EuroGTP II Guide

Good practices for evaluating quality, safety and efficacy of novel SoHO preparations

Guidance, methodologies and tools

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EuroGTP II guide

Good practices for
evaluating quality, safety and efficacy
of novel SoHO preparations

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

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During the process of integration of the EuroGTP II tool to the SoHO platform of the EDQM, the EuroGTP II tool has been redesigned, and the terminology used in the first edition of the guide updated.

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Disclaimer

The Associated and Collaborative Partners of the Good Practices for demonstrating safety and quality through recipient follow-up project (hereinafter referred to as 'EuroGTP II project') developed this guidance to provide recommendations and to improve the quality of healthcare delivery within the field of human tissues and cells.

This guidance document and associated tool represent the views of the EuroGTP II project, which were achieved after careful consideration of the scientific evidence available at the time of preparation. In the absence of scientific evidence on certain aspects, a consensus between the EuroGTP II partners has been obtained.

The aim of the methodologies and tools proposed is to aid tissue bankers and healthcare professionals in the evaluation of safety, quality and efficacy of tissue and cellular therapies and products, therefore providing effective care of their patients.

However, adherence to guidance does not guarantee a successful or specific outcome, nor does it establish a standard of care.

EuroGTP II outcomes do not override national regulations, healthcare professionals' clinical judgment and treatment of patients. Ultimately, healthcare professionals must make their own clinical decisions on a case-by-case basis, using their clinical judgment, knowledge, and expertise, taking into account the conditions and circumstances, and in consultation with Competent Authorities (CAs).

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Collaborations

EuroGTP II project is interlinked with:

VISTART Joint Action (Vigilance and Inspection for the Safety of Transfusion, Assisted Reproduction and Transplantation) – intended to support EU member states in developing and strengthening their capacity for monitoring and control of quality, safety and efficacy in the field of blood, tissue and cell transplantation[1].

ECCTR Project (European Cornea and Cell Transplantation Registry) – aims to build a common assessment methodology and establish an EU web-based registry and network for academics, health professionals and authorities to assess and verify the safety, quality and efficacy of corneal transplantation.

Collaboration with this project is considered advantageous, as the use of registries is considered an important tool for the evaluation of efficacy and safety of substances of human origin (SoHO). The criteria identified by the ECCTR are also considered to be a valuable example for the definition of follow-up and clinical evaluation principles by the EuroGTP II project.

GAPP Joint Action (facilitatinG the Authorisation of Preparation Process for blood and tissues and cells) – having in mind the need for future requirements associated with the clinical evaluation of efficacy and safety performed by national CAs, and the links needed to assure the coherence between EuroGTP II outcomes and any future tools developed, the Co-ordinator (Banc Sang i Teixits (BST)) is an Associative Partner in the JA.

These collaborations aim to develop harmonised procedures and good tissue and cell practices (GTPs), for the different European stakeholders: tissue establishments (TEs), organisations responsible for human application (ORHAs) and national CAs.

Abbreviations

AMSTAR	Assessing the Methodological Quality of Systematic Reviews
ART	assisted reproductive technologies
BTC	blood, tissues and cells
CA	Competent Authority
CBB	cord blood bank
CHAFEA	Consumers, Health, Agriculture and Food Executive Agency
CPP	critical process parameter
CQA	critical quality attribute
DNA	deoxyribonucleic acid
EC	European Commission
ECCTR	European Cornea and Cell Transplantation Registry
EDQM	European Directorate for the Quality of Medicines & HealthCare
EIM	European IVF monitoring
ESSKA	European Society for Sports Traumatology, Knee Surgery and Arthroscopy
EUTCD	European Tissue and Cell Directives
FISH	fluorescence *in situ* hybridisation
GAPP	facilitatinG the Authorisation of Preparation Process for blood and tissues and cells
GCP	good clinical practice
GRADE	Grading of Recommendations Assessment, Development and Evaluation
GTP	good tissue and cell practices
GvHD	graft-versus-host disease
HLA	human leukocyte antigen
HPC	haematopoietic progenitor cell
HSC	haematopoietic stem cell
HSCT	haematopoietic stem cell transplantation
IAT	Interactive assessment tool
ICSI	intracytoplasmic sperm injection
IVF	*in vitro* fertilisation
MAR	medically assisted reproduction
MED	minimal essential data
NICE	National Institute for Clinical Excellence (NICE)
ORHA	organisation responsible for human application
QC	quality control
RCT	randomised controlled trial
ROBINS-I	risk of bias in non-randomised studies - of interventions
SARE	serious adverse reaction and/or event
SoF	summary of findings
SoHO	substances of human origin
T&C	tissues and cells
TCD	T-cell depletion
TE	tissue establishment
TESE	testicular sperm extraction
TNC	total nucleated cell
TUNEL	terminal deoxynucleotidyl transferase deoxyuridine triphosphate nick-end labelling assay
V&S	Vigilance and Surveillance
VAS	visual analogue scale
VISTART	Vigilance and Inspection for the Safety of Transfusion, Assisted Reproduction and Transplantation
WP	work package

1. Introduction

The preparation of blood components and tissue and cell preparations (BTC) intended for human application must comply with high standards of quality and safety, according to the requirements of the European Tissue and Cell Directives (EUTCD)[2-7] in order to ensure a high level of health protection in the EU. This concept becomes even more important with novel BTCs that are applied for the first time in humans or are prepared with new and innovative methodologies.

Advances in basic science, technology and medicine continually create opportunities for new and improved BTC. These may be wholly new types of BTCs, or improved methodologies for the preparation of existing BTCs. While the objective of these changes and novelties is to prepare BTCs that are safer, clinically more effective and meet the needs of clinicians and patients, there is always a risk that any change in the processing method can result in harm in the recipient. It is therefore vital that an evaluation of the potential risk of a process is systematically evaluated whenever a significant change is made.

To date, no European regulations or standardised methodologies have been established to facilitate systematic evaluation of novel BTCs prior to introduction into a clinical setting; however, the VISTART project has produced a document for CAs responsible for tissues and cells (T&C), to introduce the first principles on this topic1. This could represent the basis of a future regulatory framework based around the need to gather clinical follow-up of recipients as a means of validating the clinical performance of T&C prepared with newly developed processing methodologies, and novel therapies. Furthermore the results of an EU survey of TEs carried out by the EuroGTP II project confirmed the need for safety and efficacy studies, based on risk-based assessment.

The European Commission, being conscious of the necessity to strengthen standards for quality, safety and efficacy of BTCs, especially those related to novel BTCs, funded the EuroGTP II project (European Good Tissue and cells Practices II) – "Good Practices for demonstrating safety and quality through recipient follow-up". The main objective is to set up good practices with regard to preclinical and clinical evaluation of human replacement tissues, haematopoietic progenitor cells (HPC) and medically assisted reproduction (MAR), including reproductive tissue and cell preparations. EuroGTP II will provide continuity with the first EuroGTP project[8], which developed European Good Tissue Practices for the activities carried out in TEs.

By using the systematic approach proposed, the users of this guide will be able to:

a) evaluate risks resulting from all aspects of the BTC supply chain (from donor selection to clinical application) of the final product;

b) design appropriate studies proportionate to the level of residual/unknown risk to confirm that the BTC is safe and effective.

The project has developed good practices, principles and reference tools applicable to BTCs and how to conduct adequate clinical follow-up studies. The methodologies proposed in this guide are intended to be systematic and consistent, in order to promote a standard approach to practices and recognition among stakeholders.

The methodologies defined in this guide aim to

provide guidance for TEs, ORHAs, CAs and professional societies, and the outcomes will be publicly available.

The good practices proposed do not override or replace national regulations, and authorisation procedures defined at national level by the CAs.

Furthermore, the content developed by the EuroGTP II project only applies to BTCs and their applications as regulated by the EUTCD. BTCs that are subject to "substantial manipulation" or that "are not intended to be used for the same essential function or functions in the recipient as in the donor" (as defined in Regulation 1394/2007/EC), are not part of the scope of this project.

1.1. Project rationale and objectives

There are three key outputs from the Euro GTP II Project:

A. Development of a systematic, risk-based mechanism and *Interactive Assessment Tool* (IAT: https://soho-guides.edqm.eu/eurogtp2tool/) to:

- evaluate if a new or changed BTC has significant novelty;
- determine the overall risk arising from the novelty
- determine an appropriate level of preclinical and clinical evaluations to address and assess the risk;
- implement the results of risk assessment into routine practice and follow up the results.

Chapter 2 provides a more detailed methodology for this objective.

B. To create a *T&C database* (http://db.goodtissuepractices.site) of tissue/cell products, preparation processes, applications and therapies.

- The purpose of this database is to provide data related to the products and therapies available, and support end users in the evaluation of BTCs for safe and efficacious use. The structure and content of the database were designed to ensure that the data collected are consistent, and to support the collection of efficacy and quality data associated with the clinical use of SoHO at European level.

Chapter 7 provides a more detailed methodology for this objective.

C. To put in place mechanisms to ensure the sustainability of the project's outcomes and propose a structure for the development of European accreditation and training programmes for TEs, MAR centres and ORHAs.

- The ***GTP's Management Model*** aims to assure the continuity and sustainability of the outcomes of the EuroGTP II project, and the future update, promotion and harmonisation of GTP standards.

This output does not form part of this guide, as it is an independent deliverable of the EuroGTP II project.

1.2. Overview of the EuroGTP II Guide

The purpose of this document is to provide structured guidance on how to use the tools and methodologies developed by the EuroGTP II project.

This guide has been developed with the collaboration of experts and representatives of EU TEs, ORHAs, scientific associations, universities, CAs, research organisations and national registries (Partners and Experts of the EuroGTP II project – Annex I).

In order to ensure alignment and coherence with existing documents dealing with patient follow-up and quality aspects, the above-mentioned stakeholders considered the following current guidelines and reference documents in the development of the guide:

- VISTART deliverable regarding regulatory principles for clinical follow-up of recipients – Principles for Competent Authorities for the evaluation and approval of clinical follow-up protocols for blood, tissues and cells prepared with newly developed and validated processing methodologies[1]
- ARTHIQS (Assisted Reproductive Technologies and Haematopoietic stem cells Improvements for Quality and Safety throughout Europe) recommendations for cord blood banks and donor follow-up registries[9]
- Outcomes of SoHO Vigilance & Surveillance (V&S) Project[10]
- Deliverables of the European Union Standards and Training for the Inspection of Tissues Establishments (EUSTITE) Project[11]

- Deliverables of the European Good Tissue Practices (EuroGTP) Project[8]
- Guide to the Quality and Safety of Tissues and Cells for Human Application, 2017, 3rd edition, Council of Europe, European Directorate for the Quality of Medicines & HealthCare (EDQM)
- The EU Coding Platform – Reference Compendia for the Application of a Single European Code for Tissues and Cells (SEC)
- Notify Library – Global Vigilance and Surveillance Database for Medical Products of Human Origin
- FACT-JACIE (Joint Accreditation Committee – ISCT & EBMT) International Standards for Hematopoietic Cellular Therapy Product Collection, Pro- cessing, and Administration[13]
- ESHRE (European Society of Human Reproduction and Embryology) Guidelines for good practice in IVF laboratories[14]

The outputs of the EuroGTP II project will also be used as a basis for the next Joint Action (GAPP) that will develop the criteria for evaluating quality aspects of preparation processes by CAs.

1.3. Structure of this document

The guide is structured in seven principle chapters:
Chapter 1 – General Introduction and overview
Chapter 2 – Methodology for BTC characterisation, assessment of novelty and risk evaluation
Chapter 3 – Instructions for the correct use of EuroGTP II methodologies and tools
Chapter 4 – Specific guidance with regard to using EuroGTP II methodologies and tools for replacement tissue preparations and therapies
Chapter 5 – Specific guidance with regard to using EuroGTP II methodologies and tools for HPC preparations and therapies
Chapter 6 – Specific guidance with regard to using EuroGTP II methodologies and tools for MAR preparations and therapies
Chapter 7 – A guide to the structure and use of the T&C database

1.4. How should the guide be used?

It is suggested that Chapters 1, 2 and 3 of this guide be read in their entirety before attempting to use the methodologies proposed by the EuroGTP II project.
Chapters 4, 5 and 6 are intended to be used as a reference, as they provide specific guidance for the use of tools and methodologies applied to the different areas of SoHO.

2. Outline methodology for BTC characterisation, assessment of novelty and risk evaluation

The assessment methodologies proposed by the EuroGTP II project can be applied on paper using the available templates (Annex II and Annex III) or online using the EuroGTP II Interactive Risk Assessment Tool (IAT).
Instructions for the correct use of these methodologies can be found in Chapter 3 and/or in the SoHO-specific Chapters: 4 – Replacement tissues, 5 – HPC and 6 – MAR of this guide.
An overview of EuroGTP II methodologies is available in Annex IV.

2.1. Characterisation of the BTC

Before commencing assessment of novelty and the associated risk, it is important that the BTC is thoroughly characterised so that the process can be performed accurately. This requires the following details to be documented (the template provided in Annex II may assist users in this process):

- Justification for the implementation of change, including the key benefits of the innovation.
- How is the BTC prepared? What, if any, changes have been made to the established preparation or treatment protocol?
- What is the origin of the BTC (autologous or allogeneic, or in the case of MAR concerning partner or non-partner donation)?
- In what format is it presented for clinical application (e.g. packaging, methodology and preservation technique)?
- What, if any, excipients or other reagents or residues could be transferred through clinical application of the BTC (such as carriers or preservatives)?
- What are the CPPs applied to the BTC preparation protocol?
- What are the CQAs necessary for the BTC to deliver its intended result?
- What clinical indication is the BTC to be used for?

Additionally, prior to the implementation of changed/new processes, the template provided in Annex II should be completed with a description of the factors that justify the developments. This may include the following information:

- existence of prior clinical data reported by other centres (if applicable);
- quality control measures and any other quality indicators evaluated;
- overview of the intended clinical effect of the BTC;
- bibliographic evidence that supports the implementation of changes;
- in-house data generated to justify the process.

