MSK allografts, such as the use of elevated-temperature water washing, physical removal of adherent tissues and bone marrow, the use of washes with solvents, acids or oxidising agents and supercritical CO_2 alone or with co-solvent, can also reduce microbial load [8-12].

Donors' bones may contain residues of functional bone growth factors. These bone morphogenic proteins (BMP) are found in the organic part of the extracellular matrix and are embedded in the bone mineral. They can be exposed by bone demineralisation.

Depending on other aspects of bone processing and the initial BMP content, the exposure of BMP may promote 'osteoinduction' (i.e. when bone healing is initiated and maintained via stem-cell recruitment) in environments lacking an adequate local population of osteoclasts and osteoblasts [13]. DBM is often ground to a powder to facilitate its application to graft sites. This powder is dry, light and difficult to handle, so it is often mixed with an excipient prior to application. This may be done at the time of application, for example mixing the graft with the recipient's blood or saline, or during the manufacturing process using carriers such as glycerol, forming a paste or gel which is easier to manipulate.

24.6 Quality control

Good Practice Guidelines

G24.7 SoHO establishments should determine which CQAs are relevant to their specific protocols and determine if they will be addressed by process validation or quality control; see Tables 24.1 and 24.2.
G24.8 Quality control tests on demineralised/mineralised bone should take at least the following additional quality criteria into account:
- **G24.8.1** *in vivo/in vitro* osteoinduction test (if the main property of the graft is osteoinductivity and not mechanical property);
- **G24.8.2** volume characterisation.

Recommendations

R24.6 For lyophilisation (freeze drying) of cancellous/corticancellous bone, cortical bone, femoral head, fascia lata or demineralised/mineralised bone, the recommended maximum limit for residual moisture is ≤ 10% (w/w) or for available water (aW) ≤ 0.5. In the case of bone mixture, the recommended limit for residual moisture is ≤ 15% (w/w) and for aW ≤ 0.5.

Supplementary information

For microbiological risk assessment and control testing please refer to Chapter 12 – Microbiology, and Chapter 13, §13.3 – Exceptional release.

24.7 Labelling and packaging

Good Practice Guidelines

G24.9 MSK tissues should be at least double packed separately in sterile pouches or in sterile sealed containers to avoid contamination.

24.8 Preservation/storage

SoHO preparations may be stored at different stages before, during and after processing at a SoHO establishment in quarantine and after release. Different

preservation methods have been developed to maintain the biological properties of tissues for long periods of time (see Chapter 10 – Processing).

Good Practice Guidelines

G24.10 Cooled grafts (e.g. meniscus) should be stored at +2-8°C in a saline solution and transported at a similar temperature as was used during storage.

G24.11 Fresh hypothermic preparations (e.g. osteochondral) should be stored between +2 and +8°C and transported at a similar temperature as was used during storage. Fresh organ-cultured grafts should be stored between +28 and +37°C and transported at room temperature (+15-25°C).

G24.12 Lyophilised/freeze-dried grafts should be stored at +4-30°C. Freezing and long-term transport temperatures above 30°C should be avoided.

G24.13 Frozen grafts should be stored between −40°C and −80°C and transported at a similar temperature as was used during storage, or alternatively transported on dry ice for a defined period.

G24.14 Cryopreserved grafts should be stored between −140 and −196°C and transported at a similar temperature as was used during storage or alternatively transported on dry ice for a defined period.

G24.15 If grafts should be stored at −20°C (or other temperature above −40°C) after distribution, the shelf life at this temperature should be supported by validation data or a documented rationale based on maintenance of the critical properties of the graft.

Recommendations

R24.7 For meniscal or tendons grafts, lyophilisation is not recommended because it produces a significant reduction of graft biomechanical properties. In the case of meniscal grafts, it also produces histological changes.

R24.8 Cryopreservation is not recommended for osteochondral grafts because it produces a drastic decrease in chondrocyte viability.

Supplementary information

Preservation and storage of MSK tissues by deep freezing without use of cryoprotectants is a common method. There is limited scientific evidence to justify particular temperature limits but, in general, it is accepted that

Table 24.2 **Critical quality attributes (CQAs) commonly evaluated by routine quality control**

Graft type	Removal of adherent soft tissues	Absence of:			No microbial growth after processing[b]	Residual calcium content under 8%	Residual moisture below defined limit[c]	Dimensions within acceptable limits[d]
		Gross pathological or morphological abnormalities[a]	Blood staining	Fibre separation				
Cancellous or cortico-cancellous bone	✓				✓		✓	✓
Cortical bone	✓				✓		✓	✓
Structural bone	✓	✓			✓			✓
Demineralised bone matrix (DBM)					✓	✓	✓	✓
MSK grafts with excipient(s)	✓				✓		✓	✓
Osteochondral allografts		✓			✓			✓[e]
Tendons and ligaments	✓	✓	✓	✓	✓			✓
Fascia lata	✓	✓		✓	✓		✓	✓
Cartilage	✓	✓	✓	✓	✓			✓
Meniscus	✓	✓	✓	✓	✓			✓
Autologous bone flap					✓			

[a] SoHO establishments should establish a list of relevant pathological and morphological abnormalities pertinent to each graft type.

[b] Acceptable limits to be defined by each SoHO establishment; see Chapter 12 for guidance.

[c] Only pertinent to lyophilised or freeze-dried grafts.

[d] According to each SoHO establishment's criteria.

[e] Donor–recipient matching in osteochondral allografts: either graft dimensions (recommended) or donor–recipient height/weight.

freezing an allograft has little impact on the mechanical properties of the tissue, and for unprocessed allografts can diminish their immunogenicity [14-16].

Cryopreservation for the storage of MSK tissues has been historically used for storage of osteochondral grafts, where donor cell viability is essential for clinical performance [17]. However, it is now known that this method does not retain donor cell viability for this type of graft [18].

Removal of water content is used to ensure stability of tissue by inhibiting degradative chemical reactions that require the presence of liquid water. As the processes required to remove water also kill donor cells, they also reduce the graft immunogenicity and permit graft storage and transport at ambient temperature, which may have operational advantages [18]. Elimination of water can be performed using different methods: lyophilisation (freeze-drying) with the aim to decrease the water content of frozen tissue under vacuum through sublimation; solvent dehydration, usually using chemical substances such as acetone or ethanol; or, more basically, the tissue can be allowed to dehydrate in a stream of air, perhaps in the presence of hygroscopic chemicals.

Fresh hypothermic preparations (osteochondral) normally have a shelf life of up to 56 days. In the case of fresh organ-cultured grafts the shelf life is normally up to 56 days [19, 20]. Lyophilised/freeze-dried grafts, frozen grafts and cryopreserved grafts normally have a shelf life up to five years.

Storage of unprocessed tissues [21] at hypothermic or near normothermic (≈ 33°C) temperatures allows maintenance of cell viability (e.g. osteochondral grafts) for a short period (1-3 months).

Fresh preservation (hypothermic or organ-cultured at 37°C) is the best method to preserve chondrocyte viability. Lyophilisation is the preferred preservation method for demineralised/mineralised bone.

24.9 Distribution and transport conditions

Transport of SoHO preparations aims to guarantee the preservation of graft-storage conditions from SoHO entity to end user. Transport systems will vary, depending on the preservation method used.

Good Practice Guidelines

G24.16 Frozen and deep-frozen grafts should be transported using a container with an adequate cooling system.

G24.17 Once the released SoHO preparation has been thawed at the organisation responsible for human application (ORHA), it should not be re-frozen.

G24.18 Cryopreserved SoHO preparations should be transported using dry-shipping containers (vapour-phase nitrogen at or below –140°C).

G24.18.1 When a SoHO preparation has been transported on dry ice, the tissue should not be returned to liquid or vapour-phase nitrogen tanks unless validated or supported by a documented scientific rationale.

G24.19 Dehydrated and freeze-dried grafts should be transported using a container sufficient to protect the integrity of the packaging system.

24.10 Biovigilance

Some of the examples below are from the Notify Library, which includes well-documented cases of adverse reactions and adverse events associated with the transplantation of MSK tissue. The database is publicly accessible and can be searched by substance type, by adverse occurrence type and by record number. For further evaluated cases of adverse outcomes associated with MSK banking, search the Notify Library at www.notifylibrary.org.

Serious adverse reactions (SARs) for MSK transplants include:

i. Transmission
   - *a.* A case of human T-cell lymphotrophic virus type-1 transmission by a deep-frozen bone allograft, resulting in asymptomatic seroconversion of the recipient, is described in Record Number 587.
   - *b.* A case of human immunodeficiency virus (HIV) transmission, through frozen femoral head used in scoliosis surgery, is documented in Record Number 19. Both donor and recipient developed acquired immunodeficiency syndrome (AIDS) 40 months after transplant; similarly, after the transplantation of lyophilised bone chip, HIV was transmitted to the recipient (Record Number 1431).
   - *c.* Tuberculosis outbreaks linked to a bone graft product are described in Record Numbers 2248 and 2255. In the first case, among 19 patients who had received grafts from the relevant batch, microbiologic testing of a vertebral, paraspinal soft tissue, or sputum specimen, showed that 15 (79%) had a positive acid-fast bacilli smear, *M. tuberculosis* nucleic acid amplification test, or culture; and in the second case a single donor was processed into 154 units of a viable cell-containing allogeneic bone product and distributed. Of these, 136 units were transplanted and evidence of tuberculosis transmission was obtained in 87 recipients, three of whom died due to tuberculosis.
   - *d.* Record Number 459 describes a donor-transmitted invasive group A streptococcal infection, with the diagnosis confirmed by *emm* gene-sequence analysis of isolates from the blood and hemi-patellar tendon tissue of the donor and recipient; a similar case was reported with the transmission of *Streptococcus pyogenes* group A in Record Number 641 and *Serratia marcescens* in three patients receiving bone from the same donor in Record Number 630.
   - *e.* Transmission of a bacterial infection (*E. coli*) to the recipient after bone filling during orthopaedic surgeries which required treatment with antibiotics

(Record Number 1102). Also, transmission of *Enterobacter cloacae* in Record Number 966.

f. A case of donor-to-recipient hepatitis C virus (HCV) transmission by patellar tendon transplantation is described in Record Number 563 and confirmed by identical HCV genotype 1a and phylogenetic nucleic acid arrangement between donor and recipient.

g. An HIV type-1 transmission from a seronegative organ-and-tissue donor was confirmed by the recipient's seroconversion 3 weeks post-transplant (Record Number 581).

h. Record 608 shows the transmission of bacterial infection to two recipients of menisci from the same donor. One of the recipients died of *C. sordellii* sepsis after knee surgery. The other recipient of a meniscus was also infected, but recovered with antibiotics.

ii. Graft failure

a. Graft failure or delayed engraftment in seven bone tissues (2021 Annual Biovigilance European Commission report).

iii. Undue exposure to risk intervention

a. Mislabelling of femur discovered in the operating room upon opening package: left femur labelled as right. Patient under anaesthesia without proper graft, operation had to be rescheduled (Record Number 164).

iv. Other

a. Toxicity:

· Intra-articular reaction associated with the use of freeze-dried, ethylene oxide-sterilised bone–patella tendon–bone allografts in the reconstruction of the anterior cruciate ligament (Record Number 176).

Serious adverse events (SAEs) for MSK transplants include:

i. Tissues and cells defect

a. Several cases of SAEs resulting from unsuitable bone allograft release are reported in the database. Record Numbers 139, 140, 141 and 142 describe bone allografts with chondrosarcoma, lymphocytic lymphoma, Paget's disease and rheumatoid arthritis respectively; all were diagnosed during histological examination of the femoral head and resulted in discarding of allografts.

ii. System failure

a. Records Numbers 173 and 174 describe meniscus allografts with anatomic abnormality and fracture, respectively; both were discovered in the hospital and resulted in discarding of allografts, thereby delaying treatment.

iii. Materials

a. Bacterial contamination of the processing solution detected after processing.

iv. Equipment failure

a. Tank containing bone: liquid nitrogen ran out – all tissues thawed and discarded (Record Number 165).

v. Human error

a. Sampling procedure not followed during processing (2023 Annual Biovigilance European Commission report).

24.11 Patient follow-up

General guidance is provided in Chapter 14, section 14.13.

Recommendations

R24.9 It is advised that patients undergoing MSK transplantation receive systematic and regular clinical follow-up to identify post-transplant complications, such as infection, suture-loosening or rejection episodes. This is the international standard for patient care.

Supplementary information

Follow-up may take place at the hospital exclusively or as shared care between the transplant centre (or hospital) and primary care. If not noted and left untreated, these events may result in failure of graft incorporation or loss of mobility.

The frequency of follow-up visits is specific to the type of MSK transplant procedure, the indication for surgery and the presence of possible risk factors in the recipient. Low-risk procedures include filling bone in dental procedures while high-risk procedures may include transplantation of structural bone, or tendons. Where a high level of potential risk related to the graft has been identified, closer follow-up is indicated.

Systematic longer-term follow-up at one and two years after high-risk MSK transplantation is typically performed in many transplant centres, either as part of an internal quality programme and/or as part of participation in national or international suggested practices. After two years, lifelong annual or biannual clinical follow-up visits are often suggested.

Systematic follow-up includes registration of some basic clinical and quality criteria. Most physicians record the main clinical indications to surgery; patient-reported outcomes measure the time for complete osseointegration, range of motion recovered etc.

One of the differences from other tissues is that MSK tissues can be used in different presentations (lyophilised, frozen demineralised etc.) in many different situations, so it is not easy to establish comparisons between different procedures and recipient diagnose.

Usually, MSK is not the unique treatment applied; in general, it is used in conjunction with other procedures, such as new prosthese in revision surgery when bone filling is necessary; there are many registries but many of them are more focused on the prosthesis

aspects than on the use of grafts. Many countries have national registries for arthroplasty as part of the Network of Orthopaedic Registries of Europe, but the use of bone is not analysed [22-25].

The same situation occurs with ligament substitution: there are national registries related to the primary diagnosis, and some of them take into consideration the graft used, but not as the primary item as in the case of cornea transplant registries [26, 27].

In another situation, bone is used as a structural element, as in the case of tumour when the mechanical strength is a crucial actor together with graft incorporation as a complementary treatment procedure [28]. Some countries, such as Denmark, have national registries but not all of them collect which kind of graft was used [29]. Countries like the UK, Sweden, Norway and Luxembourg have existing registries related to knee ligament reconstruction.

Currently, there are no specific national or international registries for MSK transplantation; the current registries are focused either on the primary diagnosis or on whether a prosthesis was used. This situation makes it difficult to record the level of risk of an SAR and the level of success in tissue integration of the different methods of preservation.

Systematic patient follow-up and participation in registries would be an important benefit for MSK transplant centres, not only by providing the best treatment of individual patients at the level of international standards, but also by comparing a SoHO establishment's own results to international standards [30].

24.12 Developing and niche applications

Developments in MSK tissue banking focus mainly on the following areas:

- developing new preservation methods that maintain the mechanical and biological properties of the grafts;
- developing new procedures such as specific cell seeding to improve graft incorporation in recipients;
- improving the safety of grafts through the development of better decontamination processes [31].

Bone grafts (predominantly cancellous and corticocancellous chips, cubes etc.) can be used as carriers for antibiotics. The idea is that impregnated bone grafts release antibiotics at high concentrations and for a prolonged time at the site of implantation. This finds an application in the prevention or treatment of postoperative infections, especially in hip revision surgery, which is among the most common procedures where bone transplants are being used [32-34].

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Chapter 25. Haematopoietic progenitor cells from bone marrow and peripheral blood

25.1 Introduction

The transplantation of haematopoietic progenitor cells (HPC) represents one of the most widely used forms of cell therapy, in part because haematopoiesis is the best-characterised biological model of somatic stem cell differentiation. Following the first case reports more than sixty years ago, the procedure rapidly established itself as a life-saving treatment for adult and paediatric patients with a variety of malignant and non-malignant diseases. The latter is the case when the haematopoietic cell is functionally damaged by congenital or acquired disorders such as severe congenital immune deficiencies, haemoglobinopathies, metabolic diseases or bone marrow failure. More recently, the use of autologous HPC transplantation in combination with immunosuppressive agents has been evaluated for patients with certain autoimmune diseases.

In its main field of application, i.e. as a component of the treatment of patients with poor-risk or advanced haematological malignancies, it is now well established that allogeneic HPC transplantation exerts its beneficial effects through the recognition of residual tumour cells in the recipient by donor-derived immune effectors (graft-*versus*-tumour effect, or GvT). Thus, allogeneic HPC transplantation represents a clinically useful, immune cellular therapy. Limits to the use of these therapeutic procedures are their intrinsic toxicity, dominated by (though not limited to) graft-*versus*-host disease (GvHD), an immune disorder in which donor-derived immune effectors recognise and harm the host's normal tissues such as skin, gut and liver, lungs and eyes. In addition, the availability of donor cells might be a limitation.

The field has tremendously developed in the past half-century, and it has integrated medicinal and technical innovations, including the use of new immunosuppressive agents, the use of different sources of HPC, such as bone marrow, mobilisation of stem cells from peripheral blood and cord blood, the collection of cells from unrelated donors and much improved supportive care for patients. During recent years, the use of volunteers as unrelated donors has dramatically increased due to the extensive improvements in human leukocyte antigen (HLA) typing and matching algorithms and the growth throughout the world of donor registries that are associated in the World Marrow Donor Association (WMDA) (Figure 25.1).

Several other biotechnological advances, including stem cell selection, lymphocyte depletion and immune effector cell activation, have entered clinical practice and made special types of transplants such as haplo-identical transplantation a valid clinical choice with rapidly increasing numbers (Figure 25.2).

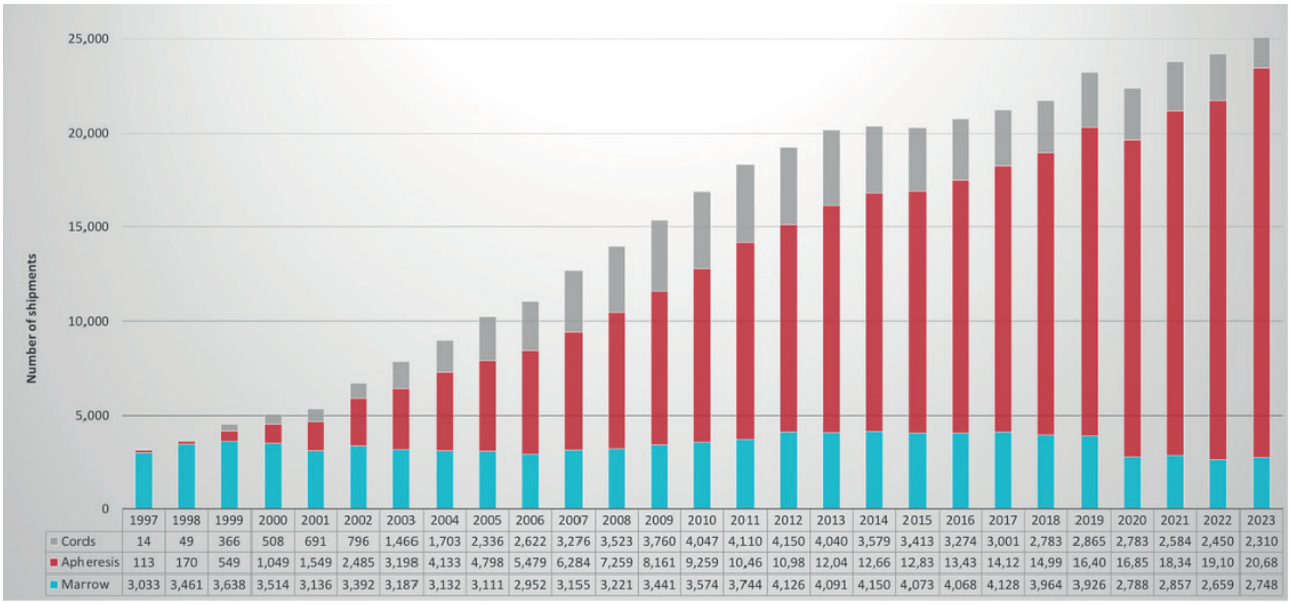
However, despite these advances, collection of HPC remains relatively unchanged. Hospitals that care for recipients often obtain autologous or family donor allogeneic HPC from collection and processing facilities at hospitals or blood establishments in their immediate vicinity. Each of the collection and processing facilities works on a typically small to medium scale. In over 50% of allogeneic HPC transplantations, grafts from unrelated donors are used, which very often have to be imported from other countries or continents. Given the high rate of international exchange of donated HPC material, harmonisation of the practices in this field is of great benefit.

This chapter defines the specific requirements for collection, processing, storage and transplantation of HPC derived from bone marrow – HPC, Marrow, known as HPC(M), or from peripheral blood – HPC, Apheresis, known as HPC(A), and the additional requirements for mononuclear cell (MNC) concentrates procured by apheresis – MNC, Apheresis, known as MNC(A) – either for immediate use or for further development of

immunocompetent cells used after or instead of HPC transplantation.

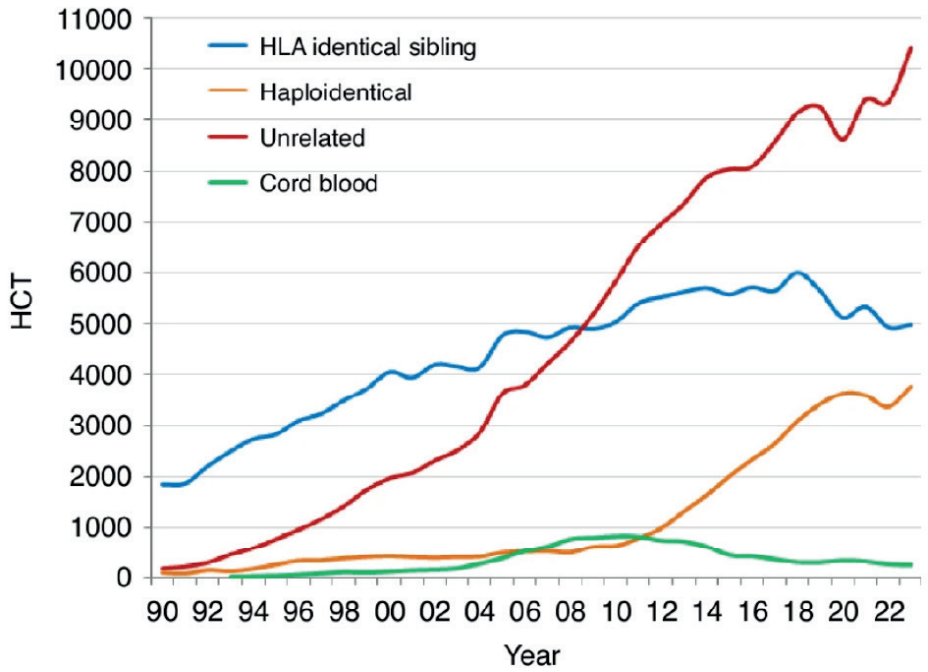
The substances of human origin (SoHO) discussed in this chapter fall under Regulation (EU) 2024/1938 of the European Parliament and of the Council of 13 June 2024 on standards of quality and safety for SoHO ('the SoHO regulation'). However, if these cells are subjected to substantial manipulation (such as expansion or genetic modification) or are used in the recipient for an essential function that is different from the original function in the donor, in the EU they are then regulated as medicinal products. This means that their processing, storage, distribution and use in patients must respect the requirements of Regulation 1394/2007/EC on advanced therapy medicinal products ('the ATMP Regulation') as well as all other relevant provisions of the EU and country-specific medicines rules. The generic chapters (Part A) of this Guide all apply to HPC transplantation.

Figure 25.1 **Unrelated HPC marrow, HPC apheresis and HPC cords shipped, 1997-2023 (WMDA Annual Report 2023)**



	1997	1998	1999	2000	2001	2002	2003	2004	2005	2006	2007	2008	2009	2010	2011	2012	2013	2014	2015	2016	2017	2018	2019	2020	2021	2022	2023
Cords	14	49	366	508	691	796	1,466	1,703	2,336	2,622	3,276	3,523	3,760	4,047	4,110	4,150	4,040	3,579	3,413	3,274	3,001	2,783	2,865	2,783	2,584	2,450	2,310
Apheresis	113	170	549	1,049	1,549	2,485	3,198	4,133	4,798	5,479	6,284	7,259	8,161	9,259	10,46	10,98	12,04	12,66	12,83	13,43	14,12	14,99	16,40	16,85	18,34	19,10	20,68
Marrow	3,033	3,461	3,638	3,514	3,136	3,392	3,187	3,132	3,111	2,952	3,155	3,221	3,441	3,574	3,744	4,126	4,091	4,150	4,073	4,068	4,128	3,964	3,926	2,788	2,857	2,659	2,748

Figure 25.2 **HPC transplantation reported to the European Bone Marrow Transplantation (EBMT) registry by donor type, 1990-2023 – patient numbers per year with first transplant**



Source: Passweg JR, Baldomero H, Atlija M *et al.* The 2023 EBMT report on hematopoietic cell transplantation and cellular therapies. Increased use of allogeneic HCT for myeloid malignancies and of CAR-T at the expense of autologous HCT. *Bone Marrow Transplantation* 2025;60:519-28.

25.2 Recruitment of potential donors, identification and consent

Most of the patients who could benefit from HPC transplantation do not have an HLA genotypically identical sibling donor. The chance of having a fully HLA-identical sibling donor is 25-30%, depending on the number of siblings. In some cases, an extended family search can provide an HLA phenotypically identical donor. This can happen in cases of consanguinity due to cultural or geographical reasons, or in cases of common haplotypes, and can increase the likelihood of finding a matched or partially matched family donor [1].

If no HLA-matched suitable donor is found in the patient's family, an alternative HPC graft can be considered. This would include searching for an unrelated donor through bone marrow or umbilical cord blood registries (see Chapter 5) and extending the search to a mismatched unrelated source or an HLA genotypically haplo-identical relative (e.g. parents, siblings, children). The most common donor-selection algorithm is described in the European Bone Marrow Transplantation (EBMT) handbook (see Figure 25.3) [2]. For haplo-identical transplantation using HPC(M) or HPC(A), promising protocols have been developed in recent years [3] and EBMT have published recommendations on haplo-identical donor selection criteria [4].

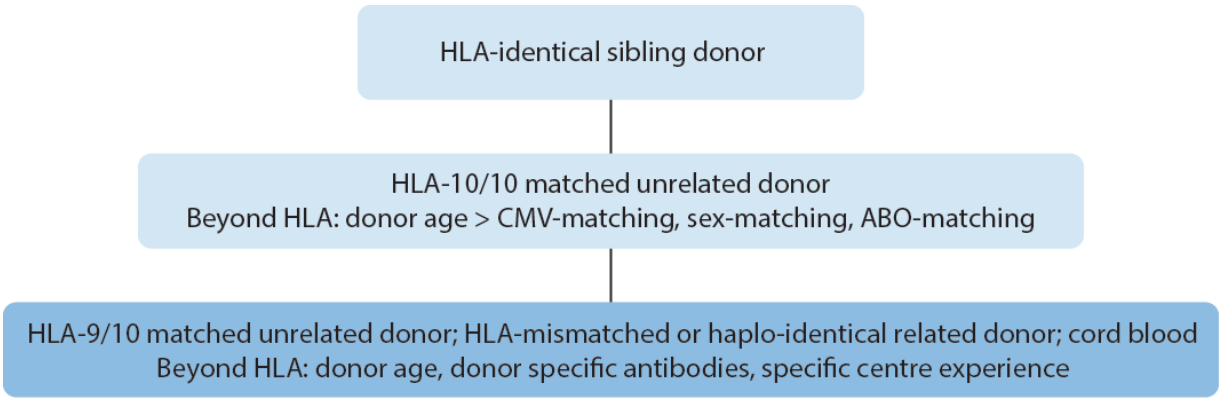
The relevance of HLA matching is dependent on a variety of factors, including but not limited to GvHD prophylaxis, transplant conditioning, graft manipulation and clinical donor characteristics such as age, sex and *Cytomegalovirus* serostatus. Biologically mismatched HLA can be recognised by alloreactive donor T-cells, leading both to toxic GvHD and to beneficial graft-*versus*-leukaemia (GvL) as counterbalancing effects [5]. Moreover, missing self-HLA on patient cells or mismatches between certain groups of HLA-C alleles in donor and recipient [6] can also lead to alloreactivity by natural killer (NK) cells, a phenomenon associated with GvL but not GvHD [7].

In unrelated stem-cell transplantation, it is generally accepted that the best donor is matched for at least 8/8 HLA-A, B, C, DRB1 alleles, with every mismatch leading to an approximate 10% reduction in survival probability [8]. Mismatches at HLA-DQB1 and DPB1 are more controversial. For HLA-DPB1, the concept of permissive, clinically well-tolerated mismatches was pioneered on the basis of experimentally defined T-cell epitope groups (TCE). This led to the increasingly accepted notion that avoidance of non-permissive HLA-DPB1 TCE mismatches improves outcome and has to be favoured when several (at least 8/8, but 10/10 if including HLA-DQB1 alleles) matched unrelated donors are available [9]. A mismatch at HLA-DQB1 seems to be unfavourable in the presence of other mismatches.

In apparent contradiction to the dramatic effect of subtle HLA mismatches in unrelated transplantation, transplants across several mismatches or an entire HLA haplotype are possible in cord blood and haplo-identical family donor transplantation, respectively [10, 11]. This is probably due to graft composition in the former and GvHD prophylaxis in the latter, with a higher proportion of regulatory and naïve T-cells in cord blood compared with adult stem-cell sources, and an important attenuating effect of agents such as post-transplant cyclophosphamide or high-dosage anti-thymocyte globulin on GvHD in the haplo-identical setting. The important question of whether and to what extent these specific characteristics will change the landscape also of unrelated stem-cell transplantation in the future will have to be answered in prospective clinical trials under way.

The first bone marrow donor registry was established in 1974 by the Anthony Nolan Bone Marrow charity in London. In 1988 the EBMT group with the Europdonor Foundation set up Bone Marrow Donors Worldwide (BMDW), based in Leiden, Netherlands. In 2017 the WMDA took over the activities of BMDW and the NetCord Foundation, and now co-ordinates the collection and listing of the HLA phenotypes and other important data of volunteer HPC donors and cord blood units. As of September 2023, the WMDA database included more than 43 million HPC donors (see https://statistics.wmda.info/)

Figure 25.3 **Scheme for donor selection for adult patients with haematologic malignancies**



CMV: Cytomegalovirus

Source: adapted from the EBMT Handbook [2].

and over 823 000 cord blood units. Since ethnic minorities are under-represented in HPC donor registries, it is very important that donor centres and cord blood banks work with these communities to explain the need to increase the ethnic diversity of the registry and to recruit new potential donors. In the case of unrelated adult donors, once the HLA-typing and personal data are entered onto the donor registry, further blood samples may be requested, leading to possible haematopoietic stem-cell donation at any time in the future.

The WMDA, an organisation of representatives of HPC donor registries, cord blood banks, other organisations and individuals with an interest in HPC transplantation (www.wmda.info), plays an important role in the field of HPC transplantation from unrelated donors, providing a forum for discussion and guidelines on the more critical aspects of collection and clinical use of HPC. Moreover, the WMDA offers to donor registries a certification programme according to internationally accepted standards.

The main role of the registries is to facilitate interactions between HPC transplant centres and donor centres. The worldwide registries share their unrelated donors and cord blood units' data in the Search, Match (& Connect) global database operated by WMDA (see Appendix 32). So far, the 'Search' functionality has been implemented in nine registries. The 'Connect' functionalities have been established by the WMDA and need to be implemented by the registries.

Emergency rescue procedures limit the consequences related to unforeseen unavailability of a donor (e.g. acute illness, accident, failed mobilisation). These procedures could be identifying a backup donor or searching for a cord blood unit, a haplo-identical donor or an autologous transplantation with previously cryopreserved autologous HPC.

25.2.1 Informed consent

Informed consent in a free decision-making process is required for allogeneic donation and for autologous donation. General considerations are described in Chapter 5.

25.2.2 Donor evaluation

General information on the activities related to donor recruitment, evaluation, screening, and the examination and collection of samples can be found in Chapters 5, 6, 7 and 8 of this Guide. The general criteria for donor selection are outlined in Chapter 6. The specific criteria for exclusion of HPC are given in the same chapter, in Table 6.2.

Table 6.4 details the general risk conditions for SoHO donors, mainly relating to communicable infectious diseases requiring careful evaluation. Donor screening is provided in Chapter 7.

25.2.2.1 *Allogeneic donor*

Good Practice Guidelines

G25.1 Donor evaluation (including a pregnancy test if applicable) should be completed before the patient starts with the preparative regimen and, in cases involving HPC from the peripheral blood, before the donor receives the first dose of the mobilisation agent.

G25.2 The criteria for donors should also include:

- **G25.2.1** suitability for anaesthesia (for collection of bone marrow);
- **G25.2.2** assurance of adequate venous access;
- **G25.2.3** additional tests if appropriate (e.g. in cases of family history or elderly donor);
- **G25.2.4** policy for making decisions in cases of 'only one' donor but who does not meet eligibility criteria (e.g. only one suitable donor but with risky behaviour).

G25.3 Specific recommendations should be available, especially for related donors who would not be eligible as unrelated donors due to age (e.g. young donors < 18 years, elderly donors > 60 years) or specific health issues [12-16].

G25.4 To ensure the safety of donor and recipient, a physician involved in the HPC(A) collection procedure should be available for consultation during the collection period, from the beginning of G-CSF (granulocyte-colony stimulating factor) injections to the post-collection period. This also applies for HPC(M) collection when G-CSF is used. Where responsibilities for HPC(A) mobilisation, cell collection and post-collection donor care are divided between entities, physicians involved should be available for consultation accordingly.

Recommendations

It is recommended that:

- R25.1 the physician who evaluates the donor be independent of the transplant team, to avoid conflict of interest;
- R25.2 the indications outlined in Table 25.1 be followed regarding the assessment of the medical suitability of adult progenitor cell donors [12, 14];
- R25.3 decisions with regard to donor safety be, as a policy, the responsibility of a physician who is not directly involved in recipient care;
- R25.4 if donors' co-morbidities result in contraindication of the person for HPC donation, the physician who decided to contraindicate this person ensure that an appropriate medical follow-up will be initiated, in accordance with the medical condition of the rejected donor.

Table 25.1 **Recommendations on assessing the medical suitability of adult progenitor cell donors (see Chapters 6 and 7) [12]**

Assessment stage (donor)	Method	Topics to consider	Specific to this stage
Recruitment/ registration (unrelated)	History/questionnaire	Malignancy Autoimmune disease Cardiovascular disease (or a combination of risk factors) Chronic disease (pulmonary/neurologic/ haematologic/serious allergies) Relevant medical history (malignancy, thrombo-embolic disease etc.) Risks of infectious diseases (behaviour) Inherited/genetic disease	Look for permanent diseases or behaviour that have a clear donor risk or unacceptable recipient risk and that are relatively easy to assess
During selection stage (unrelated)	History/questionnaire Blood tests for infectious disease markers (see Chapter 7 – Screening for markers of transmissible infections)	Update history of topics above, and also: Risks of infectious diseases – behaviour, (medical) invasive procedures, (planned) travel (Planned) medical procedures (including blood transfusion, dentist, vaccination, tattoo etc.) Serious psychosocial or psychiatric disease with impact on availability/ capacity to go through donation procedure Medication Non-prescription drug use Height and weight Blood pressure Pregnancy, pregnancy planning, breastfeeding Back problems, chronic pain	Identify contraindications for one of the two collection methods (HPC from bone marrow or peripheral blood) Provide information about (possible) transmissible disease to the transplant centre Provide information to the transplant centre about any availability issues
Prior to HLA-typing (related)	History/questionnaire	As above	Identify contraindications before concluding that the relative donor is the best match; may save time and disappointment
During work-up/PE (related and unrelated)	Complete medical history; Complete physical examination (PE); Laboratory tests: Blood tests for infectious disease markers (see Chapter 7 – Screening for markers of transmissible infections), on indication/per request transplant centre: hepatitis E, HTLV I+II, Chagas, Zika, malaria, West Nile virus, etc. Full blood count; erythrocyte sedimentation rate (ESR); if indicated: coagulation screen; blood film; haemoglobin electrophoresis; ABO and Rh typing, screening for red blood cell and HLA-antibodies in case of HLA mismatch; Biochemistry: urea, creatinine, electrolytes, liver enzymes, lactate dehydrogenase (LDH), ferritin; random glucose; β-HCG human chorionic gonadotropin; (for females of child-bearing age), protein electrophoresis; Chest X-ray, when indicated; Electrocardiogram	As during selection stage; in addition: any signs of undiagnosed disease.	US Centers for Disease Control and Prevention [CDC], European Centre for Disease Prevention and Control [ECDC]

Supplementary information

The WMDA Donor Medical Suitability Recommendations provide minimum standards for unrelated donors, which can be used for donor assessment [13]. Donors with a history of malignant disease (excluding haematological malignancies) after a minimum disease-free period of five years can be considered as suitable for sibling donation after careful assessment of the risk for malignant disease transmission (see Chapter 4) [14].

It is up to the discretion of the transplant physician whether to accept donors with previous or existing infectious diseases (e.g. hepatitis B or others) if no other donor is available, based on careful risk evaluation. The specificity of allogeneic HPC transplantation lies in the fact that, for the vast majority of patients, the HPC graft is infused immediately after collection. Thus, safety is reliant mostly on stringent evaluation of donors, which can be performed appropriately only if all needs are fully anticipated. In cases where there is a risk to a patient or product safety, a formal acceptance of that risk is signed by the transplant physician and the patient or their legal representative (urgent medical need) (see also Chapter 4).

The increasing age of recipients of allogeneic HPC transplants is related not only to the increasing age of the population but also to the introduction of less toxic conditioning regimens, allowing elderly patients and patients with co-morbidities to undergo HPC transplantation. Even if the age limit to donate is well defined for unrelated donors and is over 18 and under 55-60 years for most international registries (G25.3 and G25.6), these limits do not strictly apply for related donors. Some donors will present with co-morbidities (discovered or not) during evaluation. If the co-morbidities found during evaluation allow HPC donation, the physician in charge is responsible for managing these co-morbidities during the entire donation process, including specialist consulting as needed.

25.2.2.2 *Autologous donors*

For autologous donors, suitability criteria for HPC donation are less strict than for allogeneic donors. General information on infectious disease markers is outlined in Chapter 7. General information on donor recruitment is outlined in Chapters 5 and 6.

Good Practice Guidelines

G25.5 Criteria should include threshold values of the complete blood count before starting bone marrow collection or leukapheresis.

Supplementary information

Autologous donors can donate even if results of the required tests are reactive or positive, or if other risk factors have been identified in the patient's medical history, as long as procedures for prevention of potential cross-contamination during HPC collection, processing and storage are implemented.

25.2.2.3 *Specific considerations for paediatric donors and other persons not capable of informed consent*

If minors are being considered as allogeneic HPC donors, in addition to the criteria shown in Chapter 5, national regulations are to be followed.

Good Practice Guidelines

G25.6 Children should become donors only in very specific circumstances, as family donors only and never through public registries (see Chapter 6).

Recommendations

R25.5 The use of haematopoietic growth factors and insertion of a central venous line are not recommended for paediatric donors.

25.3 Collection

25.3.1 Haematopoietic progenitor cell graft sources

General information on donors' recruitment is provided in Chapter 5. HPC(M) and HPC(A) are obtained from living donors only, either from the patient themself (in the case of autologous transplantation) or from a fully or partly HLA-matched allogeneic related or unrelated donor. The choice of the donor is based on the best HLA matching, and other factors such as age, sex, *Cytomegalovirus* serostatus, ABO compatibility and NK alloreactivity. Local algorithms help guide donors' selection, determining the most suitable donor for each patient based on the specific circumstances [2].

Recommendations

It is recommended that:

R25.6 particular attention be paid to HLA-mismatched donor selection (parents, brothers/sisters, adult children or other family members), explaining the criteria of donor choice to the patient and potential family donors in advance;

R25.7 the discard of autologous and allogeneic grafts from related and unrelated donors comply with the signed consent and written agreements of collection centres and donor registries, where applicable.

Supplementary information

For autologous purposes, HPC are now obtained almost exclusively from peripheral blood HPC(A) and used to accelerate haematopoietic recovery after high doses of

Table 25.2 **Advantages and disadvantages of methods of haematopoietic progenitor cell collection**

Collection method	Advantages	Disadvantages
Bone marrow collection	Donor: Single collection Use of cytokines (mobilisation agents) not necessary Recipient: Less chronic GvHD	Donor: General or epidural anaesthesia Invasive procedure Considerable risk of morbidity (associated with anaesthesia, collection method, mobilisation agents if used) Potential tissue damage/infection at collection site Possible need for blood transfusion Recipient: Slower engraftment of neutrophils and platelets Potential graft contamination with skin contaminants Possible contamination with tumour cells in autologous HPC procurements
Peripheral blood apheresis	Donor: No anaesthesia Recipient: Faster engraftment of neutrophils and platelets Potentially less contamination of autologous product by tumour cells	Donor: Collection may take more than one day (i.e. several procedures may be needed) May require placement of a central venous catheter for collection (risk of haemorrhage, embolism, pneumothorax/haematothorax and infection) Potential loss of platelets Considerable risk of morbidity (associated with mobilisation agents and apheresis technique including anticoagulation) Recipient: Increased risk of chronic GvHD

Source: EBMT [17].

chemotherapy. In the allogeneic setting, the HPC graft source depends on the age and size of the donor and recipient – i.e. paediatric or adult donor, since some countries do not support G-CSF administration and apheresis in paediatric sibling donors – and the kind of disease (malignant or non-malignant) treated, as well as the transplant protocol (myeloablative, reduced intensity, T-cell replete or deplete haplo-identical transplantation).

Currently HPC(A) represents 80% of the stem cell source in allogeneic transplantation. However, HPC(M) is still the preferred source in allogeneic paediatric transplantation from HLA-matched related or unrelated donors and for patients with non-malignant diseases (e.g. bone marrow failures).

Criteria for HLA-mismatch transplantation and selection of 'alternative' donors – in the sense of donors without an 8/8 HLA match with the recipient (considered as 'standard' donors) – are constantly updated as they are evaluated in the context of biomedical research or registry studies.

25.3.2 Collection procedures

The majority of HPC are provided using one of two technologies: either collected from bone marrow or by apheresis from peripheral blood. The advantages and disadvantages of these methods are shown in Table 25.2.

Supplementary information

The International Standards for Hematopoietic Cellular Therapy Product Collection, Processing, and Administration were developed by two organisations, JACIE (the Joint Assurance Committee–ISCT & EBMT) and FACT (Foundation for the Accreditation of Cellular Therapy), and provide minimum guidelines for transplant, collection and processing facilities. These standards can serve as guidance for SoHo establishments and entities to establish and maintain quality management systems impacting on all aspects of their activities and to engage in continuous improvement in donor and patient care and laboratory performance in collection, processing, storage and transplantation [18]. The WMDA also has standards, which provide minimum guidelines for donation, collection and transport of HPC from unrelated donors [19].

Severe adverse reactions can occur in donors with the administration of the mobilisation agents, and during and after allogeneic HPC donations (see section 24.10 – Biovigilance) [20, 21].

25.3.2.1 *Collection of haematopoietic progenitor cells from bone marrow*

Good Practice Guidelines

G25.7 A pre-anaesthesia visit to the donor should be performed before collection of bone marrow.
G25.8 Provision for counselling of donors and for their routine post-donation follow-up should be ensured.
G25.9 A 24-hour blood component donor-support protocol, including the provision of *Cytomegalovirus* sero-negative or equivalent (e.g. pathogen-inactivated), irradiated and leukocyte-depleted blood components, should be available.

G25.10 For collection of bone marrow, written procedure(s) should be established, including at least:
- **G25.10.1** disinfection technique;
- **G25.10.2** preparation of media and materials used to obtain bone marrow;
- **G25.10.3** puncture technique;
- **G25.10.4** provision of containers for collected bone marrow;
- **G25.10.5** monitoring of the volume of the collected bone marrow;
- **G25.10.6** irrigation of collected syringes;
- **G25.10.7** bone marrow filtration;
- **G25.10.8** quality controls (e.g. total nucleated cell [TNC] counting, sterility, etc.);
- **G25.10.9** labelling.

G25.11 Bone marrow grafts contain bony spicules, fat and clots that should be filtered during collection or processing.

Recommendations

It is recommended that:

R25.8 the collection and anaesthesia times not exceed 120 and 150 minutes, respectively [22];

R25.9 the maximum volume of bone marrow to be collected not exceed 20 mL/kg donor weight;

R25.10 collection teams not aspirate a volume more than 5 mL if single-hole and not more than 10 mL if multiple-hole needles are used, at each aspiration to avoid dilution of the bone marrow with blood [2, 22];

R25.11 all efforts be made to manage allogeneic donors in such a way as to minimise the need for blood transfusions. Therefore, a patient blood management programme taking care of iron supplementation, if necessary, is recommended [18].

Supplementary information

Collection of bone marrow is an aseptic process that is undertaken in an operating theatre by appropriately trained personnel. Bone marrow for therapeutic use is obtained through multiple punctures, usually from the posterior iliac crests. When absolutely necessary, the anterior iliac crests can also be used. The sternum is not considered an appropriate method of bone marrow collection. Punctures are usually undertaken under general anaesthesia. Epidural anaesthesia may be considered.

The bone marrow TNC number is used to determine the adequacy of the collection. The recipient's body weight and type of post-collection manipulation determine the target TNC and volume of bone marrow to be collected [22]. The minimum target for autologous transplantation without graft manipulation is 2×10^8 TNC/kg recipient body weight. The target dose for most allogeneic transplantations is $2\text{-}3.5 \times 10^8$ TNC/kg recipient body weight.

Adverse reactions related to HPC marrow collection are associated with anaesthesia, pain at aspiration sites, bruising and, rarely, local infection (see §24.10).

25.3.2.2 *Collection of HPC from peripheral blood*

Good Practice Guidelines

G25.12 Before each apheresis procedure, the donor (autologous or allogeneic) should be evaluated. At least the following tests are necessary:
- **G25.12.1** complete blood count (including platelet count) ≤ 24 h before collection;
- **G25.12.2** vital signs and temperature;
- **G25.12.3** blood cultures at least in presence of fever to investigate possible microbial contamination of the collected product;
- **G25.12.4** update of medical history.

G25.13 Special attention should be paid to paediatric patients and the specific circumstances pertaining to apheresis in young patients, whose weight (often under 20 kg) places them at risk of haemodynamic changes, both at commencement and during the procedure.

Recommendations

It is advised that:

R25.12 in both autologous and allogeneic settings, HPC from peripheral blood be collected in an apheresis facility by healthcare professionals who have experience in:
- R25.12.1 care for patients with haematological or oncological diseases, and in
- R25.12.2 HPC mobilisation and therapeutic apheresis.

R25.13 that the apheresis device be primed with matched, irradiated red blood cell units in patients weighing less than 20 kg.

Supplementary information

Expertise to carry out apheresis is of particular importance for small children (up to 20 kg) for autologous collection (which is usually indicated in solid tumours or haematologic malignancies); the transplantation programme needs to maintain trained and experienced personnel for apheresis in paediatric units.

25.3.2.2.1 Allogeneic donors

Mobilisation of HPC from the bone marrow to the peripheral blood before collection is ensured by recombinant human G-CSF (rhG-CSF), which is administered to healthy adult donors in order to mobilise HPC.

Good Practice Guidelines

G25.14 If $CD34^+$ cell numbers do not meet the defined threshold, rescue strategies should be established (e.g. 'immediate' bone marrow collection).

G25.15 The first dose of rhG-CSF should be given under the supervision of trained healthcare professionals, who should follow up with the donor for at

least 30-60 minutes, because of a potential risk of allergic reaction.

G25.16 The physicians in charge of mobilisation and collection of HPC from the donor should be informed in detail of the necessary measures to be taken in case severe adverse reactions (SARs) occur, especially for anaphylactic shock, spleen rupture, capillary leakage and acute hepatitis.

G25.17 Side-effects and risks associated with the mobilisation procedures should be discussed with the donors and donors should be informed about all aspects of rhG-CSF administration, including known short- and long-term effects, and given the opportunity to ask questions (see also Chapter 8).

Supplementary information

Immediate and severe side-effects associated with rhG-CSF administration are rare (see Table 25.3) and raise the issue of inpatient *v.* outpatient administration (see §25.10). Donors who live far away from the transplant centre will require administration at home.

Routinely, HPC(A) collection takes place on day five after four days of rhG-CSF administration. Up to now the administration of HPC binding inhibitors in allogeneic donors is off-label use and is done with appropriate ethical permission.

Approximately 5-10% of donors may be asked to provide a subsequent donation of HPC or MNC concentrates to the same patient. The frequency of second donations seems to be higher for HPC(M) donors, and it may increase with the application of new therapeutic strategies. Individual registries or transplant centres are responsible for establishing the interval between donations, for the same or for a different recipient, on the basis of the risks to the donor and the patient. However, these limits do not strictly apply to related donors.

25.3.2.2.2 Autologous donors

Cell mobilisation before autologous collection is ensured by administration of various types of mobilisation regimens; rhG-CSF used to mobilise progenitors can be administered alone or in combination with chemotherapy or other agents (e.g. HPC binding inhibitors such as plerixafor, immunostimulants).

Recommendations

R25.14 It is recommended that collection centres have protocols that can determine the optimal number of cells to be collected, considering the patients' well-being during and after collection, as well as their needs as future recipients.

Supplementary information

Circulating levels of $CD34^+$ cells guide the commencement of apheresis. The number of cells required varies with the size of the patient and number of transplantations indicated (double grafting is indicated for some diagnoses).

25.3.2.2.3 Apheresis collection yield

Processed blood volumes vary, depending on the collection protocol and cell separator.

Recommendations

It is recommended that:

R25.15 the target number of $CD34^+$ cells be set before starting apheresis, according to institutional protocols;

R25.16 weight discrepancies between donor and recipient be considered, because this can affect the $CD34^+$ cell yield per kg recipient weight.

Supplementary information

The target will vary for autologous and allogeneic donations and will depend on clinical need and regulations (as well as best available professional practices). Target doses of $CD34^+$ cells for a single autologous transplantation range from a minimum of 2×10^6 $CD34^+$ cells/kg recipient weight to a more preferable 5×10^6 $CD34^+$ cells/kg recipient weight. The ability to achieve this goal is dependent on the underlying disease of the patient, therapy and mobilisation protocol.

The target for allogeneic donations is higher, because of the longer time to engraftment of neutrophils and platelets associated with allogeneic transplantation; HPC(A) doses above 4×10^6 $CD34^+$ cells/kg might be needed, especially when $CD34^+$ enrichment (a loss of $CD34^+$ cells is expected) or a T-cell depletion method is used. In addition to optimising HPC collection, apheresis technique ensures that collected cells have minimal contamination with other cells that could compromise subsequent processing steps or contribute to side-effects in recipients. HPC from apheresis contain small volumes of red blood cells (haematocrit < 5-10%) so the risk of donation-related anaemia is very low. Depending on the device used for collection of HPC(A), the donor may experience a marked loss of platelets, in some cases resulting in post-donation values < 100 G/L (10^9/L). Under such circumstances a consecutive donation is carefully considered. Monitoring the collection efficiency of the apheresis device can help to identify technical problems (e.g. inaccurate camera setting) if the expected apheresis yield according to the $CD34^+$ pre-cell count in the peripheral blood is not reached during collection.

Some Health Authorities do not permit the use of G-CSF in paediatric donors and so bone marrow collection might be employed.

25.3.2.3 *Mononuclear cells from peripheral blood*

To enhance immune responsiveness after HPC transplantation, specific mononuclear cells are being used and/or

evaluated in clinical trials. However, these immune effector cells are also used in patients who had not undergone an allogeneic or autologous HPC transplantation previously. As starting material for further processing, e.g. chimeric antigen-receptor T-cells (CAR-T cells), MNC concentrates are collected from an unstimulated donor.

An MNC donor can be a former HPC donor (i.e. donor lymphocytes infusion [DLI]), a third-party donor or the patient (autologous donor). In addition, MNCs could be collected for off-line extracorporeal photopheresis (ECP) for the treatment of T-cell-mediated diseases.

DLI can be administered to the selected patient after allogeneic HPC transplantation, either prophylactically to augment the anti-tumour immune response (following reduced-intensity conditioning protocols) or in cases of mixed chimerism or of relapse of an underlying disease (mostly myeloid malignancies). The goal of this therapy is either to induce complete donor chimerism or a remission of the patient's malignancy by a process called GvT effect.

The following cells are being used and/or evaluated in clinical trials:

i. DLI to enhance immune surveillance against infections in patients with poor immune recovery experiencing relapsing/resistant viral infections;
ii. T regulatory cells (Treg) for the prevention and control of GvHD;
iii. NK cells as GvT effectors by alloreactivity of killer Ig-like receptors (KIRs) in donor–recipient direction;
iv. viral- and fungal-specific T-cells for the treatment of several infections (e.g. *Cytomegalovirus*, Epstein–Barr virus, *Adenovirus*, *Aspergillus*);
v. vaccination with peptide-loaded dendritic cells (DC) for induction of tumour-specific T-cell responses for treatment of metastatic disease, or for treating GvHD;
vi. mesenchymal stem cells to enhance engraftment in allogeneic and autologous HPC transplantations, or in treatment of GvHD;
vii. autologous or allogeneic CAR-T cells;
viii. MNC for off-line ECP.

25.3.3 Temporary storage and transport to the SoHO establishment

Progressive loss of HPC viability occurs during non-frozen storage. Nevertheless, HPC(A) and HPC(M) are stored in non-frozen conditions before processing and infusion or during transportation. Survival of HPC stored in a non-frozen state is dependent on the concentration of leukocytes (TNC), buffering capacity of the HPC and anti coagulant in the graft, product volume and storage temperature.

Good Practice Guidelines

G25.18 The storage time in the non-frozen state before infusion or cryopreservation should be kept as short as possible and should not exceed 72 h.

G25.19 Fresh HPC and MNC(A) should be stored and transported for not longer than 72 hours at room temperature (15-25°C) or refrigerated (2-8°C) as requested by the organisation responsible for human application (ORHA).

Recommendations

R25.17 It is recommended to cryopreserve HPC as soon as possible to avoid cell loss and reduced viability during processing.

R25.18 It is also recommended to cryopreserve MNC(A) collected from DLI as soon as possible, for the same reasons.

Supplementary information

Cell viability decreases and the risk of microbial growth increases during storage at room temperature, as well as in refrigerators (see Chapter 12).

25.3.4 Processing of haematopoietic progenitor cells

Processing of minimally manipulated HPC is intended to provide appropriate conditions for preservation and storage or to improve the risk–benefit ratio of autologous or allogeneic HPC transplantation [2, 18]. It does not affect the main biological properties of the collected cells, which is to support the marrow re-populating ability (MRA) and the establishment of haematopoietic chimerism in myelo-ablated or immunosuppressed recipients in allogeneic transplant.

Generic requirements for processing facilities, including quality management systems, are described in Chapters 2 and 9.

Supplementary information

The current FACT–JACIE international standards for haematopoietic cellular therapy product collection, processing and administration also apply to processing facilities [18].

25.3.4.1 *Volume reduction*

Volume reduction is either a preparatory step to further processing (including cryopreservation and storage) or a means to reduce the volume of the infused cells and thus prevent recipient side-effects related to volume overload in the transplanted patient. Various centrifugation-based techniques can be used that are validated at the site.

Good Practice Guidelines

G25.20 Cell loss associated with volume reduction should be evaluated and the expected recoveries should be defined.

25.3.4.2 *Red blood cell depletion*

Red blood cell depletion is almost exclusively performed if bone marrow is used as the HPC graft source, whereas HPC(A) are usually not RBC-depleted due to the low content of RBCs. Various techniques for blood cell depletion are available, including buffy-coat centrifugation or apheresis cell separation.

Good Practice Guidelines

G25.21 The efficiency of the collection technique should be assessed by measuring the residual RBC content, which should be kept to a minimum.
G25.22 The cell loss associated with such procedures should be evaluated and the expected recoveries and amount of acceptable residual RBC should be defined.

Supplementary information

HLA identity does not preclude the existence of major or minor ABO incompatibility between HPC donor and recipient. In cases of major ABO incompatibility (i.e. isohaemagglutinins of the recipient blood group directed against RBC of the donor blood group) acute haemolysis can occur during infusion of the stem cell graft.

25.3.5 Plasma removal

Plasma removal represents a critical step in cases with minor ABO incompatibility between a donor and a recipient in the allogeneic setting. The necessity of plasma removal in cases of minor ABO incompatibility can be judged using a titration of anti-A and anti-B antibodies in the donor blood during the period that precedes the donation. Plasma removal is usually done by centrifugation of the collected cells.

Good Practice Guidelines

G25.23 The cell loss associated with plasma removal procedures should be evaluated and the expected recoveries should be defined.

Recommendations

R25.19 In minor ABO incompatibility, where anti-recipient-RBC antibodies are 1:256 or greater, it is advised to remove plasma, especially from bone marrow grafts [2].

25.3.6 Cryopreservation, thawing and infusion

25.3.6.1 *Cryopreservation of haematopoietic progenitor cells*

The purpose of cryopreservation is to preserve HPC in such a way as to ensure their viability and potency. To minimise the volume infused, peripheral blood and bone marrow may be concentrated before cryopreservation.

Good Practice Guidelines

G25.24 The volume of cell suspension per bag should be adjusted by the body weight of the patient.
G25.25 Special attention should be paid if the recipient is a small child or if a patient has impaired renal or liver function.
G25.26 The cryoprotectant should be cooled before being added to the cells (e.g. HPC).
G25.27 The process of adding the cryoprotective mixture to the cells should be slow and carried out at a reduced temperature (i.e. under cooling) because an increase in temperature is harmful to cells (exothermic reaction).
G25.28 Methods to prevent, or at least minimise, the risk of contamination or cross-contamination during cell preparation and storage using appropriate measures (e.g. secondary bag, vapour-phase liquid nitrogen; see Chapter 9) should be employed.
G25.29 A stability programme for cryopreserved grafts should be implemented in order to evaluate viability and potency at different storage durations.

Recommendations

R25.20 For the administration of cryopreserved progenitor cell preparations, it is recommended that:
- R25.20.1 the dimethyl sulfoxide (DMSO) concentration be as low as possible [2, 18] and that a maximal daily dose of DMSO of 1 g/kg body weight, which is equivalent to an infusion of 10 mL/kg of cells in 10% DMSO solution, is not exceeded;
- R25.20.2 HPC be cryopreserved as soon as possible because shelf life of HPC without cryopreservation is acceptable only up to 72 h. However, cell viability decreases if cells are frozen at the end of their shelf life;
- R25.20.3 before starting cryopreservation, the concentration of nucleated cells allow a final concentration of $< 2 \times 10^8$/mL after cryopreservation.

Supplementary information

Cryopreservation is used systematically in the autologous setting. In the allogeneic setting, cell collection from the donor is usually synchronised with administration of a conditioning regimen to the recipient and direct infusion of the HPC product (within ≤ 48-72 h after collection) without freezing. However, sometimes allogeneic HPC are cryopreserved for logistical reasons, such as unavailability of the donor at the scheduled date of transplantation (collection in advance), professional constraints, unforeseen changes in transplantation schedules or over-collection of stem cells. In a case of cryopreservation of HPC from an unrelated donor,

registries can request an additional consent from the respective donor as this approach can lead to the risk that collected cells are not used.

The cryopreservation method for HPC(A) or HPC(M), once RBC and plasma are depleted from the latter, is the same. Cryopreservation involves addition of a cryoprotectant (e.g. DMSO) to a suspension of HPC and protein-rich medium, with or without dextran or hydroxyethyl starch (HES). The final product includes 5–10% of DMSO as a cryoprotectant and 0.05–0.25 mL of acid citrate dextrose, solution A (ACD-A) stabiliser solution per mL of transplant [2]. Immediately after DMSO addition, HPC are cooled at –1°C to –2°C per minute. For most therapeutic cells, the cooling rate is controlled by a controlled-rate freezer in which vapour-phase liquid nitrogen is pumped into the freezing chamber facilitating a sudden temporary drop of the temperature in order to compensate for the thermal release caused by the solidification of the suspension (see also Chapter 17 [2]).

Although this is the commonly used method for cryopreservation of therapeutic cells, other methods may be used (e.g. freezing in a mechanical freezer) as long as they result in acceptable post-thaw viability and potency.

The final phase of cooling in a controlled-rate freezer is usually quicker, with the temperature drop adjusted to 5°C/min. When the mixture has reached approximately –100°C to –120°C, cryopreserved cells are transferred to a storage container.

Variations in cryopreservation methods include the concentration of frozen cells, the amount and source of plasma protein and the cooling rate.

Maximal shelf life has not been defined for cryopreserved HPC. HPC(M) or HPC(A) have been transplanted successfully even over 11 years after cryopreservation.

25.3.6.2 *Cryopreservation of mononuclear cells*

Processing of MNC(A) mainly involves adjustment of volume and cell number according to the clinical protocol used. The number of MNC and specifically the number of $CD3^+$ T-cells is determined by flow cytometry analysis, and further characterisation of T-cell subpopulations may be undertaken according to special requirements and needs.

Good Practice Guidelines

G25.30 The cryopreservation method chosen should be validated in order to establish the expected level of viable T-cells after thawing.

Supplementary information

For DLI, frequently a T-cell dose of 1×10^6 $CD3^+$/kg body weight of the recipient is the starting dose, and then further treatments with escalating doses may be used. This amount can be even lower in haplo-identical transplantation. Some protocols also include cell-selected preparation.

25.3.6.3 *Thawing and infusion*

Good Practice Guidelines

G25.31 HPC(M) and HPC(A) and MNC (A) can be thawed at the bedside or in a processing facility, but time to infusion should be minimised to avoid decrease of cell viability.

G25.32 HPC should be infused immediately after thawing and as fast as possible, at approximately 5-20 mL/min using standard transfusion sets or equivalent, although this interval may be longer if the HPC are washed. Bags should be thawed one after another to avoid cell loss in terms of adverse events in the recipient.

G25.33 If cells are not infused immediately after thawing or have to be transported to the clinical facility, the maximum possible time between thawing and infusion should be validated.

G25.34 Leukoreduction filters capture HPCs and should never be used.

Recommendations

R25.21 If washing of HPCs is performed it is recommended not to expose all cells to the risk of washing procedures at once, unless there is a validation that demonstrates the maintenance of morphological and functional characteristics of the cells.

Supplementary information

Washing of HPC(A) and HPC(M) may be performed for patients at a high risk of adverse reactions because of the associated risks with cell washing, such as cell clumping, cell loss, osmotic injury or contamination. Washing is normally performed in processing facilities either by manual centrifugation or by automated washing in closed systems using specific equipment.

Thawing can be performed using temperature monitored dry-warming devices or temperature-controlled water baths (G25.31).

25.3.6.4 *Cell-selected preparations*

Specific Conformité Européenne (CE)-marked devices are available to select $CD34^+$ cells or deplete other lymphocyte subpopulations from bone marrow or peripheral blood on the large scales needed for clinical transplantation.

Good Practice Guidelines

G25.35 The method chosen should be validated and should ensure sufficient purity and viability for a safe transplant and sustained engraftment.

25.3.6.5 *T-cell depletion and depletion of alloreactive immune effector cells*

T-cell depletion is associated with positive (i.e. GvHD prevention) and negative (i.e. prolonged immunosuppression) consequences that prevent its adoption in routine clinical practice, and it is rarely used in HLA-identical or HLA-matched transplantation. This is because the advantages of reducing GvHD are offset by associated increases in relapse rates and graft failures. Indications for T-cell depletion depend on the clinical protocol, such as transplantation for non-malignant diseases and use of haplotype-mismatch donors without a T-cell deplete approach (e.g. post-transplant cyclophosphamide; pTCy). In these situations, it is important that T-cell depletion is as extensive as possible.

Good Practice Guidelines

G25.36 Accurate determination of the residual T- and B-cell content should be performed.
G25.37 The highest acceptable dose of residual T- and B-cells or alloreactive T-cells should be defined in advance by the medical team in charge of the recipient.
G25.38 The collection team should seek advice from the medical team if this goal cannot be achieved.

Supplementary information

$CD34^+$-positive immunoselection can also be considered a T-cell depletion method because, as in standard $CD3^+$ depletion, almost all T-cells are eliminated, including the T-cell receptor (TCR) gamma/delta-positive T-cells not involved in GvHD and exerting anti-leukaemic activity, as demonstrated by several authors. A specific depletion of TCR alpha/beta-positive cells spares the gamma/delta T-cells and is more beneficial over the depletion of all T-cell subsets. Combining this with a $CD19^+$ B-cell depletion for preventing transmission of Epstein–Barr virus (EBV) is a very encouraging strategy, especially in haploidentical transplantation settings [23].

Other specific procedures evaluated by clinical trials include depletion of activated and alloreactive T-cells (i.e. those that can be identified by the expression of the CD25 T-cell receptor subunit). Removal of $CD25^+$ T-cells can be done using immuno-selection devices similar to those routinely used for $CD34^+$-positive cell selection or T-cell depletion.

25.3.6.6 *Tumour cell depletion in the autologous setting*

Autologous tumour cells collected with normal HPC may contribute to post-transplant relapse, but this has not been firmly established on the basis of clinical and biological observations. A definitive advantage for tumour-purging of autologous grafts has not been demonstrated by clinical trials. The use of $CD34^+$ cell-selection devices for this purpose is only applicable in a few clinical protocols (e.g. neuroblastoma).

Generic requirements for processing facilities, including the quality management system, are described in Chapters 2 and 9.

Good Practice Guidelines

G25.39 If a transplant team decides to use tumour cell depletion procedures, then detection of residual tumour cells should be as accurate as possible, using either immuno-histochemical techniques or flow cytometry analysis, or molecular biology techniques.

25.4 Quality control

Good Practice Guidelines

G25.40 Quality-control tests on HPC grafts from bone marrow and peripheral blood apheresis should take at least the following quality criteria into account:
- **G25.40.1** nucleated cell count,
- **G25.40.2** viable $CD34^+$ cell enumeration,
- **G25.40.3** microbiological testing,
- **G25.40.4** ABO Rh blood group for allogeneic products,
- **G25.40.5** measurement of residual ABO-incompatible red cell volume for allogeneic products.

G25.41 Quality-control tests on mononuclear cells from unstimulated peripheral blood apheresis should take at least the following quality criteria into account:
- **G25.41.1** nucleated and mononuclear cell count (when required, e.g. DLI),
- **G25.41.2** viability (when required, e.g. DLI),
- **G25.41.3** viable $CD3^+$ cell enumeration (when required e.g. DLI),
- **G25.41.4** microbiological testing,
- **G25.41.5** ABO Rh blood group for allogeneic products.

Supplementary information

Quality of the graft can also be measured by the detection of colony-forming units (CFU) in clonogenic assays. These functional tests are hampered by the delay required to produce results (usually two weeks). Thus, the results are usually only available long after a non-cryopreserved cell preparation has been transplanted into an allogeneic recipient. Clonogenic assays are also hampered by poor intra- and inter-laboratory reproducibility. This particular issue could be improved by using commercially available and standardised culture media and by participation in proficiency testing and external quality-assessment schemes.

25.4.1 Clinical information needed to confirm donor suitability and recruitment

Details of clinical and biological information pertaining to donor identification, screening and recruitment along with all information pertaining to processing and distribution are described in Chapters 5 and 6 of this Guide. This information needs to remain as a permanent part of the preparation and release file.

25.4.2 Safety controls

Detection of transmissible infections is undertaken through donor screening (using microbiological and other testing, as required by national, European and international guidance and regulations) and through microbiological testing of samples obtained at the different stages of cell collection, processing and distribution (see also Chapters 5, 7, 8, 10 and 12).

Detection of donor-transmissible diseases other than occult pre-neoplastic or neoplastic diseases or other disorders is through donor screening, using medical questionnaires, physical examination and biological testing, as necessary (see Chapter 6).

Good Practice Guidelines

G25.42 Removal of RBC, immune effector cells (allogeneic/autologous products) or tumour cells (autologous products only) through specific processing procedures should be validated and documented where applicable.

Recommendations

R25.22 It is recommended that the proportions of the various subpopulations of leukocytes in the collected cell preparations be measured.

Supplementary information

High numbers of mature blood cells, such as granulocytes, or contamination with RBCs may negatively affect several subsequent processing steps and may contribute to recipient side-effects at re-infusion.

25.4.3 Immunophenotyping and colony-forming unit assay

The number of TNC in combination with the number of viable $CD34^+$ cells is a widely used measure for evaluating the quality of procured bone marrow. The cell dose for recipients is usually expressed in TNC and $CD34^+$/kg of recipient weight. In addition, nucleated cell counts are largely used as in-process controls to document that technical procedures have been appropriately conducted in the processing facilities (i.e. recovery of TNC following plasma removal, volume reduction, RBC depletion).

$CD34^+$ cell counts are used as a marker for HPC, both in the peripheral blood of individuals undergoing mobilisation regimes and in the collected cells, whether from apheresis following mobilisation or from bone marrow donation. $CD34^+$ cell counts are usually measured by flow cytometry, using monoclonal antibodies that recognise one or several epitopes on the human CD34 membrane antigen. Use of a single platform, rather than a dual platform, minimises errors in calculating cell counts. The ISHAGE (International Society for Hemotherapy and Graft Engineering) algorithm provides a robust and reproducible gating strategy to measure $CD34^+$ cells [24, 25].

Combined evaluation of both $CD34^+$ cell recovery and total viability after storage and cryopreservation is an acceptable way to measure the potency of an HPC graft when the detection of CFU in clonogenic assays is not feasible. These functional tests are hampered by the delay required to produce results (usually two weeks); thus, the results are usually only available long after a non-cryopreserved cell preparation has been transplanted in an allogeneic recipient. Clonogenic assays are also hampered by poor intra- and inter-laboratory reproducibility. This particular issue could be improved by using commercially available and standardised culture media and by participation in proficiency testing and external quality-assessment schemes.

Colonies are enumerated and classified on the basis of their morphologic characteristics: CFU-GM (granulocytes and macrophages), CFU-GEMM (granulocyte-, erythrocyte-, macrophage-, megakaryocyte) and BFU-E (burst-forming units – erythroblast). The frequency of $CD34^+$ cells that form colonies differs among the sources of HPC and is higher in HPC(A) than in HPC(M).

Recommendations

It is advised that:

R25.23 a policy be defined to deal with grafts where $CD34^+$ cells are at a low frequency when clonogenic assays are used either as qualitative (e.g. growth or no growth) or as quantitative potency tests.

25.4.4 Release criteria

Generic requirements for product release, together with standards, are described in Chapter 13.

Good Practice Guidelines

G25.43 The cells should be administered only with a valid prescription for infusion. This prescription should list the type of cell preparation that is suitable for that patient and provide specific information on dosing. If necessary, the cells may be further processed before infusion (e.g. washing, dilution) and this should be recorded.

Recommendations

It is recommended that:

R25.24 specific instructions be established in SoHO establishments on how to deal with the recipient, donor and stem cells throughout the donation, through the processing and issue stages and all the way through to transplantation;

R25.25 the processing and transplant facilities agree on the cell dose (nucleated cell count, mononuclear cell count, $CD34^+$ cell count or clonogenic assays as appropriate for the source of HPC) required to achieve reliable and sustainable engraftment.

Supplementary information

Acceptance and release criteria may differ between autologous and allogeneic grafts. In autologous grafts, infectious disease-marker (IDM) test results may be positive (this is normally not the case in allogeneic grafts). In allogeneic grafts, which are mainly used directly after collection, microbiological test results are pending at the time of administration and cannot serve as release criteria (in contrast to autologous grafts).

25.4.5 Quality control for mononuclear cells

Good Practice Guidelines

G25.44 In addition to the safety controls (listed in §25.4.2) and definition of release criteria (§25.4.4), the specific requirements for allogeneic products should include the determination of the absolute number and frequency of T-cells ($CD3^+$ and/or subpopulations) and cell viability by flow cytometry analysis.

Recommendations

R25.26 It is recommended that the anti-tumour effect be evaluated at intervals as defined in the clinical protocol, where applicable.

25.5 Labelling and packaging

Generic requirements for product coding, packaging and labelling are described in Chapter 15.

Good Practice Guidelines

G25.45 Labelling should unambiguously identify the additives used and the conditions under which the cells are to be stored and distributed.

Recommendations

R25.27 It is strongly recommended that the recipient be identified when cells are distributed for administration.

Supplementary information

The names of unrelated donors are identified through the Global Registration Identifier for Donors (GRID), which is a unique global donor identifier to guarantee safe and unequivocal donor identification developed by the WMDA [26]. In all cases donors and recipients are traceable (see Chapter 15).

25.6 Storage

Generic requirements for product storage, together with standards, are described in Chapter 11. Guidance on release can be found in Chapter 13.

Good Practice Guidelines

G25.46 There should be a stability protocol that evaluates the viability and potency of long-term stored cryopreserved cellular therapy preparations.

G25.47 The time to cryopreservation should be minimised but should not exceed 72 hours, during which time the HCP and MNC(A) have preferably been stored refrigerated.

G25.48 Cryopreserved HPC and MNC(A) should be stored and transported at temperatures equal to or below –140°C.

G25.49 Thawed HPC and MNC(A) should be stored and transported refrigerated (2-8°C) and applied without undue delay.

25.7 Distribution and transport conditions

Generic requirements for product transport and documentation, together with standards, are described in Chapter 13.

For cross-border transport of HPC from unrelated donors, international standards and guidelines are available [27].

25.8 Contingency plan for HPC collections

Information on continuity planning can be found in Chapter 2, section 2.14.

Natural hazards such as earthquakes or hurricanes, pandemics or other related moments of global crisis can be challenging for the collection of HPC, their transportation and timely infusion.

During the Covid-19 pandemic and the lockdown of countries, significant problems for HPC transplant programmes emerged. Transplantation societies and donor registries recommended cryopreservation of unrelated donor HPC products before the recipient commenced conditioning therapy, to mitigate the donor and travel risks associated with the pandemic. Therefore, HPC

programmes started to cryopreserve HPCs as soon as they arrived at the SoHO establishment and started with the conditioning regimen thereafter. Registries request an additional consent from the respective donor for cryopreservation because this approach can lead to the risk that collected products will not be used, either due to the possibility of a clinical deterioration of the patient (who is then no longer eligible for transplantation) or due to poor post-thaw graft quality.

Data on post-thaw quality of allogeneic products with respect to pre-cryopreservation conditions are lacking. A recent Australian study reported a median post-thaw $CD34^+$ cell recovery of 76% (range, 6% to 122%) [28]. In this study, a longer transit time before cryopreservation, higher leukocyte count during storage and complex product manipulation before cryopreservation were independently associated with inferior post-thaw $CD34^+$ cell recovery and therefore poor product quality. This may put the recipient at risk of delayed or incomplete engraftment [28].

25.9 Biovigilance

General information on biovigilance is outlined in Chapter 18 and applies to HPC transplantation.

Adverse occurrences in transplantation of HPCs and immune effector cells can be reported to the Notify Library at www.notifylibrary.org, a project launched by the World Health Organization (WHO) in collaboration with the Italian National Transplant Centre (CNT) [29]. In addition, WMDA has set up a central global reporting system for its member organisations to report serious events and adverse reactions related to HPC grafts: Serious (Product) Event and Adverse Reactions or S(P)EAR [30]. The scope of this system is to collect information on serious events and adverse reactions that occurred during collection or processing of HPC from unrelated donors and in the follow-up period.

25.9.1 Serious complications in the recipient

25.9.1.1 *Complications related to haematopoietic stem cell infusion*

Infusion of HPC is, in general, well tolerated. Complications are consequences of immunological incompatibility, iatrogenic toxicities, microbiological contamination and manufacturing/administrative errors. Some complications are similar to the complications caused by transfusion of standard blood products, which is particularly true for allogeneic HPC preparations if they contain ABO-incompatible RBCs or plasma. Other complications are specific to HPC infusion and are related to allogeneic and autologous HPC.

Adverse reactions can be immunological or non-immunological, acute or delayed.

25.9.1.2 *Haemolysis of red blood cells*

Donor–recipient mismatching in erythrocyte antigens is not a contraindication for HPC transplantation, but haemolytic reactions due to ABO and non-ABO antibodies may occur. Reactions can be acute or delayed. The risk of haemolysis is also dependent upon the type of HPC preparation because the content of RBCs and plasma is different.

Acute haemolytic reactions are severe complications of HPC infusion. They are caused by ABO incompatibility between the donor and recipient. Usually, the risk of acute haemolysis is greater if the RBCs of the donor are incompatible with the recipient's plasma (major ABO incompatibility). However, haemolysis can also occur if the donor's plasma is incompatible with the recipient's RBCs (minor ABO incompatibility). In general, low titres (e.g. < 1:64) are associated with mild or no reactions, whereas high titres (e.g. 1:1024) are associated with acute haemolytic reactions. The volume of incompatible RBCs infused also determines reaction severity. Bone marrow contains a high amount of RBCs and can cause acute haemolysis. The risk of haemolysis can be reduced by removal of antibodies from the patient's circulation utilising extracorporeal apheresis procedures or by removal of RBCs from the bone-marrow preparations of the donor. In contrast, apheresis preparations usually have < 10–15 mL RBCs, which is not enough for significant reactions.

Signs and symptoms of acute haemolytic reactions can be: chills, fever, dyspnoea, chest pain, back pain, headache, hypotension, oliguria, anuria, bleeding and shock. If an acute haemolytic reaction is suspected, cardiovascular and renal functions have to be maintained and development of disseminated intravascular coagulation has to be prevented.

Delayed haemolytic reactions may occur if the recipient is allo-immunised on the infused RBC antigens of the donor, or if the recipient receives the donor's B lymphocytes within an HPC preparation, which can produce antibodies against the recipient's RBCs ('passenger lymphocytes syndrome'). If the recipient is allo-immunised on the donor's RBC antigens, infusion of RBCs can stimulate an anamnestic immune response of the residual B lymphocytes of the recipient. The antibodies will reach a clinically significant level within 2-14 days after HPC infusion, whereas the infused RBCs will remain in circulation. The haemolysis is limited by the amount of infused RBCs.

If the recipient receives the donor's B lymphocytes in an HPC preparation that can produce antibodies against the recipient's ABO or other RBC antigens, haemolysis can be more serious because of passenger lymphocyte syndrome. At greater risk are recipients who receive ABO minor-incompatible HPC. Typically, haemolysis will occur within 1-3 weeks after HPC infusion. Occasionally, life-threatening haemolysis can occur. Apheresis HPC preparations contain higher numbers of lymphocytes and carry a greater risk of delayed haemolysis.

Signs and symptoms of delayed haemolytic reactions are the same as for acute haemolytic reactions, but the severity differs.

In autologous settings and if cryopreserved HPC are used, passive haemolysis is more common.

25.9.1.2.1 Complications within the respiratory tract

HPC infusion frequently induces complications within the respiratory tract. During administration, patients often start coughing. Coughing is related primarily to application of cryopreserved autologous HPC and is usually accompanied by nausea and vomiting. Dyspnoea, with mild decreases in the vital capacity of the lungs, is noted quite often.

Severe respiratory complications, such as acute respiratory failure, are rare. Transfusion-related acute lung injury (TRALI) can occur if granulocyte activation in the pulmonary vasculature is caused by neutrophil antibodies or bioactive mediators, which increase microcirculation permeability and allow massive leakage of fluids and proteins into the alveolar space and interstitium. Signs and symptoms of TRALI usually occur < 6 h after HPC infusion, and include acute respiratory distress, low-grade fever, hypoxaemia (oxygen saturation < 90% on room air) and bilateral pulmonary infiltrates on frontal radiographs of the chest. If hypoxia is observed during HPC infusion, the infusion is stopped immediately, and respiratory support is initiated as intensive as dictated by the clinical picture. Supplementation is useful in almost all cases. Corticosteroids and diuretic drugs are not useful. In severe cases, transfer to an intensive care unit (ICU) may be necessary.

25.9.1.2.2 Febrile non-haemolytic complications

During HPC infusion, patients may experience febrile non-haemolytic transfusion reactions (FNHTR). These reactions may be observed in allogeneic and autologous transplantation. FNHTR are manifested by a low-grade fever during, shortly after or ≤ 2 h after infusion of cells. FNHTR can be accompanied by chills, rigor and mild dyspnoea without evidence of haemolysis. This phenomenon may reflect the action of antibodies against leukocytes or the action of cytokines (present in infused preparations or generated by the recipient) after cell infusion.

No laboratory tests are helpful in predicting and preventing FNHTR. If a patient develops fever, chills and myalgia during HPC infusion, a clinical assessment and laboratory work-up is to be performed to exclude haemolytic, septic or TRALI reactions. FNHTR are short-lived complications, and anti-pyretic agents usually provide effective symptomatic relief.

25.9.1.2.3 Dimethyl sulfoxide toxicity

DMSO is the most widely used cryoprotectant, but it can detrimentally affect cell viability and is the cause of many side-effects observed during infusion. DMSO toxicity is the most common complication of infusion of cryopreserved HPC.

Within minutes of starting the infusion, a metabolite of DMSO is excreted through the lungs and causes a garlic-like odour that can lead to a foul taste in the mouth. Infusion of DMSO can induce a wide range of other symptoms: pruritus; sedation; headache; nausea; vomiting; abdominal cramps; diarrhoea; flushing; low-grade fever; chills; dizziness; garlic-like odour; haemoglobinaemia with red-coloured urine; elevation of levels of hepatic enzymes; and elevation of levels of creatinine kinase. DMSO toxicity has been linked to cardiovascular side-effects such as bradycardia or tachycardia, hypotension and, in rare cases, myocardial infarction.

DMSO toxicity is dose-dependent. The maximum daily intravenous dose of DMSO is 1 g/kg, which is equivalent to an infusion of 10 mL/kg of cells in 10% DMSO solution. Premedication with anti histamines, slowing the infusion rate, increasing the resting time between multiple infusion aliquots, dilution of thawed HPC preparations by albumin–dextran-40 solution at a ratio of 1:2 or 1:3, or removal of DMSO by washing can prevent symptoms and reduce the risk of DMSO-related toxic effects.

25.9.1.2.4 Neurological complications

Neurological symptoms during HPC infusion vary widely. Headache is common and can be related to increased intravascular volume. Occasionally, patients experience more severe side-effects such as muscle spasms and seizures. Cerebral infarcts and acute encephalopathy are rare. Simple muscle spasms often resolve spontaneously. For patients with acute mental changes, loss of consciousness or seizures, urgent intervention is necessary. HPC infusion is interrupted and rapid neurological assessment is done as well as basic laboratory tests, including electrolytes and glucose. If seizures persist, anti-epileptic drugs are indicated and ICU transfer could be considered.

Neurological complications are probably linked to a large number of non-mononuclear cells and/or caused by DMSO, but this suspicion has not been clearly demonstrated.

25.9.1.2.5 Cardiac toxicity

Cardiac toxicity is common and manifests as bradycardia and other disorders of cardiac rhythm. It is usually mild (though severe cases of arrhythmias have been reported). Severe bradycardia occurs more often in recipients of cryopreserved bone marrow and may require aggressive supportive care.

Aetiology may be because of hypervolaemia due to extensive hydration before infusion, large volume of transplant, hyperosmolality of DMSO, hypothermia, lysis of graft cells or underlying cardiac conditions.

25.9.1.2.6 Allergic complications

Allergic reactions usually manifest as urticaria and pruritus. Most occur in patients receiving allogeneic transplants. Anaphylactic-type reactions are rare. Allergic

reactions present as bronchospasm and/or laryngospasm, hypotension, severe dyspnoea, pulmonary and/or laryngeal oedema, facial burning and flushing, abdominal pain, diaphoresis, diarrhoea and dizziness.

Causes of allergic reactions are not clear. They may be related to the substances used during cell collection, cell processing or cryopreservation, such as hydroxyethyl starch (HES), dextran or DMSO, that can react with antibodies in the donor or recipient plasma, or with anti-immunoglobulin (Ig)A antibodies in IgA-deficient recipients. No laboratory tests can help to predict or prevent allergic reactions. Therapy is dependent upon symptoms. For mild reactions, administration of anti histamines will be helpful or, in severe cases, corticosteroids, epinephrine and cardiorespiratory support.

25.9.1.2.7 Anticoagulation effects

Patients receiving non-cryopreserved and non-manipulated bone marrow have a greater risk of haemorrhage because of high concentrations of unfractionated heparin in bone-marrow grafts.

Risk of bleeding is increased in thrombocytopenic recipients.

25.9.1.2.8 Hypertension/hypotension

Hypertension is more common in cryopreserved HPC or unmanipulated bone marrow HPC, because such grafts have a higher volume. Hypertension is a result of acute volume overload due to rapid infusion, prophylactic hydration and the hyperosmolality of the infused preparation.

Hypotension is also more common in cryopreserved HPC. It is linked with vasodilatation due to histamine generation. Premedication by anti histamines decreases the incidence and severity of hypotension.

25.9.1.2.9 Acute renal failure

Acute renal failure is more common in application of a high volume of cryopreserved HPC. It is caused by a large amount of DMSO and cellular debris.

25.9.1.2.10 Microbial contamination

Microbial contamination of an HPC product is possible. Bone marrow, which is collected into an open system, has a higher rate of contamination than HPC collected from peripheral blood. Contamination may occur at several steps in the process. It can be due to occult asymptomatic bacteraemia in the donor. For autologous donation, because of the particular nature of the graft and the recipient's condition because of the treatment, it is vital that HPC are collected irrespective of the possible febrile status. In cases of sepsis, collection at a later time point may be considered. Although it is a rare occurrence, it has to be kept in mind as a potential cause of microbial contamination of grafts.

Contamination of HPC can also occur during collection, processing, storage, thawing or sampling, due to breach of sterility.

After transfusion of contaminated HPC, symptoms of a septic reaction usually develop rapidly. High fever, tachycardia and hypotension, nausea and vomiting, and a 'shock-like' clinical picture may arouse suspicion of bacterial septicaemia. If such symptoms occur during HPC administration, the infusion is stopped immediately and all infusion bags and equipment examined.

Known microbial contamination of an HPC unit is not an absolute contraindication for HPC infusion. Patients receiving culture-positive preparations require antibiotic therapy, which can be antibiogram-specific or cover a broad spectrum of bacteria, starting optimally two days before transplantation. When there are positive microbial culture results, criteria for implementation of administration of preparations can be found in Chapter 12, section 12.10.

A contingency plan is usually expected to be in place in case of urgent medical need.

25.9.1.2.11 Transmission of infectious and genetic diseases

All cases of suspected post-transplantation infection or genetic disease transmission related to HPC infusion are reported immediately to the collection site and/or donor registry, whose staff follow the requirements of the national vigilance system (see Chapter 18).

Donors of HPC preparations are tested for infectious disease markers to avoid transfusion of transmissible diseases according to national requirements. However, the potential risk of transmission of infectious agents by infected donors or cross-contamination during storage cannot be removed completely. A viral infection < 6 months after transplantation is usually due to transmission of hepatitis B or C virus or human immunodeficiency virus. For other viral infections the period will differ, depending on the incubation period.

Screening of HPC volunteer donors for genetic disease is mainly based on their medical history and on the results of laboratory tests. HPC recipients undergo extensive genetic testing to assess transplant efficacy and detect disease relapse, and these tests raise the possibility of incidentally identifying genetic or molecular findings that may be of donor origin. To address questions from donor registries and transplant centres on how to manage genetic variants of possible donor origin, the Medical Working Group of the WMDA convened an expert panel to review the evidence and propose a decision-making framework [31]. The risk of transmission of a genetic disease is higher with cord blood than with the other sources of HPC (bone marrow and peripheral blood stem cells (PBSC)), since some diseases might not be evident at birth or even some months later.

According to the Notify Library, very few cases of genetic disease transmission have been described after bone-marrow transplants (cyclic neutropenia, Gaucher's disease). Autoimmune disease transmission has also

been reported (thyroiditis, type 1 diabetes, myasthenia gravis, vitiligo, etc.) [29].

25.9.1.2.12 Engraftment failure

After HPC transplantation, recovery occurs in populations of myeloid, erythroid and immune cells. The earliest sign of haematopoietic recovery is an increase in numbers of granulocytes and platelets in peripheral blood within days and weeks after graft infusion. Engraftment of erythroid lines and immune reconstruction occurs within weeks or months. Engraftment is dependent on the dose and source of progenitor cells, method of cell preparation, function of bone-marrow stroma, intensity of the preparative regimen, donor–recipient relationship and ABO compatibility.

Measurement of granulocyte and platelet engraftments provides essential information about the success of clinical protocols as well as the quality of collection and processing of HPC.

Primary graft failure for HPC transplantation from bone marrow or peripheral blood is defined as a neutrophil count of $< 0.5 \times 10^9$/L by day +30 with associated pancytopenia. Donor chimerism testing is also done to confirm the suspicion of graft failure.

The current definition for haematological recovery includes neutrophil recovery, defined as the first of three consecutive days with an absolute neutrophil count $\geq 0.5 \times 10^9$/L, and platelet recovery which is defined as a platelet count of $\geq 20 \times 10^9$/L in the absence of platelet transfusion for 7 consecutive days.

In allogeneic transplantation, chimerism evaluation is essential to confirm that cells are donor-derived, particularly in reduced-intensity and non-myeloablative conditioning regimens. A common cutoff for complete donor chimerism is ≥ 95% of donor-derived cells (typically ranging from 90% to 97.5% in most centres).

The incidence of graft failure (GF) is < 3-5% in the autologous-HCT and matched allogeneic-HCT setting, but it increases up to 10% in the cases of haplo-identical or Cord Blood Transplantation (CBT). The prognosis of GF is poor, and most patients die due to infections or bleeding, with an overall survival rate at 3-5 years after the diagnosis of GF in the range of 20-30% [2].

The transplant unit generally reports graft failure to the SoHO establishments to enable thorough investigation of the quality and handling of grafts.

25.9.1.2.13 Graft-*versus*-host disease

GvHD is a serious and potentially lethal complication of allogeneic HPC transplantation. GvHD occurs if infused T-lymphocytes engraft in the recipient and react against the recipient's tissues. Any allogeneic HPC preparations can cause GvHD.

Classic acute GvHD occurs < 100 days after transplantation, whereas in chronic GvHD (classic chronic and overlap syndrome) no time limit exists.

As acute GvHD is a result of an alloimmune effect, the major risk for occurrence is the presence of HLA disparity and increasing degrees of HLA mismatching increase the probability of more severe disease. Other important and consistent risk factors include older patient age, use of female donors for male recipients, prior alloimmunisation of the donor, and the nature of GvHD prophylaxis. A number of publications have variously reported risk factors such as increasing donor age, increasing intensity of the preparative regimen, the use of PBSCs as opposed to bone marrow, and recipient seropositivity for *Cytomegalovirus*.

Main risk factors for chronic GvHD are unrelated and/or mismatched donor, HPC(A) as donor source, older donor age, older patient age (> 12 years) and female donor into male recipient combinations.

Clinical manifestations of GvHD typically involve the skin, liver and gastrointestinal tract in the acute setting, but can affect (among others) the eyes, oral mucosa, liver, gastrointestinal tract, genitals, lung, joints and fasciae in the chronic setting. To predict the outcome of acute GvHD, scoring based on organ involvement is important. Chronic GvHD is more likely in recipients of HPC(A) than in recipients of HPC(M).

Treatment of GvHD includes high-dose corticosteroids, T-cell-suppression drugs, monoclonal antibodies targeting T-cells, extracorporeal photopheresis or mesenchymal stem cells.

25.9.2 Serious adverse reactions and events related to the graft

Serious adverse reactions and events (SAREs) related to the graft – also referred to here as S(P)EAR (serious product events and adverse reactions) as defined by the WMDA – can be the result of: inappropriate transport, receipt of a wrong unit, receipt of a damaged unit package, incorrect/non-labelled unit, non-receipt of a transplant, inappropriate storage in hospital or infusion of a unit into the wrong recipient. The transplantation centre typically reports these incidences immediately to the SoHO establishment, to the registries if appropriate and, according to national legislation, to Health Authorities responsible for SoHO.

25.9.3 Serious adverse reactions in haematopoietic progenitor cell donors

Deaths in unrelated HPC donors are very rare, and few cases have been reported to WMDA. A small number of deaths have been reported also in related donors, from causes such as subarachnoid haemorrhage, sickle cell crisis, myocardial infarction and pulmonary embolism [21, 32, 33]. In some of these donors, pre-existing medical conditions were identified *post mortem*, highlighting the need for stringent medical suitability criteria and assessment of all HPC donors [14, 15, 34].

Bearing in mind that HPC donations are voluntary and altruistic acts of assumedly perfectly healthy individuals, it is the ethical and professional obligation of medical professionals and also good practice to notify,

document, investigate and report SARs in the living donor, and not only those influencing the quality and safety of SoHO. SARs are uncommon in healthy donors, and rare types of SAR or emerging trends are likely not to be noticed at the national level. SARs in stem-cell registry donors are followed at the international level by the WMDA through an online web application to report in a standardised way any incident occurring to a donor or donated product [35]. Unfortunately, no consistent follow-up exists at present for related donors. The EBMT has established a donor follow-up, which is included in the EBMT database [34].

25.9.3.1 *Complications in haematopoietic progenitor cell (bone marrow) donors*

Donation of bone marrow is, in general, a safe and well-tolerated procedure, but some mild symptoms related to induction of general anaesthesia are common. In most cases, donors recover fully within two weeks.

Reactions in bone-marrow donors include constitutional symptoms such as nausea, vomiting, anorexia, insomnia and fatigue (most common).

Complications related to puncture of bone marrow, such as pain upon collection, walking, sitting and climbing stairs, as well as minor infection, are rare. Bone and soft-tissue trauma at the harvest site may cause pain, bleeding, oedema or nerve compression. Damage to a lumbosacral nerve root or penetration into the pelvic cavity or internal iliac vessels may cause severe morbidity. Anaesthesia carries an unavoidable (albeit very small) risk of life-threatening cardiac or respiratory events, as well as the possibility of allergic or idiosyncratic reactions to anaesthetic agents. Removal of large volumes of blood may cause symptoms of hypovolaemia or anaemia [12, 21, 35, 36].

Cytopenias (anaemia, thrombocytopenia) and more serious reactions such as deep-vein thrombosis (DVT), thromboembolism, cerebrovascular accident and subdural bleeding have been documented. Post-donation septicaemia and anaesthesia-related complications have also been described, as well as respiratory complications such as pulmonary alveolitis and oedema [21, 32].

25.9.3.2 *Complications in haematopoietic progenitor cell (apheresis) donors*

Complications are related to apheresis and administration of rhG-CSF or other mobilising agents used. Symptoms related to citrate infusion are the most common. See Table 25.3.

Additional complications related to apheresis include haematoma, arterial punctures, delayed bleeding, pain from injury to nerves or tendons, thrombophlebitis, local allergy, generalised allergic reactions, vasovagal reactions, haemolysis and air embolism. All severe conditions requiring hospitalisation or intervention, or resulting in death < 24 h after collection, are reported immediately.

Use of rhG-CSF in mobilisation is, in general, safe. Common short-term reactions related to rhG-CSF are bone pain, headache, myalgia, nausea, vomiting, diarrhoea, fatigue, fever and irritation at injection site. Most of these effects are reversible after discontinuation of rhG-CSF administration. Other rare reactions are splenic rupture, anaphylaxis, thrombosis, gout, iritis, keratitis, autoimmune hyperthyroidism, acute lung injury, capillary leak syndrome, exacerbation of rheumatoid arthritis, insomnia and reduced numbers of thrombocytes.

Short-term reactions due to the use of HPC binding inhibitors such as plerixafor are reported to occur in approximately 30% of cases and consist of a local reaction at the injection site (erythema) and gastrointestinal side-effects (diarrhoea, abdominal distention).

Reports from long-term follow-up studies in unrelated and related apheresis HPC donors mobilised with rhG-CSF demonstrate a similar incidence of leukaemia and other malignancies to those seen in the general population. All malignant diseases in all donors treated with rhG-CSF are reported, regardless of the time of occurrence.

25.9.4 Biovigilance of mononuclear cells

The same requirements as for HPC(A) and HPC(M) apply also to MNC(A) (see above). The processing of MNC involves several steps where unexpected events may occur that have to be documented and reported (see §25.9 and Chapter 18), for instance, lower viability of frozen and thawed MNC than expected or human errors in calculating the dose of T-cells in DLI. In cases of low viability, DLI may still be used and documented, and a risk analysis is carried out. Possible adverse events associated with DLI are the development of acute and/or chronic GvHD and low blood counts. These complications may appear after one or more weeks after the administration of the $CD3^+$ cells.

As noted in section 25.9, similar complications, such as DMSO toxicity, transmission of infectious diseases or GvHD, may occur. In rare circumstances, S(P) EARs or complications in the donor are possible.

Table 25.3 **Very common adverse reactions associated with agents for haematopoietic progenitor cell mobilisation (> 10%)**

Agent	Adverse reaction
Rh-Granulocyte-colony stimulating factor (rhG-CSF)	bone pain musculoskeletal pain thrombocytopenia hyperleukocytosis transitory elevation of levels of liver enzymes elevation of levels of lactate dehydrogenase headache asthenia
Haematopoietic progenitor cell binding inhibitors (Plerixafor)	diarrhoea nausea reaction at injection site

Source: EBMT [17].

25.10 Follow-up

Information on donor and recipient protection is shown in Chapter 4.

25.10.1 HPC donor follow-up

Follow-up of the donor is described in section 25.9 and Chapter 18 of this Guide.

Recommendations

R25.28 It is recommended to perform a short-term follow-up of donors to document SAREs, and also regular long-term follow-up to document late effects of the donation or the mobilising agent [14, 30, 34].

Supplementary information

The EBMT and the WMDA recommend the long-term follow-up and care of donors for conditions related to the HPC donation for a minimum of 10 years after donation [34]. For unrelated donors this includes a mechanism for donors to contact the registry to report related medical concerns, whereas related donors contact the collection centres or the centres responsible for medical release for donation.

In addition there are other European initiatives, such as ARTHIQS [35], which presented guidelines on the set-up and regulation of haematopoietic stem-cell donor follow-up registries and banking of cord blood, and Recommendation CM/Rec(2020)6 on measures for the protection of haematopoietic progenitor cell donors [37] from the Council of Europe, which develops recommendations to member states to establish harmonised HPC donor protection measures. Among other measures, they recommend ensuring that donors (related and unrelated, adults and minors) have been offered appropriate psychological support in the event of post-donation difficulties and also medical care (see Chapter 4).

25.10.2 Patient follow-up

General information on patient follow-up is described in Chapter 14, section 14.13.

Systematic longer-term follow-up after autologous and allogeneic transplantation is typically performed lifelong in many transplant centres. Systematic follow-up includes registration of some basic clinical and quality criteria, such as quality of life of the patient.

Recommendations

It is recommended that:

R25.29 transplant centres have a policy addressing appropriate systematic follow-up of recipients after administration of autologous and allogeneic cellular therapy products;

R25.30 such patient follow-up include monitoring of the following:

- R25.30.1 blood counts and transfusion of blood products;
- R25.30.2 infections and use of antimicrobials;
- R25.30.3 organ dysfunction or failure;
- R25.30.4 graft failure;
- R25.30.5 regular assessment for evidence of acute and chronic GvHD, where applicable.

Supplementary information

Some countries have established national registries on autologous and allogeneic HPC transplantation and treatment with immune effector cells. The EBMT international registry – a database of patients who have undergone HPC transplantation – was established in 1974. It serves, among other things, as a resource helping to improve the care of patients.

Adverse occurrences in transplantation of HPCs and immune effector cells can be reported to the Notify Library and to the central global reporting system of the WMDA as mentioned before [29, 30].

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Related material

Appendix 32. Donor search through registries for haematopoietic progenitor cell transplantation

Chapter 26. **Umbilical cord blood progenitors**

26.1 Introduction

Umbilical cord blood (UCB) is a source of haematopoietic progenitor cells (HPC) that can reconstitute the haematopoietic and immune systems. These cells bear unique properties, including a high progenitor cell proliferation ability and immune naïvety. Thus, after normal deliveries, the blood remaining in the placenta after clamping can be safely collected, processed and stored for therapeutic uses.

For the purposes of this chapter, when using the term 'substances of human origin (SoHO) establishments' we are referring to 'UCB banks'.

Since the first transplant procedure, a related cord blood transplantation performed in Paris by Professor Gluckman and her team in 1988 in a child with Fanconi anaemia, more than 60 000 transplants have been reported. Subsequently, the first cord blood banks were established with voluntary donations, and the first unrelated cord blood transplant was performed. This contributed to the international development of cord blood banking. According to the World Marrow Donor Association (WMDA), over 7 600 000 units have been registered globally for any patient in need of a transplant in the WMDA Search & Match Service [1, 2].

Currently, there are two types of UCB transplantation: one type from unrelated donors, which is more common, and the related donation option. This typically occurs when there is an indication for transplantation and the patient's physician prescribes the collection of UCB for a sick sibling. The quality requirements described in this chapter apply to both types, unless the text specifies otherwise.

Compared with adult sources, the use of UCB cells offers several advantages, including no risk for the donor, prompt off-the-shelf availability and clinical benefits like low incidence of graft-*versus*-host disease (GvHD), even in partially matched transplants, which increases the patient's chance of finding a suitable donor. SoHO establishments have therefore facilitated universal access to the therapy, in particular to ethnic minorities.