2.2. Evaluation of novelty (Step 1)

It is important that the definition of 'novelty' within the context of this process is clearly established. It is not intended to encompass every change to a product or process, regardless of how minute the change is; rather it is intended to capture any change that could **significantly affect the quality and/or safety of the BTC and/or the safety of recipients**. This is the first step of the novelty and risk evaluation process (Figure 2.1).

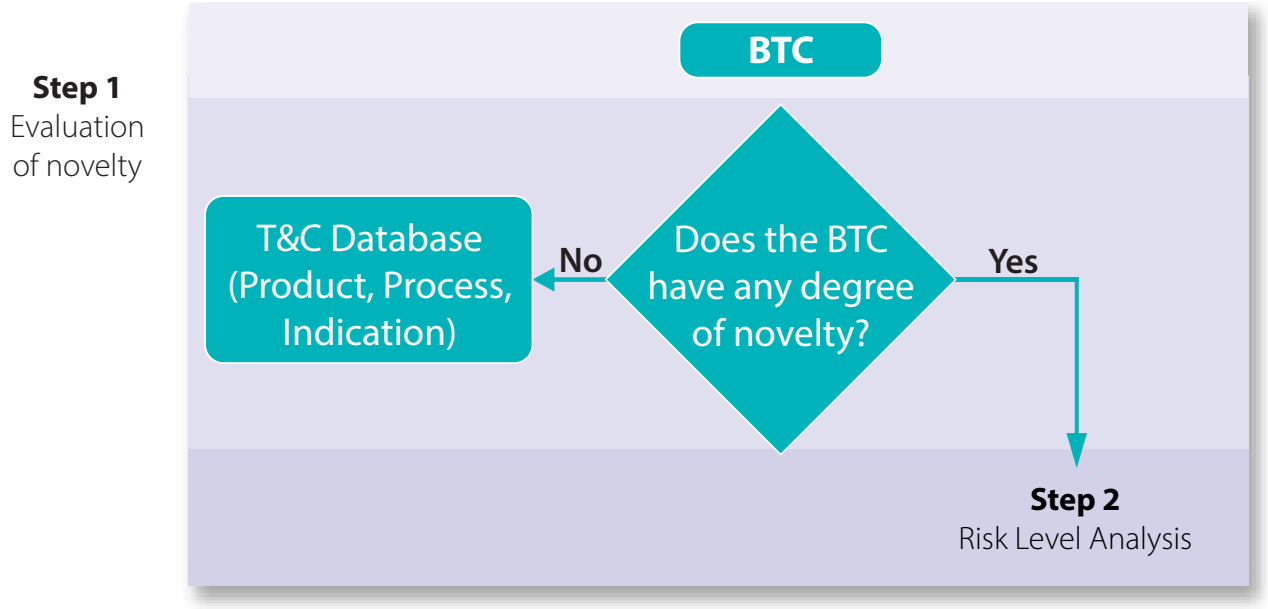

Figure 2.1. **Evaluation of novelty**

This assessment involves answering a series of seven questions, covering all aspects of BTC provision from donor selection to clinical application of the final product. If no novelty is identified (this process is discussed in detail in Chapters 3 – Generic methodologies and tools, 4 – Replacement tissues, 5 – HPC and 6 – MAR), it can be concluded that there is no significant change or innovation in the BTC being assessed and the exercise ends at this point. Users are encouraged to add their established product to the T&C Database (a guide to the structure and use of the T&C database is provided in Chapter 7 of this document).

2.3. Overview of the risk assessment process – level risk analysis (Step 2)

If Step 1 establishes that a new or changed BTC has significant novelty, a systematic risk assessment must be undertaken to identify and quantify the risks associated with it. This must be a comprehensive process that considers all aspects of the BTC supply chain, from donor selection through to implantation or clinical application of the product or therapy. This is the second step of the novelty and risk evaluation process (Figure 2.2).

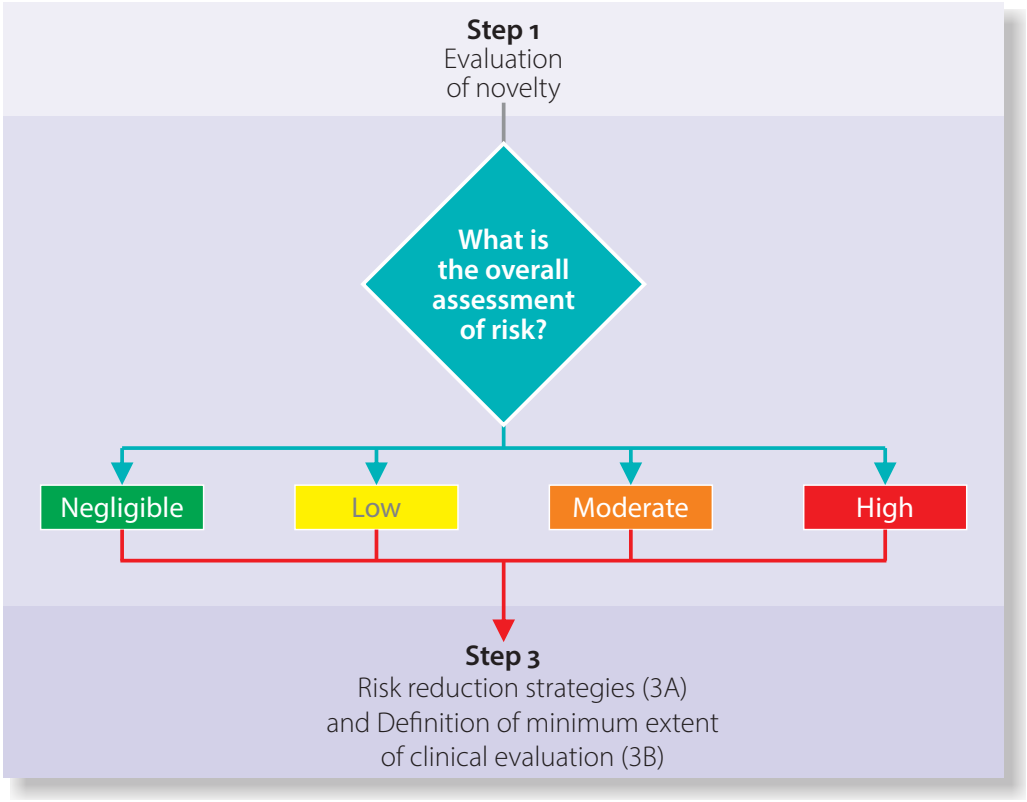

Figure 2.2. **The risk assessment process**

The risk profile is determined through the identification of potential risk factors (Figure 2.3) and analysis of risk consequences (Figure 2.4). This is further explained in Chapter 3, and in subsequent specific chapters (4 – Replacement tissues, 5 – HPC, and 6 – MAR), with some examples.

The overall process requires that specific risks are first identified in terms of the potential risk factors (Figure 2.3) and risk consequences (Figure 2.4). Each of these must be individually risk assessed to determine the residual risk of implementing the change, assessed by considering:

i) the probability of the risk occurring;

ii) the severity of the consequences should the risk occur;

iii) The probability that the source of the hazard for the risk consequences will be detected before the BTC is applied. This does not refer to detection of the consequences of the risk post-implantation;

iv) Any existing evidence that can be used to mitigate the risk.

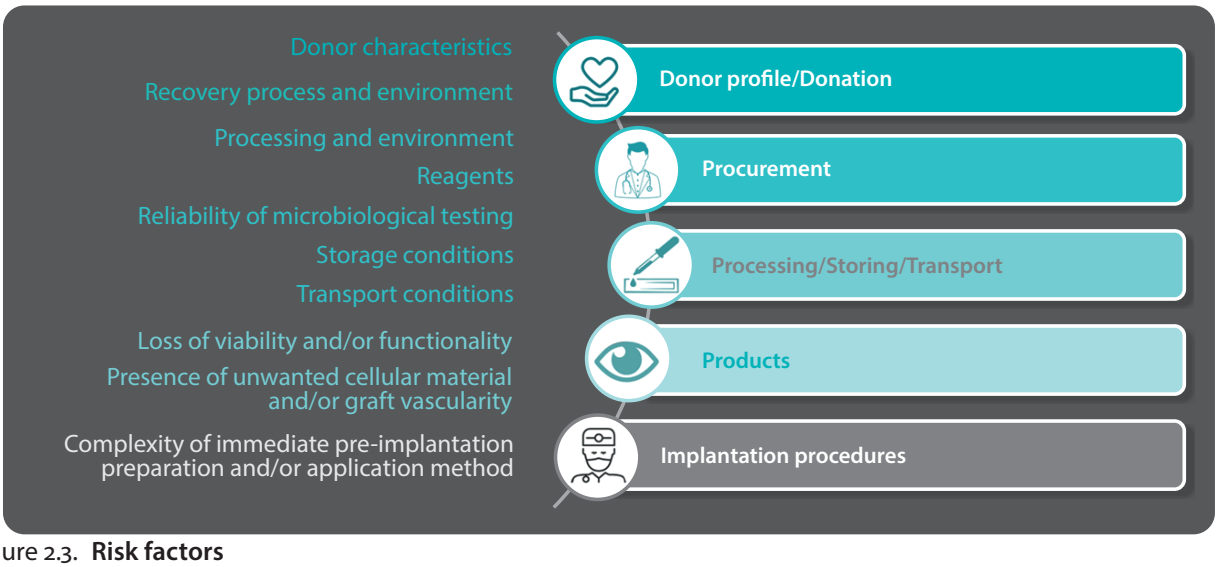

Figure 2.3. **Risk factors**

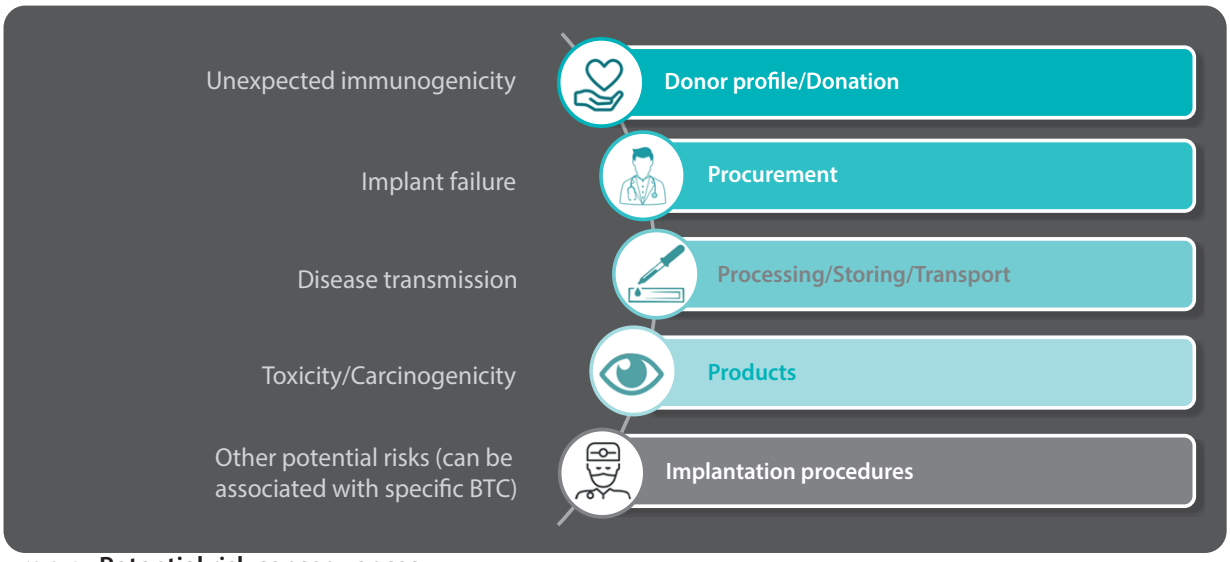

Figure 2.4. **Potential risk consequences**

The outcome of this exercise will be a single, overall risk score – Final Risk Score – (on a scale of 0 to 100) that can be used to inform the definition and extent of preclinical and clinical evaluation necessary to support the proposed novelty or change (the EuroGTP II algorithm for the calculation of the Final Risk Score is detailed in Annex V).

The tool used to quantify risk (described in detail in the next chapter) takes into account the number of individual risks assessed when calculating the proportional risk value. Thus, a process where multiple minor risks are identified could generate the same Final Risk Score as a process where only a single major risk is identified.

The quantity and quality of the available evidence, such as published data in peer-reviewed literature and internal validation reports, can be used to reduce this overall risk score. The whole risk assessment process is explained in more detail in Chapter 3.

2.4. Definition of the extent of studies (Step 3)

The Final Risk Score generated by the risk assessment process determines the corresponding extent of studies required to ensure the safety and efficacy of the BTC, in terms of the preclinical (*in vitro* and/or *in vivo*) and clinical evaluation (Figure 2.5). The specific, individual risk consequences identified further determine the precise test criteria indicated. The methodology proposed (detailed in Chapters 3 – Generic methodologies and tools, 4 – Replacement tissues, 5 – HPC and 6 – MAR of this guide), will assist users in designing these protocols.

Step 3A: Risk reduction strategies – Use preclinical studies (*in vitro* and *in vivo*) to mitigate the identified risks

After the risk assessment exercise, users should consider if the given risk score can be mitigated by performing preclinical studies.

In some scenarios, the initial risk may be negligible and the BTC may be used in humans without additional preclinical studies. However, if the risk is higher than negligible, it may be possible to perform additional *in vitro* and *in vivo* preclinical studies (if not already done) to mitigate and potentially reduce the level of risk prior to clinical application (examples of preclinical evaluations are given in Chapters: 4 – Replacement tissues, 5 – HPC and 6 – MAR of this guide).

Step 3B: Extent of clinical evaluationIn situations where the risks cannot be reduced sufficiently with preclinical studies, an internal risk-benefit exercise should be done in collaboration with the clinicians, to assess if it is justifiable to use the BTC in a clinical setting

The requirements of the clinical evaluation should be proportional to the remaining level of risk. Details of how to design and implement these studies are listed below (Table 2.1), and described in more detail in the specific chapters (4 – Replacement tissues, 5 – HPC and 6 – MAR) of this guide.

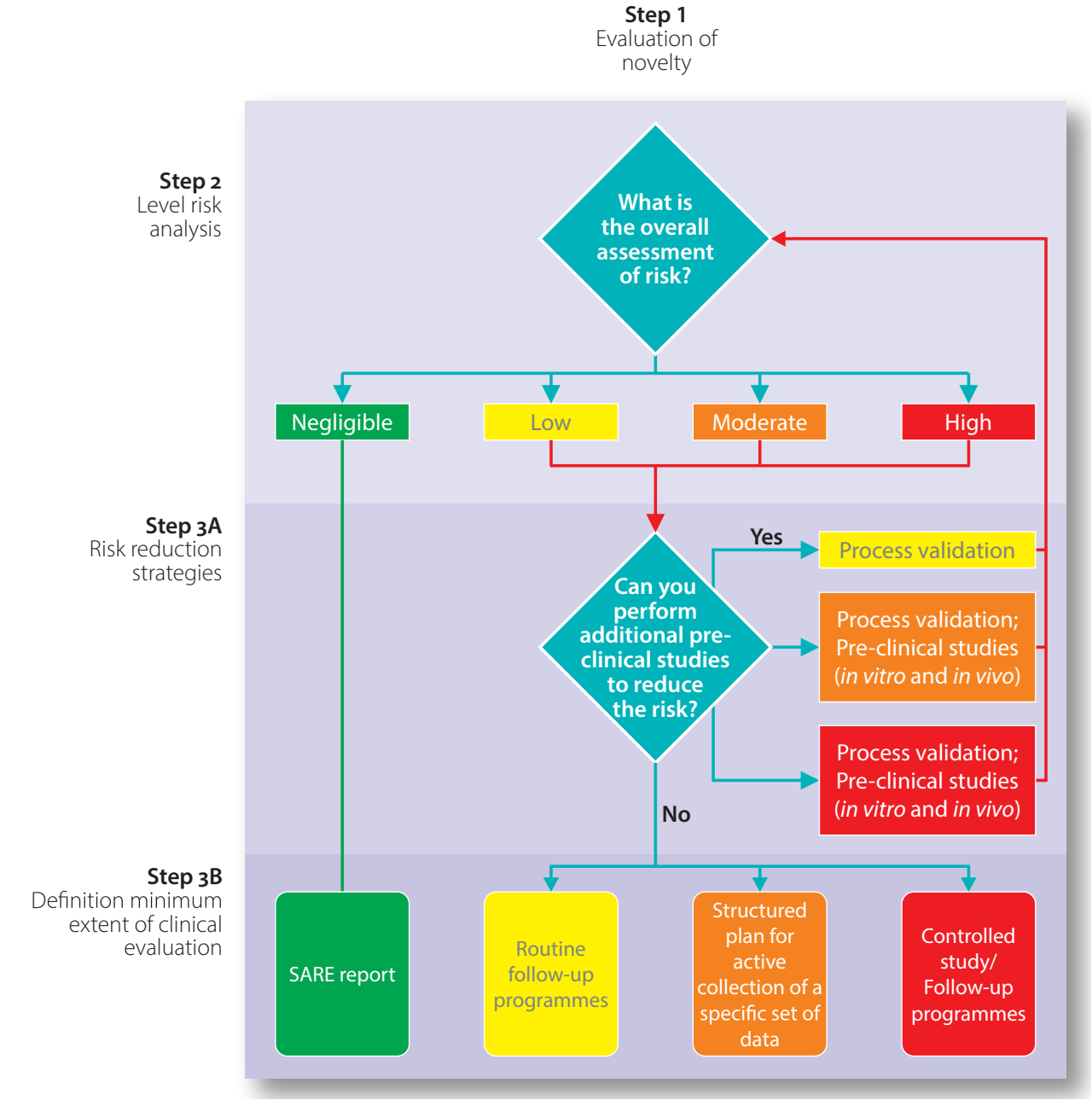

Figure 2.5. **The risk reduction and determination of the extent of studies required**

Table 2.1. **Extent of studies* required according to the level of risk determined in the assessment**

Level of risk	Extent of proposed studies*
Negligible	***Step3A: Risk reduction strategies*** The assessment indicates that the BTC is safe and efficacious for clinical use and very unlikely to cause harm to recipients; however, it may be advisable to conduct a validation of the process, if not already done. If the nature of the risk is not related to the process itself, the requirement for validation may not apply, for example where the novelty is in the method of clinical application. ***Step 3B: Extent of clinical evaluation*** No clinical follow-up over and above the mandatory requirement, such as SARE reporting.
Low	***Step3A: Risk reduction strategies*** The assessment indicates that the BTC is safe and efficacious for clinical use and unlikely to cause harm to recipients; however, a validation of the process, if not already done, should be performed. If the nature of the risk is not related to the process itself, the requirement for validation may not apply, for example where the novelty is in the method of clinical application. ***Step 3B: Extent of clinical evaluation*** In addition to the mandatory requirement for SARE reporting, feedback from immediate post-clinical application monitoring (routine clinical follow-up) may be collected for a defined period or number of procedures. Clinical audit† may also be used after an appropriate period of use.
Moderate	***Step3A: Risk reduction strategies*** The assessment indicates that more evidence is needed to support safe and effective use of this BTC and mitigate risk. Process validation should be performed; however, if the nature of the risk is not related to the process itself, the requirement for validation may not apply, for example where the novelty is in the method of clinical application. Preclinical in vitro evaluation studies, specific to the identified risks, should be performed if not already done. Preclinical in vivo evaluation using an animal model should be considered if applicable (and if not already done). Please refer to specific chapters of this guide (4 – Replacement tissues, 5 – HPC, and 6 – MAR) for additional details. ***Step 3B: Extent of clinical evaluation*** A structured plan for active collection of a specific set of data relating to the safety and efficacy of the BTC should be put in place, in addition to routine clinical follow-up. Ethical approval may be required and the principles of Good Clinical Practice (GCP)15 adhered to. Consideration should be given to restricting provision of the BTC to a limited number of patients and/or centres until the risks have been adequately mitigated Please refer to specific chapters of this guide (4 – Replacement tissues, 5 – HPC, and 6 – MAR) for additional details.
High	***Step3A: Risk reduction strategies*** The assessment indicates that significantly more evidence is needed to support safe and effective use of this BTC and mitigate risk. Process validation should be performed; however, if the nature of the risk is not related to the process itself, the requirement for validation may not apply, for example where the novelty is in the method of clinical application. Preclinical in vitro evaluation studies, specific to the identified risks, should be performed if not already done. Preclinical in vivo evaluation using an animal model should be considered if applicable (and if not already done). Please refer to specific chapters of this guide (4 – Tissues, 5 – HSC, and 6 – ART) for additional details. ***Step 3B: Extent of clinical evaluation*** The BTC should only be used clinically in the context of an ethically approved, controlled (where applicable) clinical evaluation until the residual risks have been adequately mitigated. The principles of GCP[15] must be adhered to. Clinical evaluation and follow-up programmes should be implemented and safety and efficacy must be continuously monitored. If available national and international registries are recommended for gathering follow-up data. Please refer to specific chapters of this guide (4 – Replacement tissues, 5 – HPC, and 6 – MAR) for additional details.

* Process validation, Preclinical *in vitro* and *in vivo* studies, and Clinical evaluation.
† In the context of this guide, clinical audit refers to retrospective or prospective evaluation of routinely collected clinical data.

Design considerations for clinical evaluations adapted to T&C products/therapies

The design of clinical evaluation programmes must be planned in close co-operation between the TEs and the clinicians responsible for the clinical application of the BTC. The collaboration between TEs and end users is critical to identify suitable design parameters, clinical indications, number of patients, type of follow-up proportionate to the residual risks identified and to ensure that comprehensive data are gathered to evaluate efficacy.

The design of the clinical evaluation should consider:

a) the nature of the risk (e.g. if sudden graft failure is a significant risk, then patient recruitment should allow for sufficient observation time between one patient and the next to be enrolled);

b) the number of patients required to obtain statistically significant data, where applicable. If the number needed is too high because the disease is rare or the follow-up period is very long, then alternative solutions must be proposed.

The design of clinical evaluation should take into

consideration the requirements of GCP[15], including independent ethical committee opinion and any other national or regional specific regulations.

Specific guidance relating to design of clinical evaluation for different types of BTC is provided in Chapters 4 – Replacement tissues, 5 – HPC and 6 – MAR. However, certain features relating to design of clinical evaluation protocols are common to all types of BTC. There are fundamentally two types of evaluation:

i) single-arm study (case series/registry approach);

ii) controlled study, where the BTC is directly compared to a control treatment.

The type of clinical evaluation protocol selected will depend on a number of considerations, specifically:

- the level of risk – if risk is high, a controlled study is more suitable, provided that it is feasible for the BTC in question;
- the availability of a suitable control treatment;
- the length of time that patients need to be followed up for; if long-term follow-up is required, a controlled study may not be practical, and a registry approach may be considered.

In addition to these considerations, TEs should endeavour to collaborate with fellow TEs to set up multicentre studies, to ensure that sufficient patients are recruited. Collaborations with clinical trial units should also be pursued to ensure that the requisite skills and resources are available to manage studies.

2.5. Ethical principles and considerations

Innovative and experimental therapies are often where scientific research and clinical practice meet. Understanding and applying basic ethical principles (autonomy, non-maleficence, beneficence and justice) is essential for the clinical implementation of novel treatments.

Thus, clinical application of novel BTCs must always follow the *Ethical Principles for Medical* Research *Involving Human subjects*, determined in the Declaration of Helsinki[16], namely in what concerns the careful assessment of predictable risks and burdens to the individuals, the procedures associated with informed consent of recipients and donors, and the considerations and approval of Research Ethics Committees, including the (impact of) procurement and source of SoHO.

3. Instructions for the correct use of EuroGTP II methodologies and tools

There are three distinct phases of the risk assessment process, as explained in the previous chapters. To facilitate this process, an online Interactive Assessment Tool (IAT) has been developed. The IAT addresses the first two of these phases: evaluation of novelty and analysis of risk. This generates individual risk scores for each risk consequence identified, plus a Final Risk Score for the BTC as a whole. The output from the analysis of risk is used to inform the third phase of the process, to determine whether or not the BTC can be made generally available for clinical application on request, or if further preclinical and/or clinical evaluations are required.

ACCESSING THE EUROGTP II Interactive Risk Assessment Tool (IAT)

The IAT is accessible online (https://soho-guides.edqm.eu/eurogtp2tool/).

3.1. Key principles for effective use of the EuroGTP II methodologies and IAT

The value of the outputs from the IAT will be determined by the accuracy, comprehensiveness and relevance of the information that is put into it. It is therefore advised that:

i) The process should be treated as a long-term exercise: the intention is that the IAT will provide the framework for a detailed assessment of risk. It is important that the rationale for these decisions is recorded and documented.

ii) It is unlikely that a single individual will have sufficient knowledge and expertise to complete the whole process in one go with no support. Ideally, the assessment should be performed by a group of individuals selected for their knowledge and experience who will consider all available information to generate an accurate assessment of risk. The process should be performed by a team selected to provide the requisite knowledge and experience to fully identify and evaluate all potential risks. This may include all professionals involved in the SoHO activities, namely:

- Operational staff;
- Scientists and embryologist developing BTCs;
- Quality control personnel;
- Healthcare professionals

Please note that this list is not exhaustive.

iii) The IAT may be used at any point in the process/product development cycle. The initial process can be performed at an early stage in the development of new or revised BTCs; this may identify areas of high risk that could be addressed by preclinical development work. The exercise can be repeated at different stages of the development and implemen-

tation of the BTC, in order to re-evaluate the risks based on the information recollected (by the studies performed and/or relevant references). Much of the potential risk inherent to a new product or process can generally be eliminated or ameliorated by well-planned and focused preclinical studies. It can therefore be useful to use the IAT at a very early stage, where it can pinpoint areas where there is a high level of risk that could be addressed with preclinical *in vitro* studies, or by sourcing the appropriate literature. Often, at this stage, potential risk must be assessed as high purely due to lack of data. The IAT can be re-run during the development cycle to evaluate how ongoing work is contributing to ameliorating the overall risk, and identify areas where further effort should be focused. If used in this manner, the final use of the IAT prior to providing products for clinical use will identify the residual risk that can only be addressed with clinical evaluation or follow-up. This final output, along with all associated documentation and evidence, can be used to support submissions to CAs to seek approval to provide the BTC for clinical use, either in a routine or restricted setting as indicated by the level of residual risk.

iv) There must be a clear understanding of the critical parameters of the BTC which will contribute to its safety and efficacy, to enable the risk assessment to be performed accurately.

Note also that the IAT should only be used to assess new risks resulting from the novelty. It is assumed that for existing BTCs, which are being provided for clinical use, the existing risks have been evaluated and are adequately controlled.
Specific guidance applicable to the use of EuroGTP II methodologies and tools for different BTCs is described in Chapters 4 – Replacement tissues, 5 – HPC, and 6 – MAR.

3.2. Step 1: Evaluation of novelty

The first stage of the tool is the assessment of novelty. This involves answering a series of seven questions, shown in Table 3.1, covering all aspects of the T&C supply chain from donation to clinical application. This stage is intended to generate a simple 'yes' or 'no' answer; there is either novelty or not, irrespective of the degree of novelty.

Additionally, a third option – 'Not Applicable/Not relevant' (NA) – is provided to cover situations that are not addressed for the BTC under evaluation.