However, there are also some disadvantages: the number of stem cells in UCB is relatively low and often associated with slow engraftment, and it is not possible to use a donor lymphocyte infusion after transplantation. There are interesting approaches to improve outcomes, including the use of very high cellular units, double cord and intra-bone transplantation, and promising protocols for progenitor cell *ex vivo* expansion. The most applied protocol in older patients uses reduced intensity conditioning and a double UCB graft approach. Recently, immune active properties like an enhanced graft-*versus*-leukaemia (GvL) effect have been proposed, and reconstitution of the immune system is the current research area to achieve an improvement of UCB transplant methods [3, 4].

Thus, UCB transplantation is an alternative option in the absence of a fully matched related or unrelated donor available at an appropriate time. Advantages and limitations of its use over adult stem cell sources are summarised in Table 26.1.

The regulatory framework of UCB collection and processing to make it available to patients in need has evolved considerably since the year 2000. Accreditation and regulation have instilled confidence in clinicians, allowing them to select a UCB unit from across a wide range of banks in many countries.

In addition to the legal requirements of the 2024 European Union (EU) Regulation 2024/1938 [5], some scientific societies have developed their own recommendations such as Netcord-FACT (Foundation for the Accreditation of Cellular Therapy) and Association for the Advancement of Blood & Biotherapies (AABB) international standards for cord blood banking [6, 7] and WMDA international standards [8]. They give a worldwide perspective to improving the quality of the SoHO preparations available for transplantation. The accreditation

Table 26.1 **Advantages and limitations of the use of umbilical cord blood transplantation *v.* bone marrow transplantation and peripheral blood transplantation**

Advantages of UCB	Limitations of UCB
Expanded access to transplant	Slower engraftment
Higher availability of donor	Higher risk of non-immunological rejection (graft failure)
Faster search and shorter time to transplant	Remote possibility of transmission of a genetic disease
Greater human leukocyte antigen (HLA) disparity allowed with low incidence of acute graft-*versus*-host disease	Greater delay in immune reconstitution
Lower risk of infectious disease transmission	No possibility of donor lymphocyte infusion
More versatile transplant planning	
No risk of donor refusal	

Source: The EBMT Handbook: Hematopoietic Stem Cell Transplantation and Cellular Therapies [4].

status of the SoHO establishment is one of the selection criteria for many transplant centres during the search for a UCB unit.

26.2 Donor recruitment

General information on donor recruitment is shown in Chapter 5.

Good Practice Guidelines

G26.1 Informed donor consent should be obtained from the pregnant woman prior to active labour and signed by the pregnant woman.
G26.2 Healthcare professionals at collection sites should be trained to give information about the UCB collection procedure. For further details see Chapter 2.

Recommendations

R26.1 It is recommended that donor recruitment begin during pregnancy, with information provided by the woman's healthcare provider. However, recruitment may also take place during admission to the maternity unit, provided that the mother (if not both parents) is in suitable condition to give informed consent.

Supplementary information

With regard to informed consent (G26.1), in certain circumstances like *ex utero* collection, the consent process may be conducted in two stages: initially, consent to collect may be obtained before active labour, which can be given verbally and, if possible, recorded in the woman's medical notes. Subsequently, formal written consent for donation is required prior to shipping for processing.

Information leaflets or brochures to inform mothers about UCB banking are an important part of the recruitment plan. This, supported by the right information, may bring parents to decide the final application of their donation.

As an example, Appendix 33 shows a model of informed consent.

Public initiatives promote donation of UCB units, usually to a not-for-profit organisation, with the aim of providing UCB units for transplantation or other approved clinical applications to any patient in need. Many public SoHO establishments offer also a service for family UCB banking in case of illness in one of the siblings or other members of the donor's family in response to a medical prescription [9]. Public SoHO establishments are making their inventories available for any patient in need of a transplant directly or through a national donor registry in WMDA.

The Council of Europe has been studying the issue of UCB donation for several years and has always been concerned about the proliferation of SoHO establishments dedicated to the collection and storage of UCB for autologous or family use. This concern resulted in adoption of Recommendation Rec(2004)8 of the Committee of Ministers to member states on autologous SoHO establishments, and its explanatory memorandum [10], which recommends that member states allow establishment of SoHO establishments only for altruistic and voluntary donations of UCB. In the case of autologous (or family-use) SoHO establishments, those organisations would clearly inform parents about the differences between the various medical objectives of autologous and allogeneic donations and about the uncertainties relating to the medical applications of autologous UCB preservation. SoHO establishments have to ensure the same quality and safety standards for autologous and allogeneic UCB donation and banking.

The Council of Europe has published the booklet *Umbilical cord blood banking: a guide for parents* to provide clear, accurate and balanced information about the use of UCB in medical treatment and to guide parents through their UCB storage options [11].

If a family member is affected by a malignant, genetic or immune disorder which is potentially transplantable, related UCB units can be collected prospectively and stored for later use [12]. The rationale is that if the new baby's HLA type is compatible with the affected sibling, cord blood has the potential to be a good source of cells for transplant for the donor's sibling. The

likelihood that a sibling will share both HLA haplotypes is 25%. This is especially likely in siblings that are affected by malignant, genetic or immune disorders.

26.3 Donor evaluation

The general criteria for donor selection are outlined in Chapter 6. UCB-specific donor criteria are given in the same chapter, in Table 6.2.

Table 6.4 details the general risk conditions for SoHO donors, mainly relating to communicable infectious diseases requiring careful evaluation. Donor screening is provided in Chapter 7.

Good Practice Guidelines

G26.3 The medical history evaluation should be obtained while the mother is able to concentrate on the answers to the questionnaire and is not distracted by aspects of labour.
G26.4 In the case of a woman who gives birth to an infant donor not genetically hers, her communicable disease risk history should be obtained.
G26.5 The questionnaire should include questions to obtain (at a minimum) genetic history, including malignant disease and inherited disorders that may be transmissible to the recipient.
G26.6 If the history of communicable disease risk was obtained in advance of the maternal donor's presentation for delivery, the history should be updated to include more recent information up to the time of delivery.

Recommendations

R26.2 Asking for informed consent and providing information about it is not recommended during active labour in order to avoid distraction linked to physical and emotional stress.
R26.3 It is not recommended that either family or friends serve as interpreters or translators.

Supplementary information

Appendix 34 shows an example of a maternal health questionnaire to evaluate donor eligibility.

26.4 Collection

There are two main techniques to collect blood from the cord vein: before the placenta is delivered (*in utero*) or after the placenta is delivered (*ex utero*). These two collection techniques give similar results, and which one is used will depend on the ability of the maternity unit to collect the UCB and will have to be validated by the SoHO establishment.

Good Practice Guidelines

G26.7 Collection should not interfere with labour and delivery in order to protect the mother and the newborn's safety.
G26.8 Before collection, clamping should be performed according to institution delivery practices, which should not be modified in an attempt to increase cord blood unit (CBU) volume.
G26.9 Collection bag should contain an adequate volume of anticoagulant to prevent clotting.
G26.10 Labels attached to UCB bag or accompanying documentation should contain the following information:
- **G26.10.1** unique UCB and maternal codes;
- **G26.10.2** product name;
- **G26.10.3** collection site name or identifier;
- **G26.10.4** date/time of collection;
- **G26.10.5** name and volume/concentration of anticoagulants;
- **G26.10.6** recommended storage temperature;
- **G26.10.7** biohazard sign and/or other warning labels, following national regulations;
- **G26.10.8** statement 'Related donor' where applicable;
- **G26.10.9** donor name for related UCB units;
- **G26.10.10** recipient family or individual name, if known, for related UCB units.

G26.11 After collection, the healthcare provider in charge should complete a report describing the labour phase, listing the events to be evaluated for acceptance of the unit, such as presence of fever, complications, type of delivery, etc.

Supplementary information

Collection technique typically involves the following steps:

i. the umbilical cord is clamped as distal from the placenta as possible. Nowadays, many obstetrical medical associations recommend delayed clamping. Evidence suggests that an acceptable time of one minute is compatible with UCB banking [13];
ii. a section of the cord is cleaned with a suitable disinfectant;
iii. a needle that is attached to the collection bag is inserted into the umbilical cord vein;
iv. the collection bag is filled by gravity until the cord is empty and blood has drained into the bag.

26.5 Fresh storage and transportation

General information on transportation and storage is covered in Chapters 11 and 13.

Good Practice Guidelines

G26.12 Decision for shipping will depend on quality parameters such as collection performance, volume and (if available) total nucleated cells (TNC). These parameters should be predefined and the decision documented.
G26.13 During temporary storage in non-frozen conditions, UCB units shall be maintained in a temperature range validated to protect cell viability.
G26.14 Time between collection and the start of processing should be minimised.

Recommendations

It is recommended that:
R26.4 cryopreservation of UCB units be initiated as soon as possible;
R26.5 the duration of transport to the processing laboratory be minimised, as progressive loss of HPC viability occurs during non-frozen storage.

Supplementary information

Before transport, there is an opportunity to check the units and decide whether to transport them to the lab for processing and storage. If, for example, the blood volume or TNC count is too low, it could be a criterion for not shipping UCB from a collection site to the SoHO establishment [14].

26.6 Processing

Good Practice Guidelines

G26.15 Unrelated UCB units should be processed and cryopreserved within 48 h of collection and cryopreservation of related units should occur within 72 h.
G26.16 UCB units should be stored in freezing bags designed and approved for the cryopreservation of human cells and placed into metal cassettes to provide protection during freezing, storage, transport and shipping.
G26.17 After filling, each freezing bag should be visually examined for possible leaks and breakage of seals.
G26.18 Standard operating procedures (SOPs) related to cryopreservation should specify that the following information is recorded for each unit:
G26.18.1 TNC concentration within a defined range;
G26.18.2 the type of cryoprotectant, its final concentration and the duration of the cell exposure prior to freezing;
G26.18.3 method of freezing and end-point temperature of cooling;
G26.18.4 cooling rate within a defined range;
G26.18.5 freezing curve parameter within a defined range;
G26.18.6 storage temperature.
G26.19 Representative samples intended for viability or potency analysis should be stored under the same conditions as the UCB unit. In all cases, from each UCB unit, at least two samples of suitable material for preparation of genomic DNA, and plasma, should be required; and, from the maternal donor, serum and/or plasma and suitable material for preparation of genomic DNA, should be required. All the samples should be stored at –70°C or colder.
G26.20 The duration from addition of cryoprotectant to initiation of freezing should be minimised and the maximum time allowed should be validated by the SoHO establishment.

Recommendations

R26.6 It is recommended that an appropriate number of reference samples be stored for extended or future analysis after cryopreservation to cover safety, identity, purity and potency assessments.
R26.7 For viability and potency assays it is advisable to use, at least, two contiguous segments to the main bag.

Supplementary information

On receipt of a UCB unit, a series of checks are performed on the unit, on the associated samples and on the accompanying documentation, to verify and determine whether specific acceptance criteria are met. These include parameters such as volume, TNC content, correct documentation and labelling, signed maternal donor consent, appropriate transport conditions, absence of large/multiple clots, transport temperature and acceptable time in transit from collection centre to processing laboratory. Once a UCB unit meets the initial acceptance criteria it will continue on to be processed.

Despite some loss of cells, volume reduction including red blood cell (RBC) and plasma depletion has practical and clinical benefits: it allows efficient storage of UCB in terms of space and cost, and, most importantly, it decreases the volume of cryopreserved product and the risk of haemolysis due to ABO incompatibility. The eliminated RBC and plasma components can be used for immediate or future testing, if validated test systems for this application are available, thereby minimising the loss of the actual UCB product for testing purposes [15].

The final product volume and cellular characteristics are dependent on the starting product as well as the processing/separation technique.

The selection of a suitable protocol for cryopreservation of UCB is critical to optimise the recovery of functionally viable HPC [16]. Potential causes of cell damage include type and concentration of cryoprotectant, cell concentration, and cooling and warming rates, as well as level of control of storage conditions (see also Chapter 10).

The majority of SoHO establishments use cooling rates of 1-5°C/min to allow the cells to slowly dehydrate

as the ice phase progresses and the extracellular solute concentration increases. Cryoprotectants used for UCB are those generally established for other HPC sources. In general, a concentration of 10% DMSO is considered optimal for UCB. When used in conjunction with other cryoprotectants (e.g. Dextran), they can enhance the cryoprotective effect by allowing stabilisation of the cell membrane. Importantly, any combination used is validated with the objective of achieving the best recovery rates for TNC, $CD34^+$ and colony-forming units (CFU). Prolonged exposure of cells to DMSO can result in damage to cells.

Since about 2015, three major methods have been used in large-scale banking which give reproducible results and which could be standardised. These include the manual method using different approaches for RBC sedimentation (i.e. hydroxyethyl starch or other proprietary reagents, the semi-automated bottom-and-top method, and fully automated and programmable closed systems).

There is a clear correlation between good practice, quality certification and the outcome of UCB transplantation [17]; hence setting up a high-level processing lab is encouraged.

26.7 Storage

General requirements, including the long-term storage required, are described in Chapter 10.

Good Practice Guidelines

G26.21 SoHO establishments should have an inventory management system to ensure that each UCB unit and its associated reference samples, maternal samples and records can be located in a timely manner.

G26.22 The effects of long-term storage on the viability, potency and sterility of the UCB cells should be evaluated in a stability protocol.

G26.23 Containers (for example, refrigerators and freezers) intended for storing UCB units and their associated reference samples should not be used for any alternative SoHO preparations, unless those preparations present a comparable risk of transmission and undergo identical safety testing protocols. Should this exception arise, a supplementary risk assessment will be required to ensure safety and compliance.

G26.24 UCB units are intended for long-term storage and should be stored at –140°C or below.

G26.25 Both vapour or liquid phases can be used; however, when units are immersed in liquid nitrogen, an overwrap bag should be employed to prevent cross-contamination.

G26.26 Each facility should assess the potential risk of transient warming events during processing and/or storage.

Supplementary information

The Good Practice Guideline on simultaneous storage of UCB units alongside other SoHO preparations (G26.23) is primarily aimed at minimising the risk of cross-contamination. Additionally, this measure reflects the goal of preserving UCB units for extended periods, acknowledging that storage in the liquid nitrogen (LN_2) phase is suitable for this purpose. Given the characteristics of various SoHO types, concurrent storage is feasible with other HPC preparations. However, heightened caution is warranted, as HPC preparations typically have a higher turnover rate, which increases the likelihood of transient warming events. Implementing risk-reduction strategies – such as physically separating storage areas, for instance by assigning separate racks to each preparation – can help mitigate the possibility of temperature fluctuations. Ultimately, SoHO establishments can effectively manage these risks by conducting comprehensive risk assessments tailored to their specific storage procedures.

Examples of transient warming events include transfer of UCB units from the controlled-rate freezer to the cryostorage tank, removal of segments for HLA verification testing (or other tests required) and storage of UCB units in vapour vessels that may exhibit unstable temperatures when open. Each step is generally validated to show that the viability and potency of the UCB unit have not been compromised.

The primary reason for allowing UCB units to be stored by immersion in liquid nitrogen is due to the need for long-term preservation (G26.25). A significant number of units are already maintained in this manner, and utilising liquid storage can sometimes enable tracking of each unit's specific thermal history, as LN_2 levels in the tank can be monitored over time. Overall, this approach presents itself as a practical and effective option for certain banks, offering both flexibility and assurance in maintaining optimal storage conditions.

26.8 Quality control

Maternal donor bloods of each UCB unit are assessed for evidence of transmissible and non-transmissible diseases according to the guidelines given in Chapter 7.

Before listing them to a registry or distributing for clinical application, UCB units are assessed for qualifying safety, identity, purity and potency characteristics.

Good Practice Guidelines

G26.27 UCB units for unrelated use should have a negative microbiological control.

G26.28 For related UCB units, the results of positive microbial tests should include identity and antibiotic sensitivity of the organism(s), and these results should be reported to the prospective clinical programme.

G26.29 Each UCB unit should have undergone haemoglobinopathy screening, regardless of the family's ethnic background or history.
G26.30 Each SoHO establishment should define their quality reference values according to their validation data. The quality attributes of a UCB unit should be defined by the following variables:
- **G26.30.1** HLA typing (recommended to be done in high-resolution techniques for HLA-A, -B, -C, DRB1 and, if possible, including -DQB1 and DPB1);
- **G26.30.2** ABO typing;
- **G26.30.3** TNC counts;
- **G26.30.4** TNC yield after processing;
- **G26.30.5** Viability of TNC;
- **G26.30.6** Viable $CD34^+$ cell counts;
- **G26.30.7** Viability of $CD34^+$ cells;
- **G26.30.8** CFUs or other surrogate potency assays.

Recommendations

R26.8 Table 26.2 shows a list of reference values that are recommended to determine the quality of a UCB unit stored for clinical administration.

Supplementary information

Maternal blood obtained within seven days before or after collection of the unit (see Chapter 7) is used as a surrogate test for the infant donor's infectious disease markers (IDMs). It is strongly reflective of the infectious status of the UCB units due to the shared circulation during gestation. Testing the UCB unit for IDMs provides an additional degree of safety.

26.9 Release criteria

Meeting UCB quality specifications and following good banking practice will ensure a successful UCB transplantation [18].

Good Practice Guidelines

G26.31 On verification of donor identity: UCB unit identity should be verified by performing HLA-typing using a representative sample unequivocally linked with the UCB preparation.
G26.32 On purity and potency assessment: it should be verified that the product meets the criteria for TNC, viability and CFU as established by the SoHO establishment.
G26.33 The SoHO establishment should have a policy in place for the cases where there are no remaining reference samples available.

Recommendations

It is recommended:
R26.9 on identity verification, that a contiguous segment be physically attached to the freezing bag containing cryopreserved UCB cells;
R26.10 that maternal HLA from reference samples be tested to confirm the presence of one of her haplotypes;
R26.11 on potency assessment, that CFU assay from a frozen contiguous segment be performed prior to release for administration, to confirm ability to show cellular growth.

Table 26.2 **Specification requirements for unrelated and related UCB units**

Test	Unrelated Specification		Related Specification	
	Prior to cryopreservation	Thawed representative sample	Prior to cryopreservation	Thawed representative sample
TNC count	$\geq 5.0 \times 10^8$		Enumerated	
Total nucleated cell recovery	Should be ≥ 60%		Should be ≥ 60%	
Viability of TNC	≥ 85%		≥ 70%	
Viable CD34 count	$\geq 1.25 \times 10^6$			
Viability of CD34 cells		≥ 70%		
CFU (or other potency assays)		Growth (or positive result for potency)		Growth (or positive result for potency)
Microbial screen	Negative for aerobes, anaerobes, fungi		Negative for aerobic and anaerobic bacteria and fungi – OR – identify and provide results of antibiotic sensitivities	
Donor screening and testing	Acceptable as defined by applicable law		Acceptable as defined by applicable law	
Identity		Verified		Verified

Supplementary information

Testing maternal HLA adds additional assurance in verifying UCB HLA typing and ensuring its link to the maternal sample tested [19, 20].

The functional capacity of the UCB unit is assessed prior to release to the transplant centre. CFU assay is the surrogate potency test normally used to predict engraftment. CFUs are grown from functionally viable cells, and the results of this assay increase confidence in UCB unit quality and ability to engraft.

26.10 Labelling and packaging

Packaging is designed at all stages with two objectives: to protect the SoHO preparation and to protect personnel and the environment. For SoHO collected and/or distributed in the EU, the Single European Code (SEC) is used. See also Chapter 16 and the EU coding platform [21].

Good Practice Guidelines

G26.34 The primary packaging should be sterile and made of a biologically compatible material.
G26.35 Cryopreservation should use liquid nitrogen-resistant bags and labels.
G26.36 From collection to distribution, labelling should identify the UCB unit. Each label should include at least the unique identifier, the proper name of the product, the intended recipient (if known), the type of manipulation, the anticoagulants and additives used, and the conditions under which the cells are to be stored and distributed.
G26.37 The recipient should be identified (but not the donor) when cells are distributed for administration.

Supplementary information

International standards for labelling cellular therapy products are now available (e.g. ISBT128, Eurocode) and their implementation is required by specific accreditation bodies. Global labelling systems promote consistency and traceability, aid international exchanges, and facilitate vigilance and surveillance

26.11 Distribution

After careful search and match evaluation, UCB units are selected by transplant centres. Matching criteria are based on cell dose and level of matching between donor and recipient.

Good Practice Guidelines

G26.38 Once UCB units have been requested by a transplant centre, SoHO establishments are responsible for distribution and transport.
G26.39 For shipment of the cryopreserved UCB units, a dry shipper should be used, and the temperature monitored and recorded to detect temperature excursions.
G26.40 A plan for alternative transportation or shipping in an emergency should be in place.
G26.41 Once distributed, the return of unrelated UCB units to the original inventory should not be permitted.
G26.42 UCB units that have not been red cell reduced prior to cryopreservation should be washed, while a buffy coat-enriched UCB unit can be simply diluted or directly infused.

Recommendations

R26.12 In relation to thawing and infusion, it is recommended to ensure that the transplant centre receives information on how to handle and use the UCB unit; see Chapter 13.

Supplementary information

Handling includes thawing, dilution and washing of the UCB unit. Providing information about indications, contraindications and precautions is the responsibility of the SoHO establishment.

UCB banks list UCB data that are relevant for the facilitation of the search process, either directly or through a donor registry in the global database of WMDA (Search & Match Service [22]). Import/export can be done directly by the SoHO establishment or through third party agreements with their national bone marrow registries.

For further information, guidelines for UCB selection are published in the EBMT handbook [23].

For instructions in describing the cell preparation being distributed, there is guidance online [5, 24], including a reference document, the Circular of information for the use of cellular therapy products [25].

26.12 Biovigilance

Recommendations

It is recommended that:

R26.13 SoHO establishments ask organisations responsible for human application (ORHAs) for information on defined time periods for every UCB unit released for administration of haematopoietic reconstitution, including post-thaw cellular data to evaluate graft purity and potency;

R26.14 procedures be in place to notify UCB donors in the event that (genetic) findings in a recipient might have clinically relevant implications for the donor.

Supplementary information

On the donor side, regarding serious adverse reactions (SARs) for UCB donors, there is almost no risk in UCB collection, if done without interference with labour and if it follows the policy of cord clamping that is already in practice by each collection entity. More guidance about the right time for clamping is shown in WHO guidelines on intrapartum care for a positive childbirth experience [26].

On the patient side, information on post-thaw cellular characteristics and haematopoietic reconstitution is valuable in assessing overall performance of the SoHO establishment's procedures and also in confirming products' quality and their stability over time.

Most common serious adverse events (SAEs)/SARs occurring at transplant centres are either immediate or long-term.

i. Immediate:
   Discussed in Chapter 25 (see §25.10.2). In the use of UCB the most frequent reactions are those related to effects derived from infusing incompatible RBC, cryoprotectant or other adventitious substances used for volume reduction. The most common infusion reactions are hypertension, nausea and vomiting, bradycardia and chest pain.
ii. Long-term:
   Time to neutrophil and platelet engraftment, chimerism in the case of allogeneic transplantation, GvHD incidence and survival results.
   SARs related to the UCB donation or product, or infusion, are reported on a global level to the WMDA through an online web application to ensure a standardised way of reporting and to disseminate educational information [27].

Some of the examples below are from the Notify Library, which includes some well-documented cases of adverse reactions and adverse events. The database is publicly accessible and can be searched by substance type, by adverse occurrence type and by record number. For further evaluated cases (stated below as Record Number), search the Notify Library at www.notifylibrary.org.

SARs to UCB infusion:

i. Transmissible
   a. infectious disease, e.g. human herpes virus 6 (HHV-6) (Record Number 1428)
   b. genetic disease
ii. Immune reactions
   a. febrile non-haemolytic reactions
   b. allergic reactions
iii. Immunological complications
   a. GvHD
iv. Graft failure
   a. engraftment failure (Record Number 183)
v. Systemic complications
   a. within the respiratory tract
   b. neurological complications
   c. cardiac toxicity
   d. acute renal failure (Record Number 965)
   e. anticoagulation effects
vi. Undue exposure to risk intervention
   a. receipt of a wrong unit
   b. infusion of a unit into the wrong recipient
   c. HLA typing entered incorrectly (Record Number 1146)
   d. UCB infused with washing solution (Record Number 1132).

Regarding UCB preparation, SAEs include:

i. Tissues and cells defect
   a. lower than recommended viability at time of infusion with no SAR in patient
   b. incomplete and inaccurate documentation provided along with UCB preparation
   c. insufficient cell quantity according to minimum requested
ii. System failure
   a. incorrect/non-labelled unit
   b. inappropriate transportation
iii. Materials
   a. receipt of a damaged unit package
   b. damage to a bag
iv. Equipment failure
   a. inappropriate storage conditions
v. Human error
   a. sampling procedures not followed.

26.13 Developing applications

In addition to participating in the continuous improvement of UCB transplantation, SoHO establishments can be adapted to provide cell preparations for applications beyond transplantation. Many UCB units collected may not meet minimal cell dose criteria fixed by SoHO establishments to meet current criteria for safe transplantation, rendering a substantial number of collected samples available for other applications.

In addition, only a very small number of the units that are listed in the national and international inventories are used for clinical transplantation. Thus, both fresh and frozen UCB units represent a source of SoHO well suited for other applications. These samples are also very well characterised and have an additional advantage for alternative clinical applications. For that purpose, an ethical review would develop both new informed consent for newly collected preparations and/or a re-consenting procedure for re-purposing already banked UCB units [28].

For these developments, the essential value of the altruistic gift that the donor originally expressed is maintained. Thus, the need to develop new products for advanced therapy medicinal products (ATMPs) can be considered from voluntary UCB donations.

Potential new applications are:

- UCB-derived off-the-shelf immunotherapy such as antigen-specific T-cells, UCB-derived chimeric antigen receptor (CAR) natural killer (NK) cell

therapy, UCB alloantigen-specific regulatory T-cell (allo-Treg) therapy and others.

- Regenerative medicine using UCB or related birthing tissues such as UCB mononuclear cells, cord tissue-derived mesenchymal stromal cells (MSC), endothelial progenitor cells, and UCB-derived induced pluripotent stem cells (iPSC) to create iPSC banks from their stored HLA homozygous UCB units.
- UCB multicomponents for specialised transfusion indications (i.e. extreme preterm babies), and UCB plasma and platelet-derived preparations for non-transfusional topical applications (i.e. platelet-rich plasma for wound healing and serum eye drops from UCB).

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Related material

Appendix 33. Informed consent form for cord blood donors (Italy)
Appendix 34. Cord blood donor medical history questionnaire (Croatia)

Chapter 27. **Pancreatic islets**

27.1 Introduction

Diabetes is a global health challenge, currently affecting over 540 million people, with projections suggesting this number will rise to 783 million by 2045. Despite progress in insulin therapy, many patients continue to face difficulty achieving tight glycaemic control, leading to complications and reduced life expectancy.

Pancreatic islet transplantation has emerged as an effective treatment for restoring beta-cell function in carefully selected individuals with type 1 diabetes (T1D), type 3c diabetes (T3cDM) and other forms of insulin-dependent diabetes [1-11]. The procedure involves isolating islets from a donor or from the recipient's own pancreas (autologous islet transplantation) and infusing them into the hepatic portal vein. This method offers a less invasive alternative to whole pancreas transplantation and provides the potential for long-term insulin independence. Recent advances in islet isolation, preservation and immunosuppressive protocols have further enhanced outcomes. Additionally, research into stem cell-derived islets and xenotransplantation of insulin-producing cells is under way [12-19].

Since 2000, islet transplantation has gained widespread adoption globally, with an increasing number of centres performing both allogeneic and autologous transplants [20]. A survey conducted between 2000 and 2020 revealed that Europe accounted for 59.7% of all global transplants, followed by North America at 33.8%. Smaller numbers were reported in Asia, Oceania and South America [21].

This chapter provides an overview of pancreatic islet transplantation, outlining the key aspects of donor selection, islet isolation, transplantation techniques and post-transplant monitoring. The guidelines presented here align with international best practices and regulatory requirements for ensuring the safety, efficacy and reproducibility of islet transplantation procedures.

27.2 Indications for islet transplantation

27.2.1 Allogeneic islet transplantation

Islets of Langerhans are derived from the pancreas of a deceased donor, declared dead either by neurological criteria (DBD) or by circulatory criteria (DCD). The donor pancreas is considered the starting material for the processing. The pancreas is donated through structured organ donation programmes that adhere to strict national and international medical and ethical guidelines.

The clinical indications are the following:

- Allogeneic islet transplantation is considered for individuals with insulinopenic diabetes, aged 18 to 65 years (with the possibility of extension based on a risk–benefit assessment), who are assessed as being able to adhere to lifelong immunosuppressive therapy, who have intact or stable kidney function and meet at least one of the following conditions:
  - i. frequent metabolic instability (hypoglycaemia, hyperglycaemia or ketoacidosis) despite intensive insulin therapy;
  - ii. unacceptable glucose control and/or a high risk of mortality due to poor metabolic regulation, despite comprehensive diabetes management and education;
  - iii. hypoglycaemia unawareness;
  - iv. rapid progression of chronic diabetes complications, except diabetic nephropathy with proteinuria greater than 300 mg/dL;
  - v. psychological conditions or cognitive impairment affecting adherence to insulin therapy;
  - vi. severe subcutaneous insulin resistance requiring intraperitoneal or intravenous insulin infusions.

- Allogeneic islet transplantation is also considered for individuals with insulinopenic diabetes who are already undergoing chronic immunosuppression for any reason (e.g. due to a concomitant or prior solid organ transplant, autoimmune disease or haematologic condition) regardless of kidney function or metabolic control.

Recommendations

It is recommended that:

R27.1 a multidisciplinary team, including endocrinologists, transplant surgeons, psychologists/ psychiatrists and immunologists, assess each case individually;

R27.2 the team carefully evaluate and discuss with the patient the risks and benefits of lifelong immunosuppression;

R27.3 allogeneic islet transplantation be integrated into a comprehensive diabetes management plan, ensuring long-term follow-up;

R27.4 data from allogeneic islet transplantation be recorded in national and international registries to support research, optimise outcomes and guide future developments.

27.2.2 Autologous islet transplantation

Islets of Langerhans derived from the patient's own pancreas are obtained by preserving tissue during its surgical removal, which may be necessitated by a variety of conditions. The whole or part of the patient's own pancreas serves as the starting material for processing. Resection techniques include pancreaticoduodenectomy, distal pancreatectomy or total pancreatectomy with or without splenectomy.

The clinical indications are the following:

- Autologous islet transplantation is considered for individuals of any age, either without diabetes or with C-peptide-positive diabetes (fasting level ≥ 0.5 ng/mL), who require partial or total pancreatectomy and meet at least one of the following conditions:
  - i. chronic pancreatitis where subtotal or total pancreatectomy is indicated due to intractable pain unresponsive to medical or endoscopic therapy;
  - ii. recurrent acute pancreatitis without an identifiable reversible cause requiring total pancreatectomy;
  - iii. traumatic pancreatic injury with ductal disruption necessitating partial or total pancreatectomy;
  - iv. severe post-pancreatectomy complications (e.g. life-threatening pancreatic fistula) requiring complete pancreatectomy;
  - v. lesions in the pancreatic body or neck, where extended distal pancreatectomy is preferred over central pancreatectomy;
  - vi. technical infeasibility or a high risk of anastomotic leakage during pancreaticoduodenectomy necessitating total pancreatectomy.
- Multifocal pancreatic neoplasm, renal metastasis or diffuse intraductal papillary mucinous neoplasm (IPMN), as determined by pre-operative imaging or intra-operative evaluation, contraindicate autologous islet transplantation except within scientific studies.

Recommendations

It is recommended to:

R27.5 prioritise autologous islet transplantation to prevent post-surgical diabetes when pancreatectomy is unavoidable;

R27.6 carefully select patients based on pre-operative metabolic function and pancreatic reserve;

R27.7 implement long-term metabolic monitoring to assess graft function and ensure glycaemic stability;

R27.8 record data from autologous islet transplantation in national and international registries to support research, optimise outcomes and guide future developments.

27.3 Donor evaluation

The general criteria for donor selection are outlined in Chapter 6. The specific criteria for exclusion of islet transplantation are given in the same chapter, in Table 6.2.

Table 6.4 details the general risk conditions for substances of human origin (SoHO) donors, mainly relating to communicable infectious diseases requiring careful evaluation. In situations where the evaluation of the donor deviates from this guidance, a documented risk assessment needs to be performed.

In this context, the term 'increased risk donors' refers to potential donors who, due to certain characteristics identified while performing donor evaluation, may pose a higher risk of disease transmission to recipients.

27.3.1 Allogeneic islet transplantation

Good Practice Guidelines

G27.1 Any potential deceased donor evaluated for multi-organ donation should also be assessed for islet donation, ensuring that equitable selection criteria are applied between islet and whole pancreas transplantation.

G27.2 Considering the specific requirements associated with islet processing and transplantation, the use of increased risk donors should not be excluded.

G27.3 When considering increased risk donors, a thorough risk–benefit assessment should be performed for each individual donor–recipient pair to evaluate whether proceeding with transplantation is

clinically justifiable and presents an acceptable level of risk compared to deferring the procedure.

G27.4 In situations where there is an increased risk of disease transmission, the recipient should be informed. Clear communication should be achieved and explicit, documented informed consent should be obtained.

G27.5 A negative serum cross-match and ABO compatibility should be considered the minimum requirements for transplantation.

G27.6 For DCD donors, warm ischaemia time should not exceed one hour.

Recommendations

It is recommended that:

R27.9 warm ischaemia time for DCD donors be kept under 30 minutes;

R27.10 donor characteristics, such as body surface area, body mass index (BMI) and vasopressor use, be assessed on a case-by-case basis;

R27.11 selection criteria be adapted to centre-specific protocols.

Supplementary information

Although islet transplantation is formally classified as a procedure involving SoHO, it shares critical features with organ transplantation, both in terms of clinical context and operational requirements. In particular, islet transplantation relies on an organ – specifically, the pancreas – as its starting material, and the entire process is subject to the same time sensitivity and logistical constraints associated with organ collection, preservation and processing. Moreover, the clinical indication and the potential recipient population are essentially the same as those for pancreas transplantation. Given these overlaps, it is appropriate that the evaluation of increased-risk donors in the context of islet transplantation follows principles comparable to those used in organ transplantation.

The presence of prediabetes (impaired fasting glucose, glucose intolerance) or type 2 diabetes is not considered an absolute contraindication for donation. In such cases, it is common practice to conduct a thorough assessment of residual beta-cell function, which may include fasting C-peptide measurement or documented evidence of stable glucose control without exogenous insulin.

An intensive care unit (ICU) stay of 5–10 days is generally acceptable, with possible extensions based on a risk–benefit assessment. Amylase levels up to 600 IU/L or three times the normal range may be considered acceptable, depending on institutional guidelines.

27.3.2 Autologous islet transplantation

Good Practice Guidelines

G27.7 Autologous islet transplantation should not be performed in patients with C-peptide-negative diabetes or type 1 diabetes.

G27.8 Intraportal autologous islet transplantation should not be performed in patients with specific medical conditions, including portal vein thrombosis, portal hypertension and significant liver disease.

G27.9 The presence of multifocal neoplastic disease should be excluded by pre-operative imaging or intra-operative evaluation.

Recommendations

R27.12 In cases of pancreatic (pre)malignant disease, it is recommended to ensure the exclusion of multifocal or residual disease at the pancreatic margin and to implement strategies to minimise the risk of disease spread. This may include the islet purification step after enzymatic digestion or the removal of 0.5 to 1.0 cm of the pancreatic remnant near the pancreatic margin.

Supplementary information

A diagnosis of malignant disease is not in itself an exclusion criterion for islet autologous transplantation.

27.4 Pancreas collection, preservation and packaging

Organ donation for this purpose follows the same protocols as for whole pancreas collection to ensure the viability and safety of the islets.

The collection of the pancreas for islet transplantation typically involves the same level of care and precision as that required for whole-organ pancreas transplantation. This is due to the fact that the quality, integrity and preservation of the pancreas are critical determinants of the success of downstream islet isolation, processing and transplantation. Any compromise in the surgical technique, ischaemia times, preservation methods or transport conditions can significantly impact islet yield, viability and function. For this reason, pancreas collection is typically performed by trained personnel following standardised procedures, ensuring the organ remains undamaged and optimally preserved throughout the process. Maintaining high standards during retrieval allows maximum efficiency of islet isolation and ensures the safety and clinical effectiveness of the final transplant product.

27.4.1 Allogeneic islet transplantation

Good Practice Guidelines

G27.10 When donor pancreata are collected by a third-party collection team, the SoHO establishment should have formal agreements in place with the collection centre (SoHO entity) covering the following aspects: (i) organ collection protocols; (ii) acceptable warm and cold ischaemia times; (iii) organ preservation methods, including the use of appropriate cold storage solutions; and (iv) shipping and transport conditions, such as transport on ice (see Chapter 2).

G27.11 Efficient control of temperature during the collection process should be ensured to minimise warm ischaemia damage due to autolytic processes. This may be achieved through rapid cold perfusion and topical cooling immediately following cross-clamping, or through other validated preservation strategies, including normothermic machine perfusion, where applicable.

G27.12 Direct manipulation of the pancreas during the warm ischaemia phase should be minimised to avoid haematomas and capsular damage.

G27.13 Over-perfusion of the pancreas during the cold ischaemia phase should be avoided.

G27.14 Portal vein venting should be performed at the time of donor pancreas collection to ensure effective arterial perfusion, particularly when portal and arterial perfusion occur simultaneously.

G27.15 The pancreas should be collected in conjunction with the spleen and duodenal loop, following the same protocol as for whole organ transplantation, immediately after the liver and before the kidneys.

G27.16 The pancreas should ideally arrive at the islet isolation establishment within 8 h of cross-clamping, or within 6 h for organs from DCD donors. The maximum acceptable cold ischaemia time should be 24 h.

G27.17 Static cold storage (SCS) between 2 and 8°C should be employed, but the duration of cold ischaemia should be carefully managed to avoid irreversible damage. A preservation solution should be used to reduce cellular damage during the cooling process.

G27.18 After the perfusion/preservation process, the pancreas should be carefully placed into an appropriate primary container, along with the preservation fluid, to ensure continuous exposure to the cold preservation solution and primary and transport container.

Recommendation

R27.13 The recommended preservation solution is University of Wisconsin (UW).

Supplementary information

The pancreas can be collected in block with the liver or separately. If the third-party team is accredited by the national/regional organ collection and allocation agency for liver collection, formal agreements between the SoHO establishment and collection teams are not mandatory. However, it is common practice to ensure that the team is well informed about these key aspects.

27.4.2 Autologous islet transplantation

Good Practice Guidelines

G27.19 Pancreas resection in autologous living donors should be performed using standard techniques, such as duodenum-preserving pancreatoduodenectomy, distal pancreatectomy or total pancreatectomy with or without splenectomy.

G27.20 Blood flow to the pancreas should be optimised to minimise warm ischaemia time for islet isolation.

G27.21 Arterial perfusion and venous outflow should be meticulously maintained during surgery to ensure minimal ischaemic damage.

G27.22 The splenic artery and vein should be ligated and divided at their respective origins and terminations, minimising warm ischaemia to less than one minute.

G27.23 Following resection, the pancreas should be immediately submerged in cold preservation solution, generally without arterial flush. A ductal or vascular flushing technique (e.g. gastroduodenal artery and splenic artery flush) can be employed to minimise warm ischaemia time and maintain optimal ductal integrity for enzyme delivery.

G27.24 Cold ischaemia time management, including cold storage preservation solutions and transport conditions, should follow the same indications as those established for allogeneic islet transplantation.

Supplementary information

Pancreas preservation for islet isolation presents specific challenges due to the metabolic characteristics of islet cells, which primarily rely on the respiratory pathway for glucose metabolism. As a result, pancreatic islets are particularly sensitive to ischaemic conditions that disrupt energy-dependent cellular functions, such as the sodium–potassium ATPase pump. SCS helps maintain approximately 10% of the pancreas's normal metabolic activity, though it does not completely prevent irreversible damage after prolonged cold ischaemia. During SCS, cooling is used to slow metabolic and enzymatic activity, while a preservation solution minimises cellular damage.

The UW solution is the most commonly used perfusion solution, containing phosphate buffers for pH balance, raffinose and lactobionate for osmotic regulation, allopurinol and glutathione for free radical neutralisation, and adenosine as an ATP source. Its high potassium/sodium ratio mimics the intracellular environment, helping to protect the pancreas during storage. However, UW solution is associated with high costs and a limited shelf life. Alternative preservation solutions, such

as histidine-tryptophan-ketoglutarate (HTK) and low-potassium solutions like IGL-1, have been explored, though UW remains the preferred choice. To improve pancreas preservation beyond SCS, various alternative techniques have been investigated, including hypothermic machine perfusion (HMP), normothermic machine perfusion (NMP), *in situ* regional perfusion (IRP) and persufflation. A recent consensus statement from the European Society for Organ Transplantation (ESOT) has addressed developments in these methods [22]. Among these, normothermic regional perfusion has been the only technique shown to be effective for both whole pancreas and islet transplantation in clinical settings. Other techniques have not yet been widely applied in clinical practice and are still under investigation for human use.

27.5 Processing, preservation and storage

Islets are clusters of cells in the pancreas that are highly vascularised and range in size from less than 50 µm to about 800 µm in diameter. These clusters make up the endocrine portion of the pancreas. It is estimated that a healthy pancreas contains approximately 3.2 million islets, which account for only about 1 to 5% of the total pancreatic tissue [23]. On average, each islet contains between 100 and 1 600 endocrine cells, which are closely located near capillaries and each other.

Various factors, including complex cell-to-cell interactions among different cell types, innervation, incretins, and metabolites such as sugars and amino acids in the blood and interstitial space, all play a role in regulating glucose homeostasis. Maintaining the integrity of islet cell clusters ensures their proper function. The current method for isolating islets from human pancreas is an automated procedure for the extraction of the islets of Langerhans, first developed by Ricordi *et al.* in 1987 [20, 24].

27.5.1 Autologous and allogeneic islet transplantation

Good Practice Guidelines

G27.25 Prior to islet isolation, non-essential components such as the spleen, duodenum, peripancreatic fat, lymph nodes and vessels should be meticulously removed.

G27.26 Organ quality assessment should include thorough visual inspection before and during pancreas trimming.

G27.27 After cleaning and inspection, the pancreas should be rinsed with antibiotic-containing buffers or disinfectants, weighed and prepared for enzyme injection by catheterising the pancreatic duct.

G27.28 The organ should be perfused with a cooled perfusion solution containing a collagenase/protease mix to facilitate controlled tissue digestion.

G27.29 Proper enzyme concentration, perfusion pressure and perfusion time should be standardised to ensure uniform pancreas digestion while preserving islet viability.

G27.30 After cold perfusion, the distended pancreas should be cut in small pieces and placed into the Ricordi chamber.

G27.31 A peristaltic pump should be used to maintain a continuous flow of warmed digestion solution through the system.

G27.32 The enzyme solution should be recirculated at a controlled temperature not exceeding 37°C to prevent excessive enzyme activity that may damage islets.

G27.33 Close monitoring should be performed to determine the optimal digestion endpoint – identified when:

i) most islets are freed from acinar tissue; and

ii) intact islets are visible under microscopic observation.

G27.34 Once the digestion endpoint is reached, the heating circuit should be bypassed to halt enzyme activity, the solution should be gradually cooled to prevent further enzymatic degradation, and the enzyme solution should be diluted with a cold preservation solution or cell culture medium to stabilise islets.

G27.35 Free islets should be collected, washed and resuspended in a cold organ preservation solution prior to undergoing purification using either a continuous or discontinuous density gradient. Samples from the final islet preparation should be collected and evaluated. Islet morphology and yield should be assessed using manual counting or digital analysis. Dithizone staining should be used to selectively stain insulin-producing red islets, allowing for differentiation from exocrine tissue. Standardised counting should be performed using islet equivalents (IEQ) based on 150-micron islets.

G27.36 After isolation, purified islets should be stored in media that maintain physiological insulin-producing properties.

G27.37 If storage extends beyond four days, validation through quality control testing should be conducted before release for transplantation.

Recommendations

It is recommended to:

R27.14 perform islet purification using a continuous density gradient with a cell separator; alternatively, a discontinuous gradient or other purification systems can be utilised, based on specific needs;

R27.15 maintain islet culture temperature between 22°C and 37°C, depending on the specific requirements of the culture medium;

R27.16 adjust CO_2 concentration to 3–10%, based on the sodium bicarbonate concentration in the storage medium;

R27.17 proceed with transplantation within 96 hours of isolation;

R27.18 change the first media the day after isolation, with further changes every 2–3 days, depending on storage conditions (22°C *v.* 37°C).

Supplementary information

Preserving the integrity of the pancreas capsule enhances islet yield, and moderate fat infiltration is often associated with improved isolation outcomes. Ensuring a uniform infusion with a preservation solution minimises ischaemic damage and optimises islet viability. Special attention is usually paid to addressing oedema and fibrosis, as they can obstruct enzyme distribution and reduce islet isolation efficiency.

If islet isolation is incomplete following purification, the process may be repeated or modified by adjusting the density gradient. However, the purification step can be omitted if the tissue volume after digestion is suitable for intrahepatic infusion, considering the different requirements for autotransplantation *v.* allotransplantation.

27.6 Quality controls/release criteria

27.6.1 Autologous and allogeneic islet transplantation: critical properties

27.6.1.1 *Critical property: identity*

Good Practice Guidelines

G27.38 Visual inspection should be performed before release to ensure that the islet preparation appears consistent and free from excess tissue or gross microbial contamination.
G27.39 Islet morphology should be evaluated using dithizone staining and microscopic examination. This assessment should confirm the presence of islets based on their colour, shape and size, ensuring that the islets are properly isolated and viable for transplantation.
G27.40 The estimated tissue volume should be calculated and should not exceed the maximum recommended packed tissue volume for infusion: < 0.25 mL per kg of the recipient's body weight for autologous islet transplantation and < 0.15 mL per kg for allogeneic islet transplantation. This ensures that the transplanted tissue volume remains within safe limits, reducing the risk of complications such as portal vein hypertension, thrombosis or bleeding.

27.6.1.2 *Critical property: potency*

Good Practice Guidelines

G27.41 The islet yield should be carefully assessed at the end of the isolation procedure to ensure sufficient islet mass for transplantation, as this directly impacts both the functionality and success of the transplant.
G27.42 Islet viability should be assessed using a dye exclusion test or an equivalent validated method.
G27.43 A viability threshold of ≥ 70% should be confirmed before proceeding with transplantation.

Recommendations

It is recommended to:

R27.19 in allogeneic Islet Alone Transplantation (IAT) and Islet After Kidney (IAK) or other organ transplantations, administer a total islet dose of at least 10 000 IEQ per kilogram of the recipient's body weight, delivered over one to four infusions. For each islet infusion, pooling of islet preparations from more than one donor may be performed. This does not imply simultaneous processing of multiple pancreata, as islet preparations may derive from separate and sequential isolations. To achieve this islet mass, pooling islet cells from multiple donors per infusion is advised;

R27.20 for the first infusion in IAT and IAK procedures, use a minimum of 5 000 IEQ per kilogram of recipient body weight. For subsequent infusions, it is recommended to administer at least 3 000 IEQ per kilogram. In cases where a lower islet dose is considered acceptable, the decision is recommended to be made at the discretion of the transplant physician, based on an individual risk–benefit assessment;

R27.21 in Simultaneous Islet and Kidney Transplantation (SIK), consider adopting more flexible criteria for islet yield, as the islet graft's primary role is to support kidney function rather than achieving full metabolic independence;

R27.22 although there is no upper or lower limit for the number of transplanted islets in autologous islet transplantation, start from the basis that a long-term metabolic benefit is generally achieved with ≥ 2 500 IEQ per kg of the recipient's body weight;

R27.23 consider insulin release testing as an optional, supplementary tool for assessing islet potency, where appropriate. Although not mandatory for islet transplantation, this test may help assess islet potency, despite the absence of a validated method for determining it based on insulin secretion.

27.6.1.3 *Critical property: purity*

Good Practice Guidelines

G27.44 The purity of the islet preparation should be evaluated by determining the proportion of islet cells relative to other cell types, such as ductal and acinar cells.
G27.45 Purity assessment should be performed using dithizone staining and microscopic examination.

G27.46 Islet preparations should meet minimum purity criteria: at least 10% for autologous transplants and 20% for allogeneic transplants.

27.6.1.4 *Critical property: safety*

Good Practice Guidelines

G27.47 The endotoxin level of the final islet preparation should not exceed 5 endotoxin units (EUs) per kg of recipient body weight per administered dose.

Supplementary information

Many of the biological assays currently used to assess islet function and microbiological safety are not sufficiently rapid to support real-time release decisions. With the exception of negative Gram stains and microscopic evidence of significant contamination – both recognised as having limited sensitivity – most sterility testing methods require incubation periods that exceed the short interval between pancreatic islet isolation and transplantation, which typically ranges from several hours to a few days.

Sterility testing is performed in accordance with *Ph. Eur.* chapter 5.1.6 (Alternative methods for control of microbiological quality) and, where applicable, *Ph. Eur.* chapter 2.6.27 (Microbiological examination of cell-based preparations); see Chapter 12.

As part of routine practice in islet transplantation, *post hoc* confirmation of absence of microbial contamination is performed. If sterility criteria are not met post-release a specific monitoring of the recipient is started. If no clinical signs of infection are observed, no interventions are needed.

However, if infection is suspected, targeted antibiotic therapy is initiated according to the antibiotic sensitivities. In the case of islet allotransplantation, studies have reported microbial contamination rates ranging from 0% to 16% at various stages, including pancreatic retrieval, transport media, islet isolation and culture. The primary source of microbial contamination is generally believed to be the duodenal segment of the small bowel attached to the pancreas. The *post hoc* contamination rate of the transplanted preparation is usually rare, often below 1%. This rate is not influenced by collection techniques or preservation solutions, as studies suggest that most micro-organisms are washed, diluted or eliminated during pancreas processing.

Microbiological contamination that occurs after release and during the 14 days mandatory surveillance period of the final islet preparation typically has minimal to no impact on patient health or islet survival, provided appropriate antibiotics are administered.

In autologous islet preparations, positive cultures in isolation solutions are more common, but are rarely associated with clinical infection. Nevertheless, ensuring the absence of microbial contamination in the final preparation maximises patient safety and helps prevent potential complications.

27.7 Packaging and distribution

27.7.1 Autologous and allogeneic islet transplantation

Good Practice Guidelines

G27.48 Islets should be maintained within an optimal temperature range of 20° to 26°C allowing for a tolerance of ± 2°C for < 30 min in an appropriate solution during transport to ensure their viability.

G27.49 External ambient temperature such as hypothermia (1–4°C) or high temperatures (43°C) should be avoided to maintain islet survival.

Recommendations

It is recommended to:

R27.24 set a triple packaging system to ensure the safe transport of islets, ensuring compliance with international shipping regulations for biological materials;

R27.25 transport islets in containers that ensure their viability and maintain quality during transit;

R27.26 implement methods for monitoring temperature and other environmental factors during transport, especially in cases of air travel, where islets may be exposed to temperature variations of up to ± 20°C.

Supplementary information

Approved transport containers include syringes, conical tubes, blood transfusion bags, gas-permeable bags and silicone rubber membranes.

27.8 Labelling

27.8.1 Autologous and allogeneic islet transplantation

Good Practice Guidelines

G27.50 Information that should be included:

G27.50.1 the islet cell mass or the count of insulin-producing cells and the tissue volume used for transplantation.

G27.51 Ensure the safety and effectiveness of the procedure by documenting sterility and viability data when available.

G27.52 If any pancreas condition or functionality assessments on islets are conducted, these records should also be included.

27.9 Islet infusion

27.9.1 Autologous and allogeneic islet transplantation

Islet infusion is typically performed according to the following practice:

- Islets are ideally infused within 96 hours post-isolation. The islet function remains viable within this time window, but the timing is monitored to ensure optimal viability.
- If the infusion is delayed beyond the recommended period, selected quality parameters (such as islet morphology, islet yield, islet viability) are re-validated prior to transplantation.
- The implantation site typically is the hepatic parenchyma via the portal vein system. Using this site is the standard and well-established practice for islet transplantation.
- Alternative implantation sites, such as bone marrow, subcutaneous tissue, gastric submucosa, omentum, striated muscle, etc., may be tested exclusively in clinical research and used as a standard only after proper validation.
- The access route typically is percutaneous transhepatic catheterisation under radiological guidance, which is the most common and preferred method.
- Mini-laparotomy, either with cannulation of omental or mesenteric veins, or with recanalisation of the umbilical vein, is usually considered an alternative method.
- Islet infusion is performed using a syringe or a closed gravity-fed bag system. This ensures a controlled rate of infusion and minimises the risk of sudden pressure increases.
- Islets are diluted with 40-60 mL of an appropriate solution before infusion. Heparin is added directly to the infusion bag immediately before the infusion begins, at a dose of 70 IU/kg.
- The islet infusion procedure usually takes between 15 and 60 minutes. Portal pressure is monitored before and after the infusion, particularly during sequential infusions.
  - The infusion is performed if the portal pressure is below 20 mmHg. If portal pressure rises above 22 mmHg during an ongoing infusion, the infusion is immediately interrupted to prevent complications. In the 20–22 mmHg range, the decision to proceed, pause or interrupt is left to the clinical judgement of the transplant physician, based on real-time assessment of the individual patient's risk profile.
  - A wash media bag is infused following the islet transplant to clear any remaining islet preparation from the catheter.
  - After the wash, the portal vein catheter is carefully removed, and the tract is sealed using an appropriate sealant to minimise the risk of bleeding.
  - An ultrasound scan of the liver is typically performed within 24 hours post-transplant to assess hepatic perfusion and detect any peri-hepatic haematomas. If the recipient experiences abdominal pain, the scan is performed earlier.
  - Patients are closely monitored for 2-3 days following the transplant for potential complications such as intra-abdominal bleeding or infection.

Also commonly carried out are the following procedures:

- in autotransplantation and SIK transplants, direct islet infusion without temporary storage, when possible;
- maintain blood glucose levels between 4 and 8 mmol/L during the peri- and post-transplant periods to minimise islet loss after infusion;
- maintain a variable-rate insulin infusion for at least 24 hours post-transplant to protect islets from oxidative stress and improve survival;
- follow a carbohydrate-restricted diet (< 40 g per meal) to reduce metabolic strain on the islets and promote better glycaemic control;
- an anticoagulation therapy to mitigate instant blood-mediated inflammatory reaction (IBMIR) and for thromboprophylaxis in the post-transplant period;
- for allotransplantation with immunosuppression, administer co-trimoxazole or dapsone for *Pneumocystis* pneumonia prophylaxis for six months post-transplant. Pre-transplant administration of antihistamines and paracetamol is advised to reduce inflammation and support islet viability, particularly when a depleting agent is used. Valganciclovir prophylaxis may be considered for *Cytomegalovirus* and other viral infections, especially during the initial months post-transplant when immunosuppression levels are at their peak.

27.10 Biovigilance

General information on biovigilance is provided in Chapter 18.

Paying particular attention to the risks associated with the islet infusion procedure, especially haemorrhagic and thrombotic complications, ensures patient safety and successful transplantation outcomes.

27.11 Patient follow-up

Good Practice Guidelines

G27.53 Post-transplant follow-up should be structured to allow for the standardised assessment of metabolic outcomes. A minimum dataset should include C-peptide levels (fasting and/or stimulated),

glycosylated haemoglobin (HbA1c), insulin requirements and hypoglycaemic episodes.

G27.54 Monitoring of immunosuppression-related complications should be comprehensive, with documentation of mortality events, major cardiovascular events, severe infections, renal function decline and the incidence of neoplastic events.

Recommendations

It is recommended that:

R27.27 outcomes be evaluated using established scoring systems, such as the IGLS score or other validated systems, to ensure comparability;

R27.28 follow-up timing be adapted to each SoHO entity or establishment's protocol; however, an early assessment of islet function within the first three months post-transplant is advised, as early function is linked to better long-term outcomes;

R27.29 a structured follow-up schedule, such as at 1, 3, 6 and 12 months in the first year and at least annually thereafter, be implemented; however, flexibility in timing could be considered, based on individual patient or transplant centre needs;

R27.30 metabolic studies using stimulation tests such as OGTT, MMTT or arginine be incorporated as part of the follow-up to evaluate islet function, although these tests are not recommended as routine clinical practice;

R27.31 automated insulin delivery systems and continuous glucose monitoring (CGM) be used for both pre- and post-transplant assessments to optimise metabolic control and monitor islet graft performance;

R27.32 monitoring for autoantibodies and donor-specific antibodies (DSA), especially in cases of sudden functional decline, be used to evaluate potential immune-mediated graft dysfunction or rejection;

R27.33 liver biopsies not be performed for routine monitoring, as the likelihood of obtaining sufficient diagnostic material is low.

Supplementary information

The collection and analysis of standardised transplant data allow advances in the field of islet transplantation. International registries, such as CITR (Collaborative Islet Transplant Registry) and EPITR (European Pancreas and Islet Transplant Registry), play a critical role in this effort by consolidating data from transplant centres worldwide. These registries help track clinical outcomes, assess the effectiveness of different transplantation techniques, refine donor selection criteria and improve patient care through evidence-based insights. They also support the development of best practices and inform regulatory decision-making.

SoHO entities active in islet transplantation are encouraged to contribute relevant outcome and safety data to such registries, in order to support continuous improvement and advances in the field.

27.12 Developing applications

In the near future, alternative systems and new developments for islet isolation could emerge, offering enhanced efficiency, reduced manual intervention and improved islet yield and purity [25-27]. These systems may incorporate advanced purification techniques, real-time monitoring and closed-loop processes, potentially reducing the risk of contamination and improving sterility. With the ability to optimise critical variables like enzymatic digestion and density gradient formation, these innovations could lead to higher-quality, functional islets for transplantation. After adequate evaluation, such systems could replace the current processing methods, improving the accessibility and cost-effectiveness of islet transplantation.

27.13 References

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Chapter 28. **Hepatocytes**

28.1 Indication for hepatocyte transplantation

Hepatocyte transplantation (HTx) is a procedure where human hepatocytes, usually isolated from organs deemed unsuitable for orthotopic liver transplantation (OLT), are infused and engrafted into the recipient's liver tissue to restore the missing hepatic function. HTx is less invasive than OLT and can be repeated if necessary. The safety of HTx has been demonstrated, and treatment outcomes are promising, particularly for patients with liver-related metabolic disorders [1].

HTx is considered for patients with metabolic liver diseases, severe liver failure or genetic disorders affecting liver function, particularly when liver transplantation is not immediately available or if the patient is awaiting a donor liver.

HTx has been employed to treat various liver-based disorders, including a range of metabolic diseases and acute liver failure (ALF). These diseases include Crigler–Najjar syndrome, phenylketonuria, alpha-1 antitrypsin deficiency, familial hypercholesterolaemia, factor VII deficiency, glycogen storage diseases, infantile Refsum's disease, primary oxalosis, urea cycle defects, ornithine transcarbamylase deficiency, citrullinaemia and argininosuccinate lyase (ASL) deficiency [2]. Additionally, HTx has been utilised in the treatment of ALF caused by drug toxicity, viral infections, mushroom poisoning and acute fatty liver of pregnancy [3-5]. These conditions, characterised by liver dysfunction and metabolic imbalances, have shown varying responses to hepatocyte transplantation, with some cases achieving therapeutic benefits and others requiring liver transplantation as a final solution [4, 6]. However, there are challenges to the widespread use of HTx. One of the main obstacles is the low engraftment of transplanted hepatocytes, which affects the longevity and effectiveness of the treatment.

Additionally, the requirement for immunosuppressive drugs increases the risk of infections, particularly in critically ill patients. Despite these challenges, ongoing research is working to enhance the survival and function of transplanted hepatocytes. There are efforts to refine the procedure to improve long-term outcomes and possibly make HTx a more reliable, permanent solution for liver disorders [7].

28.2 Donor evaluation

General information on donor evaluation of Chapter 6 applies, including the specific criteria for hepatocyte donor criteria shown in Table 6.2. In those situations where, due to the origin of the donated liver (organ donation), the evaluation of the donor deviates from this guidance, a risk assessment may be used to address specific circumstances but does not replace mandatory consent or donor selection criteria.

Good Practice Guidelines

G28.1 Liver-originated diseases, extracellular matrix alterations or vascular issues that may complicate hepatocyte isolation should be carefully evaluated.

G28.2 The characteristics of the donor liver, such as its size, its degree of steatosis and the history of ischaemia (both warm and cold), should be considered because they may affect the quality of the hepatocytes.

Supplementary information

Liver tissue for hepatocyte isolation can be collected through donation after death declared by neurological criteria (DBD) and donation after death by

cardiocirculatory criteria (DCD). Living donors are possible but rarely used due to potential risks.

Steatotic livers are less preferred due to reduced cell viability and metabolic function, though neonatal livers may present a viable alternative in some cases.

While neonatal livers are generally excluded from organ transplantation due to concerns about limited maturation, functional capacity and higher thrombosis risk, they can serve as a valuable source of hepatocytes, as their hepatic function can sometimes match or exceed that of hepatocytes from adult deceased donors.

Explanted organs from patients undergoing liver transplantation (e.g. the domino procedure) can also be utilised for hepatocyte isolation, provided the donor's defect and the recipient's needs are carefully matched.

28.3 Liver collection, preservation and packaging

Good Practice Guidelines

G28.3 When donor livers are collected by a third-party collection team, the substance of human origin (SoHO) establishment should have formal agreements in place with the collection centre (SoHO entity) covering the following aspects: (i) organ collection protocols; (ii) acceptable warm and cold ischaemia times; (iii) organ preservation methods, including the use of appropriate cold storage solutions; and (iv) shipping and transport conditions, such as transportation on ice (see Chapter 2, section 2.6).

G28.4 Liver tissue should be flushed either *in situ* or *ex vivo* with an appropriate organ-perfusion solution through the hepatic artery and/or portal vein.

G28.5 The collected liver should be placed in an appropriate organ preservation solution to maintain viability.

G28.6 The liver should be triple-packaged using sterile materials to ensure aseptic conditions are preserved.

G28.7 The organ should be transported in a secondary container that maintains a temperature between 2 and 8°C, to protect the tissue during transit.

Supplementary information

Organ-perfusion machines are currently being evaluated for storage and transportation of liver tissue in normothermic or hypothermic conditions, constantly perfused with oxygenated solutions, and could potentially lead to a change in practice and improve the quality of hepatocyte isolation.

If the third-party team is accredited by the national/regional organ collection and allocation agency for liver collection, formal agreements between the SoHO establishment and collection teams are not mandatory. However, it is common practice to ensure that the team is well informed about the key aspects of the procedure.

28.4 Processing and storage

Good Practice Guidelines

G28.8 The size of the organ should guide the decision on whether to isolate hepatocytes from a segment, a lobe or the entire organ. The vessels should be cannulated to ensure adequate perfusion of the liver tissue, with catheters inserted into the major portal and/or hepatic vessels to thoroughly wash the tissue of blood and re-warm the parenchyma, particularly if the tissue has been exposed to hypothermic transportation.

G28.9 Liver tissue should be perfused sequentially with different solutions using a peristaltic pump.

G28.10 The tissue should be perfused with a collagenase/protease mixture to digest the extracellular matrix.

G28.11 After enzymatic digestion is complete, the Glissonian capsule should be mechanically disrupted, and the cells should be diluted in a cold buffer solution.

G28.12 Isolated hepatocytes should be purified using low-speed centrifugation.

Recommendations

R28.1 It is recommended to add the antioxidant N-acetylcysteine to the perfusion solution when isolating hepatocytes from steatotic liver tissue, because this practice has been shown to enhance cell viability and metabolic function [8].

Supplementary information

For liver perfusion prior to collagenase digestion, the tissue is perfused with Hank's buffered salt solution (HBSS) containing ethylene glycol tetra-acetic acid (EGTA), which removes divalent calcium ions. This disruption of cell–cell connections aids in the efficient removal of blood. Afterwards, EGTA is washed out to avoid interfering with the enzymatic solution, as calcium is crucial for collagenase activity.

Standardised enzymatic preparations are used for digestion to ensure consistency, as most collagenase solutions are crude mixtures with variable enzyme activity, toxicity and endotoxin levels differing between batches. The duration of enzymatic exposure to the tissue depends on factors such as tissue size, characteristics, number of catheters used, recirculation and perfusion speed.

Purification typically requires three sets of centrifugations (80-90 × g) to remove cell debris, dead cells and any remaining collagenase.

The average hepatocyte yield after isolation varies from approximately 3×10^6 to 2×10^7 hepatocytes per

gram of tissue, with variable viability yields reported (20-85%). Hepatocytes can be utilised fresh for transplantation or cryopreserved. Various protocols have been developed for cryopreserving human hepatocytes, with optimal results achieved by cryopreserving 10^6 to 10^7 cells/mL in University of Wisconsin (UW) organ preservation solution containing 10% dimethyl sulfoxide and 5% glucose and using a controlled-rate freezer. Current cryopreservation protocols induce severe hepatocyte damage, drastically reducing both viability and function.

Quality control testing is repeated after the thawing of aliquots, if cryopreserved hepatocytes are used. Notably, the best clinical results have been reported using freshly isolated cells rather than cryopreserved hepatocytes.

28.5 Quality control/release criteria

Good Practice Guidelines

G28.13 Primary human hepatocytes cultured in 2D conditions should exhibit a polygonal morphology with a diameter of 20 to 30 µm under the optical microscope.
G28.14 The trypan blue exclusion test should be used as the primary criterion for assessing cell quality before transplantation.
G28.15 A cell viability threshold of 60% or higher should be accepted for clinical applications.

Supplementary information

Hepatocytes have various functions that can be tested, but there is no consensus on specific functional assays to assess them before transplantation.

The primary quality-control test is cell viability, usually assessed by trypan blue exclusion, which indicates cell membrane damage but does not identify apoptotic or pre-apoptotic cells. Additionally, collagenase digestion can impair surface markers.

To complement viability assessment and support broader characterisation of hepatocyte quality, various functional assays are optimised to assess the metabolic and synthetic capacities of hepatocytes. These tests evaluate the hepatocytes' ability to perform metabolic processes such as cytochrome P450 enzyme activity, drug and bile acid conjugation, and ammonia metabolism into urea. The cells' capacity to synthesise and release proteins like albumin, alpha-1-antitrypsin and lipoproteins is also assessed, reflecting their liver function. Furthermore, assays measuring apoptotic enzyme activity provide insights into the overall cell health and potential for engraftment. Plating efficacy, which tests the cells' ability to adhere to substrates like collagen, is also considered, though its clinical relevance for predicting engraftment success remains debated. While these functional assays provide valuable information, they are not considered to be the sole criteria for excluding cells from transplantation.

Currently, pre-transplant testing focuses mainly on viability and sterility [9], and matching is typically limited to ABO blood type.

28.6 Packaging and distribution

Good Practice Guidelines

G28.16 Hepatocytes should be used as soon as possible for cell transplantation within 24 h after isolation.
G28.17 Hepatocytes should be transported under hypothermic conditions (+2 to +8°C) or cryopreserved.
G28.18 When transported under hypothermic conditions, hepatocytes should be suspended in transport solutions commonly used for solid organ transport.
G28.19 Transport time should be minimised to prevent a decline in hepatocyte viability and function.
G28.20 Hepatocyte preparations should be labelled in a manner that ensures clear identification and traceability. The label should include, as a minimum, the unique donation and product identification code, the identity of the processing SoHO establishment, relevant dates (including date of isolation and, where applicable, expiry), storage conditions and any specific handling requirements necessary to preserve product quality and safety.
G28.21 For cryopreserved hepatocytes, cells should be stored or transferred in liquid nitrogen or the vapour phase in a liquid nitrogen dry shipper.
G28.22 Cells should be rapidly thawed at a rate equal to or faster than 100°C/min when preparing for transplantation.
G28.23 The cells should be diluted in saline solution supplemented with human serum albumin.
G28.24 The cryoprotectant should be reduced to less than 1% through centrifugation.
G28.25 Cells intended for transplantation should be suspended in a transplant medium at a concentration of 1 to 10 × 10^6 cells/mL.

28.7 Administration of hepatocytes

Good Practice Guidelines

G28.26 The number of hepatocytes to be transplanted should be based on the mass to be replaced, adjusted for the specific indication.
G28.27 The upper limit for safely infusing hepatocytes during transplantation should be 2 × 10^8 cells per kg of body weight.
G28.28 To achieve the desired cell count, multiple infusions should be performed. The portal vein should be accessed through percutaneous transhepatic catheterisation of a portal vein branch.
G28.29 Coagulation disorders should be thoroughly evaluated before performing the transhepatic approach, as it carries a risk of intraperitoneal bleeding and portal vein thrombosis. Alternative access should

be considered in the case of newborns through catheterisation of the umbilical vein, or in older children via a cut-down approach or laparoscopic/mini-laparotomy insertion of a central venous cannula into a tributary of the portal vein.

G28.30 Portal pressure should be continuously monitored during hepatocyte infusion and should not exceed 12 mmHg to minimise the risk of portal hypertension.

G28.31 Intrasplenic administration should be considered as an alternative to intraportal injection for patients with liver cirrhosis, where altered liver architecture may affect hepatocyte engraftment.

G28.32 Immunosuppressive treatment should be administered both before and after hepatocyte infusion. The immunosuppressive protocols used should be similar to those employed in whole organ transplantation to prevent immune rejection of the transplanted cells.

G28.33 ABO compatibility between the donor and recipient should be ensured.

Recommendations

R28.2 It is recommended that the optimised dose for HTx be 30 to 100 × 10^6 cells per kg of body weight, with an infusion rate of 5 to 10 mL per kg per hour and a concentration of 1 to 10 × 10^6 cells per mL.

Supplementary information

In current clinical protocols, up to 10% of the liver mass is transplanted (typically 5% for metabolic diseases and 10% for acute liver failure), resulting in billions of cells being infused intraportally over a period that may last up to 48 hours [10]. Due to the risk of portal thrombosis, only a limited number of cells can be infused in a single transplant session. Therefore, hepatocytes are cold-stored between repeated infusions. Studies have shown that the integrity and function of hepatocytes in suspension deteriorate after a few hours at 37°C or in culture media [11], whereas preservation in hypothermic conditions, using solutions like UW commonly used for intact liver tissue transport and preservation, has been validated. Similar hypothermic preservation techniques have been employed to isolate hepatocytes from different liver segments at different times, minimising the time that cells spend in suspension to avoid anoikis. Although hypothermia helps protect cells by reducing metabolic activity, function deteriorates even at 4°C [12].

This risk of bleeding may be higher in patients with liver failure and coagulation disorders (e.g. ALF or acute-on-chronic liver failure). If coagulation disorders are identified, corrective measures such as intravenous infusion of fresh frozen plasma and platelets may be necessary.

Once introduced into the portal bloodstream, hepatocytes embolise into the terminal branches of the portal veins, causing transient portal hypertension and mild ischaemia-reperfusion injury. This effect on the microvasculature activates Kupffer and stellate cells, leading to the release of cytokines and growth factors, and induces vascular permeability. As a result, within the first 24 hours, many infused cells migrate through the endothelial lining and integrate into the parenchyma. Preclinical studies in rats and mice indicate that cells remaining in the portal vessels beyond 24 hours are rapidly cleared by macrophages [13]. However, in some clinical transplants, hepatocytes were still observed in portal vessels at extended time points. After establishing intercellular contacts with neighbouring hepatocytes and connecting to the biliary and vascular systems, transplanted cells become functionally and histologically indistinguishable from endogenous hepatocytes.

Various preclinical strategies have been explored to improve hepatocyte engraftment, including the use of vasodilators [14], disruption of the sinusoidal endothelium and inhibition of macrophage function [13]. These approaches are particularly important in patients with metabolic liver diseases, where liver histology and cellularity are often normal or near-normal, resulting in limited repopulation of the liver by engrafted cells. Since native liver cells proliferate at low rates under normal conditions, strategies to provide donor hepatocytes with a growth advantage have been developed. One such strategy involves external beam radiation, where low-grade irradiation of the recipient's liver temporarily disrupts the sinusoidal endothelial barrier and inhibits phagocytic activity by Kupffer cells, promoting donor cell survival without systemic toxicity. Partial liver irradiation, typically targeting 30 to 40% of the liver, has shown promising long-term results in HTx [15]. Another strategy is partial hepatectomy, which induces a regenerative response and stimulates hepatocyte proliferation. This procedure has been used successfully in preclinical studies and clinical cases, such as in Crigler–Najjar syndrome, where it was combined with temporary portal ligation to improve engraftment [13].

Hepatocytes have also been transplanted into various ectopic sites, including the peritoneal cavity, pancreas, mesenteric leaves, intrapulmonary arterial tree, lung parenchyma, kidney capsule, subcutaneous fat pad and lymph nodes. Intraperitoneal infusions are particularly used as temporary liver support. Unfortunately, transplanted hepatocytes tend to survive for only a very short period of time in ectopic sites. However, the optimal extent of liver tissue removal and timing of the procedure to enhance engraftment and proliferation still need further optimisation.

28.8 Biovigilance

General guidance on Biovigilance is provided in Chapter 18.

Risks associated with the infusion procedure, particularly haemorrhagic and thrombotic complications, are commonly considered to be areas requiring careful attention.

28.9 Patient follow-up

General information on patient follow-up is described in Chapter 14, section 14.13.

Traditional markers of tissue rejection, such as circulating liver enzymes (ALT and AST) or infiltration of inflammatory cells, are not considered as primary indicators for assessing the success of hepatocyte transplantation.

Currently, there are no specific national or international registries on hepatocyte transplantation. This situation makes it difficult to record the level of risk of an serious adverse reaction and the level of success.

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Chapter 29. **Autologous adipose tissue**

29.1 Introduction

Adipose tissue is a complex and specialised connective tissue consisting of a stromal vascular fraction (SVF) and adipocytes. Functions of adipose tissue are energy storage in the form of lipids, hormone production and local tissue architecture maintenance. All the components of adipose tissue contribute to correct engraftment, regulating adipogenesis and angiogenesis and preventing adipocyte necrosis [1-3]. The purpose of using autologous adipose tissue transplantation is reconstructive.

The main advantage of autologous adipose tissue transplantation is that it is abundantly and easily available and does not induce immune response in the recipient. Most of the studies and clinical data on adipose tissue transplantation derive from patients receiving autologous adipose tissue, which is collected, processed and grafted in a one-step surgical procedure.

This chapter aims to provide guidance on autologous adipose tissue use; for allogeneic application as starting material use, please refer to Chapter 34.

The first historical reference to adipose tissue transplant was a report by Neuber in 1893, transferring autologous adipose tissue from the forearm to the face; afterwards adipose tissue was used for facial atrophy and, later on, the first adipose tissue injection through needle was reported [4].

Initially, the outcomes of adipose tissue transplantation were unclear because of the variability of tissue resorption in patients, and in 1950 the adipose tissue transplanted retention rate per year was around 40-50% [5]. Nowadays long-term results at one year remain unpredictable, due to the high rate of resorption in the grafted site (ranging between 20 and 80%) [6-7]. The consequence of resorption is the need for additional collection and grafting procedures with the increase of inherent surgical risk, costs and discomfort for the patient.

Some key points that can improve adipose tissue transplantation outcomes are based on various studies. The importance of atraumatic collection and processing to maximise viability/survival and adipose tissue transplantation success was first described in a 1993 study [8], where the viability of various fat cylinders was compared, resulting in a greater survival for those with less than 3 mm diameter/depth, observing that the central part of grafts of more than 3.5 mm was necrotised [9].

Adipose tissue banking eliminates the need for repeated liposuction and, thereby, reduces cost and the risk of morbidity. Discrepancies in adipose tissue storage efficiency have been reported [10-11]. In summary, several studies [12-15] observed diverse effectiveness in the improvement of pathological conditions when using cryopreserved or fresh adipose tissue despite the length of the follow-up (5-120 months). Nevertheless, the existing scientific evidence is insufficient to permit the standardisation of procedures.

Finally, the lipofilling technique, reinjecting adipose tissue aspirated by liposuction, has become one of the most popular procedures performed by plastic surgeons [16, 17]. Nowadays, the most common method of adipose tissue collection and transplantation is Coleman's technique [18].

29.1.1 Indications for adipose tissue transplantation

Indications in favour of adipose tissue transfer for reconstructive purposes are multiple and include any area needing volume and/or regeneration. Among reconstructive indications, breast reconstruction is an established clinical application of adipose tissue transfer [19].

Other applications are depressed scars, eyelid depression, pitting acne, post-traumatic defects, conditions such as Romberg's disease, facial microsomia and facial hemiatrophy, improvement of function and appearance

of irradiated tissues, correction of asymmetry in Poland's syndrome, soft-tissue defect correction after trauma, oncologic treatments and lipodystrophies.

29.2 Donor evaluation

The general criteria for donor selection are outlined in Chapter 6. The specific criteria for exclusion of adipose tissue are given in the same chapter, in Table 6.2.

Table 6.4 details the general risk conditions for substances of human origin (SoHO) donors, mainly relating to communicable infectious diseases requiring careful evaluation.

29.3 Collection

General information on collection is outlined in Chapter 8.

Good Practice Guidelines

G29.1 Tissue collection technique should ensure minimal manipulation of the tissue.

Recommendations

It is recommended to:

R29.1 use the superficial layer of subcutaneous adipose tissue instead of the deep layer to obtain the greatest stromal component, which ensures higher multipotency and stemness features [20];

R29.2 collect the adipose tissue with the most suitable technique chosen for the targeted use of adipose tissue, such as Coleman's technique [18, 21].

Supplementary information

There are various subcutaneous adipose areas of the body examined to identify natural adipose tissue deposits and there is no significant difference between different donor sources in cell viability or volume retention. The most common donor site is abdominal adipose tissue because it is one of the largest adipose tissue deposits. The second most common sites are the greater trochanteric region and the inside of the thighs and knee [22, 23]. Age, but not body mass index, seems to influence adipocytes' viability in relation to the choice of donor's site for collection [18]. The main inconvenience of using the superficial layer is the potential morbidity associated with it, such as irregularities and visible deformities.

Local anaesthesia (lidocaine) and tumescent solutions, applied before collection, seem to adversely affect the metabolism of adipocytes, with reduced glucose transport, lipolysis, viability and differentiation of preadipocytes or adipose-derived stromal/stem cells (ASC) [24, 25]. That effect, however, seems to disappear when the anaesthetic is removed [26] so, after washing, the tissue can fully regain its function.

Minimal tissue manipulation during collection (G29.1) allows for gentle mechanical disruption while preserving all structural elements of the tissue, including the extracellular matrix and perivascular structures.

Various collection and preparation techniques have been introduced to obtain better and more reliable survival of adipose tissue. The smaller the adipose tissue fragments, the better the retention of oxygen and nutrient supply, favouring a higher rate of engraftment. Even if several strategies have been used, one of the major issues is still standardisation of the procedures because there are no data supporting the choice of a best technique for the collection [27, 28].

The technique of Coleman aims to obtain atraumatic adipose tissue and its safe infiltration, reducing the possibility of intravascular injection. The use of a small cannula size for the liposuction also negatively influences tissue viability and finally adipose tissue engraftment, affected by flow speed and the shear stress [29, 30]. Conventional liposuction techniques, handheld syringes and new devices show no differences in adipose tissue outcomes [31-34]. Nonetheless, it has been observed that laser liposuctioning provides higher cell viability and enhances sample quality in comparison to mechanical liposuctioning [35]. A filtration bag for adipose tissue collection can be used to simplify tissue processing, because it allows rapid elimination of fluids and free lipids and the microfragmentation of the tissue with the preservation of the native architecture.

29.4 Collection and transfer to SoHO establishment

Recommendations

It is recommended that:

R29.3 adipose tissue aspirates be collected with a syringe in a specific container, refrigerated at a transport temperature of +2 to +8°C and transferred without delay to the SoHO establishment for processing and long-term storage [36].

29.5 Processing

General information on cleanroom premises and processing is provided in Chapters 9 and 10.

Good Practice Guidelines

G29.2 Adipose tissue processing protocols should be designed to minimise manipulation of the tissue before transplantation.

G29.3 Adipose tissue should be processed under aseptic conditions and in an aseptic manner.

G29.4 The validation of an adipose tissue processing protocol should consider at least the mainte-

nance of the following tissue properties: morphology, cellular composition and viability [37].

Recommendations

It is advised to:

R29.4 eliminate fluid, blood and debris during processing of adipose tissue.

Supplementary information

There are several published protocols for processing adipose tissue, but there is no evidence to prefer one technique above another. During adipose tissue processing, the collected adipose tissue undergoes a process of eliminating fluid, blood, cell fragments and oil.

Tissue processing includes washing (e.g. 0.9% NaCl) and centrifugation (e.g. 300-3400 rpm for 3 min) [38] or decanting, or procedures that allow increased concentration of the tissue, draining and separating the floating adipose tissue from the excess of fluids, blood and debris. New protocols recently suggest that the best results are obtained by washing and gentle centrifugation [39]. Centrifugation and decantation remove 50-60% of red cells and leukocytes, and filtering and washing removes 90% of erythrocytes and leukocytes [39]. The presence of blood reduces the viability of adipose tissue, causes inflammation and impairs engraftment.

Adipose tissue is filled by lipids that make it very sensitive to external treatments (centrifuge, processing methods and temperature) so both storage at room temperature and high centrifugation speed are to be avoided to reduce the loss of tissue viability [39, 40]. Centrifugation at 400 g is sufficient to remove red fraction and excess of fluids, and greater forces produce higher oil fraction.

Better survival rates (similar to fresh specimens) were found with tissue samples frozen at a slow controlled rate (1-2°C/min) in the presence of cryoprotectants.

In particular, as well as the requirements for cryoprotectants and controlled freezing/storage, the following factors can affect the viability of transplanted adipose tissue [41]:

- collection → collection method, source location, donor age;
- processing → wash solutions, centrifugation, disaggregation;
- storage → media, cryoprotectants, storage temperature;
- recipient bed → infusion solutions, growth factors;
- implantation → method, location, flexibility.

29.6 Quality control

Good Practice Guidelines

G29.5 Quality control tests on adipose tissue should consider at least the following minimum quality criterion:

G29.5.1 Defined results of final microbiological testing.

Recommendations

It is advised:

R29.5 to have a negative result from the microbiological testing before considering release of the SoHO for human application. In cases of positive results, a risk assessment is recommended before considering application of the SoHO.

Supplementary information

Overall cell viability can be assayed using different stainings, based on cell-permeant/non-permeant [42] dyes that, when combined, mark live and dead cells at the same time. In addition, SVF and adipocytes can be isolated using enzymatic digestion from the same sample, allowing the assessment of live and dead cells of both components of adipose tissue [37].

General information on microbiology testing is provided in Chapter 12.

29.7 Preservation/storage

Good Practice Guidelines

G29.6 The SoHO establishment should specify the shelf-life of adipose tissue at defined temperature(s).

G29.7 Collected tissue should be cryopreserved for long-term preservation, in presence of a cryoprotectant agent, ensuring maximum tissue viability [40, 43-51].

G29.8 If thawing is performed at SoHO establishments before distribution, to avoid loss of vital cells before lipoinjection, the transport should be done within a short time, or as soon as possible.

G29.9 The thawing and elution procedure should be validated in order to guarantee the removal of the cryoprotecting agent and any remaining toxicity.

G29.10 Validate the storage and thawing procedure considering at least the maintenance of the following tissue properties: morphology, cellular composition and viability [37].

Recommendations

It is advised to:

R29.6 store small aliquots of adipose tissue, given that the best results of defect correction are achieved by multiple tissue injections [20, 52];

R29.7 ship cryopreserved adipose tissue at a temperature below −60°C to the organisations responsible for human application (ORHAs) with written thawing procedures included for clinicians.

Supplementary information

The resorption of autologous adipose tissue graft results in the need for repeated collection procedures (with increasing cost and risks for the patient). One way to indirectly overcome this problem is cryopreservation.

The selection of cryoprotective agent is one of the key issues for obtaining optimal viability of mature adipocytes and their precursors. The most common cryoprotective agents (G29.7) used for adipose tissues are dimethyl sulfoxide (DMSO), trehalose [53, 54] and glycerol, or their use in combination. Published data report high cellular viability in the presence of DMSO [55] as cryoprotectant when cooling is controlled, and thawing is done by fast gentle warming [56].

Studies comparing different storage conditions have shown that the banking of adipose tissue at −20°C [57] is not efficient because of the loss of adipocytes' viability, and for long-term storage processed adipose tissue is kept in the vapour phase of liquid nitrogen (at or below −140°C) [44]. However, the delivery of adipose tissue between −60 and −80°C, and temporary storage of it in this condition, does not affect the tissue composition or viability [37].

After cryopreservation, the tissue can be thawed and after the removal of cryoprotective agent it can be shipped to clinicians, refrigerated in a temperature-controlled container using a transport buffer consisting of lactated Ringer's solution with 1% human serum albumin [58].

Recent literature [59] has reported that the time of cryostorage and the cryoprotective agent in use do not influence the tissue collected, even for long-term storage (more than one year), and this finding can be taken as a basis for shelf-life validation in the SoHO establishment.

29.8 Biovigilance

Some of the examples below are from the Notify Library, which includes some well-documented cases of adverse reactions and adverse events in transplantation of adipose tissue. The database is publicly accessible and can be searched by substance type, by adverse occurrence type and by record number. For further evaluated cases of adverse outcomes associated with adipose tissue, search the Notify Library at www.notifylibrary.org.

Serious adverse reactions for adipose tissue transplantation include:

i. Transmission
   a. infection or sepsis after cryopreserved adipose tissue grafting:
      • infection from a cryopreserved autologous adipose tissue preparation four months after transplantation. The culture growth showed *Aspergillus*, and *Mycobacterium fortuitum* was also identified by polymerase chain reaction (PCR). The root cause was not identified but it is not likely to be donor derived since the autologous donor had no infections at the time of collection. Suspicion of SoHO contamination was raised during cryopreservation steps, when it was stored for two months, during the thawing procedure or during implantation (Record Number 1689);
      • three cases of atypical mycobacterial infections have been reported following an adipose tissue graft; they were on a hand, a leg and a buttock (Record Number 1690);
   b. a rare case of human bacterium transmission, *Corynebacterium bovis*, which caused an infection in a patient who had undergone autologous adipose tissue-based breast augmentation using cryopreserved adipose tissue [60];
   c. malignancy possibly attributable to the transplanted tissue (mainly due to cancer stem cells in the autologous transplant).
ii. Graft failure
   a. graft failure (e.g. excessive volume loss, calcification).
iii. Others
   a. fat embolism.

Serious adverse events include:

iv. Tissue and cell defect
   a. one study focused on the safety of cryopreserved adipose tissue injection. The samples studied showed that *Staphylococcus epidermidis* was the leading cause of cryopreserved adipose tissue contamination, and its resistance to methicillin is common. Based on the results, aseptic handling of adipose tissue during collection and preservation appeared to be most important (Record Number 4730).
   b. tissue supplied was damaged or transported at wrong temperature.
v. System failure
   a. tissue supplied was beyond its expiry date.
vi. Materials
   a. wash solutions during processing were contaminated.
vii. Equipment failure
   a. thawing of adipose tissue due to lack of nitrogen.
viii. Human error
   a. wrong tissue supplied for the intended surgical procedure.

29.9 Patient follow-up

General information on patient follow up is described in Chapter 14, section 14.13.

Systematic, long-term follow-up at one year after adipose tissue transplantation is typically performed in many transplant centres but depends on the number of

times the adipose tissue had been transplanted for the reconstruction.

Systematic follow-up includes registration of some basic clinical and quality criteria. Most physicians record the main clinical indications to surgery and patient-reported outcomes.

There are no specific national or international registries on adipose tissue transplantation. This situation makes it difficult to record the level of risk of an serious adverse reaction and the level of success of the different methods of preservation.

29.10 Developing applications

Adipose tissue can be used not only as a structural support, but it may also be a source of SVF or stem cells that can be cryopreserved before or after their isolation [57, 61]. Cells can be used to improve adipose tissue grafting, as in cell-assisted lipotransfer [62], or for the immunomodulatory property of SVF, which is currently under extensive investigation for its possible use in treating sclerosis and autoimmune, inflammatory and neurologic conditions, taking into consideration that in EU countries it may fall under the scope of Advanced Therapy Medicinal Products (ATMPs). A search for clinical trials using SVF in www.clinicaltrials.gov reveals that 18 studies are currently actively recruiting for patients. Adipose-derived stem cells isolated from SVF and expanded *in vitro* are under investigation for a whole range of diseases [63, 64] (see Chapter 34).

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Chapter 30. **Medically assisted reproduction**

30.1 Introduction

This chapter refers to medical and laboratory procedures applied to achieve pregnancies and live births by medically assisted reproduction (MAR). This discipline involves the collection, processing, storage, release and distribution/application of at least one of the following reproductive substances of human origin (SoHO): oocytes, sperm, zygotes, embryos and ovarian or testicular tissues. MAR procedures are applicable to freshly collected and/or cryopreserved gametes, zygotes or embryos originating from the couple being treated ('within-relationship use') and/or from donors ('third-party donation'). Collection, processing and storage of ovarian and testicular tissues are discussed in Chapter 31 – Fertility preservation.

Within this context, MAR is a broader term including controlled ovarian stimulation and intra-uterine insemination (IUI), whereas assisted reproductive technologies (ART) concern the procedures relevant to *in vitro* handling and the culture of gametes and embryos.

MAR is carried out in clinics that specialise in the treatment of patients who mainly, but not exclusively, present with infertility. These centres are usually SoHO establishments that fulfil tasks relevant to the collection, processing and application of SoHO preparations, bringing together laboratory and clinical procedures in a multidisciplinary unit.

Collection, processing and storage – as well as release and distribution – of reproductive SoHO preparations may also be performed by SoHO establishments (e.g. cryobanks) that are not part of a fertility clinic. Regardless of how the institution is named, the recommendations of this chapter concern all entities where reproductive SoHO preparations are handled at any stage, from donation and collection to final use.

MAR comprises various procedures, among which the most relevant are [1]:

a. Processing of sperm for the purpose of IUI. Semen is processed, and isolated sperm cells are transferred into the uterus at approximately the estimated time of ovulation.

b. *In vitro* fertilisation (IVF), involving the formation *in vitro* of a zygote (a fertilised oocyte) and the culture and intra-uterine transfer of the resulting embryo. Fertilisation may be achieved either conventionally, by co-incubation of collected and prepared sperm and oocytes (an approach referred to as standard IVF), or mediated by intracytoplasmic sperm injection (ICSI), whereby a single spermatozoon is injected into a mature oocyte.

c. *In vitro* maturation (IVM), based on the maturation *in vitro* of fully-grown immature oocytes recovered from mid-sized (4-12 mm) antral follicles, with or without prior pharmacological treatment of the patient with low doses (equivalent to < 150 IU daily), for a few days, of exogenous follicle stimulating hormone (FSH) to promote follicle growth.

d. Cryopreservation and storage of gametes, zygotes and embryos.

e. Pre-implantation genetic testing (PGT), based on genetic analysis methods to screen oocytes or *in vitro* generated embryos to ascertain if they are affected by known inherited diseases or chromosomal abnormalities incompatible with the birth of a healthy child. This approach comprises different forms of testing, such as PGT-M for monogenic/single gene defects, HLA typing and mitochondrial disorders [2], PGT-SR for chromosomal structural rearrangements and PGT-A for detection of aneuploidies [3].

MAR treatments may be considered for 'within-relationship' use in infertile couples with human immunodeficiency virus (HIV) or hepatitis B and C viruses (HBV and HCV) infection, but only following thorough assessment, management and acceptance of the residual risk

of transmission. In natural conception, disease transmission can occur through sexual intercourse (horizontal transmission) and/or may involve the future child (vertical transmission). MAR procedures are applied only after risk assessment of vertical and horizontal disease transmission and considering the individuals' health conditions [4].

In some countries, MAR can be offered to single women or female–female couples. In a few countries in Europe, under very stringent conditions, MAR treatment with surrogacy is allowed for women without a uterus or with a non-functional uterus, or for male–male couples. In surrogacy, through IUI or embryo transfer, the gestational carrier carries a pregnancy and gives birth to a child for the intended parent [1].

At the international level, each year, through its European IVF Monitoring Consortium, the European Society of Human Reproduction and Embryology (ESHRE) publishes a report of MAR activity in Europe, based on voluntary data contributions of individual countries. The latest ESHRE publication [5] (relevant to 2020) includes data from 1 440 clinics in 41 countries and reports 923 318 treatment cycles, including 135 231 with IVF, 356 408 with ICSI, 305 373 with frozen embryo transfer (FET), 57 051 with PGT, 64 007 with oocyte donation, 353 with IVM of oocytes and 4 895 cycles using frozen oocytes. A total of 1 288 institutions reported data on IUI cycles using either husband/partner's semen (IUI-H; n = 112 663) or donor semen (IUI-D; n = 38 839) in 30 and 21 countries, respectively. Sixteen countries reported 29 566 interventions in pre- and post-pubertal patients for fertility preservation (FP), including oocyte, ovarian tissue, semen and testicular tissue banking [6].

This chapter provides guidance to achieve high standards of clinical efficacy, efficiency and safety aimed at conceiving singletons resulting in a healthy liveborn, which is the ultimate goal of MAR.

Specific MAR requirements described in this chapter may vary from country to country as determined by national legislation.

30.2 Quality management, validation and risk management

General information on quality management systems (QMS) and risk management is provided in Chapters 2, 3 and 4.

Good Practice Guidelines

G30.1 Each SoHO entity should select its own set of performance indicators (PIs) and key performance indicators (KPIs) based on their organisation/processes and data provided by national registries.

Recommendations

R30.1 It is recommended to consult the performance values published by relevant national and international scientific societies to allow comparison of SoHO entities' performance with recommended competence and benchmark standards.

Supplementary information

The Special Interest Group in Embryology of ESHRE, in collaboration with Alpha Scientists in Reproductive Medicine, have established minimum performance levels (competence) and aspirational values (benchmark) for the IVF laboratory [7]. PIs for clinical practice in MAR have also been published [8].

Registries' data are relevant to setting appropriate and realistic competence and benchmark performance values. In fact, due to several factors often not entirely understood, MAR success rates can significantly vary when comparing different geographical areas or countries.

The MAR process is as prone to failures and non-conformities as that of any other medical discipline. The impact of such undesired effects can be extremely high, considering that the gametes, embryos and offspring of intended parents are also involved. Risk management will help in assessing and prioritising the possible existing hazards for monitoring and control purposes, so that the probabilities of adverse reactions and events are minimised [9-13].

For risk-assessment purposes, long-interval monitoring of possible environmental contamination by micro-organisms during MAR processing may highlight the steps that are more exposed to such a risk. Together with information from registered serious adverse events and implementation of risk self-assessment tools (e.g. Microbiological Risk of Contamination Assessment [MiRCA], see Chapter 4, section 4.4.2), such monitoring can assist in assessing and mitigating the risks involved. Sperm banking involves longer operative times because specimens are cryopreserved and stored prior to use. Larger sample volumes are also available, offering an opportunity for detection of possible contamination before and after processing.

Gametes, embryos and associated processes are highly and intrinsically sensitive. Therefore, relevant analyses of qualification, validation and verification are challenging to perform. Currently there is no test system with the necessary biological sensitivity and safety. Biological surrogates for testing may be mouse embryos (only for pre-market testing of equipment [10]), embryos not suitable for clinical treatment, sperm donated for validation purposes or other appropriately selected SoHO preparations, depending on national regulations. Comparative tests may also be performed. For example, when introducing a new embryo culture incubator, sibling embryos may be cultured in parallel in the new and pre-existing incubators and resulting developmental rates may be compared, mutually and with reference to PIs or KPIs.

Ideally, randomised controlled trials are the best approach to introduce new techniques or procedural changes [14, 15] (Figure 30.1). However, their implementation is often hindered by several factors. The European Good Tissue and Cells Practices II tool may help to design appropriate studies proportionate to the level of residual/unknown risk to confirm that SoHO preparations' safety and quality are preserved (see Chapter 4).

30.3 Patient consent

General information about consent is described in Chapter 5. This section describes additional aspects of consent forms that require specific description.

Good Practice Guidelines

G30.2 Consent forms should be different for female and male patients, although for certain treatments – especially for within-relationship use – they may be combined in one document.

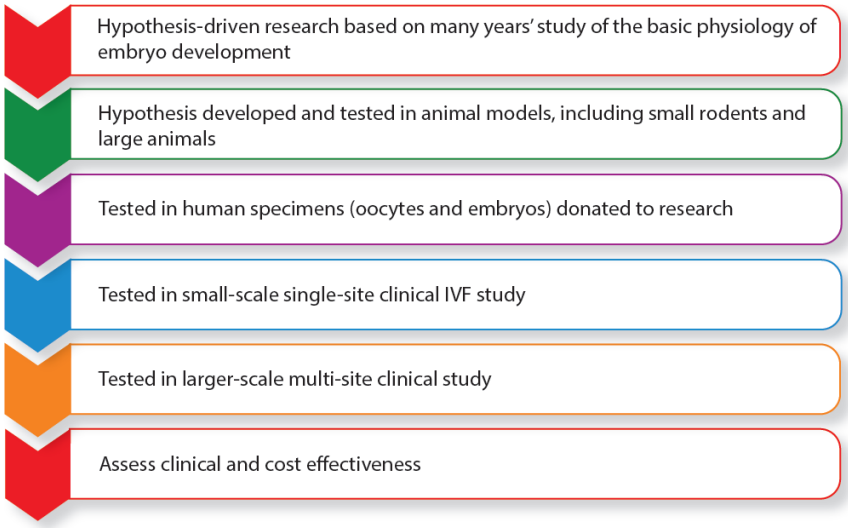
G30.3 For within-relationship use, both partners should sign these combined documents.

G30.4 The couple (or individual) subjected to MAR treatment should receive written and oral information (during medical consultation with the physician, through information sessions, leaflets, website etc.) on the following matters [16]:

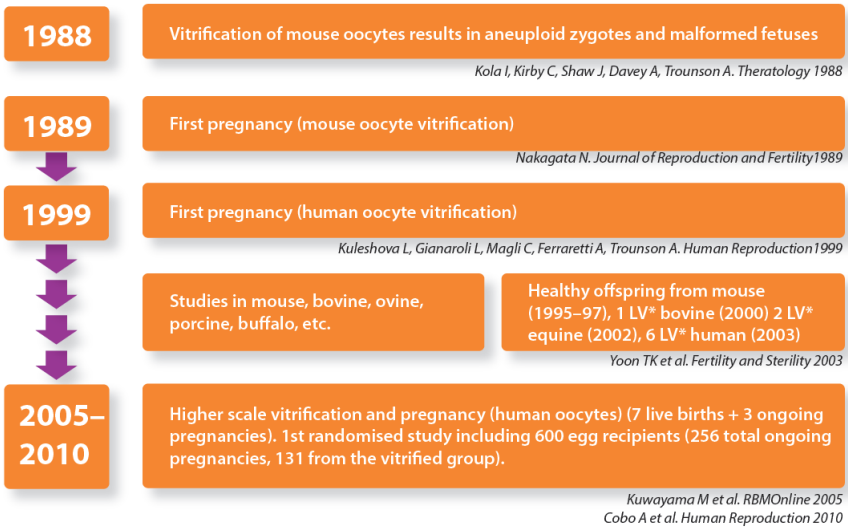
G30.4.1 national legislation governing MAR and its implications for those who have access to such treatment;

Figure 30.1 **Validation of new technologies in medically assisted reproduction**

1. Theoretical steps to be followed when introducing a new technology into clinical use in medically assisted reproduction



2. Example of a validation process in medically assisted reproduction



* LV = live-born viable offspring

Source: Adapted from Harper J *et al.* When and how should new technology be introduced into the IVF laboratory? *Human Reproduction* 2012;27(2):303-13.

G30.4.2 in cases of third-party gamete and embryo donation, the implications of current national legislation regulating the anonymity of the donor and possible right of the offspring to know their biological origins;
G30.4.3 their chances of success based on their medical history, the degree of treatment invasiveness and the possible risks associated with MAR;
G30.4.4 testing for genetic and infectious diseases, including assessments carried out in third-party donation;
G30.4.5 full description and associated risks of each stage of treatment;
G30.4.6 options to cryopreserve and store gametes and supernumerary embryos for future use according to national legislation; the couple should also be informed on the implications of national legislation regarding the fate of cryopreserved gametes or embryos, in addition to use for their own treatment: donation to another couple or individual patient depending on national legislation, donation for scientific research, donation for laboratory validation or disposal. The ultimate fate of embryos should be determined by national regulatory frameworks.
G30.4.7 possible undesired psychological effects caused by MAR treatment;
G30.4.8 possible risks to the offspring resulting from MAR treatment (particularly in multiple pregnancies) and the follow-up data on the long-term health of the offspring;
G30.4.9 if required by national regulation, the collection of information on treatment in a national registry, while protecting confidentiality and personal data.

G30.5 If within-relationship treatment is undertaken with cryopreserved gametes, then consent for thawing and subsequent embryo transfer should be given for each treatment by the partner from whom the gametes were collected and cryopreserved.

G30.6 In the case of cryopreserved embryos in within-relationship use treatments, both partners should give consent before each treatment. When the male partner has a condition or impairment that prevents him from providing valid active consent, legal assistance may be required.

G30.7 Specific consent should be collected for possible exposure to methods (add-ons) additional to mainstream IVF, ICSI and cryopreservation treatments.

G30.8 The procedure referred to as 'oocyte sharing' implies that the woman donating oocytes should be considered as both a within-relationship use patient and a third-party donor.

Recommendations

R30.2 It is recommended that the couple/patient be provided with information regarding:
R30.2.1 the total cost of a treatment cycle and existing reimbursement policies, if applicable;
R30.2.2 the possibility that treatment may be interrupted or cancelled due to medical or other reasons;
R30.2.3 the possibility of psychological counselling to the couple/patient;
R30.2.4 possible ethical issues regarding MAR.