If no novelty is identified, it can be concluded that there is no significant change or innovation in the BTC being assessed; in this case, there is no need to proceed with the rest of the IAT, and users are invited to add their BTC to the *T&C Database*.

Specific examples and explanations regarding the interpretation of these questions are provided in the specific chapters (4 – Replacement tissues, 5 – HPC and 6 – MAR).

Table 3.1. **Evaluation of novelty (Step 1)**

		Yes	No	NA
A	Has this type of BTC* previously been collected, processed/prepared and issued for clinical use by your establishment?			
B	Will the starting material used to prepare this BTC be obtained from the same donor population previously used by your establishment for this type of BTC*?			
C	Will the starting material for this BTC be procured/collected using a procedure used previously by your establishment for this type of BTC*?			
D	Will this BTC be prepared by a procedure (processing/preparation, decontamination/pathogen reduction and preservation) used previously in your establishment for this type of BTC*?			
E	Will this BTC be packaged, stored and distributed using a protocol and materials used previously in your establishment for this type of BTC*?			
F	Will this type of BTC provided by your establishment be applied/infused clinically using an application/ transfusion/infusion method used previously?			

		Yes	No	NA
G	Has your establishment provided this type of BTC* for the same clinical indication or for application/transfusion/infusion into the same anatomical site?			

* Should be interpreted as the broader category of BTC (skin, heart valves, etc.). The aim is to find out whether, despite the novelty, your organisation has experience handling this BTC

3.3. Step 2: Level risk analysis – identification and quantification of risk

If, after completing Step 1, you determine that there is some novelty resulting from your proposed change, you then proceed to Step 2 to identify and quantify the potential risks resulting from this novelty. There are a number of stages in this process:

Step 2A: Identification of risk factors

This step involves identifying the potential risk factors that are relevant to the change. The global risks that should be considered during this assessment are listed in Table 3.2. Specific risk factors, examples and explanations regarding the interpretation of these risk factors, are provided in the following chapters (4 – Replacement tissues, 5 – HPC and 6 – MAR).

Table 3.2. **Identification of risk factors**

Process	Specific risk factors	Guidance notes
Donation	Donor characteristics	Consider whether the donor population you intend to obtain the BTC from could impart any risk. For example, if the BTC is sourced from an allogeneic donor, there may be risks that immunogenicity could impact the clinical performance of the BTC, and there is a risk of disease transmission.
Procurement	Procurement/collection process and environment	Consider where and how the BTC is collected, procured or recovered, and if this process could have an influence on the BTC. How long does the process take, how complex is it and what is quality of the environment? These factors may have an impact on the probability that the BTC becomes contaminated or damaged during recovery.
Processing/ storing/ transport	Processing and environment	Consider where and how the BTC is processed/prepared, namely how long the process takes and how complex it is? (including all physical and chemical treatments applied to the product) – this may have an impact on the risk of contamination, or suggest that it may not be prepared to consistent specifications and quality. Also, consider the quality of the processing environment, which may also affect the risk of contamination. (Please note that risks associated with reagents are considered in the following specific risk factor).
	Reagents/added components	Consider any reagents used during preparation, decontamination, preservation, storage and transport of the BTC. Could they damage the BTC in any way, or could residual traces of reagent remain in the BTC that could cause toxic or immunogenic effects in recipients?
	Reliability of microbiology testing	Consider the risk that the nature of the BTC, the testing methodology and/ or the presence of residual processing reagents such as antibiotics in the finished BTC may impact the accuracy of any microbiology tests. Note: this refers specifically to bacteriology/mycology testing of the BTC, not any blood tests performed on the donor.
	Storage conditions	Consider any potential risks arising from how the starting material and BTC are stored between procurement and processing, during processing, and between processing and clinical application.
	Transport conditions	Consider any potential risks arising from how the starting material and BTC are transported, for example between the sites of procurement and processing, and between the sites of storage and clinical application.
Production/ preparation	Presence of unwanted cellular material and/or graft vascularity	This risk must be considered from the perspective that the presence of intact vital cells is desirable for some BTCs, but that this may affect tumour formation, immunogenicity and disease transmission risks. Vascular tissues may be more at risk of infiltration by pathogens or malignant cells than avascular tissues.
	Loss of viability and/or functionality (MAR)	Consider the risk that changes to procedures or processes can have on the viability or functionality of the BTC.

Process	Specific risk factors	Guidance notes
Clinical application procedures	Complexity of the immediate pre-implantation preparation and/or application method	Consider how complex the method of clinical application will be for this BTC. How long will it take, and could this introduce risks? What is the scope for errors to be made, and what could the consequences of these errors be? Low feasibility of application standardisation may influence the risks of implant failure and disease transmission, at minimum

Step 2B: Identification of risk consequences

Consider the potential consequences for the risk factors identified above.

The potential risk consequences associated with the clinical use of BTC comprise:

- Unexpected immunogenicity
- Implant failure/engraftment failure/pregnancy loss
- Disease transmission
- Toxicity/Carcinogenicity
- Other risks (associated with specific BTCs).

Examples of the combination of risk factors and specific consequences that may need to be considered are provided in the BTC type-specific chapters (4 – Replacement tissues, 5 – HPC and 6 – MAR). The purpose of the exercise is to systematically consider each risk factor and risk consequence in turn against the change. Note that for certain combinations of risk factor and risk consequence, there may be no relevant examples. It is recognised that the IAT cannot anticipate all potential types of risk; the specific risk consequences listed for each BTC type are those which it is generally agreed will be most commonly related to that type. For any risks not covered by these risk consequences, an open, 'other' category is provided and it is highly recommended to use this during the assessment.

Step 2C: Quantification of risk

The next step is to perform the risk assessment by determining the **probability, severity and detectability** for each risk factor identified for each **risk consequence**. When calculating probability, severity and detectability, you should consider the following sources of information:

- internal development and validation reports;
- previous experience and existing knowledge originating within your establishment;
- quality related data (trend analysis, indicators, product or process quality reviews, etc.);
- internal process validation studies, preclinical *in vitro* studies, preclinical *in vivo* studies, clinical evaluation protocols.

Note that there may be more than one risk consequence associated with each risk factor. If this is the case, the quantification of risk should be performed for all the risk consequence-risk factor combinations, in order to be able to address each risk specifically in future risk reduction strategies.

An explanation of the rationale behind the analysis performed should be recorded and included in the exercise. This will allow the user to keep a record of the risk consequences and risk factors evaluated.
These registers can be recorded by entering the information directly in the IAT or using the templates available in Annex II and Annex III (Replacement tissues, HPC and MAR Templates).

Assessment of probability

This assessment requires estimating the probability of any risk occurring. There are five levels of probability (Table 3.3).

Table 3.3. **Probability levels***

Level of probability	Definition
1 – Rare	Difficult to believe it could happen
2 – Unlikely	Not expected to happen but possible

Level of probability	Definition
3 – Possible	May occur occasionally
4 – Likely	Probable but not persistent
5 – Almost certain	Likely to occur on many occasions

* Definitions from V&S SoHO Project, 2009[10]

Assessment of severity

This assessment requires that you estimate the severity of the consequences of the risk, should it occur. There are four levels of severity (Table 3.4).

Table 3.4. **Severity levels***

Level of severity	Definition
1 – Non-serious	Mild clinical or psychological consequences for the recipient; however, with no hospitalisation or anticipated long-term consequences/disability
2 – Serious	Hospitalisation and/or: ◆ Persistent/significant disability or incapacity ◆ Intervention to preclude permanent damage ◆ Evidence of a serious transmitted infection ◆ Significant decrease in the expected treatment success ◆ Birth of a child with an infectious or genetic disease following MAR with donor gametes or embryos
3 – Life-threatening	◆ Major intervention necessary to prevent death ◆ Evidence of a life-threatening transmissible infection ◆ Birth of a child with life-threatening genetic disease following MAR with donor gametes of embryos
4 – Fatal	Death of the patient

* Definitions adapted from V&S SoHO Project, 2009[10]

Assessment of detectability

This assessment requires that you estimate the probability that the source of the hazard for the risk consequences will be detected before the BTC is applied (Table 3.5). This does not refer to detection of the consequences of the risk post-implantation.

Table 3.5. **Detectability levels**

Level of detectability	Definition
1 – Very high	The potential defect will almost certainly be detected before clinical application in the recipient.
2 – Moderately high	There is a reasonable chance that the potential defect will be detected before clinical application in the recipient.
3– Low	There is a low chance that the potential defect will be detected before clinical application in the recipient.
4 – Very low	It is unlikely that the potential defect will be detected before clinical application in the recipient.
5 – Cannot be detected	The potential defect will be detected only after clinical application in the recipient.

Step 2D: Assessment of risk reduction

Having calculated probability, severity and detectability, and thus an overall risk score based on 'internal' knowledge and data, it may be possible to adjust this score by taking into account other external sources of information.

These external data are not used to specifically

reduce probability, severity or detectability, rather it is used to calculate a general reduction in the overall risk score.

Data that should be taken into account when calculating risk reduction may include:

- published data in peer-reviewed literature;
- unpublished data from external sources;
- advice and information from external experts;
- clinical outcome data from external sources (e.g. registries).

When calculating the risk reduction factor, it is important that the quality and reliability of the data be considered; for example a large scale clinical trial in a high-impact, peer-reviewed journal would be considered of high quality and reliability, whereas unpublished clinical data with limited follow-up in a small number of patients less so.

An objective assessment of the quality of evidence is recommended. Available data should be reviewed in an explicit, systematic and transparent process that can be applied to both quantitative (experimental, observational and correlational) and qualitative evidence[17]. The key aim of any review is to provide a summary of the relevant evidence to ensure that assessments are performed based on adequate information.

Several methodologies are available to perform an objective evaluation of the quality and reliability of scientific data:

- To assess the risk of bias for individual studies/reviews: Assessing the Methodological Quality of Systematic Reviews (AMSTAR)[18], Risk Of Bias In Non-randomised Studies - of Interventions (ROBINS-I)[19], The Cochrane Collaboration's tool for assessing risk of bias[20] and other quality assessment methods or checklists.
- Grading of Recommendations Assessment, Development and Evaluation (GRADE) summary of findings (SoF) tables[21,22] or NICE (National Institute for Clinical Excellence) guidelines: the Manual[17].

Although this step does include some subjectivity and should be a team exercise (as noted in Section 3.1), the evidence used to justify the risk reduction should be accurately described in the rationale of the assessment. It is advisable to keep the references/documents associated with the risk assessment report (provided by the IAT or registered in the templates of Annex II and Annex III (Replacement tissues, HPC and MAR Templates)) in order to easily justify the rationale behind each risk assessment.

Based on the assessment of the data, different levels of risk reduction can be applied. This is accomplished by applying a percentage reduction to the overall risk score (probability x severity x detectability) calculated in the first three steps of the risk assessment. These levels are shown in Table 3.6.

Table 3.6. **Percentage risk reduction definitions**

Percentage risk reduction		Definition
0	None	There are no relevant data available to support reducing the calculated risk score.
25	Limited	There is a moderate amount of relevant data available to support reducing the calculated risk score, based predominantly on unpublished data.
50	Moderate	There is a moderate amount of good quality relevant data available to support reducing the calculated risk score, including published and unpublished data from external sources, and some data which have been through an independent peer-review process.
75	Substantial	There are high-quality, relevant data to support reducing the calculated risk score, including data that have been peer-reviewed and published.
95	Extensive	There is an extensive amount of high-quality, relevant data, including multiple peer-reviewed publications, that demonstrate that the probability of the risk occurring, having a significant impact and/or being undetected is negligible.

On completion of this step, a *Final Risk Score* is calculated, which will determine if the risk is **negligible**, **low**, **moderate** or **high**.

The level of residual risk will inform whether (and to what extent) preclinical (*in vitro* & *in vivo*) evaluation is indicated for the BTC, and what level of clinical evaluation and/or follow-up will be proportional to the level of risk estimated. Note that after the preliminary use of the IAT, the Final Risk Score may be in a higher risk category due to insufficient information. It may be possible to perform further preclinical (*in vitro*/*in vivo*) studies to gather new data to reduce probability/severity/detectability scores (as discussed in Section 3.4) before making a final decision to determine the level of clinical follow-up required.

3.4. Step 3: Definition of extent of studies needed based on the risks quantified

The output from Step 2 (A: Identification of risk factors, B: Identification of risks; C: Quantification of risks, D: Assessment of risk reduction) will result in the identification and quantification of one or more residual risk consequences; these can be expressed in the standard format:

'There is a risk that the BTC will …….. due to …….. resulting in……..'
E.g. - There is a risk that the BTC will be immunogenic due to the inadequate removal of donor cells resulting in an unexpected localised and systemic immune response
Or: - There is a risk that the BTC will fail due to biomechanical damage caused by the processing protocol resulting in sudden mechanical failure.

The purpose of Step 3 is to provide users with guidance to evaluate and mitigate these risks through the sequential application of preclinical (*in vitro*, *in vivo*) and clinical assessments.

Process validation

Process validation is a mandatory activity under the EUTCD, to ensure that a process is reliably achieving its stated objective. Guidance for performing process validation can be found in the Guide to the Quality and Safety of Tissues and Cells for Human Application[12], and is not within the scope of this document. If the final, overall risk is determined to be negligible, no further risk mitigation is necessary; however, it may be advisable to conduct a validation of the process. If the final overall risk is determined to be high, moderate, or even low, it is necessary that, as a minimum, the process is revalidated. However, if the nature of the risk is not related to the process itself, the requirement for validation may not apply, for example where the novelty is in the method of clinical application.

Preclinical – *in vitro* Studies

Generally, *in vitro* assessments will be performed prior to other preclinical (*in vivo*) studies. This category may also incorporate routine process validation studies. Where the overall risk is low, it is likely that it can be mitigated purely with *in vitro* assessments.