Supplementary information

Examples of separate consent forms for treatment and storage are given in Appendices 11 and 12 for the female patient and in Appendix 13 for the male patient. The Council of Europe European Committee on Organs, Tissues and Cells (CD-P-TO), has produced a brochure 'Add-ons in medically assisted reproduction treatments: A guide for those seeking fertility care.' This booklet provides information and resources to help people make informed decisions in such cases [17].

Consent of both partners for the use of cryopreserved embryos would prevent a treatment from being initiated by either of the partners without the knowledge or consent of the other. However, in several countries the woman can start a treatment with such embryos even if her partner does not give consent.

In some countries, a very specific case is the option to consent for the use of cryopreserved gametes or embryos remaining from previous treatment(s) after one of the partners has died (posthumous consent).

30.4 Donor and patient evaluation

The general criteria for donor selection are outlined in Chapter 6. The specific criteria for exclusion of MAR donors are given in the same chapter, in Table 6.2.

Table 6.4 details the general risk conditions for SoHO donors, mainly relating to communicable infectious diseases requiring careful evaluation. In situations where the evaluation of the donor deviates from this guidance, a documented risk assessment needs to be performed.

30.4.1 Evaluation of patients for within-relationship use

Good Practice Guidelines

G30.9 Male–female couples reporting an inability to conceive should be evaluated in conjunction because infertility constitutes a mutual medical condition.

30.4.1.1 *Medical history and physical examination*

Good Practice Guidelines

G30.10 Full medical history – including surgical, sexual, contraceptive, genetic, family and pregnancy history, as well as travel history to assess possible risks

of specific viral diseases and behaviours potentially affecting fertility – should be collected from both partners. They should also undergo physical examination.

G30.11 A risk–benefit analysis should be carried out case by case to determine if a couple is suitable for MAR treatment.

G30.12 Females should be assessed for:

- **G30.12.1** breast cancer, uterine cancer (endometrial, myometrial and cervical cancer);
- **G30.12.2** ovarian function, with complete menstrual history;
- **G30.12.3** ovarian reserve, including biochemical tests and/or ovarian ultrasound imaging;
- **G30.12.4** tubal patency, if appropriate;
- **G30.12.5** uterine abnormalities, such as submucous fibroids, adhesions or Müllerian malformations (septae, bicornuate uterus) seen on 2D or 3D doppler ultrasound;
- **G30.12.6** immunity to rubella; vaccination should be offered to sero-negative women before MAR treatment.

G30.13 Males should be assessed for:

- **G30.13.1** semen quality – at least one diagnostic semen analysis should be carried out before starting treatment;
- **G30.13.2** in men with azoospermia or severe oligozoospermia, if a chromosomal abnormality is detected, appropriate genetic counselling should be offered;
- **G30.13.3** in the presence of obstructive azoospermia, cystic fibrosis or renal-tract abnormalities should be screened for.

Recommendations

It is recommended:

R30.3 to screen for genetic abnormalities (e.g. Klinefelter syndrome or Y-chromosome deletions) in men with azoospermia or severe oligozoospermia;

R30.4 to offer to all women with non-iatrogenic premature ovarian insufficiency, *FMR1* premutation testing for Fragile-X;

R30.5 depending on the type of infertility problem, to perform additional specific investigation (e.g. autoimmunity, thrombophilia, haemoglobinopathies, thyroid function).

Supplementary information

If seminal parameters are abnormal, a second semen evaluation can be repeated after two to three months; procedures and reference values are described in the *WHO laboratory manual for the examination and processing of human semen* [18].

In addition to genetic testing, hormonal testing and a scrotal ultrasound can be performed to formulate a diagnosis of testicular failure or dysfunction.

Female age is associated with quantity and quality of ovarian reserve, influencing the number and quality of oocytes that may be collected for treatment. It is, therefore, considered as a major factor to estimate realistic chances of success.

The number of consecutive cycles is an important factor in assessing the ultimate probability of a live birth.

Psychological counselling before, during and after treatment is widely practised, as infertility and its investigation and treatment may cause psychological stress [19].

30.4.2 Evaluation of third-party donors

Good Practice Guidelines

G30.14 Third-party donors' evaluation should be performed to rule out any possibility that gamete or embryo donation might involve:

- **G30.14.1** an unacceptable health risk to the recipient or the offspring (e.g. due to the transmission of infectious or genetic diseases) or the donor;
- **G30.14.2** inadequate chances of treatment success.

G30.15 In cases in which embryos from a within-relationship treatment are donated, the couple from whom the gametes originate should be considered as third-party donors and should comply with the general criteria for third-party donation.

G30.16 In order to donate sperm/oocytes/embryos, the potential donor should undergo:

- **G30.16.1** consultation and counselling with a healthcare professional;
- **G30.16.2** completion of a health/medical/donor family history questionnaire;
- **G30.16.3** psychological assessment;
- **G30.16.4** medical examination: gynaecological examination and ultrasound (female donors) or genital examination (male donors);
- **G30.16.5** laboratory testing (including screening for infectious diseases);
- **G30.16.6** blood group typing;
- **G30.16.7** genetic screening:
  - **G30.16.7.1** only recessive severe genetic conditions with a well-defined phenotype, high penetrance and a high carrier frequency in the general population should be the focus of genetic screening;
  - **G30.16.7.2** screening for X-linked diseases should also be performed in female donors;
  - **G30.16.7.3** known recessive carrier status should not be an exclusion criterion except for X-linked conditions, which will be the reason for exclusion;
  - **G30.16.7.4** only variants with clear pathogenicity should be reported;
  - **G30.16.7.5** third-party donors should be encouraged to inform the SoHO entities or establishments concerned, if they are diagnosed with a genetic abnormality after the collection of donated gametes.
- **G30.16.8** karyotype analysis;

G30.16.9 semen analysis, including freeze–thaw tests to assess sperm quality after freezing and thawing (sperm donors);
G30.16.10 assessment of ovarian function and ovarian reserve, including endocrine work-up (oocyte donors);
G30.16.11 assessment of ethnic phenotype and ethnic background of the family of origin;
G30.16.12 reproductive history, including number of pregnancies, deliveries and live children, miscarriages (voluntary and/or spontaneous), children with abnormalities, stillbirths, number of previous donations.

Recommendations

R30.6 During psychological evaluation of third-party donors, it is recommended to focus on an anamnesis including, but not limited to:
- R30.6.1 motivation;
- R30.6.2 pattern of personal stability;
- R30.6.3 downstream psychological implications of being a gamete donor;
- R30.6.4 psychological guidance in the preparations for becoming a gamete donor.

R30.7 Personality and/or psychological diagnostic tests could also be considered [19].

Supplementary information

The CD-P-TO has produced a brochure 'Donation of oocytes: A guide for women to support informed decisions' to provide clear, accurate and balanced information about the donation of oocytes [20]. Examples of genetic screening include cystic fibrosis, spinal muscular atrophy and beta-thalassaemia. For genetic screening, whole genome sequencing can be performed. Sperm donors may carry pathogenic gene mutations originating from gonadal mosaicism. This prompts the possibility of screening donated sperm by whole genome sequencing.

30.4.3 Welfare of third-party gamete donors

Information on recruitment is outlined in Chapter 5 of this Guide.

Recommendations

It is recommended that:

R30.8 very young gamete donors not be included in a donation programme (even if the minimum age limit is 18 years old), but to recruit a relatively older donor group, possibly with proven fertility or fulfilled child-wish. For example, oocyte donors might preferably be accepted after having achieved a successful pregnancy of their own.

R30.9 third-party gamete donation be limited to a single SoHO establishment, especially in the absence of national third-party donor registries.

Supplementary information

In third-party gamete donation, the donors' DNA will be transmitted to future generations. Therefore, donating gametes might have an impact on the donor and his/her partner and family, including their own future children and their offspring. Although the donor identity remains unknown to the recipient, in some countries it is possible that – upon request – that identity may be disclosed to the offspring derived from the donation. Regardless of the legal possibilities for the offspring of gamete donation to learn the donor's identity, the internet and modern technology (e.g. DNA tests) now offer so many other ways to identify a person that a donor can never be given guaranteed anonymity [16].

For both male and female donors, the number of times he/she may donate depends on several factors, such as the number of children and/or families achieved with this donor's gametes, the medical and psychological risks to the donor health and the relevant legislation in the country of donation.

Limiting the number of offspring created from a single donor reduces the risk of inadvertent generation of children from genetically related parents. The determination of this limit depends on several factors, such as the population density of the area where gametes are distributed, the number of children per family and the gender distribution of children. In the absence of robust data, several countries have imposed a limit of five to ten children per donor. Limiting third-party gamete donation to a single SoHO establishment also helps facilitate compliance with limits on the number of donations and the number of recipients allowed or recommended for each donor.

If national registries for gamete/embryo donors, as well as for recipients, do not already exist, their creation is encouraged.

30.5 Testing in within-relationship use and third-party donation treatment

General information on donor screening is provided in Chapter 7.

The purpose of testing in third-party donation and within-relationship use treatments is to prevent transmission of severe infectious and genetic diseases from the person providing gametes or embryos to the recipient and the offspring derived from the treatment. Testing is also aimed at protecting staff during processing of samples contaminated with blood-borne and other transmissible pathogenic organisms.

Testing is discussed here separately for the two contexts of within-relationship use and third-party

donation. In general, less stringent biological testing is appropriate in cases of within-relationship use.

30.5.1 Within-relationship use testing

Recommendations on the time intervals between serology testing and sample collection are given in Chapter 7 (G7.12).

Recommendations

It is advised that:

R30.10 women starting MAR treatment undergo testing to detect human papillomavirus (HPV)-related cervical lesions.

R30.11 biological testing may be omitted for sperm processed for IUI and not intended for storage, provided that the SoHO establishment can demonstrate, through validated procedures, that the risks of cross-contamination and personnel exposure have been assessed and considered to be sufficiently low.

30.5.2 Third-party donation testing

General information on periodicity of testing is shown in Chapter 7 (Table 7.1, section 7.2.1.2.3.2, G7.13, G7.14).

Good Practice Guidelines

G30.17 Serological tests should be performed for *Chlamydia trachomatis* and *Neisseria gonorrhoea*, in cases of third-party sperm donation. In the EU, this should be carried out by a nucleic acid test (NAT) using a urine sample.

Supplementary information

Testing for *Cytomegalovirus*, *Mycoplasma* or *Ureaplasma* may be required by national regulations.

Latest epidemiological updates can be found at the European Centre for Disease Prevention and Control [21] and country-specific risk as reported by the Joint United Kingdom (UK) Blood Transfusion and Tissue Transplantation Services Professional Advisory Committee JPAC (transfusionguidelines.org/dsg/gdri/guidelines).

30.6 Collection

30.6.1 Collection of semen by ejaculation

Good Practice Guidelines

G30.18 Patients should be provided with clear instructions regarding the collection of the sperm sample (hygiene, sexual abstinence, timing, etc.).

G30.19 To collect the semen, patients should be provided with a sterile collection container tested for sperm toxicity, following thorough cleaning of hands and genital area.

G30.20 Patients should be instructed to deliver the sample to the laboratory as soon as possible following collection, avoiding temperatures outside the 20–37°C range.

G30.21 Semen analysis should start within one hour of collection.

G30.22 For purposes of sample traceability, records should be kept of the type of container used, the time of collection and the time interval between collection and analysis/preparation.

G30.23 Use of medications, fever during the previous months and possible partial loss of the ejaculate during collection should be documented.

Supplementary information

Ejaculated semen is usually obtained through manual stimulation; more rarely, through penile vibratory stimulation or intercourse using specially designed condoms free of spermicidal substances.

30.6.2 Surgical collection of sperm

Recommendations

R30.12 It is recommended, in patients diagnosed with non-obstructive or obstructive azoospermia, to collect the sperm surgically from the testis or epididymis in an operating room.

Supplementary information

Collection method is dependent upon the specific aetiology. A surgical method may be used for patients who cannot produce an ejaculate generally by masturbation.

- Collection of sperm from the epididymis – Percutaneous epididymal sperm aspiration (PESA) – is a method for sperm collection if the vasa deferentia are blocked. It involves the use of a sterile needle to aspirate sperm from the epididymis without a surgical incision. Sperm can also be collected from the epididymis by microsurgical sperm aspiration (MESA).
- Collection of sperm from the testis – An alternative to sperm collection from the epididymis is collection of sperm from the testis. This can be performed by testicular sperm extraction (TESE or micro-TESE, depending on the degree of invasiveness) involving tissue removal (testis biopsy). Collection may be accompanied by histopathology investigation for diagnostic purposes. TESE can also be undertaken *via* a percutaneous approach – referred to as testicular sperm aspiration (TESA) – using a sterile fine needle or a biopsy needle. This is a less invasive procedure, but it usually results in relatively less collected material.

Considering the short duration of exposure to the environment, the risk of semen contamination is

generally very low; therefore, strict environmental measures are not required when samples are produced via masturbation in semen collection rooms. If necessary, the semen sample can also be collected in a domestic environment, if allowed by national regulations, provided that the patient is appropriately instructed on how to transport the sample to the laboratory.

30.6.3 Retrograde ejaculation

In cases of retrograde ejaculation, the sperm is released in the urinary bladder after ejaculation. In these cases, sperm can be collected from the urine after voiding, if the urine pH has been increased by administration of alkaline bicarbonates via either oral intake or urinary catheterisation. Should this method yield very low-quality sperm, epididymal or testicular biopsies are alternative options.

30.6.4 Collection of semen by electro-ejaculation

In some patients (e.g. in case of spinal cord injury, pelvic surgery, multiple sclerosis, diabetes mellitus with nerve involvement, unexplained anejaculation), ejaculation by masturbation is not possible. In these cases, ejaculation can be electrically stimulated using a rectal probe. This low-voltage stimulation is usually sufficient to produce an ejaculate. However, the quality of the ejaculate is often not as good as the one obtained by masturbation. Also in this case, epididymal or testicular biopsies may represent a better option.

30.6.5 Oocytes

Oocytes can be collected following patients' natural cycle or after gonadotropin treatment to stimulate the growth and maturation of multiple ovarian follicles and associated oocytes.

Good Practice Guidelines

G30.24 During treatment, the patient should be monitored to assess the response to hormonal treatment and the associated risk of ovarian hyperstimulation syndrome (OHSS).
G30.25 Oocytes should be collected through transvaginal ultrasound-guided ovarian puncture and aspiration of follicular contents.
G30.26 The collection should be carried out under local anaesthesia (paracervical block), sedation or, rarely, general anaesthesia [22].

30.7 Processing

Good Practice Guidelines

G30.27 Reproductive SoHO preparations should be preserved in a viable and functional status before and during application; this does not allow the application of tests that are invasive or affect cell viability or DNA integrity.

Supplementary information

The following section is based largely on the *Revised guidelines for good practice in IVF laboratories* by ESHRE [23]. These guidelines were drawn up by the ESHRE Special Interest Group (SIG) Embryology and constitute the minimum requirements for any laboratory performing MAR procedures. Reproductive SoHO preparations may be contaminated during collection and processing. Contamination of the culture systems by micro-organisms could limit the success rates because of low fertilisation rates, poor embryo development, embryo degeneration etc. [24]. However, the technical specificities by which reproductive SoHO preparations are processed vastly reduce the likelihood of contamination [25]. More importantly, the probability of causing infection in the recipient is extremely limited [26, 27]. There are several risk factors relevant to microbiological contamination when considering the specifications of the laboratory air quality. The risk of contamination may not be the same for sperm cells, oocytes and embryos, due to the respective characteristics of these SoHO preparations. Microbiological sample testing is a general requisite for the clinical application of SoHO. In the case of MAR, testing each single SoHO preparation sample that is processed and released is challenging or impossible. In principle, very small volumes of spent media can be used for testing; however, relevant results are often affected by high false-negative rates and, in any case, are generated with timings that are too delayed to give an opportunity for intervention during processing. Regardless, the clinical procedures applicable to gametes and embryos involve far fewer and smaller risks compared with organ or tissue transplantation.

30.7.1 Premises for processing of gametes and embryos

30.7.1.1 *Laboratory design and management of contamination risk*

General information on risk management and premises is shown in Chapters 4 and 9.

Good Practice Guidelines

G30.28 Laboratory construction should ensure aseptic and optimal handling of SoHO preparations during all phases of treatment. To achieve this, air quality of appropriate grade, positive air pressure and high-efficiency filtration of micro-particles of the air supplied to the gamete/embryo incubators or the laboratory should be considered [23, 28].

Recommendations

R30.13 It is recommended that the laboratory handling gametes and embryos be located as close as possible to the operating room where clinical procedures are performed.

Supplementary information

Use of volatile organic compound (VOC) filters may be appropriate.

In the laboratory process of MAR, the risk of microbial contamination is intrinsically low and it is even further reduced by several conditions or procedures, such as:

a. presence of antibiotics in all media used for gamete and embryo handling and culture, which largely constrain microbial growth;
b. use of an oil overlay in association with the culture vessel, which creates a physical barrier between the medium where gametes and embryos are cultured and the surrounding atmosphere;
c. routine repeated washing steps of gametes and embryo through fresh medium.

30.7.1.2 *Laboratory equipment*

Good Practice Guidelines

G30.29 The number and type of incubators is a critical factor and should be proportional to the laboratory workload, based on the number of cycles and embryo culture strategy. Other equipment, such as cryotanks, air-flow cabinets, and micromanipulation stations, should be scaled up in proportion to the workload.

30.7.2 Processing of gametes and embryos

Good Practice Guidelines

G30.30 Reproductive SoHO preparations handling should be performed aseptically, using at all times techniques aimed at reducing the risk of microbial contamination.
G30.31 Individual pipetting devices should solely be used for one type of procedure and never used for more than one patient. If possible, preference should be given to unit-dose sterile disposable pipettes.
G30.32 Each SoHO preparation sample should be separately handled in order to prevent cross-contamination or mix-up of samples.

Supplementary information

Some laminar-flow hoods intended for IVF use cannot adhere to Grade A requirements concerning the speed of the laminar flow; however, they do adhere to Grade A requirements of particle count. It is therefore important to verify the specification of laminar-flow hoods and classify them accordingly; see Chapter 8, section 8.2.

Certain processes, such as ICSI and embryo biopsy, can be performed outside the laminar hood because they require manipulation using an inverted microscope.

30.7.3 Oocyte processing

Good Practice Guidelines

G30.33 The time between oocyte retrieval from follicles and start of culture should be minimised to avoid prolonged oocyte exposure to follicular fluid and blood.
G30.34 Follicular aspirates should be searched for the presence of oocytes using a stereomicroscope equipped with a heated stage, usually at 8-60 × magnification.
G30.35 Exposure of oocytes to high-energy light generated by microscopes should be minimised.
G30.36 Number of collected oocytes should be documented.

Supplementary information

Oocytes are very sensitive cells, requiring strict control of culture conditions and efficient and rapid handling [23].

30.7.4 Sperm processing

Good Practice Guidelines

G30.37 A pre-clinical sperm preparation test should be performed to determine the most suitable preparation and insemination technique on the day of treatment, based on sample characteristics.
G30.38 A frozen back-up sample should be requested if difficulties with sperm collection or the absence of the male partner on the day of oocyte retrieval are anticipated.
G30.39 The number of motile spermatozoa after test thawing needs to be adequate for the intended use: intra-uterine insemination (IUI), routine IVF or micro-injection (ICSI).
G30.40 Post-thawing sperm should be assessed prior to use.

Recommendations

It is recommended that:

R30.14 ejaculated sperm samples be processed to:
R30.14.1 eliminate seminal plasma, debris and contaminants;
R30.14.2 concentrate progressively motile sperm;
R30.14.3 select against morphologically abnormal and immotile sperm;
R30.14.4 promote capacitation, especially if standard IVF is indicated as insemination technique.
R30.15 in cases of azoospermia or extremely poor semen sample quality on the day of oocyte re-

trieval, a second semen sample be requested before considering alternative sperm-retrieval procedures or delaying treatment after oocyte cryopreservation;

R30.16 the sample be exposed to phosphodiesterase inhibitors (such as pentoxifylline, theophylline) or hypo-osmotic solutions when motile sperm are not observed, as these methods can identify and select live cells for use – by inducing motility and changes in tail shape, respectively [29];

R30.17 a frozen back-up sample be requested in cases presenting with very severe oligo-asthenoteratozoospermia (OAT).

Supplementary information

Swim-up technique and discontinuous density-gradient centrifugation are the most frequently used and widely accepted techniques to process sperm samples.

For surgically retrieved sperm, several techniques are available to maximise sperm recovery and select viable sperm [30]. In case of epididymal recovery, the aspirate is generally processed by swim-up or discontinuous density-gradient centrifugation, depending on sample abundance. For testicular sperm, mechanical procedures to harvest the sperm from the tissue may be combined with enzymatic treatment (e.g. collagenase) in order to facilitate release of sperm from tissue and increase recovery rates [19].

Simple 'washing', i.e. centrifugation in the absence of density gradient, can be considered in cases of severe oligoasthenoteratozoospermia.

30.7.5 Insemination of oocytes

Good Practice Guidelines

G30.41 Oocytes should be inseminated by standard IVF or ICSI. Insemination times should be compatible with the execution of fertilisation checks during working times, if time-lapse microscopy technology is not available, both in standard IVF and ICSI.

G30.42 The insemination/injection time should be decided on the basis of the number of hours elapsed from ovulation induction and/or oocyte retrieval. For example, if oocyte retrieval is performed at 36 hours from ovulation induction, oocytes should ideally be inseminated within the following four hours.

Standard IVF

G30.43 The number of progressively motile sperm used for insemination should be appropriate to optimise the chance of normal fertilisation.

G30.44 The final sperm suspension generated by the processing protocols should be prepared in the smallest possible volume using a medium compatible with the medium used for oocyte fertilisation, where the suspension will be finally diluted down to the desired sperm concentration.

ICSI

G30.45 Preparation of oocytes for ICSI:

G30.45.1 When removing cumulus cells from oocytes through enzymatic and mechanical treatment (pipetting), hyaluronidase concentration and exposure should be kept to a minimum.

G30.45.2 To prevent oocyte mechanical damage, pipettes with appropriate lumen size should be used and vigorous pipetting should be avoided.

G30.45.3 After denudation, oocytes should be thoroughly washed to remove traces of hyaluronidase.

G30.45.4 The maturation stage of the oocytes should be recorded. In standard practice, only metaphase II oocytes should be used for treatment.

G30.45.5 Both the overall time for injection and oocyte exposure to suboptimal conditions outside the incubator should be minimised. To this end, oocytes of a patient cohort are injected in groups within a single injection dish. The number of oocytes in such groups should be adjusted in accordance with the operator's skills and the sperm quality.

Sperm injection

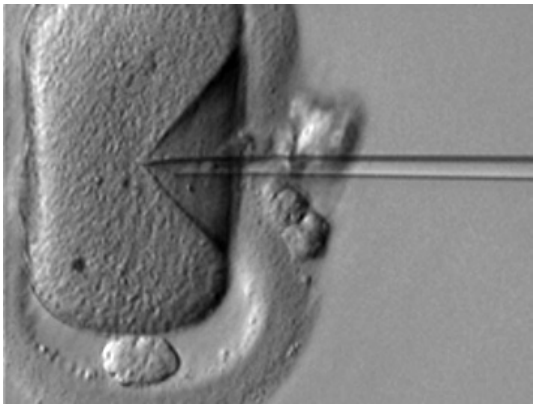
G30.46 The time for selection, immobilisation and injection of the individual spermatozoon used for each oocyte injection should be minimised.

Supplementary information

In standard IVF, co-incubation of cumulus oocyte complexes and sperm is usually performed overnight, although a shorter period may be sufficient. A progressively motile sperm concentration between 0.1 and 0.2×10^6/mL is commonly used.

In sperm injection during ICSI (Figure 30.2), viscous substances, such as polyvinylpyrrolidone (PVP) or hyaluronic acid/hyaluronan-based alternatives, are used to facilitate sperm manipulation, selection and injection.

Figure 30.2 **Intracytoplasmic sperm injection (ICSI)**



Source: Image provided by Thomas Ebner (Austria).

30.7.6 Assessment of fertilisation

Good Practice Guidelines

G30.47 Fertilisation assessment at a single time point should be performed under high magnification (at least 200 ×) at 16 to 17 hours post-insemination, using an inverted microscope equipped with Hoffman or equivalent optics to allow verification of the number and morphology of pronuclei (PN).
G30.48 In standard IVF, cumulus cells should be removed and zygotes transferred into fresh dishes containing pre-equilibrated culture medium.

Recommendations

R30.18 It is recommended that users of this Guide adopt the updated guidelines for fertilisation and embryo assessment that are included in the recently revised consensus document 'The Istanbul consensus update: a revised ESHRE/ALPHA consensus on oocyte and embryo static and dynamic morphological assessment' [31].

Supplementary information

The use of time-lapse microscopy devices allows the assessment of fertilisation independent of observing a single time point at 16-17 hours post-insemination. A normal fertilised oocyte (zygote) contains two pronuclei and two polar bodies (Figure 30.3). New PGT technologies are able accurately to identify the ploidy status of pre-implantation embryos generated from oocytes classified as atypically fertilised [32].

30.7.7 Embryo culture and transfer

Good Practice Guidelines

G30.49 Embryo assessment should be performed at high magnification (at least 200 ×, preferably 400 ×) using an inverted microscope with Hoffman or equivalent optics.
G30.50 Evaluation of cleavage-stage embryos should include cell number, relative blastomere size and symmetry, percentage of fragmentation and nuclear status (e.g. multinucleation).
G30.51 Blastocyst scoring should include expansion grade (associated with size of blastocoel cavity) and morphology of both cell populations forming the inner cell mass and the trophectoderm.
G30.52 Assessment should be performed at standardised time points post-insemination and reported accordingly.
G30.53 The decision on the number of embryos to transfer should be based on embryo quality and stage of development, female age, ovarian response, rank of treatment and, if applicable, national regulations.
G30.54 The main laboratory should be in close proximity to the embryo transfer room, and arrangements should be made to maintain medium temperature and pH while safely transporting embryos.

Recommendation

R30.19 Embryo culture to the blastocyst stage is recommended when appropriate. To this end, culture may be extended for up to seven days after insemination based on embryo quality and specific treatment requirements.

Supplementary information

Embryo selection for transfer is primarily based on developmental stage, cleavage kinetic and morphological aspects. Deselection or deprioritisation criteria, such as abnormal morphokinetics and irregular cleavages, may be considered [33]. During embryo evaluation cytoplasmic granulation and vacuolisation may also be noted, if particularly pronounced. An ESHRE/ALPHA consensus document offers comprehensive guidance for embryo assessment and prioritisation for use [31].

A recent ESHRE recommendation document has identified elective single embryo transfer as the most appropriate practice in most cases [34].

Choice and mutual compatibility of culture media, culture devices and incubations demand careful consideration to assure optimal and controlled culture conditions.

Embryo development (Figures 30.4, 30.5 and 30.6) can also be assessed using time-lapse imaging, allowing a dynamic evaluation of the timing of consecutive events during embryo culture [35], without the need of removing the embryos from the incubator. These systems also facilitate the application of more stable culture conditions.

30.7.8 Pre-implantation genetic testing (PGT)

Good Practice Guidelines

G30.55 In PGT cases, cells required for genetic analysis should be biopsied using glass microtools, and possibly a laser device, as parts of a micromanipulation apparatus.
G30.56 Trophectoderm biopsy of blastocysts should be considered the method of choice (Figure 30.7).
G30.57 Biopsy samples and embryos should be handled individually, and labelled uniquely, avoiding DNA contamination from other cells or from the operator and sample mix-up.
G30.58 Genetic counselling should be available to all couples known to carry a severe hereditary disease.
G30.59 The recipient should be informed that genetic testing on embryos does not substitute for prenatal analysis, such as amniocentesis.
G30.60 The proposal of PGT-A treatment should be accompanied by full information on its objective clinical value [35].

Supplementary information

Although not recommended, in specific cases the biopsy procedure can be also applied to:

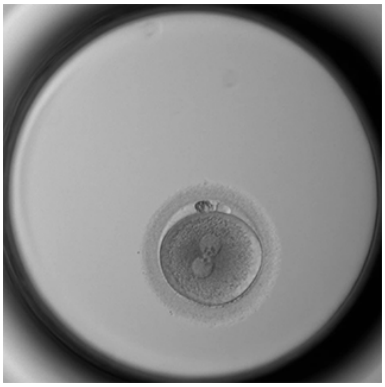
- first and second polar body;
- blastomeres of day three embryos (Figure 30.8).

Further and more detailed recommendations for biopsy in PGT may be found in the relevant ESHRE recommendation document [36].

Indications for the diverse forms of PGT are mentioned in the introduction of this chapter. PGT-A is specifically indicated for women of advanced reproductive age or with a history of recurrent miscarriages or implantation failure. It is considered as a complement to standard morphological selection of embryos for transfer. Embryo selection using PGT-A may decrease the number of embryo transfers necessary to obtain a pregnancy, thus in principle reducing the time to pregnancy. However, the cumulative results are similar compared with non-PGT-A cases [37, 38].

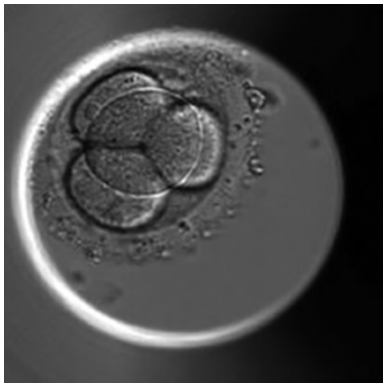
In some countries, according to national legislation, PGT may not be allowed or may only be permitted in specific cases. Other less common applications, such as the generation of histocompatible siblings for cell/tissue donation can be considered, case by case.

Figure 30.3 **Zygote with 2 pronuclei and 2 polar bodies**



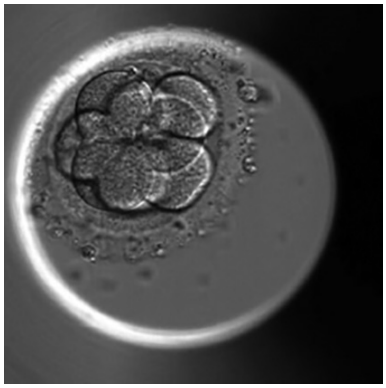
Source: Image provided by Maria Jose De los Santos Molina (Spain).

Figure 30.4 **Embryo at the 4-cell stage**



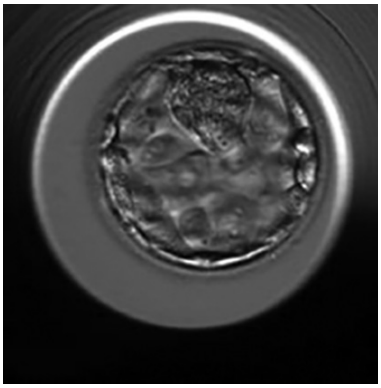
Source: Image provided by Maria Jose De los Santos Molina (Spain).

Figure 30.5 **Embryo at the 8-cell stage**



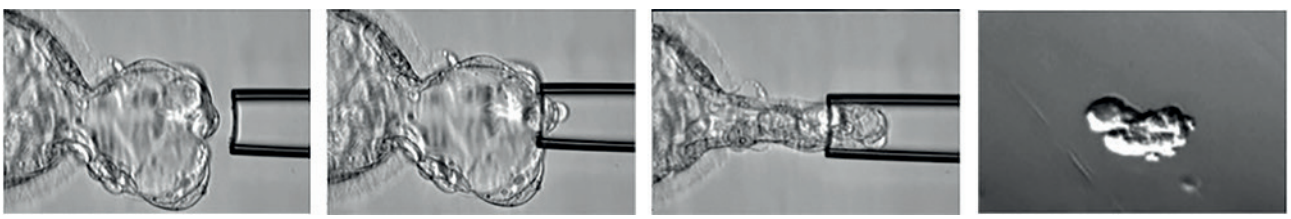
Source: Image provided by Maria Jose De los Santos Molina (Spain).

Figure 30.6 **Embryo at the blastocyst stage**



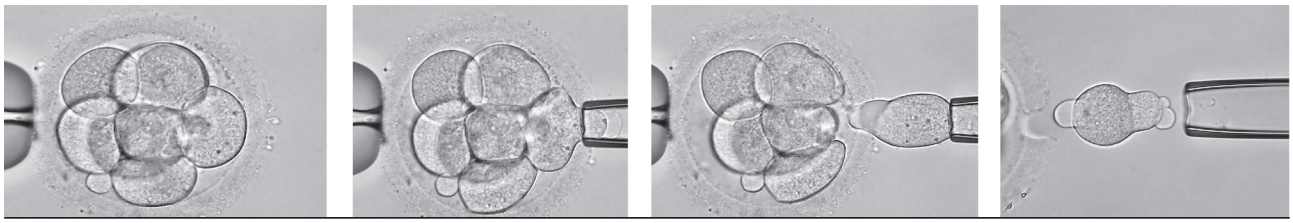
Source: Image provided by Maria Jose De los Santos Molina (Spain).

Figure 30.7 **Trophectoderm biopsy of a blastocyst**



Source: Image provided by Cristina Magli (Italy).

Figure 30.8 **Blastomere biopsy of an 8-cell stage embryo**



Source: Image provided by María José de los Santos Molina (Spain).

30.7.9 *In vitro* maturation

Recommendations

R30.20 Bearing in mind the insufficiency of data from prospective clinical trials and meta-analyses, it is recommended that IVM be considered as an innovative, although not experimental, procedure and that the couple be given full information on its current value and the lack of extensive and long-term outcome data [39].

Supplementary information

IVM refers to the maturation *in vitro* of immature oocytes cultured in specially formulated media [40]. During IVM, such oocytes progress through meiosis from prophase I to metaphase II (MII). Achieving the morphological criterion for MII classification (extrusion of the first polar body, or PB) does not necessarily imply that the oocyte is competent for normal development. IVM was introduced in MAR in the early 1990s. Since then, it has been the subject of continued scientific investigations aimed at improving the rates of mature oocytes obtained *in vitro* and ultimately clinical success. Full optimisation of oocyte IVM remains unaccomplished. Therefore, IVM has not become a mainstream treatment approach and currently remains mainly applicable to cases of fertility preservation or high ovarian follicle count, such as women presenting with polycystic ovary (PCO) or PCO syndrome (PCOS).

30.7.10 Processing of samples from seropositive subjects in a within-relationship use context

Positive results of screening for transmissible diseases do not exclude processing, as long as specific procedures are adopted to reduce the relevant risk. General information on screening is outlined in Chapter 7.

Good Practice Guidelines

G30.61 Gametes and embryos from virus-positive patients with transmissible communicable diseases should be handled separately, using dedicated equipment (cabinet or vertical laminar-flow hood) and/or scheduled for processing at the end of the daily workflow. If a Class II biosafety cabinet (BSC) is available, it should be used for semen handling.

G30.61.1 After processing, the equipment and surrounding area should be disinfected.

G30.62 Reduction in the risk of both horizontal transmission to the partner/family/healthcare providers and vertical transmission to the infant should be pursued, without affecting the effectiveness of treatments.

G30.63 Hepatitis B-seronegative individuals with seropositive partners should be offered vaccination and should consult with an infectious disease/liver disease specialist.

G30.64 For couples with seropositive males, sperm should be processed using density gradient separation of the semen sample and optional swim-up.

G30.65 If Zika virus is detected before MAR, treatment should be postponed for three months depending on whether the male or the female partner is affected [41].

Recommendations

R30.21 It is recommended, in treatment of HIV-positive couples, that the processed sample be given NAT regardless of the semen-processing procedure [4].

Supplementary information

Updated international and national guidelines have been developed for the management of MAR in cases of couples undergoing MAR that are carriers of infections/diseases (HBV, HCV, HIV, HPV, HTLV I/II, Zika, SARS-CoV-2) [4, 42]. See also Chapter 7 for guidance.

Spatial and/or temporal separation when handling specimens from virus-positive patients with transmissible communicable diseases (see Chapter 7) enables effective isolation while maintaining use of existing laminar-flow workstations, with appropriate personal protective equipment (PPE).

A class II BSC ensure appropriate biological containment through the application of negative pressure on the working area where the specimen is processed, while protecting the operator. Its use is especially pertinent to semen processing, since male patients with active viral infection may still be eligible for MAR treatment, whereas this is typically contraindicated in women.

30.8 Cryopreservation

30.8.1 Methods for gamete and embryo cryopreservation

Sperm, oocytes and embryos can be cryopreserved for future use in MAR treatments, in cases of supernumerary oocytes or embryos, fertility preservation and third-party donor gametes for banking. At present, the two most used methods for cryopreserving gametes and embryos are slow freezing and vitrification.

Slow freezing is a method based on a slow (passive or programmed) step-wise decrease in the temperature of the solution in which the specimen is cryopreserved. The programmed protocol involves specific computerised equipment and programmes for cooling different types of tissues and cells in solutions with cryoprotectant substances. Vitrification is an ultra-rapid cooling method that does not require special cooling machinery, although special consumables are required. It is performed by using cryoprotectants at much higher concentrations compared with slow freezing, although for shorter exposure time intervals. It also operates through very fast cooling and warming rates (4 000-6 000°C/s up to 10 000°C/s), depending on the volume of the vitrification solution determined by the design of the cryopreservation vessel. These conditions prevent formation of ice crystals and greatly reduce cooling-induced cell injury. Vitrification outcome can be operator-dependent.

Different types of male and female gametes and embryos at different stages of development show different sensitivity in response to low temperatures and cryoprotectant agents [43, 44].

Concerns about the safety and quality of cryopreserved human gametes and embryos have been raised, regarding cell damage (disassembly of meiotic spindles, membrane rupture), toxic effects of cryoprotectants, osmotic damage and incomplete dehydration. All these factors can potentially affect cell viability [45]. Overall, however, laboratory and clinical data converge towards the notion that gamete and embryo cryopreservation is a safe and efficient procedure [46, 47].

30.8.2 Sperm cryopreservation

Recommendations

R30.22 It is recommended that testicular biopsy samples be processed before freezing.

Supplementary information

Seminal plasma, immotile sperm and damaged sperm may be removed (by sperm processing) before freezing to select a population of sperm with higher chances of post-cryopreservation survival.

Freezing/thawing of human sperm is a well-proven application using diverse methodologies. Sperm samples are usually cryopreserved in glycerol-based cryoprotectant solutions in cryovials or straws and frozen with a slow freezing protocol using a programmed cell-freezing device. Alternatively, they can be passively cooled at 4°C, temporarily exposed to vapour nitrogen and finally plunged into liquid nitrogen.

30.8.3 Preservation of oocytes

Good Practice Guidelines

G30.66 Vitrification should be the method for oocyte cryopreservation.

Recommendations

R30.23 It is recommended that the oocytes be vitrified within two hours following collection.

Supplementary information

Since the late 2000s, successful cryopreservation of MII human oocytes has been reported worldwide, with rapidly increasing success rates due to optimisation of protocols. Specifically, much improved rates of survival, implantation and pregnancy have been obtained using vitrification, compared with slow freezing [43, 44].

30.8.4 Cryopreservation of embryos

Good Practice Guidelines

G30.67 For the cryopreservation of zygotes, cleavage-stage embryos, morulae and blastocysts, both vitrification and slow freezing can be used. Vitrification is overall superior in terms of both survival rates and clinical outcomes [44] and therefore should be preferred.

G30.68 Post-thawing embryos should have at least 50% intact cells or developmental ability after culture.

G30.69 Blastocysts should have an estimated maximum of 50% degenerated cells and/or evidence of re-expansion observed within 3–4 hours post-warming.

30.9 Storage

General information on storage is provided in Chapter 11, and information on screening for markers is provided in Chapter 7, especially Table 7.2. This table describes which results are associated with no risk of infection, which is the required level of risk before donations can be released.

Good Practice Guidelines

G30.70 Embryos should be stored individually in separate cryocarriers to encourage single frozen embryo transfer.

G30.71 Periodic emptying and cleaning of storage units at long intervals should also be considered according to the manufacturer's instructions, to extend the lifetime of the cryocarriers, as well as periodic decontamination. Regardless, based on risk assessment, all precautions should be taken to prevent damage to the samples induced by temperature changes.
G30.72 Positive serology test results should not exclude donation between partners. Robust procedures should be in place to prevent the risk of contamination, to partner or to personnel, and of cross-contamination.
G30.73 If the results of tests for HIV-1 and -2, HBV or HCV are positive, or if the donor is known to be a source of infection risk, a system of separate handling and storage should be put in place.
G30.74 If the donor is known to be a source of infection risk, a system of separate handling and storage should be put in place.
G30.75 In the case of positive serological tests, within-relationship use should be performed after consultation with a specialist on viral infections.

Recommendations

R30.24 It is recommended that the storage room with liquid nitrogen tanks be located close to the laboratory, so that cryopreserved gametes or embryos can be more safely and rapidly transferred into liquid nitrogen tanks for long-term storage.

Supplementary information

Small volumes of vitrified solutions are susceptible to temperature increases. Nevertheless, gametes, embryos and gonadal tissue kept under appropriate storage conditions can be stored for decades in liquid nitrogen. Use of frozen sperm stored for 40 years has led to the birth of healthy offspring [48]. Successful storage over a long period for oocytes and embryos has also been published [49, 50].

Sperm can be stored in straws or vials, whereas gonadal tissue is stored mostly in vials.

Oocytes and embryos are stored in carriers such as straws, strips or functionally comparable devices, whereby one carrier can hold one embryo or more/few oocytes.

Cryopreservation devices are classified as open or closed systems. Using open storage systems implies that, at some point in the processing, cryopreserved SoHO are in direct contact with liquid nitrogen [51]. In a closed system, there is no direct contact between biological material and liquid nitrogen.

Introduction of contamination in the storage vessel is due to human manipulations during processing. Viral and microbial agents may survive during long periods of time in liquid nitrogen. However, no reports have shown cross-contamination between these environmentally derived pathogens and the preserved reproductive material. Also, storage in liquid nitrogen of reproductive material originating from patients carrying infectious diseases has not led to cross-contamination of other frozen reproductive material residing in the same vessel [52, 53]. Even though evidence is lacking, it can be considered good laboratory practice to store reproductive material of patients with positive serology and negative serology separately. Vapour-phase storage containers have been proposed as an alternative to liquid nitrogen containers to decrease the risk of contamination [52].

Backup vessels are not only useful in emergency situations, but they are also beneficial for periodic maintenance of the cryo vessels.

Medical-grade liquid nitrogen is not sterile, since viral and microbial agents may survive for long periods of time in liquid nitrogen. However, microbial load, generally derived from airborne contaminants and manipulation during processing, is minimal and the presence of pathogenic agents is remote [53]. Some studies have calculated an average of less than 100 colony forming units (CFU) of aerobic micro-organisms/10 kg and less than 10 CFU of anaerobic micro-organisms/10 kg. Indeed, no reports have shown cross-contamination between these environmentally derived pathogens and the preserved reproductive material. Several approaches might be applied to further reduce the risk. The use of ceramic filters is an option, yielding very low rates of bacteria (less than 1 CFU/litre). Ultraviolet (UV) sterilisation (at 253.7 nm) has been also suggested as a modality to reduce microbial load. However, the possible advantage of implementing such measures remains unproven.

In some EU countries, national laws indicate a maximum legal storage period for cryopreserved material.

An emergency plan includes the actions to be taken in case of possible undesired situations such as tank ruptures, liquid nitrogen and power supply failures, liquid nitrogen leaks, natural disasters or even cessation of activity to safeguard the safety of samples and staff [54, 55]. General information on quality management is outlined in Chapter 2.

30.10 Distribution, import/export

General information on distribution, import and export is provided in Chapter 13.

30.11 Coding, packaging and labelling in assisted reproductive technologies

With the new SoHO legislation, an a Single European Code (SEC) is not necessary for SoHO that are intended for within-relationship use and/or that are imported or collected in the same SoHO entity where they are applied (SoHO Regulation 2024/1938 Article 43.2).

More general aspects of coding, packaging and labelling of tissues and cells are described in Chapter 16.

Good practice guidelines

G30.76 At the time of cryopreservation, documentation on biological material should include labelling of devices, cryopreservation method, date and time of cryopreservation, embryo quality and stage of development, number of oocytes or embryos per device, characteristics of semen/sperm, number of devices stored per patient, position (tank, canister) of stored samples.
G30.77 At thawing, documentation on biological material should include thawing method, date and time of thawing and post-thawing sample quality.

Recommendations

R30.25 It is recommended that colour coding of cryovials and straws be used, if available.

30.12 Traceability

General information on traceability is provided in Chapter 17. Records are currently kept for a specified period of time according to European and/or national legislation (see Chapter 17 for EU standards on traceability).

Good Practice Guidelines

G30.78 Each treatment cycle should be assigned a unique identification.
G30.79 Relevant consent forms, clinical data and details of serological exams undertaken by couples/third-party donors prior to admission to the treatment should be validated by a physician and available to the laboratory staff.
G30.80 Rules on correct identification and processing of reproductive SoHO preparations should be established in the laboratory by a system of codes and checks that considers all the following:

G30.80.1 direct verification of patient identity and correspondence with their assigned unique identification. Patients should be asked to give their own identifying information (at least full name and date of birth) before collection or assisted insemination/embryo transfer;
G30.80.2 all devices containing biological material should be clearly and permanently labelled with the unique patient and cycle identification and primarily located on the container itself, not only (if applicable) on the removable lid;
G30.80.3 biological material from different patients should not be processed in the same working area at the same time;
G30.80.4 incubators and cryostorage systems should be organised to ensure easy access and identification of the biological materials therein;
G30.80.5 during well-defined steps, especially if critical (such as gamete preparation, insemination, embryo transfer, cryopreservation and embryo biopsy) for the entire SoHO entity process, traceability should be verified and documented (reporting also time and operator identity) through correct identification of the reproductive SoHO preparations;
G30.80.6 products and materials used with SoHO preparations should be traceable. The time of each manipulation should be documented throughout the treatment. As SEC codes for embryos do not contain the gamete donor ID, the SoHO establishment engaged in distribution or import and export should ensure the traceability of the gamete donors;
G30.80.7 transport of reproductive SoHO preparations should require identification of distributing, importing and exporting institutions, as well as identification of the biological material and its conformity to clinical use. At both distributing and receiving institutions, the accompanying documentation and sample identification on the storage device should be checked to ensure that they correspond with patient records [56].

Supplementary information

During critical steps in MAR processing, SoHO preparations traceability can be executed by use of the four-eye-principle of witnessing (i.e. a double check of the identification by a second person) and/or by use of commercially available electronic identification systems (see Chapter 17 for EU standards on traceability).

30.13 Biovigilance in medically assisted reproduction procedures

General guidance on the implementation of good vigilance and surveillance practice is provided in Chapter 18.

Examples of serious adverse reactions (SARs) reported for MAR are given below. Examples of biovigilance surveillance systems in MAR are provided in Appendices 25 and 27.

In addition, the Notify Library (www.notifylibrary.org) includes many well-documented cases of adverse occurrences in MAR treatments. The database is publicly accessible and can be searched by type of SoHO, adverse occurrence and record ID number.

30.13.1 Serious adverse reactions and events

30.13.1.1 *Serious adverse events*

Serious adverse events (SAEs) may occur at any stage of clinical or laboratory processes (collection, insemination, embryo transfer, cryopreservation). Causes of mix-ups or loss of SoHO can be multiple: processing steps, mislabelling, contamination, human factor involvement, misidentification, absence/failure of witnessing and/or poor-quality systems. The consequences may include reduced or no chance of pregnancy, (genetic) disease transmission, psychological impact and ethical/legal issues.

Some examples of SAEs reported in MAR are:

i. Mix-ups/loss of traceability
   a. mix-up of sperm samples during preparation/treatment;
   b. sperm sample contaminated by another sample (e.g. with a used pipette);
   c. oocytes fertilised with spermatozoa from the wrong person and vice versa;
   d. insemination of a woman with sperm cells from wrong third-party donor;
   e. thawing and use of cryopreserved embryos not belonging to the patient under treatment;
   f. labelling error of tubes/dishes containing the oocytes/sperm/embryos causing confusion or misidentification;
   g. loss of gametes by damage, spillage or other accidental causes.
ii. Loss of gametes or embryos resulting in total loss of chance of pregnancy in one cycle (e.g. technical failure of incubator, cryo equipment or cryo tank, accident with culture dishes);
iii. Embryos destined for culture or freezing were instead destroyed (error in transmission of information);
iv. Gametes or embryos lost due to microbiological contamination;
   a. Adverse events after treatment with gametes from third-party donors.
v. Genetic condition discovered in a sperm/oocyte donor years after the gamete donation (see also section 30.13.2).

30.13.1.2 *Serious adverse reactions*

Serious Adverse Reaction (SAR) means any incident which could be reasonably associated with the quality or safety of SoHO, or their collection from a donor or human application to a recipient, that caused harm to a living donor, to a recipient or to offspring from MAR. Some examples of SARs reported in MAR are:

i. examples of SARs reported in within-relationship use and third-party donation cases:
   a. severe OHSS leading to hospitalisation;
   b. puncture of intestinal loop during oocyte retrieval;
   c. intense bleeding after oocyte retrieval;
   d. ovarian torsion after ovarian stimulation.
ii. Examples of SARs reported in MAR for recipients:
   a. salpingitis after intra-uterine insemination;
   b. bacterial infection of the recipient due to infected sperm;
   c. mix-up of samples in the genetics or IVF laboratory (PGT-M treatment), causing the birth of a baby carrying a genetic disease.

30.13.2 Transmission of genetic diseases by third-party donation

Good Practice Guidelines

G30.81 Parents should be strongly encouraged to inform the SoHO establishment or entity where they received fertility treatment, if a child conceived through a third-party donation is diagnosed with a genetic disease, so that appropriate investigations about the origin of the genetic defect can be put in place.

Supplementary information

SARs (e.g. discovery of a genetic disease in the offspring) do not always mean that all the offspring from a given donor will be affected by the disease or that they will be genetic carriers. The decision on whether to inform other involved recipients and offspring will be based on several factors: the type of genetic condition, the impact on the health of the offspring and future descendants, ethical and legal issues and the existence of a previously signed consent form with regard to the right not to be informed.

Third-party donors may unknowingly carry genetic defects causing a severe disease. Thereby, gamete banks, for example, when distributing sperm or oocytes from an affected third-party donor to multiple recipients could potentially be spreading a severe genetic disease.

The above examples emphasise that forward and backward traceability is of the utmost importance in MAR treatments. More generally, a growing interest has emerged in the right of persons conceived by third-party donation to access information on their origins, for medical or non-medical reasons. This has prompted the release of an analysis of existing legal evidence by the Council of Europe [57]. The institution of national registries would allow traceability of third-party donors and offspring and would play a key role in biovigilance activities.

30.13.3 Cross-border management of serious adverse reactions and events

Individuals travel abroad to access fertility treatment for various reasons (legal restrictions, long waiting times, treatment costs, lack of expertise, quality of treatment).

Recommendations

R30.26 If treatment takes place in countries outside the EU, once couples or individuals return to their country of origin, there is a risk that the SARE is not reported to the Health Authorities or the SoHO entity or establishment. It is advised that the medical teams involved in both countries communicate with each other to ensure adequate treatment and follow-up, ensuring investigation on potential causes and implementation of preventive measures.

30.13.4 Additional considerations

In order to ensure global consistency and harmonisation when communicating regarding MAR, a consensus and evidence-driven set of terms and definitions has been generated [1].

To that end, the use of a unique European database for third-party donor/recipient allocation would allow prompt, rapid and reliable SARE report management.

30.14 Patient follow-up

General information on patient follow-up is provided in Chapter 14, section 14.13.

Systematic follow-up requires recording of basic clinical and treatment information. Relevant records usually include clinical indications, applied procedures, information on materials used for treatment (especially for embryo culture conditions) and reported outcomes. Although achievement of a healthy live birth is the ultimate goal of MAR, other relevant outcomes following treatment are:

a. possible undesired effects of ovarian stimulation or oocyte retrieval (including OHSS and surgical complications, although rare),
b. embryo implantation,
c. biochemical pregnancy,
d. clinical pregnancy,
e. multiple pregnancy,
f. ongoing pregnancy,
g. miscarriage,
h. pregnancy and delivery complications,
i. newborn status at birth.

Short-, medium- and long term post-natal outcomes are very difficult to follow up because, in most countries' registries for MAR, treatment and child health are not integrated. Therefore, mutual linking of such sources of information ensures better monitoring of the safety of MAR.

Implementation of national registries for gamete/embryo donors, as well as for recipients, is highly encouraged.

As a major extension of the European IVF Monitoring (EIM) consortium effort, ESHRE is currently developing the European monitoring of Medically Assisted Reproduction (EuMAR) project, co-funded by the EU-4Health Framework of the European Union. EuMAR aims to develop a European registry of prospective cycle-by-cycle data collection on the application and outcomes of MAR treatments. The project addresses the need for more transparency, surveillance and biovigilance in MAR across country borders, in line with the European regulation's revision of the EU Directives on blood, tissues and cells [6].

30.15 Developing clinical applications

MAR is a rapidly evolving field. Development and implementation of new technology may affect not only donors and recipients, but also future generations. It is therefore important that these procedures are proven to be safe and efficient.

The introduction of MAR techniques into clinical practice may follow the three-levels methodology: 1. experimental, 2. innovative, 3. established [15]. To this end, a scoring tool at each level determines whether a threshold has been reached of sufficient efficacy, safety, procedure and effectiveness (see Figure 30.9). Further information on the introduction of new methodology is reported in Chapter 19.

30.15.1 Use of PRP in fertility restoration

Platelet-rich plasma (PRP) involves the isolation of autologous platelets at high concentration after centrifugation of peripheral blood. The process of centrifugation is assumed to initiate platelet degranulation, which in turn triggers the release of growth factors that may augment cell mitosis, angiogenesis, chondrogenesis, chemotaxis, proliferation and growth.

In the case of MAR it has been hypothesised that PRP may improve folliculogenesis and/or endometrial development. In the case of repeated implantation failure or thin/refractory endometrium, the use of PRP has not been associated with any risk, but there is no evidence regarding the effects in the uterus or embryos.

Although it is a promising treatment, more studies are needed to assess the efficacy and safety due to the lack of safety data [58, 59].

30.15.2 Time-lapse technology

Time-lapse technology (TLT) is a conceptually simple live cell microscopy technique, by which micrographs are acquired at short time intervals and viewed sequentially at accelerated pace in order to observe phenomena and processes that otherwise would go unnoticed. In association with phase contrast optics, TLT is particularly suited to observing dynamically and non-invasively the development of the pre-implantation embryo *in vitro*, whose ability to establish a viable pregnancy is reflected in the achievement of specific developmental stages at defined time intervals. Events such as embryo cleavage,

fragmentation, multinucleation, compaction, blastocoel formation and blastocyst development can be described temporally and dynamically with unprecedented precision and therefore interpreted in a new light.

The debate on the ability of TLT to improve the efficiency of MAR treatments, by ranking embryos of a cohort according to their developmental ability and prioritising their use for embryo transfer, remains currently unresolved [36, 60]. Regardless, TLT has revealed unsuspected developmental behaviours of the embryo, manifested in atypical modalities of second PB extrusion, deregulation of pronuclear formation and breakdown, abnormal cleavage and other uncommon phenomena. Access to such evidence has the potential to extend our appreciation of the biology of embryo development. This can bring significant advantages, irrespective of the need of embryo ranking for transfer. Indeed, TLT has significant potential to rescue for clinical use abnormally fertilised zygotes, fine-tune and streamline embryo static observation, standardise and automate embryo assessment, and develop novel and more sensitive performance indicators. In addition, TLT allows embryo assessment without removal of culture dishes from the incubator, thereby assuring ideal culture conditions, superior to standard embryo culture in conventional incubators.

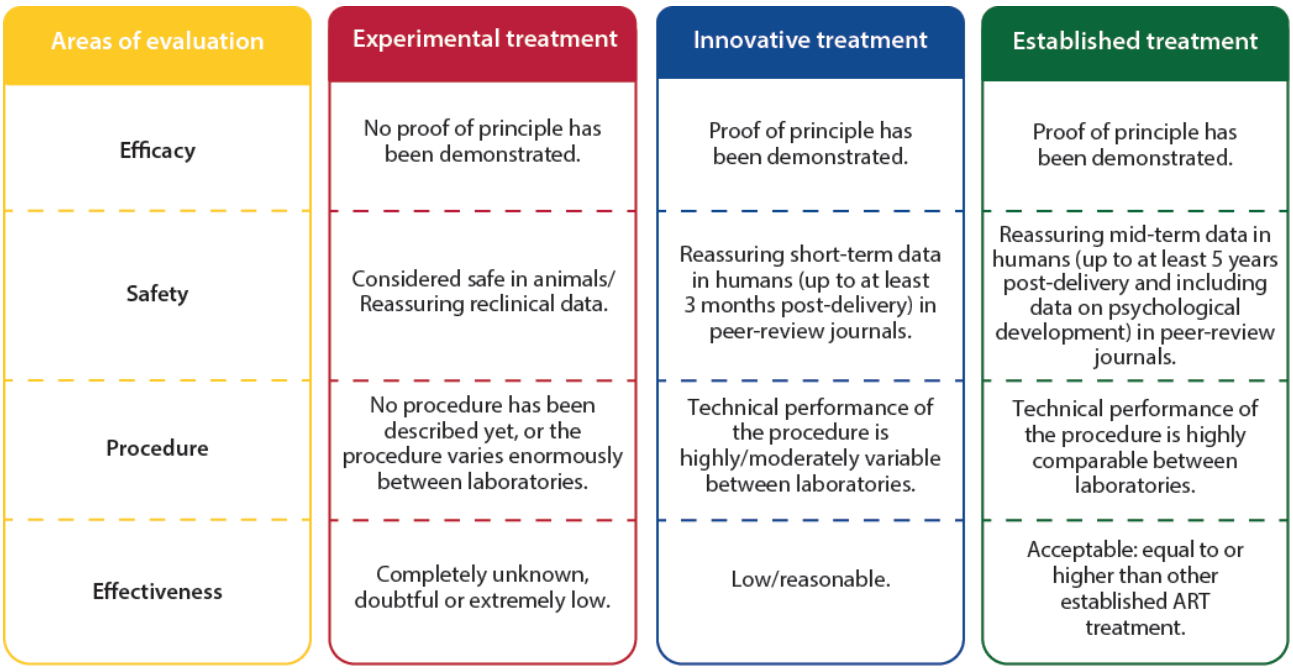
Recent studies also indicate that the full potential of TLT to predict embryo developmental ability can be achieved by combination with advanced imaging and artificial intelligence (AI) approaches [61]. The generation of large amounts of data by TLT, in terms of imaging and morphokinetic timing data, has indeed facilitated the development of AI modalities which are functionally associated with TLT systems. AI tools have been developed for the automated annotation of morphokinetic events, aiming at standardising the annotating process and saving time compared with the manual procedure. In addition, several AI algorithms have been proposed for embryo selection. These algorithms have been trained to predict implantation potential or ploidy status by analysing static photos or video sequences of developing embryos [62, 63]. AI algorithms for embryo selection, some of which are commercially available, claim to boast high predictive ability for implantation or ploidy. However, their introduction into routine clinical practice warrants caution, given that only retrospective validation studies are currently available, underscoring the need for prospective randomised trials to demonstrate clinical benefit.

30.15.3 Non-invasive genetic testing of pre-implantation embryos

Embryo morphological assessment performs relatively poorly in determining embryo quality and predicting implantation. Many embryos that do not implant or end in miscarriage are indeed carriers of chromosomal abnormalities due to inherited meiotic and mitotic errors.

Sampling spent culture media for embryonic cell-free DNA released into the culture medium from both the trophectoderm and the inner cell mass has been proposed as a biomarker to prioritise blastocysts for transfer and reduce time to pregnancy. Embryonic cell-free DNA may reflect the actual genetic status of the embryo without the need for trophectoderm biopsy [64]. This new technology could be able to determine the euploidy status or even single gene mutations of human embryos. The sensitivity and specificity for the ploidy concordance rate between PGT-A and non-invasive PGT-A (niPGT-A) has been reported to range from 76.5% to 91.3% and from 64.7% to 93.3% respectively. Although babies have been born from niPGT-A [65],

Figure 30.9 **Experimental, innovative and established treatments**



optimisation of the protocols (culture conditions, standardisation, contamination issues...) is required to increase informativity rate and accuracy. Extensive validation will also be required before it can be translated into clinical practice as a diagnostic test. Studies have shown that the detection rate of beta-thalassaemia is higher using blastocoelic fluid compared with spent culture medium [66].

30.15.4 Whole genome screening

Recent research developments in the field of genomics have made possible the comprehensive testing of the human genome by combining the methods of next-generation sequencing with advanced bioinformatics. In this way, a complete picture of each individual genome, including single nucleotide variation (SNV) and copy number variation (CNV), allows expanded DNA screening. The application of this approach permits PGT and non-invasive prenatal testing, with expanded carrier screening, but also the disclosure of gamete donor anonymity. The comprehensive information derived from whole genome screening has benefits as well as limitations and risks, and its introduction into clinical practice warrants prudence and genetic counselling [67].

30.15.5 Mitochondrial replacement therapy

Recently in the UK, although under licence by regulatory authorities, novel reproductive technologies known as mitochondrial donation or mitochondrial replacement therapy (MRT) have been introduced into clinical practice. These approaches enable women with mitochondrial DNA (mtDNA) disorders to have healthy, genetically related children [68]. Since mitochondrial transmission is uniparental and of maternal origin, the diseases caused by mitochondrial dysfunction are passed on from mother to offspring in a dominant way. Because there is no cure for mitochondrial diseases, prevention of germline transmission is a priority.

PGT is a possible option to reduce transmission of heteroplasmic mtDNA mutations, although it can be ethically challenging to determine which embryos are to be transferred [69].

MRTs, however, are able to avert transmission of mutations in the mtDNA. As the technique uses part of a donated egg to replace the maternal mutated mtDNA the child would inherit DNA from a 'third person', the mitochondrial donor.

MRTs are a modification of conventional IVF and comprise two main strategies. In the pronuclear transfer procedure, the mother's oocyte with mutated mtDNA is fertilised with the father's sperm and simultaneously a donated oocyte with wild-type mtDNA also is fertilised with the father's sperm. Then, the parental pronuclei are removed from the zygote with mutated mtDNA and transferred into the enucleated zygote with wild-type mtDNA. Finally, the embryo with wild-type mtDNA is transferred to the uterus.

The maternal spindle transfer technique starts at an earlier stage of oocyte development. The metaphase II spindle and associated chromosomes are transferred from the mother's oocyte with mutated mtDNA to an enucleated donor oocyte with wild-type mtDNA. Then, the reconstructed oocyte is fertilised with the father's sperm and the embryo with wild-type mtDNA is transferred to the uterus. Maternal spindle transfer is technically more challenging than pronuclear transfer. One zygote, however, is discarded with the pronuclear transfer technique, which could raise ethical concerns, whereas no zygote is destroyed with the spindle transfer technique.

MRT is still considered on a case-by-case basis. Few successful case reports have been published and long-term results are lacking. ESHRE released a relevant recommendation stating:

> Mitochondrial replacement therapy is considered experimental. Furthermore, there is insufficient evidence of a benefit on pregnancy outcomes or safety. Therefore, it is to be applied in strict research protocols, ensuring the safety of the patients and donors involved, as well as guaranteeing long-term follow-up of their offspring [29].

30.15.6 Gene editing

Gene editing includes a group of technologies that allow genome modification by inserting, deleting, replacing or modifying genetic material at specific locations in the DNA sequence. In the case of genes carrying a mutation, the enzyme cuts the mutation and replaces it with the correct DNA sequence, making it of great interest in the prevention and treatment of human diseases. This approach can be used for somatic gene editing for the treatment of not only genetic disorders, but also cancer and infectious diseases. Ethical concerns arise when gene editing is used to alter genes in gametes or in the embryo, introducing changes that will be passed to future generations.

In 2015, a study from China was published, reporting genome editing by CRISPR-Cas9 on non-viable human embryos [70]. The following year, the United Kingdom issued the world's first endorsement of a national regulatory authority for research on human embryos using genome editing. Additional studies have demonstrated how human germline gene modification is rapidly progressing from the experimental field to clinical research applications [71]. The results obtained so far raise high expectations regarding possible therapeutic applications in humans, but much remains to be considered before clinical applications, including the reproducibility of the technique and possible long-term consequences [72].

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Related material

Appendix 11. Example of consent form: female (HFEA, UK)
Appendix 12. Example of consent form: female (CNPMA, Portugal)
Appendix 13. Example of consent form: male (HFEA, UK)
Appendix 25. Example of serious adverse reaction (SAR) for reproductive SoHO
Appendix 27. Example of serious adverse event (SAE) for reproductive SoHO

Chapter 31. **Fertility preservation**

31.1 Introduction

Fertility preservation (FP) can be applied for both medical and non-medical reasons, despite some differences in technical approaches and expected results.

FP involves biomedical interventions undertaken in order to avoid, delay, diminish or circumvent the exhaustion of the germ-cell pool of the individual. In most current circumstances, either in anticipation of the effects of cytotoxic therapy applied to treat a severe disease or for a number of possible reasons causing postponement of parenthood, this involves cryopreservation of gonadal tissue, gametes or embryos. FP is usually offered to pre-pubertal girls and boys and to males and females of reproductive age at risk of losing their reproductive potential due to either malignant or non-malignant diseases. For non-medical conditions such as postponement of parenthood, previous vasectomy, transgender treatments or other reasons, gamete cryopreservation is also an option.

This chapter describes the indications for male and female FP and the techniques currently available for the cryopreservation of reproductive cells and germinal tissue. The multidisciplinary collaboration between all the medical specialists involved, such as paediatricians, oncologists, psychologists and reproductive specialists, ensures proper evaluation and counselling for each patient. Patient assessment and approach will depend on disease, age and treatment. FP and subsequent restoration may include experimental techniques, whose availability may be restricted according to national legislation. FP is not to be interpreted in a narrow sense, but rather as the possibility for patients to preserve or protect their own gametes or germ tissue for future use to facilitate pregnancy or restore endocrine function.

This chapter therefore defines specific requirements for FP treatments, and procedures may vary from country to country as determined by national legislation.

31.2 Female fertility preservation

The clinical indications for FP include situations in which fertility loss is predicted as a consequence of a cytotoxic treatment for a specific disease (e.g. in oncological patients) [1, 2].

Good Practice Guidelines

G31.1 Information about possible options and future use of cryopreserved gametes or germinal tissue should be discussed with patients, or with the parents/legal guardians along with the patient (in the case of patients who are minors).

G31.2 All pre-pubertal girls and women of reproductive age newly diagnosed with specific medical conditions (e.g. certain cancers or (auto)immune pathologies) and whose treatment may cause premature ovarian insufficiency should be considered for the possibility of FP, and where this is a potential option that the patient wishes to pursue they should be referred to a fertility specialist for counselling about the risk of infertility and for information about available fertility preservation options. In the case of minor patients, this discussion should take place with the patient and, as appropriate, with their parents or legal guardians.

G31.3 The potential downsides of undergoing FP, including possible delay of the cancer treatment, should be weighed against the benefits of having reproductive cells and/or tissues stored for future use. The implications should be discussed and FP (if appropriate) should be offered before cancer treatment is started.

G31.4 For the health of transgender and gender diverse people, they should be given the most up-to-date standards of care [3], and before hormone treatment they should be informed about the possibility

of FP treatment and the possible use of their gametes in the future [4].

G31.5 All patients should be made aware of relevant details, such as the expected survival rate of oocytes after cryopreservation as well as the biological limitations associated with pregnancy due to age-dependent oocyte aneuploidy.

Supplementary information

For some patients, FP can be performed after first line treatment [5, 6]. Factors such as age, ovarian reserve, gonadotoxicity of the treatment and the time available before treatment starts will help in the decision whether to undergo FP [7].

The Council of Europe European Committee on Organs, Tissues and Cells (CD-P-TO) has produced the booklet 'Fertility preservation: A guide for people facing an illness or life events that may affect their fertility', published by the European Directorate for the Quality of Medicines & HealthCare (EDQM). This booklet provides clear, accurate and balanced information about FP [8].

31.2.1 Oncological reasons for fertility preservation

Chemotherapy and radiation therapy may cause depletion of the pool of primordial follicles in the ovaries of girls or premenopausal women and thus render them infertile. Once ovaries are exhausted of follicles, the patient will experience premature ovarian insufficiency and infertility. In the case of pre-pubertal girls, loss of the entire stock of primordial follicles implies that the girl will not enter puberty spontaneously and that she will not be able to become pregnant with her own oocytes later on in life [9]. This is of course a very serious side-effect of an otherwise efficient cancer treatment and is, by many, considered a significant reduction in the quality of life. As more and more girls and women at reproductive age survive a malignant disease today, these unwanted side-effects will affect an increasing number of adults in the population.

It is well known chemotherapeutic drugs belonging to the group of alkylating agents cause the most severe damage to ovaries. Alkylating agents, such as cyclophosphamide or busulfan, are used to treat various forms of cancer, including breast cancer, lymphoma and sarcoma. They are also used in the preconditioning protocol before haematopoietic progenitor cells (HPC) transplantation. As alkylating agents cause damage to both dividing and resting cells, they are very toxic to the oocytes and granulosa cells of the primordial follicles, which are the most immature of the resting pool of ovarian follicles [10].

Radiation therapy, whether given to the abdomen or the spine, will also affect ovarian function. Radiation therapy is very toxic to oocytes, because doses as low as 2 Gy will destroy half of the pool of follicles. Whenever possible, the ovaries are shielded or moved away from the field of radiation, but scatter dose is inevitable [11].

Some genetic conditions may be also a reason for FP. Women carriers of mutations in the BReast CAncer (*BRCA*) genes may be counselled for the prophylactic removal of their ovaries due to an increased risk of developing ovarian cancer compared with the general population [11].

31.2.2 Non-oncological reasons for fertility preservation

Non-malignant diagnoses – such as kidney disease, autoimmune conditions or haematological diseases like aplastic anaemia or thalassaemia – can sometimes be life-threatening and require treatment with alkylating agents or even HPC. Women affected by any severe disease requiring these treatments may also need FP [12].

When ovarian surgery is required, as in the case of severe endometriosis or benign ovarian cysts, or borderline cysts, healthy ovarian tissue containing primordial follicles will inevitably be excised as an effect of the operative procedure. These procedures may pose some threat to the reproductive potential of the patient, and in these cases FP can also be offered [13].

Certain genetic conditions – such as Turner mosaicism, galactosaemia, Fragile X mutation carrier status or blepharophimosis, ptosis or epicanthus inversus syndrome – are associated with premature exhaustion of the pool of primordial follicles in the ovaries, and girls and women at reproductive age suffering from any of these conditions can also be potential candidates for FP [14, 15].

Several options exist to preserve fertility in post-pubertal girls and women. Oocytes, embryos and ovarian tissue can be cryopreserved depending on the characteristics of each individual case and considering the most efficient alternative for every patient [16]. Examples of consent forms for female FP are given in Appendices 11 and 12.

Also, gender affirming cross-hormone treatment for transgender persons is potentially harmful to their fertility. Therefore, transgender males may undergo oocyte collection and storage before cross-hormone treatment and sex-reassignment surgery.

31.2.3 Non-medical reasons for fertility preservation

Non-medical FP can be also considered in young women who want to postpone maternity (age-related FP) [13].

31.3 Male fertility preservation

FP can be driven by medical conditions, such as boys and men facing gonadotoxic treatment or surgical procedures affecting semen production and collection, or specific lifestyle habits.

Good Practice Guidelines

G31.6 Information about possible options and future use of cryopreserved gametes or germinal tissue should be discussed with patients, or with the parents/legal guardians along with the patient (in the case of patients who are minors).

G31.7 The potential downsides of undergoing FP, including possible delay of cancer treatment, should be weighed against the benefits of having reproductive cells and/or tissues stored for future use. The implications should be discussed and FP (if appropriate) should be offered before cancer treatment is started.

G31.8 For the health of transgender and gender diverse people, they should be given the most up-to-date standards of care [3], and before hormone treatment they should be informed about the possibility of FP treatment and the possible use of their gametes in the future [4].

G31.9 FP should be counselled whenever patients are at a high risk of infertility as a consequence of a cytotoxic treatment for a specific disease (e.g. in cancer patients) or due to a benign condition (e.g. severe autoimmune diseases) [17].

G31.10 FP in children should be advised only in cases of high gonadotoxicity treatments, due to the invasiveness of the procedures.

Supplementary information

The CD-P-TO has produced the booklet 'Fertility preservation: A guide for people facing an illness or life events that may affect their fertility', published by EDQM. This booklet provides clear, accurate and balanced information about FP [8].

31.3.1 Oncological reasons for fertility preservation

Chemotherapeutic agents and radiation treatments can adversely affect the male gonadal epithelium. Thus, therapies used to cure cancer (but also used for several non-malignant conditions) may render the patient temporarily or permanently infertile. The amount of damage depends on the regimen, the cumulative dosage of treatments used and the individual capacity of recovery. Mitotically active spermatogonia are highly sensitive to cytotoxic treatments and radiation. Low doses of these treatments deplete the pool of differentiating spermatogonia, while spermatogonial stem cells (SSC) may initially survive, and spermatocytes and spermatids can continue their maturation into sperm. However, when no new precursors are obtained from the stem cell pool, differentiation of germ cells into mature sperm does not occur, which ultimately induces testicular involution [18].

Significant damage is reported after treatment with alkylating agents, of which different threshold doses are reported in the literature (e.g. for cyclophosphamide and cisplatin-based drugs). Both alkylating and platinum-containing agents cause direct DNA and RNA damage, therefore also affecting non-dividing, reserve stem cells [19]. The gonadal epithelium is highly susceptible to radiation-induced damage. Differentiating spermatogonia are sensitive to scattered doses of radiation as low as 0.1 Gy, leading to short-term cessation of spermatogenesis. Cumulative doses above 3 Gy affect SSC and cause long-term azoospermia, whereas doses in excess of 6 Gy deplete the SSC pool and can lead to permanent infertility. Fractionation of radiotherapy increases the germ-cell toxicity. Overall, post-treatment infertility problems are reported in up to 60% of cancer patients [20].

31.3.2 Non-oncological reasons for fertility preservation

As in females, certain non-malignant pathologies such as haematological and autoimmune conditions requiring potentially gonadotoxic treatments could compromise male fertility. Also FP can be offered to men undergoing vasectomy for future reproductive options. Transgender women planning to start cross-hormone treatment and/or undergoing gender – confirming surgery can benefit from FP [21]. Analogous considerations previously explained for transgender men also apply for transgender women.

31.4 Consent in fertility preservation

Good Practice Guidelines

G31.11 It is important that, together with informed consent, in the case of FP for pre-pubertal children, care should be taken to explain to parents or legal guardians the future use of banked gonadal tissue.

Recommendations

R31.1 It is recommended that cryopreserved embryos not be used for reproductive purposes if the couple is separated, unless both members of the couple sign to give written consent for their use. This situation may also be subject to national regulations.

Supplementary information

An example of a generic consent form for cryopreservation of sperm appears in Appendix 13. The form can be used as a template that can be adjusted according to national legislation or common practice of the Medically Assisted Reproduction (MAR) centre. Information with regard to the process, legal time of storage and potential risks can be reported in the consent form or in a related information document.

31.5 Patient evaluation

In addition to the clinical examination of the disease to be treated, patients who are candidates for FP undergo evaluation similar to that for patients undergoing MAR techniques under the new Substances of Human Origin (SoHO) regulation (Chapter 30).

Good Practice Guidelines

G31.12 Close interdisciplinary co-operation between paediatric oncologists and gynaecologists, fertility specialists, urologists, andrologists, paediatric surgeons, psychologists or other medical specialists should be required [7].
G31.13 In the case of pre-pubertal girls, the assessment of the ovarian reserve by measurement of blood levels of anti-Müllerian hormone (AMH) should be investigated. AMH levels should be interpreted cautiously due to a natural, gradual increase in concentration from birth through puberty.

Supplementary information

In the case of peri-pubertal boys, measurement of testicular volume is helpful to predict the chances of successful retrieval of spermatozoa and semen production in adolescents, whose semen parameters – as soon as spermatogenesis has initiated – are comparable to those of adult patients, irrespective of the underlying disease [22]. In the case of pre-pubertal girls, no further gynaecological investigations are usually performed, since they can be perceived as intrusive and an emotional and psychological burden for these patients [7].

Gametes can be used for MAR with the aim of obtaining embryos in a donation within a relationship.

31.6 Collection

31.6.1 Female

31.6.1.1 *Ovarian tissue*

Good Practice Guidelines

G31.14 Adult women who do not have the time to undergo stimulation for collection of oocytes, either because cancer treatment is imminent or because the cancer is hormone-dependent, should be candidates for ovarian tissue cryopreservation.
G31.15 For all patients, including transgender men, ovarian tissue collection should be performed before the start of gonadotoxic treatment [17].
G31.16 Ovarian tissue collection should be offered to pre-pubertal girls, and to post-pubertal girls not ready to undergo ovarian stimulation.
G31.17 In cases where collected ovarian tissue needs to be transported for further processing, the transport should be maintained around +2-8°C for the whole journey to the SoHO establishment.

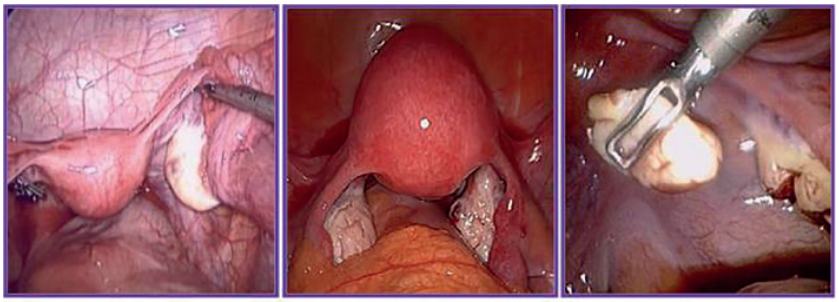
Supplementary information

The collection of ovarian tissue is performed at any time of the menstrual cycle and can be performed at short notice.

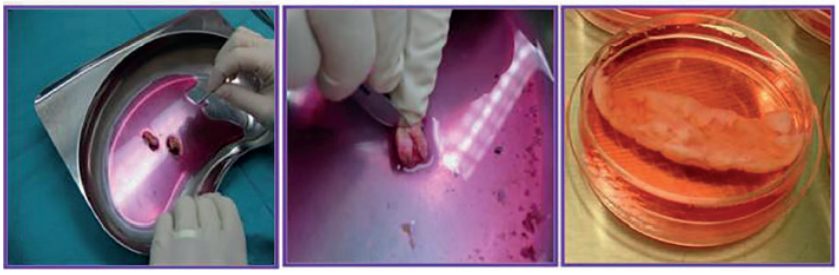
By the collection of ovarian tissue, many thousands of primordial follicles can be preserved. Such follicles lie within the cortical tissue of an ovary, with the vast majority of follicles in the outermost 1-2 mm of the ovary. An entire ovary, a portion of it or ovarian cortical biopsies are removed by an operative procedure under general anaesthesia and prepared for cryopreservation [23]. See Figures 31.1 and 31.2.

Figure 31.1 **Ovarian tissue collection by laparoscopy: stage 1**

Images in the upper panel show the surgery steps taken to obtain the tissue.



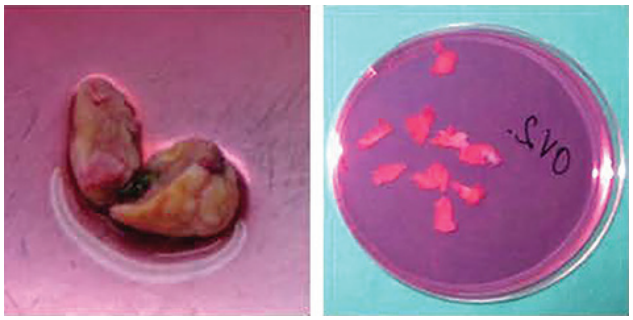
The lower panel shows processing steps: the medulla of the ovary is removed.



Source: Images provided by Sonia Herraiz, Valencia, Spain.

Figure 31.2 **Ovarian tissue collection by laparoscopy: stage 2**

After the medulla removal, the remaining ovarian cortex is then cut into small pieces of 10 mm × 5-10 mm.



Source: Images provided by Sonia Herraiz, Valencia, Spain.

From a technical standpoint, it is possible to store ovarian tissue at the time of gender-confirming surgery and thus during cross-hormone therapy, though this approach is highly experimental [21].

Ovarian tissue is transported on ice (between +2 and +8°C) in a transport medium supplemented with serum albumin in order to reduce the tissue metabolic activity and the ischaemic damage [24].

31.6.1.2 *Oocytes*

Good Practice Guidelines

G31.18 Oocyte cryopreservation should be done before the start of gonadotoxic therapies (or at least one year after its end).

Recommendations

R31.2 It is recommended to pay special attention to avoiding high oestrogen production during ovarian stimulation in patients with oestrogen-dependent diseases. For instance, in cases of breast cancer, stimulation protocols based on the use of tamoxifen or letrozole have been developed to reduce the risk of an unwanted high level of oestradiol. However, no evidence showed that 5 to 10 days of high oestradiol levels have any influence on the disease.

Supplementary information

The start of stimulation is usually on the third day of the menstrual cycle, but it can be started at any time in the menstrual cycle, including in the luteal phase, with good results. With the aim to increase the yield of oocytes, two consecutive stimulations and collections can be performed according to the approach referred to as dual stimulation [25].

In order to collect mature oocytes, controlled ovarian stimulation is performed in most cases. This stimulation is similar to the stimulation for *in vitro* fertilisation (IVF). Oocytes will be collected by aspiration via the transvaginal route following the same steps previously described in Chapter 30.

Some post-pubertal girls may be able to undergo ovarian stimulation and tolerate trans-vaginal ultrasound monitoring and oocyte retrieval.

Any pre-menopausal patient with a sufficient ovarian reserve is eligible for oocyte collection for FP (see Chapter 30). The number of oocytes that can be collected depends on the age of the patient and her ovarian reserve.

31.6.2 Male

31.6.2.1 *Testicular tissue*

Recommendations

R31.3 It is recommended that surgical recovery of gonadal tissue be combined with other planned interventions requiring anaesthesia, such as bone marrow sampling or implantation of venous ports, with the aim of minimising trauma to the patient.

Good Practice Guidelines

G31.19 Testicular biopsy should be performed at the cranial pole of the gonad, to avoid damage to the main testicular artery.

G31.20 In cases where testicular tissue has been extracted from pre-pubertal boys, immunohistochemical staining should be used to assess the presence of SSC in the collected and stored tissue.

Supplementary information

Vascular preservation is achieved through direct identification of vessels under microscopic magnification and selective dissection. If microsurgical testicular sperm extraction (micro-TESE) is used, vascular preservation is achieved through direct identification of vessels under microscopic magnification and selective dissection. Also, it allows the clinician to find specific areas with a higher chance of sperm present due to local spermatogenesis (G31.19).

After making a transverse or midline scrotal skin incision of 2-3 cm, the tunica vaginalis is opened and the lateral surface of the testis is exposed. The tunica albuginea is incised (0.5 cm in length), and the testes are compressed to make the testicular tissue protrude. A biopsy of 2-3 mm^3 is then cut with scissors. The tunica albuginea and the skin are closed over.

Besides being useful in FP, testicular biopsy in pre-pubertal boys is a minor procedure that can provide valuable information for predicting the risk of malignancy and fertility (see also Figure 31.3).

The amount of tissue collection for FP will have an effect on future testosterone production, and hormone replacement therapy could possibly be needed.

However, it has been shown that the development of the testis in boys after biopsy of gonadal tissue for FP did not have an effect on testicular growth [18, 26]. A balance between the amount of tissue collected and the amount conserved is important to achieve adequate levels of testosterone. Since the testis volume in very young pre-pubertal children can be limited, one third of the testis is generally collected in this patient population.

31.6.2.2 *Sperm*

Recommendations

R31.4 It is recommended to take special care to clearly explain to young post-pubertal boys how to produce a sample by ejaculation, since not all patients will be sexually active.

Supplementary information

Sperm samples can be collected in adult men, post-pubertal boys and in peri-pubertal boys if the patient is capable of obtaining a sample through masturbation. In cases of failure to produce a semen sample by masturbation, assisted ejaculation techniques such as penile vibratory stimulation or electro-ejaculation could be considered as a second-line treatment option (see Chapter 30).

Semen parameters in adolescents under the age of 14 years are not comparable to adults but are sufficient for FP; the underlying disease may also have an impact on sperm parameters. However, measurement of testicular volume is helpful to predict the chances of successful retrieval of spermatozoa and semen production in adolescents, whose semen parameters – as soon as spermatogenesis has been initiated – are comparable to those of adult patients, irrespective of the underlying disease.

31.7 Processing

The techniques for processing and storage are described in Chapter 30.

31.7.1 Female

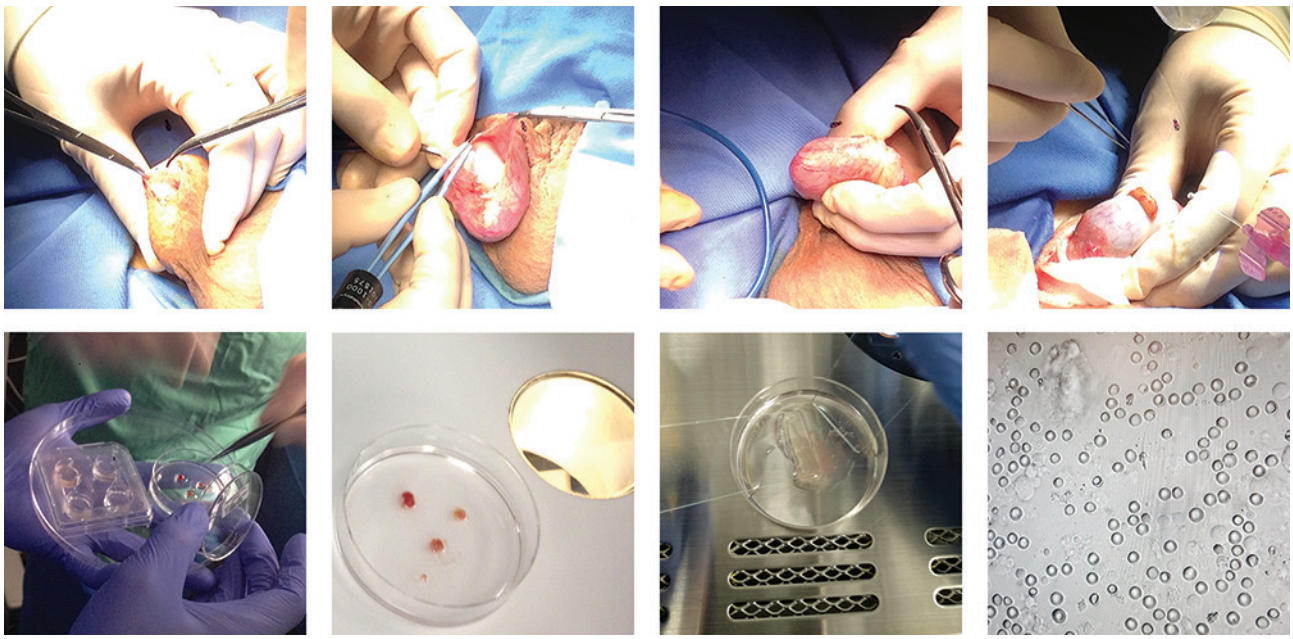
31.7.1.1 *Ovarian tissue*

Ovarian tissue is processed after collection either by ovarian biopsy or by bisection of the ovary in cases of whole ovariectomy. The medulla, the inner part of the ovarian tissue, is removed by careful scraping with a scalpel to prepare the cortical tissue. The cortex is subsequently cut into smaller fragments (5 × 5 mm or 5 x 10 mm) and one to three fragments of ovarian tissue are transferred to cryovials, immersed in cryoprotectant solution and subsequently slow frozen for cryostorage. Pre-pubertal tissue may require adjustments in the technique as medulla might be cryopreserved as well. Vitrification of the ovarian tissue is another optional methodology (see SoHO monograph 31.1).

The collected ovary is best processed immediately after collection, but successful transplantations have been published with cooling and transport of the organ lasting up to 24 h [25].

Figure 31.3 **Conventional testicular biopsy and tissue processing for sperm collection**

Images in the upper panel show a surgical procedure for testicular tissue extraction. Lower panel shows processing steps: the small pieces of tissue are cut into smaller pieces. Isolated testicular spermatozoa and/or the tissue can be cryopreserved and thawed for future use.



Source: Images provided by Dina Pabon (Spain).

Good Practice Guidelines

G31.21 Cortical thickness for adults should measure on average 1-2 mm, free of residual medulla, whenever possible.

Supplementary information

The main factors assessed while evaluating the collected tissue are:

- cellularity/viability;
- the presence of primordial follicles;
- the number of primordial follicles;
- confirmation of an intact ovarian cortex free of signs of tissue necrosis;
- verification that the pieces are of adequate size (on average 5 mm × 5 mm).

31.7.1.2 *Oocytes*

Good Practice Guidelines

G31.22 Oocyte cryopreservation should be the preferred option for FP in post-pubertal patients who can be submitted to controlled ovarian stimulation.
G31.23 Vitrification is the technique, due to the excellent results obtained in IVF patients in terms of survival, embryo development and implantation [13, 27].

31.7.1.3 *Embryos*

Recommendations

R31.5 Cryopreservation of oocytes and embryos are possible options for FP in couples. It is recommended to discuss with the patients the advantages and disadvantages of each option.

Supplementary information

With regard to reproductive cells, the possibilities of cryopreservation can be different in various countries, depending on national regulations.

31.7.1.4 *Ovarian tissue oocyte (OTO)* in vitro *maturation*

During ovarian tissue processing, the medulla can be further minced into small pieces in a petri dish with medium and examined under a stereomicroscope for the presence of cumulus oocyte complexes (COC). These COC can be collected and subjected to *in vitro* maturation (IVM) in order to obtain metaphase II oocytes that can be collected and stored. This collection, with IVM and storage of oocytes obtained during the processing of ovarian tissue, is considered a highly innovative and yet experimental FP technique, since so far only five live births have been described in Europe [28]. However, it opens up the possibility of cryopreservation of a few *in vitro* matured oocytes in the case of ovarian tissue collection and storage.

31.7.2 Male

31.7.2.1 *Ejaculate*

Recommendations

R31.6 It is recommended that patients undergoing oncology treatment wait two years after treatment before using fresh sperm samples for MAR or natural conception [29].

Supplementary information

Sperm cryopreservation is performed for male FP in post-pubertal males [30]. Semen characteristics may vary with both patient age and type of disease, with testicular cancer patients having the worst semen quality. For adolescents, in more than 80% of cases semen can be cryopreserved. However, up to 20% of adolescent or adult patients may either fail to produce a semen sample or may present with azoospermia.

The methodology for sperm cryopreservation is described in Chapter 30. Depending on the type of oncological treatment and age, gonadal toxicity may be temporary or permanent.

31.7.2.2 *Surgical sperm recovery from testicular tissue/epididymis*

Good Practice Guidelines

G31.24 For mid-pubertal boys unable to provide an ejaculate, for azoospermic patients and for anejaculation, surgical sperm collection by sperm-extraction procedures should be offered.

Supplementary information

Sperm can be retrieved by

- microsurgical aspiration of the epididymal fluid (microsurgical epididymal sperm aspiration or MESA),
- testicular biopsy/testicular sperm aspiration (TESA),
- percutaneous epididymal sperm aspiration (PESA), or
- testicular sperm extraction (TESE).

Testicular tissue is transported on ice (between +2 and +8°C) in a transport medium (e.g. Hepes-buffered DMEM/F12), which may be supplemented with serum albumin (in general, 10% HSA). Fresh TESE specimens for immediate intracytoplasmic sperm injection (ICSI)/processing can be transported at 37°C [26, 31].

31.7.2.3 *Conventional testicular biopsy and tissue processing for sperm recovery*

Since the reproductive potential of cryopreserved immature testicular tissue has still to be demonstrated in humans, the technique remains experimental.

Processing of the testicular tissue consists of cutting the tissue into small fragments, submerging the pieces in medium supplemented with a cryoprotectant to protect the cells from cryodamage and then subjecting them to controlled slow freezing [32].

No standardised protocol for cryopreservation of immature testicular tissue is available.

Most groups are using DMSO-based cryoprotectants (0.7-1.4 M DMSO) with or without the addition of sucrose. Slow-freezing protocols are mostly applied. Vitrification may also be effective when using higher doses of cryoprotectants.

31.8 Storage

The permitted storage period for cryopreserved sperm, oocytes, embryos and reproductive tissues varies according to national legislation; however, from a biological standpoint, the theoretical storage limit for maintaining viability remains undetermined (Chapter 30).

Good Practice Guidelines

G31.25 Temperature below –140°C should be guaranteed for gonadal tissue, embryos and gametes.
G31.26 Temperature below –140°C should also be maintained when handling the stored samples.
G31.27 Temperature changes when handling vitrified material stored in liquid nitrogen vapour should be avoided [33].

Supplementary information

The legislation and recommendations for FP in males differ between countries. There are no strict limitations on semen quality or sperm numbers for FP strategies and there are no international guidelines for the duration of storage of spermatozoa, whether ejaculated or collected directly from the testis.

A temperature above –140°C is detrimental to the survival and quality of the material cryopreserved. Long-term storage of ejaculated or testicular spermatozoa, ovarian cortex, oocytes or embryos does not negatively affect the quality of the frozen material, provided that it is stored at a temperature of –140°C or below [34]. With regard to ovarian cortex, a descriptive study of a limited number of cases from cancer patients has shown that after 18 years of storage ultrastructural examination demonstrated a good sub-cellular integrity of follicles and high stroma viability [34].

31.9 Clinical application

31.9.1 Female fertility restoration

Good Practice Guidelines

G31.28 When a patient wishes to use her preserved tissue or oocytes/embryos for MAR treatment, the physician who treated her with the gonadotoxic therapy should be consulted if available, or another equivalent physician, as to whether it is safe for the patient to attempt a pregnancy.
G31.29 If oocytes or embryos have been cryopreserved, an embryo replacement cycle should be planned. If the woman is menopausal, her endometrium will be prepared in a hormone replacement cycle.

Supplementary information

The efficacy of oocyte vitrification and ovarian tissue cryopreservation in women undergoing gonadotoxic treatments shows a trend towards higher live birth rates after oocyte vitrification [35].

In cases of transplantation of ovarian tissue, this can be done either orthotopically (at the remaining ovary or at the site of the removed ovary) or heterotopically to other sites such as the abdominal wall. These alternative options have implications for the modalities by which pregnancy can be achieved, spontaneously or by MAR [36]. After transplantation, the risk of surgery-related infection will depend on aspects related to vascularisation.

Normal cycle hormones are expected after 3-4 months of transplantation. During, on average, 2.5 years, the transplanted tissue will be active as demonstrated by the return of menses and oestradiol production, and the length of this endocrine activity will depend on the number of primordial follicles present in the tissue [37]. Although the primary reason for the use of stored reproductive material may be a future desire for a child, the restoration of endocrine function could also potentially be a reason for transplanting ovarian tissue. A review by Dolmans *et al.* [17] showed that, of 258 patients undergoing ovarian tissue transplantation and for whom follow-up data were available, 204 received a diagnosis of premature ovarian insufficiency. Of those, 181 (88.7%, ranging from 77.6% to 97.2% according to centre) experienced the recovery of ovarian endocrine function, as shown by the resumption of menstruation.

Spontaneous pregnancies can occur after ovarian transplantation, but many times IVF is needed. The duration of functionality varies from a few months up to 10 years, with a mean of 3-4 years.

It has to be emphasised that a significant percentage of women suffering from cancer will experience a spontaneous return of ovarian function months after chemotherapy. For these women spontaneous pregnancies may occur and they may not need their cryopreserved gametes or gonadal tissue. Hundreds of

ovarian transplantations have been performed to date, with a pregnancy rate of between 30% and 50% and over 130 live births [38].

Pre-menarcheal girls who lose all their ovarian tissue due to chemo- or radiation therapy will not enter puberty spontaneously. These girls will need to be induced with exogenous hormones in order to undergo normal pubertal development. After puberty, they will need to take hormonal replacement therapy to prevent osteoporosis and improve general well-being. Later on in life they can have their cryopreserved ovarian tissue transplanted in order to re-establish menstrual cycling and/or become pregnant [39].

31.9.2 Male fertility restoration

Good Practice Guidelines

G31.30 Regular semen analysis should be offered to patients after treatment.

Recommendations

R31.7 When cryopreserved samples are used, and in cases of male factor, ICSI is recommended to improve the chances of success.

Supplementary information

In most cases, where chemotherapy and/or radiotherapy has been applied, spontaneous recovery of spermatogenesis is possible up to 10-15 years after the end of treatment; however, it cannot be accurately foreseen. About 60% of male oncological patients will face infertility problems after the end of the cancer therapy.

Before ICSI was implemented, the success rate of MAR procedures with cryopreserved semen samples (intra-uterine insemination or IVF) was low. When ICSI procedures are applied, the success rates using cryopreserved spermatozoa are comparable to standard IVF and ICSI procedures in infertile couples using fresh sperm.

31.10 Quality control

Good Practice Guidelines

G31.31 A fresh biopsy of ovarian cortex should be taken for histological assessment of follicles before freezing.

G31.32 After storage, the success of ovarian cortex transplantation, which depends on quality and quantity of the re-introduced tissue, should be evaluated by measuring the relevant endocrine function and fertility restoration.

G31.33 Depending on the medical reason for tissue cryopreservation and the type of disease, the ovarian cortex and testicular tissue should be sampled and sent for histological examination to detect any malignant cells.

G31.34 A risk assessment for the re-introduction of malignant cells should be performed, and transplanting of tissue should be with the agreement of the treating oncologist. This is to be considered especially in the case of leukaemia, since it is known that leukaemic cells can reside in the stroma of the cortical tissue.

G31.35 Women suffering from disseminated cancer with a risk of ovarian metastases should be advised against transplanting the tissue.

G31.36 Microbiological testing should be done on the preparation medium and freezing solution that has been in contact with the SoHO preparation.

Recommendations

R31.8 It is advised to evaluate oocyte survival rates in comparison with published data.

Follicle density in the tissue destined for cryopreservation may be assessed histologically to predict the chances of post-storage restoration of ovarian function. However, since primordial follicles are often distributed irregularly throughout the ovarian cortex, their absence or scarcity in small, examined specimens does not necessarily correspond to a contraindication for cryopreservation.

Quality control before tissue transplantation includes approaches aimed at decreasing the risk of cancer recurrence while maximising the tissue viability after thawing or warming.

As the autotransplantation of cryopreserved tissue of oncological patients may be associated with a risk of re-introduction of cancer cells, different approaches to detect such cells are under development [40].

Post-cryopreservation viability of ovarian tissue depends on both possible damage caused by cryopreservation methodology and the original amount of primordial follicles. Guidance on microbiological testing is given in Chapter 12 (G31.29).

31.11 Biovigilance

General guidance on the implementation of good vigilance and surveillance practice is provided in Chapter 18.

Examples of serious adverse reactions (SARs) reported for MAR are given in Chapter 30, section 30.13.

In addition, the Notify Library (www.notifylibrary.org) includes many well-documented cases of adverse occurrences in MAR treatments. The database is publicly accessible and can be searched by type of SoHO, adverse occurrence and record ID number.

31.11.1 Female

Since such transplantations are scarce, compared with other disciplines, limited cases of adverse events and reactions have been reported. To date the surgical related complications remain low (≈3%), although reports of a major complication (intra-abdominal haemorrhage) have been published [41].

31.11.2 Male

A number of studies have examined sperm quality in the man after spontaneous recovery of spermatogenesis. Both cancer and its treatment are associated with sperm DNA damage, although treatment-induced DNA damage seems to be modest and transient. In a large cohort study of offspring from male cancer survivors, a modest but statistically significant increase in the risk of major congenital abnormalities was observed. This was independent of whether the sperm were cryopreserved pre-treatment and used for MAR or if the children were conceived naturally [42].

Apart from adverse reactions and events related to use of the gametes in MAR, other events can be considered. For instance, with regard to complications after testicular tissue removal, studies report low short-term complication rates, including testicular haematoma, wound infection, scrotal cellulitis, orchitis and excessive pain of 0 to 5.9% in small series and from 1.99 to 2.1% in larger FP programmes with over 100 patients [43].

Surgical complications may also occur after testicular biopsy. It has been reported that around 3% of men had experienced minor complications, 0.64% repeated surgery and 0.05% lost the testicle [44].

31.12 Developing applications

31.12.1 Female experimental approaches

31.12.1.1 *Oocyte regeneration*

In cases where no oocytes exist, current investigations are being carried out on the generation of oocytes derived *in vitro* from pluripotent stem cells as a promising though still incipient therapy.

31.12.1.2 *Organ culture*

When re-implantation is contraindicated, other strategies are being explored. Some of them include the *in vitro* growth of structures containing theca cells and primordial follicles together. This approach will require the use of IVM techniques and the means to determine to what extent cryopreservation will affect follicular viability [45].

Other lines of investigation could involve *in vitro* perfusion and hormonal stimulation of the patient's removed whole ovary (or ovaries), where oocytes might be matured, aspirated and cryopreserved. As no ovarian stimulation is required, these techniques would shorten the time for starting oncologic treatment of the patient, as well as avoiding the risk of re-introducing malignant cells via transplantation.