Preclinical – *in vivo* Studies

In vivo assessments will usually only be considered where the risk cannot be sufficiently mitigated with *in vitro* studies, for cost and ethical reasons. There may, however, be criteria that can only be accurately evaluated with *in vivo* models. The specific chapters give guidance on how to define which tests could be used for the different types of novel BTC regarding specific risk consequences (4 – Replacement tissues, 5 – HPC and 6 – MAR).

Clinical Evaluation Protocols

If the risk cannot be mitigated to 'negligible' or 'low' levels by *in vitro* or preclinical studies, and when ethically accepted, clinical evaluation protocols may be necessary before the BTC is made generally available.

Guidance for the correct definition of protocols to address the specific risk categories referred to in Step 2 is presented in Chapters 4 – Replacement tissues, 5 – HPC and 6 – MAR of this document.

In the context of this guide, *Clinical Evaluation* is defined as: clinical follow-up studies for monitoring predefined clinical outcome indicators to evaluate quality, safety and effectiveness/efficacy of a BTC for a defined number of patients.
The studies proposed in the specific chapters and relevant appendices are for guidance purposes, and are not intended to be an exhaustive, authoritative or mandatory list of tests that must be performed. These should be considered in conjunction with any tests already performed by the TE.

4. Replacement tissues – Specific guidance for the use of EuroGTP II methodologies and tools

Define what type of BTC you are evaluating

First it is important to define the general category of BTC for which you are going to use the tool, as this will generate specific risk factors. For tissues, choose 'Replacement tissues' and subsequently the specific category of replacement tissue under evaluation, and finally the category per tissue and cell (Annex X) (Figure 4.1).

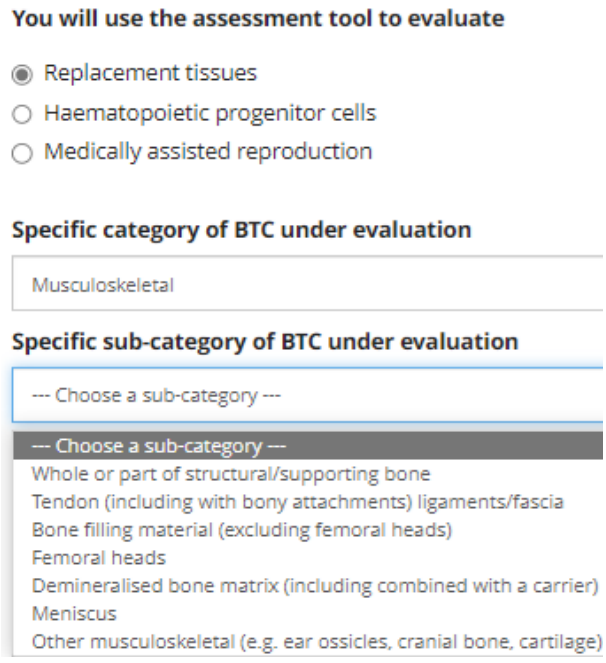

Figure 4.1. **IAT screenshot: different types of tissues**

4.1. Evaluation of novelty (Step 1)

Table 4.1 outlines the questions asked when the tool is being used, a brief explanation of the information that the question is intended to elicit, and some examples to demonstrate when novelty may or may not be present.

When performing this exercise please note the following definitions:
"this type of BTC" should be interpreted as the broader category of BTC (e.g. pulmonary valve, amniotic membrane, skin).
"this BTC" refers to the specific product or therapy under evaluation (e.g. decellularised heart valve, amniotic membrane extract, demineralised bone).

Table 4.1. **Exercise for assessing novelty**

		Yes	No	Na
A	Has this type of BTC previously been collected, processed/prepared and issued for clinical use by your establishment?			

Explanation
The purpose of this question is to determine if your establishment has previously prepared, collected, banked or provided the specific anatomical type of BTC for clinical application. It is not necessary that this BTC has been banked using the same process.

Examples
A1 – Your establishment already banks pulmonary and aortic heart valves, but you intend to start processing them in a different way. In this case, you would answer 'Yes' to this question, and there is no novelty.
A2 – Your establishment already banks Achilles tendons, and you intend to start banking peroneus longus tendons. In this case you would answer 'No'; although you already bank tendons, you do not bank this particular anatomical type of tendon, so there is novelty.
A3 – Your establishment provides pericardial graft as a dural patch, and you intend to start banking fascia lata for the same purpose. In this case, you would answer 'No'; although the graft is to be used for the same purpose for which you already provide another type of graft, you have not banked this type of tissue previously, so there is novelty.

		Yes	No	Na
B	Will the starting material used to prepare this BTC be obtained from the same donor population previously used by your establishment for this type of BTC?			

Explanation
The purpose of this question is to determine whether there may be differences in the BTC resulting from the donor population. Examples of changes that would create novelty are changing the age limits for donors of the BTC, or changing specific aspects of the donor selection criteria applicable to the BTC. Note that this does not apply to generic changes to donor selection criteria, e.g. changes to blood-borne infection screening requirements, but should be considered when making specific changes to donor selection criteria that impact specific BTCs.

Examples
B1 – Your establishment wishes to raise the age limit for donors of tendons from 65 to 70. In this case, you are clearly changing your donor population, so you would answer 'No'; there is novelty.
B2 – Your establishment implements routine screening of your donor population for a new tropical virus that has become endemic in your country. In this case, it is a systematic change which will affect donors of all tissues; while you may technically be impacting your donor population by implementing a new test, you are not changing the overall makeup of the donor population. You would therefore answer 'Yes' to this question, because there is no novelty.

		Yes	No	Na
C	Will the starting material for this BTC be procured/collected using a procedure used previously by your establishment for this type of BTC?			

Explanation
This question is to determine if a change in the way in which the BTC is procured from the donor (or patient) may impact its safety or quality.

Examples
C1 – Your establishment currently banks skin allografts, which are procured from donors using an electric dermatome. In order to improve the quality of your grafts, you are proposing to change to a different type of dermatome. In this case, there may be novelty; you would need to take a view, based on your knowledge of the process, as to whether or not this could introduce significant change.
C2 – Your establishment currently procures hearts for valve donation from deceased donors within 24 hours of death; you are considering expanding this time limit to 48 hours. In this case, there is definite novelty, as there would clearly be risks relating to contamination of the tissue and deterioration of the tissue quality resulting from the increased post-mortem retrieval time.

		Yes	No	Na
D	Will this BTC be prepared by a procedure (processing/preparation, decontamination/pathogen reduction and preservation) used previously in your establishment for this type of BTC?			

Explanation
This question covers a wide range of protocols, essentially covering all processes applied to the graft between retrieval and preservation.

Examples
D1 – Your establishment currently banks tendon allografts which are terminally sterilised with gamma irradiation; you are considering changing to gas plasma sterilisation. There would clearly be novelty here, as you are introducing a novel process which could have significant implications for graft safety and quality.
D2 – Your establishment currently uses buffered saline in many of your routine tissue processing protocols. Your current supplier has discontinued this product and you intend to switch to a new supplier who provides the reagent to the same specification. In this case, there is unlikely to be novelty; you are not proposing to make a change to the fundamental process, just replacing 'like with like'.

		Yes	No	Na
E	Will this BTC be packaged, stored and distributed using a protocol and materials used previously in your establishment for this type of BTC?			

Explanation
This question seeks to elicit whether there are any significant changes in how the BTC is packaged, stored and distributed prior to transplantation.

Examples
E1 – Your establishment currently stores bone allografts at -40 °C prior to distribution; you are considering changing this to -20 °C. In this case, there is novelty as you are making a change that could clearly affect the safety and quality of your grafts.
E2 – Your establishment currently provides morsellised bone allografts in 20 g pack sizes. You are considering changing this pack size to 40 g. In this case, there is unlikely to be novelty; the change must be one that could significantly affect the quality and/or safety of the graft.

		Yes	No	Na
F	Will this type of BTC provided by your establishment be applied clinically using an application/transfusion/infusion method used previously?			

Explanation
This question seeks to elicit whether there are any significant changes in how the BTC is clinically applied.

Example
F1 – A graft that is being used with an open surgical procedure for implantation is now to be implanted using a minimally invasive technique (e.g. arthroscopy). You need to consider if the change in the implantation method could impact the properties/performance of the graft. In this case there is novelty, and your answer would be "No".
F2 – Your establishment has been preparing cold storage corneas and is currently implementing procedures to prepare "cultured corneas". In this case there is no novelty in the implantation method, and your answer would be "Yes".

		Yes	No	Na
G	Has your establishment provided this type of BTC for the same clinical indication or for application/transfusion/infusion into the same anatomical site?			

Explanation
This question seeks to elicit whether the BTC will be implanted/applied for a new clinical indication or for patients with a clinical indication/new anatomical site never used before.

Examples
G1 – You have been providing decellularised skin to treat leg ulcers and the surgeons wish to utilise the graft for breast reconstruction. In this situation the properties required for performance of the graft have changed; you now need to consider if the graft is biomechanically suitable for this indication. In this case there is novelty, and your answer would be "No".
G2 – You have been providing heart valves for transplant and now your TE aims to prepare decellularised heart valves for the same type of pathology. In this case there is no novelty, because the anatomical site will be the same, and your answer would be "Yes".

4.2. Level risk analysis (Step 2)

Step 2A: Identification of risk factors

If, after completing part 1 of the IAT, you determine that there is some novelty resulting from your proposed change, you should now proceed to Step 2 to identify and quantify the potential risks resulting from this novelty. The risks have been subdivided into nine factors:

I) Donor characteristics
II) Procurement process and environment
III) Processing and environment
IV) Reagents
V) Reliability of microbiology testing
VI) Storage conditions
VII) Transport conditions

VIII)Presence of unwanted cellular material and/or graft vascularity

IX) Complexity of the immediate pre-implantation preparation and/or application method

You must first determine which of these risk factors are relevant to the aspect or aspects of your proposed change which result in novelty. Worked examples are provided later in this document to demonstrate how the process works.

Step 2B: Identification of risks

Having identified the appropriate risk factor(s), you should then determine which specific risk consequences are applicable. A standard set of risk consequences is applied to each factor, with an open, 'other' category for any risks not covered in the four main categories.

a) Unexpected immunogenicity
b) Implant failure
c) Disease transmission
d) Toxicity/Carcinogenicity
e) Other

Examples of the combinations of risk factors and specific risk consequences that may need to be considered are provided in Table 4.2. The purpose of the exercise is to systematically consider each risk factor and risk consequence in turn against the nature of the change. Note that for certain combinations of risk factor and specific risk, there may be no relevant examples. It is recognised that the IAT cannot anticipate all potential types of risk; the four specific risk consequences listed are those which it is generally agreed will be most commonly related to BTCs. For any risks not covered by these four categories, an open, 'other' category may be used, and is provided in the IAT.

Table 4.2. **Identification and interpretation of the risk factors and risk associated with tissues**

	Risk factors	Examples and explanation	Risk	Examples and Explanation
Donation	Donor characteristics	This factor requires that you consider whether the novelty in your donor population represents any new risk for recipients, and/or increases the previously existing risk.	Unexpected immunogenicity	If your TE decides to stop human leukocyte antigen (HLA) matching your donors for a specific BTC, you should consider whether this could affect the clinical outcome of the recipient.
			Implant failure	i) If you increase the age of your donor population, could this impact the quality of your graft? ii) Certain aspects of a donor's medical history may impact the suitability of certain grafts for transplantation; changes should be considered in this light.
			Disease transmission	If a change is made so that a graft that was previously only obtained from heart-beating donors will now be obtained from deceased donors, this may affect the risk of graft contamination and disease transmission.
			Toxicity/Carcinogenicity	This consequence is unlikely to be applicable to this risk factor; however, changes in donor selection criteria related to poisoning, for example, may create a risk.
			Other	Consider other risks if applicable.
Procurement	Procurement/ collection process and environment	This factor requires that you consider where and how the BTC, or the material used to manufacture it, are recovered. For example, how long does the process take, how complex is it, and what is quality of the environment?	Unexpected immunogenicity	Could changes to the procurement process result in elevated quantities of immunogenic material being present in the graft?
			Implant failure	Could changes to the procurement process result in the grafts being damaged during procurement?
			Disease transmission	i) Could changes to the procurement process result in an increased risk of donor-recipient disease transmission? ii) Could changes to the procurement process result in an increased risk of the graft being contaminated with environmental organisms?
			Toxicity/Carcinogenicity	Could any chemicals (e.g. disinfectants) used in the procurement process be transferred to the graft?
			Other	Consider other risks if applicable.

	Risk factors	Examples and explanation	Risk	Examples and Explanation
Processing/storing/transport	Processing and environment	This factor requires that you consider where and how the BTC is processed. For example, how long and how complex is processing, and what is the quality of the processing environment?	Unexpected immunogenicity	Could changes in cleaning or washing protocols lead to the graft retaining more residual donor cell content?
			Implant failure	i) Could the length of the process result in the quality of the graft deteriorating? ii) Could the environmental conditions applied during processing (heat, pressure, humidity, etc.) affect the graft quality?
			Disease transmission	Could the processing length, complexity or environment (e.g. heat, pressure, humidity, etc.) affect the risk of environmental contamination?
			Toxicity/Carcinogenicity	Could the BTC degrade during processing, generating toxic compounds?
			Other	Consider other risks if applicable.
	Reagents/ added components	This factor requires that you consider any reagents used during recovery, processing, decontamination and storage of the BTC. For example, could they damage the BTC in any way, or could residual traces of reagent remain in the BTC that could cause toxic or immunogenic effects in recipients?	Unexpected immunogenicity	Could any of the reagents you use, of which residual traces could remain in the final product, generate immunogenicity?
			Implant failure	Could any of the reagents alter the essential biomechanical properties of the product?
			Disease transmission	Are quality control procedures applied to reagents sufficient to avoid the risk of contamination?
			Toxicity/Carcinogenicity	Could residual traces of any of the reagents you use remain in the final product, generating toxicity/carcinogenicity?
			Other	Consider other risks if applicable.
	Reliability of microbiology testing	This factor requires that you consider the risk that the nature of the BTC, the testing methodology and/ or the presence of residual processing reagents such as antibiotics in the finished BTC may impact the accuracy of any microbiology tests. Note this refers specifically to bacteriology/mycology testing of the BTC, and not to any blood tests performed on the donor.	Unexpected immunogenicity	It is unlikely this combination of risk and risk factor could occur associated with tissues.
			Implant failure	Could undetected micro-organisms damage the graft, leading to implant failure?
			Disease transmission	Could undetected micro-organisms result in disease transmission?
			Toxicity/Carcinogenicity	It is unlikely this combination of risk and risk factor could occur associated with tissues.
			Other	Consider other risks if applicable.

	Risk factors	Examples and explanation	Risk	Examples and Explanation
Processing/storing/transport	Storage conditions	This factor requires that you consider any potential risks arising from how the starting material and BTC are stored between procurement and processing, during processing, and between processing and implantation.	Unexpected immunogenicity	Changes in storage temperature may preserve immunogenic factors more effectively.
			Implant failure	Consider how storage conditions (e.g. temperature, time) may impact the important properties of the graft.
			Disease transmission	Consider how storage conditions (e.g. temperature, time) impact the risk of the graft being contaminated due to, for example, changes in the primary packaging.
			Toxicity/Carcinogenicity	Could packaging material degrade due to time and/or temperature, generating toxic compounds? Or could the graft itself degrade due to storage conditions?
			Other	Consider other risks if applicable.
	Transport conditions	This factor requires that you consider any potential risks arising from how the starting material and BTC are transported, for example, between the sites of procurement and processing, and between the sites of storage and implantation.	Unexpected immunogenicity	Changes in transport temperature/time may preserve immunogenic factors more effectively.
			Implant failure	Consider how transport conditions (e.g. temperature, time) may impact the properties of the graft.
			Disease transmission	Consider how transport conditions (e.g. temperature, time) impact the risk of the graft being contaminated due to, for example, changes in the primary packaging.
			Toxicity/Carcinogenicity	Could solutions, packaging material or the graft itself degrade due to transport conditions (e.g. due to changes in the temperature), generating toxic or carcinogenic chemicals?
			Other	Consider other risks if applicable.
Product	Presence of unwanted cellular material and/or graft vascularity	This factor requires that you consider the risk that although the presence of intact vital cells is desirable for some BTCs, but that this may increase the risk of immunogenicity or disease transmission, for example. Consider also that vascular tissues may be more at risk of infiltration by pathogens or malignant cells than avascular tissues.	Unexpected immunogenicity	Grafts that contain donor material that is not intended to be present may be more immunogenic.
			Implant failure	Could donor cell material impact the clinical performance of the graft, perhaps by delaying integration?
			Disease transmission	Consider if the presence of donor cells could increase the risk of transmission of intracellular viruses or malignancy. The degree of tissue vascularity may also increase the risk that the tissue could harbour donor-derived infections.
			Toxicity/Carcinogenicity	It is unlikely that this risk factor could apply to this risk; however, each situation must be considered on a case-by-case basis.
			Other	Consider other risks if applicable.
Clinical application procedure	Complexity of the immediate pre-implantation preparation and/or application method	This factor requires that you consider the complexity of the pre-implantation method for this BTC. How long will it take, and could this introduce risks? What is the scope for errors to be made, and what could the consequences of these errors be?	Unexpected immunogenicity	Consider if the pre-implantation preparation procedure (e.g. washing of the graft immediately before implantation) is sufficiently robust to ensure that immunogenic reagents or donor-derived components present in the graft are removed prior to implantation.
			Implant failure	Consider the complexity of the pre-implantation and application methods and how critical these are for the clinical performance of the graft. Are they complex and potentially liable to error?
			Disease transmission	Consider if the pre-implantation and application methods could increase the risk of disease transmission due to the length and complexity of the procedures (e.g. long period of exposure to the environment during the preparation and implantation).
			Toxicity/Carcinogenicity	Consider if the pre-implantation preparation procedure (e.g. washing of the graft immediately before implantation) is sufficiently robust to ensure that reagents or donor-derived components present in the graft that could cause toxicity/carcinogenicity are removed prior to implantation.
			Other	Consider other risks if applicable.

Step 2C: Quantification of risk consequences

When the risk factors and the potential risk consequences have been identified, the potential impact of this risk analysis needs to be determined according to the definitions presented in Section 3.3 (and summarised in Annex IV).

By entering the information into the IAT users will generate a report detailing the assessment performed, which will include the identification and quantification of individual risk consequences, the *Final Risk Score* and risk classification (the detailed algorithm is described in Annex V).

Step 2D: Assessment of risk reduction

Having calculated probability, severity and detectability, and thus an overall risk based on 'internal' knowledge and data, it may be possible to adjust this score by taking into account other external sources of information. These external data are not used to specifically reduce probability, severity or detectability, but are used to calculate a general reduction in the overall risk. (More details related to risk reduction are described in Section 3.3 of this guide).

4.3. Interpretation of the outcomes of the risk analysis and definition of extent of studies needed based on the risks quantified (Step 3)

The *Final Risk Score* defines the overall level of risk inherent in the BTC. Based on this, further actions to reduce risk may or may not be necessary as described in Table 4.3.

Step 3A: Risk reduction strategies – Use of preclinical studies (*in vitro* and *in vivo*) to mitigate the identified risks

If the *Final Risk Score* is "low", "moderate" or "high" further studies may be performed, if not already done, to provide additional information to re-evaluate the level of risk (using Step 2).

Additional guidance to facilitate the implementation of Step 3A (Risk reduction strategies) is provided in Annex VI. In this annex, information is provided for each type of tissue in the form of matrices that can be used to select *in vitro* and *in vivo* tests appropriate to mitigate the risk previously identified in Step 2.

The methodology proposed by EuroGTP II suggests that this be done by reference to matrices and tables. The matrices suggest a number of different test criteria that are specific for different types of BTC, each of which are also subdivided into specific tests. It then suggests which of these tests could be applied to address specific risk consequences (Annex VI).

Tests listed in the matrices of Annex VI are for guidance only and not intended to be an exhaustive list of mandatory tests.

Step 3B: Definition of extent of clinical evaluation

In situations where the risks cannot be further reduced with preclinical studies, the BTC may be used in humans subject to authorisation by the CA, with the provision that appropriate clinical evaluation protocols (monitoring, follow-up or evaluation appropriate to the level of remaining risk) are put in place.

Table 4.3. **Extent of studies* according to the level of risk determined in the assessment**

Level of Risk	Extent of proposed studies
Negligible	***Step3A: Risk reduction strategies*** ◆ The assessment indicates that the BTC is safe and efficacious for clinical use and very unlikely to cause harm to recipients. You should conduct a validation of the process, if not already done. If the nature of the risk is not related to the process itself, the requirement for validation may not apply, for example where the novelty is in the method of clinical application.
	Step 3B: Extent of clinical evaluation ◆ Serious adverse reaction and event (SARE) reporting.
Low	***Step3A: Risk reduction strategies*** ◆ The BTC is safe and efficacious for clinical use and unlikely to cause harm to recipients. A validation of the process, if not already done, should be performed. If the nature of the risk is not related to the process itself, the requirement for validation may not apply, for example where the novelty is in the method of clinical application. Please refer to Annex VI for additional details.
	Step 3B: Extent of clinical evaluation ◆ SARE reporting. ◆ Feedback from immediate post-transplant monitoring (routine clinical follow-up) may be collected for a defined period or number of procedures. ◆ *Clinical audit** may also be used after an appropriate period of use. Please refer to Annex VI for additional details.
Moderate	***Step3A: Risk reduction strategies*** ◆ The assessment indicates that more evidence is needed to support safe and effective use of this BTC and mitigate risk. Process validation should be performed; however, if the nature of the risk is not related to the process itself, the requirement for validation may not apply, for example where the novelty is in the method of clinical application. ◆ Preclinical *in vitro* evaluation, specific to the identified risks, should be performed if not already done. ◆ Preclinical *in vivo* evaluation, specific to the identified risks, using an animal model should be done if applicable (and if not already completed). Please refer to Annex VI for additional details.
	Step 3B: Extent of clinical evaluation ◆ A structured plan for active collection of a specific set of data relating to the safety and efficacy of the BTC should be put in place, in addition to routine clinical follow-up. Ethical approval may be required and the principles of Good Clinical Practice (GCP)[15] adhered to. ◆ Consideration should be given to restricting provision of the BTC to a limited number of patients and/or centres until the risks have been adequately mitigated. Please refer to Annex VI for additional details.
High	***Step3A: Risk reduction strategies*** ◆ The assessment indicates that significantly more evidence is needed to support safe and effective use of this BTC and mitigate risk. ◆ Process validation should be performed; however, if the nature of the risk is not related to the process itself, the requirement for validation may not apply, for example where the novelty is in the method of clinical application. ◆ Preclinical *in vitro* evaluation, specific to the identified risks, should be performed if not already done. ◆ Preclinical *in vivo* evaluation, specific to the identified risks, using an animal model should be done if applicable (and if not already completed). Please refer to Annex VI for additional details.
	Step 3B: Extent of clinical evaluation ◆ The BTC should only be used clinically in the context of an ethically approved, controlled (where applicable) clinical evaluation until the residual risks have been adequately mitigated. The principles of GCP[15] must be adhered to. ◆ Clinical evaluation and follow-up programmes should be implemented and safety and efficacy must be continuously monitored. If available, national and international registries are recommended for gathering follow-up data. Please refer to Annex VI for additional details.

* In the context of this guide, clinical audit refers to retrospective or prospective evaluation of routinely collected clinical data.

A worked example demonstrating the whole process from novelty assessment to the definition of the extent of studies needed is provided in Annex VII.

5. Haematopoietic progenitor cells – Specific guidance for the use of EuroGTP II methodologies and tools

Define what type of BTC you are evaluating

First it is important to define the general category of BTC for which you are going to use the tool, as this will generate specific risk factors. For HPC, choose the type of cells under evaluation (Figure 5.1).

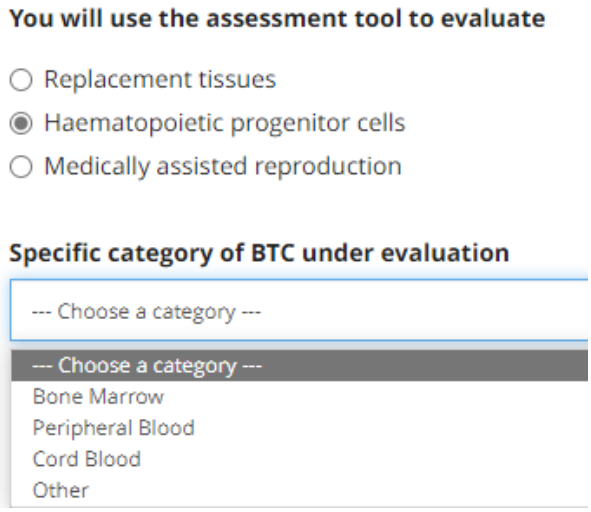

Figure 5.1. **IAT screenshot: different types of HPC**

If selecting HPC, you will also be asked to choose the origin of the specific type of cells under evaluation:

- Bone marrow
- Peripheral blood
- Cord blood
- Other sources

5.1. Evaluation of novelty (Step 1)

This chapter presents the questions asked when the tool is being used, a brief explanation of what each question is intended to elicit, and some examples to demonstrate when novelty may or may not be present. The questions as they appear in the IAT are shown in Table 5.1.

Table 5.1. **Exercise for assessing novelty**

		Yes	No	Na
A	Has this type of BTC previously been collected, processed/prepared and issued for clinical use by your establishment?			

Explanation
The purpose of this question is to determine whether your institution has previously collected, prepared and issued the specific anatomical type of BTC in clinical application for a specific indication. It does not require that the BTC has been issued and administered before for a different indication.

Examples
A1 – Your establishment is already performing T-cell depletion on haematopoietic grafts, but you intend to revise the processing. In this case you would answer 'Yes' to this question; there is no novelty.
A2 – Your establishment is performing HSCT using BM and PBSC grafts. It is decided to start a cord blood transplantation programme. In this case you answer 'No'; you have no experience in handling and issuing cord blood.

		Yes	No	Na
B	Will the starting material used to prepare this BTC be obtained from the same donor population previously used by your establishment for this type of BTC?			

Explanation
This question aims to elicit possible differences in the characteristics of the BTC caused by a change in the donor population. Examples of changes that would create novelty are changing the age limits for donors of the BTC, or changing specific aspects of the donor selection criteria applicable to the BTC. Note that this does not apply to generic changes to donor selection criteria, e.g. changes to blood-borne infection screening, but should be considered when making specific changes to donor selection criteria that have an impact on the specification of the BTC.

Examples
B1 – Your establishment wishes to raise the age limit for donors of HPC from 70 to 75. In this case, you are clearly changing your donor population, so you would answer 'No'; there is a novelty.
B2 – Your establishment implements routine screening of your donor population for a new virus that has become endemic in your country. In this case, it is a systematic change which will affect donors of all tissues; while you may technically impact your donor population by implementing a new test, you are not changing the overall makeup of the donor population. You would therefore answer 'Yes' to this question; there is no novelty.
B3 – Your organisation decides to start immunising donors prior to progenitor cell donation. This is a specific change directed at the immune system of the donor and the recipient, which will result in a change to your donor population characteristics. You would answer 'No' to this question; there is novelty.

		Yes	No	Na
C	Will the starting material for this BTC be procured/collected using a procedure used previously by your establishment for this type of BTC?			

Explanation
The question is to determine whether a change in the way in which the BTC is procured from the donor (or patient) may impact its safety or quality.