31.12.2 The use of artificial intelligence to predict the optimal number of oocytes to preserve

Assessment of oocyte quality is a determining factor for successful fertilisation, embryo development and pregnancy. Currently, embryologists assess quality by evaluating specific morphological characteristics. However, morphology evaluation is hindered by high subjectivity and has limited ability to predict embryo development and implantation outcomes. Although the development of AI systems for embryo selection has been the topic of intense research in recent years [46], studies on the use of AI for oocyte quality assessment are scarce. Some AI-based algorithms have been proposed for the evaluation of oocyte morphological features and fertilisation potential. The use of machine learning approaches seems able to predict the number of oocytes retrieved after controlled ovarian stimulation [47], but also to determine the optimal number of oocytes to optimise outcomes in the context of MAR [48]. Although the technology seems promising, it needs extensive validation.

31.12.3 Male experimental approaches

31.12.3.1 *Testicular tissue recovery*

Testicular tissue is mostly collected in pre-pubertal boys when there is no possibility to produce a sperm sample. Considered still experimental, collection of testicular tissue can be performed at any time. In general, unilateral collection takes place, with a maximum of 1/3 of the testis.

Processing of the testicular tissue consists of cutting the tissue into small fragments, submerging the pieces in medium supplemented with a cryoprotectant to protect the cells from cryodamage and then subjecting them to controlled slow freezing [26, 49]. However, no standardised protocol for cryopreservation of immature testicular tissue is available.

In the case of pre-pubertal boys, it has been proposed as a good approach to practise the examination of tissue at the time of surgery to check for the presence of haploid germ cells or sperm cells, freezing half of the tissue using a protocol that privileges the survival of sperm (if present) and the other half with a protocol favouring the survival of spermatogonia. Despite no evidence on the amount of tissue needed for future use, cryopreservation can also be offered for immature testicular tissue in pre-pubertal and adolescent boys. As has been said above, generally unilateral partial orchiectomy takes place, removing less than 50% of the testicular volume [50].

31.12.3.2 *Sperm regeneration*

Development of the procedures used for the preservation of SSC and testicular tissues from boys and adolescents is far more advanced than research into the methods needed to realise the fertile potential of these cells. In principle, fertility restoration strategies in laboratory practice will include autotransplantation of a suspension of SSC by injection into the testis to restore spermatogenesis, or autotransplantation of frozen-thawed testicular grafts back into the testis or an ectopic site. Should any risk of re-introduction of malignant cells exist via the transplant, then the only option is to grow and mature the SSC *in vitro* [51].

SSC transplantation was originally described in a mouse model and is now an established research tool. SSC are infused through the efferent ducts into the rete testis, a technique which has been successfully applied in a number of species, including humans. The procedure is best performed under ultrasound guidance and presents a relatively non-invasive strategy for stem cell transfer. However, the colonisation efficiency after infusion of enzymatically digested testicular cells remains low. For future clinical applications, SSC need to be isolated, enriched and propagated *in vitro* before they can be autotransplanted in the numbers required to efficiently recolonise the testis and reinstate spermatogenesis.

Nonetheless, the principle of the procedure has been shown, and offspring have been generated from transplanted spermatogonia in a number of species, including primates [52, 53]. While the demonstration of functional donor spermatogenesis following SSC transplantation in primates is an important milestone on the way towards using SSC to restore human fertility, it remains to be proved that the epigenetic programming and stability of SSC are not compromised following cryopreservation, culture and transplantation in humans.

Grafting of fragments of testicular tissue provides an alternative strategy to the use of cryobanked immature testis tissue. This approach maintains the SSC within their non-exposed natural niche, thus preserving the interactions between the germ cells and their supporting somatic cells. This procedure was successfully applied to retrieve sperm from ectopic and intra-testicular allografts, and insemination studies using ICSI have demonstrated that the spermatozoa were able to support full-term development of the progeny. This procedure is now tested in a number of species. To date, differentiated spermatogonia have been achieved to produce primary spermatocytes, but not complete spermatogenesis [54].

Similarly, as in the case of oocytes, when no germ cells are available in the initial testis biopsy, an alternative option may be the *in vitro* derivation of sperm cells from the patient's somatic cells, such as skin fibroblasts, by induced pluripotency or differentiation of these cells. This approach is, however, still in its infancy.

31.12.4 Organ culture

The major hurdle to overcome in patients with a haematological malignancy is the risk of re-introducing residual malignant cells via the testicular tissue. Sorting protocols using magnetic – activated cell sorting (MACS), fluorescence – activated cell sorting (FACS) or differential plating have been found to have variable efficiency when used to enrich human SSC. The risk of re-introduction of malignant cells via the SoHO preparations may be circumvented by *in vitro* spermatogenesis. *In vitro*-derived spermatozoa that are free from residual disease can then be used to inseminate oocytes using ICSI. Various strategies – including standard 2D cultures, 3-dimensional culture of testicular cells or organ culture – have been tested and showed some promise [55]. Although encouraging results have recently been obtained regarding the genetic and epigenetic stability of human SSC during long-term culture, the fertility of *in vitro*-derived sperm has still to be established before the clinical value and safety of this type of experimental approach can be fully assessed.

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Related material

Appendix 11. Example of consent form: female (HFEA, UK)
Appendix 12. Example of consent form: female (CNPMA, Portugal)
Appendix 13. Example of consent form: male (HFEA, UK)
Appendix 35. Informed legal consent for cryopreserving and storing semen from a minor (Spain)

Chapter 32. **Human milk**

32.1 Introduction

Donor human milk (DHM) is human milk (HM) expressed by a lactating mother and donated freely and voluntarily with a specific consent to a non-profit human milk bank (HMB) to be fed to another mother's child [1, 2]. For the remainder of this chapter the term 'HMB' is replaced by the term 'substances of human origin (SoHO) establishment'.

HM is produced by the human mammary glands, containing unique nutritional components and a myriad of bioactive factors, such as microbiome, human milk oligosaccharides (HMO), growth factors, hormones, enzymes, immunomodulators and anti-infective factors, which promote growth and development, shape the immune system and maintain the health of newborn children [3, 4]. Hence, HM not only covers the nutritional needs of infants, but also facilitates maturation of various organs such as the gut and the brain and builds the infant's gut microbiome, particularly through HM microbiome and HMO content [3, 5-7].

Breastfeeding is considered as a human right for each infant and mother. She may initiate breastfeeding within the first hour of birth and continue to do so exclusively for the first six months of life or even longer partially [8]. For all the above reasons, mother's own milk (MOM) is universally accepted as the optimal feeding choice for neonates and infants, and it is considered of vital importance for preterm infants.

In comparison to formula milk, HM (MOM and/or DHM) feeding confers protection for preterm infants against necrotising enterocolitis, as proven by randomised controlled trials [9, 10]. Observational studies show that feeding preterm infants with HM is also associated with a decreased risk of retinopathy of prematurity [11], decreased risk of bronchopulmonary dysplasia [12-15], improved feeding tolerance [16] and increased neurocognitive development [17, 18]. In addition, it has been shown that having access to a SoHO establishment is associated with elevated rates of exclusive breastfeeding in preterm infants at discharge from the hospital [19, 20].

The use of DHM is also recognised in term and late preterm infants as the preferable option when supplementation due to insufficient maternal milk supply is needed to prevent dehydration and severe hyperbilirubinaemia (jaundice) [21].

Therefore, official bodies and scientific associations, such as the World Health Organization (WHO) [22, 23], the American Academy of Pediatrics [8] and the European Society for Paediatric Gastroenterology, Hepatology and Nutrition (ESPGHAN) [16, 24], recommend that MOM be the first choice in the feeding of preterm infants and that, when MOM is unavailable or insufficient, processed DHM obtained from a SoHO establishment is the second-best choice.

From the early 20th century, old HMBs (now SoHO establishments) have been created and integrated into national policies for the improvement of neonatal health worldwide [25].

In the European Union (EU), HM is considered a substance of human origin (SoHO). At the EU level, an HMB is a SoHO establishment that processes and stores or releases, imports or exports donated human milk. SoHO establishments can also be in charge of the selection, collection, testing, traceability and distribution of donated human milk to the recipient infant, in response to valid medical requests [26].

The use of MOM that is only handled or stored in a communal facility (without further processing) falls outside the scope of the EU regulation. If the milk is pasteurised (classified as processing in the SoHO Regulation) in the SoHO establishment, relevant parts of the Regulation still apply, and the HM falls within the scope of the Regulation.

The scope of this chapter applies to:

1. DHM in the form of:

a. raw DHM: DHM which has not undergone processing (heat treatment or other antimicrobial treatments):
   i. refrigerated DHM at +2° to +8°C
   ii. frozen DHM at temperature below −15°C
b. pasteurised DHM: DHM that has undergone a pasteurisation process.

2. MOM:
a. that has been processed in a SoHO establishment.

32.2 Quality management & risk assessment

Chapters 2, 3 and 4 address quality management, qualification, validation and verification, and risk management respectively.

Recommendations

R32.1 It is recommended that the HACCP (Hazard Analysis and Critical Control Point) system be used for risk assessment. Other assessment tools can also be used [1, 27, 28].

32.3 Donor recruitment

Information about donor recruitment that is shown in Chapter 5 applies to HM.

Good Practice Guidelines

G32.1 The donor should be able to take the decision of donating her HM in accordance with national regulations, where they exist and are not addressed by SoHO legislation.
G32.2 Age should not be a contraindication or limitation for donation.
G32.3 Only the milk remaining after feeding their own baby/ies should be considered for donation, where applicable, provided that the baby/ies is/are adequately nourished.
G32.4 The extent and volume of HM donation should not have a negative impact on the donor's health.
G32.5 Hyperlactorrhea and breast engorgement should be avoided.
G32.6 The SoHO establishment should provide all new donors with training on collecting milk.
G32.7 A communication channel should be provided to all donors to give the opportunity to raise concerns or questions to the SoHO establishment.
G32.8 Donors should be instructed to report any information that may have an impact on the safety and quality of the milk during donation. These may include illness, use of prescribed or over-the-counter medications, or substance use (e.g. alcohol, cannabis and tobacco) to define if the donor is temporarily or permanently unsuitable. The SoHO establishment should provide the donors with a comprehensive list of reportable situations for information.

Recommendations

It is recommended that:
R32.2 donors be provided with face-to-face training, if possible, including virtual on-screen training, with additional information by telephone and in writing;
R32.3 the training cover [29]: handwashing; good personal hygiene; modality of collecting and expressing milk, including cleaning and using breast pumps and containers; home storing of DHM (including cooling and freezing); labelling DHM and documenting storage conditions; and transport of donated milk, when required;
R32.4 in all communications between the SoHO establishment and prospective donors the value of breastfeeding their own infant(s) in accordance with WHO guidance be highlighted [21, 22].

Supplementary information

Although age is not a limitation for donation, in the case of patients younger than 18 years old (G32.2 and G32.4), the minimal age has to fulfil national requirements, see Chapter 5.

Making bereaved mothers aware of the possibility of donating their previously expressed and stored milk, as well as continuing to express and collect their milk for the purposes of HM donation if desired by them, has been shown to be of benefit in navigating their grief [30, 31].

HM donation is promoted through a variety of channels: written material such as information leaflets and posters, media, social networks etc. Direct contact between pregnant mothers, physicians and midwives is important to provide balanced information and clarifications [28]. More information can be found at www.milkbanking.eu/.

32.4 Evaluation of prospective donors

The general criteria for donor selection are outlined in Chapter 6. The specific criteria for exclusion of HM are given in the same chapter, in Table 6.2.

Table 6.4 details the general risk conditions for SoHO donors, mainly relating to communicable infectious diseases requiring careful evaluation.

Good Practice Guidelines

G32.9 While a donor continues to donate, she should be contacted at least every month to ask about her general health and the exclusion criteria to assess whether repetition of serological testing is indicated

or whether a deferral period or a definitive exclusion needs to be established.

G32.10 Milk expressed prior to a donor's screening for acceptance should be segregated and clearly labelled as 'awaiting acceptance' until the donor is fully approved. The milk should only be accepted after a risk assessment has been completed, taking into account how the milk was collected, stored and identified.

Recommendations

R32.5 If a request from a mother who wishes to donate milk is declined by the SoHO establishment after evaluation, for whatever reason, it is recommended that the staff explain to the woman in an understandable manner the reasons for the rejection and how this affects, or does not affect, her own infant and/or herself [32].

Supplementary information

Routine physical examination of all donors is not necessary.

32.4.1 Donor serological screening

Screening for markers of transmissible diseases is outlined in Chapter 7.

Good Practice Guidelines

G32.11 Donor screening should be performed at the first donation and, subsequently, when infectious risk factors during donation are reported by the donor, or as in G32.12 or G32.13.

G32.12 If the milk will be pasteurised, donor testing should be repeated at least every three months or when the last donation is taking place.

G32.13 In the case of raw milk, donor testing should be repeated at least every two months or when the last donation is taking place.

G32.14 Testing for human T-lymphotrophic virus (HTLV-I/II) should be performed; at least for donors who live/d in or originate from high-prevalence areas [33] or who have known sexual partners originating from those areas, or if the donor's parents originated from those areas; see Chapter 7, section 7.6.

G32.15 If there is a suspected risk factor for an infection such as *Trypanosoma cruzi* during the health interview, additional testing for the relevant infection should be done, especially when the milk is not pasteurised.

G32.16 Seropositivity for *Cytomegalovirus* (CMV) should be considered a contraindication only in the case of non-pasteurised milk.

Supplementary information

The risk of transmission of *T. cruzi* through breastfeeding has not clearly been established; nevertheless, it should be noted that pasteurisation inactivates *T. cruzi* [34]; see section 32.8.

32.5 Human milk collection

The milk can be expressed by hand or with a breast pump.

Good Practice Guidelines

G32.17 Breast hygiene washing at least once per day, washing the breast with water and mild soap, should be encouraged.

G32.18 If the bottles in use are not disposable, they should be cleaned and sterilised before reuse. The use of ethylene oxide should be avoided for the sterilisation of milk bottles.

G32.19 The bottles should be purpose-designed and sold as intended for HM storage, including freezing (down to –25°C) and high temperatures (35°C or above).

G32.20 If the milk is to be frozen, the SoHO establishment provides guidance as to the maximum volume of milk that may be placed in the container prior to freezing, to allow for fluid expansion.

Recommendations

It is recommended that:

R32.6 the breast pump be given additional washing prior to expressing, to reduce microbial contamination of the milk, together with cleaning and drying all the components of the breast pump after each use;

R32.7 if milk extraction be performed at home, the breast pump be washed, rinsed with each use and disinfected once a day [35];

R32.8 the breast pump be stored if not used;

R32.9 if milk is to be stored in glass or rigid plastic bottles, they use breakage-resistant glass and/or rigid plastic bisphenol A-free bottles [28];

R32.10 if milk is to be stored in flexible plastic bags (breast milk storage bags), they use a double layered bag.

Supplementary information

Breast pump components are to be dried using hygienic methods, such as air drying on a clean surface or using a dedicated drying rack, avoiding contact with potentially contaminated cloths or towels (R32.7).

In the case of polyethylene bags, considering that they can easily break with the risk of loss of milk and contamination, a double bag is normally used (R32.11).

32.6 Storage

Good Practice Guidelines

G32.21 Storage at home:

G32.21.1 After collection, the bottle should be closed and labelled and kept in the storage conditions defined by the SoHO establishment.

G32.21.2 The bottle should be labelled at least with the identifying information of the donor and the date of expression.

G32.21.3 Collected milk should remain at room temperature for the shortest possible time (maximum one hour after expression) before being refrigerated or frozen to be sent to the SoHO establishment.

G32.21.4 If the milk is stored refrigerated, it should be transported to the SoHO establishment within 24 hours [27, 31, 36].

G32.21.5 If the milk is stored frozen, it should be transported to the SoHO establishment within 30 days.

G32.21.6 The storage temperature of milk collected and stored at home should be maintained during transport to the SoHO establishment.

G32.21.7 If milk is stored at home frozen or refrigerated, donors should be instructed to notify the SoHO establishment of any failure or any incident affecting their home fridge or freezer. The SoHO establishment should decide about acceptance of the milk after a risk assessment.

G32.22 Transport to the SoHO establishment:

G32.22.1 The SoHO establishment should take responsibility for arranging transport of the milk from home to the SoHO establishment.

G32.22.2 If a third party is used, an agreement should be in place with the SoHO establishment that defines the collection and transport conditions, establishing the transport temperature and the maximum transport time associated with validation of the bottle.

G32.22.3 The bottles used in transport should be either validated to maintain the designated temperature range in the worst-case transport conditions or independently monitored.

G32.23 Arrival at the SoHO establishment:

G32.23.1 When the milk arrives, the SoHO establishment should check the label of each bottle as well as the accompanying documentation.

G32.23.2 The SoHO establishment should check the integrity of the biological product and the bottle(s). A record of these checks should be maintained as part of the SoHO establishment records.

G32.23.3 Visual inspection should be carried out.

G32.23.3.1 If the milk exhibits unusual colouring, or if it contains any foreign bodies or visible impurities, these should be noted.

Recommendations

R32.11 It is recommended that milk be transported by donors:

R32.11.1 according to validated protocols (G10.5) and using transport freezers or bags provided by the SoHO establishment;

R32.11.2 without using wet ice.

Supplementary information

Minimising the time that the collected HM remains at room temperature following expression reduces the risk of microbial growth and degradation of labile components [37, 38].

32.6.1 Milk storage in the SoHO establishment prior to processing

Good Practice Guidelines

G32.24 DHM should be stored on receipt by the SoHO establishment and frozen at −15°C or below until pasteurisation or, in the case of raw milk, until it is issued.

G32.25 If DHM is stored at −15°C or below, it should be processed within three months from the date of collection.

G32.26 The period of storage at −15°C or below prior to pasteurisation of DHM should be minimised to prevent free fatty acid increase. Alternatively, if it is possible, DHM should be frozen and stored at the lowest possible temperature (ideally −60°C or below) prior to pasteurisation for a maximum period of 12 months.

Recommendations

R32.12 It is recommended that the volume of expression received be recorded.

Supplementary information

The lipase activity (bile salt-stimulated lipase) present in DHM is active while stored at a temperature of −20°C or below and therefore there is active lipolysis, which degrades triglycerides, reducing their content and increasing the content of diglycerides, monoglycerides and free fatty acids [39]. In contrast, when milk is stored at −70°C or below, no measurable lipolysis is observed [39]. In addition, fat and energy content is significantly higher in the samples stored at −70°C or below compared with the paired samples at −20°C or below, whereas there is no significant difference when protein and carbohydrate concentrations are compared [40].

It has been shown that, due to heat inactivation of the breast milk lipases, the increase of free fatty acid levels is not observed after pasteurisation [41].

32.7 Processing

Pasteurised HM is known to retain many beneficial and protective components of HM [42]. However, pasteurisation also affects some of the nutritional and biological properties of HM and eliminates the beneficial microbiota of HM, thus resulting in the reduction of some bacteriostatic mechanisms that render milk more susceptible to post-heating microbial contamination and to a decrease in its nutritional and biological value [43-45].

Although pasteurisation inactivates the majority of viruses, such as HIV, CMV, human papilloma virus (HPV), SARS-CoV-2, Ebola and Marburg, there are not yet enough data to consider this methodology as a full viral inactivation procedure [46-50].

General information about processing is outlined in Chapter 10. General information about premises is outlined in Chapter 9. Premises requirements for sterile procedures do not apply to DHM handling; see Chapter 9, section 9.2.

Good Practice Guidelines

G32.27 Visual inspection should be carried out if the milk is suspect:

- **G32.27.1** if the milk exhibits unusual colouring or smell, or
- **G32.27.2** if it contains any foreign bodies or visible impurities; these should be noted, and the milk should be discarded.

G32.28 Staff handling milk in a SoHO establishment should use appropriate clothing and hygiene conditions suitable for the environment where the milk is processed.

G32.29 In cases where milk from multiple donors is pooled, a risk assessment should be performed that includes risk–benefit analysis, ensuring full traceability of donors and reducing the risk of cross-contamination from the milk of other donors.

G32.30 Each SoHO establishment should state in their protocol the maximum number of donors that may be allowed in one pool.

G32.31 In cases where raw milk is received from non-high-risk donors with all negative infectious determinations, each SoHO establishment should conduct a risk analysis before acceptance.

- **G32.31.1** The risk analysis should include the initial level of bacterial count of every batch of donated milk.
- **G32.31.2** The decision taken should be documented and the parents/donor informed.

G32.32 During pasteurisation, bottle caps should remain above water level to prevent contamination, unless caps and equipment designed for submersion are used. In these cases, additional checks should be put in place to ensure seals are effective and end users should be cautioned to discard containers with an incomplete seal and should notify the milk bank immediately.

G32.33 A control bottle containing the same amount of milk or water as all the other bottles in the batch should be fitted with a calibrated probe thermometer to record milk temperature during pasteurisation.

G32.34 The control bottle should follow the same process as the rest of the batch at all times.

G32.35 The control bottle temperature and the water bath temperature should be monitored and recorded.

G32.36 Microbial testing after pasteurisation should be performed in a regular way based on a risk evaluation.

G32.37 The correct functioning of the pasteurisers should be checked regularly, at least once a year.

Recommendations

It is recommended that:

R32.13 pasteurisation be performed at a temperature of 62.5 ± 0.5°C [45] for 30 minutes, a process which is known as Holder pasteurisation (HoP). The HoP method is recommended in all international guidelines for the establishment and management of SoHO establishments [1, 27, 29, 36]. A few quality criteria for the HoP process have been proposed: temperature plateau in the range of 62-64.5°C, duration of the plateau between 30 and 35 minutes, exposure time over 58°C < 50 min, and rapid cooling from 62.5°C to 10°C (≤ 1 h);

R32.14 a macronutrient analysis of the milk be performed and a sample of each batch of pasteurised milk is kept for further tests if needed;

R32.15 the earliest expression date among the different milk extractions in a pool be used to determine the shelf life and establish the expiry date of the pool;

R32.16 while pooling and aliquoting, exposure of the milk to room temperature be as short as possible and no longer than 4 hours.

Supplementary information

The first check for donated milk, before processing, is the evaluation of its organoleptic characteristics (colour and smell) (G32.26). HM varies widely in colour and may be clear (early colostrum), yellow (colostrum and early transitional milk), creamy white or white tinged with blue or green, depending on the period of lactation, the extent to which the breast has been emptied and the mother's diet. Unusual colouring that requires discarding of the milk is colouring due to contact with blood (pinkish, reddish or brown). When working in a cleanroom and/or when wearing a mask while the bottle is opened inside a laminar flow cabinet, it is difficult to perform an olfactory inspection.

Macronutrient content from single-donor pools of preterm infants' mothers is variable [46]. Pooling of milk prior to pasteurisation from a limited number of donors (usually three to five) [36] can be performed in an effort to create a relatively consistent product in regard to macronutrient content. Studies show that, as the number

of donors is increased, the nutrient variability decreases and a better macronutrient content is obtained [47-50]. The disadvantage is that it can be more difficult to identify the origin of any contamination [27]. The milk of a healthy woman contains many micro-organisms; these are mainly symbiotic, but pathogenic micro-organisms can also be found. However, the possibility of infection through milk and any harmful effects is low, thanks to the anti-infective substances present in milk.

It is common practice in SoHO establishments to increase safety and reduce the risk of contamination by pasteurising the milk. This process can be carried out in a shaker water bath, followed by immediate cooling or with equipment specifically designed for pasteurising HM. Pasteurised HM is known to retain many beneficial and protective components of HM [51]. However, pasteurisation also affects some of the nutritional and biological properties of HM and eliminates the beneficial microbiota of HM, thus resulting in the reduction of some bacteriostatic mechanisms that render milk more susceptible to post-heating microbial contamination and to a decrease in its nutritional and biological value [52, 53].

In order to provide the same level of safety with improved preservation of the nutritional and bioactive components of HM, new techniques of processing are under development, as presented in the last section of this chapter (section 32.13).

32.8 Microbiological evaluation

The microbiological evaluation of DHM that is processed includes the testing of each pool or donation before pasteurisation, to identify unusual or heavy contamination and pathogens [1]. See G32.35.

Good Practice Guidelines

G32.38 The milk should be discarded if any microbial growth is present after pasteurisation [1, 27].

Recommendations

It is advised:

R32.17 to perform a pre-pasteurisation test on the first donation from a new donor and whenever milk from a donor has been discarded due to a positive culture post-pasteurisation. In that case, it is advised to perform a pre-pasteurisation microbiological test on the next batch from that donor;

R32.18 the European Milk Bank Association recommends discarding the milk if, prior to pasteurisation, total bacterial growth is $> 10^5$ colony-forming units (CFU)/mL, or if *Enterobacteriaceae* total $> 10^4$ CFU/mL, or if *Staphylococcus aureus* is $> 10^4$ CFU/mL [1, 27, 31];

R32.19 that each SoHO establishment defines the list of micro-organisms in the pre-processing samples that will result in discarding the milk before pasteurisation; see Chapter 12.

32.9 Milk storage in the SoHO establishment after processing

Recommendations

R32.20 After pasteurisation, if the milk is not expected to be used within 24 hours, it is recommended to refreeze and store DHM at –15°C or below for up to six months [27] or at –60°C or below for up to 12 months [38, 54].

Supplementary information

The criteria of refrozen after pasteurisation may vary depending on national guidelines, although similar recommendations can be found in the guidelines of the Human Milk Bank Association of North America [35, 55] and similar bodies in Australia [56], Italy [27], Spain [36] and France [57], among others [58, 59].

32.10 Labelling and packaging

In addition to the information about labelling set out in Chapter 16, labels for packaging pasteurised milk may contain also ascertained information about the nutritional value of the milk, such as concentration of protein, fats and carbohydrates. These data, if not present on the bottle, can be included in the accompanying information. The volume of the final storage containers may vary depending on the needs of the recipient.

32.11 Distribution and traceability

Distribution of milk is conducted in such a way as to ensure product traceability between donor and recipient, as described in Chapter 17.

Good Practice Guidelines

G32.39 During distribution the milk should remain frozen (except in the case of lyophilised milk).

G32.40 Thawing should be done slowly, within a refrigerator between +2 and +8°C, or quickly, in a water bath with water at a temperature not exceeding 37°C, or under lukewarm running water, or with an air thawing device suitable for use with human milk or with a validated defrosting device. Care should be taken to avoid the cap area and ensuring water does not ingress the bottles.

G32.41 Pasteurised milk, when distributed after being thawed, should be used for feeding as soon as possible; it can be kept in the refrigerator for a maximum period of 24 h [27] and cannot be refrozen.

G32.42 HM should not be thawed in a microwave oven because, if the milk is used within a short time, there is a risk of causing burns to the recipient, as the

temperature of the milk itself is uneven and may be higher than estimated by handling the container [27].

G32.43 The receiving hospital should document when and how the milk has been used and to whom it has been administered.

G32.44 The registry data of the donor mother should be kept separately from her health data.

G32.45 The identity of the recipient should not be revealed to the donor and vice versa.

32.12 Biovigilance

General information about biovigilance is described in Chapter 18. HM-derived reactions and events are described below.

In addition, the Notify Library (www.notifylibrary.org) includes documented cases of adverse occurrences. The database is publicly accessible and can be searched by type of SoHO, adverse occurrence and record ID number.

32.12.1 Donor adverse reactions

One study retrospectively reviewed [50] donor adverse reactions related to milk donation and found three adverse reactions (three out of 976 donors, 0.31%) during blood drawing for serology testing. Of those three, two were mild-to-moderate reactions from donors feeling faint and bruised and one was a serious vasovagal syncope with seizure activity.

32.12.2 Recipient adverse reactions

As for the recipients, there were 10 (10/3 350 = 0.3%) potential adverse reactions reported by clinicians; but, after assessment, all reported reactions were considered unlikely to be related, or definitely not related, to receipt of pasteurised donor HM [52].

In the Notify Library [53] there are two cases described:

- Record Number 1977: a case report of an infant with haemolytic disease of the fetus and newborn treated with intra-uterine transfusion and exchange transfusion but subsequently developing hyperbilirubinaemia attributed to passively acquired anti-D absorbed through the gastrointestinal tract via milk feeding containing high titre anti-D. The imputability for this case was classified as probable.
- Record Number 1824: infection caused by maternal CMV strain was demonstrated, but some doubts remain as to the exact route of infection; this could have happened *in utero* or by breastfeeding.

32.12.3 Serious adverse events

A study [60] shows that the most frequent contaminants in DHM are microbial and environmental chemical pollutants, but there is no clarity about the impact on health outcomes.

The Notify Library has three records related to a study conducted in 2012 by purchasing DHM via internet from individual donors and not via HMBs and it was found that:

- Record Number 1592: 89% of the milk arrived above the recommended frozen temperature; 45% was above the recommended refrigerated temperature (4°C). Of the milk received, a quarter had severe packaging or container damage.
- Record Number 1591: 11% of the samples had a level of bovine DNA.
- Record Number 1590: 74% of milk samples were colonised with Gram-negative bacteria or had more than 10^4 CFU/mL.

Even though donor adverse reactions and events related to pasteurised DHM are rare (it is generally a low-risk biological product), ongoing use of biovigilance systems remains essential.

32.13 Patient follow-up

General information on patient follow-up is described in Chapter 14, section 14.13.

Actually, there are no specific national or international registries for donated HM. This situation makes it difficult to record the level of risk of a serious adverse reaction and the level of success of the different methods of preservation.

32.14 New technologies for processing donor human milk

Due to the limitations of HoP, several new techniques have been developed and are under investigation with the purpose of eliminating pathogens in milk and better preserving the biological properties of HM [61-64]. Among these techniques, high temperature short time is used in a few SoHO establishments [65, 66]:

- High temperature short time (HTST)

  HTST pasteurisation is usually performed by heating thin-layered milk in continuous flow systems at 72°C for 10/15 seconds. In the past, processes were sometimes performed with shorter heating times, or higher temperatures (5 seconds instead of 15; 75°C instead of 72°C).
  Presently, three different HTST pasteurisers, specifically designed and validated for HM treatment, are available.
  Recent evidence confirms that HTST pasteurisation of HM allows a higher retention of bioactive factors, such as immunoglobulins (secretory IgAs) and lipase, when compared with HoP.

In addition, other promising technologies include:

- High pressure processing (HPP) [67]

  HPP is usually performed, as a non-thermal pasteurisation method, by applying high hydrostatic pressure, from 400 to 800 MPa, for a short period of time (5-10 minutes).
  Research so far has been able to show that HPP would ensure comparable or even improved safety of HM compared with HoP: most bacteria currently present in HM are destroyed by HPP, and antimicrobial activity is preserved. Sporulating bacteria such as *Bacillus cereus* can be a problem for SoHO establishments, and optimising the HPP process to destroy such bacteria is still a challenge.
  In comparison to HoP, HPP allows better preservation of nutrients and bioactive components.
  At present no SoHO establishment uses HPP as a pasteurisation method. All the devices available on the market are used for research and development purposes only. The cost of the device is a major concern, but a full economic evaluation is needed.

- Ultraviolet (UV) irradiation [62]

  Ultraviolet-C (UV-C) irradiation is based on utilisation of short wavelength ultraviolet radiation to treat a thin milk layer, resulting in irreparable DNA damage, thus leading to bacterial and viral cell destruction. Studies have shown that DHM irradiated with UV-C light maintains several active biological components, such as immunoglobulin A, bile salt stimulating lipase and alkaline phosphatase, preserving also their function, without altering fatty acid composition. The challenge of treating milk effectively with short wavelength UV radiation is the limited penetration of UV light through non-transparent, thick fluids. Experience from the food industry and in the experimental setting with DHM showed that milk could be treated with UV-C light, provided it was presented to the light source in a thin layer. Currently, no UV-C devices are commercially available for HM treatment.

- Ultra high-pressure homogenisation (UHPH) [68, 69]

  UHPH is based on the principles of conventional homogenisation, but thanks to new designs and the use of new construction materials in the valves, pressures of up to 300 MPa have been achieved. UHPH extends shelf life by inactivating micro-organisms due to shear forces, impact, pressure and cavitation, among other effects that occur in the homogenisation valve, allowing for the production of sterile products depending on the applied process. Improvements in the functionality of food matrices have also been described due to increased emulsion capacity and stability, without affecting nutritional value and sensory characteristics. Although UHPH treatment is not usually associated with thermal treatment, during the process the matrix undergoes temperature changes as it passes through the valve due to the previously described forces, with temperature increasing by 2-2.5°C for every 10 MPa of pressure, but with ultra-short residence times (< 0.5 s). Currently, there is evidence regarding the effect of this UHPH treatment on HM to inactivate non-spore-forming micro-organisms and on some of the bioactive components associated with this technology using UHPH treatments at 250 MPa and an inlet temperature of 20°C.

- Despite its advantages, very few SoHO establishments use lyophilisation after pasteurisation as a method of preservation. Donor milk that has been lyophilised after pasteurisation can be stored at ambient temperature as a powder for up to 18 months. In France, this technology is used to supply donor HM to French overseas territories. The SoHO establishment of Bordeaux-Pessac in France uses HoP, followed by lyophilisation, and processes more than 12 000 litres of milk per year [70].

32.15 References

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Chapter 33. **Intestinal microbiota**

33.1 Introduction

The intestinal microbiota (IM) constitutes a resilient, stable and integral part of the human organism. It includes bacteria, viruses, fungi, archaea and parasites [1]. Normal bowel function and metabolism rely on a functional intestinal microbiome.

Faecal microbiota transplantation (FMT) is the transfer of IM preparations, with no intended changes of composition or properties, from a healthy donor to a recipient with the intent to modulate the recipient's microbiome for therapeutic purposes [2].

The use of FMT was first reported in modern science in 1958 by Eiseman and co-workers, who successfully treated four patients who suffered from pseudomembranous colitis [3]. Since then, observational studies, randomised clinical trials and systematic reviews and meta-analyses have provided high-grade clinical evidence that FMT is effective in preventing recurrence of *Clostridioides* (formerly *Clostridium*) *difficile* infection (CDI) in patients who have been treated with standard antimicrobial therapy [4-8]. Also, FMT is effective for CDI that is refractory to antimicrobial therapy where other causes of diarrhoea have been ruled out [9]. Alongside the increased incidence, severity and mortality of this infection, embedding the use of FMT in publicly available healthcare saves human lives and decreases the economic burden on healthcare systems.

Based on the current evidence, both the European Society for Microbiology and Infectious Disease and the American Gastroenterology Association, as well as numerous national guidelines, recommend FMT as a treatment for recurrent *C. difficile* infection (rCDI) [10, 11]. Accordingly, FMT services have been implemented throughout Europe [12]. The processing of IM preparations and the clinical application, FMT, follow evidence-based international consensus reports [9, 13-15]. Transplanting IM from a healthy donor with the aim of restoring a patient's perturbed microbiota appears promising for several other disorders, such as ulcerative colitis, graft-*versus*-host disease (GvHD) or hepatic encephalopathy.

Concurrent with the commercial development of human microbiota-based medicinal products, strict adherence to the ethical principles described in Chapter 1 of this Guide is essential to safeguard donors' rights and prevent the commodification of human biological materials.

33.2 Donor recruitment and consent

Details of donor recruitment and consent, including principles for donor anonymity and voluntary unpaid donation, are described in detail in Chapter 5.

Good Practice Guidelines

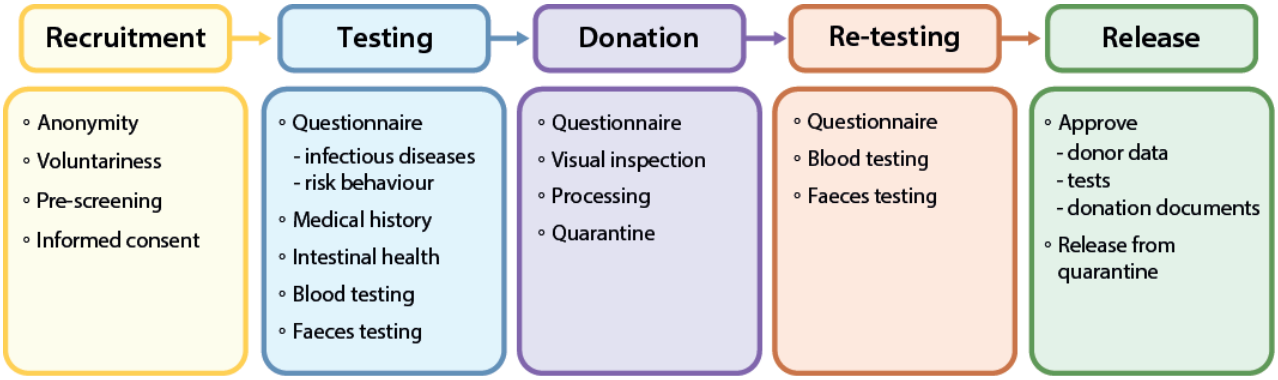
G33.1 The requirements to be fulfilled for donor recruitment, donor screening, collection, processing and release of IM preparations should follow the pattern illustrated in Figure 33.1.

Recommendations

R33.1 It is advised to recruit donors who:

- R33.1.1 are older than 18 years but younger than 65 years, because older individuals are more likely to suffer from chronic diseases and/or may have a less diverse microbiome;
- R33.1.2 have a normal body mass index (BMI), i.e. between 18 and 30 kg/m^2.

Figure 33.1 **Tasks in donor recruitment, screening, collection, processing and release of intestinal microbiota for faecal microbiota transplantation (FMT)**



Supplementary information

Safety parameters and precautionary principles guide the current selection of faeces (stool) donors for IM preparations for FMT. The aim is to minimise the risk of adverse reactions caused by the transfer of pathogenic micro-organisms from donor faeces. The structural setup of a donor recruitment and screening service influences eligibility e.g. by recruiting among donors already approved for donation of other substances of human origin (SoHO) [15-17].

Eligibility rates vary from 3% following public advertising to 30% when recruiting donors from healthy subgroups of the general population, such as blood donors [16, 18]. Data from *C. difficile* patients receiving FMT show no superiority of related donors over unrelated ones [19].

Recent data demonstrate that healthcare workers, when rigorously screened for enteropathogenic micro-organisms and antimicrobial-resistant bacteria, can be considered suitable stool donors for FMT programmes. The prevalence of pathogens and resistant organisms among healthcare workers is comparable to their prevalence in the general population, and exclusion is based on individual screening results rather than occupational status [20]. Although certain microbiota signatures may impact efficacy of FMT in chronic inflammatory bowel diseases (IBD) [21, 22], no validated selection criteria based on specific microbial properties have yet been developed.

33.3 Donor evaluation and screening

The general criteria for donor selection are outlined in Chapter 6. The specific criteria for exclusion of IM are given in the same chapter, in Table 6.2.

Table 6.4 details the general risk conditions for SoHO donors, mainly relating to communicable infectious diseases requiring careful evaluation. Chapter 7 details guidance on screening for markers.

In alignment with the donor evaluation and screening principles outlined in Chapters 6 and 7, potential faeces donors usually complete two steps for approval:

1. an in-person interview to assess medical history and risk factors,
2. laboratory testing of blood and faeces.

Good Practice Guidelines

G33.2 In addition to an evaluation of medical history and risk behaviours, and general blood screening for pathogens as described in Chapter 7, additional tests specific for IM preparation for FMT should be performed as shown in Table 33.1.

G33.3 In immediate relation to each donation, the donor should complete a short donation-specific questionnaire to assess any events that may have occurred between donor approval and each donation. The results should be evaluated by a qualified health professional.

G33.4 A donation period should have a defined maximum duration, not exceeding three months.

G33.5 Complete donor screening, including blood and faeces tests, should be done at the beginning and after the end of each donation period.

G33.6 IM preparations processed during a donation period should be quarantined until the validation steps during donation, and all post-donation donor screening results, are available.

G33.7 Blood and faeces tests conducted at the post-donation screening should consider the window period of potential pathogens.

Recommendations

It is advised to:

R33.2 perform a pre-screening of eligible IM donors using standardised criteria for donor age, BMI (see R33.1.2), absence of chronic disease, food allergy, medication allergy or any kind of anaphylactic allergy. Persons who are not excluded by these pre-screening criteria are approached for information and a request for informed consent to undergo detailed donor screening as outlined in this section. See also Chapter 6, Table 6.2;

R33.3 provide a structured workflow for donor registration, screening and donation logistics, as outlined in Figure 33.2;

R33.4 address new potential issues on the day of the donation (new since selection or previous donation) (see G33.3) such as [14]:

R33.4.1 any new gastrointestinal symptoms (e.g. diarrhoea, nausea, vomiting, abdominal pain);

R33.4.2 any new systemic symptoms (e.g. fever, throat pain, swollen lymph nodes);

R33.4.3 vaccination with a live attenuated vaccine;

R33.4.4 active mucocutaneous infections (e.g. genital herpes);

R33.4.5 any new mild disease (e.g. runny nose, cold sore, skin rash, genital complaints);

R33.4.6 direct contact with a person with gastroenteritis (since last screening);

R33.4.7 use of antibiotics or other drugs that may alter gut microbiota (since last screening);

R33.4.8 ingestion of a substance that may harm the recipients (medication, drug use) (since last screening);

R33.4.9 new sexual partner (since last screening);

R33.4.10 travel abroad, especially in tropical areas (since last screening);

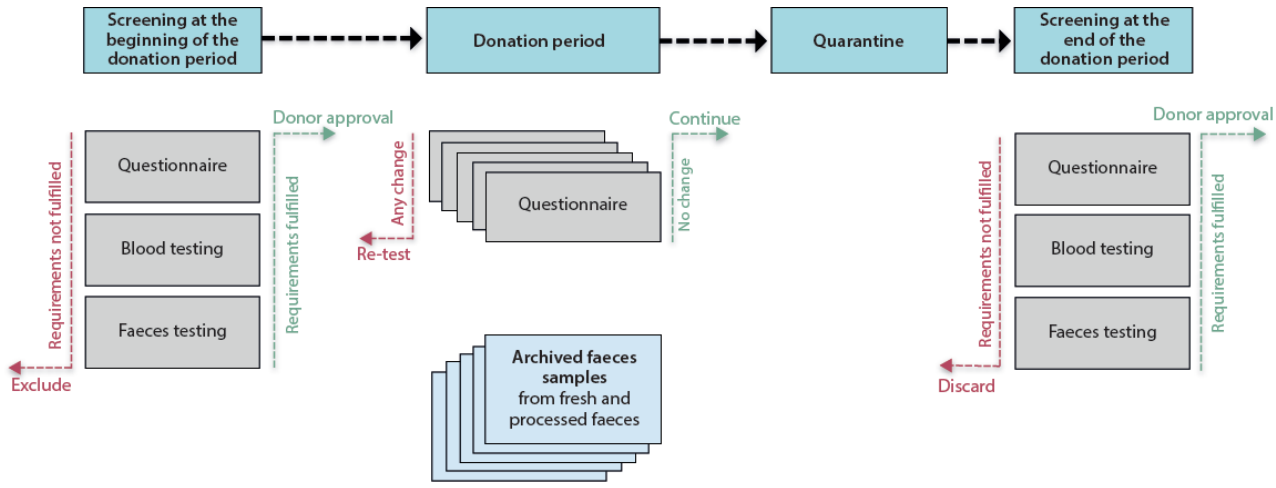
R33.4.11 contact with human blood (wound, piercing, tattoo);

Table 33.1 **Specific blood and faeces screening for faecal microbiota transplantation**[a]

Risk of transmission	Blood and faeces tests to consider	Good Practice Guidelines
Important and established risk	***Blood*** Virus: Hepatitis A (IgM) Human T-lymphotropic virus-1 (HTLV1) for donors born in, or having travelled to, endemic regions ***Faeces*** Bacteria: Shiga-like toxin-producing *Escherichia coli* (STEC), *Shigella* species, *Campylobacter jejuni* and *C. coli*, *Salmonella species*, *Yersinia enterocolitica*, toxigenic *Clostridioides difficile* Antibiotic-resistant bacteria: Extended-spectrum beta-lactamase-producing bacteria (ESBL), multidrug-resistant Gram-negative bacteria (MRGN) including carbapenemase-producing *Enterobacterales* (CPE), vancomycin-resistant *Enterococci* (VRE) Virus: *Norovirus, Rotavirus* Parasites: *Cryptosporidium* species, *Giardia lamblia*, *Entamoeba histolytica*	Testing should be done. Positive test should lead to (temporary) exclusion of the donor depending on the infectivity status of the donor
Important and potential risk; transmission via FMT is debatable and has not been documented	***Blood*** Virus: Epstein–Barr Virus (EBV IgM and IgG), *Cytomegalovirus* (CMV IgM and IgG) ***Faeces*** *Adenovirus, Astrovirus, Enterovirus, Parechovirus, Sapovirus*	Testing should be done. For EBV and CMV, positive IgG with negative IgM should not lead to exclusion.

[a] Refer to Chapter 7, Table 7.1 for guidance on transmissible pathogens (e.g. HBV, HCV and HIV)

Figure 33.2 **Safe collection of intestinal microbiota for faecal microbiota transplantation**



R33.4.12 travel abroad, especially in tropical areas or countries at risk for communicable diseases or travellers' diarrhoea (countries listed by the European Centre for Disease Prevention and Control (ECDC) at the following address: https://atlas.ecdc.europa.eu/public/index.aspx); see Chapter 6.

R33.5 in severely immunocompromised CDI patients, consider documenting additional micro-organisms (e.g. CMV, EBV, *Helicobacter pylori*, intimin-producing *E. coli* and *Dientamoeba fragilis*) in IM preparations to support an individual risk–benefit assessment; however, in the absence of evidence-based criteria, such findings are not to be used as automatic exclusion criteria;

R33.6 when considering blood and faeces screening, certain additional risks may be relevant in specific clinical or epidemiological contexts. Blood and faeces tests that are not considered necessary in relation to FMT and the rationales for not considering testing are listed in Table 33.2. Routine screening for these agents is not recommended; however, targeted testing may be appropriate in selected cases based on individual risk assessment.

Supplementary information

Because donor faeces inherently contain micro-organisms and are therefore never 'sterile', Table 12.4, listing micro-organisms that usually result in discard of a SoHO preparation, does not apply in the evaluation of faeces donors.

Furthermore, numerous micro-organisms have been considered for donor–recipient matching or donor exclusion, but no evidence-based criteria have been published. This applies to but is not restricted to CMV, EBV, *H. pylori*, intimin-producing *E. coli* and *D. fragilis*. Recent prospective and retrospective studies have shown that *D. fragilis* is rarely transmitted through FMT and does not negatively affect clinical outcomes or cause a rise in adverse event rates in recipients – including immunocompromised patients – even when donors test positive for *D. fragilis*, similar to current practice for *Blastocystis hominis* [23, 24].

The blood and faeces test panels are dynamic and are to be updated regularly in line with known risk factors for disease transmission. In light of emerging diseases, additional tests or methods may need to be incorporated. Local adaptations may be applied, and members of the screening panel are to be subject to regular review.

33.4 Collection, temporary storage and transport to SoHO establishment

General information about collection is outlined in Chapter 8.

Good Practice Guidelines

G33.8 At the collection site, measures should be taken to ensure that the donated faeces are fresh and derived from the particular donor. Visual inspection of the donor faeces should be undertaken to document consistency (Bristol score 3-5) [25] and to confirm the absence of blood, pus and urine.

G33.9 The SoHO entity should provide clean, pathogen-free, faecal containers appropriate for transportation of human material.

G33.10 If defaecation takes place at the donor's home, faeces should be placed in a pathogen-free, faecal container during transport to the SoHO establishment to minimise any changes in microbial content or characteristics. The SoHO entity should provide instructions for stool collection and storage at home (temperature conditions and time limits).

Recommendations

It is recommended that:

R33.7 faecal samples be provided by the donor directly at the SoHO establishment; alternatively, the donor may collect the sample at home and transfer it to the SoHO establishment;

R33.8 donors be given the option to deliver their donation directly to the SoHO establishment within two hours following defaecation [26];

Table 33.2 **Blood and faeces tests that are NOT considered necessary in relation to faecal microbiota transplantation (FMT)**

Micro-organism, specimen (test)	Rationale for not recommending testing
Methicillin-resistant *Staphylococcus aureus* (MRSA), faeces (PCR) *Helicobacter pylori*, faeces (antigen) SARS-CoV2, faeces (PCR)	Transmission with FMT has not been documented
Blastocystis hominis, faeces (PCR) *Dientamoeba fragilis*, faeces (PCR)	Transmission may be possible but would not have negative consequences for the recipient
Strongyloides stercoralis, blood (serology) or faeces (PCR) *Vibrio* spp., faeces (PCR)	Testing may be considered only in specific circumstances, e.g. after travel to endemic areas, if not clarified by travel history

R33.9 cooling elements be used to protect faecal samples during transportation to the SoHO establishment.

33.5 Processing

General information about processing is outlined in Chapter 10. In the past, fresh donor faeces were processed for immediate use. Because the waiting time for post-donation screening results abrogates the use of fresh donor faeces, FMT that complies with the present Guide can only be performed using frozen IM preparation. In addition, the use of frozen IM has several logistic advantages.

Good Practice Guidelines

G33.11 Visual inspection of the donor faeces should be undertaken to document consistency and to confirm the absence of blood and urine. Stool consistency should be evaluated using an established scale, e.g. Bristol stool chart scale [25].
G33.12 To preserve the donated IM, faeces should be processed within six hours following defaecation [13].
G33.13 If a faeces donation cannot be processed immediately after arrival in the SoHO establishment, it should be stored at +2-8°C until processing begins, still adhering to the maximal six hours total time of processing.
G33.14 Prior to processing, the designated spaces and non-disposable equipment should be cleaned, using measures effective against sporulating bacteria [14].
G33.15 Donor faeces for IM preparation should be homogenised with sterile 0.9% saline, using a blender, smasher or stomacher bag [27] or manually using a spatula [28].
G33.16 Archived samples from fresh unprocessed faeces from each donation should be stored at −80°C for at least one year after distribution of the last IM preparation from the donor for look-back and quality improvement purposes.
G33.17 Archived samples of IM preparation should be kept for at least one year after the last application to allow any investigation of adverse events/reactions.

Recommendations

It is advised to:
R33.10 use around 30-50 g per IM preparation;
R33.11 add a cryoprotectant before freezing, such as glycerol.

Supplementary information

Processing of faeces into an IM preparation under aerobic conditions is considered suitable when preparing them for use in patients with rCDI, because anaerobic processing does not increase clinical cure rates [29]. This may be explained by findings that a considerable proportion of the bacterial genera tolerate oxygen for a limited amount of time or produce resilient spores that allow transfer of at least a proportion of anaerobic intestinal bacteria. Nevertheless, precautions are generally taken to minimise contact with oxygen during processing.

The amount of added saline depends on the viscosity required for clinical application. Donor faeces intended for infusion may be sieved or centrifugated to remove debris and thereby prevent clogging during administration. Donations for encapsulated IM may undergo further centrifugation steps to concentrate the donated sample and reduce the required number of capsules.

Processing into liquid suspensions and packaging into bags, syringes or capsules dictates the content of saline and glycerol in the final IM preparation. The amount of glycerol used for cryopreservation has no independent effect on the recipient.

Lyophilisation using a lyoprotectant such as trehalose may further reduce water content without changing microbiota composition or properties, and lyophilised donor faeces may be encapsulated similarly to glycerol-conserved microbiota [30, 31]. Whereas liquid or encapsulated glycerol-conserved faeces suspensions have been validated for clinical use, no protocols for collection of equally effective lyophilisation have yet been published.

The use of frozen IM preparation with added cryoprotectant is equally effective for rCDI as fresh faeces [27, 32].

33.6 Quality control

Good Practice Guidelines

G33.18 For IM preparations, data should be recorded in the processing records. See also Chapter 2.

Supplementary information

Processing records may include specifications for quality control, such as the time from donation to completion of processing, completeness of documentation for donor assessment and release, intact appearance of the IM preparation (both primary and secondary packaging), and weight of the donation and final IM preparation, as well as adequate storage conditions.

33.7 Organisation, personnel and premises

General information about premises is outlined in Chapter 9.

Recommendations

R33.12 The use of a dedicated processing area for IM processing is recommended.

Supplementary information

Premises requirements for sterile procedures do not apply to IM handling and FMT; see Chapter 9, section 9.2.

33.8 Computerised systems

Maintenance of computerised systems is described in Chapter 15.

SoHO establishments that handle IM have often been established on a small scale and often for research purposes, but computerised systems enable appropriate upscaling over time. Computerised systems may further allow local, national and international registries to ensure donor and recipient traceability and monitor adverse events/reactions and clinical effectiveness of IM preparations.

33.9 Labelling and packaging

General information on labelling and packaging is described in Chapter 16.

Good Practice Guidelines

G33.19 Primary packaging, including bags, syringes or other receptacles, should be suitable for liquid IM preparations for administration via colonoscopy, upper gastrointestinal (GI) endoscopy, tube delivery or enema.
G33.20 For orally administered liquid IM in capsules, acid-resistant capsules should be used as primary packaging.
G33.21 The containers used for storing the frozen IM preparations should be sealed and clean.
G33.22 The containers used for storing the frozen faecal preparations should be sealed and clean.
G33.23 IM preparations should be stored deep frozen (–60°C to –80°C).
G33.24 IM preparations can be stored without loss of effect for up to two years if stored at deep-frozen temperatures (–60°C to –80°C).
G33.25 Storage of IM preparations at frozen temperatures (at or below –15°C) should not exceed eight weeks, unless sufficient stability data are available to support a longer storage duration [33].
G33.26 Storage at +2-8°C, if directly related to application after thawing, should not exceed six hours.

Recommendations

R33.13 It is recommended to store encapsulated IM preparations at –60°C to –80°C to prevent moisture permeation and to ensure optimal stability.

33.10 Clinical application

FMT may be performed in either an inpatient or an outpatient setting, depending on clinical patient assessment and procedural requirements.

Recommendations

It is recommended that:
R33.14 clinicians, SoHO entities and SoHO establishments who are new to IM preparation and FMT consult with organisations who have more experience in the area, and this consultation may include indications and any other related practices;
R33.15 an expert panel be created, linked to each SoHO establishment, that can provide consultation, particularly for expanded access or experimental indications. As FMT indications increase, the panel may include relevant medical specialists to assess suitability and advise on further diagnostics or alternatives.

33.10.1 Indications for FMT treatment

Modulation of human IM is regarded as a medical treatment carried out by physician specialists who have the associated medical qualifications and obligations.

Good Practice Guidelines

G33.27 FMT should be considered for recurrent *Clostridioides difficile* infection following recommended antimicrobial therapy.
G33.28 FMT should be considered for severe or fulminant *C. difficile* infection, regardless of the number of occurrences, in appropriately selected patients.

Recommendations

R33.16 FMT is recommended for rCDI, where alternative explanations for persistent diarrhoea should also be carefully considered and investigated (G33.27) [34].

Supplementary information

Current clinical guidelines recommend FMT for a second CDI recurrence (i.e. a third episode) [8, 10, 35]. In selected patients, FMT can be also considered at an earlier stage [36]. FMT can be lifesaving and thus indicated in patients with a first episode of refractory or severe or fulminant CDI [10, 37-40].

FMT modulates the indigenous host IM with potential long-term engraftment of donor microbiota [41].

In the context of CDI, a standardised patient questionnaire may be used with key information about the current episode of CDI, previous episodes of CDI, treatment effects and microbiological testing, and previous medical history and medication use. A standardised

treatment protocol for rCDI will enable the comparison of outcomes among centres and subsequently increase safety and facilitate quality assurance.

Expanded access denotes the application as an individualised healing attempt, according to Article 37 of the World Medical Association Declaration of Helsinki [42]. Three principles may be applied when evaluating a patient for expanded access to FMT. First, the patient condition that leads to considering FMT is related to disruption of the IM. Second, the use is to be supported by clinical or experimental evidence. Third and last, an expert panel evaluates and documents the indication.

33.10.2 Preparation of the recipient

Good Practice Guidelines

G33.29 Patients with CDI should receive pre-treatment with *C. difficile*-specific antimicrobials for at least three days and until 12 to 48 hours before FMT.
G33.30 Patients scheduled to receive FMT via colonoscopy should undergo bowel cleansing prior to the procedure.
G33.31 Before using FMT in *C. difficile*, risk factors for treatment failure should be minimised: where possible, patients should stop taking proton pump inhibitors and non-*C. difficile* antibiotics [13].
G33.32 Special attention should be paid to patients with a history of anaphylactic reactions secondary to food allergy because of a theoretical risk of transplanting allergenic material from donor faeces.

Recommendations

R33.17 In cases where FMT is administered via enema or capsules, no specific pre-treatment other than anti-CDI antimicrobials is recommended, as it does not improve clinical efficacy.

Supplementary information

In patients who are stabilised on initial *C. difficile*-specific antibiotic treatment, this treatment may be continued until FMT can be performed.

Pre-treatment is an important bridging therapy for recipients while they are waiting for the procedure. It aims to reduce general and bowel-related symptoms and to facilitate engraftment of the donor microbiota in the bowel of the recipient. Inadequate bowel preparation has been identified as a risk factor for failure of colonoscopically administered FMT [37].

For upper GI delivery of liquid IM preparations, bowel lavage may be performed [4], although evidence for its use is lacking.

33.10.3 Thawing of intestinal microbiota preparations

Good Practice Guidelines

G33.33 The IM preparation should be kept at room temperature for a maximum of 4 hours when applied to the recipient.
G33.34 Thawed IM preparations should be used the same day and should not be refrozen.
G33.35 Unused thawed IM preparations should be discarded with appropriate documentation.
G33.36 If water baths are used for thawing, only clean water baths with fresh water should be used to avoid contamination of IM preparations.

Supplementary information

Freeze–thaw cycles may adversely affect the viability of microbial communities in the IM preparation [38].

Thaw times depend on the volume and type of IM preparations. A 200 mL IM preparation may be thawed overnight in a +2-8°C refrigerator or for approximately five hours at room temperature. The thaw time for FMT capsules is usually 15 minutes from –20°C or –80°C. Warm water baths or incubators (28°C to 37°C) can be used to speed up the thawing of IM preparations. Warm baths to accelerate thawing are to be avoided whenever possible. However, if rapid thawing is required for an urgent procedure, the sample is to be thawed with continuous temperature monitoring, ensuring it remains in the water bath for the shortest time possible.

33.10.4 Application methods

FMT can be performed through different delivery routes for IM preparations, including lower delivery such as colonoscopy or enema, upper GI delivery with endoscopy or tube, or by capsules for oral ingestion. Liquid or encapsulated IM preparations are chosen accordingly.

For each delivery route, application of IM preparations from the same donor may be repeated if a single treatment fails to cure *C. difficile* or prevent recurrence.

Good Practice Guidelines

G33.37 During colonoscopy, the IM preparation should be administered into the right part of the colon of the recipient, when possible [43].
G33.38 For upper GI administration by gastroscopy, nasoduodenal or nasojejunal tube, or gastrostomy, the recipient should be positioned at least 45° upright, and remain in this position for at least two hours after the procedure to reduce the risk of aspiration [44].
G33.39 Capsules should be visually inspected before application, and all capsules should have intact shape. Capsules that have visible cracks should be discarded. In case of damaged packaging, capsules should not be used.

G33.40 Patient with swallowing difficulties that prevent capsule ingestion should not be offered encapsulated IM preparations.
G33.41 In patients with severe small bowel dysmotility, upper GI administration should be avoided or used with caution.

Recommendations

R33.18 When repeated FMTs are necessary, further administrations may be indicated.
R33.19 It is recommended to use faeces from the same donor in cases of serial application and to change the donor in any case of recurrence following initial cure [12].

Supplementary information

In patients with severe *C. difficile* infection and high risk of colonic perforation, donor faeces may be infused into the left hemicolon or via the upper GI route for safety reasons.

Capsule ingestion confers minimal procedural cost and risk and may be equally effective as other application methods. Usually, 15-30 capsules per application are preferred without significant differences in clinical effect [45].

Differences in clinical efficacy have been reported between delivery routes (capsule, endoscopy, enema). While gastrointestinal endoscopic delivery generally shows high efficacy after a single administration, enema-based delivery often requires repeated treatments to achieve sustained clinical response, as reflected in available meta-analyses and clinical experience [46].

The ideal volume for instillation of liquid faeces preparation has not been established. Volumes of 25-200 mL may be used for upper GI delivery; larger volumes (e.g. 250-500 mL) could be used for instillation via colonoscopy.

A clinical treatment plan for patients with rCDI is adjusted for disease severity and comorbidities. Outpatient treatment or in-hospital treatment is planned in accordance with the patient's underlying diseases and clinical condition. Post-procedure observation is planned according to the route of delivery, the underlying diseases and the general condition of the patient.

33.10.5 Use of FMT in severely immunocompromised patients

FMT appears safe in immunocompromised patients [47, 48]. The need to follow strict donor screening is emphasised by a report of a severely immunocompromised patient's death following transfer of multidrug-resistant organisms related to a non-compliant donor screening process [49].

For less severely immunocompromised patients (e.g. < 200 CD_4 T-cells/µL or prolonged use of corticosteroids at a mean dose of ≥ 0.3 mg/kg/d of prednisone equivalent for at least three weeks) or treatment with other recognised T-cell immunosuppressants, such as cyclosporine, tumour necrosis factor (TNF)-alpha blockers, specific monoclonal antibodies (e.g. alemtuzumab), methotrexate or nucleoside analogues during the last 90 days), or inherited severe immunodeficiency (e.g. chronic granulomatous disease or severe combined immunodeficiency), normal donor screening appears sufficient.

Recent data indicate that FMT is safe in severely immunocompromised patients, including those with GvHD and profound immunosuppression, provided it is performed with rigorous donor screening and appropriate clinical monitoring. The risk of developing complications after transfer of potential pathogens may be increased in severely immunocompromised patients, such as:

- current or foreseeable neutropenia (< 500 neutrophils/µL) within the next 14 days;
- scheduled or recent (< 100 days) allogeneic stem cell transplantation (SCT); or
- active GvHD requiring immunosuppressive treatment.

33.11 Biovigilance

General information about biovigilance is outlined in Chapter 18.

FMT is generally considered safe and, when used to treat life-threatening CDI, the immediate benefit outweighs the manageable and sometimes hypothetical risks.

Adverse reactions (ARs) following FMT include abdominal pain, dyspepsia, diarrhoea, fever or constipation. These are usually self-limiting and require no specific treatment. Serious AEs and reactions (SAREs) related to the procedure include aspiration, ileus, perforation, sepsis or even death.

Serious adverse reactions following FMT, such as death or hospitalisation due to viral or bacterial infections or relapse of IBD, have been reported in several studies and may even be underreported [50, 51]. Rare SAREs include hyperammonaemic hepatic encephalopathy as previously described [52]. This knowledge mandates reporting to biovigilance registries at the local level and to the relevant health authority, with the recording of well-defined and standardised data.

33.12 Patient follow-up

General information on patient follow up is described in Chapter 14, section 14.13.

Recommendations

R33.20 It is advised to apply more stringent follow-up to all immunocompromised patients, and careful reporting of outcomes and side effects is needed to confirm the safety of FMT in those patients.

Supplementary information

Reporting may be summarised on an annual basis and includes the number of patients, the treatment modalities used (e.g. colonoscopy, enema, nasoenteric tube, gastroscopy, capsules), as well as follow-up data including safety and clinical outcomes, adverse events and adverse reactions. Post-FMT follow-up of up to two years is commonly performed by a SoHO entity.

At present, there are no specific national or international registries on FMT. This situation makes it difficult to record the level of risk of an SARE and the level of success of the different methods of preservation. A prospective registry could enable monitoring of individual FMT services and at national and international levels. Providing data to a European FMT registry is encouraged, allowing comparison of outcomes between FMT services and facilitation of quality improvement.

In addition, general information on donor protections is described in Chapter 5, section 5.2.

33.13 Developing applications

While the use of FMT is established for *C. difficile* infection, potential future indications are being investigated in clinical trials. These include, but are not restricted to, ulcerative colitis, irritable bowel syndrome, relapsing pouchitis, carrier status with multidrug-resistant organisms, hepatic encephalopathy, GvHD and immune checkpoint inhibitor treatment or its complications.

The question of an optimal donor–recipient match, in particular the hypothesis of the existence of so-called super donors, remains elusive [53]. No established definition of the normal IM exists, and several attempts have been made to define a disrupted IM, generally termed dysbiosis [40]. While faeces donor selection for FMT in patients with *C. difficile* infection may mainly be based on the absence of risk factors, optimised donor selection might influence the effectiveness of SoHO in conditions such as IBD and cancer therapy. It is also important to consider avoiding the use of impaired microbiota that could be ineffective or even harmful to the recipient.

The presence of particular micro-organisms and other donor-specific characteristics may confer beneficial therapeutic outcomes even in patients with *C. difficile* infection [54].

The use of multi-donor preparations may ensure a high microbial diversity in the treatment preparation [55], but it challenges basic principles of traceability and the concept of transplanting a complete and balanced viable microbial ecosystem from a single donor to the recipient.

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Chapter 34. **SoHO as starting materials**

34.1 Introduction

In recent years, novel therapies have been and are being developed involving substances of human origin (SoHO) and/or using them as starting materials for the manufacture of new therapeutic products. These new applications or products can be difficult to classify because they are subject to different regulatory frameworks at different stages of their manufacturing process, from donor registration to clinical application.

This chapter aims to provide guidance on the quality and safety of SoHO used as starting materials. It applies to SoHO entities that undertake:

- donor registration;
- donor history review and medical examination;
- testing of donors or of persons from whom SoHO are collected for autologous or within-relationship use;
- or the collection or release of SoHO in connection with the manufacture of:
  - medical devices, regulated by Regulation (EU) 2017/745,
  - medicinal products, regulated by Directive 2001/83/EC,
  - advanced therapy medicinal products (ATMPs), regulated by Regulation (EC) No 1394/2007,
  - investigational medicinal products, regulated by Regulation (EU) No 536/2014.

If activities like storage, distribution, import and export are carried out in SoHO establishments prior to the start of manufacturing processes, they are also within scope of this Guide and this chapter.

The general quality and safety requirements of the chapters in Part A apply here, but some specific considerations are further developed in this chapter. An overview of the process workflow from SoHO collection to ATMP manufacturing is provided in Figure 34.1.

It is important to note that, in different countries outside the European Union (EU), these SoHO-based therapies may fall under different regulatory frameworks, including those for transplantation, medicinal products, ATMPs or medical devices.

Any operator active in the field needs to carefully consider the legal requirements that apply to the activities they are undertaking, and it is advisable that, before starting any activities, they consult with the relevant authorities to understand the regulatory environment and seek any licence/authorisation that may be required. An overview of the legal framework for the development of ATMPs in the EU is provided in Appendix 36 [1, 2].

Another important aspect to consider is distribution and import/export. Many SoHO are collected and then cross borders. According to the SoHO Regulation 2024/1938, the SoHO establishment is responsible for such procedures. Guidelines on performing these activities are outlined in Chapter 13.

Importantly, although SoHO establishments are authorised in accordance with Regulation (EU) 2024/1938, ATMP manufacturers may request quality audits to ensure that all steps performed in SoHO establishments follow the specifications of their ATMPs and their applicable directive.

Lastly, good communication between biovigilance and pharmacovigilance systems facilitates effective investigation and corrective/preventive actions if ATMPs are associated with adverse outcomes, including those reported by the donor or identified through patient follow-up.

34.2 Donor for starting materials

General information about recruitment, consent, evaluation and testing of SoHO donors who provide

starting materials for ATMPs is outlined in Chapters 5 (section 5.3), 6 and 7 of this Guide.

Good Practice Guidelines

G34.1 SoHO files documenting the development of an ATMP should ensure that consent remains valid at all stages of the process.

Recommendations

R34.1 Currently, the recommendation is that no routine genetic screening be carried out on donors, provided that relevant genetic tests will be done on the stem cell lines or derived ATMP.

Supplementary information

Recent and rapid advances in biological and medical research are allowing SoHO to be used increasingly in new ways. However, their expanding therapeutic potential and unique source present their own challenges, requiring a delicate balance between three factors: the duty of care owed to donors; the duty of care owed to recipients; and the development and regulation of the technologies themselves.

SoHO can only be obtained from the body of a person, making ethical principles the essential cornerstone of their use. The person providing the material may be living or deceased, and the material may be used almost immediately or stored for long periods. It may also be used following minimal manipulations or heavily processed. Due to this diversity of circumstances, the limits of consent are clearly defined. If starting materials are used to manufacture products regulated by other legislation, specific consent will be required.

In this regard, some professional associations and other concerned bodies, such as the European Committee on Organs, Tissues and Cells (CD-P-TO) of the Council of Europe and the World Marrow Donor Association (WMDA), have offered guidance [3, 4]. The WMDA has published an update of the principles that guide donation for cell and genetic therapies, and this supports non-remuneration as the best way to ensure the safety and wellbeing of donors and patients alike. The principle of prohibiting financial gain from the human body and its parts, as such, whether the donor is living or deceased (and by extension the need to ensure voluntary and unpaid donation), remains central in any consideration of the donation or use of SoHO.

With respect to intermediate cell banks, when SoHO is processed for storage or intended to be repurposed for the manufacture of different ATMPs in the future, the original consent is taken into consideration in any decisions regarding its subsequent use. If the initial consent does not permit a specific new application, a new consent procedure may be required for that additional use, and if the consent process allows (G34.1).

The reason for not recommending routine genetic screening (R34.1) of the donor is that there is a significant genetic distance between the donor and the final ATMP. Trace-back is not performed routinely but is personalised at the discretion of the attending physician and in agreement with the person(s) responsible for the care of the donor.

34.3 Collection of starting materials

The process flow for the collection of starting material for the manufacture of novel therapies involving SoHO usually starts with the referral of a donor (living or deceased) by the clinical site or donor team to the collection entity.

Collection of SoHO as starting material for ATMPs is described in Chapter 8 of this Guide and, depending on the nature of the SoHO collected, some additional tests may be performed either for quality or safety of the future ATMPs, according to the manufacturer's instructions.

Aspects of labelling, storage (if necessary) and transport are outlined in Chapters 16, 11 and 13, respectively.

Good Practice Guidelines

G34.2 The findings from any tests should be communicated to the ATMP manufacturer to ensure their requirements are met.

G34.3 Collection should be carried out according to the general requirements detailed in Chapter 8, taking into account any additional instructions that might have been detailed in the manufacturer's protocol.

G34.4 Acceptance criteria for SoHO used as starting materials for the manufacture of ATMPs, such as minimal cell number, viability or cell composition or tissue characteristics, should be established by the ATMP manufacturer, who may also require additional checks.

G34.5 Specifications requested by ATMP manufacturers related to these SoHO should dictate whether and to what stage substances and materials can have a defined level of bioburden or whether they need to be sterile.

G34.6 Collected SoHO should be transported by SoHO entities to an affiliated SoHO establishment for direct distribution to the ATMP manufacturer or for further processing, including minimal manipulations, if required.

G34.7 SoHO entities and establishments involved and the ATMP manufacturer should agree in advance on the extent of additional documentation that should accompany the collection and distribution.

G34.8 Adverse events associated with the collection step should be recorded, reviewed and reported through the biovigilance process in accordance with Chapter 18 of this Guide.

G34.9 Living donors should undergo post-collection follow-up in accordance with a defined procedure.

34.4 Processing of starting material

After collection, SoHO are transferred to a SoHO establishment where they can be directly released or undergo processing by non-substantial manipulations. The following processing methods are considered non-substantial manipulations according to annex 1, EC 1394/2007: cutting, grinding, shaping, centrifugation, soaking in antibiotic or antimicrobial solutions, sterilisation, irradiation, cell separation, concentration or purification, filtering, lyophilisation, freezing, cryopreservation and vitrification.

Figure 34.1 shows an example of process flow from collection by SoHO entities to distribution to ATMP manufacturers. Potential activities covered by SoHO regulation are described.

Good Practice Guidelines

G34.10 Any non-substantial manipulation performed at the level of the SoHO establishment should be defined following specifications agreed with the ATMP manufacturer. Those should be reflected in an appropriate third-party agreement between them.

G34.11 All SoHO-derived starting materials should be released before being distributed to the ATMP manufacturer.

Supplementary information

Many types of SoHO are currently being used or researched for the development of new therapeutic solutions (see Table 34.1). Due to their undifferentiated nature, stem cells can be expanded *in vitro* and differentiated into various tissue-specific cells. This unique characteristic can be exploited to support the development of new therapies for the treatment of several conditions. However, before considering a new cell-based therapy, it is necessary to understand the physiological properties of each progenitor cell type. In addition, it is important to take into consideration that in some countries the use of some of these cells may not be permitted by law. Within cells, lymphocytes are used as a source for immunotherapy, and this is probably the most frequently used cell type for developing ATMPs, currently.

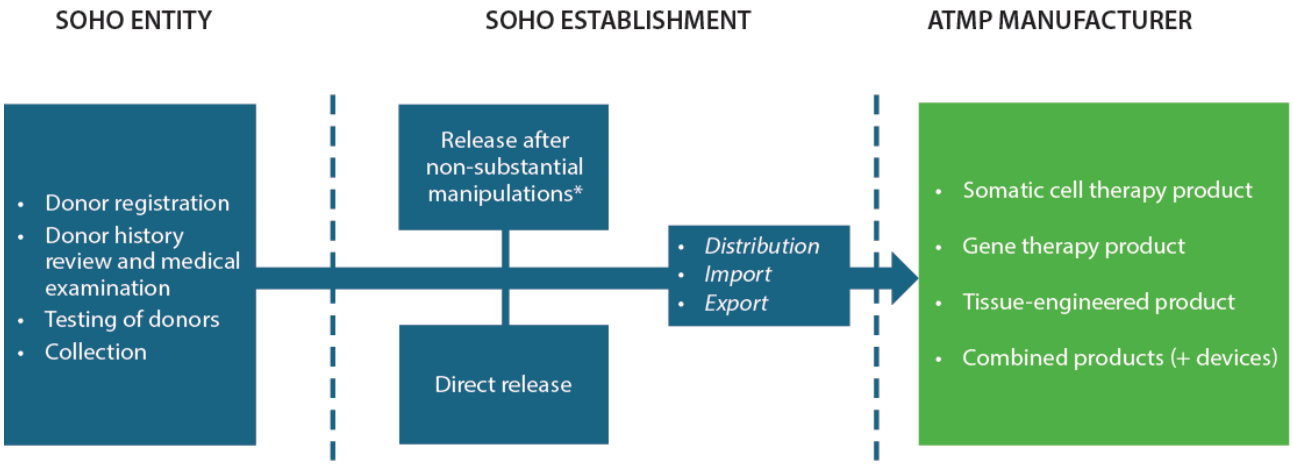
In addition to cell sources, tissues can also serve as scaffolds to mimic extracellular matrix (ECM) in the context of tissue engineering. The ECM has a heterogeneous composition of proteoglycans, proteins and signalling molecules, and can thus influence cell differentiation, proliferation, survival and migration. These structures, alone or in combination with cells and bioactive agents, provide mechanical support during *in vitro* cell growth and *in vivo* implantation. A diversity of biomaterials and manufacturing methods, including 3D bioprinting, have been used to create novel alternatives to traditional tissue engineering strategies (see §34.4.7 below). Natural and synthetic polymers, bioceramics or combinations of these as composites have been used.

Below, sections 34.4.1 to 34.4.7 list seven examples of the most common starting materials.

34.4.1 Starting material from blood-derived mononuclear cells

Several immunotherapy applications – such as chimeric antigen receptor (CAR)-T-cells [5], virus-specific T-cells [6, 7], 'natural killer' (NK) cells [8] or dendritic cells [9], among others – are increasingly used for therapy. Normally, they use the patient's own blood-derived cells; however, registered stem-cell donors, cord blood banks or other third-party donor registries can also be employed to create personalised therapies for recipients of allogeneic haematopoietic progenitor cells (HPC) transplantation, or to develop off-the-shelf therapies for universal use. For more information see Chapters 25 and 26.

Figure 34.1 **Process flow and SoHO activities for starting materials**



*Annex 1, EC 1394/2007: List of non-substantial manipulations (as above, §34.4).

Donors are evaluated for their eligibility for an apheresis procedure, bone marrow collection or blood donations. Currently, cell preparations from leukapheresis procedures are the most common source of starting material, and collection sites specialising in HPC collection are normally used for these procedures. For eligibility, the medical evaluation of the donor considers the burden associated with the collection of a large number of leukocytes using apheresis devices. Special procedures would be in place for paediatric donors. Depending on the method used to obtain blood cells, a predetermined level of target cells may be relevant before collection (see section on HPC collection in Chapter 25).

The mononuclear cell fraction isolated through an apheresis procedure is normally used as starting material to ensure a higher dose of target cells for cell culture. The blood volume processed, to obtain enough cells, depends on the patient's peripheral blood counts and is calculated to avoid unnecessary apheresis time with the increasing risks of serious adverse reactions (see also Chapter 25).

34.4.2 Starting materials from mesenchymal stromal cells

Mesenchymal stromal cells (MSCs) are multipotent stem cells with immuno-regulatory and regenerative properties [10, 11]. They can normally be isolated from bone marrow and, recently, from other non-bone-marrow sources, such as umbilical cord blood, adipose tissue, adult muscle or the dental pulp of deciduous baby teeth. After growing in adherence cultures, they consist of a defined population with the ability to differentiate into a variety of cell types, including osteoblasts, chondrocytes and adipocytes. Due to their unique characteristics, they can be used either in immunotherapy through their modulating properties, or in regenerative medicine through their paracrine effects on tissue repair.

Considering their regenerative capacity function, MSCs are used preferably from an autologous source, although they can also be used in allogeneic settings.

The three main sources of MSCs presently used for human application are bone marrow, adipose tissue and umbilical cord, and can be obtained in brief as follows. Bone marrow is normally obtained after aspiration from the anterior iliac crest with the patient in a supine position. The aspiration syringe is loaded with heparin to prevent clotting of the marrow sample. The technique is like that used in bone marrow collection for HPC transplantation. In the case of adipose tissue, MSCs are found in subdermal adipose tissue and are normally obtained through abdominal liposuction using the techniques of aesthetic surgery. Finally, MSCs can be isolated from all perinatal tissues, such as placenta, amniotic membrane, umbilical cord and Wharton's jelly. These tissues, which are the less invasive source for MSCs, are aseptically collected during caesarean section and can be obtained from different areas of cord and placental tissues, which are dissected before further processing.

Table 34.1 **Some cell types being used to develop novel cell-based therapies**

	Cell type	Source	Processing
Pluripotent	Embryonic stem cells (ESC)	Cells obtained from the inner cell mass of blastocysts which have been cryopreserved and are no longer to be used for fertility treatment.	Cultured embryos (maximum 14 days) are dissociated, and the inner cell mass is removed and cultured for a few more days. Then inner cell mass outgrowths consisting of potential ESC are isolated and expanded to create stem cell lines.
	Reprogrammed stem cells	Somatic cells which are reprogrammed to an embryonic stem cell-like state.	Reprogramming is achieved by introducing into adult cells (e.g. epithelial cells) a defined and limited set of transcription factors (e.g. stemness transcription factors) giving rise to induced pluripotent stem cells (iPSC). Cell reprogramming can also be achieved by nuclear transfer, giving rise to nuclear-transfer pluripotent stem cells (ntPSC).
Multipotent (lineage-restricted)	Somatic stem cells	Found in various tissues and may be isolated from extra-embryonic tissues, fetal specimens and adult tissues. Generally referred to by their tissue origin (mesenchymal stem cell, adipose-derived stem cell, endothelial stem cell, dental pulp stem cell, neural stem cells, etc.)	Specific protocols have been developed, depending on the cell type.
Lineage-committed	Progenitor cells (e.g. haematopoietic progenitor cells), immune cells	Somatic cells that are committed to a specific mature cell fate and can divide only a limited number of times.	Can be transplanted without *in vitro* expansion (e.g. bone marrow, peripheral blood, cord blood) or cultured *in vitro* and differentiated into more restricted cell types for clinical application. SoHO used for advanced therapies could be applied to two main areas: (1) to restore or produce immunological functions in patients (immunotherapy) and (2) to restore organ- or tissue-specific functions (regenerative medicine).

Besides the sources described above, MSCs can also be derived from other solid organs/tissues, such as the liver, the kidney, the lung, the pancreas and others.

34.4.3 Starting material from chondrocytes

In cartilage repair, autologous chondrocyte implantation (ACI) therapy is widely used for the treatment of isolated cartilage defects [12]. This technique is based on an implantation of a suspension of *in vitro* expanded chondrocytes.

For donor evaluation, the patient is examined by an arthroscopic procedure where the location, depth and size of the defect and the quality of the surrounding cartilage are evaluated. A typical patient is a young patient with large (> 2 cm^2) full-thickness chondral or osteochondral defect surrounded by healthy cartilage. The ACI technique includes a two-stage procedure, with an initial collection of a cartilage biopsy, which is sent for chondrocyte culture, followed by a second-stage operation that includes the cell application. A full-thickness cartilage biopsy (about 200-400 mg) is procured from a low-weight-bearing area of the knee during arthroscopy. The biopsy is transferred to a sterile transport tube with biopsy medium. The biopsy tube and blood tubes are then placed in an outer secondary packaging that ensures the sterility and maintenance of the temperature and is approved for transport of biological substances (see also Chapter 16). The biopsy is kept cold – at about 5-15°C – during transport to ensure the quality of the biopsy specimen. Then, it is sent to the appropriate laboratory for further processing.

34.4.4 Starting material from keratinocytes

Ex vivo expanded keratinocytes can be used in skin repair [13]. They are derived from skin biopsies that can be obtained as follows. In the autologous setting, donor-site selection and timing are important. To get access to as many adult stem and progenitor cells as possible, the donor site is preferably in a hair-bearing area of healthy skin. The sooner (after trauma) the skin biopsy is taken, the better because the patient (and tissue) might be contaminated by microbes, which will affect the subsequent cell culture. However, the skin areas available for donor-site selection are principally determined by the extent and location of the injured skin.

For allogeneic application, donor selection includes – apart from general donor evaluation criteria (see Chapters 6 and 7) – the tissue-specific criteria defined in Chapter 22. The transplanted allogeneic keratinocytes act as a temporary wound coverage, stimulating wound healing; thus, human leukocyte antigen (HLA) typing or ABO blood grouping are not necessary. When collecting the skin for culture of keratinocytes, it is beneficial that the site for the biopsy is in an area with healthy skin as remote from the lesional skin as possible. The biopsy can be either full-thickness or split-thickness. A full-thickness skin biopsy is preferred because of the number of stem and progenitor cells in the appendages (hair follicles, sweat glands, etc.). The collection goal is to yield as many non-differentiated keratinocytes as possible.

The biopsy site is first cleaned properly with disinfectant – i.e. ethanol (70%) with no additives – and, after the site has dried, it is given a second wash with sterile saline solution before the biopsy is done (e.g. with a scalpel). Local anaesthetics can be used *ad lib*.

Immediately after collection, the biopsy is placed in a medium suitable for transportation (basal culture medium with or without serum – or similar – and antibiotics in normal cell-culture concentrations). Then, use a sterile primary container, closed and appropriately labelled. Transport the biopsy to the culture facility and initiate the cell-isolation process as soon as possible (for example, < 24-48 hours after surgical removal of the biopsy). Minimising the time will increase the likelihood of the successful culture of cells. Handling and culturing the skin biopsy/keratinocytes *in vitro* opens a risk of contributing (microbiological) contaminants to the cells, both from the skin itself and from culture conditions. Those risks can only be avoided by adequate facilities with a controlled environment (see Chapter 9) and skilful, excellent handling techniques by the staff (see Chapter 2).

34.4.5 Starting material from limbal stem cells

Limbal stem cell (LSC) deficiency refers to acquired pathological deficiencies such as chemical burns and inherited deficiencies such as aniridia, which may lead to ocular surface disease, including persistent epithelial defects with chronic inflammatory conditions, vascularisation and scarring of the cornea and conjunctiva, and corneal conjunctivalisation [14-15]. LSCs are being used to treat these conditions. Donors are tested for infectious diseases as described in Chapter 7, although in the case of autologous use a positive test result may not be an exclusion criterion.

The treatment of unilateral LSC deficiency may involve *ex vivo* expansion of a tissue explant or isolated LSCs from the unaffected limbal region of a patient's healthy eye. In this case the autologous cell population is isolated, and the final aim is to expand the limbal epithelial cells for application into the affected eye. Bilateral LSC deficiency, on the other hand, is a devastating pathological condition affecting both eyes; in this case, autologous limbal tissue cannot be sourced from the same patient since both eyes are affected. Alternative sources of tissue include the culture of epithelial cells lining the autologous oral mucosa. Allogeneic sources of tissue, from deceased donors, may be an option for restoring the function of the ocular surface, although procedures with autologous healthy tissue are always preferable to those using heterologous sources [16, 17].

34.4.6 Starting material from stromal vascular fraction

Stromal vascular fraction (SVF) is isolated from adipose tissue; see Chapter 29. SVF may be used either directly or as a source material to isolate regenerative cells for treating various clinical conditions including musculoskeletal, neurological, immunological, cardio-pulmonary and immunological disorders, as well as soft tissue defects [18, 19]. Commonly, SVF cells are of autologous origin. Donor testing includes assays for transmissible diseases as described in Chapter 7; although their presence is not an exclusion criterion, there is benefit in documenting any deviation, and the operating procedures need to describe any special actions to avoid cross-contamination to other cells and to ensure the safety of personnel.

SVF can be isolated from either resected adipose tissue or aspirated adipose tissue using tumescent liposuction. Further details can be found in Chapter 29 on adipose tissue. There is no consensus on what the best area is to obtain these cells. Although a common procedure is still lacking, in general, minced adipose tissue is digested by enzymes including collagenase, dispase, trypsin or similar [19]. However, mechanical procedures have also been reported [20]. After neutralising the enzymes, the released elements defined as SVF are separated from mature adipocytes by differential centrifugation.

34.4.7 Starting materials for bioprinting

Bioprinting is an emerging technology in the regenerative medicine field, and it is being explored to tackle the need for tissues and organs suitable for the treatment of patients. It allows the generation of 3D structures by controlled disposition of biological materials and supporting components, and functional elements as biochemicals or living cells. The technique itself arranges these components layer-by-layer with a particular spatial placement of functional components [21].

The final aim of the technique is to mimic, to the maximum extent, the natural environment of living tissues at both structural and cellular levels to generate and use complex 3D functional living tissues. For example, multilayered skin, bone, vascular grafts, tracheal splints, heart tissue and cartilaginous structures have been implanted [22, 23]. Importantly, the combination of natural biomaterials (that possess the appropriate biochemical and biomechanical signals) with bioprinting (which can design complex structures with specific shape and size at macroscopic and microscopic levels) opens the door to obtaining a new set of tissues with defined and specific characteristics.

Natural tissue matrices, also known as decellularised ECMs, have been used for bioprinting [22-25] and are very useful as source material for the 'building blocks' required for bioprinting; many of the attributes of decellularised tissues – such as their biocompatibility, their ability to support the attachment and differentiation of cells, and their capacity to be remodelled by the recipient's innate biology – make them ideal for this purpose. SoHO establishments are developing awareness and appreciation of this novel use of donated tissues.

The ability to collect an effective custom-made SoHO preparation, with specific shape, size, porosity and mechanical properties, is of great interest for personalised medicine in treating specific patient pathologies. Ideally, the composition of this *de novo*-built SoHO preparation will accomplish certain specific needs, such as the induction of new tissue formation and regeneration, without activating the immunological response [26]. Several studies show that decellularised ECM from different tissues can promote regeneration in damaged areas, such as demineralised bone [27], amniotic membrane [28], nerve [29] and skin [30, 31]. In recent years, due to the biological composition of ECM, the use of decellularised tissues as starting material has been considered for the generation of an ECM-based biomaterials pool to develop bio-inks in 3D bioprinting. The main hypothesis is that, if decellularised ECM promotes tissue regeneration, so the ECM pool from digested tissue could be used as the starting materials in a 3D bioprinting technique to develop *de novo* personalised grafts.

Moreover, the bioprinting technique offers the opportunity to combine different natural materials, such as collagens [32], fibrin [33], gelatin [34] or fibrinogen [20], to obtain hybrid scaffolds, to enhance bioactivity in the implantation site. The advantage of using natural polymers is that they fully satisfy the biochemical requirements of the tissue in terms of composition and biochemical signalling. Nowadays, the natural polymers used are mainly collagen and gelatine obtained from tendons and ligaments from SoHO establishments. This approach allows clinicians and patients to benefit from the biological properties of human SoHO preparations that have been increasingly used in recent decades and gives the opportunity to SoHO establishments to integrate the new 3D bioprinting technologies for more personalised SoHO preparations.

Of note, these approaches have not yet been deeply studied for human application. Therefore, a lot of effort is still needed in this field.

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Related material

Appendix 36. Legal framework for the development of advanced therapy medicinal products in the European Union

Part C.

SoHO monographs

20.1 Organ-cultured corneal donor tissue for (deep) anterior lamellar keratoplasty (ALK/DALK)

SoHO preparation	Organ-cultured corneal donor tissue for (deep) anterior lamellar keratoplasty (ALK/DALK)
Definition	Organ-cultured corneal donor tissue to be used for anterior lamellar keratoplasty: cornea, full thickness (low endothelial cell density).
Established clinical indications	• Stromal disease caused by keratoconus, corneal dystrophies, scars and keratitis, or similar diseases compromising corneal function or the integrity of the cornea, when the endothelium of the recipient is assumed to have normal function.
Critical properties	• Absence of stromal abnormalities. • Endothelial cell density measurement may be required for DALK if surgeon needs to switch to penetrating keratoplasty (PK) when anterior chamber is penetrated. • No evidence of microbial growth.
Quality control requirements	• Normal morphology and integrity of the corneal stroma. • Not to be used if the organ-culture medium is turbid or becoming yellow, or if samples of medium are culture-positive for micro-organisms.
Storage and transport	• The SoHO preparation is stored in organ-culture medium with an osmotically active agent to keep the tissue thin (Dextran or similar component). • The SoHO preparation is not refrigerated and needs to be transported at 10-38°C. • The SoHO preparation can be stored up to seven days depending on the concentration and type of osmotic thinning agent used in the medium.
Special labelling and accompanying information	• Placed in a container appropriately labelled with a unique identifying code. This code is included in the accompanying documentation. • In the EU, when SoHO preparations are distributed for human application, they must be labelled with the Single European Code (SEC) as applicable. • Specific information not coded in the SEC that should be included in the accompanying documentation: – Endothelial cell density, if applicable; – Diameter of the central clear zone; – Expiration date.
Special warnings	• Not to be used for penetrating keratoplasty unless surgeon needs to switch to PK and the endothelial cell density is as specified in the quality control requirements (above).

20.2 Cold-stored corneal tissue for (deep) anterior lamellar keratoplasty (ALK/DALK)

SoHO preparation	Cold-stored corneal tissue for (deep) anterior lamellar keratoplasty (ALK/DALK)
Definition	Cold-stored corneal donor tissue to be used for anterior lamellar keratoplasty: cornea, full thickness (low endothelial cell density).
Established clinical indications	• Stromal disease caused by keratoconus, corneal dystrophies, scars and keratitis, or similar diseases compromising corneal function or the integrity of the cornea, when the endothelium of the recipient is assumed to have normal function.
Critical properties	• Absence of stromal opacities. • Endothelial cell density measurement may be required for DALK if surgeon needs to switch to penetrating keratoplasty (PK) when anterior chamber is penetrated. • No evidence of microbial growth.
Quality control requirements	• Morphology and integrity of the corneal stroma. • Not to be used if the preservation medium is turbid or becoming yellow.
Storage and transport	• The SoHO preparation is stored in cold-storage medium. • The SoHO preparation is refrigerated (1-10°C). • The SoHO preparation can be stored up to 21 days according to the manufacturer's instructions (precise duration of storage depends on the type of storage medium). • The SoHO preparation is transported to the ORHA at 1-10°C.
Special labelling and accompanying information	• Placed in a container appropriately labelled with a unique identifying code. This code is included in the accompanying documentation. • In the EU, when SoHO preparations are distributed for human application, they must be labelled with the Single European Code (SEC) as applicable. • Specific information not coded in the SEC that should be included in the accompanying documentation: – Endothelial cell density, if applicable; – Diameter of the central clear zone; – Expiration date.
Special warnings	• Not to be used for penetrating keratoplasty unless surgeon needs to switch to PK and the endothelial cell density is as specified in the quality control requirements (above).

20.3 Organ-cultured corneal tissue for Descemet membrane endothelial keratoplasty (DMEK)

SoHO preparation	Organ-cultured corneal tissue for Descemet membrane endothelial keratoplasty (DMEK)
Definition	Organ-cultured corneal donor tissue to be used for DMEK: cornea for endothelial keratoplasty (precut/peeled in the substances of human origin (SoHO) establishment).
Established clinical indications	• Primary endothelial failure (mainly Fuchs corneal dystrophy). • Secondary endothelial failure (mainly pseudophakic bullous keratopathy). • Regraft for endothelial decompensation.
Critical properties	• Endothelial cell density. • No evidence of microbial growth.
Quality control requirements	• Descemet membrane is manually peeled off the corneal stroma with the endothelium attached, either completely (free roll) or attached centrally or peripherally to the corneal stroma. • Endothelial cell density measurement by microscopy after organ culture but before Descemet membrane peeling to ensure sufficient endothelial quality. • Graft diameter measurement with calliper or trephine. Peripheral tears (damage) in Descemet membrane should be noted in the accompanying documentation. • Not to be used if the organ-culture medium is turbid or becoming yellow, or if samples of the medium are culture-positive for bacteria or fungi.
Storage and transport	• The SoHO preparation is stored in organ-culture medium with or without an osmotically active agent. • The SoHO preparation should not be refrigerated and should be transported at 10-38°C. • The SoHO preparation can be stored up to seven days depending on the concentration and type of osmotic thinning agent used in the medium.
Special labelling and accompanying information	• Placed in a container appropriately labelled with a unique identifying code. This code is included in the accompanying documentation. • In the EU, when SoHO preparations are distributed for human application, they must be labelled with the Single European Code (SEC) as applicable. • Specific information not coded in the SEC that should be included in the accompanying documentation: – Donor age; – Endothelial cell density; – Expiration date; – Graft diameter if already trephined and presence of tears (damage); – Whether the TCP is attached to the stroma, free-flowing as a roll, or preloaded in an injector.
Special warnings	• Not to be used for penetrating keratoplasty.

20.4 Cold-stored corneal tissue for Descemet membrane endothelial keratoplasty (DMEK)

SoHO preparation	Cold-stored corneal tissue for Descemet membrane endothelial keratoplasty (DMEK)
Definition	Cold-stored corneal donor tissue to be used for DMEK: cornea for endothelial keratoplasty (precut/peeled in the SoHO establishment).
Established clinical indications	• Primary endothelial failure (mainly Fuchs corneal dystrophy). • Secondary endothelial failure (mainly pseudophakic bullous keratopathy). • Regraft for endothelial decompensation.
Critical properties	• Endothelial cell density. • No evidence of microbial growth.
Quality control requirements	• Descemet membrane is manually peeled off the corneal stroma with attached endothelium, either completely (free roll) or attached centrally or peripherally to the corneal stroma. • Endothelial cell density measurement by microscopy before Descemet membrane peeling to ensure sufficient endothelial quality. • Graft diameter measurement with calliper or trephine. • Peripheral tears (damage) in Descemet membrane should be noted in accompanying documentation. • Not to be used if the storage medium is turbid or becoming yellow.
Storage and transport	• The SoHO preparation is stored in cold-storage medium. • The SoHO preparation is refrigerated (1-10°C). • The SoHO preparation can be stored up to 21 days according to the manufacturer's instructions (precise duration of storage depends on the type of storage medium). • The SoHO preparation is transported to the ORHA at 1-10°C.
Special labelling and accompanying information	• Placed in a container appropriately labelled with a unique identifying code. This code is included in the accompanying documentation. • In the EU, when SoHO preparations are distributed for human application, they must be labelled with the Single European Code (SEC) as applicable. • Specific information not coded in the SEC that should be included in the accompanying documentation: – Donor age; – Endothelial cell density; – Expiration date; – Graft diameter if already trephined and presence of tears (damage); – Whether the graft is attached to the corneal stroma, free-flowing as a roll, or preloaded in an injector.
Special warnings	• Not to be used for penetrating keratoplasty.

20.5 Organ-cultured corneal tissue for Descemet stripping automated endothelial keratoplasty (DSAEK)

SoHO preparation	Organ-cultured corneal tissue for Descemet stripping automated endothelial keratoplasty (DSAEK)
Definition	Organ-cultured corneal donor tissue to be used for DSAEK: cornea for endothelial keratoplasty (precut/peeled) in the SoHO establishment.
Established clinical indications	• Primary endothelial failure (mainly Fuchs corneal dystrophy). • Secondary endothelial failure (mainly pseudophakic bullous keratopathy). • Regraft for endothelial decompensation.
Critical properties	• Endothelial cell density. • Absence of stromal abnormalities. • No evidence of microbial growth.
Quality control requirements	• Endothelial cell density measurement by microscopy after organ culture but before pre-cutting. • No stromal opacities in the central zone. • Graft diameter measurement with calliper. • Not to be used if the organ-culture medium is turbid or becoming yellow, or if samples of medium are culture-positive for micro-organisms.
Storage and transport	• The SoHO preparation is stored in organ-culture medium with an osmotically active agent to keep the tissue thin (Dextran or similar component). • The SoHO preparation should not be refrigerated and should be transported at 10-38°C. • The SoHO preparation can be stored up to 7 days, depending on the concentration and type of osmotic thinning agent used in the medium.
Special labelling and accompanying information	• Placed in a container appropriately labelled with a unique identifying code. This code is included in the accompanying documentation. • In the EU, when SoHO preparations are distributed for human application, they must be labelled with the Single European Code (SEC) as applicable. • Specific information not coded in the SEC that should be included in the accompanying documentation: – Endothelial cell density; – Expiration date; – Central SoHO preparation thickness; – Cap diameter.
Special warnings	• Not to be used for penetrating keratoplasty.

20.6 Cold-stored corneal tissue for Descemet stripping automated endothelial keratoplasty (DSAEK)

SoHO preparation	Cold-stored corneal tissue for Descemet stripping automated endothelial keratoplasty (DSAEK)
Definition	Cold-stored corneal donor tissue to be used for DSAEK: cornea for endothelial keratoplasty (precut/peeled) in the SoHO establishment.
Established clinical indications	• Primary endothelial failure (mainly Fuchs corneal dystrophy). • Secondary endothelial failure (mainly pseudophakic bullous keratopathy). • Regraft for endothelial decompensation.
Critical properties	• Endothelial cell density. • Absence of stromal opacities. • No evidence of microbial growth.
Quality control requirements	• Endothelial cell density measurement by microscopy before pre-cutting. • No stromal opacities in the central zone. • Graft diameter measurement with calliper. • Not to be used if the storage medium is turbid or becoming yellow.
Storage and transport	• The SoHO preparation is stored in cold-storage medium. • The SoHO preparation is refrigerated (1-10°C). • The SoHO preparation can be stored up to 21 days according to the manufacturer's instructions (precise duration of storage depends on the type of storage medium). • The SoHO preparation is transported to the ORHA at 1-10°C.
Special labelling and accompanying information	• Placed in a container appropriately labelled with a unique identifying code. This code is included in the accompanying documentation. • In the EU, when SoHO preparations are distributed for human application, they must be labelled with the Single European Code (SEC) as applicable. • Specific information not coded in the SEC that should be included in the accompanying documentation: – Endothelial cell density; – Expiration date; – Central SoHO preparation thickness; – Cap diameter.
Special warnings	• Not to be used for penetrating keratoplasty.

20.7 Organ-cultured corneal tissue for penetrating keratoplasty (PK)

SoHO preparation	Organ-cultured corneal tissue for penetrating keratoplasty (PK)
Definition	Organ-cultured corneal donor tissue to be used for PK: cornea, full thickness (high endothelial cell density).
Established clinical indications	Primary endothelial failure (mainly Fuchs corneal dystrophy). Secondary endothelial failure (mainly pseudophakic bullous keratopathy). • Stromal disease (keratoconus, corneal dystrophies with stromal involvement, scars). • Keratitis and similar diseases compromising corneal functions or the integrity of the eye. • Regraft.
Critical properties	• Endothelial cell density. • Absence of stromal opacities. • No evidence of microbial growth.
Quality control requirements	• Endothelial cell density measurement by microscopy. • No stromal scars/opacities in the central zone. • Not to be used if the organ-culture medium is turbid or becoming yellow, or if samples of medium are culture-positive for micro-organisms.
Storage and transport	• The SoHO preparation is stored in organ-culture medium with an osmotically active agent to keep the tissue thin (Dextran or similar component). • The SoHO preparation should not be refrigerated and should be stored at 10-38°C. • The SoHO preparation can be stored up to seven days depending on the concentration and type of osmotic thinning agent used in the medium.
Special labelling and accompanying information	• Placed in a container appropriately labelled with a unique identifying code. This code is included in the accompanying documentation. • In the EU, when SoHO preparations are distributed for human application, they must be labelled with the Single European Code (SEC) as applicable. • Specific information not coded in the SEC that should be included in the accompanying documentation: – Endothelial cell density; – Expiration date; – Diameter of the clear zone.
Special warnings	• None.

20.8 Cold-stored corneal tissue for penetrating keratoplasty (PK)

SoHO preparation	Cold-stored corneal tissue for penetrating keratoplasty (PK)
Definition	Cold-stored corneal donor tissue to be used for PK: cornea, full thickness (high endothelial cell density).
Established clinical indications	• Primary endothelial failure (mainly Fuchs corneal dystrophy). • Secondary endothelial failure (mainly pseudophakic bullous keratopathy). • Stromal disease (keratoconus, corneal dystrophies with stromal involvement, scars). • Keratitis and similar diseases compromising corneal functions or the integrity of the eye. • Regraft.
Critical properties	• Endothelial cell density. • Absence of stromal opacities. • No evidence of microbial growth.
Quality control requirements	• Endothelial cell density measurement by microscopy before cold storage. • No stromal scars or opacities in the central zone. • Not to be used if the storage medium is turbid or becoming yellow.
Storage and transport	• The SoHO preparation is stored in cold-storage medium. • The SoHO preparation is refrigerated (1-10°C). • The SoHO preparation can be stored up to 21 days according to the manufacturer's instructions (precise duration of storage depends on the type of storage medium). • The SoHO preparation is transported to the ORHA at 1-10°C.
Special labelling and accompanying information	• Placed in a container appropriately labelled with a unique identifying code. This code is included in the accompanying documentation. • In the EU, when SoHO preparations are distributed for human application, they must be labelled with the Single European Code (SEC) as applicable. • Specific information not coded in the SEC that should be included in the accompanying documentation: – Endothelial cell density; – Expiration date; – Diameter of the clear zone.
Special warnings	• None.

20.9 Sclera

SoHO preparation	Sclera for use as a patch SoHO preparation in scleral reinforcement or as coverage of synthetic ocular draining devices
Definition	Stored scleral tissue prepared in a SoHO establishment.
Established clinical indications	• Scleral defects needing reinforcement. • Coverage of synthetic ocular draining devices. • Coverage of prosthetic eye globes after enucleation.
Critical properties	• Scleral tissue devoid of intraocular contents (vitreous, lens, iris, choroidal and retinal tissue) and adnexa (remnants of muscles, conjunctiva). • No evidence of microbial growth.
Quality control requirements	• No signs of intraocular contents or adnexa. • No presence of the additional donor exclusion criteria as related to vascularised tissues.
Storage and transport	• Sclera stored in saline with antibiotics in a refrigerator has only to be kept for short periods (≤ 7 days).
Special labelling and accompanying information	• Placed in a container appropriately labelled with a unique identifying code. This code is included in the accompanying documentation. • In the EU, when SoHO preparations are distributed for human application, they must be labelled with the Single European Code (SEC) as applicable. • Specific information not coded in the SEC that should include in accompanying documentation: – Donor age; – Size of the scleral SoHO preparation, either whole or size of individually packaged pieces; – Expiration date.

21.1 Amniotic membrane sheet

SoHO preparation	Amniotic membrane (AM) sheet
Definition	Amniotic membrane (AM) obtained from placenta processed in a substances of human origin (SoHO) establishment and preserved for use as biological dressing and substrate for cell growth in different clinical applications.
Established clinical indications	• Ophthalmological indications (e.g. ophthalmic corneal ulcerations, persistent epithelial defects, conjunctival defects, limbal stem cell deficiency, chemical or thermal burns, traumatic macular hole). • Burns, as a temporary or permanent wound dressing. • Wound healing (e.g. vascular ulcers, epidermolysis bullosa, surgical wounds, radiation burns). • In neurosurgery as dural substitute. • In orthopaedic surgery. • Intra-abdominal and reconstructive surgery. • Oral and maxillofacial surgery. • Gynecological/urological indications (e.g. nerve bundle protection, vaginal and urethral reconstructions).
Critical properties	• Preserved structural integrity (barrier function). • No evidence of microbial growth. • Adequate AM SoHO preparation size. • For freeze-dried AM: residual moisture (water).
Quality control requirements	• Structural integrity of the tissue (e.g. absence of visible holes, coloured membrane or abnormal thickness). • Microbiological testing.
Storage and transport	**Cryopreservation** • Cryopreserved AM SoHO preparations are stored in liquid or vapour phase of nitrogen at temperatures below – 140°C. Distribution is to be done in dry ice (solid carbon dioxide) or in a liquid nitrogen dry-shipper. **Freezing** • Deep frozen AM (with or without cryoprotectant) are to be stored between – 60° and – 80°C and transported at a similar temperature as was used during storage. Transport temperatures of cryoprotected AM above – 60°C are to be avoided to ensure the stability of the product and maximum safety for the recipient. • If grafts are stored at a temperature above – 60°C after distribution, the shelf life at this temperature should be supported by validation data or a documented rationale based on maintenance of the critical properties of the graft. **Lyophilisation/freeze-drying** • Lyophilised/freeze-dried AM are stored and transported at room temperature (15-25°C).
Special labelling and accompanying information	• Placed in a container appropriately labelled with a unique identifying code. This code is included in the accompanying documentation. • In the EU, when SoHO preparations are distributed for human application, they must be labelled with the Single European Code (SEC) as applicable. • Specific information not coded in the SEC that should be included in accompanying documentation: – Key specifications (e.g. SoHO preparation dimensions in cm^2, appropriate radiation-sensitive labels); – Instructions for appropriate rehydration or thawing according to the different storage conditions; – Information on the composition of antibiotic decontamination cocktail or other residues; – Information on the composition of cryoprotective solution when/if used; – Orientation of amnion (epithelial or stromal side on carrier material).
Special warnings	• Rehydration time of freeze-dried preparations or thawing time of frozen/cryopreserved SoHO preparations, additionally to expiry date after preparation, is defined by the originating SoHO establishment, and rehydration or thawing should be performed under sterile conditions. • For AM immersed in a storage solution, the liquid should be removed and the SoHO preparations rinsed with sterile saline solution or equivalent. • Do not re-freeze thawed AM preparations. • Information about possible residues from processing. • Single recipient use.

21.2 Derivatives of the amniotic membrane

SoHO preparation	Amniotic membrane in liquid form
Definition	Amniotic membrane (AM) derivates obtained from placental tissues, processed in a SoHO establishment and preserved for use: liquid amniotic membrane suspension for drops or for injection; amniotic membrane homogenate and extract; injectable matrix/gel.
Established clinical indications	• Orthopaedic indications (e.g. osteoarthritis, plantar fasciitis, tendinopathy, cartilage and bone injuries). • Ophthalmological indications (e.g. corneal ulcerations, epithelial defects, chemical burns, dry eye disease). • Wound healing (e.g. ulcers, radiation burns).
Critical properties	• No evidence of microbial growth. • Suitable consistency/texture for each specific application. • For freeze-dried AM: residual moisture (water).
Quality control requirements	• Microbiological testing.
Storage and transport	**Freezing** • Deep frozen AM derivates (with or without cryoprotectant) are to be stored between – 60° and – 80°C and transported at a similar temperature as was used during storage. • Lyophilised/freeze-dried AM derivates are stored and transported at room temperature (15-25°C). Freezing and high temperatures (above 30°C) should be avoided.
Special labelling and accompanying information	• Placed in a container appropriately labelled with a unique identifying code. This code is included in the accompanying documentation. • In the EU, when SoHO preparations are distributed for human application, they must be labelled with the Single European Code (SEC) as applicable. • Specific information not coded in the SEC that should be included in accompanying documentation: – Key specifications (e.g. appropriate radiation-sensitive labels); – Instructions for dilution, reconstitution, rehydration or thawing (appropriate solutions, timing); – Volume to use; – Instruction for injection or drop application; – Information on the composition of antibiotic decontamination cocktail or other residues.
Special warnings	• Lyophilised/frozen allografts are to be used within appropriate timing after rehydration/thawing following instructions. • Rehydration time of freeze-dried products or thawing time of frozen products is to be defined by originating SoHO establishment and rehydration or thawing should be performed under sterile conditions. • Do not re-freeze thawed AM products. • Single recipient use.

22.1 Acellular dermal matrix (ADM)

SoHO preparation	Acellular dermal matrix (ADM)
Definition	Decellularised dermis from donor skin.
Established clinical indications	• Burn injuries. As dermal substitute in deep partial- or full-thickness burns to improve scar quality and to prevent post-burn joint contracture. • Reconstructive surgery. As a valid reconstructive tool for any surgical wound in general surgery (e.g. abdominal wall repair), orthopaedic (e.g. rotator cuff reconstruction), oncologic (e.g. breast-conserving surgery), ear nose and throat surgery (e.g. myringoplasty, rhinoplasty), reconstruction of ocular region (fornix, eyelid), bariatric surgery, neurosurgery (duraplasty, Chiari surgery), dental surgery (coverage procedure and reconstruction) and urological surgery (treatment of stress incontinence). • Ulcers. Uninfected, chronic ulcers and diabetic foot ulcers, to accelerate the closure and healing rate. • Full-thickness acute wounds: as a scaffold to support cell ingrowth and granulation tissue formation and to achieve durable coverage of exposed critical structures (bone, tendons). • Composite graft technique to restore the dermal component by application of ADM, and thin autologous grafts or keratinocytes.
Critical properties	• Depletion of donor cells (and genetic material) below a level to be determined by the SoHO establishment. • Graft dimensions and thickness measured and recorded (plain or meshed). • Preserved structural integrity. • Flexibility/pliability. • Resistance to mechanical stress and suturability. • No evidence of microbial growth. • Absence of cytotoxicity.
Quality control requirements	• Microbiological testing. • Graft dimensions and thickness measured and recorded. • Biocompatibility test (cytotoxicity tests).* • Mechanical resistance test.* • Morpho-structure evaluation by histological staining (e.g. orcein, Masson, haematoxylin-eosin) or electron microscopy (EM).* • Decellularisation process by specific histological staining e.g. DAPI, Hoersch and DNA quantification assay (ng DNA/mg dry tissue).* • Residual water test – for lyophilised ADM. *At least as part of the validation process. All critical quality attributes should be checked, both as part of initial validation and periodically.
Storage and transport	Final product storage and transport conditions depend on SoHO preparation's final preservation method: • **Lyophilised/freeze dried ADM** are stored at room temperature (15-25°C) or refrigerated (2-8°C). • **Deep-frozen grafts** are stored between – 60°C and – 80°C and transported at a similar temperature as used during storage or alternatively transported on dry ice for defined period. • **Cryopreserved grafts** are stored at – 140°C or below and transported at a similar temperature as was used during storage or alternatively transported on dry ice for defined period. • **Glycerol-preserved grafts** are stored and transported at refrigerated temperature (2-8°C); at room temperature 15-25°C (after appropriate validation to ensure that any critical quality attributes of the tissue (other than viability) are maintained (i.e. normal morphological structure).

SoHO preparation	Acellular dermal matrix (ADM)
Special labelling and accompanying information	• Placed in a container appropriately labelled with a unique identifying code. This code is included in the accompanying documentation. • In the EU, when SoHO preparations are distributed for human application, they must be labelled with the Single European Code (SEC) as applicable. • Specific information not coded in the SEC that should be included in accompanying documentation: – Appropriate radiation-sensitive labels should be used for irradiated ADM; – Thickness and size; – Instructions for appropriate manipulation by end-users; – Information on the composition of antibiotic decontamination cocktail or other residues.
Special warnings	• Rehydration of glycerol-preserved or lyophilised allografts is recommended before use (e.g. by washing in saline solution for 20-30 min).

22.2 Deep-frozen skin allografts

SoHO preparation	Deep-frozen skin allografts
Definition	Viable skin allografts preserved in a cryoprotective solution.
Established clinical indications	• Temporary/semipermanent covering of burns. • Temporary covering of epidermolytic diseases (e.g. toxic epidermolytic necrosis, staphylococcal scalded skin syndrome). • Treatment of hard-to-heal ulcers. • Temporary covering of wounds with exposed bone and/or tendons. • Wound-bed preparation (promoting neovascularisation).
Critical properties	• Cell viability: it is maintained by deep-frozen skin grafts to a lesser degree if compared to cryopreserved skin grafts; to consider a graft as viable, a minimum of 20% of residual cell viability should be achieved. • No evidence of microbial growth. • Preserved structural integrity (normal epidermal/dermal structure). • Graft dimensions and thickness measured and recorded (plain or meshed). • Mechanical resistance. • Capable of engraftment.
Quality control requirements	• Microbiological testing. • Cell viability assessment (if required, depending on the intended application).
Storage and transport	• Deep-frozen skin grafts are stored in ultra-low-temperature refrigerators, which generally maintain a temperature between – 60° and – 80°C. • Transport on dry ice.
Special labelling and accompanying information	• Placed in a container appropriately labelled with a unique identifying code. This code is included in the accompanying documentation. • In the EU, when SoHO preparations are distributed for human application, they must be labelled with the Single European Code (SEC) as applicable. • Specific information not coded in the SEC that should be included in accompanying documentation: – Graft area in cm^2; – Graft thickness; – Number of sheets; – Decontamination solution composition; – Cryoprotective solution composition; – Cell viability; – Instructions for appropriate manipulation by end-users.
Special warnings	• Do not re-freeze thawed skin graft. • Do not irradiate viable skin graft. • Rinse out cryoprotectants before use. • Rinse with an appropriate solution (saline solution or balanced salt solution).

22.3 Glycerol-preserved skin allograft

SoHO preparation	Glycerol-preserved skin allograft
Definition	Split-thickness, glycerol-preserved, de-vitalised skin grafts, with epidermis and upper dermis components.
Established clinical indications	• Temporary biological dressing: – in partial-thickness burns; – on meshed autografts (sandwich technique); – on donor site; – after application of *in vitro* cultured keratinocytes. • Temporary biological dressing: in partial/full-thickness burns. • Temporary coverage in toxic epidermolytic necrolysis, GvHD. • Temporary biological dressing for difficult, non-healing wounds, pyoderma gangrenosum to protect and preserve the viable granulation tissue from desiccation and necrosis (antalgic and antibacterial effect). • Wound-bed preparation (promoting wound healing).
Critical properties	• Graft dimensions and thickness measured and recorded (plain or meshed). • Preserved structural integrity (normal epidermal/dermal structure). • No evidence of microbial growth.
Quality control requirements	• Intact epidermis and upper dermis (normal morphological structure). • Microbiological testing.
Storage and transport	• The graft is stored in glycerol 85% solution to keep the SoHO preserved before use. • Glycerol-preserved grafts are stored and transported at refrigerated temperature (2-8°C); at room temperature 15-25°C (after appropriate validation to ensure that any critical quality attributes of the SoHO, other than viability, are maintained, i.e. normal morphological structure).
Special labelling and accompanying information	• Placed in a container appropriately labelled with a unique identifying code. This code is included in the accompanying documentation. • In the EU, when SoHO preparations are distributed for human application, they must be labelled with the Single European Code (SEC) as applicable. • Specific information not coded in the SEC that should be included in accompanying documentation: – Size of graft, width and length; – Graft thickness; – Plain or meshed; – Instructions for appropriate manipulation by end-users.
Special warnings	• Rinse out glycerol before use (incubation in a large volume of sterile 0.9% NaCl solution for 10 min at room temperature). • Not to be used if the storage medium is opaque.

22.4 Cryopreserved skin allografts

SoHO preparation	Cryopreserved skin allografts
Definition	Skin allografts preserved in a cryoprotective solution.
Established clinical indications	• Temporary/semipermanent covering of burns. • Temporary covering of epidermolytic diseases (e.g. toxic epidermolytic necrosis, staphylococcal scalded skin syndrome). • Treatment of hard-to-heal ulcers. • Acantholytic dermatoses (Darier disease). • Treatment of cutaneous loss due to meningococcal septicaemia and purpura fulminans. • Temporary covering of wounds with exposed bone and/or tendons. • Wound-bed preparation (promoting neovascularisation and providing dermal template for epidermal grafts).
Critical properties	• Cell viability assessment (if required, depending on the intended application). • No evidence of microbial growth. • Preserved structural integrity (normal epidermal/dermal structure). • Graft dimensions and thickness measured and recorded (plain or meshed). • Mechanical resistance. • Capable of engraftment. • Long-term storage.
Quality control requirements	• Cell viability assessment (if required, depending on the intended application). • Microbiological testing. Absence of microbial contamination (the presence of microbial contamination may not preclude release but agreement with clinical end users and risk assessment if required).
Storage and transport	• Cryopreserved skin grafts are stored at temperature – 140°C or below in liquid nitrogen tanks (vapour phase) or ultralow temperature mechanical freezers. • Transport in a nitrogen dry-shipper or on dry ice (according to the SoHO establishment procedures).
Special labelling and accompanying information	• Placed in a container appropriately labelled with a unique identifying code. This code is included in the accompanying documentation. • In the EU, when SoHO preparations are distributed for human application, they must be labelled with the Single European Code (SEC) as applicable. • Specific information not coded in the SEC that should be included in accompanying documentation: – Graft area in cm^2; – Graft thickness in mm; – Number of sheets; – Decontamination solution composition; – Cryoprotective solution composition; – Cell viability; – Instructions for appropriate manipulation by end-users.
Special warnings	• Do not re-freeze thawed skin graft. • Do not irradiate viable skin graft. • Rinse out cryoprotectants before use with an appropriate solution (saline solution or balanced salt solution).

23.1 Cryopreserved aortic heart valve allograft, antibiotic decontamination

SoHO preparation	Cryopreserved aortic heart valve allograft, antibiotic decontamination
Definition	Aortic heart valve, including the root of the aorta and variable length of associated artery. Decontaminated by incubation with one or more antibiotics and cryopreserved using controlled rate cooling in the presence of a cryoprotectant.
Established clinical indications	• Double output right ventricle. • Truncus arteriosus. • Transposition of the great vessels. • Pulmonary valve stenosis or insufficiency. • Aortic valve stenosis or insufficiency. • Aortic valve or pulmonary valve endocarditis. • Congenital pulmonary or aortic valve malformation. • Replacement of prosthetic valve (mechanical/bioprosthetic) in aortic position.
Critical properties	• No visible (or minimal) calcification or malformations present in the valve or associated vessel. • A rim of myocardium (or mitral leaflet) of at least 2 mm depth surrounding the root of the aortic valve. • The associated aortic artery should not be cut below the level of 1-2 mm of the height above the commissure. • The native biomechanical and hydrodynamic properties of the valve should not be altered by the decontamination and preservation protocols applied, as demonstrated by a functionality test. • Pre-decontamination, no pathogenic micro-organisms detectable on the graft. • Post-decontamination, no viable micro-organisms detectable on the graft.
Quality control requirements	• Microbiological testing. • Functionality tests (such as competency) should be within the acceptable criteria.
Storage and transport	• SoHO preparations are stored at < – 140°C for long-term storage. • SoHO preparations are transported to the point of use using either liquid nitrogen cooled shippers (dry shipper) or solid carbon dioxide refrigeration (dry ice). • If SoHO preparations are stored at – 80°C (or other temperature > – 140°C) after distribution, the shelf life at this temperature should be supported by validation data or a documented rationale based on maintenance of the critical properties of the graft. • If SoHO preparations are shipped on dry ice, they should not be returned to storage at < – 140°C unless this is supported by validation data or a documented rationale based on maintenance of the critical properties of the graft.
Special labelling and accompanying information	• Placed in a container appropriately labelled with a unique identifying code. This code is included in the accompanying documentation. • In the EU, when SoHO preparations are distributed for human application, they must be labelled with the Single European Code (SEC) as applicable. • Specific information not coded in the SEC that should be included in accompanying documentation: – Donor age (if requested by the surgeon); – Key dimensions (annular diameter and length/diameter of associated vascular conduit); – Identity of any residual processing chemicals (antibiotics and cryoprotectants); – Instructions for appropriate thawing and dilution of the cryoprotectant.
Special warnings	• Use as soon as possible after thawing. The maximum permissible period between thawing and transplantation should be defined based on validation data or a documented rationale. • Do not re-freeze the thawed heart valve graft. • Rinse with an appropriate solution, as recommended by the SoHO establishment (e.g. saline solution or balanced salt solution).

23.2 Cryopreserved pulmonary heart valve allograft, antibiotic decontamination

SoHO preparation	Cryopreserved pulmonary heart valve allograft, antibiotic decontamination
Definition	Pulmonary heart valve, including pulmonary trunk and variable length of associated artery. Decontaminated by incubation with one or more antibiotics and cryopreserved using controlled rate cooling in the presence of a cryoprotectant. Patches of the pulmonary valve (e.g. monocuspid patch) can be preserved for established surgical procedures.
Established clinical indications	• Tetralogy of Fallot. • Double outlet right ventricle. • Truncus arteriosus. • Transposition of the great vessels. • Pulmonary valve stenosis or insufficiency. • Pulmonary valve atresia. • Absent pulmonary valve syndrome. • Ross procedure. • Congenital pulmonary valve malformation. • Endocarditis of the native or prosthetic pulmonary valve. • Prosthetic valve degeneration in the pulmonary valve position. • Complications of trans-catheter pulmonary valve replacement. • Right ventricular outflow tract reconstruction in complex congenital heart disease (monocuspid patch).
Critical properties	• No visible (or minimal) calcification or other malformations present in the valve or associated vessel. • A rim of myocardium of at least 2 mm depth surrounding the root of the pulmonary valve. • The associated pulmonary artery should not be cut below the level of 1-2 mm above the commissure. • The native biomechanical and hydrodynamic properties of the valve should not be altered by the decontamination and preservation protocols applied, as demonstrated by a functionality test. • Pre-decontamination, no pathogenic micro-organisms detectable on the graft. • Post-decontamination, no viable micro-organisms detectable on the graft.
Quality control requirements	• Microbiological testing. • Functionality tests (such as competency) should be within the acceptable criteria.
Storage and transport	• SoHO preparations are stored at below – 140°C for long-term storage. • SoHO preparations are transported to the point of use using either liquid nitrogen cooled shippers (dry shipper) or solid carbon dioxide refrigeration (dry ice). • If SoHO preparations are to be stored at – 80°C (or other temperature above – 140°C) after distribution, the shelf life at this temperature should be supported by validation data or a documented rationale based on maintenance of the critical properties of the graft. • If the SoHO preparations is shipped on dry ice, it should not be returned to storage at below – 140°C unless this is supported by validation data or a documented rationale based on maintenance of the critical properties of the graft.

Special labelling and accompanying information	• Placed in a container appropriately labelled with a unique identifying code. This code is included in the accompanying documentation. • In the EU, when SoHO preparations are distributed for human application, they must be labelled with the Single European Code (SEC) as applicable. • Specific information not coded in the SEC that should include in accompanying documentation: – Donor age (if required by the surgeon); – Key dimensions (annular diameter and length/diameter of associated vascular conduit); – Identity of any residual processing chemicals (antibiotics and cryoprotectants); – Instructions for appropriate thawing and dilution of the cryoprotectant.
Special warnings	• Use as soon as possible after thawing. The maximum permissible period between thawing and transplantation should be defined based on validation data or a documented rationale. • Do not re-freeze the thawed heart valve graft. • Rinse with an appropriate solution, as recommended by the SoHO establishment (e.g. saline solution or balanced salt solution).

23.3 Cryopreserved vascular allograft, antibiotic decontamination

SoHO preparation	Cryopreserved vascular allograft, antibiotic decontamination
Definition	Artery, e.g. iliac, femoral artery or descending aorta. Vein, e.g. saphenous vein. Decontaminated by incubation with one or more antibiotics and cryopreserved using controlled rate cooling in the presence of a cryoprotectant.
Established clinical indications	• Replacement of infected prosthetic vascular allografts. • Mycotic arterial aneurysm. • Chronic limb ischaemia. • Critical acute limb ischaemia. • Vascular trauma. • Malignant infiltration of the vascular wall. • Vascular dissection. • Bypass (CABG) or as a patch for reconstruction.
Critical properties	• No visible (or minimal) atheroma or calcification present. • Intact structure of the tissue matrix and integrity of the vascular wall. • No stenosis or dilation present. • Branching arteries should be 2-3 mm in length. • No damage or significant haematomas in the vessel wall. • Pre-decontamination, no pathogenic micro-organisms detectable on the graft. • Post-decontamination, no viable micro-organisms detectable on the graft.
Quality control requirements	• Microbiological testing.
Storage and transport	• SoHO preparations are stored at or below – 140°C for long-term storage. • SoHO preparations are transported to the point of use using either liquid nitrogen cooled shippers (dry shipper) or solid carbon dioxide refrigeration (dry ice). • If SoHO preparations are to be stored at – 80°C (or other temperature > – 140°C) after distribution, the shelf life at this temperature should be supported by validation data or a documented rationale based on maintenance of the critical properties of the graft. • If the SoHO preparation is shipped on dry ice, it should not be returned to storage at < – 140°C unless this is supported by validation data or a documented rationale based on maintenance of the critical properties of the graft.
Special labelling and accompanying information	• Placed in a container appropriately labelled with a unique identifying code. This code is included in the accompanying documentation. • In the EU, when SoHO preparations are distributed for human application, they must be labelled with the Single European Code (SEC) as applicable. • Specific information not coded in the SEC that should be included in the accompanying documentation: – Donor age (if requested by the surgeon); – Key dimensions (proximal and distal annular diameter and length); – Identity of any residual processing chemicals (antibiotics and cryoprotectants); – Instructions for appropriate thawing and dilution of the cryoprotectant.
Special warnings	• Use as soon as possible after thawing. The maximum permissible period between thawing and transplantation should be defined based on validation data or a documented rationale. • Do not re-freeze the thawed vascular graft. • Rinse with an appropriate solution, as recommended by the SoHO establishment (e.g. saline solution or balanced salt solution).

23.4 Cryopreserved non-valved patches and conduits, antibiotic decontamination

SoHO preparation	Cryopreserved non-valved patches and conduits, antibiotic decontamination
Definition	Artery wall, either patches or conduits. Decontaminated by incubation with one or more antibiotics and cryopreserved using controlled rate cooling in the presence of a cryoprotectant.
Established clinical indications	• Tetralogy of Fallot. • Double output right ventricle. • Truncus arteriosus. • Transposition of the great vessels. • Ventricular septal defect. • Pulmonary stenosis. • Hypoplastic heart (L/R). • Heart transplant (plasty of the vessels).
Critical properties	• No visible (or minimal) atheroma or calcification present. • No visible stenosis or dilation present. • No damage or significant haematomas in the vessel wall. • Pre-decontamination, no pathogenic micro-organisms detectable on the graft. • Post-decontamination, no viable micro-organisms detectable on the graft.
Quality control requirements	• Microbiological testing.
Storage and transport	• SoHO preparations are stored at or below – 140°C for long-term storage. • SoHO preparations are transported to the point of use using either liquid nitrogen cooled shippers (dry shipper) or solid carbon dioxide refrigeration (dry ice). • If SoHO preparations are to be stored at – 80°C (or other temperature > – 140°C) after distribution, the shelf life at this temperature should be supported by validation data or a documented rationale based on maintenance of the critical properties of the graft. • If the SoHO preparation is shipped on dry ice, it should not be returned to storage at < – 140°C unless this is supported by validation data or a documented rationale based on maintenance of the critical properties of the graft.
Special labelling and accompanying information	• Placed in a container appropriately labelled with a unique identifying code. This code is included in the accompanying documentation. • In the EU, when SoHO preparations are distributed for human application, they must be labelled with the Single European Code (SEC) as applicable. • Specific information not coded in the SEC that should be included in the accompanying documentation: – Donor age (if requested by the surgeon); – Key dimensions (e.g. proximal (annular) and distal diameter, width and length); – Identity of any residual processing chemicals (antibiotics and cryoprotectants); – Instructions for appropriate thawing and dilution of the cryoprotectant.
Special warnings	• Use as soon as possible after thawing. The maximum permissible period between thawing and transplantation should be defined based on validation data or a documented rationale. • Do not re-freeze the thawed patch or conduit graft. • Rinse with an appropriate solution, as recommended by the SoHO establishment (e.g. saline solution or balanced salt solution).

23.5 Decellularised aortic heart valve allograft, antibiotic decontamination

SoHO preparation	Decellularised aortic heart valve allograft, antibiotic decontamination
Definition	Aortic heart valve, including the aortic root and variable length of associated ascending aorta. Decontaminated by incubation with one or more antibiotics and decellularised according to validated procedures.
Established clinical indications	• Double output right ventricle. • Truncus arteriosus. • Transposition of the great vessels. • Pulmonary valve stenosis or insufficiency. • Aortic valve stenosis or insufficiency. • Aortic valve or pulmonary valve endocarditis. • Congenital pulmonary or aortic valve malformation. • Replacement of prosthetic valve (mechanical/bioprosthetic) in aortic position.
Critical properties	• No visible (or minimal) calcification present in the valve or associated vessel. • A rim of myocardium (or mitral leaflet) of at least 2 mm depth surrounding the root of the aortic valve. • The associated aortic artery should not be cut below the level of 1-2 mm above the commissure. • The native biomechanical and hydrodynamic properties of the valve should not be altered by decellularisation, decontamination and preservation protocols applied, as demonstrated by a functionality test. • Pre-decontamination, no pathogenic micro-organisms detectable on the graft. • Post-decontamination and decellularisation, no viable micro-organisms detectable on the graft.
Quality control requirements	• Microbiological testing. • Functionality tests (such as competency) within the acceptable criteria.
Storage and transport	• SoHO preparations are stored frozen or at 2-8°C for long-term storage based on the decellularisation protocol used. • SoHO preparations are transported based on the decellularisation protocol used. • The SoHO preparations are to be stored frozen or at 2-8°C after distribution.
Special labelling and accompanying information	• Placed in a container appropriately labelled with a unique identifying code. This code is included in the accompanying documentation. • In the EU, when SoHO preparations are distributed for human application, they must be labelled with the Single European Code (SEC) as applicable. • Specific information not coded in the SEC that should be included in the accompanying documentation: – Donor age (if requested by the surgeon); – Key dimensions (annular diameter and length/diameter of associated conduit); – Identity of any residual processing chemicals (antibiotics and decellularisation agents); – Instructions for appropriate handling.
Special warnings	• Use as soon as possible after thawing or removing from cold storage. The maximum permissible period between thawing and transplantation should be defined based on validation data or a documented rationale. • Do not re-freeze the thawed heart valve graft. • Rinse with an appropriate solution, as recommended by the SoHO establishment (e.g. saline solution or balanced salt solution).

23.6 Decellularised pulmonary heart valve allograft, antibiotic decontamination

SoHO preparation	Decellularised pulmonary heart valve allograft, antibiotic decontamination
Definition	Pulmonary heart valve, including pulmonary trunk and variable length of associated artery. Decontaminated by incubation with one or more antibiotics and decellularised according to validated procedures. Patches of the pulmonary valve (e.g. monocuspid patch) can be preserved for established surgical procedures.
Established clinical indications	• Tetralogy of Fallot. • Double output right ventricle. • Truncus arteriosus. • Transposition of the great vessels. • Pulmonary valve stenosis or insufficiency. • Pulmonary valve atresia. • Absent pulmonary valve syndrome. • Ross procedure. • Congenital pulmonary valve malformation. • Endocarditis of the native or prosthetic pulmonary valve. • Prosthetic valve degeneration in the pulmonary valve position. • Complications of trans-catheter pulmonary valve replacement. • Right ventricular outflow tract reconstruction in complex congenital heart disease (monocuspid patch).
Critical properties	• No visible (or minimal) calcification or leaflet malformation present in the valve or associated vessel. • A rim of myocardium of at least 2 mm depth surrounding the root of the pulmonary valve. • The associated pulmonary artery should not be cut below the level of 1-2 mm above the commissure. • The native biomechanical and hydrodynamic properties of the valve should not be altered by the decellularisation, decontamination and preservation protocols applied, as demonstrated by a functionality test. • Pre-decontamination, no pathogenic micro-organisms detectable on the graft. • Post-decontamination and decellularisation, no viable micro-organisms detectable on the graft.
Quality control requirements	• Microbiological testing. • Functionality tests (such as competency) within the acceptable criteria.
Storage and transport	• SoHO preparations are stored frozen or at 2-8°C for long-term storage based on the decellularisation protocol used. • SoHO preparations are transported based on the decellularisation protocol used. • The SoHO preparations are to be stored frozen or at 2-8°C after distribution.
Special labelling and accompanying information	• Placed in a container appropriately labelled with a unique identifying code. This code is included in the accompanying documentation. • In the EU, when SoHO preparations are distributed for human application, they must be labelled with the Single European Code (SEC) as applicable. • Specific information not coded in the SEC that should be included in the accompanying documentation: – Donor age (if requested by the surgeon); – Key dimensions (annular diameter and length/diameter of associated conduit); – Identity of any residual processing chemicals (antibiotics and decellularisation agents); – Instructions for appropriate handling.
Special warnings	• Use as soon as possible after thawing or removing from cold storage. The maximum permissible period between thawing and transplantation should be defined based on validation data or a documented rationale. • Do not re-freeze the thawed heart valve graft. • Rinse with an appropriate solution, as recommended by the SoHO establishment (e.g. saline solution or balanced salt solution).

23.7 Cryopreserved pericardium, antibiotic decontamination

SoHO preparation	Cryopreserved pericardium, antibiotic decontamination
Definition	Pericardium patches. Decontaminated by incubation with one or more antibiotics and cryopreserved using controlled rate cooling in the presence of a cryoprotectant.
Established clinical indications	• Vascular patch plasty or reconstruction. • Ventricular septal defect (VSD) closure. • Atrial septal defect (ASD) closure. • Widening plasty during valve replacement. • Reconstruction of ventricular aneurysms. • Dural substitute.
Critical properties	• No visible tears or cuts to be present in the patch. • Mechanical properties preserved after the processing procedure (decontamination and cryopreservation). • Post-decontamination, no viable micro-organisms detectable on the graft. • Pre-decontamination, no pathogenic micro-organisms detectable on the graft.
Quality control requirements	• Microbiological testing.
Storage and transport	• SoHO preparations are stored at or below – 140 °C for long-term storage. • SoHO preparations are transported to the point of use using either liquid nitrogen cooled shippers (dry shipper) or solid carbon dioxide refrigeration (dry ice). • If SoHO preparations are to be stored at – 80°C (or other temperature > – 140°C) after distribution, the shelf life at this temperature should be supported by validation data or a documented rationale based on maintenance of the critical properties of the graft. • If the SoHO preparation is shipped on dry ice, it should not be returned to storage at < – 140°C unless this is supported by validation data or a documented rationale based on maintenance of the critical properties of the graft.
Special labelling and accompanying information	• Placed in a container appropriately labelled with a unique identifying code. This code is included in the accompanying documentation. • In the EU, when SoHO preparations are distributed for human application, they must be labelled with the Single European Code (SEC) as applicable. • Specific information not coded in the SEC that should be included in the accompanying documentation: – Donor age (if requested by the surgeon); – Key dimensions; – Identity of any residual processing chemicals (antibiotics and cryoprotectants); – Instructions for appropriate thawing and dilution of cryoprotectant.
Special warnings	• Use as soon as possible after thawing. The maximum permissible period between thawing and transplantation should be defined based on validation data or a documented rationale. • Do not re-freeze thawed pericardium graft. • Rinse with an appropriate solution, as recommended by the SoHO establishment (e.g. saline solution or balanced salt solution).

24.1 Cancellous/corticocancellous bone

SoHO preparation	Cancellous/corticocancellous bone
Definition	Cancellous bone, also referred to as trabecular bone or spongy bone, forms the inner part of the bone. Cancellous bone is the porous mineralised component of bone that has a honeycomb or sponge-like appearance. The bone matrix is organised into a three-dimensional lattice of bony processes (trabeculae) arranged along lines of stress. Cancellous bone provides an osteoconductive matrix for bone remodelling and healing. Cortical bone, also referred to as compact bone or lamellar bone, forms the cortex of most bones and is much denser, harder and stiffer than cancellous bone. It consists of multiple microscopic columns (osteons) conferring excellent structural integrity. Cancellous and corticocancellous bone grafts include different presentations, ranging from chips (sawn or ground to various sizes, generally 1-10 mm diameter/edge length) and geometrical pieces (discs, wedges, blocks, dowels) to customised bone grafts, particles, granules and powder.
Established clinical indications	• Bony defects to be filled. • Bone loss in fractures. • Prosthetic replacement surgery. • Filling after tumour resection. • Spinal surgery. • Malunion. • Maxillofacial and periodontal surgery.
Critical properties	• Cancellous bone free of muscle, periosteum and cortical bone. • Corticocancellous bone free of muscle. Cartilage can be preserved or removed in the case of geometrical pieces if necessary (femoral head, femoral condyle, tibial plateau, etc.). • Reduction or removal of fat, blood cells and bone marrow. • No evidence of microbial growth. • Adequate size and volume. • For geometrical pieces in cases of indicated supporting load constraints: the essential native biomechanical properties of the bone should not be significantly altered (including terminal sterilisation if applied). • Absence of cytotoxicity due to the processing protocol.
Quality control requirements	• Microbiological testing. • In cases of lyophilisation (freeze-drying): residual moisture or available water. • Size and shape characterisation.
Storage and transport	There are different approaches to preserving cancellous/corticocancellous grafts. Final product storage and transport conditions depend on the product's final preservation method: • **Lyophilised/freeze-dried grafts** are stored at 4-30°C. Freezing and long-term transport temperatures above 30°C should be avoided. • **Frozen grafts** are stored between – 40°C and – 80°C and transported at a similar temperature as used during storage or alternatively transported on dry ice for a defined period. If grafts are to be stored at – 20°C (or other temperature above – 40°C) after distribution, the shelf life at this temperature should be supported by validation data or a documented rationale based on maintenance of the critical properties of the graft. • **Cryopreserved grafts** are stored at – 140°C or below and transported at a similar temperature as was used during storage or alternatively transported on dry ice for a defined period.

SoHO preparation	Cancellous/corticocancellous bone
Special labelling and accompanying information	• Placed in a container appropriately labelled with a unique identifying code. This code is included in the accompanying documentation. • In the EU, when SoHO preparations are distributed for human application, they must be labelled with the Single European Code (SEC) as applicable. • Specific information not coded in the SEC that should be included in the accompanying documentation: – Key specifications (e.g. appropriate radiation-sensitive labels); – Instructions for use, including reconstitution, rehydration or thawing (appropriate solutions, timing); – Volume, particle size, graft size if applicable; – Information on the composition of antibiotic decontamination cocktail or other residues if applicable.
Special warnings	• Single recipient use. • Lyophilised, frozen or cryopreserved allografts should be used within appropriate timings after rehydration or thawing following instructions. Timings are defined by originating SoHO establishment and rehydration/thawing procedures are performed under sterile conditions. • Do not re-freeze thawed grafts. • Do not freeze or heat lyophilised/freeze-dried grafts.

24.2 Cortical bone

SoHO preparation	Cortical bone
Definition	Cortical bone, also referred to as compact bone or lamellar bone, forms the cortex of most bones and is much denser, harder and stiffer than cancellous bone. It consists of multiple microscopic columns (osteons) conferring excellent structural integrity. Cortical bone graft is obtained from long bone shafts (usually femur, tibia, humerus and fibula). It includes a range of different presentations: e.g shaft segments, hemicylinders, plates, struts, pins, screws, particles, granules and powder.
Established clinical indications	• Bony defects to be filled. • Bone loss in fractures. • Prosthetic replacement surgery. • Filling after tumour resection. • Spinal surgery. • Malunion. • Maxillofacial and periodontal surgery.
Critical properties	• Free of muscle and periosteum. • Reduction or removal of fat, blood cells and bone marrow. • No evidence of microbial growth. • Adequate size and volume. • For grafts supporting load constraints: the essential native biomechanical properties of the bone should not be significantly altered. • Absence of cytotoxicity due to the processing protocol.
Quality control requirements	• Microbiological testing. • In cases of lyophilisation (freeze drying): residual moisture or available water. • Size and shape characterisation.
Storage and transport	Final product storage and transport conditions depend on the product's final preservation method: • **Lyophilised/freeze-dried grafts** are stored at 4-30°C. Freezing and long-term transport temperatures above 30°C should be avoided. • **Frozen grafts** are stored between – 40 and – 80°C and transported at a similar temperature as was used during storage or alternatively transported on dry ice for a defined period. If grafts are to be stored at – 20°C (or other temperature above – 40°C) after distribution, the shelf life at this temperature should be supported by validation data or a documented rationale based on maintenance of the critical properties of the graft. • **Cryopreserved grafts** are stored at – 140°C or below and transported at a similar temperature as was used during storage or alternatively transported on dry ice for a defined period.
Special labelling and accompanying information	• Placed in a container appropriately labelled with a unique identifying code. This code is included in the accompanying documentation. • In the EU, when SoHO preparations are distributed for human application, they must be labelled with the Single European Code (SEC) as applicable. • Specific information not coded in the SEC that should be included in the accompanying documentation: – Key specifications (e.g. appropriate radiation-sensitive labels); – Instructions for use, including reconstitution, rehydration or thawing (appropriate solutions, timing); – Volume, particle size, graft size if applicable; – Information on the composition of antibiotic decontamination cocktail or other residues if applicable.
Special warnings	• Single recipient use. • Lyophilised, frozen or cryopreserved allografts should be used within appropriate timings, after rehydration or thawing following instructions. Timings are defined by originating SoHO establishment and rehydration/thawing procedures are performed under sterile conditions. • Do not re-freeze thawed grafts. • Do not freeze or heat lyophilised/freeze-dried grafts.

24.3 Femoral head

SoHO preparation	Femoral head
Definition	The femoral head is a corticocancellous human graft. It provides structural support to restore segmental bone loss. It is often pre-sized by surgeons directly before use, making it fit for various applications. It includes a range of different presentations: whole, halved and quartered femoral heads, with or without femoral neck.
Established clinical indications	• Bony defects to be filled. • Bone loss in fractures. • Prosthetic replacement surgery. • Filling after tumour resection. • Spinal surgery. • Malunion. • Maxillofacial and periodontal surgery.
Critical properties	• Corticocancellous bone free of muscle and periosteum. Cartilage can be preserved or removed in the case of geometrical pieces if necessary. • No evidence of microbial growth.
Quality control requirements	• Microbiological testing. • In cases of lyophilisation (freeze-drying): residual moisture or available water. • Size and shape characterisation.
Storage and transport	Final product storage and transport conditions depend on the product's final preservation method: • **Lyophilised/freeze-dried grafts** are stored at 4-30°C. Freezing and long-term transport temperatures above 30°C should be avoided. • **Frozen grafts** are stored between – 80°C and – 40°C and transported at a similar temperature as was used during storage, or alternatively transported on dry ice for a defined period. If grafts are to be stored at – 20°C (or other temperature above – 40°C) after distribution, the shelf life at this temperature should be supported by validation data or a documented rationale based on maintenance of the critical properties of the graft. • **Cryopreserved grafts** are stored at – 140°C or below and transported at a similar temperature as was used during storage or alternatively transported on dry ice for a defined period.
Special labelling and accompanying information	• Placed in a container appropriately labelled with a unique identifying code. This code is included in the accompanying documentation. • In the EU, when SoHO preparations are distributed for human application, they must be labelled with the Single European Code (SEC) as applicable. • Specific information not coded in the SEC that should be included in the accompanying documentation: – Key specifications (e.g. appropriate radiation-sensitive labels); – Instructions for use, including reconstitution, rehydration or thawing (appropriate solutions, timing); – Volume, particle size, graft size if applicable; – Information on the composition of antibiotic decontamination cocktail or other residues if applicable.
Special warnings	• Single recipient use. • Lyophilised, frozen or cryopreserved allografts should be used within appropriate timings, after rehydration or thawing following instructions. Timings are defined by the originating SoHO establishment and rehydration/thawing procedures are performed under sterile conditions. • Do not re-freeze thawed grafts. • Do not freeze or heat lyophilised/freeze-dried grafts.

24.4 Structural bone

SoHO preparation	Structural bone
Definition	Structural bone is a corticocancellous human graft; its main purpose is to replace a bone segment in the recipient. It can be processed with or without ligament or muscular attachments, and with or without articular cartilage. It can be a whole bone or a part from it (distal, proximal, half, third).
Established clinical indications	• Bone loss in fractures. • Prosthetic replacement surgery. • Filling after tumour resection. • Malunion. • Maxillofacial surgery.
Critical properties	• Free of periosteum. Ligament and muscular attachments as well as cartilage can be preserved if applicable. • No evidence of microbial growth. • No morphology alterations (e.g. deformities, fracture callus). • Absence of significant alteration of the native biomechanical properties (including terminal sterilisation if applied). • Absence of cytotoxicity due to the processing protocol.
Quality control requirements	• Microbiological testing. • Side, size and shape characterisation.
Storage and transport	Final product storage and transport conditions depend on the product's final preservation method: • **Frozen grafts** are stored between – 40 and – 80°C and transported at a similar temperature as was used during storage, or alternatively transported on dry ice for a defined period. If grafts are to be stored at – 20°C (or any other temperature above – 40°C) after distribution, the shelf life at this temperature should be supported by validation data or a documented rationale based on maintenance of the critical properties of the graft. • **Cryopreserved grafts** are stored at – 140°C or below and transported at a similar temperature as was used during storage, or alternatively transported on dry ice for a defined period.
Special labelling and accompanying information	• Placed in a container appropriately labelled with a unique identifying code. This code is included in the accompanying documentation. • In the EU, when SoHO preparations are distributed for human application, they must be labelled with the Single European Code (SEC) as applicable. • Specific information not coded in the SEC that should be included in the accompanying documentation: – Key specifications (e.g. appropriate radiation-sensitive labels); – Instructions for use, including thawing (appropriate solutions, timing); – Side, size and/or shape characteristics; – Information on the composition of antibiotic decontamination cocktail or other residues.
Special warnings	• Single recipient use. • Frozen or cryopreserved allografts should be used within an appropriate time after thawing, following instructions. Timings are defined by the originating SoHO establishment and thawing procedures are performed under sterile conditions. • Do not re-freeze thawed grafts.

24.5 Meniscus

SoHO preparation	Meniscus
Definition	Meniscus is a crescent-shaped fibrocartilaginous structure located at the knee joint. Its main function is to provide stability to the femorotibial join, distribute axial load and absorb shock. It can be processed with or without bone block from tibia plateau.
Established clinical indications	• Meniscus replacement: orthopaedics, sports medicine.
Critical properties	• Absence of meniscal surgery antecedent on medical history. Absence of blood straining and degenerative or traumatic lesions. • No evidence of microbiological growth after processing. • Absence of significant alteration of the native biomechanical properties. • Absence of cartilage surgery antecedent on medical history. • Processing steps should largely reduce or remove fat, blood cells and bone marrow from bone block if applicable. • Absence of cytotoxicity due to the processing protocol. • Donor–recipient matching: meniscus dimensions (recommended) or donor–recipient height/weight.
Quality control requirements	• Microbiological testing. • Side and size characterisation (meniscus: anterior-posterior distance, medial-lateral distance).
Storage and transport	Final product storage and transport conditions depend on the product's final preservation method: • **Frozen grafts** are stored between – 40 and – 80°C and transported at a similar temperature as was used during storage or alternatively transported on dry ice for a defined period. If grafts are to be stored at – 20°C (or other temperature above – 40°C) after distribution, the shelf life at this temperature should be supported by validation data or a documented rationale based on maintenance of the critical properties of the graft. • **Cryopreserved grafts** are stored at – 140°C or below and transported at a similar temperature as was used during storage or alternatively transported on dry ice for a defined period.
Special labelling and accompanying information	• Placed in a container appropriately labelled with a unique identifying code. This code is included in the accompanying documentation. • In the EU, when SoHO preparations are distributed for human application, they must be labelled with the Single European Code (SEC) as applicable. • Specific information not coded in the SEC that should be included in the accompanying documentation: – Key specifications (e.g. appropriate radiation-sensitive labels); – Instructions for use, including thawing (appropriate solutions, timing); – Side and size characteristics; – Information on the composition of antibiotic decontamination cocktail or other residues.
Special warnings	• Single recipient use. • Frozen or cryopreserved allografts should be used within appropriate timing after thawing, following instructions. Timings are defined by originating SoHO establishment and thawing procedures are performed under sterile conditions. • Do not re-freeze thawed grafts.

24.6 Cartilage

SoHO preparation	Cartilage
Definition	Cartilage parts of rib bones (costal cartilage).
Established clinical indications	• Replacement for lost or insufficient cartilage tissue and mechanical stabilisation, e.g. reconstructive surgery, thoracic, trauma and paediatric surgery, otorhinolaryngology, maxillofacial periodontal surgery.
Critical properties	• Absence of blood straining, calcification and degenerative or traumatic lesions. • No evidence of microbial growth. • Absence of cartilage surgery antecedent on medical history. • Processing steps should largely reduce or remove fat, blood cells and bone marrow from bone block if applicable. • Absence of cytotoxicity due to the processing protocol.
Quality control requirements	• Microbiological testing. • Size characterisation.
Storage and transport	Final product storage and transport conditions depend on the product's final preservation method: • **Cooled grafts** are stored at 1-10°C in a saline solution and transported at similar temperature as was used during storage. • **Frozen grafts** are stored between – 40 and – 80°C and transported at a similar temperature as was used during storage or alternatively transported on dry ice for a defined period. If grafts are to be stored at – 20°C (or any other temperature above – 40°C) after distribution, the shelf life at this temperature should be supported by validation data or a documented rationale based on maintenance of the critical properties of the graft. • **Cryopreserved grafts** are stored at – 140°C or below and transported at a similar temperature as was used during storage, or alternatively transported on dry ice for a defined period.
Special labelling and accompanying information	• Placed in a container appropriately labelled with a unique identifying code. This code is included in the accompanying documentation. • In the EU, when SoHO preparations are distributed for human application, they must be labelled with the Single European Code (SEC) as applicable. • Specific information not coded in the SEC that should be included in the accompanying documentation: – Key specifications (e.g. appropriate radiation-sensitive labels); – Instructions for use, including thawing (appropriate solutions, timing); – Side and size characteristics; – Information on the composition of antibiotic decontamination cocktail or other residues.
Special warnings	• Single recipient use. • Frozen or cryopreserved allografts should be used within appropriate timing after thawing, following instructions. Timings are defined by the originating SoHO establishment and thawing procedures are performed under sterile conditions. • Do not re-freeze thawed grafts.

24.7 Tendon and ligament

SoHO preparation	Tendon and ligament
Definition	Ligament is a fibrous connective tissue that connects bones. The patellar ligament is considered the distal portion of the common tendon of the Quadriceps femoris muscle, which continues from the patella to the tibial tuberosity, and it is also called the patellar tendon. Tendon is a dense fibrous connective tissue that connects muscle to bone. Tendons can be processed with bone block (patellar ligament, Achilles tendon) or without it (i.e. anterior tibialis tendon, posterior tibialis tendon, peroneus longus tendon, semitendinosus tendon, gracilis tendon, Achilles tendon).
Established clinical indications	• Joint instability. • Injuries of the extensor mechanism of the knee. • Bridging a gap in tendon injuries. • Repair of chronic muscle tendon ruptures. • Muscle transpositions.
Critical properties	• Absence of tendinous pathology antecedent on medical history. • Absence of muscle and peritendon. • Absence of blood staining, fibre separation, stiffness and degenerative or traumatic lesions. • No evidence of microbial growth after processing. • Absence of significant alteration of the native biomechanical properties (e.g. if terminal sterilisation is applied). • Processing steps should largely reduce or remove fat, blood cells and bone marrow from bone block, if applicable. • Absence of cytotoxicity due to the processing protocol.
Quality control requirements	• Microbiological testing. • Size characterisation (length, thickness, double loop thickness if applicable).
Storage and transport	Final product storage and transport conditions depend on the product's final preservation method: • **Frozen grafts** are stored between – 40 and – 80°C and transported at a similar temperature as was used during storage or alternatively transported on dry ice for a defined period. If grafts are to be stored at – 20°C (or any other temperature above – 40°C) after distribution, the shelf life at this temperature should be supported by validation data or a documented rationale based on maintenance of the critical properties of the graft. • **Cryopreserved grafts** are stored at – 140°C or below and transported at a similar temperature as was used during storage, or alternatively transported on dry ice for a defined period.
Special labelling and accompanying information	• Placed in a container appropriately labelled with a unique identifying code. This code is also included in the accompanying documentation. • In the EU, when SoHO preparations are distributed for human application, they must be labelled with the Single European Code (SEC) as applicable. • Specific information not coded in the SEC that should be included in the accompanying documentation: – Key specifications (e.g. appropriate radiation-sensitive labels); – Instructions for use, including thawing (appropriate solutions, timing); – Side and size characteristics; – Information on the composition of antibiotic decontamination cocktail or other residues.
Special warnings	• Single recipient use. • Frozen or cryopreserved allografts should be used within appropriate timings after thawing, following instructions. Timings are defined by originating SoHO establishment and thawing procedures are performed under sterile conditions. • Do not re-freeze thawed grafts.

24.8 Fascia lata

SoHO preparation	Fascia lata
Definition	Fascia is a fibrous tissue that covers muscles. It can be processed in various sizes for different clinical applications.
Established clinical indications	• Replacement for lost or insufficient soft tissue, e.g. dental medicine, sports medicine, reconstructive surgery, visceral surgery, rhinoplasty.
Critical properties	• Free of muscle, fat and connective tissue. • Absence of significant holes or fibre separation. • No evidence of microbial growth. • Absence of cytotoxicity due to the processing protocol.
Quality control requirements	• Microbiological testing. • Size characterisation. • In cases of lyophilisation (freeze-drying): determine residual moisture or available water.
Storage and transport	Final product storage and transport conditions depend on the product's final preservation method: • **Lyophilised/freeze-dried grafts** are stored at 4-30°C. Freezing and long-term transport temperatures above 30°C should be avoided. • **Frozen grafts** are stored between – 40 and – 80°C and transported at a similar temperature as was used during storage, or alternatively transported on dry ice for a defined period. If grafts should be stored at – 20°C (or any other temperature above – 40°C) after distribution, the shelf life at this temperature should be supported by validation data or a documented rationale based on maintenance of the critical properties of the graft. • **Cryopreserved grafts** are stored at – 140°C or below and transported at a similar temperature as was used during storage, or alternatively transported on dry ice for a defined period.
Special labelling and accompanying information	• Placed in a container appropriately labelled with a unique identifying code. This code is included in the accompanying documentation. • In the EU, when SoHO preparations are distributed for human application, they must be labelled with the Single European Code (SEC) as applicable. • Specific information not coded in the SEC that should be included in the accompanying documentation: – Key specifications (e.g. dimensions, appropriate radiation-sensitive labels if applicable); – Instructions for use, including thawing (appropriate solutions, timing); – Side and size characteristics; – Information on the composition of antibiotic decontamination cocktail or other residues.
Special warnings	• Single recipient use. • Frozen or cryopreserved: rinsing with an appropriate solution before use is recommended. • Lyophilised, frozen or cryopreserved allografts should be used within appropriate timings after rehydration or thawing following instructions. Timings are defined by the originating SoHO establishment and rehydration/thawing procedures are performed under sterile conditions. • Do not freeze or heat lyophilised/freeze-dried grafts. • Do not re-freeze thawed grafts.

24.9 Osteochondral allograft

SoHO preparation	Osteochondral allograft
Definition	Hyaline cartilage results from a combination of a semi-translucent matrix, mainly composed of randomly oriented collagen fibres and some elastin, and chondrocytes. It is normally found on joint surfaces. Osteochondral graft includes hyaline cartilage and subchondral bone. It is mainly obtained from knee (most common), ankle, shoulder or hip.
Established clinical indications	• Treatment of chondral or osteochondral lesions. • Sequel of joint fractures.
Critical properties	• Absence of chondral pathology antecedent on medical history. • Absence of blood staining and degenerative or traumatic lesions. • No evidence of microbiological growth. • Chondrocyte viability > 70%. • Donor–recipient matching: either graft dimensions (recommended) or donor–recipient height/weight.
Quality control requirements	• Microbiological testing. • Side and size characterisation.
Storage and transport	Final product storage and transport conditions depend on the product's final preservation method: • **Fresh hypothermic grafts** are stored between 2 and 8°C and transported at a similar temperature as was used during storage. • **Fresh organ-cultured grafts** are stored between 28 and 37°C and transported at room temperature (15-25°C).
Special labelling and accompanying information	• Placed in a container appropriately labelled with a unique identifying code. This code is included in the accompanying documentation. • In the EU, when SoHO preparations are distributed for human application, they must be labelled with the Single European Code (SEC) as applicable. • Specific information not coded in the SEC that should be included in the accompanying documentation: – Key specifications (e.g. storage temperature); – Instructions for use, including rinsing (appropriate solutions, timing); – Side and size characteristics; – Information on the composition of antibiotic decontamination cocktail or other residues.
Special warnings	• Single recipient use. • Fresh allografts should be used within appropriate timings, following instructions. Rinsing steps and timings are defined by the originating SoHO establishment and rinsing is performed under sterile conditions. • Do not freeze grafts.

24.10 Demineralised bone

SoHO preparation	Demineralised bone
Definition	Demineralised bone matrix (DBM) graft can be composed of cortical bone, cancellous bone or a combination of both. They are submitted to a demineralisation process to reduce calcium content and expose bone morphogenetic proteins (BMP). Cancellous bone (mineralised) provides an osteoconductive matrix for bone remodelling and healing. Cortical bone (mineralised) consists of multiple microscopic columns (osteons) conferring excellent structural integrity. DBM can be mixed with mineralised bone (cancellous or/and cortical) = DBM mixture. For putty and paste a carrier may be contained in DBM. It includes a range of different presentations: chips sawn, fibres milled or ground to particles, geometrical pieces, putty or paste.
Established clinical indications	• Bony defects to be filled. • Bone loss in fractures. • Prosthetic replacement surgery. • Spinal surgery. • Malunion. • Maxillofacial and periodontal surgery. • Osteosynthesis. • Arthrodesis. • Osteotomies.
Critical properties	• Free of muscle, periosteum and cartilage. • Reduction or removal of fat, blood cells and bone marrow. • No evidence of microbial growth. • Osteoinductive capability (if claimed). • Adequate size and volume. • Absence of cytotoxicity due to the processing protocol.
Quality control requirements	• Microbiological testing. • *In vivo/in vitro* osteoinduction test (if the main property of the graft is osteoinductivity and not a mechanical property). • Residual calcium content: maximum 8%. • Determine residual moisture or available water. • Volume characterisation.
Storage and transport	• **Lyophilised/freeze-dried grafts** are stored at 4-30°C. • Freezing and long-term transport temperatures above 30°C should be avoided.
Special labelling and accompanying information	• Placed in a container appropriately labelled with a unique identifying code. This code is included in the accompanying documentation. • In the EU, when SoHO preparations are distributed for human application, they must be labelled with the Single European Code (SEC) as applicable. • Specific information not coded in the SEC that should be included in the accompanying documentation: – Key specifications (e.g. storage temperature); – Instructions for use, including reconstitution, rehydration or handling (appropriate solutions, timing); – Volume and/or particle size if applicable; – Information on the composition of antibiotic decontamination cocktail or other residues if applicable.
Special warnings	• Single recipient use. • Lyophilised allografts should be used within appropriate timings after rehydration, following instructions. Timings are defined by originating SoHO establishment and rehydration procedures are performed under sterile conditions. • Do not freeze or heat dehydrated/freeze-dried grafts.

24.11 Bone mixture

SoHO preparation	Mixture of bone with excipients
Definition	Cancellous, corticocancellous or cortical bone which is mixed with an excipient (= additive); bone has main function.
Established clinical indications	• Bony defects to be filled. • Bone loss in fractures. • Prosthetic replacement surgery. • Spinal surgery. • Malunion. • Maxillofacial and periodontal surgery.
Critical properties	• Free of muscle, fat and connective tissue. • No evidence of microbial growth. • Absence of cytotoxicity due to the processing protocol. • Bone has main clinical function, excipient has only supportive function. • Excipient has known clinical safety (e.g. antibiotics).
Quality control requirements	• Microbiological testing. • Size characterisation. • Determine residual moisture or available water.
Storage and transport	Final product storage and transport conditions depend on the product's final preservation method: • **Lyophilised/freeze-dried grafts** are stored at 4-30°C. Freezing and long-term transport temperatures above 30°C should be avoided. • **Frozen grafts** are stored between – 40°C and – 80°C and transported at a similar temperature as was used during storage or alternatively transported on dry ice for a defined period. If grafts are to be stored at – 20°C (or any other temperature above – 40°C) after distribution, the shelf life at this temperature should be supported by validation data or a documented rationale based on maintenance of the critical properties of the graft. • **Cryopreserved grafts** are stored at – 140°C or below and transported at a similar temperature as was used during storage, or alternatively transported on dry ice for a defined period.
Special labelling and accompanying information	• Placed in a container appropriately labelled with a unique identifying code. This code is included in the accompanying documentation. • In the EU, when SoHO preparations are distributed for human application, they must be labelled with the Single European Code (SEC) as applicable. • Specific information not coded in the SEC that should be included in the accompanying documentation: – Key specifications (e.g. appropriate radiation-sensitive labels); – Instructions for use, including thawing (appropriate solutions, timing); – Side and size characteristics; – Information on the composition of antibiotic decontamination cocktail or other residues; – Information on excipient.
Special warnings	• Single recipient use. • Lyophilised allografts should be used within appropriate timings after rehydration, following instructions. Timings are defined by the originating SoHO establishment and rehydration procedures are performed under sterile conditions. • The use of excipients should be clarified in advance for allergic patients. • Do not freeze or heat lyophilised/freeze-dried grafts.

24.12 Autologous bone flap

SoHO preparation	Autologous bone flap
Definition	Skull bone flap debrided of soft tissues for autologous use only.
Established clinical indications	• Cranioplasty after decompressive craniectomy.
Critical properties	• No evidence of microbial growth (the presence of microbial contamination may not preclude release but may indicate the need for antibiotic treatment in the recipient). • Maintenance of dimensions and shape.
Quality control requirements	• Microbiological testing.
Storage and transport	• Frozen grafts can be stored between – 40°C and – 80°C for long-term storage and transported at a similar temperature as was used during storage, or alternatively transported on dry ice for a defined period.
Special labelling and accompanying information	• Placed in a container appropriately labelled with a unique identifying code. This code is included in the accompanying documentation. • In the EU, when SoHO preparations are distributed for human application, they must be labelled with the Single European Code (SEC) as applicable. • Specific information not coded in the SEC that should be included in the accompanying documentation: – Key specifications (e.g. storage temperature, dimensions); – Instructions for appropriate thawing; – Information on the composition of antibiotic decontamination cocktail or other residues.
Special warnings	• For autologous use only. • Do not sterilise irradiated bone flaps. • Use immediately after thawing. • Thawing time should be defined by the SoHO establishment and thawing procedures should be performed under sterile conditions.

25.1 Haematopoietic progenitor cells from bone marrow – HPC(M)

SoHO preparation	Haematopoietic progenitor cells from bone marrow – HPC(M)
Definition	HPC are found in small numbers in bone marrow. The infused HPC(M) can originate from the recipient (autologous) or from another individual (allogeneic). HPC(M) can be used as fresh unmanipulated product or can be further processed (e.g. buffy-coat preparation, cell selection, cryopreservation).
Established clinical indications	• Restoration of haematopoiesis after chemo- and/or radiation therapy (autologous and allogeneic transplantation). • Establishment of donor chimerism (allogeneic transplantation).
Critical properties	• Cellularity/viability a. for autologous transplantation: – Determine the number of viable nucleated cells; – Determine the number of viable $CD34^+$ cells. b. for allogeneic transplantation: – Determine the number of viable nucleated cells; – Determine the number of viable $CD34^+$ cells. • Absence of microbial contamination – The presence of microbial contamination may (or may not) preclude release but may indicate the need for antibiotic treatment in the recipient.
Quality control requirements	• Nucleated cell count. • Enumeration of viable $CD34^+$ cells. • Microbiological testing. • ABO Rh blood group for allogeneic products. • Measurement of residual ABO-incompatible red cell volume.
Storage and transport	• **Fresh HPC(M)** can be stored and transported up to 72 hours at room temperature (15-25°C) or refrigerated (2-8°C) as requested by the ORHA/SoHO entity. – The time to cryopreservation should be minimised. • **Cryopreserved HPC(M)** are stored and transported at temperatures equal to or below – 140°C. • **Thawed HPC(M)** are stored and transported refrigerated (2-8°C) and applied without undue delay.
Special labelling and accompanying information	• Placed in a container appropriately labelled with a unique identifying code. When applicable, this code is included in the accompanying documentation. • In the EU, when SoHO preparations are distributed for human application, they must be labelled with the Single European Code (SEC) as applicable. • Specific information not coded in the SEC that should be included in the accompanying documentation: – donor name (autologous or related donors) or donor GRID (unrelated donors); – recipient name (if permitted), recipient ID (if applicable); – nucleated cell count and viable $CD34^+$ cell enumeration; – ABO Rh blood group; – volume; – anticoagulation volume; – identity of the collection facility and/or donor registry (SoHO entity); – identity of processing and distribution facility (SoHO establishment); – instructions for appropriate thawing, if applicable.
Special warnings (if needed)	• Do not irradiate. • Properly identify intended recipient and product. • For use by intended recipient only. • For autologous use only, if applicable. • Do not use leukoreduction filters. • Use immediately after thawing. • If microbial contamination is present, consider antibiotic treatment in the recipient.

25.2 Haematopoietic progenitor cells from peripheral blood apheresis – HPC(A)

SoHO preparation	Haematopoietic progenitor cells from peripheral blood apheresis – HPC(A)
Definition	HPC(A) are collected by apheresis from the mononuclear cell fraction of circulating blood after their mobilisation from the bone marrow. The infused HPC(A) can originate from the recipient (autologous) or from another individual (allogeneic). HPC(A) can be used as fresh unmanipulated product or further processed (e.g. cell selection, cryopreservation).
Established clinical indications	• Restoration of haematopoiesis after chemo- and/or radiation therapy (autologous and allogeneic transplantation). • Establishment of donor chimerism (allogeneic transplantation).
Critical properties	• Cellularity/viability a. for autologous transplantation: – Determine the number of viable $CD34^+$ cells. b. for allogeneic transplantation: – Determine the number of viable $CD34^+$ cells. • Absence of microbial contamination – The presence of microbial contamination may (or may not) preclude release but may indicate the need for antibiotic treatment in the recipient.
Quality control requirements	• Nucleated cell count. • Viable $CD34^+$ cell enumeration. • Viable $CD33^+$ for allogeneic products. • Microbiological testing. • ABO RhD blood group for allogeneic products. • Measurement of residual ABO-incompatible red cell volume.
Storage and transport	• **Fresh HPC(A)** can be stored and transported up to 72 hours at room temperature (15-25°C) or refrigerated (2-8°C), as requested by the ORHA. • **Fresh HPC(A)** can be stored up to 72 hours without cryopreservation. – The time to cryopreservation should be minimised. • **Cryopreserved HPC(A)** are stored and transported at temperatures equal to or below – 140°C. • **Thawed HPC(A)** are stored and transported refrigerated (2-8°C) and applied without undue delay.
Special labelling and accompanying information	• Placed in a container appropriately labelled with a unique identifying code. When applicable, this code is included in the accompanying documentation. • In the EU, when SoHO preparations are distributed for human application, they must be labelled with the Single European Code (SEC) as applicable. • Specific information not coded in the SEC that should be included in the accompanying documentation: – donor name (autologous or related donors) or donor GRID (unrelated donors); – recipient name (if permitted), recipient ID (if applicable); – viable $CD34^+$ cell enumeration; – ABO Rh blood group; – volume; – anticoagulation volume; – identity of the collection facility and/or donor registry (SoHO entity); – identity of processing and distribution facility (SoHO establishment); – instructions for appropriate thawing (if applicable).
Special warnings	• Do not irradiate. • Properly identify intended recipient and product. • For use by intended recipient only. • For autologous use only, if applicable. • Do not use leukoreduction filters. • Use immediately after thawing. • If microbial contamination is present, consider antibiotic treatment in the recipient.

25.3 Mononuclear cells from unstimulated peripheral blood apheresis – MNC(A)

SoHO preparation	Mononuclear cells from unstimulated peripheral blood apheresis – MNC(A)
Definition	Unstimulated mononuclear cells are collected by apheresis from the circulating blood. The collected cells can originate from the recipient (autologous) or from another individual (allogeneic). Unstimulated mononuclear cells can be used as fresh non-manipulated products or further processed (e.g. cryopreservation, cell selection, starting material for ATMPs).
Established clinical indications	• MNC(A) after allogeneic stem cell transplantation from the original HPC donor are used in cases of relapse and mixed chimerism or as relapse prophylaxis to enhance the graft-versus-malignancy effect, to promote immune reconstitution and prevent infection complications. • MNC(A) for generation of cellular therapies and ATMPs (e.g. NK-cell therapy, virus-specific T-cells, CAR-T cells) and for off-line extracorporeal photopheresis (ECP).
Critical properties	• Cellularity/viability: – Determine the number of viable nucleated cells, mononuclear cell count, viable $CD3^+$ cells (when required). • Absence of microbial contamination – The presence of microbial contamination may (or may not) preclude release but may indicate the need for antibiotic treatment in the recipient.
Quality control requirements	• Nucleated and mononuclear cell count. • Viability. • Viable $CD3^+$ cells enumeration. • Microbiological testing. • ABO Rh blood group for allogeneic products.
Storage and transport	• **Fresh MNC(A)** can be stored and transported up to 72 hours at room temperature (15-25°C) or refrigerated (2-8°C) as requested by the transplant centre. – The time to cryopreservation should be minimised. – Fresh MNC(A) can be stored up to 72 hours without cryopreservation. • **Cryopreserved MNC(A)** are stored and transported at temperatures equal to or below – 140°C. • **Thawed MNC(A)** are stored and transported refrigerated (2-8°C).
Special labelling and accompanying information	• Placed in a container appropriately labelled with a unique identifying code. When applicable, this code is included in the accompanying documentation. • In the EU, when SoHO preparations are distributed for human application, they must be labelled with the Single European Code (SEC) as applicable. • If applicable: warning statements and/or biohazard label. • Specific information not coded in the SEC that should be included in the accompanying documentation: – donor name (autologous or related donors) or donor GRID (unrelated donors); – recipient name, recipient ID (if applicable); – total nucleated and mononuclear cell count; – viable $CD3^+$ cell count; – ABO Rh blood group (allogeneic products); – volume; – anticoagulation volume; – identity of the collection facility and /or donor registry (SoHO entity); – identity of processing and distribution facility (SoHO establishment); – instructions for appropriate thawing, if applicable.
Special warnings	• Do not irradiate. • Properly identify intended recipient and product. • For use by intended recipient only. • For autologous use only, if applicable. • For further processing, if applicable (if collected as starting material for generation of cellular therapies or ATMPs). • Do not use leukoreduction filters. • Use immediately after thawing (if applicable). • If microbial contamination is present, consider antibiotic treatment in the recipient.

26.1 Haematopoietic progenitor cells from umbilical cord blood – HPC(CB)

SoHO preparation	Haematopoietic progenitor cells from umbilical cord blood – HPC(CB)
Definition	HPC are found in umbilical cord blood (UCB). SoHO establishments collect, transport, process and store UCB and, after validation of the UCB, transfer the data to a stem cell registry (SoHO entity). Upon request, UCB units are released and distributed, cryopreserved as whole blood or buffy-coat enriched.
Established clinical indications	• Alternative source for haematopoietic progenitor cell transplantation when no sibling or family HLA-match donors are available.
Critical properties	• Cellularity: – Cell dose will depend on clinical indication, age and patient's body weight. It is suggested to increase cell dose if there is HLA mismatch increase or if the patient has a non-malignant disorder. • Absence of microbial contamination: – The presence of microbial contamination will not preclude release but may indicate the need for antibiotic treatment in the recipient.
Quality control requirements	• Total nucleated cell (TNC) count. • Total viable $CD34^+$ cell enumeration. • Viability (%) of $CD45^+$ and $CD34^+$ cells. • Maternal infectious disease markers. • Microbiological testing. • ABO Rh blood group and verification of HLA typing. • Measurement of residual ABO-incompatible red cell volume. • Colony-forming units (CFU) or other validated potency assay.
Storage and transport	• **Cryopreserved HPC(CB)** are stored and transported at temperatures equal to or below – 140°C. • **Fresh and thawed HPC(CB)** are stored and transported refrigerated (2-8°C). Each SoHO establishment fixes the fresh and post-thaw expiry time.
Special labelling and accompanying information	• Placed in a container appropriately labelled with a unique identifying code. This code is included in the accompanying documentation. • In the EU, when SoHO preparations are distributed for human application, they must be labelled with the Single European Code (SEC) as applicable. • If applicable: warning statements and/or biohazard label. • Specific information not coded in the SEC that should be included in the accompanying documentation: – Donor ID (recipient ID, if applicable); – TNC count and viable $CD34^+$ cell enumeration; – Result of a potency assay (i.e. CFU); – ABO Rh blood group and HLA; – Volume; – Identity of the collection facility and/or donor registry; – Identity of processing and distribution facility; – Instructions for appropriate thawing and washing (if units have not been red cell reduced prior to cryopreservation); – Information brochure (including handling instructions for the use of cellular therapy products, with indications, contraindications, side-effects and hazards, dosage and infusion recommendations).
Special warnings	• Do not irradiate. • Properly identify intended recipient and product. • For use by intended recipient only. • For autologous use only, if applicable. • Do not use leukoreduction filters. • Use immediately after thawing.

27.1 Allogeneic pancreatic islet

SoHO preparation	Allogeneic pancreatic islet
Definition	Islets of Langerhans derived from the pancreas of a deceased donor (declared dead by either neurological criteria [DBD] or circulatory criteria [DCD]). The donor pancreas is considered the starting material for the processing. Organ donation for this purpose follows the same protocols for whole pancreas retrieval, to ensure the viability and safety of the islets. In the case of pancreata collected from DCD donors, warm ischaemia time is not to exceed one hour. The pancreas is donated through structured organ donation programmes that adhere to strict national and international medical and ethical guidelines.
Established clinical indications	- Insulinopenic diabetes with intact/stable kidney function, characterised by at least one of the following conditions: • Frequent metabolic instability (hypoglycaemia, hyperglycaemia or ketoacidosis) despite intensive insulin therapy. • Unacceptable glucose control and/or a high risk of mortality due to poor metabolic regulation, despite comprehensive diabetes management and education. • Hypoglycaemia unawareness. • Rapid progression of chronic diabetes complications, except diabetic nephropathy with proteinuria greater than 300 mg/dL. • Psychological conditions or cognitive impairment affecting adherence to insulin therapy. • Severe subcutaneous insulin resistance requiring intraperitoneal or intravenous insulin infusions. - Insulinopenic diabetes with any level of kidney function, under chronic immunosuppression for any reason (such as a concomitant or previous solid organ transplant, autoimmune disease, haematologic conditions etc.).
Critical properties	Identity: • Islet morphology: – Dithizone stain (DTZ) and microscopic evaluation: presence of islets confirmed by colour, shape and islet size. • Estimated tissue volume: – Maximum recommended packed tissue volume for the infusion: < 0.15 mL per kg recipient body weight. • Viability: – Viable islets: ≥ 70%. • Purity: – Islet purity: ≥ 20% at DTZ stain and microscopic quantification. • Safety: – Gram stains or equivalent rapid microbiological tests may be performed prior to transplantation. – Absence of microbial contamination: no evidence of microbial contamination at the time of release for transplantation, acknowledging that culture results may not yet be finalised. – Endotoxin levels: endotoxin content in the islet preparation should be within acceptable limits (≤ 5 endotoxin units per kg recipient body weight). ABO compatibility: • Verification of ABO compatibility.

SoHO preparation	Allogeneic pancreatic islet
Quality control requirements	• Visual inspection: – Parameters: appearance, pellet volume. • Dithizone stain (DTZ): – Stain and microscopic evaluation: colour, shape, islet size, islet yield, islet purity. • Viability testing: – Dye exclusion test: viability. • Sterility testing: – Endotoxin testing: sterility, purity; – Microbiological testing: rapid sterility testing method or pharmacopoeia sterility test (post-release result; sterility). • Blood group and HLA typing: – ABO Rh Blood Group: verification; – HLA typing: verification.
Storage and transport	• Transportation at 20°C to 26°C.
Special labelling and accompanying information	• Placed in a container appropriately labelled with a unique identifying code. This code is included in the accompanying documentation. • In the EU, when SoHO preparations are distributed for human application, they must be labelled with the Single European Code (SEC) as applicable. • Specific information not coded in the SEC that should be included in the accompanying documentation: – Identity of the collection facility and/or donor registry (SoHO entity); – Identity of processing and distribution facility (SoHO establishment); – Manufactured date and time; – Release time; – Recipient name and ID if applicable; – Donor ABO Rh blood group; – Tissue volume; – Islet yield; – Purity; – Viability.
Special warnings	• Do not irradiate. • Do not use leukodepleting filters. • Include biohazard labels or warning statements if applicable. • Administer within 24 hours after product release. • Do not use if product time exceeds 24 hours post-product release or if temperature is not maintained between 20 and 26°C. • Administer cells through the hepatic portal vein, with the estimated tissue volume not exceeding 0.15 mL/kg recipient body weight. • Monitor portal venous pressure peri-infusion. • Monitor for bleeding and portal vein thrombosis following infusion. • For use by intended recipient only.

27.2 Autologous pancreatic islet

SoHO preparation	Autologous pancreatic islet
Definition	Islets of Langerhans derived from the patient's own pancreas, obtained by preserving tissue during its collection, which may be necessitated by a variety of conditions. The whole or part of the pancreas serves as the starting material for processing.
Established clinical indications	Individuals of any age, with or without diabetes, including those with C-peptide–positive diabetes (fasting C-peptide ≥ 0.5 ng/mL), who require partial or total pancreatectomy and meet at least one of the following conditions: • Chronic pancreatitis, when subtotal or total pancreatectomy is indicated due to intractable pain unresponsive to medical and/or endoscopic therapy. • Recurrent acute pancreatitis, in the absence of anidentifiable reversible cause, when total pancreatectomy is indicated. • Traumatic pancreatic injury with main pancreatic duct disruption, when partial or total pancreatectomy is indicated. • Severe complications following pancreatic surgery (e.g. life-threatening pancreatic fistula) requiring completion (total) pancreatectomy. • Lesions located in the pancreatic body or neck, when an extended distal pancreatectomy is preferred over central pancreatectomy. • Technical infeasibility or high risk of anastomotic leakage during pancreaticoduodenectomy, necessitating total pancreatectomy. **Contraindications** • Multifocal pancreatic neoplasms (e.g. MEN-associated disease), • Renal cell carcinoma metastases to the pancreas, or • Diffuse intraductal papillary mucinous neoplasm (IPMN), as determined by preoperative imaging and/or intraoperative evaluation, contraindicate autologous islet transplantation outside of scientific studies.
Critical properties	Identity: • Islet morphology: – Dithizone stain (DTZ) and microscopic evaluation: presence of islets confirmed by colour, shape and islet size. • Estimated tissue volume: – Maximum recommended packed tissue volume for the infusion: < 0.25 mL per kg recipient body weight. Potency: • Viability: – Viable islets: ≥ 70%. Purity: • Islet purity: ≥ 10% at DTZ stain and microscopic quantification. Safety: • Absence of microbial contamination: No microbial growth.
Quality control requirements	Visual inspection: • Parameters: Appearance, pellet volume. Dithizone stain (DTZ): • Stain and Microscopic Evaluation: colour, shape, islet size, islet yield, islet purity. Viability testing: • Dye exclusion test: viability. Sterility testing: • Endotoxin testing: sterility, purity. • Microbiological testing: rapid sterility testing method or pharmacopoeia sterility test (post-release result; sterility).
Storage and transport	• Transport at 20°C to 26°C.

SoHO preparation	Autologous pancreatic islet
Special labelling and accompanying information	Placed in a container appropriately labelled with a unique identifying code. This code is included in the accompanying documentation. In the EU, when SoHO preparations are distributed for human application, they must be labelled with the Single European Code (SEC) as applicable. Specific information not coded in the SEC that should be included in the accompanying documentation: • Identity of the collection facility and/or donor registry (SoHO entity); • Identity of processing and distribution facility (SoHO establishment); • Manufactured date and time; • Release time; • Recipient name and ID if applicable; • Tissue volume; • Islet yield; • Purity; • Viability; • Endotoxin.
Special warnings	• Do not irradiate. • Do not use leukodepleting filters. • Include biohazard labels or warning statements if applicable. • Administer within 24 hours after product release. • Do not use if product time exceeds 24 hours post-product release or if temperature is not maintained between 20 and 26°C. • Administer cells through the hepatic portal vein, with the estimated tissue volume not exceeding 0.25 mL per kg recipient body weight. • Properly identify intended recipient and product. • For use by intended recipient only.

29.1 Cryopreserved adipose tissue for autologous use

SoHO preparation	Cryopreserved adipose tissue for autologous use
Definition	Complex and specialised connective tissue consisting of a stromal vascular fraction (SVF) and adipocytes, cryopreserved for autologous use, antibiotic decontamination.
Established clinical indications	• Reconstructive breast surgery.
Critical properties	• Minimally processed. • Maintenance of cellular composition, morphology and viability. • Absence of microbial contamination: – The presence of microbial contamination may (or may not) preclude release but may indicate the need for antibiotic treatment in the recipient.
Quality control requirements	• Microbiological testing.
Storage and transport	• SoHO preparations are stored at or below – 140°C for long-term storage. • Distribution is in dry ice (solid carbon dioxide) or in a liquid nitrogen dry-shipper. • If the SoHO preparation is shipped in dry ice, it should not be returned to storage at or below – 140°C unless this is supported by validation data on maintenance of the critical properties.
Special labelling and accompanying information	• Placed in a container appropriately labelled with a unique identifying code. This code is included in the accompanying documentation. • In the EU, when SoHO preparations are distributed for human application, they must be labelled with the Single European Code (SEC) as applicable. • Specific information not coded in the SEC that should be included in accompanying information: – Key specifications (volume); – Information on the composition of cryopreserving solution; – Identity of any residual processing chemicals (antibiotics, cryoprotectants); – Instruction for appropriate rinsing.
Special warnings	• Do not re-freeze thawed graft. • Use immediately after thawing. • Information about possible residues from processing and storage (antibiotics, cryopreservation media). • Instructions for appropriate thawing and washing. • For autologous use only.

30.1 Vitrified oocytes for third-party donation

SoHO preparation	Vitrified oocytes for third-party donation
Definition	Vitrified human oocytes obtained after controlled ovarian hyperstimulation, to be used for third-party oocyte donation in medically assisted reproduction (MAR). Vitrification is an ultra-rapid cooling method consisting of a very fast temperature drop (4 000-6 000°C/s up to 10 000°C/s, depending on the volume and device used) of the solution in which the specimen is cryopreserved without formation of ice crystals and where the potential toxicity of cryoprotectants is minimised due to shorter exposure in comparison with other cryopreservation approaches.
Established clinical indications	Women needing a third-party oocyte donation to achieve a pregnancy. The most common indications are: • Premature ovarian failure, either primary or secondary, including surgical oophorectomy, irreversible gonadal damage after chemotherapy or radiotherapy, Turner syndrome and other chromosomal disorders causing gonadal dysgenesis. • Ovaries inaccessible, or inaccessible for oocyte collection. • Start of natural menopause, or other age-related infertility. • Carriers of genetic diseases that cannot be avoided by pre-implantation genetic diagnosis for monogenic/single gene defects (PGT-M). • Carriers of structural abnormalities that cannot be treated by pre- implantation genetic diagnosis for chromosomal structural rearrangements (PGT-SR). • Carriers of mitochondrial diseases.
Critical properties	• Maturation status of the oocytes is metaphase II (MII).
Quality control requirements	• Morphological assessment of oocyte size and maturation status.
Storage and transport	• Temperature below – 140°C.
Special labelling and accompanying information	• Placed in a container appropriately labelled with a unique identifying code. This code is included in the accompanying documentation. • In the EU, in the context of third-party donation, when SoHO preparations are distributed for human application, they must be labelled with the Single European Code (SEC) as applicable. The SEC code is also needed for embryos generated after third-party donation. • Specific information not coded in the SEC that should be included in the accompanying documentation: – Number of shipped samples; – Type of medium used for storage, including batch information; – Type of storage device; – Number of oocytes per storage device; – Instructions for warming. • In cases when the vitrification device is too small to include the donation identification sequence (DIS) on the label, the DIS should be included in the accompanying documentation, permitting traceability between the sample code and the DIS.
Special warnings	• Any registered events and reactions in the donor file that may have implications for usage.

30.2 Vitrified oocytes for within-relationship use

SoHO preparation	Vitrified oocytes for within-relationship use
Definition	Vitrified human oocytes obtained after controlled ovarian hyperstimulation, to be used for fertility preservation and/or oocyte within-relationship use in medically assisted reproduction (MAR). Vitrification is an ultra-rapid cooling method consisting of a very fast temperature drop (4 000-6 000°C/s up to 10 000°C/s depending on the volume and device used) of the solution in which the specimen is cryopreserved without formation of ice crystals and where the potential toxicity of cryoprotectants is minimised due to shorter exposure in comparison with other cryopreservation approaches.
Established clinical indications	Women using their own cryopreserved oocytes to achieve a pregnancy. The most common indications are: • Fertility preservation for medical and non-medical reasons. • Avoidance of surplus embryos in culture for legal/ethical reasons. • Oocyte accumulation for cycle optimisation.
Critical properties	• Maturation status of the oocytes is metaphase II (MII).
Quality control requirements	• Morphological assessment of oocyte size and maturation status.
Storage and transport	• Temperature below – 140°C.
Special labelling and accompanying information	• Placed in a container appropriately labelled with a unique identifying code. This code is included in the accompanying documentation. • The Single European Code (SEC) is not necessary for vitrified oocytes intended for within-relationship use, and/or that are imported or collected in the same SoHO entity where they are applied (SoHO Regulation 2024/1938 Article 43.2). • In the context of within-relationship use, when SoHO preparations are transported for human application, the code will allow tracing of the identity of the person from whom SoHO are collected. • Specific information that should be included in the accompanying documentation: – Number of shipped samples; – Type of medium used for storage, including batch information; – Type of storage device; – Number of oocytes per storage device; – Instructions for warming. • In cases when the vitrification device is too small to include the donation identification sequence (DIS) on the label, the DIS should be included in the accompanying documentation, permitting traceability between the sample code and the DIS.
Special warnings	• Any registered events and reactions in the patient file that may have implications for usage.

30.3 Cryopreserved sperm for third-party donation

SoHO preparation	Cryopreserved sperm for third-party donation
Definition	Cryopreserved human spermatozoa obtained by ejaculation, to be used in non-partner sperm donation for medically assisted reproduction (MAR). Processed by equilibrating the sperm sample with cryoprotectants (with or without previous washing) followed by controlled cooling rate down to a temperature of approximately – 100°C and thereafter transferred to liquid nitrogen.
Established clinical indications	Couples or individuals in need of a non-partner sperm donation to achieve a pregnancy, either by intra-uterine insemination or by *in vitro* fertilisation of oocytes. The most common indications are: • Azoospermia. • Recurrent fertilisation failure. • Single women. • Same sex female couple. • Carriers of genetic diseases that cannot be avoided by pre-implantation genetic diagnosis for monogenic/single gene defects (PGT-M). • Carriers of structural abnormalities that cannot be treated by pre-implantation genetic diagnosis for chromosomal structural re-arrangements (PGT-SR).
Critical properties	• Presence of post-thaw viable and motile sperm.
Quality control requirements	• The number of motile spermatozoa after test thawing should be adequate for the intended use: intra-uterine insemination (IUI), conventional *in vitro* fertilisation (IVF) or micro-injection (ICSI).
Storage and transport	• Temperature below – 140°C. • Cryopreserved sperm is shipped in dry shipper in liquid nitrogen or on carbon dioxide ice.
Special labelling and accompanying information	• Placed in a container appropriately labelled with a unique identifying code. This code is included in the accompanying documentation. • In the EU, in the context of third party-donation when SoHO preparations are distributed for human application, they must be labelled with the Single European Code (SEC) as applicable. The SEC code is also needed for embryos generated after third-party donation. • Specific information, not coded in the SEC, that should be included in the accompanying documentation: – Number of shipped samples; – Pre-cryopreservation sperm concentration and motility; – Number of motile spermatozoa per device; – Type of medium used for storage, including batch information; – Type of storage device; – Instructions for thawing. • In cases when the cryopreservation device is too small to include the donation identification sequence (DIS) on the label, the DIS should be included in the accompanying documentation, permitting traceability between the sample code and the DIS.
Special warnings	• Any registered event and reactions in the donor file that may have implications for usage.

30.4 Cryopreserved sperm for within-relationship use

SoHO preparation	Cryopreserved sperm for within-relationship use
Definition	Cryopreserved human spermatozoa obtained by ejaculation or lavage, to be used in partner sperm donation for intra-uterine insemination (IUI), *in vitro* fertilisation (IVF) or micro-injection (ICSI). Processed by equilibrating the sperm sample with cryoprotectants (with or without previous washing) followed by controlled cooling rate down to a temperature of approximately – 100°C and thereafter transferred to liquid nitrogen.
Established clinical indications	Couples or individuals in need of their own cryopreserved sperm to achieve a pregnancy, either by intra-uterine insemination, IVF or ICSI. The most common indications are: • Retrograde ejaculation. • Erectile dysfunction. • Regular absence of the male partner from the location of treatment. • Collection by electro-ejaculation. • Fertility preservation for medical and non-medical reasons.
Critical properties	• Presence of post-thawing viable sperm.
Quality control requirements	• The number of motile spermatozoa after test thawing should be adequate for the intended use: IUI, IVF or ICSI.
Storage and transport	• Temperature below – 140°C. • Cryopreserved sperm is shipped in dry shipper in liquid nitrogen or on carbon dioxide ice.
Special labelling and accompanying information	• Placed in a container appropriately labelled with a unique identifying code. This code is included in the accompanying documentation. • The Single European Code (SEC) is not necessary for cryopreserved sperm intended for within-relationship use and/or that are imported or collected in the same SoHO entity where they are applied (SoHO Regulation 2024/1938 Article 43.2). • In the context of within-relationship use, when SoHO preparations are transported for human application, the code will allow tracing of the identity of the person from whom SoHO are collected. • Specific information that should be included in the accompanying documentation: – Number of shipped samples; – Pre-cryopreservation sperm concentration and motility; – Number of motile spermatozoa per device; – Type of medium used for storage, including batch information; – Type of storage device; – Instructions for thawing. • In cases when the cryopreservation device is too small to include the donation identification sequence (DIS) on the label, the DIS should be included in the accompanying documentation, permitting traceability between the sample code and the DIS.
Special warnings	• Any registered event and reactions in the patient file that may have implications for usage.

30.5 Vitrified embryos

SoHO preparation	Vitrified embryos for fertility preservation, within-relationship use or third-party donation
Definition	Vitrification of human embryos obtained after *in vitro* fertilisation (IVF) or micro-injection (ICSI), to be used for fertility preservation, partner and/or non-partner embryo donation. Vitrification is an ultra-rapid cooling method consisting of a very fast temperature drop (4 000-6 000°C/s up to 10 000°C/s depending on the volume and device used) of the solution in which the specimen is cryopreserved without formation of ice crystals and where the potential toxicity of cryoprotectants is minimised due to shorter exposure in comparison with other cryopreservation approaches.
Established clinical indications	The most common indications are: • Women in the context of within-relationship use facing gonadotoxic treatment. • Freeze-all strategy in case of suboptimal endometrium. • Storage of supernumerary embryos for later usage. • PGT-cases of blastocyst biopsy. • Fertility preservation. • Embryo donation.
Critical properties	• Post-thawing cleavage-stage embryos should have at least 50% intact cells or developmental ability after culture. • Blastocysts should have an estimate of < 50% degenerated cells and/or evidence of re-expansion observed 3–4 hours post-warming.
Quality control requirements	• Morphological and/or morphokinetical assessment of embryos.
Storage and transport	• Temperature below – 140°C.
Special labelling and accompanying information	• Placed in a container appropriately labelled with a unique identifying code. This code is included in the accompanying documentation. • In the EU, when SoHO preparations are distributed for human application, they must be labelled with the Single European Code (SEC) as applicable. • The SEC is not necessary for vitrified embryos intended for within-relationship use and/or that are imported or collected in the same SoHO entity where they are applied (SoHO Regulation 2024/1938 Article 43.2). • In the context of within-relationship use, when SoHO preparations are transported for human application, the code will allow tracing of the identity of the person from whom SoHO are collected. • Specific information not coded in the SEC that should be included in the accompanying documentation: – Number of shipped samples; – Type of medium used for storage, including batch information; – Type of storage device; – Number of embryos per storage device; – Instructions for warming. • In cases where the vitrification device is too small to include the donation identification sequence (DIS) on the label, the DIS should be included in the accompanying documentation, permitting traceability between the sample code and the DIS.
Special warnings	• Any registered events and reactions in the donor or patient file that may have implications for usage.

31.1 Cryopreserved ovarian tissue for autologous transplantation

SoHO preparation	Cryopreserved ovarian tissue for autologous transplantation
Definition	Fragments of ovarian cortical tissue preserved in a cryoprotective solution. An entire ovary, a semi-ovary or ovarian cortical biopsies can be collected. In cases of using the whole ovary, it will be bisected and the medulla discarded. Cortical pieces are cryopreserved in the presence of cryoprotectants, followed by controlled slow freezing down to a temperature of approximately – 140°C and thereafter transferred to liquid nitrogen. For prepubertal ovaries, the medulla can also be cryopreserved either separately from the cortex or together with the cortex.
Established clinical indications	• Fertility preservation for pubertal patients and women in need of urgent gonadotoxic treatments like chemotherapy where ovarian stimulation and subsequent oocyte cryopreservation is not possible. • Fertility preservation for prepubertal and peripubertal girls in need of gonadotoxic treatments like chemotherapy. • Genetic disease for prepubertal and adult patients.
Critical properties	• Cortical thickness on average 1-2 mm.
Quality control requirements	• Microbiological testing on the preparation medium and freezing solution that has been in contact with the SoHO preparation. • Testing for presence of malignant cells by standard haematoxylin-eosin staining only in cases where there is a considerable risk of malignant cell involvement in the ovary. • A fresh biopsy of ovarian cortex should be taken for histological assessment of follicles before freezing. • After transplantation: the resumption of oestrogen production (within 2-6 months after transplantation or until graft function has been re-established).
Storage and transport	• The collected ovary is best processed immediately after collection, but successful transplantations have been published with cooling (+2-8°C) and transport of the organ lasting up to 24 h. • Cryopreserved cortical pieces are stored and transported at temperatures below – 140°C.
Special labelling and accompanying information	• Placed in a container appropriately labelled with a unique identifying code. This code is included in the accompanying documentation. • In the EU in the context of SoHO preparations that are imported to or collected in the same SoHO entity where they are applied, SEC is not necessary (Art 43.2.e). • Specific information not coded that should be included in the accompanying documentation: – Number of shipped vials; – Results of microbiological testing, when applicable; – Results of testing for presence of malignant cells, when applicable; – Number of tissue pieces per vial; – Type of medium used for storage, including batch information; – Type of storage device; – Instructions for thawing. • Any remarks concerning abnormalities of the ovary and/or characteristics of the cortex while preparing the tissue, not limited to the tightness or fragility of the cortex. • In cases when the container is too small to include the donation identification sequence (DIS) on the label, the DIS should be included in the accompanying documentation, permitting traceability between the sample code and the DIS.
Special warnings	• Do not irradiate. • Properly identify intended recipient and product. • For use by intended recipient only. • Use immediately after thawing. • If presence of microbial contamination is detected, consider prophylactic antibiotic treatment in the recipient. • Donor risk of metastases despite tissue sampling for search to find malignant cells. • Any registered event and reactions in the donor file that may have implications for usage.

31.2 Sperm suspension from testicular tissue for within-relationship use

SoHO preparation	Sperm cell suspension from testicular tissue for within-relationship use
Definition	Cryopreserved cellular suspension containing human spermatozoa – accompanied by immature sperm cells, Sertoli cells, Leydig cells, epithelial cells and blood cells, obtained from testicular biopsy or testicular aspiration, to be used in within-relationship-use sperm donation for micro-injection (ICSI). Processed for cryostorage by equilibrating the sperm sample with cryoprotectants (with or without previous washing) followed by controlled or manual cooling rate down to a temperature of approximately – 100°C and thereafter transferred to liquid nitrogen.
Established clinical indications	Couples or individuals in need of their own cryopreserved sperm to achieve a pregnancy, by ICSI. The most common indications are: • Obstructive and non-obstructive azoospermia; • Previous failure with sperm from ejaculate in cases of severe male factor; • Fertility preservation.
Critical properties	• Presence of viable and/or motile sperm.
Quality control requirements	• The number of motile spermatozoa after test thawing needs to be adequate for the intended use: ICSI.
Storage and transport	• Temperature below – 140°C. • Cryopreserved cell suspension can be shipped in a dry shipper in liquid nitrogen or on carbon dioxide ice.
Special labelling and accompanying information	• Placed in a container appropriately labelled with a unique identifying code. This code is included in the accompanying documentation. • In the EU, in the context of within-relationship use, when SoHO preparations are transported for human application, the code will not reveal the identity of the person from whom SoHO are collected. • Specific information not coded that should be included in the accompanying documentation: – Number of shipped samples; – Pre-cryopreservation sperm concentration and motility; – Number of motile spermatozoa per device; – Type of medium used for storage, including batch information; – Type of storage device; – Instructions for thawing. • In cases when the cryopreservation device is too small to include the donation identification sequence (DIS) on the label, the DIS should be included in the accompanying documentation, permitting traceability between the sample code and the DIS.
Special warnings	• Do not irradiate. • Properly identify intended recipient and product. • For use by intended recipient only. • Use immediately after thawing. • Any registered event and reactions in the patient file that may have implications for usage.

32.1 Frozen raw donor human milk

SoHO preparation	Frozen raw donor human milk
Definition	Human milk, expressed by a donor, that has been stored frozen in a SoHO establishment, following specific indications for use by a recipient, and which has not undergone any processing (heat treatment or other antimicrobial treatments).
Established clinical indications	• First feeding option when mother's own milk is not available. • Prevention of necrotising enterocolitis in premature infants. • Prevention of feeding intolerance.
Critical properties	• CMV negative donor.
Quality control requirements	• Microbiological testing.
Storage and transport	• Storage at – 15°C or below for a maximum period of 6 months; and at – 60°C or below, for a maximum of 12 months. • Donor human milk should be transported frozen to the ORHA/SoHO entity.
Special labelling and accompanying information	• To be placed in a bottle, which is appropriately labelled with a unique identifying code. When applicable, this code is to be included in the accompanying documentation. • In the EU, when SoHO preparations are distributed for human application, they need to be labelled with the Single European Code (SEC) as applicable. • Specific information not coded in the SEC that should be included in accompanying documentation or on the label: volume, storage temperature, nutritional information (if available), instructions for appropriate thawing and date of expiry. • Raw human milk for nutritional use.
Special warnings	• Do not thaw in a microwave oven. • After thawing it should be kept between + 2 and + 4°C and administered within 24 h. • Do not heat with microwave oven. • Do not refreeze.

32.2 Frozen pasteurised donor human milk

SoHO preparation	Frozen pasteurised donor human milk
Definition	Human milk expressed by a donor, processed and stored frozen in a SoHO establishment, following specific indications, for use by a recipient.
Established clinical indications	• First feeding option when mother's own milk is not available. • Prevention of necrotising enterocolitis in premature infants. • Prevention of feeding intolerance.
Critical properties	• No bacterial growth at the end of the process.
Quality control requirements	• Microbiological testing pre-pasteurisation and post-pasteurisation.
Storage and transport	• Storage at – 15°C or below for a maximum duration of 6 months; and at – 60°C or below for a maximum of 12 months. • Donor human milk should be transported frozen to the ORHA/SoHO entity.
Special labelling and accompanying information	• To be placed in a bottle, which is appropriately labelled with a unique identifying code. When applicable, this code is to be included in the accompanying documentation. • In the EU, when SoHO preparations are distributed for human application, they need to be labelled with the Single European Code (SEC) as applicable. • Specific information not coded in the SEC that should be included in accompanying documentation or on the label: volume, storage temperature, nutritional information (if available), instructions for appropriate thawing and date of expiry. • Pasteurised human milk for nutritional use.
Special warnings	• Do not thaw in a microwave oven. • After thawing it should be kept between + 2 and + 4°C and administered within 24 h. • Do not heat with microwave oven. • Do not refreeze.

33.1 Frozen donor intestinal microbiota capsules

SoHO preparation	Frozen donor intestinal microbiota (IM) capsules
Definition	Frozen and encapsulated human faeces (Intestinal microbiota, IM) from a single healthy donor, prepared in a SoHO establishment to be used for faecal microbiota transplantation (FMT) with no intent to change composition or properties of the donor microbiome.
Established clinical indications	• Recurrent infection with *Clostridioides difficile* following recommended antibiotic therapy. • Fulminant or refractory infection with *Clostridioides difficile*.
Critical properties	• Acid-resistant capsules containing cryoprotectant, e.g. glycerol.
Quality control requirements	• Microbiological testing of donor before donation. • Re-screening of the donor after a quarantine period before release of the IM preparation. • Freezing of the IM preparations should be documented according to standard protocols in the SoHO establishment.
Storage and transport	• Storage at +2-8°C for up to six hours. • Storage at – 15 °C or below for up to eight weeks. • Storage at – 80°C for up to 24 months.
Special labelling and accompanying information	• Placed in a container appropriately labelled with a unique identifying code. This code is included in the accompanying documentation. • In the EU, when SoHO preparations are distributed for human application, they must be labelled with the Single European Code (SEC) as applicable.
Special warnings	• Do not administer capsules to patients with swallowing difficulties. • Capsules should be visually inspected before application, and all capsules need to have intact shape. Capsules that have visible cracks should be discarded. In case of damaged packaging, capsules should not be used. • Thawed IM preparations should be used the same day. • IM preparations should be kept at room temperature when applied to the recipient. • Do not refreeze thawed IM preparations. • Capsules should not be administered to patients with a history of anaphylactic reaction secondary to food allergy.