Examples
C1 – Your establishment is currently administering filgrastim (G-CSF) for the mobilisation of HPC in donors. It is decided to start using a biosimilar for this purpose. In this case, there may be a novelty, because the nature of the cells and composition of the graft could have been changed in a way that it influences the quality and efficacy.
C2 – Your establishment decides to change the apheresis kits/system from brand A to brand B. Both devices have CE marking for collection of progenitor cells and are used in other establishments. The collection technique is based on the same principles. This is not a novelty, because the procedure has been shown to be suitable for the purpose and the technique is not new in your hands.

		Yes	No	Na
D	Will this BTC be prepared by a procedure (processing/preparation, decontamination/pathogen reduction and preservation) used previously in your establishment for this type of BTC?			

Explanation
This question covers a wide range of protocols, essentially covering all processes applied to the graft between retrieval and preservation.

Examples
D1 – Your establishment currently stores autologous PBSC grafts in liquid nitrogen storage, after controlled-rate freezing. You are considering changing to mechanical freezing and storage. There would clearly be novelty here, as you are introducing a novel process which could have significant implications for graft safety and quality.
D2 – Your establishment currently uses buffered saline in many of your routine cell processing protocols. Your current supplier has discontinued this product, and you intend to switch to a new supplier who provides the reagent to the same specification. In this case, there is unlikely to be a novelty; you are not proposing to make a change to the fundamental process, just exchanging 'like with like'.

	Yes	No	Na
E Will this BTC be packaged, stored and distributed using a protocol and materials used previously in your establishment for this type of BTC?			

Explanation
This question seeks to elicit whether there are any significant changes in how the BTC is packaged, stored and distributed prior to transplantation.

Examples
E1 – Your establishment currently transports BM grafts at room temperature. You are considering changing the procedure and transporting all HPC-BM and A products cooled (4-10 °C). There would clearly be a novelty, as you are making a change that could affect the safety and quality of your grafts.
E2 – Your establishment is adding a tempex box to protect the progenitor cell bag during transport. There is no novelty because the box does not influence the essential characteristics of the product.

	Yes	No	Na
F Will this type of BTC provided by your establishment be applied/infused clinically using an application/ transfusion/infusion method used previously?			

Explanation
This question seeks to elicit whether there are any significant changes in how the BTC is clinically applied.

Examples
F1 – Your establishment currently administers cord blood progenitor cells intravenously. Direct intra-bone infusion is being considered. In this case there is a novelty; the safety and efficacy of the changed method has to be proved.
F2 – Your establishment has infused cord blood from related donors. They are considering using cord blood from unrelated donors. There is no novelty in the infusion method, and your answer would be 'Yes'.

	Yes	No	Na
G Has your establishment provided this type of BTC for the same clinical indication or application/transfusion/infusion to the same anatomical site before?			

Explanation
This question seeks to elicit whether there are any significant changes in how the BTC is clinically applied.

Examples
G1 – Your establishment currently provides the BTC for patients suffering from haematological malignancies via intravenous infusion. A programme for the use of this BTC for cardiovascular repair by direct infusion into affected areas of the heart muscles is being considered. In this case you answer is 'No'; there is a novelty.
G2 – Your establishment currently provides progenitor cells for haematological malignancies via intravenous infusion. They are considering starting a programme to treat patients with haemoglobinopathies. Progenitor cells are administered via intravenous infusion. Your answer would be 'Yes'; there is no novelty.

5.2. Level risk analysis (Step 2)

The second exercise aims to determine the risk associated with the novelties identified in the process being evaluated.

Every modification in the processes associated with the donation, procurement, testing, processing, storage and distribution of BTC may have potential consequences for the quality of these products and safety of recipients. Moreover, different levels of novelty represent different risks and have a distinct impact on the quality and safety of the tissue and cell products. The evaluation of the different levels of these risks can be performed using the methodology proposed in the current rationale.

Step 2A: Identification of risk factors

At first, the risk factors associated with the changes in the process are selected. There are nine risk factors that could apply to HPC (Table 5.2).

You must first determine which of these risk factors are relevant to the aspect or aspects of your proposed change which result in novelty. Worked examples are provided later in this document to demonstrate how the process works.

Step 2B: Identification of risk consequences

Having identified the appropriate risk factor(s), you should then determine which specific risk consequences are applicable. A standard set of risk consequences is applied to each factor, with an open, 'other' category for any risks not covered in the four main categories.

a) Unexpected immunogenicity
b) Engraftment failure
c) Disease transmission
d) Toxicity/Carcinogenicity
e) Other

Examples of the combination of risk factors and specific risk consequences that may need to be con-

sidered are provided in Table 5.2. The purpose of the exercise is to systematically consider each risk factor and risk consequence in turn against the nature of the change. Note that for certain combinations of risk factor and specific risk consequence, there may be no relevant examples.

Table 5.2. **Identification of the risk factors and risks associated with HPC**

	Risk factors	Explanation	Risk	Examples
Donation	Donor characteristics	Consider whether the novelty in your process has an impact at the time of the donation. This factor requires that you consider whether the donor population from which you intend to obtain the BTC could pose any risk for the recipient.	Unexpected immunogenicity	Could adjustment of donor selection criteria (age, HLA match grade), induce (severe) GvHD?
			Engraftment failure	Could increasing the age of the donor population impact the quality of the graft? Could certain aspects of a donor's medical history impact the number of HPCs before transplantation?
			Disease transmission	Is the risk for transmission of infectious diseases increased if you accept donors who have travelled to endemic areas.
			Toxicity/Carcinogenicity	Could the use of a new type of bag to collect the graft induce toxicity?
			Other	Will the use of a new apheresis device affect the number of HPCs collected?
Procurement	Procurement/ collection process and environment	Consider where and how the BTC is currently recovered/ collected and whether the changes proposed with the novel method change the recovery time, complexity or quality of the environment. For example, how long does the process take, how complex is it, and how do the procurement devices affect the quality of the HPC?	Unexpected immunogenicity	Could changes to the procurement process result in elevated quantities of immunogenic material being present in the graft?
			Engraftment failure	Could the use of new haematopoietic growth factors affect the composition of the graft, resulting in poor engraftment?
			Disease transmission	Could changes to the procurement process result in an increased risk of donor-recipient disease transmission?
			Toxicity/Carcinogenicity	Could any chemicals (e.g. disinfectants) used in the procurement process be transferred to the graft?
			Other	Does a different collection needle influence the number of the specific type of cells?

	Risk factors	Explanation	Risk	Examples
Processing/storing/transport	Processing and environment	Consider the current processing method for the BTC and how the change in processing can affect the product. How long does the novel preparation process take and how complex is it? This may have an impact on the risk of contamination or cell characteristics that may not be consistent with product specifications. Also consider the quality of the processing environment, which may also affect the risk of contamination.	Unexpected immunogenicity	Could the process change lead to the introduction of unwanted cellular components?
			Engraftment failure	Could the length of the process result in the quality of the graft deteriorating? Could the environmental conditions applied during processing (e.g. temperature, pressure, humidity) affect the graft quality?
			Disease transmission	Could the length, complexity or environment where the processing takes place affect the risk of environmental contamination? Could changes to the processing result in an increased risk of the graft being contaminated with environmental organisms?
			Toxicity/Carcinogenicity	Could the BTC degrade during processing, generating toxic compounds?
			Other	Can the devices used in the processing influence the quality of the HPC?
	Reagents	Consider any reagents used during recovery, processing, preparation, decontamination and storage of the BTC. Could they damage the BTC in any way, or could residual traces of reagent remain in the BTC that could cause toxic or immunogenic effects in recipients?	Unexpected immunogenicity	Could a change of cryoprotectant induce an unexpected immunogenic reaction?
			Engraftment failure	Could change of cryoprotectant affect engraftment?
			Disease transmission	Could the use of reagents lead to decontamination of the graft?
			Toxicity/Carcinogenicity	Could the use of reagents cause toxic effects in the recipient?
			Other	Could the use of reagents cause any other effects in the recipient?
	Reliability of microbiology testing.	Consider the risk that the testing methodology and/or presence of residual processing reagents such as antibiotics in the finished BTC may impact the accuracy of any microbiology testing of the BTC. This risk factor is not about blood tests on the donor.	Unexpected immunogenicity	Could the residual presence of antibiotics lead to anaphylactic/allergic reactions?
			Engraftment failure	Could the reaction to the presence of microbiological agents lead to non-engraftment of rejection of the graft?
			Disease transmission	Could the change of processing medium mask a positive outcome of current microbiology testing?
			Toxicity/Carcinogenicity	Could the presence of toxin-producing bacteria cause a reaction in the recipient?
			Other	Consider other risks if applicable.

	Risk factors	Explanation	Risk	Examples
Processing/storing/transport	Storage conditions	Consider any potential risks arising from how the starting material and BTC are stored, between procurement and processing, during processing, and between processing and clinical application.	Unexpected immunogenicity	Can a change in the plastics of primary packaging cause enhanced immunogenic material in the grafts?
			Engraftment failure	Could the storage temperature affect the viability of the cells?
			Disease transmission	Could the storage temperature affect the risk of contamination?
			Toxicity/Carcinogenicity	Can the cryoprotectant cause toxic reactions in the recipient of the graft?
			Other	Could storage conditions cause any other risk to the recipient?
	Transport conditions	Consider any potential risks arising from how the starting material and BTC are transported, for example between the sites of procurement and processing, and between the sites of storage and clinical application.	Unexpected immunogenicity	Unlikely that this factor could apply risk.
			Engraftment failure	Can the duration of the shipment influence the number of relevant cells present in the graft?
			Disease transmission	Could the duration of the transport create a risk of contamination?
			Toxicity/Carcinogenicity	Could transport conditions (e.g. heavy shaking) lead to damage of the packaging and chemical contamination of the product.
			Other	Can shaking and mechanical movements caused by a new transport method hamper the integrity of the packaging?
Product	Presence of unwanted cellular material	Consider the risk of the presence of inactivated cells, debris or cell components which may cause immunogenicity or disease transmission.	Unexpected immunogenicit	Do centrifugation forces during apheresis cause the presence of cell debris?
			Engraftment failure	Could the presence of inactivated cells lead to engraftment failure?
			Disease transmission	Can the recipient be infected by contaminated cord blood during procurement?
			Toxicity/Carcinogenicity	Unlikely that this factor could apply risk.
			Other	Consider other risks if applicable.
Clinical application procedure	Complexity of the pre-implantation preparation and/or application method	Consider how complex the method of transplantation will be for this BTC. How long will it take, and could this introduce risks? What is the scope for errors to be made, and what could the consequences of these errors be?	Unexpected immunogenicity	Does the preparation/application of the product involve handling that could cause critical change to the specifications of the final product?
			Engraftment failure	Does the preparation/application of the product involve handling that could cause engraftment failure?
			Disease transmission	Does the preparation/application the product involve handling that could cause bacterial contamination of the product?
			Toxicity/Carcinogenicity	Does the preparation/application of the product involve handling that could cause introduction of chemical substances?
			Other	Consider other risks if applicable.

Step 2C: Quantification of risk consequences

When the risk factors are selected and the potential risks are identified, the potential impact of this risk analysis needs to be determined according to the definitions present in Section 3.4 and summarised in Annex IV.

5.3. Interpretation of the outcomes of risk analysis and definition of extent of studies needed based on the risks quantified (Step 3)

Using the EuroGTP II methodologies you will be able to perform a risk analysis, determine the risk profile and the level of risk associated with the novel product, process or procedure. As a result, the tools (IAT/EuroGTP II algorithm) will provide the value of the individual risks and the *Final Risk Score* which is proportional to the number of risks evaluated (in the form of a level of risk).

It is important to state that HPC transplant centres should be prepared to invalidate treatments when they prove problematic (in terms of safety and effectiveness), even when a novelty of negligible risk was implemented. HPC transplant centres should collect data and register of follow-up in a systematic way and make them available to the scientific community regardless of the success of the treatment: not withholding results that point to a negative outcome or that turn out to be inconclusive. Therefore, it is important in all processes, regardless of the level of risk, to monitor and register SARE.

The table below provides general guidance on the follow-up studies needed in terms of the level of risk determined (adjusted according to Provoost V. *et al.* 2014) (Table 5.3).

Table 5.3. **Generic review of extent of studies needed**

Level of Risk	Extent of studies needed
Negligible	***Step3A: Risk reduction strategies*** A change in process could have a negligible level of risk because it is part of a therapy or procedure that is considered as established or standard. In this case multi-centre studies (ideally randomised controlled trials (RCTs)) are published in peer-reviewed journals and the procedures are performed according to a validated and/or standard protocol. Minimal process validation is needed. The technical performance of staff should be monitored and comparable with other TEs or published studies; therefore, standard key performance indicators (KPIs) should be monitored on the technical quality of the staff performing the procedures. Dropping KPIs indicating protocol drift must lead to investigation of both the procedural steps and/or the possibility to retrain staff.
	Step 3B: Extent of clinical evaluation A routine/safety follow-up programme (e.g. EBMT Patient Registry[23]) is sufficient as good practice states. Follow-up procedures should be focused on assessing efficacy, comparing the clinical follow-up with the results obtained before the implementation of the change in the process. Long-term (ideally transgenerational) health effects, including aspects such as fertility, oncology and mental health, should be monitored.
Low	***Step3A: Risk reduction strategies*** Implementing a standard procedure or treatment in an HPC centre that has never performed it requires an intensive validation. Training of staff (as required by Joint Accreditation Committee ISCT-Europe & EBMT (JACIE)) is necessary in order to reach the outcomes published in scientific literature. A learning curve may be expected and should be part of the validation report. When implementing the procedure, additional quality controls must be performed to monitor critical process parameters (CPPs) and critical quality attributes (CQAs). For example, when a TE is switching from T-cell depletion (TCD) to CD34+ selection (which they have never performed before), engraftment rate, and graft-versus-host reactions should be carefully monitored.
	Step 3B: Extent of clinical evaluation A safety follow-up programme is necessary. Follow-up procedures (forms EBMT Med-A, Med-B or Med-A cellular) should focus on assessing efficacy, comparing the clinical follow-up with the results obtained before the implementation of the change in the process and in relation to the results published in scientific literature. The expected learning curve should be kept as short as possible and put in relation to the follow-up programme. Likewise, established techniques are subject to long-term (ideally transgenerational) follow-up of the health effects, as established by EBMT.