33.2 Frozen donor intestinal microbiota suspension

SoHO preparation	Frozen donor intestinal microbiota (IM) suspension
Definition	Liquid suspension of processed human faeces from a single healthy donor, prepared in a SoHO establishment to be used for FMT, with no intent to change composition or properties of the donor microbiome.
Established clinical indications	• Recurrent infection with *Clostridioides difficile* following recommended antibiotic therapy. • Refractory or fulminant infection with *Clostridioides difficile*.
Critical properties	• Liquid IM preparation.
Quality control requirements	• Microbiological testing of donor before donation. • Re-screening of the donor after a quarantine period before release of the IM preparation. • Freezing of the suspension to be documented according to standard protocols in the SoHO establishment.
Storage and transport	• Storage at +2-8°C for up to six hours. • Storage at –15°C for up to eight weeks. • Storage at –80°C for up to 24 months.
Special labelling and accompanying information	• Placed in a container (cryobag or syringes) appropriately labelled with a unique identifying code. This code is included in the accompanying documentation. • In the EU, when SoHO preparations are distributed for human application, they must be labelled with the Single European Code (SEC) as applicable.
Special warnings	• Thawing may be done overnight at +2-8°C. • Thawing time from –20°C at ambient temperature is 15-30 minutes. • Thawed IM preparation should be used within four hours and always the same day. • IM preparations should be kept at room temperature when applied to the recipient. • Do not administer via upper GI route in case of severe small bowel dysmotility. • Do not refreeze thawed donor faeces suspensions. • IM suspensions should not be administered to patients with a history of anaphylactic reaction secondary to food allergy. • Only clean water baths with fresh water should be used, to avoid contamination.

Part D.

Appendices

Appendix 1. A brief history of the preparation of tissues and cells for human application

The best documented accounts of early transplants deal with skin transplantation, though the success or failure of these procedures has not been well documented. The first reliable account is that of the Indian surgeon Sushruta, who in the 2nd century BCE used autografted skin transplantation for a nose reconstruction (rhinoplasty). Later, in the 16th century CE, the Italian surgeon Gasparo Tagliacozzi carried out successful skin autografts, but he consistently failed with allografts, offering the first suggestion of rejection several centuries before that mechanism could be understood. He attributed it to the "force and power of individuality" in his 1596 work *De Curtorum Chirurgia per Insitionem*. Orthopaedic surgeons refer to the origin of their discipline as 1668, when Job van Meekeren reported on the grafting of bone from a dog's skull to correct a defect in a soldier's cranium. It was not until 1869 that the first completely documented fresh human-skin allograft was carried out by the Swiss surgeon Jacques Reverdin.

The first successful full-thickness corneal transplant, a keratoplastic operation, was carried out in 1905 by Eduard Zirm at Olomouc Eye Clinic in Moravia (now in Czech Republic). Pioneering work in the surgical technique of transplantation was done in the early 1900s by the French surgeon Alexis Carrel, together with Charles Guthrie, who developed techniques for suturing arteries and veins. Their skilful anastomosis operations and new suturing techniques laid the groundwork for later transplant surgery, and Alexis Carrel won the 1912 Nobel Prize in Physiology or Medicine for his work in the field. Major steps in skin transplant occurred during the First World War, notably through the work of Harold Gillies in Aldershot, UK. Among his advances was the tubed pedicle graft, which maintained a fleshy connection from the donor site until the graft established its own blood supply.

Bone is the oldest tissue transplant on record and the most common tissue transplanted today. The first bone transplant recorded in modern times occurred in Scotland in 1878 when Sir William Macewen removed an infected humerus from a 12-year-old boy and replaced it with three allografts from an amputated tibia from another child with rickets. In 1907, Erich Lexer in Berlin developed a procedure to remove a whole knee joint from an amputee in one operating room and transport the 'warm' graft to an adjacent operating room for immediate transplant into the recipient. Five years later, Alexis Carrel's work predicted the storage of tissues for future transplantation, and surgeons began to use bones and developed their own 'bone banks'. These pioneers included Inclan in Cuba, Bush, Wilson and Hibbs in the USA, Hult working in Sweden, Judet in France, and Klen in what was then Czechoslovakia. Most of these early bone banks were simply refrigerators and, later, freezers, but greater sophistication was developed by bone banks in Leeds (UK), Berlin, Athens and Warsaw. When long-term freezer storage of long bones became feasible, limb-sparing surgery using this type of bone allograft to avoid amputations in the treatment of malignant skeletal tumours became popular. Burrwell (UK), Parrish and Mankin (USA) and Ottolenghi (Argentina) published their results.

The orthopaedic profession realised that, if very large segments of bone could be transplanted successfully, smaller segments could also be used. This resulted in a very large increase in the use of bone allografts. Tissue-storage methods were developed further during the 1950s by Hyatt at the US Navy Tissue Bank in Bethesda, Maryland, where they adapted methods of lyophilisation from the food preservation industry and applied the process to the preservation of bone and skin, which could then be easily stored, transported

and reconstituted for use when needed. This method of preservation allowed bone to be stored and transported easily without any electrical or mechanical requirements and has had a profound effect on the availability and use of bone allografts. By the end of the 1990s, use of musculoskeletal tissue allografts (i.e. bone, cartilage, soft tissue) had become commonplace in many clinical areas. In 1944, Paton had established the world's first eye bank, the Eye-Bank for Sight Restoration, in New York. Subsequently, the first deceased-donor eye bank was established in Odessa using eyes (packed in glass containers) sent by rail from a trauma centre in Moscow.

The first recorded cardiac valve transplantation was carried out in Toronto by Gordon Murray, who implanted an aortic allograft in the descending thoracic aorta to relieve aortic insufficiency in 1956. The first orthotopic transplantation of the aortic valve was undertaken by Donald Ross in London in 1962 and independently by Brian Barratt-Boyes in Auckland, New Zealand, a few weeks later. Pulmonary and mitral valves were first used as allografts in subsequent years, with the pulmonary autograft procedure being carried out first in 1967.

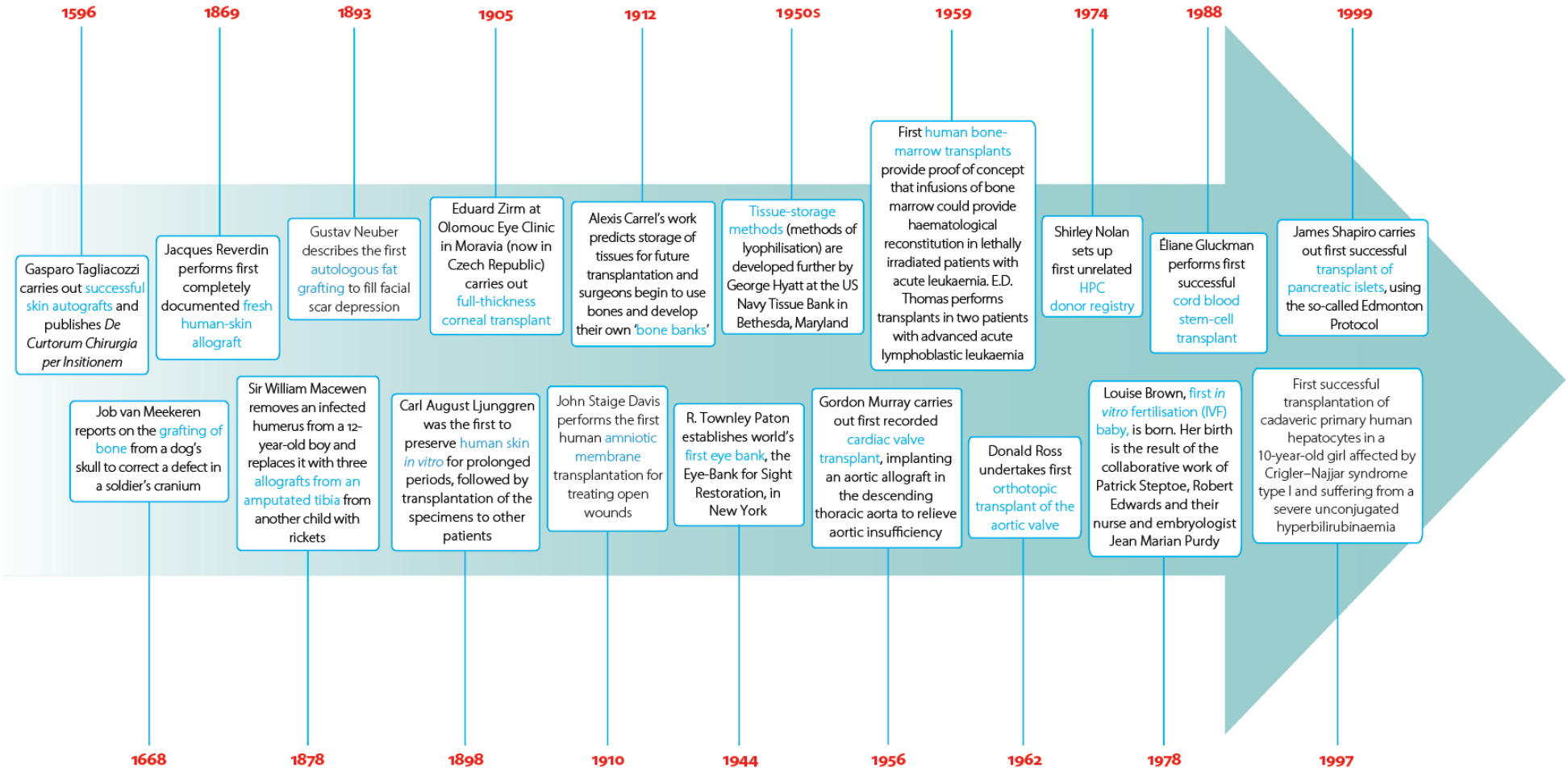
After the atomic bomb explosion in Japan that ended the Second World War, many scientists began to explore ways of protecting humans from radiation. The first experiments were done in mice and later in dogs by E.D. Thomas. As early as 1956, the idea that bone-marrow transplants might exert a therapeutic effect against malignancies was proposed by Barnes and Loutit, who observed an anti-leukaemic effect of transplanted spleen cells in experimental murine models. In 1959, the first human bone-marrow transplants gave proof of concept that infusions of bone marrow could provide haematological reconstitution in lethally irradiated patients with acute leukaemia. E.D. Thomas performed transplants in two patients with advanced acute lymphoblastic leukaemia, with a syngeneic graft after high-dose total body irradiation; the grafts were successful, but the patients died a few months later of relapse. G. Mathé administered allogeneic bone marrow for the treatment of several patients who had suffered accidental exposure to irradiation, and most survived with autologous reconstitution. In 1965, Mathé was the first to describe long-term engraftment of sibling bone marrow, thereby demonstrating chimerism, tolerance and an anti-leukaemic effect. Although the transplant itself was successful, the patient eventually died of varicella with chronic graft-*versus*-host disease (GvHD). In 1970, M. Bortin reported 203 transplants carried out between 1958 and 1968, with only three patients alive at the time of the report. The major causes of death were graft failure, GvHD and relapse. After these disappointing results, few centres persisted and the number of transplants declined sharply.

Major progress came from the discovery of the human leukocyte antigen (HLA) system by J. Dausset and J.J. Van Rood. Selection of HLA-identical siblings as bone-marrow donors diminished the risk of rejection and GvHD. Using animal models, R. Storb and E.D. Thomas developed the model of total body irradiation for conditioning (in dogs) and the use of methotrexate for GvHD prevention. In mice, G. Santos showed that the use of cyclophosphamide could add immuno-suppression to the myeloablation of total body irradiation. He was also the first to use busulfan instead of total body irradiation. In 1988, the first successful cord blood stem-cell transplant was done by E. Gluckman to treat a child with Fanconi's anaemia with cells from his healthy HLA-identical sibling (related) donor. The first unrelated donor registry was established in London in 1974 by Shirley Nolan, trying to find an HLA-match donor to her son who was diagnosed with Wiskott–Aldrich syndrome. After this first donor recruitment drive, the number of bone-marrow and peripheral haematopoietic progenitor cell donors increased all over the world, with more than 40 million volunteer donors now registered, including more than 800 000 cord blood units [1].

Transplantation of pancreatic islets has been carried out in humans since 1990 [2]. However, it was not until 1999 that the first successful transplant of pancreatic islets, using the so-called Edmonton Protocol, was undertaken by James Shapiro [3]. European centres became active around the same period, but their transplant recipients had complications of type-I diabetes that could not be managed with insulin injections. The advantage of the Edmonton Protocol was that it allowed restoration of the finely tuned regulation of glucose metabolism through appropriate insulin production by transplanted islets. In 2005, the first pancreatic islet transplant from a living donor – from a 56-year-old woman to her 27-year-old diabetic daughter – resulted in transplanted cells producing insulin within minutes after transplantation.

On 25 July 1978, Louise Brown, the first *in vitro* fertilisation (IVF) baby, was born in Oldham, UK [4]. Her birth was the result of the collaborative work of Patrick Steptoe, Robert Edwards and their nurse and embryologist Jean Marian Purdy. Since then, this research area has seen major improvements in the laboratory – e.g. cryopreservation of gametes and embryos, intracytoplasmic sperm injection (ICSI) [5], pre-implantation genetic diagnosis [6] and improved clinical management (such as methods for ovarian stimulation and embryo culture conditions) – thereby leading to a considerable increase in the use of assisted reproductive technologies (ART) [7]. A brief summary of the main relevant events in tissues and cells transplantation is shown in Figure A1.1.

Figure A1.1 **Summary of the timeline of the main relevant events in tissues and cells transplantation**



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Appendix 2. **Acronyms and other abbreviations**

3D	three-dimensional
AAS	ascending aorta
AATB	American Association of Tissue Banks
AAV	*adeno*-associated virus
Ab	antibodies
ACI	autologous chondrocyte implantation
AE	adverse event
AFC	antral follicle count
AIDS	acquired immunodeficiency syndrome
ALK	anterior lamellar keratoplasty
AMH	anti-Müllerian hormone
Anti-CMV	antibody to *Cytomegalovirus*
Anti-EBV	antibody to Epstein–Barr virus
Anti-HBc	antibody to hepatitis B core antigen
Anti-HCV	antibody to hepatitis C virus
Anti-HIV-1	antibody to HIV-1
Anti-HIV-2	antibody to HIV-2
ARE	adverse reactions and events
ART	assisted reproductive technology [*see also* MAR]
ARTHIQS	Assisted Reproductive Technologies and Haematopoietic stem cells for transplantation Improvements for Quality and Safety throughout Europe [*joint action*]
ATMP	advanced therapy medicinal product
aW	available water
BET	bacterial endotoxin test
BFU-E	burst-forming units – erythroblast
BM	bone marrow
BMDW	Bone Marrow Donors Worldwide [organisation: now part of WMDA]
BMP	bone morphogenetic proteins
BMSC	bone marrow stromal cells
BRCA	BReast CAncer gene
BSS	balanced salt solution
CAPA	corrective and/or preventive action
CAR	chimeric antigen receptor
CAT	Committee for Advanced Therapies
CBC	complete blood count
CD	cluster of differentiation
CD-BIO	Committee on Bioethics of the Council of Europe
CDC	Centers for Disease Control and Prevention [*USA*]
CDI	*Clostridioides difficile* infection
CD-P-TO	European Committee (Partial Agreement) on Organs, Tissues and Cells of the Council of Europe
CE (*marked*)	Conformité Européenne
CEA	cultured epithelial autografts
CFU	colony-forming units
CFU-GM	colony-forming units – granulocyte/ monocyte
CHAPS	3-[(3-cholamidopropyl) dimethyl-ammonio]-1-propanesulfonate
CJD	Creutzfeldt–Jakob disease
CLET	cultivated limbal epithelial transplantation
CMV	*Cytomegalovirus*
CNS	central nervous system
CNT	Centro Nazionale Trapianti (*Italy*)
CNV	copy number variation
COC	cumulus enclosed oocyte
COD	cause of death
COH	controlled ovarian hyperstimulation
COMET	cultivated oral mucosal epithelial transplantation

DALK	deep anterior lamellar keratoplasty
DBD	deceased by neurological determination of death
DBM	demineralised bone matrix
DC	dendritic cells
DCD	deceased by circulatory determination of death
DHM	donor human milk
DLI	donor lymphocyte infusions
DM	*Diabetes mellitus*
DMEK	Descemet membrane endothelial keratoplasty
DMSO	dimethyl sulfoxide
DQ	design qualification
DSAEK	Descemet stripping automated endothelial keratoplasty
DVT	deep-vein thrombosis
EATCB	European Association of Tissue and Cell Banks
EBMT	European Society for Blood and Marrow Transplantation
EBV	Epstein–Barr virus
EC	European Commission
ECCTR	European Cornea and Cell Transplant Registry
ECDC	European Centre for Disease Prevention and Control [*EU*]
ECM	extracellular matrix
ECVAM	European Centre for the Validation of Alternative Methods
EDQM	European Directorate for the Quality of Medicines & HealthCare
EDTA	ethylenediamine tetra-acetic acid
EEBA	European Eye Bank Association
EGF	endothelial growth factor
EGTA	ethylene glycol tetra-acetic acid
EIA	enzyme immunoassay
EK	endothelial keratoplasty
ELISA	enzyme-linked immunosorbent assay
EMA	European Medicines Agency
EQSTB	European Quality System for Tissue Banking [*EU project*]
ESBL	extended-spectrum beta lactamases
ESC	embryonic stem cell
ESHRE	European Society for Human Reproduction and Embryology
EU	European Union *not* endotoxin units (*see* IU)
EUROCET	European Registry for Organs, Tissues and Cells
Eurocode IBLS	Eurocode International Blood Labelling Systems
EuroGTP	Euro Good Tissue Practices [*EU project]*
EuroGTP II	Good [Tissue] Practices for demonstrating safety and quality through recipient follow-up [*EU project*]
Eustite	European Standards and Training in the Inspection of Tissue Establishments [*EU project*]
EUTC	European Code for Tissues and Cells
FACS	fluorescence-activated cell sorting
FACT	Foundation for the Accreditation of Cellular Therapy
FDA	Food and Drug Administration [*USA*]
FED	Fuchs endothelial dystrophy
FET	frozen embryo transfer
FIPS	fingerprints
FMEA	failure mode and effects analysis
FMECA	failure mode, effects and criticality analysis
FMT	faecal microbiota transplantation
FNHTR	febrile non-haemolytic reactions
FOS	fastidious organism supplement
FSH	follicle-stimulating hormone
FTSG	full-thickness skin graft
GAG	glycosaminoglycans
G-CSF	granulocyte-colony stimulating factor
GEMM	granulocyte-erythrocyte-macrophage-megakaryocyte
GF	growth factors
GM	granulocytes and macrophages
GM-CSF	granulocyte macrophage-colony stimulating factor
GMP	Good manufacturing practice *[EU document*]
GPA	glycerol-preserved allografts
GTP	good tissue practice
GV	germinal vesicle
GvHD	graft-*versus*-host disease
GvT	graft-*versus*-tumour
HACCP	hazard analysis and critical control points
HAV	hepatitis A virus
HBc	hepatitis B core antigen
HBsAg	hepatitis B surface antigen
HBV	hepatitis B virus
HCV	hepatitis C virus
HEPA	high-efficiency particulate air
HES	hydroxyethyl starch
HHV	human *Herpes* virus
HIV	human immunodeficiency virus
HLA	human leukocyte antigen
HM	human milk
hMG	human menopausal gonadotrophin
HoP	Holder pasteurisation
HPC	haematopoietic progenitor cells
HPP	high-pressure processing

HPV	human *papilloma* virus
HRV	human *rotavirus*
HSC	haematopoietic stem cells
HSG	hysterosalpingography
HSV	*Herpes simplex* virus
HTLV	human T-lymphotropic virus
HTST	high temperature short time pasteurisation
HVAC	heating, ventilating and air conditioning
HyCoSy	hysterosalpingo-contrast sonography
IAK	islet after kidney transplantation
IATA	International Air Transport Association
ICCBBA	International Council for Commonality in Blood Banking Automation
ICMART	International Committee Monitoring Assisted Reproductive Technologies
ICSI	intracytoplasmic sperm injection
ICU	intensive care unit
IDM	infectious disease marker
IEC	Independent Ethics Committee
IEQ	islet equivalents
IFN	interferon
Ig	immunoglobulin
IL	interleukin
iPSC	induced pluripotent stem cell
IQ	installation qualification
ISCT	International Society for Cellular Therapy
ISN	International Society for Nephrology
ISO	International Organization for Standardization
ISPE	International Society for Pharmaceutical Engineering
ISSCR	International Society for Stem Cell Research
IT	information technology
ITA	islet alone transplantation
IU	international unit (*for endotoxins*)
IUI	intra-uterine insemination
IVF	*in vitro* fertilisation
IVM	*in vitro* maturation
JACIE	Joint Accreditation Committee–ISCT & EBMT
KIR	killer immunoglobulin-like receptors
KLAL	keratolimbal allograft
KPI	key performance indicator
LAL	limulus amoebocyte lysate
LH	luteinising hormone
LSC	limbal stem cells
MAR	medically assisted reproduction
MCM	metastatic cutaneous melanoma
MESA	microsurgical epididymal sperm aspiration
MII	metaphase II
MNC	mononuclear cells
MPHO	medical products of human origin
MRA	marrow re-populating ability
MRSA	methicillin-resistant *Staphylococcus aureus*
MRT	mitochondrial replacement therapy
MSC	mesenchymal stromal (stem) cells
mtDNA	mitochondrial DNA
MTT	tetrazolium salt assay
NAC	nipple–areola complex
NAT	nucleic acid amplification technique/ nucleic acid test
NEC	necrotising enterocolitis
NICE	National Institute of Health and Clinical Excellence [*UK*]
niPGT	non-invasive pre-implantation genetic testing
NK	natural killer
NPC	non-valved pulmonary conduit
NRT	neutral red test
NtPSC	nuclear-transfer pluripotent stem cells
OA	osteoarthritis
OECD	Organisation for Economic Co-operation and Development
OHSS	ovarian hyperstimulation syndrome
ONT	Organización Nacional de Trasplantes [*Spain*]
OQ	operational qualification
ORHA	organisation responsible for human application
Parvo-B19	*Parvovirus* B19
PBK	pseudophakic bullous keratopathy
PBSC	peripheral blood stem cells
PCR	polymerase chain reaction
PESA	percutaneous epididymal sperm aspiration
PGD	pre-implantation genetic diagnosis, *see* PGT
PGS	pre-implantation genetic screening, *see* PGT
PGT	pre-implantation genetic testing (formerly known as PGD and PGS: *see above*)
PGT-A	pre-implantation genetic testing for aneuploidies
PGT-M	pre-implantation genetic testing for monogenic/single gene defects
PGT-SR	pre-implantation genetic testing for chromosomal structural re-arrangements
Ph. Eur.	*European Pharmacopoeia*, 11th edn, Strasbourg: Council of Europe 2025
PIC/S	Pharmaceutical Inspection Co-operation Scheme
PK	penetrating keratoplasty
PN	ProNucleus
PO	procurement organisation
POI	premature ovarian insufficiency

POSEIDON	Promoting Optimisation, Safety, Experience sharing and quality Implementation for Donation organisation and networking in unrelated haematopoietic stem cell transplantation in Europe [*EU project*]
PPA	preparation process authorisation
PQ	performance qualification
PRF	platelet-rich fibrin
PRIVILEGED	Privacy in Law, Ethics and Genetic Data [*EU project*]
PROH	1,3-propanedial
PRP	platelet-rich plasma
PVP	PolyVinylPyrrolidone
QC	quality control
QM	quality manager
QMS	quality management system
QRM	quality risk management
RABS	Restricted Access Barrier System
RANTES	regulated on activation, normal T-cell expressed and secreted
RATC	rapid alerts for tissues and cells
RBC	red blood cell
RCT	randomised control trial
RhD	Rhesus D antigen
rhG-CSF	recombinant human granulocyte colony-stimulating factor
RP	responsible person
RPN	risk priority number
RUO	research use only
SAE	serious adverse event
SAL	sterility assurance level
SAR	serious adverse reaction
SARE	severe adverse reactions and events
SCS	static cold storage
SDS	sodium dodecyl sulfate
SEC	Single European Code
SIG	special interest group
SIK	simultaneous islet and kidney transplantation
SLA	service-level agreement
SNV	single nucleotide variation
SoHO	substance(s) of human origin
SoHO V&S	vigilance and surveillance of substances of human origin
SOP	standard operating procedure
SP-CTO	Council of Europe Committee of Experts on the Organisational Aspects of Co-operation in Organ Transplantation
S(P)EAR	serious (product) events and reactions
SSC	spermatogonial stem cell
STSG	split-thickness skin graft
SVF	stromal vascular fraction
SVR	sustained virological response
T1DM	Type-1 *Diabetes mellitus*
TAMC	total aerobic microbial count
TBV	total blood volume
TCP	tissue or cell preparation
TCR	T-cell receptor
TESA	testicular sperm aspiration
TESE	testicular sperm extraction
TGF	tumour growth factor/transforming growth factor
TLT	time-lapse technology
TNC	total nucleated cells
TNF	tumour necrosis factor
TPV	total plasma volume
TRALI	transfusion-related acute lung injury
TSB	tryptic soy broth
TSE	transmissible spongiform encephalopathy
TTS	The Transplantation Society
TYMC	total combined yeasts/moulds count
UCB	umbilical cord blood
UHPH	ultra-high-pressure homogenisation
UPS	uninterrupted power supply
UW	University of Wisconsin
V&S	vigilance and surveillance
vCJD	variant Creutzfeldt–Jakob disease
VEGF	vascular endothelial growth factor
VISTART	Vigilance and Inspection for the Safety of Transfusion, Assisted Reproduction and Transplantation [*joint action*]
VMP	validation master plan
VOC	volatile organic compound
VRE	Vancomycin-resistant *enterococci*
WHO	World Health Organization
WMDA	World Marrow Donor Association
WNV	West Nile virus

Appendix 3. **Glossary**

Acceptance criteria	Requirements needed to meet the relevant pre-defined quality and safety standards in order to ensure an acceptable final product for human application.
Adipose tissue	Loose connective tissue, composed of adipocytes and stromal vascular fraction, which serves as energy storage and endocrine organ.
Advanced therapy medicinal product	A medicinal product that can be a gene therapy medicinal product, a somatic cell therapy medicinal product, a tissue-engineered product or a combined advanced therapy medicinal product, which incorporates cells and medical devices (and these may be actively implantable).
Adverse event	Any untoward occurrence associated with the collection, testing, processing, storage or distribution of tissues and cells. *See also*: Serious adverse event.
Adverse reaction	Any unintended response, including a communicable disease, in the donor or the recipient that is associated with the procurement or human application of tissues and cells. *See also*: Serious adverse reaction.
Agarose gel electrophoresis	Diagnostic tool used to visually separate DNA fragments.
Allogeneic	Refers to SoHO removed from one individual and applied to another of the same species
Allogeneic islet	Islets of Langerhans derived from the pancreas of a deceased donor (death by neurological criteria [DBD] or death by cardiocirculatory criteria [DCD]).
Allograft	SoHO transplanted between two genetically different individuals of the same species. The term is synonymous with 'homograft'.
Ambient temperature	The temperature of the surrounding environment.
Amniotic membrane	The innermost layer of the placental membrane; it surrounds the fetus during pregnancy.
Angiogenesis	Physiological process by which new blood vessels form from pre-existing vessels.
Antibiogram	*See*: Resistogram.
Antibiotic/ antimicrobial	An agent that inhibits the growth of, or destroys, micro-organisms.
Apheresis	A medical technique in which peripheral blood of a donor or patient is passed through an apparatus that separates one particular constituent and returns the remaining constituents to the donor or patient.
Aseptic techniques	Procedures designed to prevent contamination from micro-organisms and spread of infection.
Assisted reproductive technology	All interventions that include *in vitro* handling of both human oocytes and sperm, or of embryos, for the purpose of reproduction. This includes, but is not limited to, *in vitro* fertilisation (IVF), embryo transfer (ET), intracytoplasmic sperm injection (ICSI), embryo biopsy, preimplantation genetic testing (PGT), assisted hatching, gamete intrafallopian transfer (GIFT), zygote intrafallopian transfer, gamete and embryo cryopreservation, semen, oocyte or embryo donation and gestational carrier cycles. Thus, ART does not, and ART-only registries do not, include assisted insemination using sperm from a woman's partner or a sperm donor. *See also*: Medically assisted reproduction.

Audit	Periodic, independent and documented examination and verification of activities, records, processes and other elements of a quality system to determine their conformity with specific internal or external requirements. They may be conducted by professional peers, internal quality system auditors or auditors from certification or accreditation bodies.
Autologous	Refers to SoHO removed from and applied in the same individual.
Autologous islet transplantation	Islets of Langerhans derived from the patient's own pancreas, obtained by preserving tissue during its surgical collection.
Azoospermia	Absence of spermatozoa in the ejaculate.
Bacteraemia	The presence of viable bacteria in the circulating blood.
Banking	Processing, preservation, storage and distribution of SoHO for human application or other purposes, including research and training.
Barcode	An optical machine-readable representation of data relating to the object to which it is attached.
Batch	A defined quantity of starting material, packaging material or product processed in one process (or series of processes) so that it can be considered to be homogeneous.
Bioactivity	The effect of a substance upon a living organism.
Bioburden	Total number of viable micro-organisms or total microbial count present, on or in SoHO or in the environment, usually measured before the application of a decontamination or sterilisation process.
Biochemical cue	Chemical signal that occurs in a biological organism and causes a biological response.
Biocompatibility	Property of a material being compatible with living tissue. Biocompatible materials do not produce a toxic or immunological response when exposed to the body or body fluids.
Biodegradability	Disintegration of materials by biological processes.
Biomechanical cue	Mechanical signal that occurs in a biological organism and causes a biological response.
Bionics	Biologically inspired engineering is the application of biological methods and systems found in nature to the study and design of engineering systems and modern technology.
Biophysical cue	Physical signal that occurs in a biological organism and causes a biological response.
Bioprinting	Combination of cells, growth factors and biomaterials using layer-by-layer deposition to fabricate biomedical parts that maximally imitate natural tissue characteristics, including structures that are later used in medical and tissue engineering fields.
bisBenzimide	Organic compound used as a fluorescent stain for DNA in molecular biology applications.
Blastocyst	An embryo, around 5-6 days after fertilisation, with an inner cell mass, an outer layer of trophectoderm and a fluid-filled blastocoele cavity.
Blastomere	A cell in a cleavage-stage embryo.
Blood groups	ABO or ABo. Both forms are widely used, but this Guide uses O. The O is from German *ohne* ('without') and means the same as o ('zero'): these are red blood cells without A or B antigens on the cell surface.
Blood vessels	Any allograft prepared from arteries or veins except the pulmonary trunk and ascending aorta.
Bone	The hard, rigid, mineralised form of connective tissue constituting most of the skeleton of vertebrates and composed primarily of calcium salts. There are two types of osseous tissue that form bones: cortical bone (the compact bone of the shaft of a bone that surrounds the marrow cavity) and cancellous or trabecular bone (typically occurs at the ends of long bones, proximal to joints and within the interior of vertebrae). Cancellous bone is highly vascular and frequently contains bone marrow.
Bone marrow	Tissue at the centre of large bones. It is the place where new blood cells are produced. Bone marrow contains two types of stem cell: haematopoietic (which can produce blood cells) and stromal (which can produce fat, cartilage and bone).
Calibration	An operation that, under specified conditions, first establishes a relation between the quantity values (with measurement uncertainties provided by measurement standards) and the corresponding indications (with their associated measurement uncertainties); and, second, uses this information to establish a relation from which to obtain a measurement result from an indication.
Cardiac vascular tissues	Allografts consisting of heart valves, non-valved conduits prepared from the pulmonary trunk (with or without bifurcation) or from the ascending aorta (with or without arch), or patch grafts prepared from these conduits.
Cell	The smallest transplantable and functional unit of life.
Cell culture	Growth of cells in a nutrient medium *in vitro*.
Cell migration	Movement of cells in particular directions, often in response to specific external signals, including chemical signals and mechanical signals.
Circulation	Transfer of tissues or cells from a tissue establishment to another operator for further processing.

Clean area	A locally controlled environment designed to provide a defined level of particulate and microbiological cleanliness which provides a physical barrier from surrounding working area. Clean zones are typically generated by dedicated contamination control technologies such as laminar air flow cabinets, isolators, or Restricted Access Barrier Systems (RABS), which create unidirectional airflow.
Cleavage-stage embryo	Embryo, beginning with the 2-cell stage and up to, but not including the morula stage.
Clinical evaluation	Clinical follow-up studies for monitoring predefined clinical outcome indicators to evaluate quality, safety and effectiveness/ efficacy of SoHO preparation for a defined number of patients.
Clinical-outcome monitoring plan	A programme for evaluating the safety and effectiveness of a SoHO preparation following human application.
Clinical-outcome registration	The management of a clinical registry where information on the results from the implementation of a clinical-outcome monitoring plan are recorded, including transferring such information to other registries.
Clinical study	An experimental evaluation of a SoHO preparation, with the objective of drawing conclusions regarding its safety and effectiveness.
Closed system	A system that employs a physical barrier that completely separates the internal environment from the external environment and allows contact between them only through irreversible measures.
Coding	A system for unique identification of SoHO for human application, comprising a donation identifier and product identifier for the specific type of tissue or cell.
Cold ischaemia time	The duration between the initiation of cold preservation and the start of processing.
Collagen	Main structural protein.
Collagenase/ protease mixture	Enzymatic solution used to digest the extracellular matrix of liver tissue, allowing hepatocyte isolation.
Collection	A process by which SoHO are obtained from a person, including any preparatory steps, such as hormone treatment, needed to facilitate the process at, or under the supervision of a SoHO entity.
Colonisation	The natural, biological presence or spread of micro-organisms.
Combined advanced therapy medicinal product	A medicinal product incorporating cells and medical devices (which may be actively implantable).
Compatibility testing	Testing for the presence or absence of recipient antibodies to HLA and to blood group antigens present on the tissues or cells for transplantation.
Compensation	Making good of any losses or the reimbursement of expenses associated with donation.
Competent authority	*See*: Health Authority.
Computerised system	A system including the input of data, electronic processing and the output of information, to be used either for reporting or for automatic control.
Consent	Lawful permission or authorisation for collection of SoHO: (a) the permission given freely without coercion by a living donor or a recipient for an action affecting them to proceed; (b) the permission given freely without coercion by any person granting consent on behalf of a living donor or a recipient who has no capacity to consent, or the authorisation granted under national legislation, for an action to proceed in relation to the living donor or the recipient; or (c) the permission given freely without coercion by any person granting consent, or the authorisation granted under national legislation, for an action to proceed in the case of a deceased donor in accordance with national legislation.
Consent to donation	Lawful permission or authorisation for removal of human cells, tissues and organs for transplantation. *See also*: Opt-in donation; Opt-out donation.
Contained laboratory, contained area	According to EU GMP, an area constructed and operated in such a manner (and equipped with appropriate air handling and filtration) as to prevent contamination of the external environment by biological agents from within the area.
Contamination	Accidental inclusion or growth of harmful micro-organisms, such as bacteria, yeast, mould, fungi, virus, prions, protozoa or their toxins and by-products. Contamination is different from colonisation, which is the natural, biological presence of micro-organisms.
Contract acceptor	The entity or contracted third party that performs particular arrangements (activities, work or services) under an agreement for a SoHO entity.
Contract giver	The entity that subcontracts particular arrangements (activities, work or services) to a different entity or contracted third party and is responsible for setting up an agreement defining the duties and responsibilities of each party.
Controlled ovarian stimulation	Pharmacological treatment in which women are stimulated to induce the development of multiple ovarian follicles to obtain multiple oocytes.
Cord blood	Blood collected from placental vessels and umbilical cord blood vessels after the umbilical cord is clamped and/or severed as a source of haematopoietic progenitor cells.

Cord blood bank	A specific type of SoHO establishment in which haematopoietic progenitor cells collected from placental and umbilical cord blood vessels are processed, cryopreserved and stored. It may also be responsible for collection, testing or distribution.
Cornea	The transparent anterior part of the outer fibrous coat of the eye. A collagenous tissue bounded by an outer stratified epithelium and an inner monolayer of endothelial cells. The major refractive component of the eye.
Critical	Potentially having an effect on the quality and/or safety of (or having contact with) SoHO.
Critical consumables/ reagents/ materials	Any consumable/reagent/material used in the collection, transport, preparation, storage or issue of SoHO that could significantly impact the safety and/ or efficacy of the SoHO it is applied to should it not meet its designated specification.
Critical SoHO	SoHO for which an insufficient supply will result in serious harm or risk of serious harm to recipients' health or in a serious interruption in the manufacture of products regulated by other (EU) legislation, where an insufficient supply of such products will result in serious harm or risk of serious harm to human health.
Critical SoHO entity	SoHO entity that carries out activities contributing to the supply of critical SoHO, where the scale of those activities is such that a failure to carry them out cannot be compensated by activities of other SoHO entities or alternative substances or products for recipients.
Cross-contamination	Unintentional transfer of micro-organisms and/or other material from one donation or processing batch to another.
Cryopreservation	Preservation and storage at sub-zero temperature of viable tissues and cells (including gametes and embryos) using a cryoprotectant to preserve viability, either by controlled slow freezing or by vitrification.
Cryoprotectant	A chemical compound that is used to protect cells and tissues against freezing injury.
Cumulus cell	The multi-layered mass of granulosa cells surrounding the oocyte.
Cycle	The process (including medication or not) that leads to a unique collection of gametes (for donation or own use), to the single (at a unique time) use of gametes or to the transfer of one or more embryos in a single procedure.
Cytotoxicity	Quality of being toxic to cells.
Death by cardiocirculatory criteria	A donor who is declared dead based on circulatory criteria. Also known as deceased non-heart-beating donor.
Death by neurological criteria	A donor who is declared dead based on the irreversible loss of neurological functions. Also known as deceased heart-beating donor.
Deceased donor	Deceased person who has been referred to a SoHO entity or establishment with a view to SoHO collection, and from whom consent had been granted in that respect or from whom collection is permitted, in accordance with national rules. *See also*: Death by neurological criteria; Death by cardiovascular criteria.
Decontamination	The process of removing or neutralising contaminants.
De-epidermisation	Process by which epidermis is removed from skin.
Delivery rate	Number of deliveries of neonates expressed per 100 initiated cycles, aspiration cycles or embryo-transfer cycles. It includes deliveries that resulted in the birth of one or more live and/or stillborn babies.
Denudation	The removal or stripping of the cumulus cells from the oocyte.
Design qualification	The first step in the qualification of new equipment or facilities. The first phase of qualification consisting of a documented process that verifies that the design specifications of facilities, equipment or computerised systems meet the intended regulatory and operational performance characteristics before implementation or construction.
Deviation	Departure from an approved instruction/ protocol or established standard.
Differentiation	Process by which a less specialised cell becomes a more specialised cell type.
Diminished ovarian reserve	A term generally used to indicate a reduced number and/or reduced quality of oocytes, such that the ability to reproduce is decreased.
Direct use	Any procedure in which tissues and cells are donated and used without banking or storage.
Discontinuous gradient centrifugation	Sperm-preparation technique based on sedimentation of sperm at different rates depending on density.
Disinfection	A process that reduces the number of viable micro-organisms, but does not necessarily destroy all microbial forms, such as spores and viruses.
Disposal (of tissues/cells)	The act or means of discarding tissues and/or cells.

Distribution	Providing released SoHO: (a) intended for human application to a specific recipient in the same or another entity: (b) intended for human application in general, without the prior identification of a specific recipient, in the same or in another entity; (c) intended for the manufacture of products regulated by other legislation to a manufacturer of products regulated by other legislation to a manufacturer of such products.
Dithizone (DTZ) staining	A method used to selectively stain insulin-producing islets red, allowing for differentiation from exocrine tissue and assessment of islet morphology and purity.
Donation	A process by which a person voluntarily and altruistically gives SoHO from their own body for persons in need, or authorises the use of such SoHO after their death; it includes the necessary medical formalities, examination and treatments and monitoring of the donor, irrespective of whether that donation is successful or not; it also includes, where applicable, the consent given by an authorised person in accordance with national legislation.
Donor	An individual, living or deceased, who is a source of tissues or cells for human application and for other purposes, including research.
Donor advocate	An individual distinct from the recipient's primary treating physician whose main obligation is to protect the interests, wellbeing and safety of the donor. The donor advocate may help the donor understand the process, the procedures and the potential risks and benefits of donation.
Donor evaluation	The procedure for determining the suitability of an individual, living or deceased, as a donor of cells or tissues.
Donor human milk	The human milk expressed by a breastfeeding mother and donated voluntarily and freely to a SoHO establishment.
Donor recruitment	Any activity aimed at informing persons about activities related to donation or at encouraging them to donate SoHO.
Donor registration	Recording in a registry, and transferring to other registries, where appropriate, information on a donor that is essential for identifying a match with a prospective recipient.
Donor selection	*See*: Donor evaluation.
Double embryo transfer	Transfer of two embryos.
Effectiveness	Presence of functionality proven by *in vitro* analytics (e.g. potency assays) depending on the mode of action of the SoHO preparation; the extent to which the use of SoHO reaches the intended biological or clinical outcome in the recipient.
Effectiveness of SoHO	The extent to which the human application of SoHO achieves the intended biological or clinical outcome in the SoHO recipient. Any reference to effectiveness should be considered to include an expected response in a SoHO recipient, either a response that is measurable in terms of degree, such as an engraftment after transplant, or an expected result in a SoHO recipient that is successful or not, but cannot be measured in terms of degree, and which is evaluated in accordance with a previously approved clinical-outcome monitoring plan, when such a plan is required.
Efficacy	The presence of desired clinical effects or desired patient outcome from treatment.
Elastin	Highly elastic protein in connective tissue that allows many tissues in the body to resume their shape after stretching or contracting.
Electron beam irradiation	Use of beta irradiation, usually of high energy under elevated temperatures and nitrogen atmosphere, for sterilisation or cross-linking of polymers.
Eligible/ Eligible donor	A potential consented SoHO donor who is medically suitable and meets specific criteria for the donation of at least one type of SoHO.
Embryo	The result of continued development of the zygote to 8 completed weeks after fertilisation, equivalent to 10 weeks of gestational age.
Embryo biopsy	The removal of cells (blastomeres or trophectoderm cells) from the embryo for the purpose of genetic analysis.
Embryo donation	Transfer of an embryo resulting from gametes (spermatozoa and oocytes) that did not originate from the recipient and her partner.
Embryo transfer	Procedure in which one or more embryos are placed in the uterus or Fallopian tube.
Emerging disease	A disease that has recently appeared in a population for the first time, or that may have existed previously but is rapidly increasing in incidence or geographic range.
End user	A healthcare practitioner who undertakes human application procedures.
Endotoxins	Large molecules consisting of a lipid and a polysaccharide, which are found in the outer membrane of Gram-negative bacteria.
Error	A mistake or failure to carry out a planned action as intended, or application of an incorrect plan that may or may not cause harm to patients.

Escharectomy	Surgical procedure based on removal of necrotic skin tissue from a full-thickness burn.
Ethylene oxide	Organic compound and toxic gas, being a surface disinfectant widely used in hospitals and the medical equipment industry for sterilisation.
Exceptional release	The distribution for clinical use of a unit of tissues and/or cells/SoHO that does not fully comply with the defined safety and quality criteria for release. The release is justified by a specific clinical need in which the benefit outweighs the risk associated with the non-compliance. *See also*: Negative-to-date release.
Excipient	Inactive substance that supports or enhances function or handling of tissue, e.g. carrier.
Expert	Individual with the appropriate qualifications and experience to provide technical advice to a Health Authority inspector.
Expiry date	The date after which tissues or cells are no longer suitable for use. Also known as 'expiration date'.
Export	Activities carried out to send SoHO from one country to a third country. *See also*: Third countries.
Facility	A physical building or part of a building.
Faecal microbiota transplantation	Transfer of minimally processed donor faeces from one donor to a patient.
Fallopian tube	A long duct in the female abdomen that transports the oocytes that have been released from the ovary to the uterus.
Fascia	A layer of fibrous connective tissue that surrounds muscles, groups of muscles, blood vessels and nerves; it binds some structures together while permitting others to slide smoothly over each other.
Fertilisation	Entry of the oocyte by a spermatozoon followed by combination of their genetic material, resulting in the formation of a zygote.
Fertility	The capacity to establish a clinical pregnancy.
Fertility preservation	Cryopreservation of reproductive tissues or cells to preserve reproductive capacity.
Fibronectin	High-molecular glycoprotein of the extra-cellular matrix (ECM) that plays a major role in cell adhesion, growth, migration and differentiation.
Field safety corrective action	Corrective action taken by a manufacturer for technical or medical reasons to prevent or reduce the risk of a serious incident in relation to a device made available on the market.
Final product	Any SoHO preparation intended to be transplanted or administered after the final release step.
Financial neutrality of donation	The principle or requirement that no financial gain or loss will be incurred by the donor as a result of the donation.
Follow-up	Subsequent evaluation of the health of a patient, living donor or recipient, for the purpose of monitoring the results of the donation or human application, maintaining care and initiating post-donation or post-application interventions.
Freeze drying	*See*: Lyophilisation.
Freezing	As used in this guide, it means storage of SoHO at sub-zero temperatures, with or without cryoprotectant.
Full-thickness skin grafts (FTSG)	Graft composed of epidermis and full-thickness dermis (with adnexal structure).
Fungaemia	The presence of fungi in the circulating blood.
Gamma irradiation	Penetrating electromagnetic radiation arising from the radioactive decay of atomic nuclei. It is used for medical equipment sterilisation.
Gestational carrier	A woman who carries a pregnancy with an agreement that she will give the offspring to the intended parent(s). Gametes can originate from the intended parent(s) and/or a third party (or parties).
Glycosaminoglycans (GAG)	Long unbranched polysaccharides that are highly polar and thus attract water; useful as a lubricant or shock absorber.
Good laboratory practice	Set of principles that provides a framework within which studies are planned, carried out, monitored, recorded, reported and archived by laboratories conducting testing of all kinds.
Good Manufacturing Practice (GMP)	An EU standard applied internationally for the safe manufacture of medicinal products. Although the processing of tissues and cells is not normally regulated under medicinal manufacturing legislation, many of the principles of GMP can be applied usefully to tissues and cells for human application.
Good practice	A method or technique that has consistently shown results superior to those achieved by other means and which is currently used as a benchmark.
Graft	Part of the human body that is transplanted in the same or another person to replace a damaged part or to compensate for a defect.
Grafting	*See*: Transplantation.
Haematopoietic progenitor cells	Primitive haematopoietic cells capable of self-renewal as well as maturation into any of the haematopoietic lineages, including committed and lineage-restricted progenitor cells, unless otherwise specified and regardless of tissue source. Also known as 'haematopoietic stem cells'.
Haematoxylin-Eosin	Histology staining used in medical diagnosis.

Haemodilution	In reference to blood samples from a donor, a decrease in the concentration of the donor's plasma proteins, circulating antigens or antibodies and any infectious agent present, resulting from the transfusion of blood or blood components and/or infusion of fluids, e.g., colloid(s) and/or crystalloid(s). Also known as 'plasma dilution'.
Haemolysis	Damage to red cells resulting in the release of haemoglobin into serum/plasma.
Health Authority	In the context of this Guide, the body which has been delegated with the responsibility for ensuring that SoHO donation, banking and human application are appropriately promoted, regulated and monitored in the interests of donor and patient safety and public transparency on a national or regional basis by their government. Other terms, such as 'regulatory authority', 'regulatory agency' or, in the EU, 'competent authority', are equivalent to it.
Heart valve	One of the four structures within the heart that prevent the backflow of blood by opening and closing with each heartbeat. They include two semilunar valves (aortic and pulmonary), the mitral (or bicuspid) valve and the tricuspid valve. They permit blood flow in only one direction.
Hepatocyte transplantation	Infusion of hepatocytes to restore liver function.
Homograft	*See*: Allograft.
Human application	Use of SoHO on or in a human recipient (being inserted, implanted, injected, infused, transfused, transplanted, ingested, transferred, inseminated or otherwise added to the human body) in order to create a biological interaction with that body.
Human error	A mistake made by a person rather than being caused by a poorly designed process or by the malfunctioning of a machine such as a computer.
Human Milk Bank	A facility established with the purpose of selecting, collecting, processing, storing and distributing human milk donated by lactating mothers who are not biologically related to the recipient infant, to meet the specific needs of individuals for whom donor human milk is prescribed by healthcare providers.
Human tissues and cells for human application	Material containing or consisting of human tissues and/or cells intended for implantation, transplantation, infusion or transfer into or onto a human recipient. *See also*: SoHO.
Hybrid scaffold	Scaffold obtained using different types of materials.
Hydroxyproline quantification	Hydroxyproline is a non-essential amino acid (proline derivative) which results from collagen acid hydrolysis.
Hypoglycaemia unawareness	A condition where an individual with diabetes does not experience or recognise the warning signs of low blood sugar (hypoglycaemia).
Identification of SoHO	The labelling of SoHO to uniquely designate their origin, use or destination. *See also*: Labelling.
Immune-privileged niche	A certain site of the human body able to tolerate the introduction of antigens without eliciting an inflammatory immune response.
Implantation (in the context of assisted reproductive technologies)	Attachment and subsequent penetration by the zona-free blastocyst (usually in the endometrium) that starts 5-7 days after fertilisation. *See also*: Transplantation.
Import	Activities carried out to bring SoHO into one country from a third country before their release. *See also*: Third countries.
Importing SoHO establishment	An establishment or a unit of a hospital or another body established within the EU which is a party to a contractual agreement with a third-country supplier for the import into the EU of SoHO coming from a third country and intended for human application.
Imputability	The likelihood that an adverse reaction, in a living SoHO donor, is associated with the collection process or that such a reaction, in a SoHO recipient or offspring from medically assisted reproduction, is associated with the human application of the SoHO.
Incident	A generic term for an adverse reaction or adverse event.
Incident reporting (adverse event reporting, serious/critical incident reporting)	A system in a healthcare organisation for collecting, reporting and documenting adverse occurrences that affect patients and are inconsistent with planned care (e.g. medication errors, equipment failures, violations).
Informed consent	A person's voluntary agreement, based upon adequate knowledge and understanding of relevant information, to donate, to participate in research or to undergo a diagnostic, therapeutic or preventive procedure.
Inner cell mass	A group of cells in the blastocyst that give rise to the embryonic structures: the fetus, the yolk sac, the allantois and the amnion.
In-process control	Checks undertaken during processing to monitor and, if necessary, to adjust the process to ensure that a product conforms to its specification. Control of the environment or equipment may also be regarded as a part of in-process control.
Inspection	On-site assessment of compliance with local/national regulations on tissues and cells, carried out by officials of the relevant Health Authority.

Installation qualification	The documented verification of the 2nd phase of qualification, ensuring that facilities, equipment and computerised systems used in critical processes by SoHO establishments are appropriately selected and installed in accordance with the design, the manufacturer's recommendations and/or user requirements.
Insulinopenic diabetes	A condition characterised by a deficiency in insulin production.
Intracytoplasmic sperm injection	A procedure in which a single spermatozoon is injected into the oocyte cytoplasm.
Intraportal infusion	Preferred method for the clinical infusion of hepatocytes, where the portal vein is typically accessed via percutaneous transhepatic catheterisation.
Intrasplenic administration	Alternative route to intraportal infusion considered for patients with liver cirrhosis.
Intra-uterine insemination	Procedure in which processed sperm cells are transferred transcervically into the uterine cavity.
In vitro fertilisation	Assisted reproductive technology procedure that involves extracorporeal fertilisation. It includes conventional *in vitro* insemination and ICSI, for which see: Intracytoplasmic sperm injection.
In vitro maturation	The maturation of oocytes recovered from non-pre-ovulatory follicles that may have been primed (or not) with exogenous gonadotrophins. Also, the *in vitro* process of maturation from immature dendritic cells (DC) to mature DC.
Islet equivalents (IEQ)	A standardised counting unit based on 150-micron islets, used to assess islet yield.
Islet infusion	The procedure of transplanting the isolated islets, typically into the hepatic parenchyma via the portal vein system.
Islets of Langerhans	Clusters of cells in the pancreas that are highly vascularised and range in size from less than 50 µm to about 800 µm in diameter.
Keratoplasty	Corneal transplantation.
Key performance indicator (KPI)	A quantifiable measure or a set of quantifiable measures used to trace critically significant performance over time.
Labelling	Includes steps taken to identify packaged material by attaching the appropriate information to the container or package so it is clearly visible on or through the immediate carton, receptacle or packaging. *See also*: Identification of tissues and cells.
Laminin	High-molecular-weight protein of the extracellular matrix with important roles in cell differentiation, migration and adhesion.
Laparoscopy	A surgical procedure in which a small incision is made through which a viewing tube (laparoscope) is inserted.
Limbal stem cells	The population of stem cells residing in the basal epithelium of the limbus, giving rise to the corneal epithelium.
Limbal tissue	Tissue bridging the junction between the cornea and sclera. Site of the limbal stem cells that renew the corneal epithelium. Limbal stem cell deficiency causes ocular surface disease.
Limbus	The area bridging the junction between the cornea and sclera.
Live birth rate	Delivery of one or more infants with any signs of life expressed per 100 initiated cycles, aspiration cycles or embryo-transfer cycles.
Living donor	A living person who has volunteered to a SoHO establishment, or has been presented by a person granting consent on their behalf, in accordance with national legislation, with a view to making a donation of SoHO, for the purpose of use in a person other than themselves, and other than *in situ*ations of within-relationship use in the setting of MAR.
Lyophilisation	A controlled freezing and dehydration process through the sublimation of water under vacuum from ice directly to vapour to a residual water content of < 5%. Typically used to preserve a non-viable perishable material or to make the material more convenient for transport. Also known as freeze drying.
Malignancy	Presence of cancerous cells or tumours with a tendency to metastasise, potentially resulting in death.
Manipulation	Preparation of retrieved tissues or cells to make them suitable for human application. In the context of processing of haematopoietic progenitor cells, this is a laboratory procedure that selectively removes, enriches, expands or functionally alters the cells.
Masson trichrome	Staining protocol used in histology for distinguishing cells from the surrounding connective tissue.
Materials	A collective term that encompasses reagents, instruments, medicines, implants and consumables used in various critical processes.
Measurement accuracy	Closeness of agreement between a measured quantity value and a true quantity value of a measurand. The concept 'measurement accuracy' is not a quantity and is not given a numerical quantity value. A measurement is said to be more accurate when it offers a smaller measurement error.
Measurement uncertainty	Non-negative parameter characterising the dispersion of the quantity values being attributed to a measurand.

Medically assisted reproduction (MAR)	Reproduction brought about through various interventions, procedures, surgeries and technologies to treat different forms of fertility impairment and infertility. These include ovulation induction, ovarian stimulation, ovulation triggering, all ART procedures, uterine transplantation and intra-uterine, intracervical and intravaginal insemination with semen of husband/partner or donor. *See also*: Assisted reproductive technology (ART).
Medicinal product	Any substance or combination of substances presented as having properties for treating or preventing disease in human beings, or which may be used in or administered to human beings with a view to either making a medical diagnosis, or restoring, correcting or modifying physiological functions by exerting a pharmacological, immunological or metabolic action.
Meiotic spindle	Spindle apparatus composed of microtubules that support and segregate chromosomes during meiotic division.
Metaphase II oocyte	Mature oocyte at the metaphase of the second meiotic division.
MHC II antigen	Class of major histocompatibility complex (MHC) molecules normally found only on antigen-presenting cells such as dendritic cells, mononuclear phagocytes, some endothelial cells, thymic epithelial cells and B cells.
Micromanipulation in ART	Technology that allows micro-operative procedures to be done on the spermatozoon, oocyte, zygote or pre-implantation embryo.
MTT	3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide is a colorimetric assay for assessing cell metabolic activity.
Musculoskeletal	Tissues that are part of the skeleton and muscular system, including muscles, bones, cartilage, tendons and ligaments, which function in the support and movement of the body.
Negative-to-date release	The release of tissues or cells for human application before completion of testing for bacterial or fungal cultures. The cultures are negative at the time of release.
Next of kin	A person's closest living blood relative or relatives.
Non-compliance	Failure to comply with accepted standards, requirements, rules or laws.
Non-partner donation	A donation of reproductive SoHO to be used for medically assisted reproduction in a SoHO recipient with whom the SoHO donor does not have an intimate physical relationship; also called 'third-party donation'.
Novelty	Any new SoHO preparation or change that could significantly affect the quality and/or safety of tissues and cells and/or the safety of recipients.
Oligozoospermia	Total concentration of spermatozoa $< 15 \times 10^6$/mL (low sperm count).
Oocyte	The female gamete (egg).
Oocyte cumulus complex	Oocyte surrounded by the granulosa and corona radiate cells.
Oocyte sharing	Refers to a female partner who enters MAR treatment and decides to donate a specified number of her retrieved oocytes. Also known as egg sharing.
Operational qualification	Documented verification of the 3rd phase of qualification where the performance of critical facilities, equipment and computerised systems is tested under normal operating conditions to ensure it meets predefined performance characteristics and acceptance criteria.
Opt-in donation	System for determining voluntary consent to donate where consent has been given by an individual during their lifetime or by an individual's family after their death. Also known as explicit or express consent.
Opt-out donation	System for determining voluntary consent to donate where donation may proceed unless an individual has expressed an objection during their lifetime. Also known as presumed or deemed consent.
Organ	Differentiated and vital part of the human body, formed by different tissues, that maintains its structure, vascularisation and capacity to develop physiological functions with a significant level of autonomy.
Organ culture	Culture of the whole or parts of an organ in medium *in vitro* to preserve cell–cell and cell–matrix interactions and to maintain structure and function.
Organisation responsible for human application (ORHA)	A healthcare establishment or unit of a hospital or another body that carries out human application of SoHO. In the EU, a SoHO entity.
Osteoconductivity	A capability of providing a scaffold that facilitates the growth of new bone on a surface or into pores or channels.
Osteoinductivity	A capability of inducing osteogenesis.
Ovarian hyperstimulation syndrome	An exaggerated systemic response to ovarian stimulation characterised by a wide spectrum of clinical and laboratory manifestations. It is classified as 'mild', 'moderate' or 'severe' according to the degree of abdominal distension, ovarian enlargement and respiratory, haemodynamic and metabolic complications.
Package insert	A document included in the packaging of a distributed tissue or cell product that includes important supplementary information for the end users on handling, storage, traceability and adverse outcome reporting and, in some cases, on the product's properties or characteristics.

Packaging	Packaging, including primary and secondary packaging, aims to protect tissues and cells and to present them to the operator (initial or in-process packaging) or to the clinical user (final packaging) in a suitable manner. Also refers to containers. *See also*: Primary packaging; Secondary packaging.
Packaging material	Any material employed in the packaging of tissues or cells, excluding any outer packaging used for transportation or shipment. Packaging materials are referred to as 'primary' or 'secondary' according to whether or not they are intended to be in direct contact with the product. Also refers to containers.
Partner donation	Donation of reproductive cells between a couple who declare that they have an intimate physical relationship. *See also*: Within-relationship use.
Peracetic acid	Organic compound used for the disinfection of medical supplies to prevent biofilm formation.
Percutaneous epididymal sperm aspiration	Sperm aspiration by percutaneous puncture of the epididymis by a fine-needle technique.
Performance qualification	Documented verification of the final phase of qualification during which the integrated systems (facilities, physical and computerised equipment) are tested to confirm their consistent and satisfactory performance of specified critical processes conducted under operational conditions in SoHO entities and establishments and that the finished product meets all release requirements.
Pericardium	A double-walled sac that contains the heart and the roots of the great vessels.
PicoGreen assay	Selective dsDNA quantification of as little as 25 pg/mL.
Placenta	An organ that connects the developing fetus to the uterine wall to allow nutrient uptake, waste elimination and gas exchange via the mother's blood supply.
Plasma dilution	*See*: Haemodilution.
Polar body	A haploid cell with very little cytoplasm that is formed and is separated from the oocyte during meiosis and that contains a nucleus produced in the first or second meiotic division.
Pooling	Physical contact between, or any process that could result in cross-contamination between, SoHO from more than one collection from the same donor, or from two or more donors.
Portal vein	The blood vessel that carries blood from the digestive organs to the liver, which is the standard implantation site for islet infusion.
Posthumous donation	The donation of tissue or cells after the donor's death with prior written consent of the donor.
Pre-implantation genetic testing (PGT)	A test performed to analyse the DNA from oocytes (polar bodies) or embryos (cleavage stage or blastocyst) for HLA-typing or for determining genetic abnormalities. Such tests include: PGT for aneuploidies (PGT-A); PGT for monogenic/single gene defects (PGT-M); and PGT for chromosomal structural rearrangements (PGT-SR).
Preservation	Use of chemical agents, alterations in environmental conditions or other means during processing to prevent or retard biological or physical deterioration of tissues or cells.
Primary packaging	Any material employed in the packaging of tissues and cells that is intended to be in direct contact with the graft, excluding any outer packaging used for transportation or shipment. Also refers to containers.
Procedures	Description of all the tasks, operations and processes to be carried out, the precautions to be taken and the measures to be applied to ensure the quality and safety of tissues and cells from procurement through processing, testing and storage to human application.
Processing	Any operation involved in the handling of SoHO, including (but not limited to) washing, shaping, separation, decontamination, sterilisation, preservation and packaging, except for preparatory handling of SoHO for immediate human application during surgical intervention without the SoHO being removed from the surgical field before they are applied. (Regulation EU 2024/1938).
Proliferation	Rapid reproduction of a cell.
Pronucleus	The nucleus of the sperm or the oocytes during the process of fertilisation, after the sperm has entered the oocytes but before they fuse.
Prophase I oocyte	Immature oocyte at the prophase of the first meiotic division.
Propidium iodide	Fluorescent intercalating agent used to evaluate cell viability or DNA content in cell cycle analysis.
Pyrogen	A substance, typically produced by a bacterium, which induces fever when introduced or released into the blood.
Pyrogenic	Producing or produced by heat or fever.
Qualification	The process of demonstrating that personnel, facilities, equipment, materials and computerised systems meet specified requirements and perform reliably under intended conditions.
Quality	Fulfilment of a specific set of standards, characteristics and requirements.
Quality assurance	The actions planned and performed to provide confidence that all systems and elements that influence the quality of the product are working as expected, both individually and collectively.

Quality control	The part of quality management that is focused on fulfilling quality requirements. In terms of preparation, it concerns sampling specifications and testing; for an organisation, it relates to documentation and release procedures, which together ensure that the necessary and relevant tests have actually been carried out and that materials have not been released for use until their quality has been judged to be satisfactory.
Quality improvement	The actions planned and performed to develop a system to review and improve the quality of a product or process.
Quality management system	The organisational structure, with defined responsibilities, procedures, processes and resources, for implementing quality management, including all activities that contribute to quality, directly or indirectly.
Quarantine	The initial status of collected SoHO while awaiting a decision on their acceptance or rejection, or of tissues or cells isolated physically or by other effective means from other donated material for other reasons until their suitability for use is established.
Randomised control trial	A study in which samples or subjects are allocated at random into groups, called the 'study' and 'control' groups, to receive or not receive an experimental therapeutic intervention.
Rapid alert	An urgent communication to relevant individuals/organisations to ensure the protection of donors or recipients when an unexpected risk has been identified.
Raw donated human milk (DHM)	DHM which has not undergone processing (heat treatment or other antimicrobial treatments); raw DHM can be refrigerated DHM at +2° to +8°C or frozen DHM at temperature < –15°C.
Recall	Removal from use of specific stored or distributed tissues and cells that are suspected or known to be potentially harmful. *See also*: Return; Withdrawal.
Recipient	The person to whom SoHO are applied or for whom the human application of SoHO is envisaged, whether by allogeneic, autologous or within-relationship use in the setting of MAR.
Recovery	*See*: Collection.
Registry	A repository of data collected on SoHO donors and/or recipients for the purpose of audit, clinical outcome assessment, quality assurance, validation, healthcare organisation and planning, research and surveillance
Regulatory authority	*See*: Health Authority.
Release	A process through which it is verified that a SoHO meets defined quality and safety criteria and fulfils the conditions of any applicable authorisation, before distribution or export.
Remodelling	Change of the micrometric structure.
Reproductive cells	Oocytes and spermatozoa – in this Guide, those oocytes and spermatozoa collected to be used for the purpose of assisted reproduction or fertility preservation. It also includes embryos.
Resazurin	Indicator of cell viability based on oxidation-reduction reactions.
Resistogram	The result of a test for the sensitivity of an isolated bacterial strain to different antibiotics. Also known as an antibiogram.
Retrieval	*See*: Collection.
Return	Sending back recalled SoHO to the SoHO establishment that supplied them for human application.
Risk assessment	Identification of potential hazards with an estimation of the likelihood that they will cause harm and of the severity of the harm if it did occur.
Risk mapping	Risk mapping is a way of representing and prioritising the risks of an organisation. It enables a diagnosis of the vulnerabilities of a system, an organisation or a process (major risks) to be established and to objectively assist in decision-making by ranking the risks according to their criticality. Finally, it enables the implementation of actions to manage the risk.
Room temperature	In temperature-controlled facilities, room temperature ranges from a minimum of +15°C to a maximum of +25°C.
Root cause analysis	A structured approach to investigating and identifying the factors that resulted in a harmful or potentially harmful outcome, with its nature, magnitude, location and timing.
Roughness	Quality or state of having an uneven or irregular surface.
Scaffold	A chemical structure made using biological scaffolding.
Sclera	Fibrous white outer coat of the eye.
Secondary packaging	Any material employed in the packaging of tissues and cells that is not intended to be in direct contact with the graft, and excluding any outer packaging used for transportation or shipment. Also refers to containers.
Semen analysis	A description of the ejaculate to assess function of the male reproductive tract. Characteristic parameters may include volume and pH, the concentration, motility, vitality and morphology of spermatozoa, and the presence of other cells.
Septicaemia	A systemic disease caused by the spread of pathogenic micro-organisms or their toxins via the circulating blood.

Serious adverse event	Any incident or error that poses a risk of any of the following: (a) harm to a living SoHO donor, to a SoHO recipient or to offspring from medically assisted reproduction; (b) a defect detected in one SoHO entity that would have implications in that entity or another for SoHO recipients or SoHO donors because of shared practices, services, supplies or critical equipment; (c) loss of a quantity of SoHO that causes human applications to be postponed or cancelled; (d) loss of highly matched SoHO or of SoHO for autologous use; (e) a mix-up of reproductive SoHO in such a way that an oocyte is fertilised with sperm from a person other than the intended person, or reproductive SoHO are applied to a SoHO recipient other than the intended SoHO recipient; (f) inappropriate SoHO distribution; (g) loss of traceability of SoHO.
Serious adverse reaction	An adverse reaction that results in any of the following: (a) death; (b) life-threatening, disabling or incapacitating condition, including transmission of a pathogen or of a toxic substance that might cause such a condition; (c) transmission of a genetic disorder that: (i) in the case of medically assisted reproduction with third-party donation, resulted in pregnancy loss or might result in a life-threatening, disabling or incapacitating condition in the offspring from medically assisted reproduction; or (ii) in the case of medically assisted reproduction in the context of within-relationship use, resulted in pregnancy loss or might result in a life-threatening, disabling or incapacitating condition in the offspring from medically assisted reproduction, due to a pre-implantation genetic test error; (d) hospitalisation or prolongation of hospitalisation; (e) the need for a major clinical intervention to prevent or reduce the effects of any of the results referred to in points (a) to (d); (f) prolonged sub-optimal health of a SoHO donor following single or multiple SoHO donations.
Seriousness	The degree of severity of an adverse reaction, involving harm to a living SoHO donor, a SoHO recipient or offspring from medically assisted reproduction, or for public health in general, or the degree of severity of an adverse event involving a risk of such harm.
Shipment	A type of transport where the transfer of SoHO from the distributing to the receiving facilities is carried out by means of a contract with a third party, usually a specialised logistics company.
Skin	Thin layer of tissue forming the natural outer covering of the human body. Skin is composed of two primary layers: the epidermis and dermis. These layers are separated by a thin sheet of fibres, the basement membrane. Keratinocytes constitute 95% of the epidermis. The dermis provides tensile strength and elasticity to the skin through an extracellular matrix composed of collagen fibrils, microfibrils and elastic fibres, embedded in proteoglycans.
SoHO (substances of human origin)	[In the EU,] any substance collected from the human body, whether it contains cells or not and whether those cells are living or not, including SoHO preparations resulting from the processing of such substances.
SoHO activities	Donor registration, donor history review, medical examination, testing, collection, processing, quality control, storage, release, distribution, import, export, human application to the patient and clinical outcome registration.
SoHO clinical study	An experimental evaluation of a SoHO preparation, with the objective of drawing conclusions regarding its safety and effectiveness.
SoHO entity	An entity (legally established in the EU) that carries out one or more of the SoHO activities: donor registration, donor history review and medical examination, testing, collection, processing, quality control, storage, release, distribution, import, export, human application and clinical-outcome registration.
SoHO establishment	A SoHO entity that carries out any of the following SoHO activities: (a) both processing and storage; (b) release; (c) import; (d) export.
SoHO preparation	A type of SoHO that: (a) has been subjected to processing and, where relevant, one or more other SoHO; (b) has a specific clinical indication; and (c) is intended for human application to a recipient or is intended for distribution. *See also*: SoHO preparation authorisation.
SoHO preparation authorisation	The formal approval by a SoHO competent authority (in the EU) of a SoHO preparation.
Somatic cells	Any cell of a living organism other than the reproductive.
Spermatozoon	The mature male reproductive cell.
Split-thickness skin graft (STSG)	Graft composed of epidermis and partial-thickness dermis.
Sporicidal	Refers to a substance, agent or product used for killing bacterial spores.
Standard operating procedure	Written instructions describing the steps in a specific process, including the materials and methods to be used and the expected result. *See also*: Procedures.

Starting material	Any collected SoHO that will constitute an integral part of an advanced therapy medicinal product active substance.
Static cold storage	Method of preserving the pancreas at 4°C to slow metabolic and enzymatic activity.
Sterilisation	Any process that eliminates or inactivates transmissible infectious agents (pathogens) containing nucleic acids, e.g. vegetative and spore forms of bacteria and fungi, parasites or viruses, present on a surface, in a fluid, in medication or in a compound such as biological culture media. Sterilisation can be achieved by applying the proper combinations or conditions of heat, chemicals, irradiation, high pressure and filtration.
Sterility assurance level (SAL)	The expected probability of a micro-organism surviving on an individual unit of product after exposure to a sterilisation process. SAL 10^{-6} has been established as the standard for allografts and indicates a probability of one chance in a million that one unit of product will be contaminated with a single organism after a sterilisation process. If the product meets or exceeds this standard, it is then considered sterile.
Storage	The maintenance of SoHO under appropriate controlled conditions until distribution.
Storage temperature	The temperature at which SoHO must be stored to maintain their required properties.
Supercritical carbon dioxide	Fluid state of carbon dioxide where it is held at or above its critical temperature and critical pressure. It is an alternative for terminal sterilisation of biological materials and medical devices in combination with peracetic acid.
Supernumerary embryos	Excess embryos after embryo transfer.
Surveillance	Systematic collection, collation and analysis of data for public health purposes and the timely dissemination of public health information for assessment and public health responses, as necessary.
Sustained virological response (SVR)	Undetectable HCV RNA in blood at 12 weeks (SVR12) and 24 weeks (SVR24) after the end of therapy, assessed by HCV RNA nucleic acid test (NAT) with a LOD ≤15 international units (IU) per mL.
Swim up	A preparation technique based on the ability of spermatozoa to swim in the culture medium.
TCID50	The concentration of virus required to infect 50% of cells in a monolayer.
Tendon	A tough band of fibrous connective tissue that usually connects muscle to bone and which can withstand tension.
Terminal sterilisation	A method for achieving the sterility of a product in its sealed container and with a sterility assurance level of 10^{-6} or better.
Testicular sperm extraction/ aspiration	A surgical procedure involving testicular biopsies or needle aspirations to obtain sperm for use in IVF and/or ICSI.
Third countries	Term used within the EU to refer to countries that are not members of the EU.
Third-country supplier	An organisation, located outside the EU, which is contracted to supply SoHO or to perform activities that might influence the quality and safety of the SoHO imported.
Third party	Any organisation that provides a service to a SoHO entity or establishment on the basis of a contract or written agreement.
Third-party donation	A donation of reproductive SoHO to be used for medically assisted reproduction in a SoHO recipient with whom the SoHO donor does not have an intimate physical relationship. *See:* non-partner donation.
Time-lapse imaging	The photographic recording of microscope image sequences. In this Guide, used for documentation of gametes, zygotes, cleavage-stage embryos or blastocysts at regular intervals.
Tissue	An aggregate of cells joined together by, for example, connective structures and performing a particular function.
Tissue and cell activities	*See*: SoHO activities.
Tissue bank	*See*: SoHO establishment.
Toxicity	The degree to which a substance can damage an organism.
Traceability	Ability to locate and identify a specific tissue/cell during any step from collection, through processing, testing and storage, to distribution to the recipient or disposal. This implies the ability to identify: the donor; the SoHO establishment or processing facility that receives, processes or stores the tissue and cells; and the recipient(s) at the medical facility/facilities applying the tissues and cells to the recipient(s). Traceability also covers the ability to locate and identify all relevant data relating to products and materials coming into contact with those tissues and cells.
Transmissible communicable disease	Infection (caused by pathogens such as HIV, HBV or HCV) that is transmissible from an infected SoHO donor to SoHO recipients through SoHO preparations.
Transmissible disease	Comprises all clinically evident illnesses (i.e. characteristic medical signs and/ or symptoms of disease) resulting from the infection, presence and growth of micro-organisms in an individual or the transmission of genetic conditions to the offspring. In the context of transplantation, malignancies and autoimmune diseases may also be transmitted from donor to recipient.
Transplantation, implantation or grafting	Transfer (engraftment) of human tissues or cells from a donor to a recipient with the aim of restoring function(s) in the body. *See also*: Implantation (in the context of assisted reproductive technologies).

Transport	The act of transferring SoHO from one facility to another, either within one SoHO entity or between SoHO entities, within defined conditions that maintain the SoHO properties.
Trophectoderm	Outer layer of cells in a blastocyst (composed of trophectoderm and inner cell mass cells). A group of cells in the blastocyst that do not produce any embryonic structures but give rise to the chorion, the embryonic portion of the placenta.
Trypan blue exclusion test	Primary test used to assess cell viability of hepatocytes before transplantation.
Unique identification code	A code that unambiguously identifies a particular donor and donation (e.g. a unique donation + tissue product code). *See also*: Coding.
Validation	Documented evidence giving a high degree of assurance that a specific process or system, including pieces of equipment or the environmental conditions, will perform consistently to deliver a product meeting its pre-determined specifications and quality attributes, based on intended use. or Confirmation, through the provision of objective evidence, that the requirements for a specific intended use or application have been fulfilled (ISO9000:2015).
Vas deferens (*plural* vasa deferentia)	Tube(s) that transport(s) sperm from the epididymis to the ejaculatory ducts.
Verification	Preferred term for the validation or qualification of IT systems/software. or Confirmation, through the provision of objective evidence, that specified requirements have been fulfilled (ISO9000:2015).
Vigilance	Alertness to and/or awareness of serious adverse events, serious adverse reactions or complications related to donation and human application of SoHO, involving an established process for reporting at local, regional, national or international level. or A set of organised surveillance and reporting procedures relating to adverse reactions and adverse events. *See also*: Surveillance.
Viraemia	The presence of viruses in the blood.
Vitrification	Method of ice-free cryopreservation achieved through an extreme elevation in solution viscosity sufficient to suppress the crystallisation of water. Requires rapid cooling and/or high concentrations of solutes, such as the conventional cryoprotectants, to reach the glass transition temperature without ice formation.
Wettability	Tendency of one fluid to spread on, or adhere to, a solid surface in the presence of other immiscible fluids. Wettability refers to the interaction between fluid and solid phases.
Window period	Period of time before infection can be detected by a specific testing method.
Withdrawal	Process instigated by a SoHO establishment to recall tissues or cells that have been distributed.
Within-relationship use	The use of reproductive SoHO for medically assisted reproduction between persons with an intimate physical relationship. *See also*: Partner donation.
Xenograft	Graft of tissue taken from a donor of one species and grafted into a recipient of another species.
Xenotransplant-ation	Any procedure that involves the transplantation, implantation or infusion into a human recipient of either (a) live tissues, cells or organs from a non-human animal source, or (b) human body fluids, tissues, cells or organs that have had *ex vivo* contact with live non-human animal cells, tissues or organs.
Zygote	A diploid cell resulting from the fertilisation of an oocyte by a spermatozoon, before completion of the first mitotic division.

Appendix 4. **Example of cleanroom qualification**

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Short description of equipment or process being validated.

Initial qualification of clean rooms for use in regulated environments

This VAL script is for the initial qualification of a new cleanroom; qualification of a cleanroom following repair/refurbishment is detailed in VAL848

Details of equipment used in the validation.

An active Environmental Monitoring System (EMS)

Settle plates

Contact / air sampling plates

Particle counter

Active air sampler

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Details of testing levels and methods used in validation
Eudralex Volume 4 "The Rules Governing Medicinal Products in the European Union" Annex 1 Manufacture of Sterile Medicinal Products
SOP254 – Environmental Monitoring using Contact Plates
SOP975 – Environmental Monitoring using Active Air Sampling
SOP978 – Environmental Monitoring using Settle Plates
SOP2382 – Environmental Monitoring Equipment
MPD382 – National Environmental Monitoring System for Controlled Rooms
MPD1358 – HACCP Study Protocol and Plan
MPD1360 – Establishment of an Environmental Monitoring Programme
MPD1361 – Handling Out-of-Specification Environmental Monitoring Results
SOP5497 – Guidance on How to Implement and Document HACCP Study Outcomes
SOP5051 – Using the MiniColt 4 for Undertaking Cleanroom Smoke Tests
SOP6756 – Using the AFM Cube Cleanroom Fogger for Undertaking Cleanroom Smoke Studies
ISO14644 – BSEN14644 and EU GMP Cleanroom Standards

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INSTALLATION QUALIFICATION

No	Description	Acceptance Criteria	Results	Pass/ Fail	Comments	Signature & Date
1.	Clean room designed in accordance with required operating specifications	Appropriate specifications available and clean room designed to meet specifications. Current drawings for clean room layout and air handling unit(s) are available.				
2.	Clean room layout, fixtures and finishes are installed according to the current drawings and are of an appropriate standard.	Clean room finishes are smooth, impervious, non-shedding and crack and crevice free. Floor to wall, wall to wall and wall to ceiling junctions are coved and finished in vinyl and are defect free. All wall and ceiling penetrations are fully sealed with silicone sealant and are defect free. Light fittings and filter housings are surface mounted and are fully sealed with silicone sealant and are defect free. There are no un-cleanable recesses and minimal projecting ledges, shelves, cupboards and equipment. Fixtures, fittings and clean room furniture are all present, secure and free of rust and defects. Clean room entry / exit doors and pass-through hatch doors are interlocked or otherwise controlled to prevent both doors being opened simultaneously.				

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3.	Confirm access to the EMS system data is available	Records must be accessible during the validation process				
4.	Confirm that particle counters and differential pressure monitoring systems are calibrated and available	In date calibration certificates must be available and equipment free for use during the entire validation period				
5.	Ensure clean room and associated air handling unit(s) is registered as an asset in QPulse	QPulse asset number must be generated				
6.	QA Approval	QA approval of completed IQ stage				

OPERATIONAL QUALIFICATION

No	Description	Acceptance Criteria	Results	Pass/ Fail	Comments	Signature & Date
1.	Particle Challenge Leak Test for testing of each installed HEPA filter using DOP	External contractor to perform DOP testing of facilities in accordance with ISO 14644-3. Aerosol concentrations must be ≤ 0.01% of the upstream concentration				
2.	Air exchange rate testing. Tested in accordance with BSEN 14644-3-2005	External contractor to measure airflow volume or airflow velocity. Air change rate in compliance with design specification				
3.	Particle Counting for classification of the clean room – at rest	External contractor to perform particle counting in clean room to meet EU GMP Annex 1 “at rest” limits for particulates (working to ISO 14644-1)				
4.	Particle Counting for classification of the clean room – in operation	External contractor to perform particle counting in clean room to meet EU GMP Annex 1 “in operation” limits for particulates (working to ISO 14644-1)				

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5.	Air flow distribution testing using smoke visualisation	External contractor to perform smoke visualisation test or follow SOP5051/SOP6756 in accordance with ISO14644-3 demonstrating: • Flow distribution is satisfactory within each room • Any dead spots within each room have been identified • No areas of excessive turbulence below working height exist (that could lead to particulate contamination)				
6.	Determine environmental monitoring locations	Locations for EM have been determined and support the findings of the smoke visualisation studies				
7.	Perform deep clean of the clean room(s)	Documented clean by either an external third party or NHSBT via a documented methodology	Date of clean:			
8.	Perform baseline environmental monitoring of the clean rooms as per the requirements of MPD1360	Determine the baseline microflora present within the clean room prior to operational use	Date of baseline monitoring exercise:			
9.	Perform three routine cleans of the clean room	Clean room cleaning must be easy to facilitate and unobstructed	Date of first clean: Date of second clean: Date of third clean:			

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10.	Perform dynamic no-manufacture environmental monitoring as per MPD1360. For both particulate counts and viable monitoring.	Full set of plates must be exposed and results shown to not exceed EU GMP Annex 1 limits for microbial contamination (see MPD382) Full set of counts must be obtained in accordance with MPD382 and checked for compliance with EU GMP Annex 1 “at rest” limits. Identification of any organisms detected in grade A and B areas is required, identification of any organisms detected in grade C and D areas is only required if action/alert levels for the area are breached as MPD1361.	Date of first monitoring exercise: Date of first clean: Date of second monitoring exercise: Date of second clean: Date of third monitoring exercise: Date of third clean:			
11.	Record the differential pressures for clean room facilities during dynamic no-manufacture environmental monitoring	Daily records must be obtained for differential pressures, and compliance with the design specification and EU GMP confirmed (10-15 Pa between adjacent rooms of different grades at rest).				

12.	Perform dynamic manufacture environmental monitoring as per MPD1360. For both particulate counts and viable monitoring.	Full set of plates must be exposed whilst non-clinical clean room work is performed, and results shown not to exceed EU GMP Annex 1 limits for microbial contamination (see MPD382) Full set of counts must be obtained whilst non-clinical clean room work is performed and checked to ensure compliance with EU GMP Annex 1 "in operation" limits (see MPD382) Identification of any organisms detected in grade A and B areas is required, identification of any organisms detected in grade C and D areas is only required if action/alert levels for the area are breached as MPD1361.	Date of first monitoring exercise: Date of first clean: Date of second monitoring exercise: Date of second clean: Date of third monitoring exercise: Date of third clean:			
13.	Record the differential pressures for clean room facilities dynamic manufacture environmental monitoring.	Records must be obtained for differential pressures, and compliance with the design specification and EU GMP confirmed (10-15 Pa between adjacent rooms of different grades during working).				
14.	Review data for non-dynamic manufacture environmental monitoring and dynamic manufacture environmental monitoring	Results should not highlight any problems or trends. All results must not exceed the upper limits for the relevant grades of room, in compliance with EU GMP Annex 1 (see MPD382)				
15.	At rest recovery rate monitoring within stipulated limits - this test is not required for grade D cleanrooms due to the high concentration of particles required for testing	Particulate air recovery/cleanup rate must be achieved within 20 minutes in the "at rest" state and after operators/working have left the room (and after simulated operation tests).				

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16.	QA Approval	QA approval of completed OQ stage				

PERFORMANCE QUALIFICATION

No	Description	Acceptance Criteria	Results	Pass/ Fail	Comments	Signature & Date
1.	HACCP study undertaken as SOP5485	Perform HACCP study. Follow SOP5497 for interpretation of results. **Note**: If the clean rooms are being qualified prior to product work streams being identified a deviation may be raised to mitigate non completion of this step. The HACCP study must be performed during set up of product processing in the clean room.				
2.	Document operational go live date	All other validation activities completed.	Go live date:			
3.	Perform a weekly clean of the clean room for a minimum of an 8 week period.	Cleaning is performed and documented on local forms				
4.	Perform routine viable environmental monitoring as per MPD382 and monitor for at least 13 months to capture seasonal trends	Routine monitoring should be performed, results examined for trends including seasonal trends as MPD1262 and no adverse trends detected.				
5.	Perform particle counting monitoring as per MPD382 and monitor for at least 13 months to capture seasonal trends	Routine monitoring should be performed, results examined for trends including seasonal trends as MPD1262 and no adverse trends detected.				
6.	Record the differential pressures for clean room facilities for a minimum of 8 weeks of continued monitoring.	Records must be obtained for differential pressures for each day that environmental monitoring is performed and checked for compliance with the design specification and EU GMP (10-15 Pa between adjacent rooms of different grades.				

7.	Review results and data. Identify any issues and trends	Results should not highlight any problems or trends. All results must not exceed the action limits for the relevant grades of room, in compliance with EU GMP Annex 1.				
8.	QA Approval	QA approval of completed PQ stage				

NOTE: Each Validation Phase must be signed off before commencing the next phase of testing and before go-live

Deviations and Adverse Events

QPulse no.	Details	Date raised	Date closed

FURTHER TESTING DETAILS: (If applicable)

Appendix 5. **Examples of operational qualification protocols**

This appendix provides two examples of how the operational qualification (OQ) of equipment may be performed. Before performing operational qualification, Substances of Human Origin (SoHO) establishments or entities usually establish which operating parameters, and parameter characteristics, are relevant to the safety and efficacy of the tissue or cell preparations (TCPs) that the equipment will be used to process, transport or store, and for each parameter and parameter characteristic. They also establish acceptable ranges, limits or other criteria. It is important that the parameters selected can be measured using clear and unambiguous metrics.

When measuring specific parameters, it is best practice to keep other parameters constant to ensure the consistency of results. The operating parameters and acceptable ranges used in these examples are given as suggestions and are not intended to be definitive for the equipment in question.

Operational qualification tests for CO_2 incubators

In this example, three relevant operating parameters (temperature, CO_2 concentration and humidity) have been selected; and for each parameter two characteristics (uniformity and recovery) have been identified.

It is also good practice to ensure that the tests are performed under simulated real-life conditions; for example, the presence of objects within the incubator may impact both uniformity and recovery studies, and consideration is given to repeating the qualification with a simulated minimum and maximum load.

Uniformity tests can also provide helpful mapping data to inform where best to place the independent sensors that will be used to monitor the different parameters during routine use.

Temperature uniformity test

- **Purpose**: To verify that the CO_2 incubator maintains a uniform temperature across different areas inside the chamber.
- **Procedure**: Place multiple calibrated temperature probes at various locations within the incubator (e.g. corners, centre, top and bottom shelves). Set the incubator to a specified temperature, such as 37°C, and allow it to stabilise. Record the temperature readings at regular intervals (e.g. every minute) for a defined period (e.g. 24 hours).
- **Acceptance criteria**: The temperature variation across all probe locations falls within the specified range (e.g. ±0.5°C from the set point).

CO_2 concentration uniformity test

- **Purpose**: To ensure that the CO_2 concentration is consistent throughout the incubator chamber.
- **Procedure**: Similar to the temperature test, place calibrated CO_2 sensors at different locations inside the incubator. Set the CO_2 level to a specific value, such as 5%, and allow the incubator to stabilise. Measure the CO_2 concentration at each sensor location over a set period.
- **Acceptance criteria**: The CO_2 concentration remains within the defined range (e.g. ±0.2%) across all probe locations.

CO_2 recovery time test

- **Purpose**: To verify how quickly the incubator can return to the set CO_2 concentration after the door has been opened and closed.
- **Procedure**: Briefly open the incubator door for a specified duration (e.g. 1 minute) to simulate routine access. Close the door and start measuring

the time taken for the CO_2 concentration to return to the set level (e.g. 5%).
- **Acceptance criteria**: The CO_2 concentration returns to the set level within a predefined time frame (e.g., within 10 minutes).

Temperature recovery time test

- **Purpose**: To verify how quickly the incubator can return to the set temperature after the door has been opened and closed.
- **Procedure**: Briefly open the incubator door for a specified duration (e.g. 1 minute) to simulate routine access. Close the door and start measuring the time taken for the temperature to return to the set level (e.g. 37°C).
- **Acceptance criteria**: The temperature returns to the set level within a predefined time frame (e.g. within 10 minutes).

Humidity level verification

- **Purpose**: To confirm that the incubator maintains the appropriate humidity level for cell culture conditions.
- **Procedure**: Place a calibrated humidity sensor inside the incubator. Fill the water reservoir according to the manufacturer's instructions and allow the incubator to stabilise. Measure and record the humidity level over a specified period.
- **Acceptance criteria**: The humidity level remains within the acceptable range for cell culture (e.g. 90-95% relative humidity).

Humidity recovery time test

- Purpose: To verify how quickly the incubator can return to the set humidity after the door has been opened and closed.
- Procedure: Briefly open the incubator door for a specified duration (e.g. 1 minute) to simulate routine access. Close the door and start measuring the time taken for the humidity to return to the set level (e.g. 90-95% rH).
- Acceptance criteria: The humidity returns to the set level within a predefined time frame (e.g. within 30 minutes).

Other considerations regarding temperature monitoring sensors

- Where the intended use of the sensor is known, it is good practice to focus the sensor calibration over the specific range of temperatures that it would be expected to monitor.
- Where sensors are located in very low (or high) temperature environments, they may be subject to very rapid and large temperature changes during routine use, for example when the door of an ultra-low temperature freezer is opened. In these cases, monitoring of the ambient temperature may not provide an accurate reflection of items stored in the location, and the use of a heat-sink around the sensor is considered, to buffer rapid temperature changes and prevent the generation of multiple false alarms. Heat sinks mimic as closely as possible the minimum size, shape and thermal mass of the smallest of the items kept in the monitoring location. Typically, they may be made from machined aluminium blocks, or more simply containers of glycerol.
- Alternatively, consideration is given to setting an alarm delay (if the system has this capacity) so that an alarm is not generated until the temperature has been continually out of specification for a defined period.

Operational qualification tests for a temperature monitoring system

The second example in this appendix looks at qualification of an independent temperature monitoring system. These can vary in scope from single sensors used to monitor a remote refrigerator or freezer, to multiple sensors monitoring multiple locations, or they can even be integrated into a larger environmental monitoring system, including different types of sensors. However, the core principles are the same.

Sensor accuracy verification

- **Purpose**: To confirm that the temperature sensors within the monitoring system accurately measure the temperature.
- **Procedure**: Place each sensor in a controlled temperature environment, such as a calibrated temperature bath or a dry block calibrator. Set the temperature to various points across the expected operating range (e.g. −20°C, 0°C, 25°C and 37°C). Compare the readings from the temperature sensors to those of a calibrated reference thermometer at each set point.
- **Acceptance criteria**: The temperature readings from the system's sensors are within a specified tolerance (e.g. ± 0.5°C) of the reference thermometer.

System response time test

- **Purpose**: To verify the response time of the temperature monitoring system when there is a change in temperature.
- **Procedure**: Subject the sensors to a rapid temperature change by moving them from one controlled temperature environment to another (e.g. from a 25°C room to a 5°C refrigerator). Measure the time taken for the monitoring system to detect and report the change in temperature.
- **Acceptance criteria**: The system detects and records the temperature change within a specified response time (e.g. within 2 minutes).

Alarm functionality test

- **Purpose**: To ensure that the monitoring system correctly triggers alarms when temperatures fall outside the predefined acceptable range.
- **Procedure**: Set up the system with alarm thresholds (e.g. 2°C to 8°C for a cold storage area). Manually manipulate the environment to exceed these thresholds by either heating or cooling the area. Observe whether the system generates alarms (visual, auditory or digital alerts) when the temperature crosses the set limits.
- **Acceptance criteria**: Alarms activate immediately upon the temperature breaching the set thresholds and deactivate once the temperature returns to the acceptable range.

Data logging and reporting verification

- **Purpose**: To confirm that the temperature monitoring system accurately logs and reports temperature data over time.
- **Procedure**: Operate the system in a controlled environment over a set period (e.g. 24 hours) and allow it to record temperature data. After the monitoring period, review the data logs to ensure that all readings are recorded at the specified intervals (e.g. every 5 minutes) and that the system generates accurate reports.
- **Acceptance criteria**: The recorded data accurately reflect the temperature conditions over the monitoring period, with no missing or incorrect entries. Reports generated include all relevant information, such as timestamps, sensor locations and recorded temperatures.

Power failure and recovery test

- **Purpose**: To test the system's ability to recover and maintain data integrity after a power failure.
- **Procedure**: Simulate a power outage by disconnecting the power supply to the monitoring system. After a brief period, restore power and observe whether the system resumes normal operation. Check if the system has maintained data integrity and logs during the outage and verify if it alerts users to the power failure.
- **Acceptance criteria**: The system automatically resumes operation after power is restored, retains all recorded data and provides a notification of the power failure event.

User role verification test

- **Purpose**: To ensure that the temperature monitoring software correctly enforces user roles and permissions, allowing users to access only the functions and data appropriate to their assigned roles. Identify the different user roles within the software, such as Administrator, Manager, Technician and Viewer. List the specific permissions associated with each role, such as access to temperature data and ability to edit settings, generate reports, acknowledge alarms and manage user accounts.
- **Procedure**: Set up test accounts for each user role within the software. Assign the appropriate permissions according to the role definitions.
- **Login and access verification**: Log in with different roles. Verify access to all areas of the software, including the ability to manage user accounts, modify system settings, generate and delete reports and acknowledge alarms. Perform several actions within the system (e.g. acknowledging an alarm, changing settings) while logged in under different user roles. Review the audit trail to verify that all actions are correctly logged with the appropriate user role, timestamp and action details.
- **Acceptance criteria**: Each user has access only to the functions and data specified in their role definition. The software correctly restricts access to unauthorised actions, providing appropriate notifications or errors. The audit trail accurately records all actions with the correct user role, timestamp and details. Role changes are reflected immediately, with permissions updated accordingly.

Appendix 6. Example of process validation – SoHO transportation

The example of a process validation outlined below describes a process that will be common to most, if not all, Substances of Human Origin (SoHO) entities or establishments. That is, the need to transport SoHO from one place to another (for example, from the site of collection to the processing facility or from the SoHO establishment to the end user or SoHO entity). Control of the conditions of transport is critical for ensuring tissue quality.

The example below refers specifically to the transport of skin allografts from the collection site to a SoHO establishment at refrigerated temperatures. However, the principles are identical for all types of transport.

The first stage was to define the process in detail. This was achieved by addressing the following questions:

- Which type of tissue and what maximum volume will be transported?
- How is the tissue contained? What is the nature, volume and temperature of any transport solution to be used? Which type of packaging has been used?
- Which refrigerant has been used and what is its specification and volume?
- What are the specifications of the transport container (i.e. dimensions, insulation etc.)?
- What are the most extreme transport conditions allowable in terms of transport time and ambient temperature?

Once the process had been defined, the acceptance criteria needed to be defined. In our example, they were that the:

- temperature of the skin allograft has to remain at 0-10°C for the duration of the transit;
- integrity of the tissue packaging has to be maintained during transit;
- integrity of the transport container has to be maintained during transit;
- pH of the transportation fluid has to be 7.0-7.5 at the end of the transport operation.

For some tissues, it may be advisable to go further and validate the quality of the tissue after transit (e.g. by assessment of its viability or histological structure).

It was determined that the maximum amount of skin that would be transported would be 6 000 cm^2, immersed in a minimum volume of 300 mL of transport fluid. Specifications of the packaging, transport container and refrigerant were also documented. The most extreme acceptable transport conditions were defined as an ambient temperature of 40°C (e.g. a hot summer day in a vehicle) for a maximum of 12 h, with the minimum volume of refrigerant and transport solution, and the maximum volume of tissue.

A protocol was written and a model prepared using skin obtained from donors unsuitable for clinical donation. This protocol was based on the defined transport solution, refrigerant, packaging and container specifications. A calibrated data-logging thermometer was used to record the temperature on the external surface of the tissue packaging. The container was placed into a shaking incubator, set at an ambient temperature of 40°C. A shaking incubator was used to model the agitation of the container during vehicular transit (the model needs to approximate real-life conditions as closely as possible).

The study was repeated in triplicate. Acceptable results were obtained on each occasion. All results were well within the pre-defined acceptance criteria, so the process was accepted based on the results of the three replicates.

Note, however, that it may be necessary to find a compromise between an 'ideal' validation and some

operational practicalities that cannot be avoided. For example, it may not be possible or ethical to obtain and sacrifice large amounts of tissue for validation studies. In these cases, an acceptable compromise needs to be reached using risk-assessment principles (e.g. use of animal tissue as a substitute).

Application of sufficiently robust process validations (e.g. by challenging a transport process with extremes of time and temperature) obviates the need for routine temperature monitoring of the process. Thus, if the physical conditions identified by the validation study are complied with (e.g. the correct container, containing at least the minimum amount of refrigerant, in transit for less than the maximum modelled time), then it can be reliably concluded that the process itself has been carried out correctly. Therefore, to demonstrate compliance with the validated process, all that the operatives need to do is confirm that they have complied with the relevant standard operating procedures.

Appendix 7. Example of method validation – oocyte vitrification

Preservation of substances of human origin (SoHO) is used to maintain cell viability for a long period of time. However, the survival rate after thawing represents a critical step affecting the success of the treatment.

The following example refers to the validation of a new method of cryopreservation in human oocytes.

For prospective validation, a randomised clinical study with specific endpoints (primary and secondary) and acceptance criteria could be performed. See Figure A7.1.

As an alternative approach, the validation can be performed in parallel by comparing the new method with the established one, as follows. See Figure A7.2.

If and when a validation is performed in a randomised clinical study with defined endpoints (see Figure A7.1), usually a multicentre study is needed, and the endpoints (primary and secondary) are defined and agreed upon between the medical assisted reproduction (MAR) centres.

Figure A7.1 **Validation of a new oocyte vitrification method in a multicentre study**

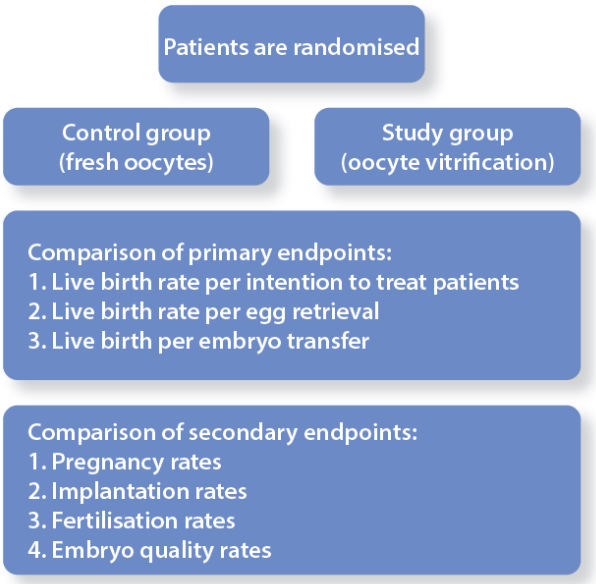
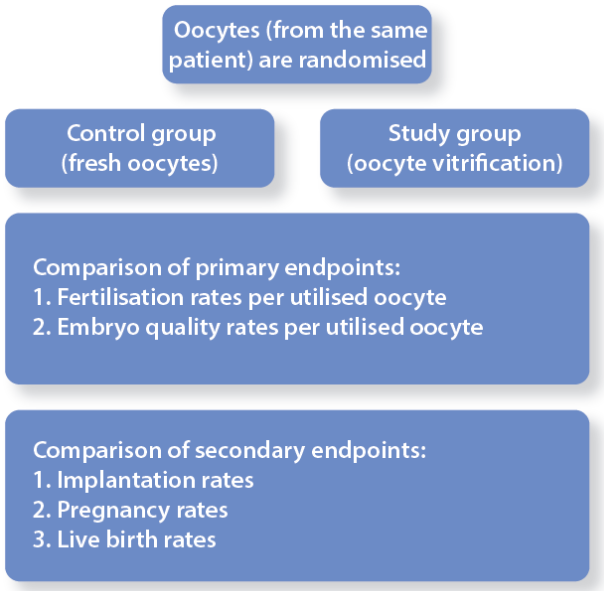
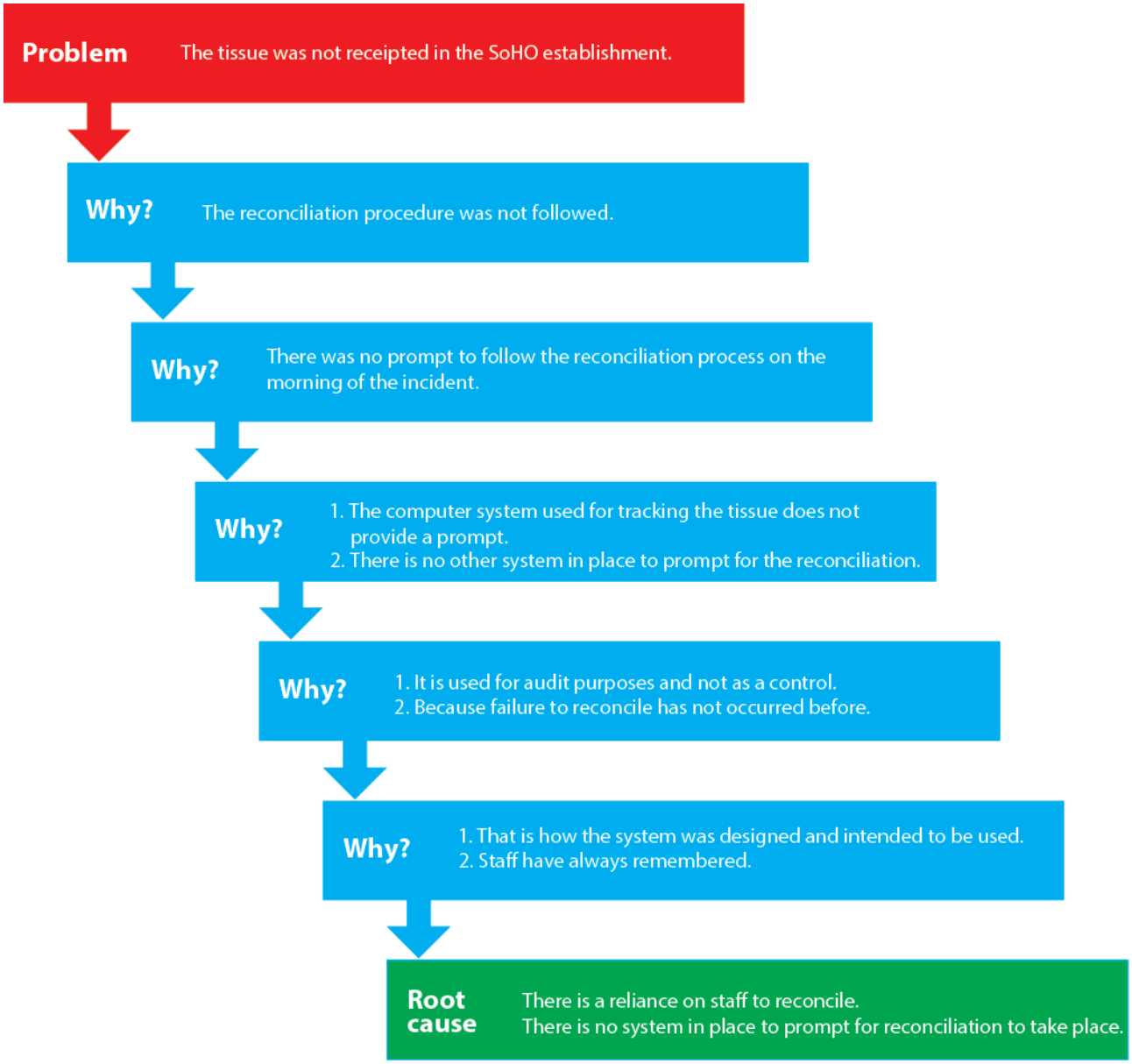


Figure A7.2 **Validation of a new oocyte vitrification method in a single centre**



Appendix 8. Example of root cause analysis: 5 whys – receipt of tissue



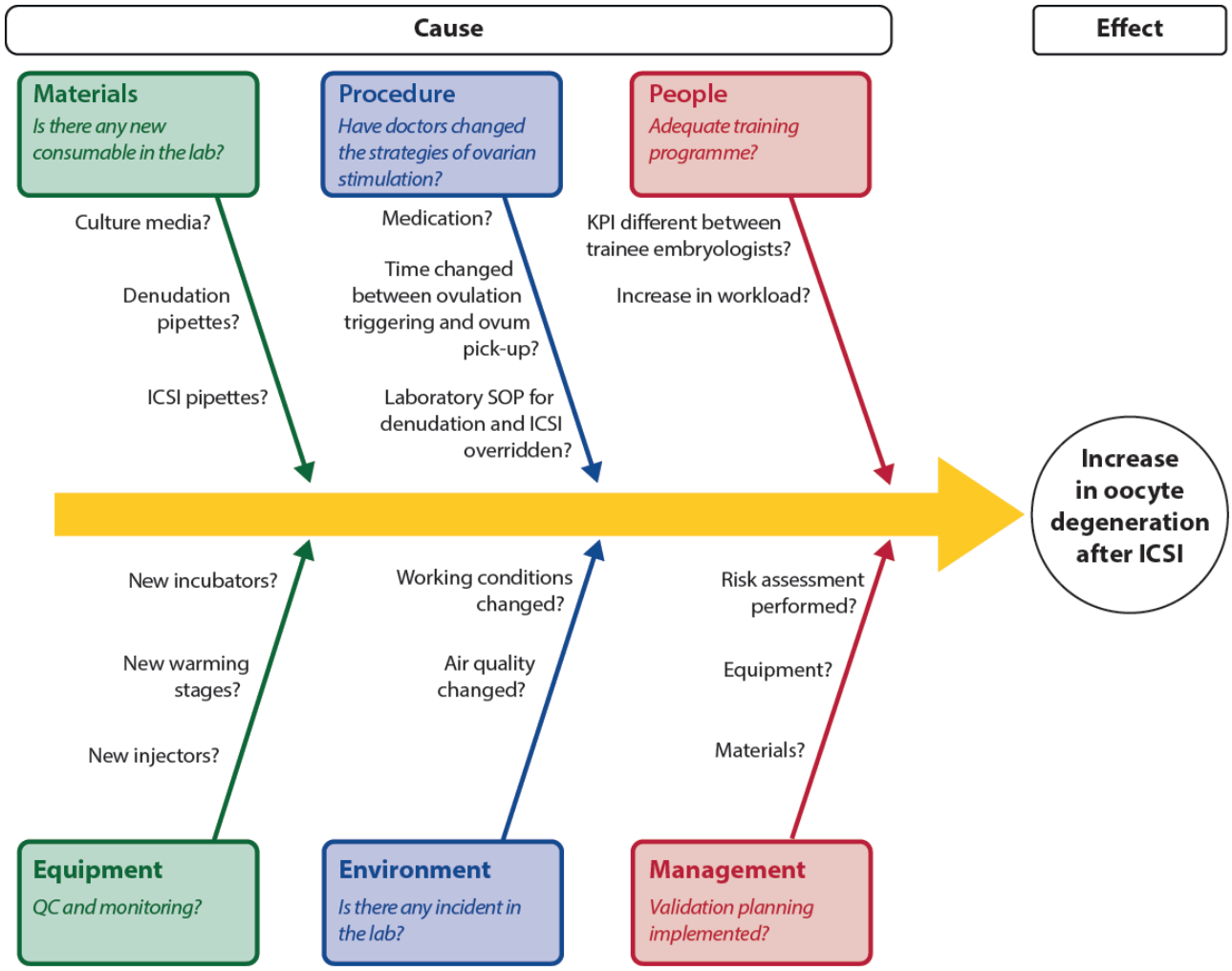
Appendix 9. Example of root cause analysis: fishbone diagram – receipt of SoHO



Root causes

1. Reconciliation procedure not followed by the SoHO establishment staff following the receipt and storage of SoHO by the porters.
2. Computer system does not alert if reconciliation is not completed.

Appendix 10. **Example of root cause analysis: fishbone diagram – medically assisted reproduction**



Appendix 11. **Example of consent form: female (HFEA, UK)**

HFEA WT Form

Human Fertilisation & Embryology Authority

Your consent to your eggs and embryos created using your eggs being used in treatment (IVF and ICSI) or stored

About this form

This form is produced by the Human Fertilisation and Embryology Authority (HFEA), the UK's independent regulator of fertility treatment and human embryo research. For more information about the HFEA, visit **www.hfea.gov.uk**.

In this form, the terms **'your embryos'** or **'embryos'** means embryos which have been created outside the body using your eggs.

When an embryo is created outside the body using your eggs with sperm from your partner or a sperm donor, then your partner or the sperm donor must also give consent to the use or storage of the embryo.

Who should fill in this form?

Fill in this form if you are having fertility treatment using embryos created outside the body (in vitro) with your eggs. This may be in vitro fertilisation (IVF) or intra-cytoplasmic sperm injection (ICSI).

If you are unable to complete this form because of physical illness, injury or disability you may direct someone else to complete and sign it for you in your presence and at your direction.

Why do I have to fill in this form?

By law (the Human Fertilisation and Embryology Act 1990 (as amended)), you need to give your written consent if you want your eggs or embryos to be used (eg, for IVF or ICSI treatment) or stored for later use. If you are storing your eggs or embryos, you must state in writing how long you consent to them being stored.

You are also legally required to record what you would like to happen to your eggs or embryos if you were to die or lose the ability to decide for yourself (become mentally incapacitated). While this is perhaps not something you have considered, if you do not record your consent on this form, in the event that you die or become mentally incapacitated, your partner would not be able to use your eggs or embryos in their own treatment or in treatment with a surrogate. If you are unsure of anything in relation to this or need further information about treatment options in the event of your death or mental incapacity, please ask your clinic.

This form will record your consent to:

- Use of your eggs to create embryos for treatment;
- Storage of your eggs for treatment purposes;
- Storage and use of your embryos for treatment;
- Use and storage of your eggs or embryos for training purposes; and
- Use and storage of your eggs or embryos in the event of your death or mental incapacity.

Depending on your circumstances, you may wish for your named partner to be able to use your eggs or embryos created using your eggs in treatment in order to carry the pregnancy in the event of your death. If this applies, you are not married or in a civil partnership with your partner, and you and your partner wish to be registered as the legal parents of any child born from the treatment, then you will both need to complete the appropriate legal parenthood forms (WP and PP). If this applies, you are married or in a civil partnership with your partner, and you wish to be registered as the legal parent of any child born if you die before embryos (that were created before your death) are transferred to your partner, then you will need to complete an additional form, 'Your consent to being registered as the legal parent in the event of your death' (PBR form). Please speak to your clinic if they have not already discussed this with you.

What do I need to know before filling in this form?

Before you fill in this form, you should be certain that your clinic has given you all the relevant information you need to make fully informed decisions. This includes:

- information about:
  - the different options set out in this form
  - the implications of giving your consent and what will happen if you do not renew your consent when prompted by your clinic
  - the consequences of withdrawing this consent, and
  - how you can make changes to, or withdraw, your consent.
- A suitable opportunity to have proper counselling about the implications of treatment and storage.

If you are unsure, or think that you have not been given all

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Patient number ☐☐☐☐☐☐☐☐☐☐

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of this information, please speak to your clinic. There is a declaration at the end of this form which you must sign to confirm you have received this information. **If you do not receive this information before filling in this form, your consent may be invalid.**

What other uses are there for my eggs or embryos during treatment?

During your treatment, you may have eggs or embryos that you do not want to use (for example, because the eggs or embryos are not needed, or are not suitable, for treatment). On this form, you can consent to your eggs or embryos being used and stored for potential use by designated healthcare professionals to practice the techniques involved in fertility treatment.

Giving your consent to your eggs or embryos being used and stored for training purposes means that eggs or embryos which are not used, or not suitable for use, in each fresh cycle of treatment can be either used immediately for training or stored for potential future use in training to allow designated healthcare professionals to learn about, and practice, the techniques involved in fertility treatment.

The decision to consent to your eggs or embryos being used for training purposes will not affect your treatment in any way.

What other uses are there for my eggs or embryos after I have finished my treatment?

After treatment, you may have stored eggs or embryos. If you no longer wish to keep your eggs or embryos for your own treatment, you can withdraw your consent to storage, and they will be disposed of. Your clinic will provide you with the necessary form.

Your other options when withdrawing your consent to use and storage for your own treatment include giving your consent to:

- the donation of unused eggs or embryos for use in someone else's treatment. Before doing this, there are lots of issues to consider, which your clinic should discuss with you. This would involve further screening tests, counselling and further consent.
- your unused eggs or embryos being used for research purposes, with the aim of helping to increase knowledge about diseases and serious illnesses and potentially develop new treatments. Research projects take place at HFEA licenced research facilities. Your clinic can give you more information about this.
- your stored unused eggs or embryos being used for training purposes to allow designated healthcare professionals to learn about, and practice, the techniques involved in fertility treatment.

Your clinic will provide you with further information regarding these options and it may be necessary to complete further consent forms.

When filling in this form, make sure you sign the declaration on every page to confirm that you have read the page and fully agree with the information provided and the consent that you are giving. When you have completed the form, you may request a copy of it from your clinic.

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HFEA centre reference ☐☐☐☐

Patient number ☐

Version 13, 19 February 2024

1 About you

1.1 **Your first name(s)** Place sticker here

1.2 **Your surname**

1.3 **Your date of birth** D D M M Y Y

1.4 **Your NHS/CHI/HCN/passport number** (please select)

2 About your partner

2.1 **Your partner's first name(s)** Place sticker here

2.2 **Your partner's surname**

2.3 **Your partner's date of birth** D D M M Y Y

2.4 **Your partner's NHS/CHI/HCN/passport number** (please select)

3 Your treatment

3.1 **Do you consent to your eggs being used to create embryos outside the body and the embryos used for your treatment (eg, through IVF or ICSI treatment)?**

In order to create embryos for your treatment you must provide your consent by ticking the 'yes' box below. You should be aware that the sperm provider (your partner or sperm donor) also has to give consent for embryos to be created.

☐ Yes ☐ No

Page declaration

Your signature **Date** D D M M Y Y

For clinic use only (optional) Patient number

4 Storing eggs or embryos

Storage periods

If not used immediately in treatment, you may wish to store your eggs or embryos so they can be used for treatment in the future. To be stored, eggs or embryos are frozen. You should think about how far in the future you might want or be able to use stored eggs or embryos and the potential costs of storing – you should discuss this with your clinic.

You should be aware that embryos can only be stored if the sperm provider (your partner or sperm donor) has also given consent.

The law permits you to store eggs or embryos for use in your treatment for any period up to a maximum of 55 years from the date that the eggs or embryos are first placed in storage. However, you will need to renew your consent every 10 years.

You can give your consent to storage on this form. You will be contacted by your clinic regarding an additional period of storage or renewal of your consent to storage at the appropriate time.

Because your clinic needs to contact you about your consent to storage, you should always inform your clinic if your contact details change or if your circumstances change (eg, in the event of separation from your named partner). If your clinic is unable to contact you to obtain your consent, then your eggs or embryos will be removed from storage and disposed of when they can no longer be lawfully stored.

You should be aware that any arrangements you need to make regarding the practicalities of storage with your clinic or funding body are separate from this consent. For example, your clinic may only continue to store embryos for the period you have specified in this form if you, or your funding provider, continue to pay the storage fees.

Storage for the first time

4.1 **Do you consent to your eggs being stored?**

☐ Yes ☐ No

If you have answered 'yes', indicate how long you consent to storage:

☐ For 10 years, or

☐ For a shorter period - specify the number of years (not exceeding 10 years):

4.2 **Do you consent to your embryos being stored?**

You should be aware that embryos can only be stored if the sperm provider (your partner or sperm donor) has also given consent.

☐ Yes ☐ No

If you have answered 'yes', indicate how long you consent to storage:

☐ For 10 years, or

☐ For a shorter period - specify the number of years (not exceeding 10 years):

You can change your storage period or withdraw your consent to storage at any time by contacting your clinic.

The consent periods above will start from the date that your eggs or embryos are first placed in storage.

Continues on the next page »

Page declaration

Your signature

Date D D M M Y Y

For clinic use only (optional) Patient number

4 Storing eggs or embryos continued

Additional storage prior to renewal

Only complete this section if you have already:

- Given your initial consent to storage of your eggs or embryos for a period less than 10 years from the date that your eggs or embryos were first placed in storage, or
- If you have renewed your consent, but consented to a storage period of less than 10 years, and now wish to request an additional period of storage for up to another 10 years before a further renewal is required.

You will be required to formally renew your consent to storage (on a renewal of consent form) no later than every 10 years after your eggs or embryos were first placed in storage. Your clinic will contact you about this at the appropriate time. If your clinic is unable to contact you, then your eggs or embryos will be removed from storage and disposed of when they can no longer be lawfully stored.

4.3 **Do you wish to consent to an additional period of storage of your eggs?**

☐ Yes ☐ No

If you have answered 'yes', indicate how long you wish your additional period of storage to last. Any period you specify below will be in addition to your existing storage period. The total number of years of storage should not exceed 10 years from date of first storage or most recent renewal.

Specify the number of years:

4.4 **Do you wish to consent to an additional period of storage of your embryos?**

You should be aware that embryos can only be stored if the sperm provider (your partner or sperm donor) has also given consent.

☐ Yes ☐ No

If you have answered 'yes', indicate how long you wish your additional period of storage to last. Any period you specify below will be in addition to your existing storage period. The total number of years of storage should not exceed 10 years from date of first storage or most recent renewal.

Specify the number of years:

You can change your storage period or withdraw your consent to storage at any time by contacting your clinic.

Page declaration

Your signature

Date D D M M Y Y

For clinic use only (optional) Patient number

5 Using eggs or embryos for training

During your treatment, you may have eggs or embryos that you do not want to use (for example, because the eggs or embryos are not needed, or are not suitable, for treatment). On this form, you can consent to your eggs or embryos being used and stored to allow designated healthcare staff to practice the techniques involved in fertility treatment.

Giving your consent to your eggs or embryos being used and stored for training purposes means that eggs or embryos which are not used, or are not suitable for use, in each fresh cycle of treatment can be either used immediately for training or stored for potential future use in training.

The decision to consent to your eggs or embryos being used for training purposes will not affect your treatment in any way.

Eggs

5.1 **Do you consent to your unused eggs being used for training purposes?**

☐ Yes ☐ No

If you give your consent to storing your eggs for training purposes, your clinic may store your eggs for these purposes for up to **55 years** from the date that your eggs are first placed in storage.

5.2 **If you consent to your eggs being used for training purposes, how long do you consent to storage?**

☐ For 55 years, or

☐ For a shorter period - specify the number of years (not exceeding 55 years): ☐☐

Embryos

5.3 **Do you consent to your unused embryos being used for training purposes?**

You should be aware that embryos can only be used if the sperm provider (your partner or sperm donor) has also given consent.

☐ Yes ☐ No

If you give your consent to storing your embryos for training purposes, your clinic may store your embryos for these purposes for a maximum of **10 years** from the date that you give consent on this form. This period cannot be extended.

5.4 **If you consent to your embryos being used for training purposes, how long do you consent to storage?**

☐ For 10 years, or

☐ For a shorter period - specify the number of years (not exceeding 10 years): ☐☐

Page declaration

Your signature **Date**

D D M M Y Y

For clinic use only (optional) Patient number

6 In the event of your death

As part of your consent, you also need to decide what you would like to happen to your eggs or embryos if you die.

In the event of your death, if you would like your partner to be able to use your eggs or embryos in their own treatment or in treatment with a surrogate, your partner must be named in section 2 of this form. If a surrogacy arrangement would be required, you will need to receive relevant information, be offered counselling, undergo further screening tests and complete additional consent forms before you die. It is therefore vitally important that you and your clinic discuss posthumous use and the different treatment options in those circumstances. Please ask your clinic if they have not already discussed this with you.

The person named at section 2 of this form will be the only person able to use your stored eggs or embryos for treatment after your death. If you do not name a person at section 2 of this form, then no one will be permitted to use your eggs or embryos for treatment after your death.

If your circumstances change after you have completed this form (for example, if you separate from the partner you have named on this form), or if you wish to withdraw your consent, you will need to contact your clinic to complete further forms.

Treatment in the event of your death

If you consent to your eggs or embryos being used **for treatment** after your death, the law permits your eggs or embryos to be stored for your named partner's **use** for 10 years from the date of your death. You must consent to both use and storage of your eggs or embryos continuing after death. This storage period cannot be extended.

If your named partner does not use your eggs within this 10 year period, then 10 years after your death, your clinic will be required to remove all your eggs from storage and dispose of them.

If your named partner does not use your embryos, whether created before or after your death, within this 10 year period, then 10 years and six months after your death, your clinic will be required to remove all your embryos from storage and dispose of them.

Your embryos can only be used and stored during this 10 year period if there is also effective consent for use and storage from the sperm provider (your partner or sperm donor).

6.1 **In the event of your death, do you consent to your eggs being used to create embryos outside the body and those embryos being used and stored for your partner's treatment?**

You should be aware that the sperm provider (your partner or sperm donor) also has to give consent for embryos to be created.

If treatment would involve a surrogate, then additional consent forms and screening tests must have been completed before you die to allow treatment to take place. It is important to speak to your clinic about this.

☐ Yes ☐ No

If you have answered 'yes', indicate how long you consent to storage of your eggs after your death:

☐ For 10 years from the date of your death, or

☐ For a shorter period - specify the number of years (not exceeding 10 years after your death): ☐

Continues on the next page »

Page declaration

Your signature **Date**

D D M M Y Y

For clinic use only (optional) Patient number

6 In the event of your death continued

6.2 **In the event of your death, do you consent to your embryos created before your death being used and stored for your partner's treatment?**

You should be aware that embryos can only be used if the sperm provider (your partner or sperm donor) has also given consent.

If treatment would involve a surrogate, then additional consent forms and screening tests **must** have been completed before you die to allow treatment to take place. It is important to speak to your clinic about this.

☐ Yes ☐ No

If you have answered 'yes', indicate how long you consent to storage of your embryos after your death:

☐ For 10 years from the date of your death, or

☐ For a shorter period - specify the number of years (not exceeding 10 years after your death):

Use of your eggs or embryos for training purposes in the event of your death

In the event of your death, you may have eggs or embryos that your named partner does not want to use (for example, because the eggs or embryos are not needed, or are not suitable, for treatment). On this form, you can consent to your eggs or embryos being used or stored after your death for potential use by designated healthcare professionals to practice the techniques involved in fertility treatment.

6.3 **In the event of your death, do you consent to your unused eggs being used and stored for training purposes?**

☐ Yes ☐ No

If you have answered 'yes', indicate how long you consent to storage of your eggs for training purposes after your death:

☐ For 55 years from the date of first storage, or

☐ For a shorter period - specify the number of years (not exceeding 55 years from the date of first storage):

6.4 **In the event of your death, do you consent to your unused embryos that were being stored for treatment purposes being used and stored for training purposes?**

You should be aware that embryos can only be used or stored for training purposes if the sperm provider (your partner or sperm donor) has also given consent.

☐ Yes ☐ No

If you have answered 'yes', indicate how long you consent to storage of your embryos for training purposes after your death:

☐ For 10 years from the date you sign this form, or

☐ For a shorter period - specify the number of years (not exceeding 10 years from the date you sign this form):

Page declaration

Your signature

Date D D M M Y Y

For clinic use only (optional) Patient number

7 In the event of your mental incapacity

As part of your consent, you also need to decide what you would like to happen to your eggs or embryos if you lose the ability to decide for yourself (become mentally incapacitated).

In the event that you lose mental capacity, if you would like your partner to be able to use your eggs or embryos in their own treatment or treatment with a surrogate, your partner must be named in section 2 of this form. If a surrogacy arrangement would be required, you will need to receive relevant information, be offered counselling, undergo further screening tests and complete additional consent forms whilst you have capacity. It is therefore vitally important that you and your clinic discuss your wishes in the event of your mental incapacity and the different treatment options in those circumstances. Please ask your clinic if they have not already discussed this with you.

The person named at section 2 of this form will be the only person able to use your stored eggs or embryos in treatment if you lose mental capacity. If you do not name a person at section 2 of this form, then no one will be permitted to use your eggs or embryos for treatment purposes if you lose mental capacity (unless you subsequently regain capacity).

If your circumstances change after you have completed this form (for example, if you separate from the partner you have named on this form), or if you wish to withdraw your consent, you will need to contact your clinic to complete further forms.

Treatment and storage in the event of your mental incapacity

You can use this form to:

- **Consent to the continued storage of your eggs or embryos without consenting to the use of your eggs or embryos in treatment.** Then, in the event that you regain capacity, you may be able to use your eggs or embryos in treatment.

  OR

- **Consent to your eggs or embryos being stored and used for treatment in the event that you lose mental capacity.** If you have a partner and you would like them to be able to use your eggs or embryos in their own treatment or in treatment with a surrogate in the event that you lose mental capacity, your partner **must** be named in section 2 of this form. If you wish to consent for your eggs or embryos to be **used** in the event that you lose capacity, you must also consent to continued **storage** of your eggs or embryos after your loss of capacity.

The law permits your eggs or embryos to be stored for treatment purposes for up to 10 years from the date that a medical practitioner certifies in writing that you lack capacity. This storage period cannot be extended.

If you **select on this form** that you do **not** consent to **either storage or use** of your eggs or embryos in the event of your mental incapacity, then:

- Your eggs or embryos will be removed from storage and disposed of when you lose mental capacity.

If you consent **either** to storage **or** to storage and use of your eggs or embryos in the event of your mental incapacity:

- If you **do not** regain mental capacity within 10 years of being certified as lacking capacity:
  - Your clinic will be required to remove all your unused eggs from storage 10 years, or in the case of embryos 10 years and 6 months, from the date that a medical practitioner certified in writing that you lack capacity and dispose of them.

Continues on the next page »

Page declaration

Your signature

Date D D M M Y Y

For clinic use only (optional) Patient number

7 In the event of your mental incapacity continued

- If you **do** regain mental capacity within 10 years of being certified as lacking capacity:
  - **You may be able to use** your eggs or embryos in treatment.
  - **You may be able to renew** your consent to storage of your eggs or embryos for your treatment. You will need to notify your clinic as soon as possible if you are certified as having regained mental capacity and wish to renew your consent to storage of your unused eggs or embryos.
  - If you **do not** notify your clinic, then it will be required to remove all your unused eggs from storage 10 years, or in the case of embryos 10 years and 6 months, from the date that a medical practitioner certified in writing that you lack capacity and dispose of them.
  - Your embryos can only be used and stored during this 10 year period if there is also effective consent for use and storage from the sperm provider (your partner or sperm donor).

Use of your eggs or embryos for training purposes in the event of your mental incapacity

You can also consent to your eggs or embryos being used and stored after your loss of capacity for potential use by designated healthcare professionals to practice the techniques involved in fertility treatment. **To do this, you will need to speak to your clinic who will be able to provide you with more information.** If you do not consent to your eggs or embryos being used in training, if they can no longer be lawfully stored they will be removed from storage and disposed of.

Eggs

7.1 **Please tick the box next to the appropriate statement below to confirm your choice.**

Select **one** of either **A, B** or **C**.

☐ **A. In the event that I lose mental capacity, I consent to my eggs being stored for possible use in future treatment, but not used by another person whilst I have lost capacity.**

Please indicate how long you consent to storage of your eggs after you lose capacity:

☐ For 10 years from the date of being certified as lacking capacity, or

☐ For a shorter period - specify the number of years (not exceeding 10 years from the date loss of capacity was certified): ____

If you have selected 7.1A, the next question you need to answer is 7.2. Remember to sign the declaration on every page.

☐ **B. In the event that I lose mental capacity, I consent to my eggs being stored and used to create embryos outside the body, and to those embryos being stored and used in my named partner's treatment whilst I have lost capacity.**

Only select option B if you have a partner named at section 2 of this form.

Continues on the next page »

Page declaration

Your signature **Date**

D D M M Y Y

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7 In the event of your mental incapacity continued

If treatment would involve a surrogate, then additional consent forms and screening tests must have been completed before you lose capacity to allow treatment to take place.

Please indicate how long you consent to storage of your eggs after you lose capacity:

☐ For 10 years from the date of being certified as lacking capacity, or

☐ For a shorter period - specify the number of years (not exceeding 10 years from the date loss of capacity was certified): ____

Please indicate how long you consent to storage of embryos created from your eggs after your loss of capacity:

You should be aware that embryos can only be stored and used if the sperm provider (your partner or sperm donor) has also given consent.

☐ For 10 years from the date of being certified as lacking capacity, or

☐ For a shorter period - specify the number of years (not exceeding 10 years from the date loss of capacity was certified): ____

If you have selected question 7.1B, the next question you need to answer is 7.2. Remember to sign the declaration on every page.

☐ **C. In the event that I lose mental capacity, my eggs should no longer be stored for my (or my partner's) treatment.**

Embryos (created before your loss of capacity)

7.2 **Please tick the box next to the appropriate statement below to confirm your choice.**

Select **one** of either **A, B** or **C**.

☐ **A. In the event that I lose mental capacity, I consent to my embryos created before my loss of capacity being stored for possible use in future treatment, but <u>not</u> used by another person whilst I have lost capacity.**

You should be aware that embryos can only be stored if the sperm provider (your partner or sperm donor) has also given consent.

Please indicate how long you consent to storage of your embryos after you lose capacity:

☐ For 10 years from the date of being certified as lacking capacity, or

☐ For a shorter period - specify the number of years (not exceeding 10 years from the date loss of capacity was certified): ____

If you have selected 7.2A, the next section you need to complete is the declaration at the end of the form. Remember to sign the declaration on every page.

Continues on the next page »

Page declaration

Your signature **Date**

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7 In the event of your mental incapacity continued

☐ **B. In the event that I lose mental capacity, I consent to my embryos created before my loss of capacity being stored and used by my named partner whilst I have lost capacity.**

Only select option B if you have a partner named at section 2 of this form.

You should be aware that embryos can only be stored and used if the sperm provider (your partner or sperm donor) has also given consent.

If treatment would involve a surrogate, then additional consent forms and screening tests must have been completed before you lose capacity to allow treatment to take place.

Please indicate how long you consent to storage of your embryos after you lose capacity:

☐ For 10 years from the date of being certified as lacking capacity, or

☐ For a shorter period - specify the number of years (not exceeding 10 years from the date loss of capacity was certified):

If you have selected question 7.2B, the next section you need to complete is the declaration at the end of the form. Remember to sign the declaration on every page.

☐ **C. In the event that I lose mental capacity, my embryos created before my loss of capacity should should no longer be stored for my (or my partner's) treatment.**

Other uses for your eggs or embryos if you die or become mentally incapacitated

If you wish your eggs or embryos to be used in the treatment of someone else (including your partner's treatment with a surrogate) if you die or become mentally incapacitated, please speak to your clinic for more information.

Depending on your circumstances, you will need to undergo additional screening, receive relevant information, be offered counselling and complete one or more of the following consent forms:

- 'Your consent to donating your eggs' (WD form)'
- 'Your consent to donating embryos' (ED form)
- 'Your consent to the use and storage of eggs or embryos for surrogacy' (WSG form).

Page declaration

Your signature

Date D D M M Y Y

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8 Declaration

Please sign and date the declaration

Your declaration

- I declare that I am the person named in section one of this form.
- I declare that:
  - before I completed this form, I was given information about the different options set out in this form, and I was given an opportunity to have counselling.
  - the implications of giving my consent, and the consequences of withdrawing this consent, have been fully explained to me.
  - I understand that I can make changes to, or withdraw, my consent to the use and storage of my eggs for the purposes I have indicated above at any point until the time that my eggs have been used for treatment or training, or my eggs have been disposed of.
  - I understand that I can make changes to, or withdraw, my consent to the use and storage of my embryos for the purposes I have indicated above at any point before embryo transfer, use of embryos in training, or my embryos have been disposed of.
  - I understand that before the end of the consent period that I have indicated at section 4 above, I will be contacted by my clinic regarding additional consent to storage or renewal of my consent to storage, if applicable.
  - I understand that if I do not request an additional period of storage or renew my consent, my consent will be taken as withdrawn and my eggs or embryos will be removed from storage and disposed of.
- I understand that I must notify my clinic if my contact details or personal circumstances change. I understand it is my responsibility to keep contact details up to date.
- I declare that the information I have given on this form is correct and complete.
- I consent to the clinic (or any subsequent HFEA-licensed clinic that may become involved in my treatment, donation or storage, or a data controller in line with the Data Protection Act 2018 and UK General Data Protection Regulation) using the information on this form in the process of providing licensed activities (in accordance with the provisions of the Human Fertilisation and Embryology Act 1990, (as amended)), or for record storage and archiving purposes.

Your signature | **Date** D D M M Y Y

If signing at the direction of the person consenting

If you have completed this form at the direction of the person consenting (because they are unable to sign for themselves due to physical illness, injury or disability), you must sign and date below. There must also be a witness confirming that the person consenting is present when you sign the form.

Representative's declaration

I declare that the person named in section one of this form is present at the time of signing this form and I am signing it in accordance with their direction.

Representative's name | **Representative's signature**

Relationship to the person consenting | **Date** D D M M Y Y

Witness' name | **Witness' signature**

Date D D M M Y Y

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Source: Human Fertilisation & Embryology Authority, United Kingdom.

Appendix 12. **Example of consent form: female (CNPMA, Portugal)**

cnpma conselho nacional de procriação medicamente assistida

CRYOPRESERVATION OF OOCYTES AND/OR OVARIAN TISSUE

Informed Consent

Oocytes are female reproductive cells that, in their immature form, are already present in the ovaries of the newborn. From puberty onwards, during each normal menstrual cycle, groups of oocytes will undergo maturation that will culminate in the release by the ovary of a mature oocyte that can be fertilised.

In certain clinical situations – for example, when required therapies may compromise the survival of reproductive cells - cryopreservation of oocytes and/or ovarian tissue has been proposed in an attempt to preserve future fertility.

Cryopreservation of oocytes may also be considered whenever a woman considers carrying out a reproductive project at an advanced reproductive age, when oocyte production/viability will likely be very low or absent.

A number of key points should be highlighted:

- Cryopreservation of oocytes does not guarantee a pregnancy; it only ensures a stock of female reproductive cells that may be used in the future through In Vitro Fertilization (IVF) or Intracytoplasmic Sperm Injection (ICSI).
- IVF and ICSI include stimulating the development and maturation of ovarian follicles containing the oocytes through the use of injectable drugs; the response of the ovaries to these drugs varies from woman to woman and will be monitored with blood tests and/or ultrasound at intervals to be defined on a case-by-case basis.
- Puncture of the ovaries for collection (aspiration) of oocytes is performed vaginally, under local anesthesia and/ or sedation.
- For various reasons, most often a deficient response of the ovaries to the medication, the treatment cycle may need to be cancelled before the puncture to obtain the oocytes.
- In rare situations, stimulation of the ovaries may trigger an excessive response, giving rise to the so-called “ovarian hyperstimulation syndrome”, which, in certain circumstances, will require specific treatment in an inpatient setting; in truly exceptional circumstances, ovarian hyperstimulation syndrome can be life-threatening.
- The administration of drugs for sedation or anesthesia may cause unexpected individual reactions, allergic or otherwise.
- As the collection of oocytes is a surgical procedure, in very rare cases, complications (for example, bleeding or infection) may require hospital admission; in truly exceptional circumstances, these complications can be life-threatening.
- The rate of pregnancy achieved with *in vitro* fertilisation (IVF) or intracytoplasmic sperm injection (ICSI) of cryopreserved oocytes varies and will depend on the clinical situation of the patient to be treated.
- Although no increase in foetal abnormalities has been reported, a possible increased risk of foetal abnormalities or regarding children development cannot be excluded at this time.
- Cryopreservation of oocytes does not entail any obligation on this centre concerning the future use of those cells, nor does it grant the person from whom they originated any type of preferential treatment; clinical criteria will determine the appropriate clinical conduct at all times.
- The collection of ovarian tissue for cryopreservation and its possible subsequent transplantation require surgical procedures with the inherent risks of the respective procedures.
- Unforeseen accidents, such as fires or calamities of other types, may, despite the safety precautions taken, lead to loss or destruction of the cryopreserved oocytes.

cnpma conselho nacional de procriação medicamente assistida

CONSENT

I, the undersigned, declare that:

- I have read and understood this document and the additional information provided.
- The queries and questions I have raised have been answered.
- I recognise that this text cannot describe exhaustively all situations that can occur in the future.
- I understand and accept that oocytes and/or ovarian tissue will be cryopreserved for a maximum period of five years and that I may revoke this authorisation at any time during this period.
- I understand and accept the conditions, risks and limitations of these techniques, namely that no guarantee can be given as far as the development and outcome of a pregnancy achieved with in vitro fertilization (IVF) or intracytoplasmic sperm injection (ICSI).
- I am aware that I alone have the right to use these frozen oocytes and/or ovarian tissue.
- I understand that, under the current legislation, until the end of this five-year period, I will have to sign a consent form in the centre for this cryostorage to continue. In the absence of a signed declaration requesting a further period of cryopreservation, I declare that I have been clearly informed that the oocytes and/or ovarian tissue will be thawed and destroyed, unless I have granted express authorisation for their use for scientific purposes. In these circumstances (write **Yes** or **No**):

  - I consent to the use of my oocytes in scientific research projects ☐

  - I consent to the donation of my oocytes to other patients I fully understand and accept the conditions, risks and limitations enumerated above. ☐

I declare that in case of donation to other patients, people born from one of these oocytes may obtain from the competent health services information regarding their genetic nature, and from CNPMA the civil identification of the donor, as from the age of 18.

Therefore, having been fully informed, I freely acknowledge the obligations implied in this agreement and give my consent for the use of these techniques for the preservation of my oocytes/ovarian tissue.

NAME _____

SIGNATURE _____

CIVIL ID/PASSPORT NO. _____

Medical Doctor: _____ ___/___/_____

cnpma conselho nacional de procriação medicamente assistida

PROTECTION OF PERSONAL DATA

CNPMA is responsible for the processing of personal data collected within the scope of the treatment for which consent is expressed through this document. These data will be processed within the scope of the CNPMA's attributions to regulate, supervise and monitor the practice of Medically Assisted Reproduction in Portugal, established by Law No. 32/2006, of 26 July, in its current wording. Your personal data will be treated securely, subject to technical and organizational measures aimed at safeguarding its confidentiality, integrity and availability. The data will be kept in compliance with the applicable legal retention period. The CNPMA guarantees data subjects the exercise of legally enshrined rights. To exercise these rights or to clarify doubts regarding the processing of your personal data, you must submit a written request to the CNPMA, namely by email to dpo.cnpma@ar.parlamento.pt or by mail to the National Council for Medical Assisted Reproduction, Assembleia da República, Palácio de São Bento, 1249-068 Lisboa.

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Source: Cryopreservation of oocytes and/or ovarian tissue, Portugal (CNPMA).

Appendix 13. **Example of consent form: male (HFEA, UK)**

HFEA MT Form

Human Fertilisation & Embryology Authority

Your consent to your sperm and embryos created outside the body using your sperm being used in treatment (IVF and ICSI) or stored

About this form

This form is produced by the Human Fertilisation and Embryology Authority (HFEA), the UK's independent regulator of fertility treatment and human embryo research. For more information about the HFEA, visit **www.hfea.gov.uk**.

In this form, the terms **'your embryos'** or **'embryos'** means embryos which have been created outside the body using your sperm.

When an embryo is created outside the body using your sperm with eggs from your partner or an egg donor, then your partner or the egg donor must also give consent to the use or storage of the embryo.

Who should fill in this form?

Fill in this form if your partner is having fertility treatment using embryos created outside the body (in vitro) with your sperm. This may be in vitro fertilisation (IVF) or intra-cytoplasmic sperm injection (ICSI).

If you are unable to complete this form because of physical illness, injury or disability you may direct someone else to complete and sign it for you in your presence and at your direction.

Why do I have to fill in this form?

By law (the Human Fertilisation and Embryology Act 1990 (as amended)), you need to give your written consent if you want your sperm or embryos to be used (eg, for IVF or ICSI treatment) or stored for later use. If you are storing your sperm or embryos, you must state in writing how long you consent to them being stored.

You are also legally required to record what you would like to happen to your sperm or embryos if you were to die or lose the ability to decide for yourself (become mentally incapacitated). While this is perhaps not something you have considered, if you do not record your consent on this form, in the event that you die or become mentally incapacitated, your partner would not be able to use your sperm or embryos in their own treatment or in treatment with a surrogate. If you are unsure of anything in relation to this or need further information about treatment options in the event of your death or mental incapacity, please ask your clinic.

This form will record your consent to:

- Use of your sperm to create embryos for treatment;
- Storage of your sperm for treatment purposes;
- Storage and use of your embryos for treatment;
- Use and storage of your sperm and embryos for training purposes;
- Use and storage of your sperm or embryos in the event of your death or mental incapacity; and
- Being registered as the legal parent of a child born as a result of your partner's treatment, if treatment occurred after your death.

What do I need to know before filling in this form?

Before you fill in this form, you should be certain that your clinic has given you all the relevant information you need to make fully informed decisions. This includes:

- information about:
  - the different options set out in this form
  - the implications of giving your consent and what will happen if you do not renew your consent when prompted to by your clinic
  - the consequences of withdrawing this consent, and
  - how you can make changes to, or withdraw, your consent.
- a suitable opportunity to have proper counselling about the implications of treatment and storage.

If you are unsure, or think that you have not been given all of this information, please speak to your clinic. There is a declaration at the end of this form which you must sign to confirm you have received this information. **If you do not receive this information before filling in this form, your consent may be invalid.**

What other uses are there for my sperm or embryos during treatment?

During your treatment, you may have sperm or embryos that you do not want to use (for example, because the sperm or embryos are not needed, or are not suitable, for treatment). On this form, you can consent to your sperm or embryos

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HFEA centre reference ☐☐☐☐

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being used and stored for potential use by designated healthcare professionals to practice the techniques involved in fertility treatment.

Giving your consent to your sperm or embryos being used and stored for training purposes means that sperm or embryos which are not used, or not suitable for use, in each fresh cycle of treatment can be either used immediately for training or stored for potential future use in training to allow designated healthcare professionals to learn about, and practice, the techniques involved in fertility treatment.

The decision to consent to your sperm or embryos being used for training purposes will not affect your treatment in any way.

What other uses are there for my sperm or embryos after treatment has finished?

After treatment, you may have stored sperm or embryos. If you no longer wish to keep your sperm or embryos for your own treatment, you can withdraw your consent to storage, and they will be disposed of. Your clinic will provide you with the necessary form.

Your other options when withdrawing your consent to use and storage for your own treatment include giving your consent to:

- the donation of unused sperm or embryos for use in someone else's treatment. Before doing this, there are lots of issues to consider, which your clinic should discuss with you. This would involve further screening tests, counselling and further consent.
- your unused sperm or embryos being used for research purposes, with the aim of helping to increase knowledge about diseases and serious illnesses and potentially develop new treatments. Research projects take place at HFEA licenced research facilities. Your clinic can give you more information about this.
- your stored unused sperm or embryos being used for training purposes to allow designated healthcare professionals to learn about, and practice, the techniques involved in fertility treatment.

Your clinic will provide you with further information regarding these options and it may be necessary to complete further consent forms.

When filling in this form, make sure you sign the declaration on every page to confirm that you have read the page and fully agree with the information provided and the consent that you are giving. When you have completed the form, you may request a copy of it from your clinic.

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Version 9, 19 February 2024

1 About you

1.1 **Your first name(s)**

Place sticker here

1.2 **Your surname**

1.3 **Your date of birth**

D D M M Y Y

1.4 **Your NHS/CHI/HCN/passport number** (please select)

2 About your partner

2.1 **Your partner's first name(s)**

Place sticker here

2.2 **Your partner's surname**

2.3 **Your partner's date of birth**

D D M M Y Y

2.4 **Your partner's NHS/CHI/HCN/passport number** (please select)

3 Your treatment

3.1 **Do you consent to your sperm being used to create embryos outside the body and the embryos being used for your partner's treatment (eg, through IVF or ICSI treatment)?**

In order to create embryos for your partner's treatment you must provide your consent by ticking the 'yes' box below. You should be aware that the egg provider (your partner or egg donor) also has to give consent for embryos to be created.

☐ Yes ☐ No

Page declaration

Your signature

Date

D D M M Y Y

For clinic use only (optional) Patient number

4 Storing sperm or embryos

Storage periods

If not used immediately in treatment, you may wish to store your sperm or embryos so they can be used for treatment in the future. To be stored, sperm or embryos are frozen. You should think about how far in the future you might want or be able to use stored sperm or embryos and the potential costs of storing – you should discuss this with your clinic.

You should be aware that embryos can only be stored if the egg provider (your partner or egg donor) has also given consent.

The law permits you to store sperm or embryos for use in your treatment for any period up to a maximum of 55 years from the date that the sperm or embryos are first placed in storage. However, you will need to renew your consent every 10 years.

You can give your consent to storage on this form. You will be contacted by your clinic regarding an additional period of storage or renewal of your consent to storage at the appropriate time.

Because your clinic needs to contact you about your consent to storage, you should always inform your clinic if your contact details change or if your circumstances change (eg, in the event of separation from your named partner). If your clinic is unable to contact you to obtain your consent, then your sperm or embryos will be removed from storage and disposed of when they can no longer be lawfully stored.

You should be aware that any arrangements you need to make regarding the practicalities of storage with your clinic or funding body are separate from this consent. For example, your clinic may only continue to store embryos for the period you have specified in this form if you, or your funding provider, continue to pay the storage fees.

Storage for the first time

4.1 **Do you consent to your sperm being stored?**

☐ Yes ☐ No

If you have answered 'yes', indicate how long you consent to storage:

☐ For 10 years, or

☐ For a shorter period - specify the number of years (not exceeding 10 years): ____

4.2 **Do you consent to your embryos being stored?**

You should be aware that embryos can only be stored if the egg provider (your partner or egg donor) has also given consent.

☐ Yes ☐ No

If you have answered 'yes', indicate how long you consent to storage:

☐ For 10 years, or

☐ For a shorter period - specify the number of years (not exceeding 10 years): ____

You can change your storage period or withdraw your consent to storage at any time by contacting your clinic.

The consent periods above will start from the date that your sperm or embryos are first placed in storage.

Continues on the next page »

Page declaration

Your signature _____ **Date** D D M M Y Y

For clinic use only (optional) Patient number _____

4 Storing sperm or embryos continued

Additional storage prior to renewal

Only complete this section if you have already:

- Given your initial consent to storage of your sperm or embryos for a period less than 10 years from the date that your sperm or embryos were first placed in storage, or
- If you have renewed your consent, but consented to a storage period of less than 10 years, and now wish to request an additional period of storage for up to another 10 years before a further renewal is required.

You will be required to formally renew your consent to storage (on a renewal of consent form) no later than every 10 years after your sperm or embryos were first placed in storage. Your clinic will contact you about this at the appropriate time. If your clinic is unable to contact you, then your sperm or embryos will be removed from storage and disposed of when they can no longer be lawfully stored

4.3 **Do you wish to consent to an additional period of storage of your sperm?**

☐ Yes ☐ No

If you have answered 'yes', indicate how long you wish your additional period of storage to last. Any period you specify below will be in addition to your existing storage period. The total number of years of storage should not exceed 10 years from date of first storage or most recent renewal.

Specify the number of years:

4.4 **Do you wish to consent to an additional period of storage of your embryos?**

You should be aware that embryos can only be stored if the egg provider (your partner or egg donor) has also given consent.

☐ Yes ☐ No

If you have answered 'yes', indicate how long you wish your additional period of storage to last. Any period you specify below will be in addition to your existing storage period. The total number of years of storage should not exceed 10 years from date of first storage or most recent renewal.

Specify the number of years:

You can change your storage period or withdraw your consent to storage at any time by contacting your clinic.

Page declaration

Your signature

Date D D M M Y Y

For clinic use only (optional) Patient number

5 Using sperm or embryos for training

During your treatment, you may have sperm or embryos that you do not want to use (for example, because the sperm or embryos are not needed, or are not suitable, for treatment). On this form, you can consent to your sperm or embryos being used and stored to allow designated healthcare staff to practice the techniques involved in fertility treatment.

Giving your consent to your sperm or embryos being used and stored for training purposes means that sperm or embryos which are not used, or not suitable for use, in each fresh cycle of treatment can be either used immediately for training or stored for potential future use in training.

The decision to consent to your sperm or embryos being used for training purposes will not affect your treatment in any way.

Sperm

5.1 **Do you consent to your unused sperm being used for training purposes?**

☐ Yes ☐ No

If you give your consent to storing your sperm for training purposes, your clinic may store your sperm for these purposes for up to **55 years** from the date that your sperm is first placed in storage.

5.2 **If you consent to your sperm being used for training purposes, how long do you consent to storage?**

☐ For 55 years, or

☐ For a shorter period - specify the number of years (not exceeding 55 years): ____

Embryos

5.3 **Do you consent to your unused embryos being used for training purposes?**

You should be aware that embryos can only be used if the egg provider (your partner or egg donor) has also given consent.

☐ Yes ☐ No

If you give your consent to storing your embryos for training purposes, your clinic may store your embryos for these purposes for maximum of **10 years** from the date that you give consent on this form. This period cannot be extended.

5.4 **If you consent to your embryos being used for training purposes, how long do you consent to storage?**

☐ For 10 years, or

☐ For a shorter period - specify the number of years (not exceeding 10 years): ____

Page declaration

Your signature _____ **Date** D D M M Y Y

For clinic use only (optional) Patient number _____

6 In the event of your death

As part of your consent, you also need to decide what you would like to happen to your sperm or embryos if you die.

In the event of your death, if you would like your partner to be able to use your sperm or embryos in their own treatment or in treatment with a surrogate, your partner must be named in section 2 of this form. If a surrogacy arrangement would be required, you will need to receive relevant information, be offered counselling, undergo further screening tests and complete additional consent forms before you die. It is therefore vitally important that you and your clinic discuss posthumous use and the different treatment options in those circumstances. Please ask your clinic if they have not already discussed this with you.

The person named at section 2 of this form will be the only person able to use your stored sperm or embryos for treatment after your death. If you do not name a person at section 2 of this form, then no one will be permitted to use your sperm or embryos for treatment after your death.

If your circumstances change after you have completed this form (for example, if you separate from the partner you have named on this form), or if you wish to withdraw your consent, you will need to contact your clinic to complete further forms.

Treatment in the event of your death

If you consent to your sperm or embryos being used **for treatment** after your death, the law permits your sperm or embryos to be stored for your named partner's **use** for 10 years from the date of your death. You must consent to both use and storage of your sperm or embryos continuing after death. This storage period cannot be extended.

If your named partner does not use your sperm within this 10 year period, then 10 years after your death, your clinic will be required to remove all your sperm from storage and dispose of them.

If your named partner does not use your embryos, whether created before or after your death, within this 10 year period, then 10 years and six months after your death, your clinic will be required to remove all your embryos from storage and dispose of them.

Your embryos can only be used and stored during this 10 year period if there is also effective consent for use and storage from the egg provider (your partner or egg donor).

6.1 **In the event of your death do you consent to your sperm being used to create embryos outside the body and those embryos being used and stored for your partner's treatment?**

You should be aware that the egg provider (your partner or egg donor) also has to give consent for embryos to be created.

If treatment would involve a surrogate, then additional consent forms and screening tests **must** have been completed before you die to allow treatment to take place. It is important to speak to your clinic about this.

☐ Yes ☐ No

If you have answered 'yes', indicate how long you consent to storage of your sperm after your death:

☐ For 10 years from the date of your death, or

☐ For a shorter period - specify the number of years (not exceeding 10 years after your death): ☐

Continues on the next page »

Page declaration

Your signature **Date**

D D M M Y Y

For clinic use only (optional) Patient number

6 In the event of your death continued

6.2 **In the event of your death do you consent to your embryos created before your death being used and stored for your partner's treatment?**

You should be aware that embryos can only be used if the egg provider (your partner or egg donor) has also given consent.

If treatment would involve a surrogate, then additional consent forms and screening tests **must** have been completed before you die to allow treatment to take place. It is important to speak to your clinic about this.

☐ Yes ☐ No

If you have answered 'yes', indicate how long you consent to storage of your embryos after your death:

☐ For 10 years from the date of your death, or

☐ For a shorter period - specify the number of years (not exceeding 10 years after your death): _____

Use of your sperm or embryos for training purposes in the event of your death

In the event of your death, you may have sperm or embryos that your named partner does not want to use (for example, because the sperm or embryos are not needed, or are not suitable, for treatment). On this form, you can consent to your sperm or embryos being used or stored after your death for potential use by designated healthcare professionals to practice the techniques involved in fertility treatment.

6.3 **In the event of your death, do you consent to your unused sperm being used and stored for training purposes?**

☐ Yes ☐ No

If you have answered 'yes', indicate how long you consent to storage of your sperm for training purposes after your death:

☐ For 55 years from the date of first storage, or

☐ For a shorter period - specify the number of years (not exceeding 55 years from date of first storage): _____

6.4 **In the event of your death, do you consent to your unused embryos that were being stored for treatment purposes being used and stored for training purposes?**

You should be aware that embryos can only be used or stored for training purposes if the egg provider (your partner or egg donor) has also given consent.

☐ Yes ☐ No

If you have answered 'yes', indicate how long you consent to storage of your embryos for training purposes after your death:

☐ For 10 years from the date you sign this form, or

☐ For a shorter period - specify the number of years (not exceeding 10 years from the date you sign this form): _____

Page declaration

Your signature _____ **Date** D D M M Y Y

For clinic use only (optional) Patient number _____

7 In the event of your mental incapacity

As part of your consent, you also need to decide what you would like to happen to your sperm or embryos if you lose the ability to decide for yourself (become mentally incapacitated).

In the event that you lose mental capacity, if you would like your partner to be able to use your sperm or embryos in their own treatment or treatment with a surrogate, your partner must be named in section 2 of this form. If a surrogacy arrangement would be required, you will need to receive relevant information, be offered counselling, undergo further screening tests and complete additional consent forms whilst you have capacity. It is therefore vitally important that you and your clinic discuss your wishes in the event of your mental incapacity and the different treatment options in those circumstances. Please ask your clinic if they have not already discussed this with you.

The person named at section 2 of this form will be the only person able to use your stored sperm or embryos in treatment if you lose mental capacity. If you do not name a person at section 2 of this form, then no one will be permitted to use your sperm or embryos for treatment purposes if you lose mental capacity (unless you subsequently regain capacity).

If your circumstances change after you have completed this form (for example, if you separate from the partner you have named on this form), or if you wish to withdraw your consent, you will need to contact your clinic to complete further forms.

Treatment and storage in the event of your mental incapacity

You can use this form to:

- **Consent to the continued storage of your sperm or embryos without consenting to the use of your sperm or embryos in treatment.** Then, in the event that you regain capacity, you may be able to use your sperm or embryos in treatment.

  OR

- **Consent to your sperm or embryos being stored and used for treatment in the event that you lose mental capacity.** If you have a partner and you would like them to be able to use your sperm or embryos in their own treatment or in treatment with a surrogate in the event that you lose mental capacity, your partner **must** be named in section 2 of this form. If you wish to consent for your sperm or embryos to be **used** in the event that you lose capacity, you must also consent to continued **storage** of your sperm or embryos after your loss of capacity.

The law permits your sperm or embryos to be stored for treatment purposes for up to 10 years from the date that a medical practitioner certifies in writing that you lack capacity. This storage period cannot be extended.

If you **select on this form** that you do **not** consent to **either storage or use** of your sperm or embryos in the event of your mental incapacity, then:

- Your sperm or embryos will be removed from storage and disposed of when you lose mental capacity.

If you consent **either** to storage **or** to storage and use of your sperm or embryos in the event of your mental incapacity:

- If you **do not** regain mental capacity within 10 years of being certified as lacking capacity:
  - Your clinic will be required to remove all your unused sperm from storage 10 years, or in the case of embryos 10 years and 6 months, from the date that a medical practitioner certified in writing that you lack capacity and dispose of them.

Continues on the next page »

Page declaration

Your signature

Date D D M M Y Y

For clinic use only (optional) Patient number

7 In the event of your mental incapacity

- If you **do** regain mental capacity within 10 years of being certified as lacking capacity:
  - **You may be able to use** your sperm or embryos in treatment.
  - **You may be able to renew** your consent to storage of your sperm or embryos for your treatment. You will need to notify your clinic as soon as possible if you are certified as having regained mental capacity and wish to renew your consent to storage of your unused sperm or embryos.
  - If you **do not** notify your clinic, then it will be required to remove all your unused sperm from storage 10 years, or in the case of embryos 10 years and 6 months, from the date that a medical practitioner certified in writing that you lack capacity and dispose of them.
  - Your embryos can only be used and stored during this 10 year period if there is also effective consent for use and storage from the egg provider (your partner or egg donor).

Use of your sperm or embryos for training purposes in the event of your mental incapacity

You can also consent to your sperm or embryos being used and stored after your loss of capacity for potential use by designated healthcare professionals to practice the techniques involved in fertility treatment. **To do this, you will need to speak to your clinic who will be able to provide you with more information.** If you do not consent to your sperm or embryos being used in training, if they can no longer be lawfully stored they will be removed from storage and disposed of.

Sperm

7.1 **Please tick the box next to the appropriate statement below to confirm your choice.**

Select **one** of either **A, B** or **C**.

☐ **A. In the event that I lose mental capacity, I consent to my sperm being stored for possible use in future treatment, but not used by another person whilst I have lost capacity.**

Please indicate how long you consent to storage of your sperm after you lose capacity:

☐ For 10 years from the date of being certified as lacking capacity, or

☐ For a shorter period - specify the number of years (not exceeding 10 years from the date loss of capacity was certified): _____

If you have selected 7.1A, the next question you need to answer is 7.2. Remember to sign the declaration on every page.

☐ **B. In the event that I lose mental capacity, I consent to my sperm being stored and used to create embryos outside the body, and to those embryos being stored and used in my named partner's treatment whilst I have lost capacity.**

Only select option B if you have a partner named at section 2 of this form.

Continues on the next page »

Page declaration

Your signature _____ **Date** D D M M Y Y

For clinic use only (optional) Patient number _____

7 In the event of your mental incapacity continued

If treatment would involve a surrogate, then additional consent forms and screening tests must have been completed before you lose capacity to allow treatment to take place.

Please indicate how long you consent to storage of your sperm after you lose capacity:

☐ For 10 years from the date of being certified as lacking capacity, or

☐ For a shorter period - specify the number of years (not exceeding 10 years from the date loss of capacity was certified):

Please indicate how long you consent to storage of embryos created from your sperm after your loss of capacity:

You should be aware that embryos can only be stored and used if the egg provider (your partner or egg donor) has also given consent.

☐ For 10 years from the date of being certified as lacking capacity, or

☐ For a shorter period - specify the number of years (not exceeding 10 years from the date loss of capacity was certified):

If you have selected question 7.1B, the next question you need to answer is 7.2. Remember to sign the declaration on every page.

☐ **C. In the event that I lose mental capacity, my sperm should no longer be stored for my (or my partner's) treatment.**

Embryos (created before your loss of capacity)

7.2 **Please tick the box next to the appropriate statement below to confirm your choice.**

Select **one** of either **A, B** or **C**.

☐ **A. In the event that I lose mental capacity, I consent to my embryos created before my loss of capacity being stored for possible use in future treatment, but not used by another person whilst I have lost capacity.**

You should be aware that embryos can only be stored if the egg provider (your partner or egg donor) has also given consent.

Please indicate how long you consent to storage of your embryos after you lose capacity:

☐ For 10 years from the date of being certified as lacking capacity, or

☐ For a shorter period - specify the number of years (not exceeding 10 years from the date loss of capacity was certified):

If you have selected 7.2A, please go to section 8. Remember to sign the declaration on every page.

Continues on the next page »

Page declaration

Your signature **Date**

D D M M Y Y

For clinic use only (optional) Patient number

7 In the event of your mental incapacity continued

B. In the event that I lose mental capacity, I consent to my embryos created before my loss of capacity being stored and used by my named partner whilst I have lost capacity.

Only select option B if you have a partner named at section 2 of this form.

You should be aware that embryos can only be stored and used if the egg provider (your partner or egg donor) has also given consent.

If treatment would involve a surrogate, then additional consent forms and screening tests must have been completed before you lose capacity to allow treatment to take place.

Please indicate how long you consent to storage of your embryos after you lose capacity:

- For 10 years from the date of being certified as lacking capacity, or
- For a shorter period - specify the number of years (not exceeding 10 years from the date loss of capacity was certified):

If you have selected 7.2B, please go to section 8. Remember to sign the declaration on every page.

C. In the event that I lose mental capacity, my embryos created before my loss of capacity should no longer be stored for my (or my partner's) treatment.

Other uses for your sperm or embryos if you die or become mentally incapacitated

If you wish your sperm or embryos to be used in the treatment of someone else (including your partner's treatment with a surrogate) if you die or become mentally incapacitated, please speak to your clinic for more information.

Depending on your circumstances, you will need to undergo additional screening, receive relevant information, be offered counselling and complete one or more of the following consent forms:

- 'Your consent to donating your sperm' (MD form)
- 'Your consent to donating embryos' (ED form)
- 'Your consent to the use and storage of sperm or embryos for surrogacy' (MSG form).

Page declaration

Your signature

Date D D M M Y Y

For clinic use only (optional) Patient number

8 Registration as legal parent after death

Complete section eight if you consented to your sperm, or embryos created outside the body with your sperm, being used in your partner's treatment after your death. If you have given your consent to your sperm or embryos (to be created outside the body with your sperm) being used after your death, you may also wish to consent to being registered as the legal parent of any child that is born as a result of your partner's treatment.

8.1 **Do you consent to being registered as the legal parent of any child born as a result of your partner's treatment with your sperm or embryos after your death?**

By ticking yes, you consent to the following:

- I consent to my name, place of birth and occupation being entered on the register of births as the legal parent of any child born from my partner's treatment using my sperm or embryos.

This register is kept under the Births and Deaths Registration Act 1953, or the Births and Deaths Registration (Northern Ireland) Order 1976, or the Registration of Births, Deaths and Marriages (Scotland) Act 1965.

- I also consent to information about my or my partner's treatment being disclosed to my partner and one of the following registrars:
  - the Registrar General for England and Wales
  - the Registrar General for Scotland
  - the Registrar for Northern Ireland.

You should be aware that being recorded in the register of births as the legal parent of a child born from your partner's treatment using your sperm or embryos does not transfer any inheritance or other legal rights to the child.

☐ Yes ☐ No

Page declaration

Your signature

Date D D M M Y Y

For clinic use only (optional) Patient number

9 Declaration

Please sign and date the declaration

Your declaration

- I declare that I am the person named in section one of this form.
- I declare that:
  - before I completed this form, I was given information about the different options set out in this form, and I was given an opportunity to have counselling.
  - the implications of giving my consent, and the consequences of withdrawing this consent, have been fully explained to me.
  - I understand that I can make changes to, or withdraw, my consent to the use and storage of my sperm for the purposes I have indicated above at any point until the time that my sperm has been used for treatment or training, or my sperm has been disposed of.
  - I understand that I can make changes to, or withdraw, my consent to the use and storage of my embryos for the purposes I have indicated above at any point before embryo transfer, use of embryos in training, or my embryos have been disposed of.
  - I understand that before the end of the consent period that I have indicated at section 4 above, I will be contacted by my clinic regarding additional consent to storage or renewal of my consent to storage, if applicable.
  - I understand that if I do not request an additional period of storage or renew my consent, my consent will be taken as withdrawn and my sperm or embryos will be removed from storage and disposed of.
- I understand that I must notify my clinic if my contact details or personal circumstances change. I understand it is my responsibility to keep contact details up to date.
- I declare that the information I have given on this form is correct and complete.
- I consent to the clinic (or any subsequent HFEA-licensed clinic that may become involved in my treatment, donation or storage, or a data controller in line with the Data Protection Act 2018 and UK General Data Protection Regulation) using the information on this form in the process of providing licensed activities (in accordance with the provisions of the Human Fertilisation and Embryology Act 1990 (as amended)), or for record storage and archiving purposes.

Your signature

Date

D D M M Y Y

For clinic use only (optional) Patient number

9 Declaration

If signing at the direction of the person consenting

If you have completed this form at the direction of the person consenting (because they are unable to sign for themselves due to physical illness, injury or disability), you must sign and date below. There must also be a witness confirming that the person consenting is present when you sign the form.

However, if the person is consenting to being registered as the legal parent after death (that is if they ticked yes to question 8.1), they **must** sign the form for themselves.

Representative's declaration

I declare that the person named in section one of this form is present at the time of signing this form and I am signing it in accordance with their direction.

Representative's name

Representative's signature

Relationship to the person consenting

Date

D D M M Y Y

Witness' name

Witness' signature

Date

D D M M Y Y

For clinic use only (optional) Patient number

Source: Human Fertilisation & Embryology Authority, United Kingdom.

Appendix 14. **Medical and social history questionnaire (NHS, UK)**

FRM4211/5.1 – Medical and Social History Questionnaire

NHS Blood and Transplant

Effective date: 18/03/2021

Tissue Donor Number | | | | | | | | | |

ODT Donor Number | | | | | | |

Medical and Social History Questionnaire

Directions for completion

1. This form must be completed in **black or dark blue ink** by the Specialist Requestor (SR)/Specialist Nurse – Organ Donation (SNOD)/Specialist Nurse – Tissue Donation (SNTD)/Tissue Donor Co-ordinator (TDC) and signed where required.

2. The original copy should be retained by the **SR/SNOD/SNTD/TDC** for the donor file.

3. In the event of organ and tissue donation, a legible copy should be sent to the relevant **Tissue Establishment**, where required.

NOTE: The term patient is used throughout the form to refer to the potential donor.

The term relative is used throughout the form to refer to the relationship between the patient and the interviewee.

FRM4211/5.1 – Medical and Social History Questionnaire

NHS Blood and Transplant

Effective date: 18/03/2021

Tissue Donor Number | | | | | | | | | | ODT Donor Number | | | | | | |

In order to ensure the safety of organs and tissue for transplant I will need to ask you some questions about *(name of patient)* medical and lifestyle history. Some of the questions are of a sensitive and personal nature. They are similar questions to those asked when someone donates blood. I will read and discuss each question with you and ask that you answer to the best of your knowledge with either a "Yes" or "No."

PATIENT INFORMATION

Patient's Forename(s) Please Print

Patient's Surname Please Print

Donating Hospital

NHS/CHI Number

Hospital Number

Date of Birth (dd/mm/yyyy)

Cause of Death

Occupation

Country of Birth

Country of residence

INTERVIEWEE INFORMATION

Information discussed with

Name Please Print

Relationship Please Print

For patients under the age of 18 months, or those who have been breast-fed or fed breast milk by a donor in the last 12 months, the mother is required to answer these questions with regard to her own and her child's health.

For children: has your child been breast-fed in the past 12 months? N/A ☐ No ☐ Yes ☐ Unknown ☐

NOTE: for all patients under the age of 18 months and any child who has been breast-fed in the last 12 months, a blood sample for microbiological testing is required from the mother, as well as from the patient.

For ALL female patients between 12 and 55 years of age*: Is there a possibility that your relative could be pregnant? N/A ☐ No ☐ Yes ☐ Unknown ☐

*DonorPath currently displays age range 13-53 years. This does not reflect current clinical guidance of 12-55 years documented in **MPD891**

FRM4211/5.1 – Medical and Social History Questionnaire

NHS Blood and Transplant

Effective date: 18/03/2021

Tissue Donor Number | | | | | | | | | | ODT Donor Number | | | | | | |

GENERAL HEALTH INFORMATION

1) Did your relative visit a general practitioner in the last two years? No ☐ Yes ☐ Unknown ☐

If YES, give details

2) Was your relative currently seeing or waiting to see a general practitioner or any other healthcare professional? No ☐ Yes ☐ Unknown ☐

If YES, give details

3) Did your relative ever take regular medication? No ☐ Yes ☐ Unknown ☐

If YES, give details of any current or previous medication including any medication for acne, prostate or psoriasis

4a) Did your relative have a history of allergies to medication, food or other substances? No ☐ Yes ☐ Unknown ☐

If YES, please provide details of the substance they were allergic to and describe the reaction

4b) Did your relative have any health problems due to exposure to toxic substances such as pesticides, lead, mercury, gold, asbestos, cyanide, agent orange etc? No ☐ Yes ☐ Unknown ☐

If YES, please provide details of the toxic substance and treatment

5a) Was your relative a diabetic? No ☐ Yes ☐ Unknown ☐

If YES, were they on insulin? N/A ☐ No ☐ Yes ☐ Unknown ☐

5b) Is there a family history of diabetes? No ☐ Yes ☐ Unknown ☐

If YES, is it insulin-dependent diabetes? N/A ☐ No ☐ Yes ☐ Unknown ☐

6. Did your relative suffer from any chronic or autoimmune illness or disease of unknown cause? No ☐ Yes ☐ Unknown ☐

If YES, give details including hospital name and dates of treatment if possible

7. Did your relative ever suffer from any bone, joint, skin or heart disease? No ☐ Yes ☐ Unknown ☐

If YES, specify which and give details

FRM4211/5.1 – Medical and Social History Questionnaire

NHS Blood and Transplant

Effective date: 18/03/2021

Tissue Donor Number | | | | | | | | | | ODT Donor Number | | | | | | |

8) Did your relative ever have hepatitis, jaundice or liver disease? No ☐ Yes ☐ Unknown ☐

If YES, give dates, diagnosis, treatment and hospital /clinic name if known

9) Did your relative recently suffer from significant unplanned weight loss? No ☐ Yes ☐ Unknown ☐

If YES, give details

10) Did your relative ever undergo any investigations for cancer or were they ever diagnosed with cancer? No ☐ Yes ☐ Unknown ☐

If YES, give details including hospital name and dates of treatment, if possible

11) Did your relative have a history of eye disease, receive any medications for eye problems (e.g. eye drops), or undergo eye surgery or laser treatment? No ☐ Yes ☐ Unknown ☐

If YES, give details including hospital name and dates of treatment, if possible

12) Did your relative ever have any operations? ***If NO go to question 15*** No ☐ Yes ☐ Unknown ☐

If YES, give details including hospital name and dates of treatment, if possible

13) Did your relative ever have any surgery on the brain or spine? N/A ☐ No ☐ Yes ☐ Unknown ☐

If YES, give details including hospital name and dates of treatment if possible. **Surgery before 1993** is particularly significant

14) Did your relative ever have an organ or tissue transplant? N/A ☐ No ☐ Yes ☐ Unknown ☐

If YES, give details including hospital name and dates of treatment if known

15) Was your relative ever told not to donate blood? No ☐ Yes ☐ Unknown ☐

If YES, give details of where, when and the reason

16) Did your relative receive a transfusion of blood or blood product(s) at any time? No ☐ Yes ☐ Unknown ☐

If YES, give details including country, hospital name, dates and reason for transfusion

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17) Did your relative suffer from any type of brain disease such as Parkinson or Alzheimer disease or dementia? No ☐ Yes ☐ Unknown ☐

If YES, give details including hospital name and dates of treatment if possible

18) Did your relative suffer from any one or more of the following problems: memory problems or confusion, change in personality or behaviour, or were they unsteady on their feet? ***If NO go to Question 19, if YES*** No ☐ Yes ☐ Unknown ☐

18a) When did these symptoms start?

Please give details

18b) Did they worsen noticeably over time? No ☐ Yes ☐ Unknown ☐

Please give details

18c) Was your relative able to live independently? No ☐ Yes ☐ Unknown ☐

Please give details

18d) Were you aware of a condition causing these symptoms? No ☐ Yes ☐ Unknown ☐

If YES, please specify condition

19) Did your relative have a family history of prion disease, such as CJD, or were they ever told that they were at risk of prion disease? No ☐ Yes ☐ Unknown ☐

If YES, give details

20) Did your relative ever receive human pituitary extracts, e.g. growth hormones or fertility treatment or test injections for hormone imbalance? No ☐ Yes ☐ Unknown ☐

If YES, give details including dates and hospital/clinic name if known

21) Did your relative ever have any significant infection? No ☐ Yes ☐ Unknown ☐

If YES, give details, and any treatment received and hospital/clinic name if known

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RECENT HISTORY

22) Did your relative come into contact with an individual with an infectious disease within the last month? No ☐ Yes ☐ Unknown ☐

If YES, please specify details, dates, symptoms, diagnosis, and treatment

23) Did your relative have any signs of infection, e.g. colds, flu, fever, night sweats, swollen glands, diarrhoea, vomiting or skin rash within the last month? No ☐ Yes ☐ Unknown ☐

If YES, please specify dates, symptoms, diagnosis, and treatment

24) Did your relative have any immunisations within the last 2 months? No ☐ Yes ☐ Unknown ☐

If YES, give details including travel vaccinations and flu vaccination or flu nasal spray

25) Did your relative have tattooing, body piercing, botox injections, acupuncture, colonic irrigation, faecal transplantation, or any other cosmetic treatments or injuries that involve piercing the skin in the last 3 months? No ☐ Yes ☐ Unknown ☐

If YES, give details including where and when including unlicensed clinics in UK or abroad

26) In the last 12 months has your relative been bitten or scratched by any animal (strays, pets, wild, farm or ticks) or been bitten by a human? Or, has your relative ever been bitten or in close contact with bats anywhere in the world or been bitten by a mammal outside the UK? No ☐ Yes ☐ Unknown ☐

If YES, give details of incident, circumstances, animal, place, dates and treatment

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TRAVEL HISTORY

27) Did your relative ever travel or live outside the UK (including business trips)? No ☐ Yes ☐ Unknown ☐

If NO go to question 33

28) In the last 12 months did your relative go outside the UK (including business trips)? N/A ☐ No ☐ Yes ☐ Unknown ☐

Give details of dates and destinations visited

29) Did your relative ever have malaria or an unexplained fever which they could have picked up whilst abroad? N/A ☐ No ☐ Yes ☐ Unknown ☐

If YES, give date of fever/illness, places visited, duration and dates

30) Was your relative ever unwell whilst abroad or in the first month of their return to the UK? N/A ☐ No ☐ Yes ☐ Unknown ☐

If YES, give details

31) Did your relative ever live or travel outside the UK for a continuous period of 6 months or more? N/A ☐ No ☐ Yes ☐ Unknown ☐

If YES, give details of dates and destinations

32) Did your relative ever go to Central America, Mexico or South America for a continuous period of 1 month or more? N/A ☐ No ☐ Yes ☐ Unknown ☐

If YES, give details of dates, places (remote/rural/urban areas), nature of visit

33) Was your relative's mother born in Central America, Mexico or South America? N/A ☐ No ☐ Yes ☐ Unknown ☐

If YES, give details (including date)

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Tissue Donor Number | | | | | | | | | | ODT Donor Number | | | | | | |

BEHAVIOURAL RISK ASSESSMENT

34) Did your relative

a) Consume alcohol? No ☐ Yes ☐ Unknown ☐

If YES, give details (including type and amount)

b) Smoke tobacco or any other substance? No ☐ Yes ☐ Unknown ☐

If YES, give details of substance, frequency, history of smoking time and time elapsed since giving up.

c) Take any recreational drugs? No ☐ Yes ☐ Unknown ☐

If YES, give details including route of administration and dates

35) Is it possible that any of the following apply to your relative?

a) Was or may have been infected with HIV, hepatitis or HTLV? No ☐ Yes ☐ Unknown ☐

b) Within the last 12 months have they injected, or been injected, with non-prescription drugs, including performance enhancing drugs or injectable tanning agents? No ☐ Yes ☐ Unknown ☐

c) Been in prison or a juvenile detention centre for more than 3 consecutive days in the last 12 months? No ☐ Yes ☐ Unknown ☐

d) Taken medication to prevent HIV infection e.g. (PrEP Pre/Post exposure prophylaxis)? No ☐ Yes ☐ Unknown ☐

If YES to any of the above questions a-d, give details, including dates.

36) Has your relative **ever** had sex – consensual or otherwise? N/A ☐ No ☐ Yes ☐ Unknown ☐

If NO, go to question 38.

If **YES**, is it possible that your relative:

a) Was given payment for sex with money or drugs in the last 3 months? N/A ☐ No ☐ Yes ☐ Unknown ☐

b) Ever had a sexually transmitted disease? N/A ☐ No ☐ Yes ☐ Unknown ☐

c) Ever had sex with anyone who was diagnosed with a Viral Haemorrhagic Fever N/A ☐ No ☐ Yes ☐ Unknown ☐

If YES, to any of the above, give details, including dates and where appropriate hospital/clinics, treatments.

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37)	Did your relative have sex, consensual or otherwise, in the last 3 months?	N/A		No		Yes		Unknown	
	If no, go to question 38. If yes, is it possible that in the last 3 months your relative had sex with								
	a) *(for male patients only)* another man?	N/A		No		Yes		Unknown	
	b) *(for female patients only)* a man who has ever had sex with another man?	N/A		No		Yes		Unknown	
	c) Anyone who is HIV or HTLV positive?	N/A		No		Yes		Unknown	
	d) Anyone who has hepatitis?	N/A		No		Yes		Unknown	
	e) Anyone who had a sexually transmitted disease?	N/A		No		Yes		Unknown	
	f) Anyone who has ever been given payment for sex with money or drugs?	N/A		No		Yes		Unknown	
	g) Anyone who in the last 12 months has injected or been injected with non-prescription drugs including performance enhancing drugs or injectable tanning agents?	N/A		No		Yes		Unknown	
	h) Anyone who could have had sex, in any part of the world, where AIDS/HIV is very common (this includes most countries in Africa)?	N/A		No		Yes		Unknown	
	i) Anyone who has developed an illness related to travel such as Zika?	N/A		No		Yes		Unknown	
	If YES, give details								
38)	Having answered all the previous questions, is there anyone else who you think may provide more information?	N/A		No		Yes		Unknown	
	If YES, please specify								

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ODT Donor Number | | | | | | |

Question number	Relevant additional information. If any questions have been answered as unknown, give an explanation

Signature of healthcare professional obtaining information

Please Print Name

Designation of healthcare professional obtaining information

Date of interview | | | | | | | | |

Time of Interview | | | | |

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Tissue Donor Number | | | | | | | | | |

ODT Donor Number | | | | | | |

CALCULATION FOR PLASMA DILUTION:		
Record ALL fluid administered in the 48 hours prior to taking sample for microbiology screening		
Fluid given. (Please give name, not just colloid or crystalloid)	Date and time administered	Vol. in millilitres

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ODT Donor Number | | | | | | |

Does a haemodilution calculation need to be carried out?	Yes		No		Reason	

A haemodilution calculation must be performed if the patient has:

1. Been transfused with blood or blood products.
2. Received infusions of synthetic colloids and/or crystalloids following blood loss*.

A - If the donor has received blood products/components, a pre-transfusion sample must be sought irrespective of volume of blood or blood product. This sample must be submitted for microbiological characterisation of the donor.

B - If haemodilution calculation is ≥50%, a pre-dilution sample must be sought.
If pre-transfusion or pre-dilution samples cannot be found, the Microbiology laboratory and all recipient centres/ tissue establishments must be informed and this documented.
* Decision on significance of blood loss should be discussed with clinical ITU team and made on a case by case basis.

CALCULATION FOR PLASMA DILUTION:

CRYSTALLOID INFUSED:

INTERVAL PRIOR TO SAMPLING	VOLUME INFUSED (ml)	% RETAINED	VOLUME RETAINED (ml)
>24 HOURS		0	None
2-24 HOURS		25	
1-2 HOURS		50	
<1 HOUR		75	
		TOTAL CRYSTALLOID RETAINED :	

BLOOD / COLLOID INFUSED:

INTERVAL PRIOR TO SAMPLING	VOLUME INFUSED (ml)	% RETAINED	VOLUME RETAINED (ml)
24-48 HOURS		100 (Blood) 50 (Colloid)	
0-24 HOURS		100	
TOTAL BLOOD / COLLOID RETAINED:			

Patient's weight: _____

ESTIMATED TOTAL BLOOD VOLUME:
70ml per kilogram of body weight
50ml per kilogram of body weight in ICU

% HAEMODILUTION

$$\frac{\text{(CRYSTALLOID RETAINED + BLOOD/COLLOID RETAINED) X 100}}{\text{BLOOD VOLUME}}$$

ACCEPT (<49%)/REJECT (≥50%)

Signature of healthcare professional obtaining information

Please Print Name

Date of interview | | | | | | | | |

Time of Interview | | | | |

Source : National Health Service, United Kingdom.

Appendix 15. **Physical assessment form (NHS, UK)**

FRM5545/5.2 – OTDT Body Map

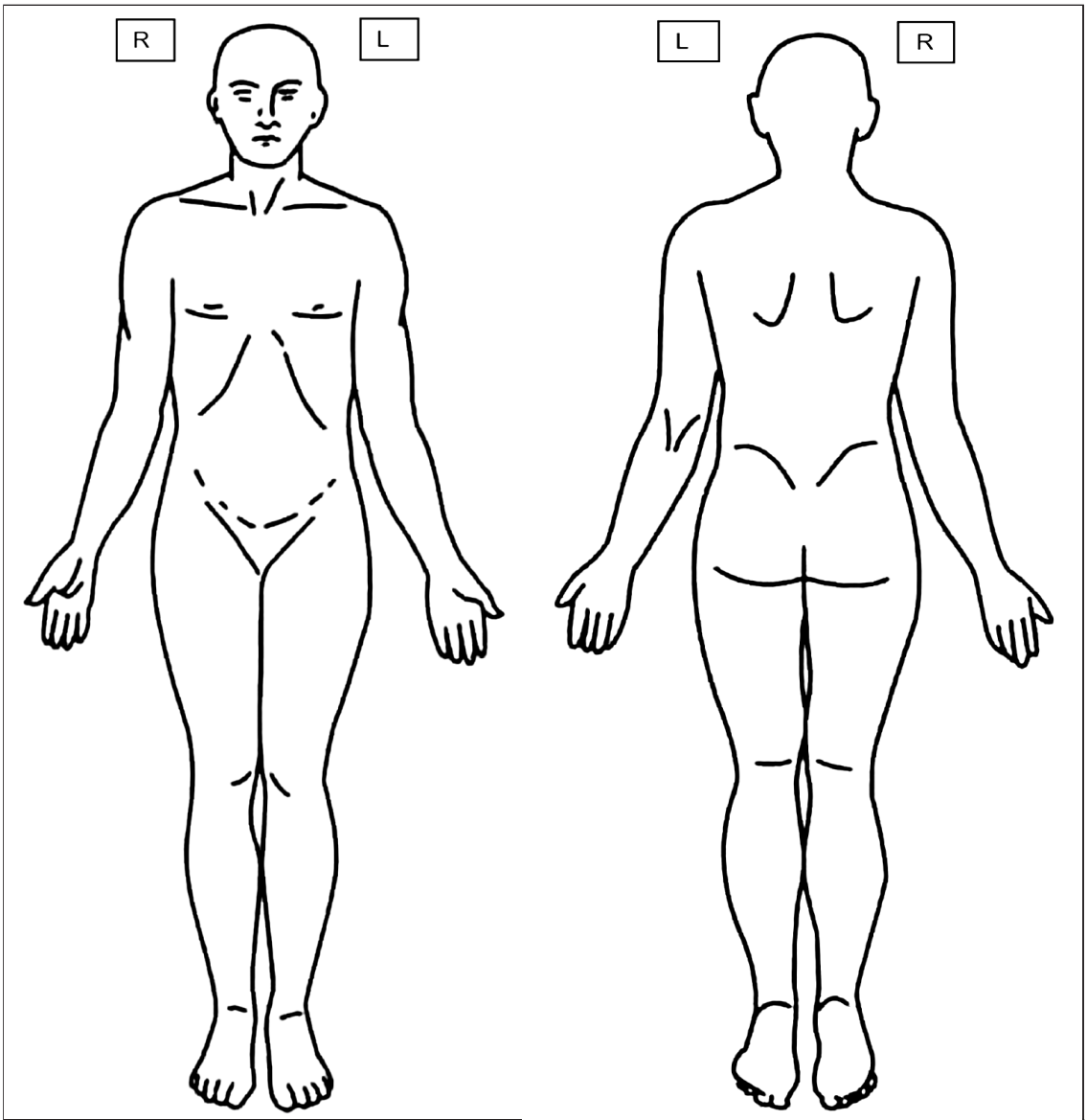
NHS
Blood and Transplant
Effective date: DRAFT

ODT Donor Number: | | | | | | |

NHSBT Donor Number: | K | | | | | | | | |

TES Donation Number: Affix Barcode Here

Date: _____ Time: _____



PHYSICAL EXAMINATION

Please mark areas examined, however limited, and note relevant features using the codes given in the key below. Please also note any areas that have not been examined.

REPORT SIGNIFICANT/ UNUSUAL FINDINGS TO THE NATIONAL REFERRAL CENTRE

KEY:

A = ET Tube	B = Incision Closed	C = Bruise	D = I.V Point
E = Dressing	F = Scar	G = Tattoo	H = Jewellery
I = Open wound	J = Body Piercing	K = Infection site	L = GI Tubing
M = ID Band	N = Puncture	O = Abrasion	P = NG Tubing
Q = Catheter	R = CVC/C line	S = Arterial line	

Text highlight for ODT only

Controlled if copy number stated on document and issued by QA
(Template Version 03/02/2020)

Cross-Referenced in Primary Document: SOP6405

FRM5545/5.2 – OTDT Body Map

NHS
Blood and Transplant
Effective date: DRAFT

ODT Donor Number: | | | | | | |

NHSBT Donor Number: | K | | | | | | | | |

TES Donation Number: Affix Barcode Here

Height (cms)	**ODT only**
Height (inches)	**ODT only**
Girth (cms)	**ODT only**
Weight (kgs) (est/actual)	**ODT only**
Chest (cms) (on request)	**ODT only**
Is there any evidence of jaundice?	**TES only** **Yes / No**

Witness (ODT only): .....

Confirmation of areas that physical exam was performed:

HEAD AND FRONT of BODY / WHOLE BODY

In accordance with NHSBT guidelines and HTA regulations, I confirm that I have conducted the donor's physical examination to the best of my ability and with the highest possible accuracy.

Print Name: ..... **Sign:** .....

Any Additional Comments:

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(Template Version 03/02/2020)

Page 2 of 2

Cross-Referenced in Primary Document: SOP6405

Source: National Health Service, United Kingdom.

Appendix 16. **Evaluation of pigmented skin lesions**

During physical examination of the donor, particular attention is given to the skin, looking for potential neoplasms or scars of previous surgical procedures (see Chapter 6).

The 'ABCDE rule' is an easy guide to detecting usual signs that may be indicative of melanoma [1-3]:

A. Asymmetry

A lesion should be considered suspicious if one half does not match the other in shape, colour, or thickness.

B. Border irregularity

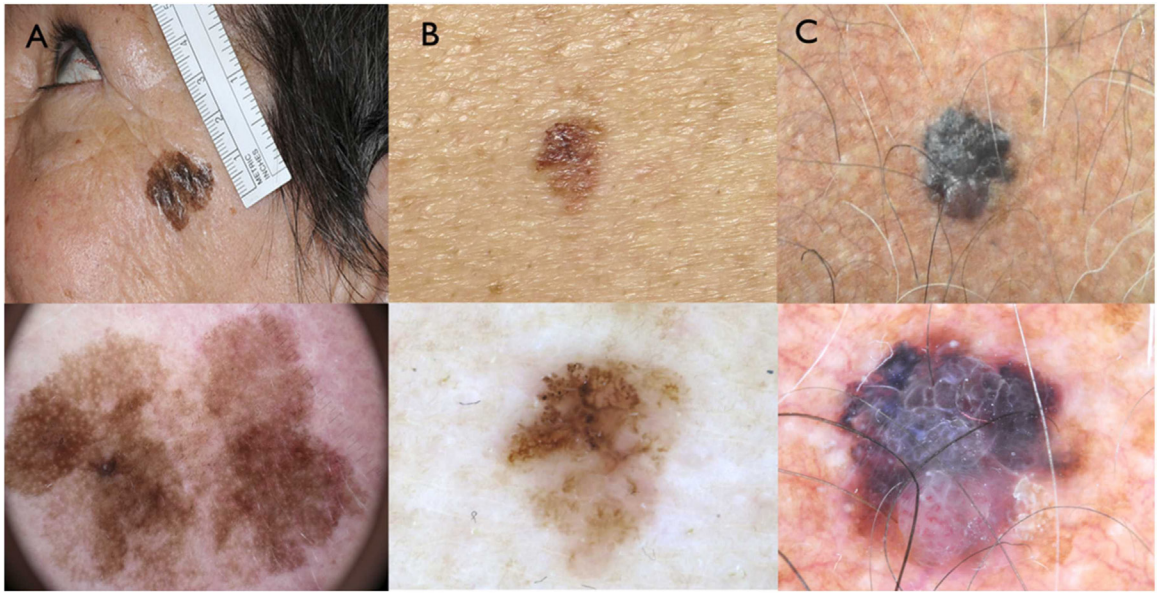
Borders that are uneven, notched, scalloped, blurred, or poorly defined raise concern for melanoma.

C. Colour

Naevi usually have a uniform colour; if there is colour variability from black-brown to red-blue-grey or white suspect melanoma.

D. Diameter/Difference

If the diameter is > 6 mm, warrant attention. Small lesions with some of the previous characteristics also lead us to suspect melanoma. If there are multiple lesions with a more or less regular aspect but among them there is one that has a very "ugly" aspect compared to the rest (ugly duckling sign), suspect melanoma.



Source: Claus G, *et al.* European consensus based interdisciplinary guideline for melanoma. Part 1: Diagnostics – update 2024. *Eur J Cancer* 2025;215:115152. https://doi.org/10.1016/j.ejca.2024.115152.

E. Evolution

Any change in a lesion over time is a key warning sign. Changes in size, shape, colour, elevation, or the appearance of new symptoms such as bleeding, itching, or crusting may indicate malignancy.

Other warning signs are:

- Naevi are the most powerful predictor of risk of melanoma. An individual with more than 100 common naevi or more than two atypical naevi has a 5- to 20-fold increased risk of melanoma.
- People with a first-degree relative with melanoma are at increased risk of developing melanoma; and 5-10% of individuals with melanoma have a family history of melanoma. If there is a suspicious lesion and there is family history of melanoma, suspect melanoma.

References

1. Friedman RJ, Rigel DS, Kopf AW. Early detection of malignant melanoma: the role of physician examination and self-examination of the skin. *CA Cancer J Clin* 1985;35(3):130-51.
2. Whited JD, Grichnik JM. The rational clinical examination. Does this patient have a mole or a melanoma? *JAMA* 1998;279(9):696-701.
3. Gachon J, Beaulieu P, Sei JF *et al*. First prospective study of the recognition process of melanoma in dermatological practice. *Arch Dermatol* 2005;141(4):434-8.

Appendix 17. **Evaluation of malignancies for risk assessment in SoHO donors**

Table A17.1 Grading of selected central nervous system tumours
Table A17.2 Recommendations on the use of organs from donors with CNS tumours
Table A17.3 Recommendations on the use of organs from donors with non-CNS cancers
Table A17.4 Carcinoma ***in situ*** denomination depends on the location
Table A17.5 Risk assessment in the case of a donor's heart for heart valves with diagnosed CNS tumour in the donor
Table A17.6 Four steps to risk assessment

Table A17.1 **Grading of selected central nervous system tumours**[a]

Diffuse astrocytic and oligodendroglial tumours	**1**	**2**	**3**	**4**
Astrocytoma, IDH-mutant		■	■	■
Diffuse astrocytoma, MYB- or MYBL1-altered	■			
Anaplastic astrocytoma, IDH-mutant			■	
Glioblastoma, IDH-wildtype				■
Glioblastoma, IDH-mutant				■
Diffuse midline glioma, H3K27M-mutant				■
Oligodendroglioma, IDH-mutant and 1p/19q-codeleted		■	■	
Anaplastic oligodendroglioma, IDH-mutant and 1p/19q-codeleted			■	

Other astrocytic tumours	**1**	**2**	**3**	**4**
Pilocytic astrocytoma	■			
Subependymal giant cell astrocytoma	■			
Pleomorphic xanthoastrocytoma		■		
Anaplastic pleomorphic xanthoastrocytoma			■	

[a] *Source*: adapted from: Louis DN, Ohgaki H, Wiestler OD *et al.* (2016), *World Health Organization histological classification of tumours of the central nervous system*. Geneva: WHO Press/Lyon: International Agency for Research on Cancer; and Louis DN, Wesseling P, Brat DJ *et al.* The 2021 WHO classification of tumors of the Central Nervous System: a summary. *Neuro-Oncology* 2021;23(8):1231-51.

Ependymal tumours	1	2	3	4
Supratentorial ependymoma		■	■	
Posterior fossa ependymoma		■	■	
Myxopapillary ependymoma		■		
Ependymoma, RELA fusion-positive		■	■	
Anaplastic ependymoma			■	

Other gliomas	1	2	3	4
Angiocentric glioma	■			
Chordoid glioma of third ventricle		■		

Choroid plexus tumours	1	2	3	4
Choroid plexus papilloma	■			
Atypical choroid plexus papilloma		■		
Choroid plexus carcinoma			■	

Tumours of the pineal region	1	2	3	4
Pineocytoma	■			
Pineal parenchymal tumour of intermediate differentiation		■	■	
Pineoblastoma				■
Papillary tumour of the pineal region		■	■	

Meningiomas	1	2	3	4
Meningioma	■	■	■	
Atypical meningioma		■		
Anaplastic (malignant) meningioma			■	

Embryonal tumours	1	2	3	4
Medulloblastoma (all subtypes)				■
Embryonal tumour with multi-layered rosettes, C19MC-altered				■
Medulloepithelioma				■
CNS embryonal tumour, NOS				■
Atypical teratoid/rhabdoid tumour				■
CNS embryonal tumour with rhabdoid features				■

Neuronal and mixed neuronal-glial tumours	1	2	3	4
Dysembryoplastic neuroepithelial tumour	■			
Gangliocytoma	■			
Ganglioglioma	■			
Anaplastic ganglioglioma			■	
Dysplastic gangliocytoma of cerebellum (Lhermitte–Duclos)	■			
Desmoplastic infantile astrocytoma and ganglioglioma	■			
Papillary glioneuronal tumour	■			
Rosette-forming glioneuronal tumour	■			
Central neurocytoma		■		
Extraventricular neurocytoma		■		
Cerebellar liponeurocytoma		■		

Tumours of the cranial and paraspinal nerves	**1**	**2**	**3**	**4**
Schwannoma	■			
Neurofibroma	■			
Perineurioma	■			
Malignant peripheral nerve sheath tumour (MPNST)		■	■	■

Mesenchymal, non-meningothelial tumours	**1**	**2**	**3**	**4**
Solitary fibrous tumour/haemangiopericytoma	■	■	■	
Haemangioblastoma	■			

Tumours of the sellar region	**1**	**2**	**3**	**4**
Craniopharyngioma	■			
Granular cell tumour	■			
Pituicytoma	■			
Spindle cell oncocytoma	■			

Table A17.2 **Recommendations on the use of organs from donors with CNS tumours**[b]

Absolute contraindications
• Primary cerebral lymphoma
• All secondary intracranial tumours
• Any cancer with metastasic spread

Intracranial tumours with an intermediate risk of cancer transmission
(2.2% with an upper 95% CI of 6.4%) include WHO grade 4 tumours and equivalents:
• Glioblastoma (IDH wild type and IDH mutant)
• Giant cell glioblastoma
• Gliosarcoma
• Pineoblastoma
• Medulloblastoma (all subtypes)
• Medulloepithelioma
• Atypical teratoid/rhabdoid tumour
• Malignant peripheral nerve sheath tumour (MPNST) – grade 4
• Embryonal tumour (all subtypes)
• Choriocarcinoma
• Diffuse midline glioma, H3K27 M-mutant

[b] *Source*: Advisory Committee on the Safety of Blood Tissues and Organs (SaBTO). Transplantation of organs from deceased donors with cancer or a history of cancer. London: Department of Health and Social Care, December 2020.

Intracranial tumours with a low risk of transmission
(< 2%) include WHO Grade 3 and equivalents:
• Anaplastic astrocytoma, IDH mutant
• Anaplastic oligodendroglioma, IDH mutant and 1p/19q deleted
• Ependymoma, RELA fusion-positive
• Choroid plexus carcinoma
• Anaplastic ganglioglioma
• Pineal parenchymal tumour of intermediate differentiation
• Papillary tumour of the pineal region
• Malignant peripheral sheath tumour grade 3
• Anaplastic/malignant meningioma
• Papillary tumour of the pineal region
• Haemangiopericytoma/ solitary fibrous tumour
• Anasplastic ependymoma
• Anaplastic pleomorphic xanthoastrocytoma

Table A17.3 **Recommendations on the use of organs from donors with non-CNS cancers**[c]

Absolute contraindications
• Active cancer with spread outside the organ
• Active haematological malignancy

High risk (> 10% risk of transmission)
• Melanoma: without spread (except as below)
• Breast: cancer other than those identified below
• Colon: cancer other than those identified below
• Kidney: renal cell cancer > 7 cm or stages 2-4
• Sarcoma: > 5 years previously and resected
• Small cell cancer: lung/neuroendocrine
• Lung cancer: stage 1

Intermediate risk (2-10% risk of transmission)
Kidney: renal cell carcinoma stage 1b (pT1b) (4-7 cm, Fuhrman/nucleolar grade 1 or 2) with curative surgery and cancer-free period of > 5 years
Breast: stage 1A (pT1, No; < 20 mm tumour size) with curative surgery and cancer-free period of between 5 and 10 years

Low risk (0.1-2% risk of transmission)
• Melanoma: superficial spreading type, non-ulcerated, with tumour thickness < 0.8 mm with curative surgery and cancer-free period of > 5 years
• Breast: stage 1A (pT1 No; < 20 mm tumour size), with curative surgery and cancer-free period of > 10 years
• Colon: stage 1A (pT1 or pT2, No) adenocarcinoma with curative surgery and cancer-free period of > 5 years
• Thyroid: solitary papillary carcinoma 0.5-2.0 cm
• Thyroid: minimally invasive follicular carcinoma 1.0-2.0 cm
• Kidney: resected solitary renal cell carcinoma > 1.0cm and < 4 cm and Fuhrman (nucleolar) grade 1 or 2 with curative treatment and cancer free > 3 years
• Prostate: Gleason =7, with curative treatment and cancer free > 5 years
• Treated gastrointestinal stromal cancers: < 2 cm, < 5% mitotic count, in stomach or duodenum

[c] *Source*: Advisory Committee on the Safety of Blood Tissues and Organs (SaBTO). Transplantation of organs from deceased donors with cancer or a history of cancer. London: Department of Health and Social Care, December 2020.

Minimal risk (< 0.1% risk of transmission)
• Skin: basal cell carcinoma
• Skin: squamous cell carcinoma with no metastases
• Skin: non-melanoma skin cancer *in situ*
• Uterine cervix: *in situ* cancer
• Thyroid: solitary papillary carcinoma (< 0.5 cm)
• Thyroid: minimally invasive follicular carcinoma (< 1.0 cm)
• Bladder: superficial non-invasive papillary carcinoma (pTa, G1/G2)
• Kidney: resected solitary renal cell carcinoma < 1.0 cm and Fuhrman (nucleolar) grade 1/2
• Prostate: Gleason < 6, or < 7 with curative treatment and cancer free > 5 years

Note. Only those cancers where evidence is available for analysis have been classified. Cancers not included in this guidance have to considered on a case-by-case basis following appropriate professional consultation.

Table A17.4 **Carcinoma *in situ* denomination depends on the location**[d]

Organ	**Type of tumour**	**Carcinoma *in situ***
Oesophagus		High-grade dysplasia, defined as malignant cells confined to the epithelium by the basement membrane
Small intestine		High-grade dysplasia/carcinoma *in situ*
Appendix	Carcinoma	Intramucosal carcinoma, invasion of the lamina propria into but not through the muscularis mucosa
	(LAMN)	• Low-grade appendiceal mucinous neoplasm confined by the muscularis propria • Acellular mucin or mucinous epithelium may invade into the muscularis propia
Anus		High-grade squamous intraepithelial lesion (previously termed carcinoma *in situ*, Bowen disease, anal intraepithelial neoplasia II-III, high-grade intraepithelial neoplasia)
Exocrine pancreas		• High-grade intraepithelial neoplasia (Panin-3) • Intraductal papillary mucinous neoplasm with high-grade dysplasia • Intraductal tubulopapillary neoplasm with high-grade dysplasia • Mucinous cystic neoplasm with high grade dysplasia
Lung		• Squamous cell carcinoma *in situ* (SCIS) • Adenocarcinoma *in situ* (AIS): adenocarcinoma with pure lepidic pattern, ≤ 3 cm in greatest dimension
Breast	LCIS	Lobular carcinoma *in situ* is considered a benign entity
	DCIS	Ductal carcinoma *in situ*
	Paget	Paget disease of the nipple not associated with carcinoma or DCIS in the underlying breast parenchyma. Carcinomas in the breast parenchyma associated with Paget disease are categorised based on the size and characteristics of the parenchymal disease, although the presence of Paget disease should still be noted
Penis	Tis	Carcinoma *in situ* (penile intraepithelial neoplasia PeIN)
	Ta	Non-invasive located squamous cell carcinoma
Renal pelvis and ureter/ urinary bladder/urethra/ prostatic urethra	Ta	Papillary non-invasive carcinoma
	Tis	Urothelial carcinoma *in situ* "flat tumour"
Parathyroid		Atypical parathyroid neoplasm (neoplasm of uncertain malignancy)

Table A17.5 **Risk assessment in the case of a donor's heart for heart valves with diagnosed CNS tumour in the donor**
See Figure 6.1 (Chapter 6) – Decision tree algorithm; for non-haematological and non-CNS tumour in deceased tissue donors.

[d] *Source*: American Joint Committee on Cancer. *AJCC Cancer Staging Manuals*, 8th edition, 2016. Last update 5 June 2018. See www.cancerstaging.org. Last accessed 5 April 2022.

In general, the tissues that can be collected from deceased donors are corneas, heart valves, arteries, musculoskeletal and skin. A previous history or a presence of malignancy requires a careful donor evaluation to avoid any risk to recipients.

Due to the special characteristics of corneas, they are excluded from this risk assessment. See Chapters 6 and 20 for more information.

Because haematological malignancies are a general contraindication for the acceptance of these tissues, the focus here is on non-haematological malignancies and specifically those that are not carcinoma *in situ*.

For this risk assessment, it is important to know in detail the exact histology of the tumour entity, its stage and grade, its previous treatment and the cancer-free period. Another point that could influence the final decision is whether there is a scarcity of the donated tissue that could increase the benefit to the recipient, putting this benefit in front of the risk of waiting more time for the next available tissue.

When there is a tumour with dissemination (metastasis) it is considered an absolute contraindication for tissue donation. In the case of Central Nervous System (CNS) tumours, those grade 3 or 4 tumours (such as glioblastoma multiform) have low or intermediate risk in the case of organ transplantation. In the past, the general idea discussed among the scientific community has been that CNS tumours did not produce metastasis outside the brain. At present, although metastasis is a rare phenomenon, it has been documented from different brain tumours and numerous paediatric brain tumours in different locations [1].

Therefore, the next question would be: In the case of a tissue donor with any CNS tumour, could the heart be donated for heart valves? Is there a risk of finding metastases in the heart from a CNS tumour?

Several aspects are considered during this risk assessment:

- Metastases to the heart from other primary tumours are 30 times more common than primary cardiac tumours [2].
- In patients with known malignancies, the frequency of cardiac metastases in the general population in autopsy is 0.7%-3.5% and up to 9.1% [3].
- Cardiac metastases mostly appear in patients with a disseminated tumour, and isolated metastases to the heart are very rare [4].
- The dissemination routes are: haematogenous spread; lymphatic spread; transvenous extension and direct extension [3].
- Two thirds of all cardiac metastases affect the pericardium, one third affect the epicardium or the myocardium and only 5% affect the endocardium [5].
- The most frequent metastases come from malignant melanoma, mesothelioma, malignant germ cell tumours, lung cancer, undifferentiated carcinoma, breast carcinoma, ovarian carcinoma, lymphoproliferative neoplasm (lymphoma, leukaemia), gastric carcinomas, renal carcinomas, pancreatic carcinoma and oesophageal cancer [2-9].
- Malignant cells can be identified in the majority of malignant pericardial effusions drained by pericardiocentesis, and the cytology of malignant cells has an extraordinary correlation with the primary histological diagnosis [3, 5].
- Tumour nodules may be found within the myocardium and on the endocardial surface. Individual masses may range from 1-2 mm to > 2 cm [7].
- In all previous references, there is no case of CNS tumour metastases to the heart; in some publications, this issue is remarked upon [10, 11].
- In paediatric donors, the epidemiology of cardiac metastases is very similar to that in adults. Although it is not a CNS tumour, one case of cardiac metastases with neuroblastoma has been described [12].
- Cardiac or pericardial metastases can be considered whenever a patient with a known malignancy develops cardiovascular symptoms [4].

Using this information, a risk assessment will proceed. The first question to answer is:

- Is there any risk of tumour transmission through the use of heart valves from a donor with CNS tumours (including glioblastoma multiforme)? See Table A17.6 Four steps to risk assessment.

Table A17.6 **Four steps to risk assessment**

The first step is to assess the probability of this situation happening.

With the previous information the score is 1.

In the case of paediatric donors with neuroblastoma, the score could be 2.

Probability score	Description	Criteria
1	improbable	unlikely but possible
2	remote	unlikely but can be reasonably expected to occur
3	occasional	will occur from time to time
4	probable	occurs often
5	frequent	continually experienced

The second step is to assess severity.

In the case that there are unknown malignant cells in the heart valves and a tumour transmission has occurred, what is the severity of this in the recipient?

If the transmission occurred, the score could be 3 or 4.

Severity score	Impact	Criteria
1	negligible	temporary discomfort or inconvenience to user or patient
2	minor	temporary injury to patient or user (e.g. minor temporary irritation) not requiring medical intervention
3	serious	significant injury or side effect requiring unanticipated medical intervention
4	critical	permanent impairment, life-threatening injury
5	catastrophic	death

The next step is the classification of the risk.

With a probability of 1 and severity of 3 or 4, the risk is low.

In the case of a paediatric patient with neuroblastoma, probability of 2 and severity 3 or 4, the risk is medium.

	Severity 1	Severity 2	Severity 3	Severity 4	Severity 5
Probability 1	1 Low	2 Low	3 Low	4 Low	5 Medium
Probability 2	2 Low	4 Low	6 Medium	8 Medium	10 High
Probability 3	3 Low	6 Medium	9 Medium	12 High	15 Very high
Probability 4	4 Low	8 Medium	12 High	16 Very high	20 Extreme
Probability 5	5 Medium	10 High	15 Very high	20 Extreme	25 Extreme

The risk management action by risk category is shown on the right.

Risk category	Action
Low	Acceptable if there is no cost-effective option to reduce further the risks
Medium	Make efforts to reduce risks within defined timescales
High/Very High	Unacceptable, unless huge benefit and no safer and equally effective option is available. Have to take all measures to decrease risk if activity is to proceed
Extreme	Stop activity or do not start

To calculate the Priority Risk Number (PRN) in the FMECA mode, we need detectability:

- Obvious
- Easy to detect
- Difficult to detect
- Very difficult to detect
- Impossible to detect

If only a macroscopic examination is performed, small metastases cannot be appreciated. In that case, the detectability could be 4. The PRN would be:

Probability of 1 × severity of 4 × detectability 4 = 16

If we examine the pericardium liquid and a pathologist does a whole heart examination, the detectability could be 2. The PRN would be:

Probability of 1 × severity of 4 × detectability 2 = 8

Applying the same criteria with children, with only macroscopic examination, the PRN would be:

Probability of 2 × severity of 4 × detectability 4 = 32

With macroscopic examination, the PRN would be:

Probability of 2 × severity of 4 × detectability 2 = 16

In conclusion, after performing the risk assessment explained above, accepting any donor with a CNS tumour has a low risk for tumour transmission when considering the heart valves, and the donation can be accepted but, to reduce the risk, a whole examination by a pathologist can be considered.

References

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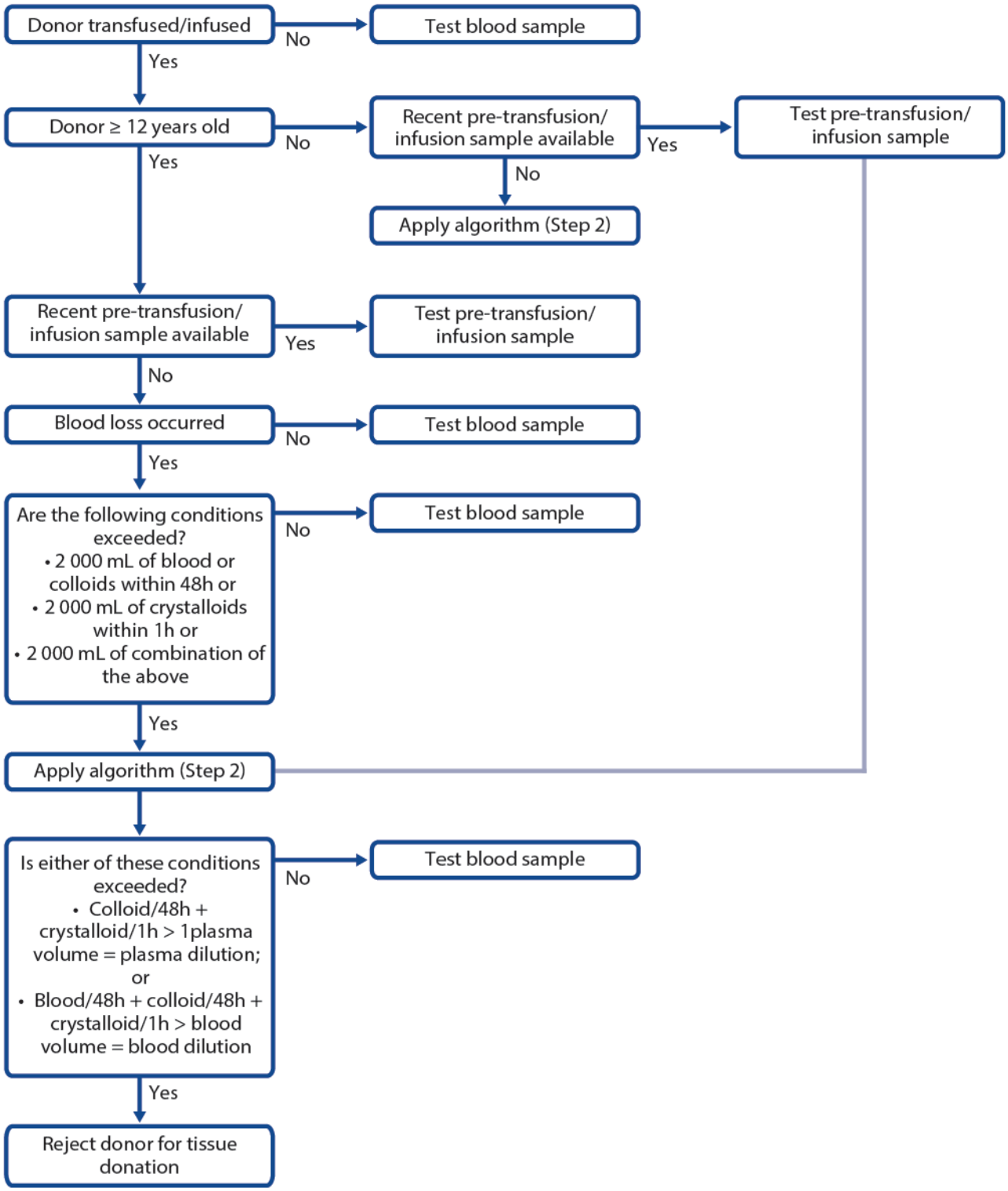
Appendix 18. **Sample haemodilution algorithm**

This sample haemodilution algorithm is based on the US Food and Drug Administration (FDA) Guidance document cited at the end of this appendix.

The following definitions, as used in this sample haemodilution algorithm, are taken from the same source (US FDA Guidance, Chapter V, p. 31):

> Blood component means a product containing a part of human blood separated by physical or mechanical means (§1271.3i).
>
> Colloid means: (1) a protein or polysaccharide solution, such as albumin, dextran or hetastarch, that can be used to increase or maintain osmotic (oncotic) pressure in the intravascular compartment; or (2) blood components such as plasma and platelets (§1271.3j).
>
> Crystalloid means an isotonic salt and/or glucose solution used for electrolyte replacement or to increase intravascular volume, such as saline solution, Ringer's lactate solution, 5% dextrose in water (§1271.3k) or total parenteral nutrition.
>
> Plasma dilution means a decrease in the concentration of the donor's plasma proteins and circulating antigens or antibodies resulting from the transfusion of blood or blood components and/or infusion of fluids (§1271.3p).

Step 1. Donor evaluation pathway



Donor ID:		Date & Time blood sample taken:	

Step 2A

Calculate plasma volume	Donor weight (kg) _____ 0.025	_____ mL
Calculate blood volume	Donor weight (kg) _____ 0.015	_____ mL
A) Record total volume of blood transfused in the **48 h** prior to death or sample collection (whichever comes first)	_____ mL of RBC transfused/48 h _____ mLof whole blood transfused/48 h _____ mL of reconstituted blood/48 h	Sum A:_____ mL
B) Record total volume of colloid infused in the **48 h** prior to death or sample collection (whichever comes first)	_____ mL plasma/48 h _____ mL platelets/48 h _____ mL albumin/48 h _____ mL HES or other colloids/48 h	Sum B: _____ mL
C) Record total volume of crystalloid infused in the **1 h** prior to death or sample collection (whichever comes first)	_____mL	Sum C:_____ mL

Step 2B

Calculate plasma volume	_____mL	Sum B + Sum C	_____mL
Calculate blood volume	_____mL	Sum A + Sum B + Sum C	_____mL

Step 2C

Calculate plasma dilution	Is Sum B + Sum C > plasma volume?	No	Yes
Calculate blood dilution	Is Sum A + Sum B + Sum C > blood volume?	No	Yes

Determination of sample acceptability for infectious disease tests

If the answers in step 2C to **both questions** are NO, the post-transfusion/infusion sample is acceptable.

If the answer in step 2C to **either** 1 **or** 2 is YES, the post-transfusion/infusion sample is not acceptable; use a pre-transfusion/infusion sample or reject the donor.

HES: hydroxyethyl starch

RBC: red blood cells

Source: US Food and Drug Administration. Guidance for industry: eligibility determination for donors of human cells, tissues, and cellular and tissue-based products (HCT/Ps), August 2007, Appendix 2 at www.fda.gov/downloads/biologicsbloodvaccines/guidancecomplianceregulatoryinformation/guidances/tissue/ucm091345.pdf, accessed 13 September 2026.

Appendix 19. **Example of validation screening: infectious disease assays of blood from deceased donors**

Introduction

The reliability of the results of screening for infectious diseases in blood samples from deceased individuals is critical and can be enhanced substantially if appropriately validated assays are used. Assays which have not been validated by the manufacturers for use with blood samples from deceased donors are expected to be individually validated with such blood samples to support performance of deceased donor screening.

There are three key issues to consider before any validation work. The potential issues associated with screening blood from deceased individuals are:

- occurrence of *post mortem* degradation, or fall in detectable level, of a screening target (a marker of infectious disease such as an antigen or antibody related to the infectious agent);
- inhibition of the assay by substances accumulating from *post mortem* changes in blood;
- the potential for a blood sample from a deceased donor to be haemodiluted.

In all three cases there is a possibility for a false-negative test result.

Validation assay

To validate assay performance when using blood samples from deceased donors, the following apply:

a. The collection times for the blood samples from deceased donors used for assay validation are expected to be representative of the full range of time points typically encountered during tissue collection, specifically from immediately after death up to 24 h after death.

b. All information about storage and handling conditions for blood tubes from time of blood collection to time of testing is documented and meets any assay sample-handling requirements stated.

c. Each blood sample from a deceased donor used for validation is evaluated for haemodilution using an approved algorithm.

d. Use a dilution series prepared in deceased donor material, or use spiked specimens inoculated with the relevant infectious-disease marker at a potency near the assay's cutoff and vary the sources used for spiking. In both cases, test in parallel with the same material diluted in serum or plasma from a living individual.

e. Test a sufficient number of samples from different deceased donors (≥20), include haemolysed samples.

f. The sample storage methods (e.g. refrigerated, frozen) used for validation mimic the method of storage that is routine for that laboratory.

Assay evaluation is undertaken to determine the overall performance of an assay, specifically including its core sensitivity and specificity.

Validation is done to determine the suitability of an already evaluated assay for use for a specific purpose in a laboratory; for example, for use with a blood specimen type (in this case, from a deceased donor) not specifically stated as known to be acceptable by the manufacturer.

Evaluations are undertaken using panels of provenanced samples of known status and importantly include seroconversion panels (i.e. sets of sequential samples from infected individuals following the course of infection from pre-exposure to late infection). However, such samples are almost all obtained from living patients. Suitable comparable 'positive' specimens

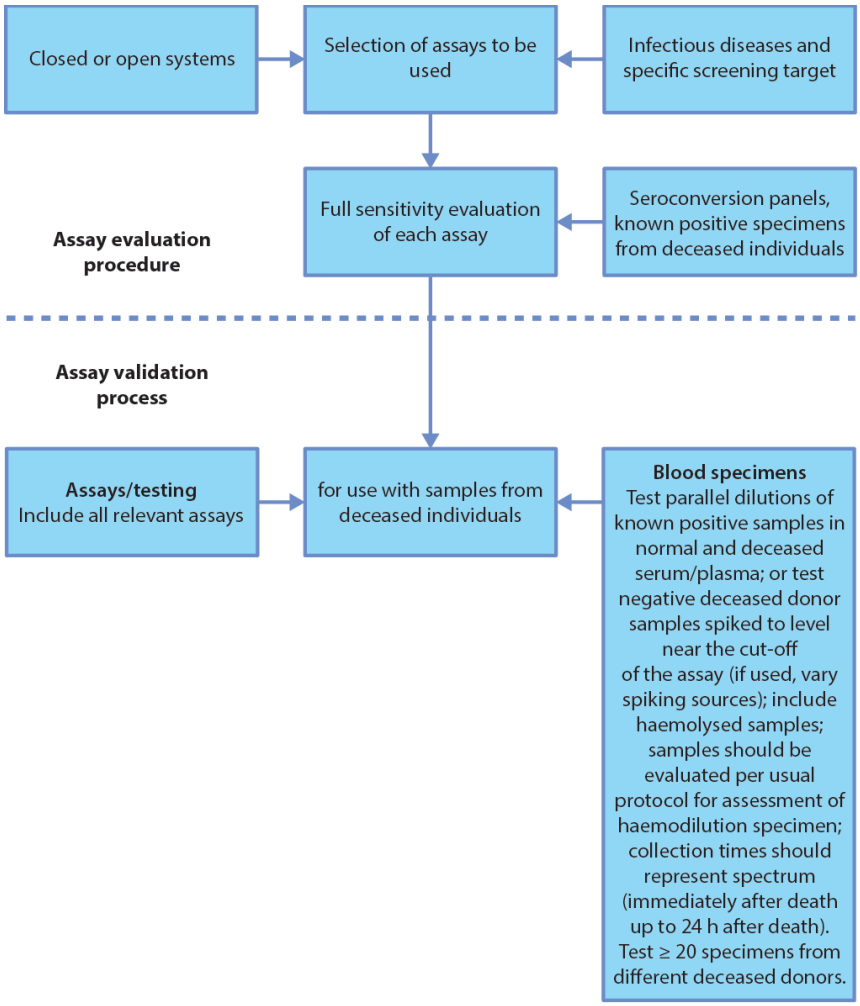
in a series from deceased individuals are not available, making this specific type of evaluation not possible. An alternative, less realistic approach but one that has been accepted by licensing authorities is to spike non-reactive blood specimens from deceased donors with known and varying levels of virus to attempt to mimic a 'true' positive specimen [1, 2].

Before using assays with blood specimens from deceased individuals, the performance of the assays is assessed in some way to make attempts to demonstrate there is no loss of, or other change in, the expected performance of the assay.

Validation of assays for use with blood from deceased individuals is critical, both for serological assays that detect antigens or antibodies and for molecular assays that detect viral nucleic acid. However, this is simultaneously problematic due to the key issues stated above and a lack of suitable blood samples from deceased individuals to support such work. The issue of the degradation of any screening target that may be present is the hardest to deal with, due to lack of suitable published studies. However, if the timeframe for sample collection *post mortem* is limited by those carrying out tissue collection, it can be theorised that it would be unlikely for any markers of a previously unidentified and relevant infection to have been degraded to a level that is undetectable using the high-quality assays available from major international diagnostics manufacturers.

If this aspect is controlled, the next issue to consider is the potential 'inhibitory effect' of the sample as a result of any *post mortem* changes. To some degree the same argument applies in regard to degradation of the screening target *post mortem* because specimen collection within a suitable timeframe minimises the extent of any *post mortem* changes, whatever they are (e.g. red cell haemolysis, precipitants/byproducts of cell death, etc.). There are ways in which an inhibitory effect can be examined in serological and molecular assays. Known positive serum/plasma samples can be diluted in serum plasma from living individuals in parallel with serum/plasma from deceased individuals and from living individuals, either to a fixed point or to extinction, and the outcomes compared. In this aspect, validation of molecular assays is slightly easier than that of serological assays because the inclusion of an internal control in molecular assays validates each test result with respect to the presence of any 'inhibitory substance' that may be present in a sample. If the internal control is not amplified, the test result for the sample is deemed to be invalid. Therefore, although not definitive, such approaches would identify

Figure A19.1 **Validation scheme**



Flowchart courtesy of Alan Kitchen with input from Susan Best

any gross effects, which would most likely result in false-negative results.

Summary

To meet donor/donation screening expectations, validation of infectious-disease assays for use with blood from deceased individuals is undertaken. If the assays have been evaluated appropriately by the testing laboratory, then there is clear understanding of the baseline performance of each assay, and this can be used as the basis of additional assay validation work for use with blood samples from deceased donors.

Lack of suitable samples to mirror evaluation panels is a particular problem, leaving comparative dilutional studies and/or spiked sample studies as feasible approaches to determine any problems associated with testing blood from deceased individuals.

References

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Appendix 20. **Sample form to assess working environment (NHS, UK)**

FRM3831/14.1 – Tissue Services Tissue Donation From Deceased Donors

NHS Blood and Transplant

Effective date: 19JAN2026

Venue:	Donation Number and Donor Number:

The intent of the below is to assess the suitability of the working environment for safety to both staff and tissue.

Minimum Requirements:

	Yes	No	Comments/Action
Do you believe that tissue can be retrieved with minimal or no environmental contamination?			
Are there specific donor related risks and actions taken to mitigate these risks e.g. large donor?			
Is there good lighting and a sink with running water available?			
Are the fire exits and evacuation procedures known?			
Is there suitable access to minimise carrying and handling e.g. parking?			
Can unauthorised people view the donation? e.g. doors open, blinds open, clear glassed windows?			
Is there unauthorised filming / photography equipment in use? E.g. if cameras present, detail if live or recorded, who can view?			

Site Assessment:

	Yes	No	Comments / Action
Are the floors wet?			
Do any surfaces appear dirty / contaminated?			
Are there any sharp objects/dangerous equipment/clutter around i.e. hazards to avoid/move?			
Is the donation area a clean environment (if necessary clean with detergent prior to use)?			
Is a post-mortem being carried out at the same time as the donation?			
Is the support equipment working and used where applicable? e.g. trolleys, control panels, 'in use' signs etc.			

Was the Donors face protected prior to moving to donation area?	YES / NO If No, explain:
Person Responsible for moving donor to / from donation area:	Porters / APTs / TS Staff / Others Others please specify:
Date and time donor removed from fridge	Date: Time: Information given by:

Authorisation to proceed:

Are you satisfied that this is a safe / clean working environment? Yes / No
If No, contact Retrieval Team Manager / Duty Manager ASAP
Name of Manager contacted:

Signed: **Date:**

Source: National Health Service (NHS), United Kingdom.

Appendix 21. **Sample donor-identification form (NHS, UK)**

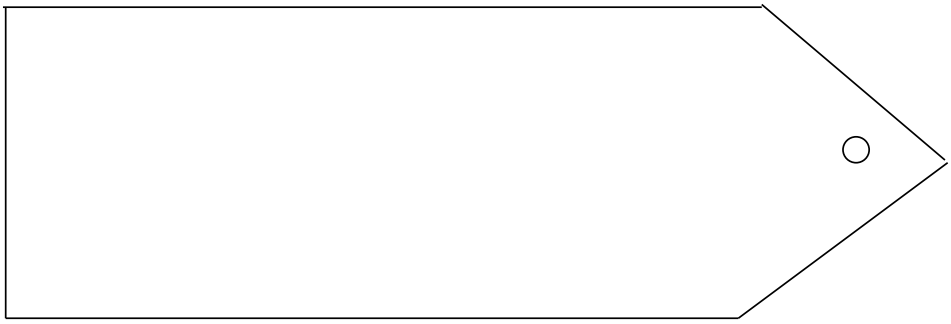
FRM3831/14.1 – Tissue Services Tissue Donation From Deceased Donors

NHS
Blood and Transplant
Effective date: 19JAN2026

Donation Number:

Confirm correct donor by transcribing identification details directly from identity band/label. You **must** have 3 points of matching ID (e.g. name, dob, hospital number, address, or (exceptionally) circumstances of death) which correspond with authorisation.

Method of ID: Toe tag / Identity band / Mortuary label / Other……



COPY TAG

3 points of matching ID confirmed by (PRINT NAME)	Sign:	Date:
Identification Double checked by (PRINT NAME)	Sign:	Date:

ID queries and resolution / outcome	SIGNATURE AND DATE

Source: National Health Service, United Kingdom.

Appendix 22. **Checklist for system audit of computerised systems**

Establishment .....

Date.....

Signature of person responsible for the revision .....

1. Identification of the system and function		Comments
Name of the system		
Version		
Supplier		
Platform		
Function		
Connected with other computerised systems		

2. Organisation	Comments
☐ Is the organisation for function and maintenance described in the QM system (system owner, system manager, person responsible)? ☐ Are these functions placed in an organisation scheme?	
☐ Is the responsibility of the supplier described in the QM system and in written agreements? ☐ Is it clear that the user is responsible for validation when data are transferred between different systems?	
☐ Have the responsible persons (functions) received enough and documented training in case of malfunction of the system?	
☐ Is the computerised system included in the scheme for internal revision?	

3. Written agreements	Comments
☐ Are responsibilities for support (software and hardware) clearly defined? ☐ Are functions/responsibilities of subcontractors included?	
☐ Are instructions for documentation of unexpected events included? ☐ Is time-limit for corrective actions by responsible support defined?	
If data are transferred between different computerised systems: ☐ Are platforms and protocols described? ☐ Are obligations to inform each other about changes and events that may influence information transfer included? ☐ Are responsibilities for the different parts in the chain between the systems clearly defined?	

4. Documentation of the system	Comments
☐ Is complete and updated documentation of the system accessible? ☐ Does the documentation contain measures for managing malfunctions and fallbacks? Is a user guide with version number accessible: ☐ in paper copy? ☐ as electronic 'help-function'?	

5. Maintenance	Comments
☐ Are standard operating procedures available for measures in case of malfunction/total downtime? ☐ Back-up system? ☐ Reset of data? ☐ Are back-up system and read-back functions tested?	

6. Changes	Comments
☐ Is a test environment available? ☐ Are validation procedures defined and performed before updates, changes, new versions in the system?	

7. Information security	Comments
☐ Is access to the computers protected by locked doors (physical data protection)?	
☐ Is a virus protection system active (if applicable)?	
Is access to the computerised system protected by personal login? ☐ Single-level login ☐ Double-level login	
☐ Is access to the system (and login) associated with a certificate of authorisation? ☐ Who decides on, and keep records of, access to the system? ☐ Are records for access to the system updated (i.e. access removed when no longer needed)?	
☐ Does the system provide traceability of the user? ☐ Does the system provide traceability of changes in manually added data/text (with the original text still readable)?	
If data are manually inserted/transferred from another system: How is correctness of the data verified: ☐ by data insert of two individuals independently? ☐ by saving the original (paper) result? ☐ by signature(s) of the individual(s) inserting the data?	
If data are automatically transferred from another computerised system: ☐ Are 'check points' to verify the correctness of data transfer available in the system or as standard operating procedures?	

Adapted from SWEDAC DOC 10:5, 2025 (3rd edn), Guide for information security work (available from www.swedac.se/wp-content/uploads/2025/06/swedac-doc-10-5-en.pdf) with interpretation of ISO/IEC 17025:2017, 'General requirements for the competence of testing and calibration laboratories' (available from www.iso.org/standard/66912.html) and ISO/IEC 27007:2020 (available from www.iso.org/standard/77802.html).

Appendix 23. **Serious adverse reaction or event: impact assessment tool**

This impact assessment tool assists practitioners and regulators in planning their response to a given serious adverse reaction or event (SARE), taking into account the broader consequences beyond the individual patient affected or potentially affected. The assessment is to be based on available data, past experience and scientific expertise.

Although the assessment is common to all types of SoHO, it can be divided into non-reproductive and reproductive.

Assessment for non-reproductive SoHO

Step 1: Assessment of the likelihood of occurrence/recurrence of the SARE

1	Rare	Difficult to believe it could happen again
2	Unlikely	Not expected to happen again
3	Possible	May occur occasionally
4	Likely	Expected to happen again, but not persistently
5	Probable	Expected to happen again on many occasions

Step 2: Assessment of the impact/consequences of the SARE should it recur

Impact level		On individual(s)		On the system		On SoHO supply
0	Insignificant	Nil	OR	No effect	OR	Insignificant
1	Minor	Non-serious	OR	Minor damage	OR	Some applications postponed
2	Moderate	Serious	OR	Damage for short period	OR	Many cancellations or postponements
3	Major	Life-threatening	OR	Major damage to system – significant delay to repair	OR	Significant cancellations – importation required
4	Catastrophic or extreme	Death	OR	System destroyed – need to rebuild	OR	All allogeneic applications cancelled

Step 3: Application of the impact matrix

Likelihood of recurrence ▶ / Impact of recurrence ▼	1 Rare	2 Unlikely	3 Possible	4 Likely	5 Certain/almost certain
0 Insignificant	0	0	0	0	0
1 Minor	1	2	3	4	5
2 Moderate	2	4	6	8	10
3 Major	3	6	9	12	15
4 Catastrophic/extreme	4	8	12	16	20

Step 4: The response

The response of a SoHO establishment or Health Authority to a specific SARE is to be proportionate to the potential impact, as assessed by the matrix shown in Step 3 above and described below.

White — The SoHO establishment is to manage the corrective and preventive actions; the Health Authority is to file the report and keep a 'watching brief'.

Pale shading — Requires interaction between the SoHO establishment and the Health Authority, which may request an inspection that focuses on the SARE and the corrective and preventive actions to be followed up, including evidence of effective recall, where necessary. Written communication to professionals working in the field might be appropriate.

Dark shading — The Health Authority will, in general, designate representatives to participate in developing or approving the corrective and preventive action plan (possibly a task force to address broader implications). Inspection, follow-up and written communication is done at the previous level; and possibly notification of health authorities in other countries where relevant.

Effectiveness of the response can be assessed by re-applying the impact matrix following implementation of corrective and preventive actions. The impact can be reduced by:

- reducing the probability of recurrence through preventive measures;
- increasing the detectability of the risk; or
- reducing the severity of the consequences, if it should recur.

Source: Adapted from SoHO V&S Guidance for Competent Authorities.

Assessment for reproductive SoHO

Step 1: Assessment of the likelihood of occurrence/recurrence of the SARE

1	Rare	Difficult to believe it could happen again
2	Unlikely	Not expected to happen again
3	Possible	May occur occasionally
4	Likely	Expected to happen again, but not persistently
5	Probable	Expected to happen again on many occasions

Step 2: Assessment of the impact/consequences of the SARE should it recur

Impact level		On individual(s)		On the system		On SoHO supply
0	Insignificant	Nil	OR	No effect	OR	Insignificant
1	Minor	Non-serious	OR	Minor damage or some procedures postponed	OR	Partial loss of gametes/embryos for one couple
2	Moderate	Serious	OR	Damage for short period. Many procedures cancelled or postponed	OR	Partial loss of gametes/embryos for one couple
3	Major	Life-threatening	OR	Major damage to system – significant delay to repair. Significant numbers of procedures cancelled	OR	Partial loss of gametes/embryos for one couple
4	Catastrophic or extreme	Death	OR	System destroyed – need to rebuild. All procedures cancelled	OR	Total loss of gametes/embryos for all couples

Step 3: Application of the impact matrix

Likelihood of recurrence ▶ / Impact of recurrence ▼	1 Rare	2 Unlikely	3 Possible	4 Likely	5 Certain/almost certain
0 Insignificant	0	0	0	0	0
1 Minor	1	2	3	4	5
2 Moderate	2	4	6	8	10
3 Major	3	6	9	12	15
4 Catastrophic/extreme	4	8	12	16	20

Step 4: The response

The response of a SoHO Establishment or Health Authority to a specific SARE is proportionate to the potential impact, as assessed by the matrix shown in Step 3 above and described below.

White — The SoHO establishment is to manage the corrective and preventive actions; the Health Authority is to file the report and keep a 'watching brief'.

Pale shading — Requires interaction between the SoHO Establishment and the Health Authority, which may request an inspection that focuses on the SARE and

the corrective and preventive actions to be followed up, including evidence of effective recall, where necessary. Written communication to professionals working in the field might be appropriate.

Dark shading The Health Authority will, in general, designate representatives to participate in developing or approving the corrective and preventive action plan (possibly a task force to address broader implications). Inspection, follow-up and written communication is done as at the previous level; and possibly notification of health authorities in other countries where relevant.

Effectiveness of the response can be assessed by re-applying the impact matrix following implementation of corrective and preventive actions. The impact can be reduced by:

- reducing the probability of recurrence through preventive measures;
- increasing the detectability of the risk; or
- reducing the severity of the consequences, if it recurs.

Source: Adapted from SoHO V&S Deliverables 5 and 8.

Appendix 24. Example of serious adverse reaction (SAR) for non-reproductive SoHO

Vigilance notification to the SoHO establishment

An orthopaedic surgeon calls the SoHO establishment because the recipient of a peroneal longus tendon has an infection in the surgical site. The culture of the surgical site is positive for *Staphylococcus epidermidis* sensible to oxacilin, ceftaroline, amikacyn, clindamicyn, rifampicyn, quinupristin-Dalfoprostin. The orthopaedic surgeon suspects that the infection originates from the graft because the recipient had no previous infection or risk factors to develop an infection.

The recipient is receiving antibiotic treatment: theicoplanin 800 mg 1c/24 h and ceftazidime 2gc/8 h.

After 3 days the infection required wound debridement and graft removal.

The SoHO establishment sends the notification of the adverse reaction to the biovigilance officer and starts the investigation as a potential serious adverse reaction (SAR).

History of donor

A 55-year-old tissue donor. Admitted at the hospital due to myocardial infarction and died of cardiac arrest 48 hours after admission. Medical antecedents: high blood pressure and smoker. Family accepted tissue donation (corneas, musculoskeletal and skin). Collection took place 8 hours after asystole.

Immediate actions and investigation

Immediately after receiving the notification, the SoHO establishment puts into quarantine all the tissues still available in the SoHO establishment from the same donor.

The SoHO establishment's investigation/ review

1. Assessment of information about the donor and collection.

   - The medical records of the donor: two blood cultures obtained at the collection time were negative.
   - Musculoskeletal collection: 12 cultures were negative.
   - Skin collection: 2 positive cultures (non-pathogenic sp.) out of 6 at the time of collection.
   - Microbiological and quality control of the clean room during the processing of the musculoskeletal tissue: no deviation found.
   - Swab culture of the graft at the moment of implant performed by the orthopaedic surgeon in the hospital laboratory: negative.

2. Assessment of the outcome of other transplantations with tissues from the same donor.

   - Check the clinical outcome of the transplantation procedure in other recipients that received tissues from the same donor (6 tissues to 5 recipients):
     - two cornea recipients: no incident, in particular no infectious complications;
     - two tendons to one recipient: tissue culture negative at the moment of transplant and the recipient without signs of infection;

- two femoral heads to two recipients: tissue cultures negative at the moment of transplant and both recipients without signs of infection.

Conclusions

After investigation the case is considered a SAR. The rationale used for the analysis of this SAR has been performed based on the information provided in section 18.6.4 of Chapter 18 and Appendix 23:

- Imputability: was judged as 1 (possible) because the evidence is indeterminate. There are no clear alternative causes (0. Excluded) but neither is there evidence in favour of attributing the reaction to the tissue (2. Probable); see Table 18.2.
- The probability of the likelihood of occurrence/recurrence was judged as 3 (possible), i.e. may occur occasionally.

Step 1: Assessment of the likelihood of occurrence/recurrence of the SARE

1	Rare	Difficult to believe it could happen again
2	Unlikely	Not expected to happen again
3	Possible	May occur occasionally
4	Likely	Expected to happen again, but not persistently
5	Probable	Expected to happen again on many occasions

The impact or the consequence was judged as follows:

- On the individual level the impact was 3 (major) for the recipient/individual (the graft had to be removed).
- On the system level the impact was 1 (minor), based on the follow-up of other recipients of a graft from this donor.
- On tissues and cell supply level, the impact was 2 (moderate) because this event had a negative impact on tissues availability and assignment because the tissues of this donor were in quarantine for a period of time while investigation was ongoing.

Step 2: Assessment of the impact/consequences of the SARE should it recur

Impact level		On individual(s)		On the system		On SoHO supply
0	Insignificant	Nil	OR	No effect	OR	Insignificant
1	Minor	Non-serious	OR	Minor damage	OR	Some applications postponed
2	Moderate	Serious	OR	Damage for short period	OR	Many cancellations or postponements
3	Major	Life-threatening	OR	Major damage to system – significant delay to repair	OR	Significant cancellations – importation required
4	Catastrophic or extreme	Death	OR	System destroyed – need to rebuild	OR	All allogeneic applications cancelled

Using the impact matrix gave a total score of 6, resulting in classification of this reaction in the pale shading on the table. This meant that the SoHO establishment and Health Authority had to interact and analyse potential corrective and preventive measures. Also required was a written communication of the investigation to the orthopaedic surgeon who instigated the notification (please see Appendix 23 for more detailed information).

Step 3: Application of the impact matrix

Likelihood of recurrence ▶ / Impact of recurrence ▼	1 Rare	2 Unlikely	3 Possible	4 Likely	5 Certain/almost certain
0 Insignificant	0	0	0	0	0
1 Minor	1	2	3	4	5
2 Moderate	2	4	6	8	10
3 Major	3	6	9	12	15
4 Catastrophic/extreme	4	8	12	16	20

Preventive measures and corrective measures implemented

- Initially tissues were quarantined as a preventive measure. In cases of suspected contamination at collection, tissues from the same donor were also cultured after thawing to check for microbial growth, even if they tested negative after processing.
- Once investigation was finished, the SoHO establishment decided to unblock the tissue still in their care.
- The SoHO establishment will reinforce the need to notify any incident or reaction among final users and authorised centres.
- The SoHO establishment has not been able to establish a corrective measure because there were no findings of protocol deviations or uncontrolled risk during the donation process.

Learning outcome

Once the graft is removed, we might culture the removed graft (at the transplant centre or the SoHO establishment) to check if the graft once explanted is positive for the same micro-organism that was found at the surgical site or for other micro-organisms.

References

1. Shabana NS, Seeber G, Soriano A, Jutte PC, Westermann S, Mithoe G, *et al*. The clinical outcome of early periprosthetic joint infections caused by *Staphylococcus epidermidis* and managed by surgical debridement in an era of increasing resistance. *Antibiotics (Basel)* 2022;12(1):40. https://doi.org/10.3390/antibiotics12010040.
2. W. Mamane, E. Masmejean. Phlegmon des gaines des tendons fléchisseurs des doigts. Pages 250-257.
3. Fiche technique bactériologie 191. *Staphylococcus epidermidis*. Centre Toulousain pour le Contrôle de qualité en Biologie clinique. EN.FTBAC. 23-06-19.01, émis le 9 avril 2019.

Appendix 25. **Example of serious adverse reaction (SAR) for reproductive SoHO**

Vigilance notification to the SoHO Establishment

A few months after birth, Leigh Syndrome (autosomal recessive/Ndufv1 mutation) was detected in a baby born following a double non-partner gamete donation with intracytoplasmic sperm injection.

The paediatrician who diagnosed the genetic abnormality considered that it might be linked to the donors. Reporting this case might prevent further distribution of gametes/embryos from that donor and the transmission of this genetic disease to other potential children born by using the gametes. The investigation started and the case was also communicated to the Health Authority.

History of donor

- The oocyte donor was 28 years of age. She had a normal karyotype and was asymptomatic. A total of five healthy babies were born from previous donations to other couples.
- The sperm donor was 27 years of age. He had a normal karyotype. A total of three healthy babies were born from his previous donations.

Investigation and immediate actions

It was the first time that this particular combination of oocyte donor and sperm donor was used.

The specific Ndufv1 gene mutation was not included in the expanded carrier genetic test at that time.

Analysis of the incidence of the serious adverse reaction

The respective SoHO establishments were informed about the potential serious adverse reactions (SAR) and advised to hold the remaining sperm cells and oocytes in quarantine pending investigation.

There were no remaining sperm nor oocytes stored, but there were three embryos that had been created with the donated gametes.

A repeated analysis of germline DNA of both donors for this specific gene mutation showed that both donors were carriers of the Ndufv1 gene mutation c.248 C> T, Ser 83 Leu.

Conclusions

After investigation and confirmation of the genetic link to the donated gametes, the case was considered a SAR. The rationale used for the analysis of this SAR was based on the information provided in section 18.6.4 of Chapter 18 and Appendix 23:

- Imputability: Definitive/certain (3), as the expanded genetic test confirms that the transmission is linked to the donors, see Table 18.2.
- The probability of the likelihood of occurrence/recurrence was judged as 2 (unlikely).

Step 1: Assessment of the likelihood of occurrence/recurrence of the SARE

1	Rare	Difficult to believe it could happen again
2	Unlikely	Not expected to happen again
3	Possible	May occur occasionally
4	Likely	Expected to happen again, but not persistently
5	Probable	Expected to happen again on many occasions

- The impact of the consequences: a recent multicentre study on Leigh syndrome survival shows that the mean age before death occurs is 2.4 years, and only 20% survived, with a maximum age of 20 years old. Thus, the impact was judged as 4 (catastrophic or extreme).

Step 2: Assessment of the impact/consequences of the SARE should it recur

Impact level		On individual(s)		On the system		On SoHO supply
0	Insignificant	Nil	OR	No effect	OR	Insignificant
1	Minor	Non-serious	OR	Minor damage or some procedures postponed	OR	Partial loss of gametes/ embryos for one couple
2	Moderate	Serious	OR	Damage for short period. Many procedures cancelled or postponed	OR	Partial loss of gametes/ embryos for one couple
3	Major	Life-threatening	OR	Major damage to system – significant delay to repair. Significant numbers of procedures cancelled	OR	Partial loss of gametes/ embryos for one couple
4	Catastrophic or extreme	Death	OR	System destroyed – need to rebuild. All procedures cancelled	OR	Total loss of gametes/ embryos for all couples

Note for the reader: This first approach was confirmed after the investigation because the child died at 2 years of age.

The impact matrix shows that a total score of 8 (2 × 4) locates this SAR in the pale shading of the table. This means that interaction between the SoHO establishment and the Health Authority would be required (please see Appendix 23 for more detailed information).

Step 3: Application of the impact matrix

Likelihood of recurrence ▶ / Impact of recurrence ▼	1 Rare	2 Unlikely	3 Possible	4 Likely	5 Certain/almost certain
0 Insignificant	0	0	0	0	0
1 Minor	1	2	3	4	5
2 Moderate	2	4	6	8	10
3 Major	3	6	9	12	15
4 Catastrophic/extreme	4	8	12	16	20

Preventive measures and corrective measures implemented

- All the donations were performed within the same SoHO establishment, so there was no need to alert other centres involved.
- Despite very low incidence of this recessive genetic disorder, the SoHO establishment decided to expand the carrier genetic screening tests to include Ndufv1 gene mutation.
- Donors were informed about the new gene carrier condition.
- These gamete donors would be eligible for donation only under the following premises:
  - Only one of the donors tested positive.
  - Both donors are positive; but, in that case, the already existing supernumerary cryopreserved embryos must be tested for Ndufv1 gene mutation using PGT-M technique.
- The case was closed.

Appendix 26. **Example of serious adverse event (SAE) for non-reproductive SoHO**

Vigilance notification

A surgical team asks for a left humerus for a planned surgery. The tissue prescribed is sent to the hospital by the SoHO establishment.

During the surgical operation, the surgeon discovers that the humerus received is a right humerus.

Immediate action

The incident was considered a serious adverse event (SAE). The surgery was cancelled; the incident was seen during the preparation of the surgery room, before patient anaesthesia.

Investigation

First step: History of the organ/tissue collection

Organs and tissues were collected simultaneously from two donors in two different surgery rooms, in the same institution during the same night. Two nurses were in charge of these collections.

- In the first surgery room, organs and tissues, including a right humerus, were collected (donor 1, nurse 1).
- In the second surgery room, organs and tissues, including a left tibia with extensor items, were collected (donor 2, nurse 2).

Second step: Context of the organ/tissue collection

The organ and tissue collections happened in a busy summer weekend during which five organ and tissue donors were managed in four days. During this period, it was necessary to increase the duty staff.

Third step: Details of the organ/tissue collection management

Room 1: The organ and tissue collection process was finished before the collection process in room 2 was finished.

Nurse (from room 1) offered to help her colleague nurse 2 (room 2) and performed the conditioning and packaging of all tissues from both room 1 (donor 1) and room 2 (donor 2).

Four containers were prepared following these two different donor collections:

- a container with the heart for valves collection from donor 1;
- a container with the heart for valves collection from donor 2;
- a container with corneas from donor 1 and corneas from donor 2;
- a container with the bones from donor 1 (right humerus) and from donor 2 (left tibia with extensor items) together.

(Not enough containers were available at that moment for multiple tissue collections.)

Only one file from each donor, including medical history, and clinical and biological parameters, was sent with all the containers. Normally, one file is sent with each container with tissues and organs collected from one donor. Note that the packaging of organs or tissue of different donors in one container is unusual.

Moreover, a specific sheet explaining the details of the tissues collections was also sent with each tissue

collected and put in the container with the tissues concerned. No sheet was included in the various containers.

All tissues were labelled with the unique donor number for traceability. The laterality was also mentioned for the tibia, but the laterality of the humerus was not mentioned.

At the end of the tissue packaging steps, the nurses' services were finished and the nurses were replaced by a new one (nurse 3).

Fourth step: at the SoHO establishment

A technician received the various containers and mistakenly believed that both the left tibia and right humerus had come from the same donor (donor 2). As the laterality of the humerus was not mentioned, he called the duty nurse (nurse 3). She was busy on a new organs and tissues collection from another donor and she answered that the humerus was a left humerus.

The conversation was by phone, and no written document was sent to confirm this information.

Fifth step: discovery of the incident

Seven months later, a left humerus was requested from the SoHO establishment by a surgeon for planned surgery.

In the surgery room, the surgeon noticed that the donor humerus sent by the SoHO establishment was a right humerus instead of a left humerus (as was requested).

He contacted the SoHO establishment, which subsequently contacted the collection team, and confirmed that no humerus was retrieved from donor 2 and that a right humerus was collected from donor 1.

Following the confirmation of the incident, a biovigilance notification for an SAE was sent to the Health Authority (HA) by the SoHO establishment.

Sixth step: analysis and conclusions

The incident is considered an SAE. The rationale used for the analysis of this SAE has been performed based on the information provided in section 18.7 in Chapter 18 and Appendix 23.

Root causes analysis

Immediate causes (human factors)

- work overload,
- tiredness,
- difference in practice for tissues packaging and labelling between the two nurses on the same organs and tissues collection site,
- no adherence to protocol.

Root causes (organisational factors)

- absence of detailed standard operational procedures for simultaneous organ and tissue collection,
- no organisational practice defined when two nurses are together on organ and tissue collection and packaging/labelling;
- no common practices on the conditioning and labelling of tissues sent to the SoHO establishment;
- not enough containers available for multiple tissue collection.

The probability or likelihood of occurrence/recurrence is considered as 3. Possibly, because corrective measures have been put in place. However, all human factors that led to this incident cannot be totally avoided, notably work overload and tiredness.

Step 1: Assessment of the likelihood of occurrence/recurrence of the SARE

1	Rare	Difficult to believe it could happen again
2	Unlikely	Not expected to happen again
3	Possible	May occur occasionally
4	Likely	Expected to happen again, but not persistently
5	Probable	Expected to happen again on many occasions

The worst impact consequences expected could be: 1. Minor damage because the surgery was postponed and the patient did not have anaesthesia until a new adapted bone was provided by the SoHO establishment.

Step 2: Assessment of the impact/consequences of the SARE should it recur

Impact level		On individual(s)		On the system		On SoHO supply
0	Insignificant	Nil	OR	No effect	OR	Insignificant
1	Minor	Non-serious	OR	Minor damage	OR	Some applications postponed
2	Moderate	Serious	OR	Damage for short period.	OR	Many cancellations or postponements
3	Major	Life-threatening	OR	Major damage to system – significant delay to repair	OR	Significant cancellations – importation required
4	Catastrophic or extreme	Death	OR	System destroyed – need to rebuild	OR	All allogeneic applications cancelled

The impact matrix gave a total score of 3 (3 × 1) locating the incident in the blank area.

This means that an interaction was not required between the SoHO establishment and the HA (please see Appendix 23 for more detailed information).

Step 3: Application of the impact matrix

Likelihood of recurrence ▶ / Impact of recurrence ▼	1 Rare	2 Unlikely	3 Possible	4 Likely	5 Certain/almost certain
0 Insignificant	0	0	0	0	0
1 Minor	1	2	3	4	5
2 Moderate	2	4	6	8	10
3 Major	3	6	9	12	15
4 Catastrophic/extreme	4	8	12	16	20

Preventive and corrective measures implemented

- Check the labelling of all tissues collected from these two donors, the ones distributed and those still stored;
- Communication with all healthcare teams involved in organs and tissues collection, about the new practices;
- Update of tissues collection procedures;
- Creation of labels for each tissue collected;
- Purchase of containers;
- Use of label sheets of different colour when tissues from different donors are simultaneously collected.

Appendix 27. Example of serious adverse event (SAE) for reproductive SoHO

Vigilance notification to the SoHO establishment

At inspection on day 1 post-fertilisation, the staff of the laboratory noticed that all the oocytes of three women undergoing medically assisted reproduction treatment on sequential days showed no signs of fertilisation. Hence, the full treatment cycle was lost because no embryos were obtained.

Immediate action

- The incident was considered a potential serious adverse event (SAE) and the laboratory informed the head practitioner, who in turn informed the patients about the case.
- The laboratory initiated an internal investigation to analyse this case and its potential causes and informed the Health Authority.

Investigation

- The laboratory investigated fertilisation rates of the oocytes that were processed by intracytoplasmic sperm injection (ICSI) on the same microscope on the days before the incident. The percentage of fertilisation was within the normal range on those days. This is considered a look-back procedure to identify other patients that might have suffered from a possible technical failure of the heating device.
- They reviewed the temperature records of the heating device on the ICSI stations, and the recorded temperatures were as defined previously.
- The maintenance and verification records of the heating device were reviewed and showed no abnormalities.
- Because oocytes of different women were affected, the problem could potentially originate from a step common to all patients. One of the problems described widely in the literature is that fertilisation rate is affected by temperature.
- Although verification suggested that the heating device had worked correctly, the SoHO establishment checked the heating device with a calibrated temperature probe to verify if there were discrepancies. The second device recorded on average a temperature that was 4°C higher, implying that the temperature recorded by the initial heating device was not correct due to a malfunction of the temperature sensor.

Conclusions

The incident was considered a SAE. The rationale used for the analysis of this SAE was based on the information provided in section 18.7 of Chapter 18 and Appendix 23:

- In the situation of an SAE, it is not possible to assess "imputability".
- The probability or likelihood of occurrence/recurrence: the decision is considered as 2 (**unlikely**).

Step 1: Assessment of the likelihood of occurrence/recurrence of the SARE

1	Rare	Difficult to believe it could happen again
2	Unlikely	Not expected to happen again
3	Possible	May occur occasionally
4	Likely	Expected to happen again, but not persistently
5	Probable	Expected to happen again on many occasions

- The worst impact consequences appeared on the system level, because the malfunction of one of the heating devices resulted in damage for a short period of time, which results in a score of 2 (**moderate**).

Step 2: Assessment of the impact/consequences of the SARE should it recur

Impact level		**On individual(s)**		**On the system**		**On tissue/cell supply**
0	Insignificant	Nil	OR	No effect	OR	Insignificant
1	Minor	Non-serious	OR	Minor damage or some procedures postponed	OR	Partial loss of gametes/ embryos for one couple
2	Moderate	Serious	OR	Damage for short period. Many procedures cancelled or postponed	OR	Partial loss of gametes/ embryos for one couple
3	Major	Life-threatening	OR	Major damage to system – significant delay to repair. Significant numbers of procedures cancelled	OR	Partial loss of gametes/ embryos for one couple
4	Catastrophic or extreme	Death	OR	System destroyed – need to rebuild. All procedures cancelled	OR	Total loss of gametes/ embryos for all couples

The impact matrix gave a total score of 4 (2 × 2), locating the incident in the pale shading area of the table.

This means that interaction between the SoHO establishment and the Health Authority is required (please see Appendix 23 for more detailed information).

Step 3: Application of the impact matrix

Likelihood of recurrence ▶ / **Impact of recurrence ▼**	**1 Rare**	**2 Unlikely**	**3 Possible**	**4 Likely**	**5 Certain/almost certain**
0 Insignificant	0	0	0	0	0
1 Minor	1	2	3	4	5
2 Moderate	2	4	6	8	10
3 Major	3	6	9	12	15
4 Catastrophic/extreme	4	8	12	16	20

Preventive and corrective measures implemented

- Update the information to the head practitioner with the results of the investigation.
- Take the heating device out of service.
- Replace faulty sensor and implement new validation of heating device before taking it back into service.
- Safety checks on the three similar heating devices that were in use in the lab (nothing abnormal detected).
- Update the heating device maintenance SOP, e.g. using a calibrated temperature probe, increasing the frequency of quality control.
- Contact the manufacturer to inform them about the defect in the heating device.
- Send out warning about technical problem with heating device to the other *in vitro* fertilisation centres in the country through the national embryology society.

Appendix 28. Serious adverse reaction: notification form for ocular tissues (Agence de la Biomédecine, France)

Notification form for SAR on ocular tissues

SEC ____ Recipient code ____ Date of birth *(dd/mm/yyyy)* ____

Date of transplant *(dd/mm/yyyy)* ____ Sex ☐ M ☐ F Eye involved ☐ Right ☐ Left

Type of graft ☐ DALK ☐ DSAEK ☐ DMEK ☐ PK

SAR

1. Short term (pre-graft until 1 month post-graft)

- ☐ Primary graft failure (endothelial decompensation)
- ☐ Ocular infection (from bacterial, fungal, parasitic or viral origin including endophthalmitis)
- ☐ Irreversible rejection (specific immunologic response)
- ☐ Systemic infection (compatible with a donor-recipient transmission)
- ☐ Persistent ulceration or corneal perforation

2. Mid- to long term	1 month to 1 year	> 1 year
☐ Any ocular pathology that could suggest transmission from the donor (for example: tumoral pathologies of the anterior segment)	☐	☐
☐ Any systemic pathology that could suggest transmission from the donor (transmissible viral diseases (rabies, hepatitis, etc.), malignant diseases, prion disease...)	☐	☐
☐ Unrecognised donor corneal diseases (history of refractive surgery, corneal dystrophy including FUCHS, keratoconus, scars...)	☐	☐
☐ Endothelial cell decompensation including cornea guttata	☐	☐
☐ Chronic endophthalmitis	☐	☐

☐ Late-onset local infection including bacterial, fungal, viral, or parasitic keratitis or kerato-endotheliitis	☐	☐
☐ Failure (leading to re-graft). Specify	☐	
☐ Persistence of complicated epithelial defects (epithelial ulcerations) (visual decline, infection, rejection, stromal ulceration, perforation...) or development of epithelial tumour, dysplasia or epithelial hyperplasia	☐	☐
☐ Defect of corneal transparency (corneal opacification, calcifications, corneal transparency delay, inflammatory infiltrate...)	☐	☐
☐ Astigmatism induced by the graft or giant astigmatism (> 8 D) linked or likely to be linked to recurrence or appearance of keratoconus on the graft	☐	☐
☐ Loss of eyeball (anatomical or functional)	☐	☐
☐ Death of patient linked to the ocular graft	☐	☐

Risk factors

☐ No risk factor identified

Risk factor identified (tick the one concerned)

☐ Neovascularisation – History of autoimmune disease (scarring pemphigoid)	☐ Neurotrophic history	☐ Atopic ground
☐ Graft size outside the range of 8.5 diameter	☐ Palpebral alterations (statics/ kinetics)	☐ Paediatric patient or less than 16 years old
☐ Hypertonia/glaucoma	☐ Emergency cornea grafting or therapeutic keratoplasty	☐ Re-graft – how many?
☐ Ocular inflammation/uveitis	☐ History of rejection on either ipsi or contralateral eye	☐ Herpes virus infection (HSV, VZV)
		☐ Dry eye syndrome
		☐ Chemical burn

☐ Other – give details

Source : Agence de la Biomédecine, France.

Appendix 29. **Serious adverse reaction: notification form for ocular tissues (NHS, UK)**

FRM4159/5 – Ocular Tissue Transplantation – Serious Adverse Reactions/Events

NHS Blood and Transplant

Effective date: 01/12/2023

NOTIFICATION OF ADVERSE REACTIONS AND EVENTS

NHSBT SERIOUS ADVERSE REACTIONS/EVENTS REPORTING MECHANISM (i.e., this form) – To be completed by the person notifying NHSBT of an Adverse Reaction/Event, Serious Adverse Reactions/Events will be reviewed by NHSBT and OTAG[1].

Please return this form as soon as possible, but within 24 hours of the incident being identified or confirmed.

TISSUE REFERENCE No.	**(e.g. G170...)**		**LEFT / RIGHT**
DATE OF TRANSPLANT		**RECIPIENT HOSPITAL**	
DATE OF EVENT		**CONSULTANT** (please print):	
DATE OF REPORTING		**CONTACT DETAILS**	**Email:** **Tel:**

EYE BANK SUPPLYING THE TISSUE (please tick)	□ NHSBT □ Moorfields □ East Grinstead
TYPE OF TISSUE OR CELLS (please tick)	□ Cornea (Whole) □ Cornea (Pre-Cut) □ Sclera □ Cornea in Ethanol □ Other – please specify……
TRANSPLANT TYPE	□ PK □ DALK □ SALK □ DMEK □ DS(A)EK □ KLAL □ Other
INDICATION/URGENCY OF TRANSPLANT:	□ Urgent □ Elective

ADVERSE EVENT:

DEFINITIONS[2]

'Serious adverse event'– means any untoward occurrence associated with the procurement, testing, processing, storage and distribution of tissues and cells that might lead to the transmission of a communicable disease, to death or life-threatening, disabling or incapacitating conditions for patients or which might result in, or prolong, hospitalisation or morbidity.

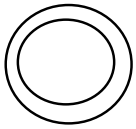
Serious Adverse Events

There are potentially numerous serious adverse events. Specific serious adverse events that are peculiar to ophthalmology include

1. Wrong material supplied
2. Tissue supplied is out of date
3. Tissue supplied is damaged, scarred or has evidence of previous donor eye surgery
4. Other[5]

For grafts supplied by NHSBT: Please contact TES customer care to arrange for return of all unused tissue in the original container to the issuing eye bank for investigation.

- Contact Tissue and Eye Services Customer Care on Tel: **0151 268 7228**
- Send one copy of this form to Tissue and Eye Services Customer Care either by e-mail to tscustserv@nhsbt.nhs.uk, or by post to Tissue and Eye Services Customer Care Dept., NHSBT, 14 Estuary Banks, Speke, Liverpool L24 8RB;
- Retain original in the patient's notes

Wrong material supplied	**YES / NO**	**Tissue supplied is damaged, scarred or has evidence of previous donor eye surgery** *(Please indicate on diagram, and provide photographic evidence if possible)*	**YES / NO** 
Tissue supplied is out of date	**YES / NO**	**Was the tissue used?**	**YES / NO**
Any surgical complications, related to tissue quality *(Please provide details below)*	**YES / NO**	**Had the patient already been anaesthetised?**	**YES / NO**
Any other adverse impact on the patient *(Please provide details below)*	**YES / NO**		

Other / Additional Details:

Controlled if copy number stated on document and issued by QA

(Template Version 03/02/2020)

Cross-Referenced in Primary Document: SOP5464

FRM4159/5 – Ocular Tissue Transplantation – Serious Adverse Reactions/Events

NHS Blood and Transplant

Effective date: 01/12/2023

ADVERSE REACTION:

DEFINITIONS[2]
'Serious adverse reaction'– means an unintended response, including a communicable disease, in the donor or in the recipient associated with the procurement or human application of tissues and cells that is fatal, life-threatening, disabling, incapacitating or which results in, or prolongs, hospitalisation or morbidity

Serious Adverse Reactions
Corneal Transplantation
1. Primary graft failure (graft never cleared)
2. Endophthalmitis or other serious ophthalmic infection likely to be attributable to the transplanted tissue[3]
3. Graft failure due to donor tissue which was out of date, damaged, scarred or had evidence of previous surgery
4. Malignancy likely to be attributable to the transplanted tissue
5. Systemic infection likely to be attributable to the transplant tissue
6. Other[4]

Ocular Tissue Stem Cell Graft
1. Endophthalmitis or other serious ophthalmic infection likely to be attributable to the transplanted tissue/cells[3]
2. Graft failure due to donor tissue/cells that were out of date or damaged
3. Malignancy likely to be attributable to the transplanted tissue/cells
4. Systemic infection likely to be attributable to the transplanted tissue/cells
5. Other[4]

Scleral Grafts
1. Endophthalmitis or other serious ophthalmic infection likely to be attributable to the transplanted tissue
2. Graft failure due to donor tissue which was out of date or damaged
3. Malignancy likely to be attributable to the transplanted tissue
4. Systemic infection likely to be attributable to the transplanted tissue
5. Other

For grafts supplied by NHSBT: Please contact TES customer care in advance to arrange for return of any explanted tissue. Please discuss redo-operation, to allow for any necessary arrangements and sample requirements

- Contact Tissue and Eye Services Customer Care on Tel: **0151 268 7228**
- Send one copy of this form to Tissue and Eye Services Customer Care either by e-mail to tscustserv@nhsbt.nhs.uk, or by post to Tissue and Eye Services Customer Care Dept., NHSBT, 14 Estuary Banks, Speke, Liverpool L24 8RB;
- Retain original in the patient's notes

Graft Failure		**YES / NO**	
Graft Failure suspected due to donor tissue quality? *(Provide further details below)*	**YES / NO**	**Graft Failure due to, or possibly due to, underlying condition/ surgical complications** *(Provide further details below)*	**YES / NO**
Has the patient had previous corneal graft(s) in this eye?	**YES/ NO** **If Yes, how many?**	**Is this suspected to be Primary Graft Failure?** (Graft never cleared, *please provide further details below*)	**YES / NO**
Endophthalmitis or other serious ophthalmic infection?		**YES / NO** **If yes, is the organism known at this time** **YES / NO** **Identified as:**	
Malignancy likely to be attributable to the transplanted tissue	**YES / NO**	**Systemic infection possibly attributable to the transplanted tissue**	**YES / NO**
Other / Additional Details:			
Date of Replacement Graft if Known:			

Notes:
1. OTAG Adverse Reactions and Events Reporting subgroup
2. EU Tissues and Cells Directive 2004/23/EC
3. This relates to the development of a severe infection likely to be attributable to the transplanted tissue. It does not include for example the occurrence of a microbial keratitis related to a suture abscess.
4. Other refers to any unexpected adverse reaction which is considered by the surgeon to be serious and possibly attributable to the transplanted tissue
5. Other refers to any adverse event which is considered to be serious and could potentially lead to a serious adverse reaction if the tissue is used in a patient.

Source : National Health Service, United Kingdom.

Appendix 30. **Decellularisation**

Decellularisation is defined as the process of removing cellular components from a scaffold while preserving the extracellular matrix (ECM)'s macrostructure and microstructure, primarily to create a nonimmunogenic scaffold suitable for repopulation with patient-derived cells. Clinical-grade ECM may ideally preserve the intrinsic structural and biological properties of the SoHO while removing cells, cellular debries and alloantigen (to minimise immunogenicity). Several procedures, combining different methods, can be applied to decellularise human tissues for clinical applications [1]. The choice of method needs to consider the intended use of the scaffold and the specific requirements of that application. Depending on the decellularisation procedure, the final properties of the graft may vary. Mechanical properties are particularly important, especially in cases where resistance to physical stresses such as traction, compression or shearing is needed to maintain structural integrity and proper functioning *in vivo*. In addition, other factors should also be taken into consideration, such as the preservation of biochemical signals, porosity and biocompatibility, in order to ensure optimal performance of the decellularised graft.

Decellularisation agents

As ECM properties vary between tissues and organs, it is suggested that the main properties to safeguard need to be clearly identified and determined in order to choose the correct decellularisation protocol. There are three general methods used to decellularise tissues or organs: physical, biological (enzymatic) and chemical [2, 3]. Each of these methods has a different mode of action and different effects on the ECM [1, 4] (see Table A30.1), which can be critical for the functionality of the final decellularised ECM. These methods are usually combined to ensure complete decellularisation and can be also combined with different techniques to improve their effectiveness (see Table A30.2).

Aside from the selected decellularisation agents, the efficiency of tissues or organs decellularisation will depend on the intrinsic tissue properties, such as the specific cell density, thickness, compaction and lipid content. The maintenance of each scaffold's mechanical properties is directly related to the maintenance of one or more ECM components. For example, structural 3D maintenance is related to structural molecules such as collagens, elasticity depends on elastic fibres [5-8] and tensile strength is associated with structural proteins and chondroitin sulphate, while the ability to resist compressions is also associated with structural proteins conformation and hyaluronic acid [9].

Table A30.1 **Techniques used to apply decellularisation agents**

Technique	Advantages	Disadvantages
Perfusion	Facilitates chemical exposure and removal of cellular material	Pressure associated with perfusion can disrupt ECM
Pressure gradient across tissue	Facilitates chemical exposure and removal of cellular material	Pressure gradient can disrupt ECM
Supercritical fluid	Pressure can burst cells – facilitates chemical exposure and removal of cellular material	Pressure necessary for supercritical phase can disrupt ECM
Agitation	Can lyse cells – facilitates chemical exposure and removal of cellular material	Aggressive agitation or sonication can disrupt ECM

Table A30.2 **Modes of action and the effect of different decellularisation agents**

Physical			
Method		***Mode of action***	***Effects on ECM***
Snap freezing		Intracellular ice crystals disrupt cell membranes	Rapid freezing can disrupt or fracture ECM
Mechanical force		Pressure can burst cells, and tissue removal eliminates cells	Mechanical force can damage the ECM
Mechanical agitation		Cell membrane lysis; facilitates chemical exposure and removal of cellular material	Aggressive agitation or sonication can disrupt ECM
Non-thermal irreversible electroporation		Selective damage on cell membrane while sparing the other tissue components	ECM preservation and produces a functional recellularisable scaffold
Biological (enzymatic)			
Method		***Mode of action***	***Effects on ECM***
Trypsin		Cleaves peptide bonds on the C-side of Arg and Lys	Prolonged exposure can disrupt ECM structure; removes laminin, fibronectin, elastin and glycosaminoglycans (GAG)
Endonucleases		Catalyse the hydrolysis of the interior bonds of ribonucleotide and deoxyribonucleotide chains	Difficult to remove from the tissue and could invoke an immune response
Exonucleases		Catalyse the hydrolysis of the terminal bonds of ribonucleotide and deoxyribonucleotide chains	Difficult to remove from the tissue and could invoke an immune response
Chemical			
Method		***Mode of action***	***Effects on ECM***
Alkaline/acid		Solubilises cytoplasmic components of cells; disrupts nucleic acids	Removes GAG
Hypo/hyperosmotic solutions		Cell lysis by osmotic shock	Efficient for cell lysis, but does not effectively remove cellular remnants
Alcohols		Dehydrate and lyse cells. Lipid removal	Fixative properties, protein precipitation and ECM ultrastructure damage.
Other solvents (acetone)		Lipid removal	Fixative properties and ECM ultrastructure damage
EDTA/EGTA		Chelating agents that bind divalent metallic ions, thereby disrupting cell adhesion to ECM	Typically used with enzymatic methods
Non-ionic detergents	Triton X-100	Disrupt lipid–lipid and lipid–protein interactions, while leaving protein–protein interactions intact	Mixed results; efficiency dependent on tissues; removes GAG
Ionic detergents	Sodium dodecyl sulfate (SDS)	Solubilise cytoplasmic and nuclear cellular membranes; tend to denature proteins	Removes nuclear remnants and cytoplasmic proteins; tends to disrupt native tissue structure, to remove GAG and to damage collagen
	Sodium deoxycolate		More disruptive to tissue structure than SDS and GAG removal
	Triton X-200		Achieves efficient cell removal when used with zwitterionic detergents
Zwitterionic detergents	3-[(3-cholamidopropyl) dimethylammonio]-1-propanesulfonate (CHAPS)	Exhibit properties of non-ionic and ionic detergents	Efficient cell removal with ECM disruption similar to that of Triton X-100
	Sulfobetaine-10 and -16		Achieves cell removal and mild ECM disruption with Triton X-200
	Tri(*n*-butyl)phosphate	Organic solvent that disrupts protein–protein interactions	Variable cell removal; loss of collagen content, although effect on mechanical properties is minimal

Physical methods (such as agitation, pressure, freeze/thaw steps, sonication, etc.) can be applied but have limited efficacy and are to be carefully evaluated to assess any possible damage to the ECM.

Usually they are combined with chemical and enzymatic methods. For the maintenance of ECM structure, ionic detergents could be the optimal choice, and it is suggested to avoid enzymatic or alkaline/acid methods because the damage to the collagen can be limited with time and temperature using an ionic detergent; but the disruption provoked by enzymatic or alkaline/acid methods is highly aggressive. This consideration is valid for the preservation of any protein.

On the other hand, osmotic buffers are a milder method to obtain decellularised ECM but they are slower and cannot penetrate into thicker, compact organs. For dense tissues or intact organs, detergents can help buffers to penetrate, but they will affect the protein ultra-structure due to disruption of protein–protein interactions. Furthermore, any residual detergents can have cytotoxic effects, and it is imperative to assure their elimination.

With the aim of removing specific proteins, it is suggested to use enzymatic treatment. However, it may cause the unspecific digestion of desired constituents and may not be sufficient for complete decellularisation of the whole tissue. Alcohols and other solvents can remove lipids very efficiently, but in the process, they also crosslink proteins and modify the ECM ultrastructure. A compromise between lipid removal and crosslinking can be achieved to decide time and temperature of the treatment with these solvents.

In conclusion, it is suggested to test, validate and monitor specific combinations of mild physical, biological and chemical methods, along with the type of administration, to obtain the best results for each tissue or organ with the aim of obtaining a scaffold that presents the desired characteristics needed for a specific clinical application. Moreover, if the scaffold is a future starting material for a medicinal product, the manufacturer is encouraged to refer to pharmaceutical guidelines for the development, validation and monitoring of its products.

References

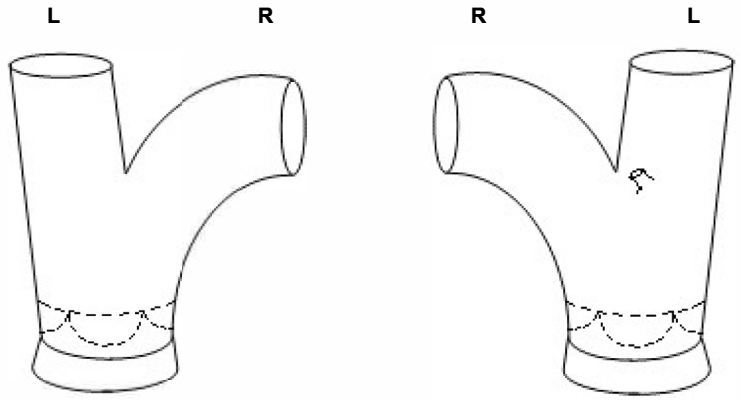
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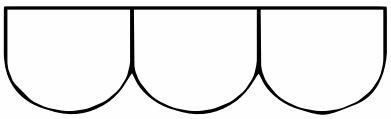
Appendix 31. **Sample forms for the evaluation of heart valves (NHS, UK)**

FRM925/5 – Pulmonary Heart Valve Information

NHS
Blood and Transplant
Effective date: 06NOV2025

Heart Valve Bank:	Liverpool/Brompton/Birmingham		**Donation Number:**
ODT Number (if applicable):			**Valve Number:**
Expiry Date of Valve:			**Photograph Available on request:** YES/NO
Age	**Sex**	**Cause of Death**	



Pathology noted on cusps	**Key**			
	Atheroma	.....	Endothelial Shedding	ES
	Fibrosis	xxx	Other *(see comments)*	
	Fenestration	ooo	Other *(see comments)*	

Pulmonary Inner Annular Diameter	mm	Left artery inner annular diameter	mm
Length of Vessel	mm	Left artery length	mm
Length of muscle skirt (Min / max)	mm	Right artery inner annular diameter	mm
Total Length (min/max)	mm	Right artery length	mm
Condition: Excellent/Good/Fair			
Comments:			

Information Entered By:	**Date:**

Return completed form to NHSBT via secure email: Heartvalve.fulfillment.liverpool@nhsbt.nhs.uk

Controlled if copy number stated on document and issued by QA
(Template Version 03/02/2020)

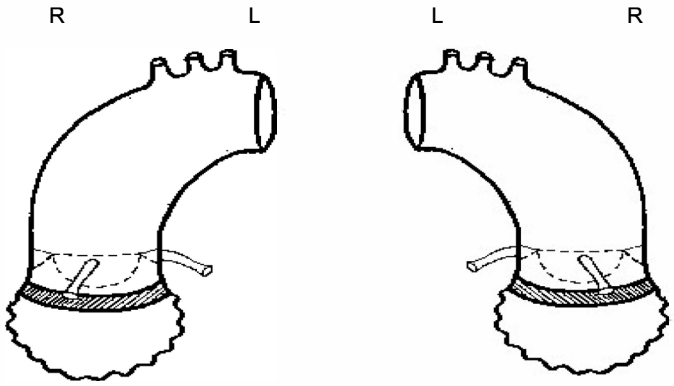
Cross-Referenced in Primary Document: SOP922

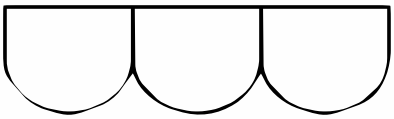
Page 1 of 2

FRM1385/5 – Aortic Heart Valve Information

NHS
Blood and Transplant
Effective date: 06NOV2025

Heart Valve Bank: Choose an item.			**Donation Number:**
ODT Number (if applicable):			**Valve Number:**
Expiry Date of Valve:			**Photograph Available on request:** YES
Age	**Sex**	**Cause of Death**	



Pathology noted on cusps	**Key**			
	Atheroma	.....	Endothelial Shedding	ES
	Fibrosis	xxx	Other *(see comments)*	
	Fenestration	ooo	Other *(see comments)*	

Aorta Inner Annular Diameter	mm	Length of muscle skirt (Min / max)	mm
Length of Aorta	mm	Total Length (min/max)	mm
BIS Code: ☐01 / ☐02 / ☐03 / ☐04 / ☐05		**Condition**: Excellent	
Comments:			

Information Entered By:	**Date:**

Return completed form to NHSBT via secure email: Heartvalve.fulfillment.liverpool@nhsbt.nhs.uk

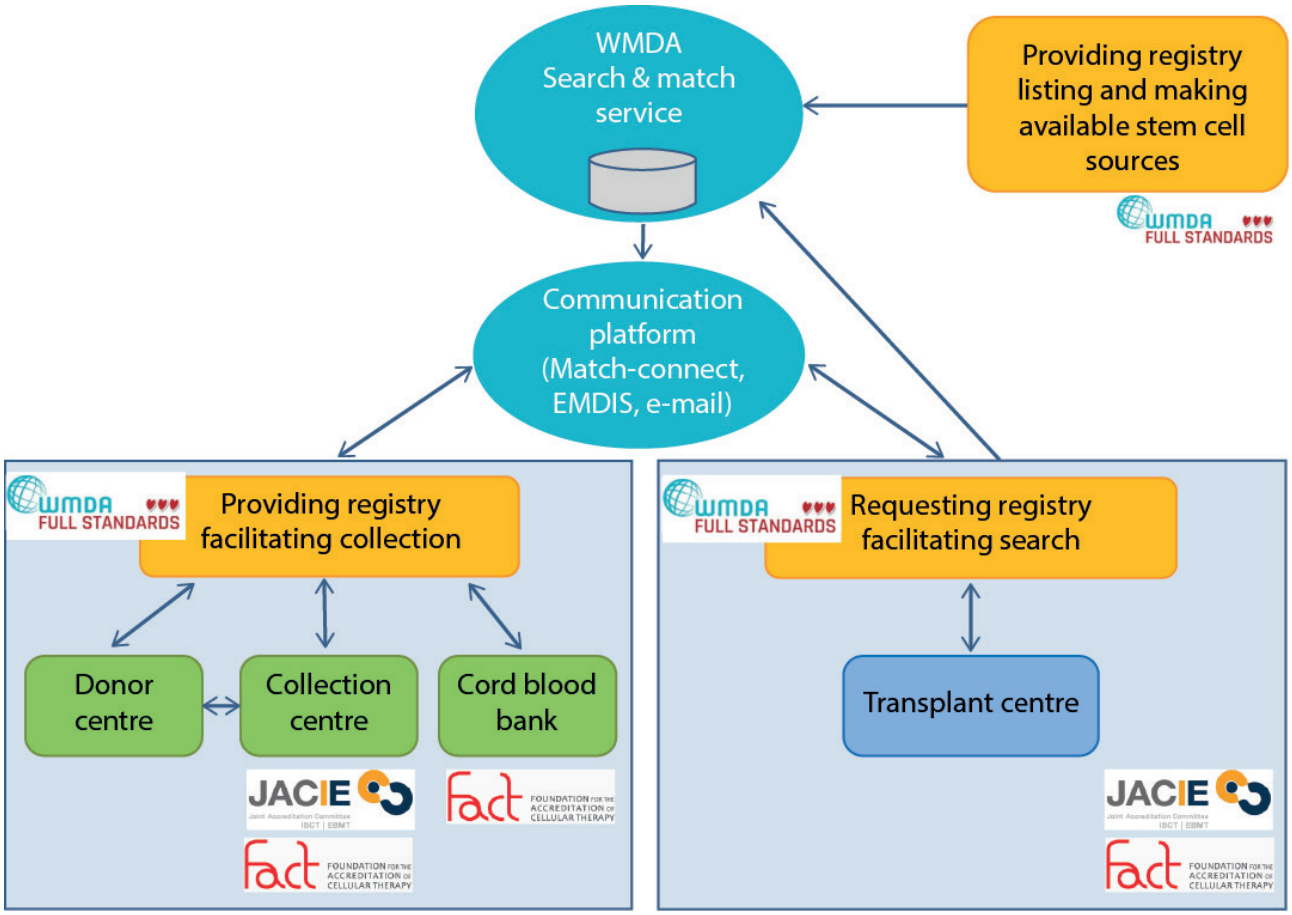
Controlled if copy number stated on document and issued by QA
(Template Version 03/02/2020)

Cross-Referenced in Primary Document: SOP922

Page 2 of 2

Source: National Health Service, United Kingdom.

Appendix 32. **Donor search through registries for haematopoietic progenitor cell transplantation**



WMDA: World Marrow Donor Association
JACIE: Joint Accreditation Committee ISCT-Europe & European Society for Blood and Marrow Transplantation
FACT: Foundation for the Accreditation of Cellular Therapy
Search, Match & Connect Service: global database of unrelated donors, and communication platform operated by WMDA

WMDA operates a global database of potential stem cell sources (Search & Match Service) and a standardised protocol and communication platform to request potential stem cell sources for further testing and collection (Match-Connect). The registries can be connected to each other independently.

- **Providing Registry**: the organisation that acts as the registry providing the stem cell source. An organisation responsible for listing and making available potential stem cell sources for patients through the Search & Match Service of WMDA, co-ordinating the search requests, provision and collection of potential stem cell sources. Depending on the registry, the registry may utilise donor centres, collection centres and cord blood banks for donor recruitment, consent, testing, management and collection.
- **Requesting Registry**: the organisation that acts as the registry requesting a search is the registry that acts on behalf of their transplant centres. An organisation responsible for co-ordinating the search and requests for patients that search for an unrelated stem cell source.
- **Transplant Centre** initiates searches for their patients through the local registry.

Appendix 33. **Informed consent form for cord blood donors (Italy)**

IBMDR – Italian Cord Blood Network

Form SCO101 (V4 1/5 mar. 2019)

WMDA Accredited

Informed consent to unrelated CB donation

CBU ID assigned by CDD .....

Mother's data

I, the undersigned declarant, *(full name)* _____,
born on *(date of birth)*: __/__/____in *(place of birth)*:_____ (Province _____),
nationality _____ resident in (Province_____), ZIP CODE_____
full address _____-_____,
phone number.:_____ mobile phone number _____
e-mail _____

Father's data

I, the undersigned declarant, *(full name)* _____,
born on *(date of birth)*: __/__/____in *(place of birth)*:_____ (Province _____),
nationality _____ resident in (Province_____), ZIP CODE_____
full address _____-_____,
phone number.:_____ mobile phone number _____
e-mail _____

I have read with attention the information included in the attachment A-sco and

- I declare to have been informed about the possible usage of the CBU;
- I declare that I will donate the CB of my child, and I know that it is finalized for unrelated transplantation use, so it may never been asked back only for related use.
- I read all the information and I was able to ask for any additional questions and I have received comprehensive explanation;
- I know that if I will deny the consent to CB collection, this will have no impact on the quality and modality of my delivery;
- I understood that CB collection doesn't imply risk for the mother and the newborn;
- I understood that collection could be done in case of natural or cesareum delivery;
- I understood that if the unit will be considered elegible and fit to be criopreserved, it will be made available through IBMDR to national and international patients in need of a hemopoietic stem cell transplant;
- I understood that I will not receive advantages from this donation;
- I agree to provide some information in order to run an anamnesis of myself and relatives and if necessary, I will be contacted in the future for further information;
- I agree to undergo blood being drawn, that will be used for testing according to the national regulation and requirements (HIV included) and I will be recontacted in case of a positive results (applicable only to the mother);
- I agree that a blood sample is stored at the CBB and it will be used for testing in case of a specific request for a patient in search;
- I agree that some testing, including genetic testing, will be done on CB samples;
- I agree to be contacted after the delivery in order to check the newborn health status;
- I know that any important information on the health status of my child that might have an impact on the recipient must be provided to the CBB.

Data:..... Mother signature.....

Father signaure (if applicable):.....

Information of the health personnel who informed mother/father and checked their identity:

Last name and name ……..……..role…….....

Hospital ……..……..

Date and time: ..... Signature.....

Donation is anonymous, unpaid and voluntary

Source : Italian Cord Blood Network.

Appendix 34. **Cord blood donor medical history questionnaire (Croatia)**

1. **Are you currently taking antibiotics?** ☐ Yes ☐ No

   Specify: _____ Why? _____

2. **Are you currently taking any other medication?** ☐ Yes ☐ No

   Specify _____ Why? _____

3. **Within the past few days have you …**

   a. … had ☐ fever, ☐ cold, ☐ cough, ☐ diarrhoea, ☐ vomiting, ☐ abscess, ☐ dental procedure, ☐ dental extraction, ☐ open wounds, ☐ any other sickness or infection?

   Specify _____

   b. … taken antibiotics? ☐ Yes ☐ No

   Specify _____ Why? _____

   c. … taken any other medication? ☐ Yes ☐ No

   Specify _____ Why? _____

4. **In the past 2 months have you …**

   a. … received any shots or vaccinations? ☐ Yes ☐ No

   Specify _____

   b. … been in contact with a person vaccinated for smallpox? ☐ Yes ☐ No

5. **In the past 6 months have you had …**

   ☐ an unexplained weight loss? ☐ an unexplained fever? ☐ frequent diarrhoea? ☐ swollen lymph nodes?

6. **Have you had any of the following diseases?**

   a. ☐ rheumatic fever, ☐ malaria, ☐ tuberculosis

   b. ☐ diabetes, ☐ renal disease, ☐ heart disease ☐ liver disease

   c. ☐ inflammatory bowel disease, ☐ ulcerative colitis, ☐ Chron's disease

   d. ☐ autoimmune disease Specify _____

   e. ☐ blood clotting problems

f. ☐ epileptic seizures

g. ☐ tumour ☐ carcinoma Specify

h. ☐ contagious jaundice ☐ hepatitis A ☐ hepatitis B ☐ hepatitis C

i. ☐ psychiatric disorder ☐ neurologic disease

7. **Are there any hereditary diseases in your family?**

a. ☐ enzymatic/metabolic disorders

b. ☐ immune system disorders

c. ☐ blood clotting problems/coagulopathy

d. ☐ haematological disorders

e. ☐ any other serious disease

Please specify who has it and which disease.

8. **Have you ever had any serious disease or surgery?** ☐ Yes ☐ No

Specify what exactly and when.

9. **In the past 12 months have you …**

a. … ☐ received a blood transfusion ☐ had acupuncture ☐ had a tattoo ☐ had a piercing?

Specify when:

b. … ☐ had a medical needle stick incident ☐ had contact with someone else's blood?

Specify when:

c. … been in contact with: ☐ a person who has hepatitis ☐ a person who had received a hepatitis B hyperimmune globulin?

Please specify: ☐ household member ☐ your sexual partner ☐ professional exposure ☐ other:

d. … used retinoids: ☐ Tegison ☐ Soriatane ☐ Accutane?

10. **Have you ever …**

a. … had a positive blood test for ☐ HIV/AIDS virus ☐ hepatitis B virus ☐ hepatitis C virus?

b. … been rejected as a blood donor? ☐ Yes ☐ No

Why? ☐ low body weight ☐ anaemia ☐ risk behaviour ☐ other:

c. … been treated with blood clotting factors concentrate?

Which?

When?

d. … received a transplant: ☐ tissue ☐ organ? Specify which and when.

e. … received a *dura mater* transplant? When?

f. … *(or members of your household)* had a transplant or a medical procedure that involves exposure to animal cells, organs or tissues? ☐ Yes ☐ No

Specify who and which procedure:

11. **Have you …**

a. … stayed in the United Kingdom between 1986 and 1997 for a period longer than 6 months? ☐ Yes ☐ No

b. … stayed in an area with HIV subtype O virus (West and Central Africa)? ☐ Yes ☐ No

c. … or your partner stayed in an area endemic for the HTLV virus (Japan, Caribbean, South America, West and Central Africa)? ☐ Yes ☐ No

d. … in the past 12 months stayed in an area where you could have contracted West Nile Virus (North America, Italy, Austria, Hungary, Serbia, Romania)? ☐ Yes ☐ No

… in the past 12 months travelled outside the country? ☐ Yes ☐ No

If yes, when and where?

12. **Do you belong in any of the risk-behaviour groups listed below?**

a. Addiction to alcohol or drugs ☐ Yes ☐ No

b. Consumer of intravenous drugs ☐ Yes ☐ No

c. Persons who frequently change sexual partners ☐ Yes ☐ No

d. Persons who take drugs or money in exchange for sex ☐ Yes ☐ No

e. Sexual partners of persons from risk behaviour groups ☐ Yes ☐ No

f. Sexual intercourse with a man who has had sex with another man ☐ Yes ☐ No

g. Sexual intercourse with a person who has taken intravenous drugs ☐ Yes ☐ No

h. Sexual intercourse with a person who has haemophilia and has received human derived clotting factor concentrates ☐ Yes ☐ No

i. Person who has syphilis or has been treated for syphilis ☐ Yes ☐ No

j. Sexual intercourse with an HIV-positive person ☐ Yes ☐ No

k. Persons who have been in jail longer than 72 hours ☐ Yes ☐ No

If you answered yes to any of questions 12.a-k, please give additional explanations:

13. **Does the child's father belong to any of the risk-behaviour groups?**
Specify:

14. **Has anyone in your family had Creutzfeldt–Jakob disease?**
Who? When?

15. **Has anyone in the child's immediate family**
(☐ mother, ☐ father, ☐ brothers, ☐ sisters)
had a: ☐ chromosome disorder, ☐ genetic disease?
Specify:

16. **Has anyone in the child's immediate family**
(☐ mother, ☐ father, ☐ brothers, ☐ sisters)
had: ☐ cancer, ☐ leukaemia, ☐ haematological disorder?

17. **Has anyone in the child's extended family**
(☐ mother, ☐ father, ☐ brothers, ☐ sisters ☐ grandparents) had …

a. … any genetic disease that can influence the lymphatic or haematopoietic system:
☐ spherocytosis ☐ thalassaemia ☐ sickle cell anaemia ☐ elliptocytosis ☐ Fanconi anaemia ☐ Diamond-Blackfan sy. ☐ SCID ☐ leukodystrophy ☐ chronic granulomatosis ☐ DiGeorge sy. ☐ Wiskott-Aldrich ☐ Nezelhof sy. ☐ hereditary thrombocytopenia ☐ Glanzmann Alport ☐ Tay-Sachs ☐ Ataxia-Telangiectasia ☐ Gaucher ☐ Hurler ☐ Hunter?

b. … ☐ autoimmune diseases, ☐ muscular dystrophy, ☐ multiple sclerosis or ☐ other severe hereditary skin disease (e.g. neurofibromatosis)?

18. **Is the child´s father your blood relative?** ☐ Yes ☐ No

19. **Are you the child's biological mother?** ☐ Yes ☐ No

20. **Have you had previous pregnancies?** ☐ Yes ☐ No

Number of: Caesarean sections Vaginal births

a. **Have you had any complications in previous pregnancies?** Specify:

b. **Have you had preterm deliveries?** Which week?

c. **Have you had a positive blood test in this pregnancy?**
☐ HIV/AIDS virus, ☐ hepatitis B virus, ☐ hepatitis C virus, ☐ syphilis

I have understood the questions and responded to the best of my knowledge. My answers are truthful.

Mother's full name		**Mother's signature**		Date:	
Health questionnaire checked by		**Signature**		Date:	

Source : Ana Rukavina Cord Blood Bank, Clinical Department of Transfusion Medicine and Transplantation Biology, UHC Zagreb, Croatia.

Appendix 35. **Informed legal consent for cryopreserving and storing semen from a minor (Spain)**

as a precautionary measure under medical circumstances with a risk of reduction of fertility

Informed consent declaration (Spanish Fertility Society – SEF) model

	Date dd mm yyyy	History no.	
Mr	Name and surname of father		
ID/Passport no.		Age	
Resident at			

	Date dd mm yyyy	History no.	
Mrs	Name and surname of mother		
ID/Passport no.		Age	
Resident at			

	Date dd mm yyyy	History no.	
Mr	Name and surname of child		
ID/Passport no.		Age	
Resident at			

Due to the present circumstances of the under-age patient (illness, intervention or medical treatment that may cause sterility, and/or the advisability of preserving a sample of his semen for use in medically assisted reproduction), we wish to cryopreserve (freeze) a sample of his semen, which, through this act, we put at the disposal of the clinic so that it may be used at a later date.

For these purposes, we, and in particular the minor, who is intellectually and emotionally capable of understanding the scope of the circumstances, have been informed of the following dispositions: Law 14/2006 of 26 May on Assisted Reproduction Techniques (ART) and Law 41/2002 of 14 November, governing the independence of the patient and the rights and obligations to clinical information and documentation.

The cryopreserved semen may be kept in authorised SoHO establishments during the lifetime of the male to whom it belongs.

The present commitment to preserve the cryopreserved semen is agreed upon for a period of years, during which Instituto Valenciano de Infertilidad (IVI) is committed to adequately maintaining the samples. On expiry of the period agreed upon, we agree to pay the annual amount that we have been informed of through the corresponding financial information, in order to cover maintenance and preservation costs.

In the event that we be interested in possessing the cryopreserved samples, we are committed to contacting IVI to inform them of our intentions. If we do not inform them of our desire to maintain the samples longer, IVI will understand that we are no longer interested in maintaining them, the cryopreservation and storage commitment, as well as all the other obligations that IVI has assumed, then being automatically dissolved, so resulting in the destruction of the sample.

Furthermore, we are committed to informing IVI of any change of address that we, or the minor, may make in the future for the appropriate purposes.

Finally, we have been informed of the absence of risk to the gametes, resulting from their cryopreservation (with the exception of catastrophe or other justified causes outside the control of this centre) and of the economic cost of their cryopreservation and storage.

We (and in particular the minor) have understood the explanations that have been provided to us in clear and simple language and the physician who has attended to us has allowed us to ask as many questions as we like and has clarified all the doubts that we have expressed.

The information has been provided to us in sufficient time for us to think it over calmly and to reach a decision freely and responsibly, and it has been communicated to us 24 hours before the standard procedure.

We have been informed that pursuant to Royal Decree 9/2014, following blood collection, serum shall be stored for a specific period of time as provided in the applicable regulations.

We also understand that at any moment and without the need for any explanation, we may revoke the consent which we are now presenting.

We, therefore, declare ourselves to be satisfied with the information received and that we understand the scope and risks of the treatment.

Authorisation

We authorise the Reproduction Unit staff to cryopreserve the semen sample that, through this act, we put at the disposal of the clinic, from our son Mr [ ], [ ] years of age, ID no. [ ] for the purposes referred to and during the maximum time stated.

Signed at . . . . . .

Date . . . . . .

Parents' signatures . . . . . .

Patient's signature . . . . . .

Laboratory director's signature. . . . . .

This consent form needs to be signed by the patient on **all** pages and on both sides.

Consent revocation

For the exercise of patients' rights and for the revocation of this consent, patients may fill in a form requiring the enforcement of such rights at any time during the procedure. This fact will be reflected in the medical history.

Source: Reproducción humana asistida, protocolos de consentimiento informado de la Sociedad española de fertilidad, Editorial Comares año 2002. (Human assisted reproduction – informed consent models, Spanish Fertility Society Model, 2002.)

Appendix 36. Legal framework for the development of advanced therapy medicinal products in the European Union

In the European Union (EU), substances of human origin (SoHO) for human application can be subject to different regulatory frameworks (depending on the intended use, mode of action and degree of manipulation). To provide a common framework for the marketing of advanced therapy medicinal products (ATMPs) in the EU, Regulation No. 1394/2007/EC of the European Parliament and of the Council on advanced therapy medicinal products (hereafter 'the ATMP Regulation') was adopted in 2007. Specifically, cells used in human application that have been subject to substantial manipulation, and/or cells that are used for an essential function or functions in the recipient different from their function in the donor, are regulated as medicinal products in the EU.

According to Article 1 (a) of the ATMP Regulation, an ATMP is any of the following medicinal products for human use:

a. a gene therapy medicinal product;
b. a somatic cell therapy medicinal product;
c. a tissue-engineered product.

A gene therapy medicinal product is a biological medicinal product that fulfils these two characteristics:

a. it contains an active substance that contains or consists of a recombinant nucleic acid used in or administered to human beings with a view to regulating, repairing, replacing, adding or deleting a genetic sequence;
b. its therapeutic, prophylactic or diagnostic effect relates directly to the recombinant nucleic acid sequence it contains, or to the product of genetic expression of this sequence.

Gene therapy medicinal products must not include vaccines against infectious diseases.

A somatic cell therapy medicinal product is a biological medicinal product that has two characteristics:

a. it contains or consists of tissues or cells that have been subject to substantial manipulation, or tissues or cells that are not intended to be used for the same essential function(s) in the recipient as in the donor;
b. it is presented as having properties for (or is used in humans with a view to) treating, preventing or diagnosing a disease through the pharmacological, immunological or metabolic action of its tissues or cells.

For the purposes of point (a), the manipulations listed in Annex I to Regulation 1394/2007/EC are not considered as substantial: cutting, grinding, shaping, centrifugation, soaking in antibiotic or antimicrobial solutions, sterilisation, irradiation, cell separation, concentration or purification, filtering, lyophilisation, freezing, cryopreservation and vitrification. Note that this list is non-exhaustive and that other processes may also fall outside the scope of Regulation 1394/2007/EC. Thus, based on scientific considerations, other manipulations may also be judged 'non-substantial' (e.g. manipulations that have been used in clinical practice in a hospital setting over many years).

A tissue-engineered product is a product that:

a. contains or consists of tissues or cells that have been subject to substantial manipulation, or tissues or cells that are not intended to be used for the same essential function(s) in the recipient as in the donor;
b. is presented as having properties for (or it is used in humans with a view to) regenerating, repairing or replacing human tissue.

A tissue-engineered product may contain cells or tissues of human or animal origin, or both. The cells or tissues may be viable or non-viable. It may also contain additional substances, such as cell and tissue preparations, bio-molecules, biomaterials, chemical substances, scaffolds or matrices. Products containing or consisting exclusively of non-viable human or animal cells and/or tissues, which do not contain any viable cells or tissues and which do not act principally by pharmacological, immunological or metabolic action, are excluded from this definition.

A combined advanced therapy medicinal product means an ATMP that fulfils the following conditions:

a. It must incorporate, as an integral part of the product, one or more medical devices within the meaning of Article 1(2)(a) of Directive 93/42/EEC or one or more active implantable medical devices within the meaning of the Article 1(2)(c) of Directive 90/385/EEC, and
b. Its cellular or tissue part must contain viable cells or tissues, or
c. Its cellular or tissue part containing non-viable cells or tissues must be liable to act upon the human body with action that can be considered as primary to that of the devices referred to.

Considerations related to the advanced therapy medicinal products regulation

Where questions arise as to the regulatory status of a substance, product or activity taht falls under the SoHO framework, a consultation within the Member State (MS) concerned must be initiated in accordance with Article 13(2) of Regulation (EU) 2024/1938. This consultation is co-ordinated among SoHO Competent Authorities (CAs) and authorities of other regulatory sectors. In the course of these consultations the CAs may also send a request for an opinion on the regulatory status of the substance, product or activity to the SoHO Coordination Board (SCB), in accordance with Article 13 (3) of Regulation (EU) 2024/1938.

The SCB is responsible for ensuring effective co-ordination and communication between the assessments and opinions issued by the SCB and other European Committees or Advisory groups, namely (and not restricted to) the Committee for Advanced Therapies (CAT), the Committee for Medicinal Products for Human Use (CHMP) and the Regulatory Committee on Medical Devices (CMTD). This includes facilitating exchanges of information during the process of issuing opinions by the SCB.

In scenarios where developers are uncertain if their activities, products or therapies are classified as gene therapies, somatic cell therapies or tissue-engineered therapies, developers go first to their CA at national level, and afterwards ask the SCB or CAT for advice, on whether a specific product is an ATMP.

Marketing authorisation for an ATMP can only be granted if, after a scientific assessment of the product's quality, efficacy and safety profile, it is demonstrated that the benefits outweigh the risks. The CAT is a specialised and multidisciplinary committee at the European Medicines Agency (EMA) responsible for assessing the quality, safety and efficacy of ATMPs that follow the centralised procedure for marketing authorisation, and it assists in the preparation of any documents related to fulfilment of the objectives of Regulation 1394/2007, where relevant. The application for a marketing authorisation must be submitted to the EMA, and the final decision is taken by the European Commission (EC).

As ATMPs derive from cell- or tissue-based starting material, Regulation (EU) 2024/1938 applies to the donation, collection and testing of the cells or tissue, and release before they are received for further processing at the ATMP manufacturer's premises, as well as to a range of other activities connected with starting material, including storage, distribution and import and export.

The ATMP Regulation gives member states the power to authorise the use of custom-made ATMPs prepared on a non-routine basis in the absence of a centralised marketing authorisation, provided that the product is used for individual patients in a hospital and under the professional responsibility of a medical practitioner. This 'hospital exemption' requires the application of national requirements on quality, traceability and pharmacovigilance equivalent to those required for authorised medicinal products.

It is important to stress that, in the EU, ATMPs are medicinal products. It follows that the overall regulatory framework governing medicines (including, but not limited to, rules on manufacture, distribution, packaging, labelling, evaluation of risks and benefits, determination of the data needed to demonstrate efficacy and safety, pharmacovigilance and advertising of medicines) applies to ATMPs. Furthermore, use of ATMPs in an investigational setting is also subject to EU rules on clinical trials.

However, flexibility in the development of ATMPs is important to anticipate the rapid evolution of science and technology in the field. To facilitate the process, the EU introduced the risk-based approach [1], which is based on the identification of various risks associated with the clinical use of an ATMP and risk factors inherent to the ATMP with respect to quality, safety and efficacy.

Additional information about the EU regulatory framework for ATMPs can be found at the EMA and EC Internet websites [1, 2].

This guidance is not intended to affect the scope of the EU rules on medicines, and any operator who intends to process, store, distribute or use cells in humans should first seek advice from national authorities on the appropriate, applicable legal framework.

In some countries, the regulatory body is the same for all products whereas, in others, those classified as ATMPs are regulated by a different body/agency from those classified as cells for transplantation.

As well as the regulatory oversight, the ethical and legal position on the use of human stem cells or progenitor cells differs in countries around the world [3]. In the EU, each member state is able to make decisions on the use of progenitor stem cells for basic research. However,

they must be compliant with the requirements stated in the ATMP Regulation if they are to be used for the manufacture of ATMPs for treating patients.

Some countries have national legislation on paediatric donors that must be taken into account when cells from children are considered.

Consideration of ethics

Local/regional/national independent ethics committees (IECs) are important bodies designated to review and approve biomedical and behavioural research involving humans, including the scientific rationale for the clinical application of a new therapy. For the latter, IECs should consider the irreversible nature of some gene/cell therapies and address the acceptability of exposing a donor to a research protocol for the benefit of the recipient, in particular where the donor is a relative of the subject, especially a parent or a sibling, to be included in the trial. IECs should also check appropriate traceability and guarantees regarding subject data protection and confidentiality. Written informed consent for receiving a cellular therapy is considered a prerequisite, as in any clinical trial.

The International Society for Stem Cell Research (ISSCR) guidelines recommend that special emphasis be placed on the risks of stem cell-based clinical research during the informed consent process. The risks include tumour formation, immunological reactions, unexpected behaviour of cells and tissues, unknown long-term health effects and sensitivities around the source of SoHO preparations [4].

Considerations in the conduct of clinical research

Clinical research, including trials of experimental interventions, is essential in translating cell-based applications into wider use and it requires the participation of human subjects, whose rights and welfare must be protected [2]. This appendix is not related to innovation with SoHO; under the new SoHO Regulation, clinical research with SoHO falls under SoHO Clinical Studies, for which see Chapter 19. Such research also follows the good practice of clinical settings for SoHO establishments defined by GAPP [5].

All people involved, including sponsors, investigators, oversight bodies and regulators, are responsible for ensuring the ethical conduct of clinical trials. As with all clinical research, clinical trials follow internationally accepted principles governing the ethical design and conduct of clinical research and the protection of human subjects [6, 7].

In the EU, a specific directive regulates clinical trials [8]. The EMA published a guideline to help professionals [9]. The EU has published Guidelines on Good Clinical Practice (GCP) specific to Advanced Therapy Medicinal Products [10].

Finally, in view of the need to ensure a consistently high level of protection for SoHO donors, and sufficient availability of SoHO for recipients, the donation of SoHO that will be exclusively for use in research without any human application must also comply with the standards concerning voluntary and unpaid donation set out in the SoHO Regulation.

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Human tissues and cells are being used in an increasing variety of ways, and advances in transplantation therapy have unquestionable benefits. Human cells and tissues for transplantation can save lives or restore essential functions, but the use of human tissues and cells also raises questions of safety and quality. Only tissues and cells collected, processed and stored following strict quality and safety standards are likely to function satisfactorily. Careful evaluation of donors is essential to minimise the risk of transmission of infections or malignancies. Furthermore, since human tissues and cells can currently only be derived from the body of a person, strong ethical principles need to be associated with their donation and use. The Council of Europe approaches tissue and cell transplantation in compliance with the principles of non-commercialisation and voluntary donation of materials of human origin.

This 6th Edition of the *Guide to the quality and safety of tissues and cells for human application* contains information and guidance for all professionals involved in donation, banking, transplantation and other clinical applications of these medical products of exceptional nature.

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