Level of Risk	Extent of studies needed
Moderate	***Step3A: Risk reduction strategies*** Novel procedures or treatments that exert a moderate risk and are considered innovative. The treatment has shown proof of principle and there are reassuring data in literature in terms of both safety and effectiveness, at least in animal studies, and preclinical data show normal engraftment or response. The studies that have published this data should have a sound methodology and be published in peer-reviewed journals. In order to implement an innovative treatment, an enhanced validation is necessary, including a range of additional quality controls performed to monitor critical process parameters (CPPs), critical quality attributes (CQAs) and the impact of the implemented BTC should be carefully monitored. Since reassuring data of this innovative treatment is already available, more specific monitoring of the published critical parameters can be performed instead of a registration of all critical parameters.
	Step 3B: Extent of clinical evaluation Clinical evaluation and follow-up programmes, conforming with the EBMT Patient Registry[23], should be implemented to assess mid-term safety (3 months up to life-long post-transplantation, including data on psychological wellbeing). These data collections should refer to patients undergoing the procedure as well as the donors, where applicable.
High	***Step3A: Risk reduction strategies*** A new procedure can be offered to patients in an experimental design aimed at showing proof of principle, short-term safety and/or effectiveness. An extensive validation including a range of additional quality controls performed to monitor critical process parameters (CPPs), critical quality attributes (CQAs) and the impact of the implemented changes is required. This extensive validation should include: Non-clinical studies: preferably there should be studies showing the experimental procedure is safe in animals. Preclinical Studies: when experimental treatments encompass a laboratory phase, then at minimum the viability of cells should be looked at in detail, monitored and registered.
	Step 3B: Extent of clinical evaluation Follow-up programme: experimental treatments should only be offered to a selected and limited patient cohort and these patients should be clearly informed about the experimental status and should receive information about (the lack of knowledge about) possible risks, alternative treatments etc. ORHAs should only offer experimental treatments or treatments based on experimental procedures after approval by a commission of medical ethics.

A worked example demonstrating the whole process from novelty assessment to the definition of extent of studies needed is provided in Annex VIII.

Step 3A: Risk reduction strategies – Use preclinical studies (*in vitro* and *in vivo*) to mitigate the identified risks

If the *Final Risk Score* is "low", "moderate" or "high", further studies may be performed, if not already done, to provide additional information to re-evaluate the level of risk (using Step 2).

Additional guidance to facilitate the implementation of Step 3A (Risk reduction strategies) is provided in the form of matrices that can be used to select *in vitro* and *in vivo* tests appropriate to mitigate the risk previously identified in Step 2.

The matrices suggest a number of different test criteria, which are specific for different types of BTC, each of which are also subdivided into specific tests. It then suggests which of these tests could be applied to address specific risk consequences (Tables 5.4 and 5.5).

Tests listed in the matrices are for guidance only and not intended to be an exhaustive list of mandatory tests.

Table 5.4. **Preclinical evaluation – Examples of in vitro tests to assist in potentially reducing the risk consequences identified (blue cells represent the tests that may be used to address the respective risk consequences)**

		Immunogenicity		Engraftment failure	Toxicity/ Carcinogenicity			Disease transmission	
Criteria	**Specific test**	**Systemic Immune response**	**Anaphylaxis**	**Engraftment failure**	**Cytotoxicity**	**Carcinogenicity**	**Teratogenicity**	**Blood-borne infections**	**Infections acquired during procurement or processing**
Sterility	Test for the presence of microbiological agents (according to JACIE standards)								
	Review environmental monitoring								
	Stability (according to JACIE standards)								
	Validation of test suitability (of all analytical methods applied)								
Identity*	Confirmation of product specifications (e.g. HLA, blood group, genetic markers, JACIE standards)								
Purity**	Quantification of the target cells at various stages: flow cytometry (e.g. CD34 +/CD 45 + cells; or CD 3) to monitor GvHD								
	Quantification of the target cells at various stages: total nucleated cell (TNC) count								
Potency*	Viability: apoptosis and/necrosis (e.g. annexin 5/7 AAD staining or TUNEL assay; trypan blue)								
	Functionality: Cytological evaluation leukocytes (diff)								
	Functionality: CFU in clonogenic assays								
	Functionality: long-term culture initiating cell assay								
	Functionality: lymphocyte subsets by flow cytometry								
Safety**	Stability test packaging: in cases of novel packaging								
	Presence of viruses: to be tested before receipt of material; according to JACIE standards								
	Residual agents: mass spectrometry, chromatography								
	Residual cell/DNA: fluorescence in situ hybridisation (FISH), cytomorphological evaluations								

* Characteristics of a product (HLA, blood group, etc.)

** Relative freedom from extraneous matter in the finished product, whether or not harmful to the recipient or deleterious to the product.

Table 5.5. **Preclinical evaluation – Examples of in vivo tests to assist in potentially reducing the risk consequences identified (green cells represent the tests that may be used to address the respective risk consequences)**

		Immunogenicity			Graft failure	Toxicity/ Carcinogenicity			Disease transmission	
Criteria	Specific test	Systemic immune response	Localised immune response	Anaphylaxis	Graft failure	Systemic cytotoxicity	Carcinogenicity	Teratogenicity	Blood-borne infections	Infections acquired during procurement or processing
Repopulation capacity	Immune deficient mouse/ small animal models e.g. cell labelling and imaging techniques									
Stem cell functionality	Histology sections for immunohistochemistry-based assays (e.g. evaluation of the expression of specific proteins important for cellular function)									
	In vivo functional assessment									
Safety of compounds	Haematopoietic colony forming cell assays									

Step 3B: Extent of clinical evaluation

In order to determine safety and efficacy in the clinical application of novel BTCs, it is necessary to evaluate and record the follow-up of patient treatment outcomes. If, after preclinical evaluation of risk-reducing steps, a certain or poorly defined risk remains, clinical follow-up is indicated. Depending on the type of risk remaining with the novel aspects of the stem cell product, specific parameters in patients should be monitored to evaluate the safety and efficacy of the novelty. Currently, the outcome of treatment of all stem cell recipients is monitored systematically by clinicians (EBMT members and non-members) using the EBMT Minimal Essential Data (MED) forms. With this registration of all stem cell treatments via a centralised database, scientific research can be performed to evaluate best practices in the treatment of haematological disorders with HPC. The MED-AB forms cover all relevant items that are required to assess the clinical outcome in patients and to detect adverse reactions and complications; the registration of variables collected by the MED-A form are essential for each HSCT. When more detailed aspects need to be monitored to evaluate patient outcome after stem cell transplantation, additional medical parameters can be found in the MED-B form. Recently, a special MED-A form for Cell Therapy has been made available.

To perform a clinical evaluation of stem cell treatment novelties, it is essential to collect information on the medical aspects mentioned in the MED-A form. To guide the evaluation it is recommended that the outcome data are not only used to establish the safety and efficacy of the treatment within a single establishment, but that the data are also uploaded to the EBMT Patient Registry[23]. The forms can be easily

downloaded from the EBMT website. In the following table (Table 5.6), an overview is given of the medical aspects that are considered essential for clinical follow-up and that are covered in MED-AB forms.

After using the IAT to determine the level of risk of the application of the novelty, please decide whether a MED-A form would cover the evaluation of the risk or if you need to complete the more extensive MED-B form. Aspects that are not covered in the form can be collected using elements of the MED-A Cell Therapy form (see Tables 5.7 and 5.8), although the novelties that are covered by this guide are not cellular therapies.

Table 5.6. **Clinical evaluation and follow-up - HPC: Bone marrow, Peripheral blood, Cord blood, as stated in EBMT Minimal Essential Data forms**

Test category	Detailed investigational options	EBMT – Form
Recovery & graft performance	Absolute neutrophil recovery	MED-A form 100 d
	Platelet reconstitution	MED-A form 100 d
	Date of last platelet transfusion	MED-B form 100 d
	Early graft loss	MED-A form 100 d
	Haematopoietic chimaerism	MED-B form 100 d
	Treatment for early graft loss or non-recovery	MED-B form 100 d
Acute GvHD	Maximum grade	MED-A form 100 d
	Stage	MED-A form 100 d
Immediate treatment post-transfusion	Growth factors	MED-B form 100 d
	Additional cell infusions	MED-A form 100 d
	Cell therapy (specified)	MED-A form 100 d
Cell therapy	Source of cells (autologous/allogeneic)	MED-A form 100 d
	Type of cells	MED-A form 100 d
	Chronological number of infusion	MED-A form 100 d
	Indication	MED-A form 100 d
	Number of infusions within 10 weeks	MED-A form 100 d
Additional disease treatment	Yes/no	MED-A form 100 d
	Reason (prophylaxis; relapse)	MED-A form 100 d
	Chemotherapy/drug administered	MED-A form 100 d
	Radiotherapy	MED-A form 100 d
Complications within the first 100 days	Infection-related complications (bacterial, fungal, viral, parasites)	MED-B form 100 d
	Systemic symptoms of infection	MED-B form 100 d
	End-organ diseases	MED- B form 100 d
	Documented pathogens	MED-B form 100 d
	Non-infection-related complications (specify)	MED-B form 100 d
Best response	Best disease status (response) after HSCT	MED-A form 100 d
	Date of death (< 100 d)	MED-A form 100 d
Chronic GvHD at day 100	Yes/no (date onset)	MED-A form 100 d
	Maximum extent (during this period)	MED-A form 100 d
	Maximum NIH score (during this period)	MED-A form 100 d
First relapse/progression	First relapse or progression after HSCT	MED-A form 100 d

Test category	Detailed investigational options	EBMT – Form
Relapse of leukaemias: method of detection	Clinical/haematological method: increase in blast cell count over 5 % in the bone marrow	MED-A form 100 d
	Cytogenetic method: reappearance of chromosome anomalies detected earlier in history of disease	MED-A form 100 d
	Molecular method: reappearance of acute leukaemia specific molecular markers detected earlier in the history of the disease	MED-A form 100 d
	Donor cell leukaemia?	MED-A form 100 d
Disease status at 100 days	Clinical/haematological	MED-B form 100 d
	Cytogenetic/FISH	MED-A form 100 d
	Detection by molecular method	MED-A form 100 d
Survival status at 100 days	Alive/deceased	MED-A form 100 d
	Main cause of death	MED-A form 100 d
	Contributory cause of death	MED-A form 100 d

Table 5.7. **Clinical Evaluation and follow-up cell therapy, as stated in EBMT Cell Therapy Minimal Essential Data A form**

Test category	Detailed investigational options	EBMT – Form
Indication for cell therapy treatment	Treatment of a primary disease, including infections or infection prevention	MED-A form 100 d
	Treatment or prevention of complications derived or expected from previous treatment including HSCT	MED-A form 100 d
	Other	MED-A form 100 d
Therapy	Clinical trial	MED-A form 100 d
	Institutional guidelines/standard treatment	MED-A form 100 d
	Hospital exemption	MED-A form 100 d
	Compassionate use	MED-A form 100 d
	Performance score of patient at initiation of treatment	MED-A form 100 d
	Cell origin	MED-A form 100 d
Donor HLA match type	HLA identical sibling (including non-monozygotic twin)	MED-A form 100 d
	Syngeneic (monozygotic twin)	MED-A form 100 d
	HLA-matched other relative	MED-A form 100 d
	HLA-mismatched relative (degree of mm 1 HLA locus mm, 2 HLA locus mm)	MED-A form 100 d
	Unrelated donor	MED-A form 100 d
Cell therapy infusion unit – description & collection	Identification	MED-A form 100 d
	Tissue source	MED-A form 100 d
	Collection procedure (incl. mobilising agents)	MED-A form 100 d

Table 5.8. **Explanation and examples of the test categories**

Test category	Explanation and examples
Cell therapy infusion unit - manipulation	*Ex vivo* manipulation of the products contained in the cell therapy infusion unit (drugs, gene manipulation, recognition of specific target/antigen, selection, expansion, induced differentiation)

Test category	Explanation and examples
Therapy and cell infusions	Chronological number of cell therapy treatment for this patient
	Primary aim of the cell therapy treatment
	Patient preparative treatment (if yes, specify)
Cell infusion episodes	Was there more than one cell infusion episode during this treatment or procedure
	Cell type and number of cells infused
	Did the treatment that includes this cell therapy episode also include other type(s) of treatment?
Response	Best clinical/biological response after the entire cell therapy treatment
	Complications & response
	First relapse or progression or significant worsening of organ function of the primary disease
	Last disease status
Toxicity during first 6 months after cell therapy was initiated	Acute GvHD (maximum grade)
	Chronic GvHD present (maximum extent & NIH score)
	Other complications or toxicities during this period (if yes, specify)
Secondary malignancy	Did a secondary malignancy, lymphoproliferative or myeloproliferative disorder occur? If yes, donor cell leukaemia or malignancy of the cellular product?
Graft assessment	Graft loss
Survival status	Alive/deceased
	Survival status
	Contributory cause of death
Persistence of the infused cells	Were tests performed to detect the persistence of the cellular products during this period?

6. Medically assisted reproduction – Specific guidance for the use of EuroGTP II methodologies

There are three steps that need to be completed in order to determine the novelty, risks and extent of studies needed before the process is implemented in the TE.

Define what type of BTC you are evaluating

First, it is important to define the general category of BTC for which you are going to use the tool, as this will generate specific risk factors. For MAR, choose 'medically assisted reproduction' and subsequently the type of reproductive BTC under evaluation (Figure 6.1).

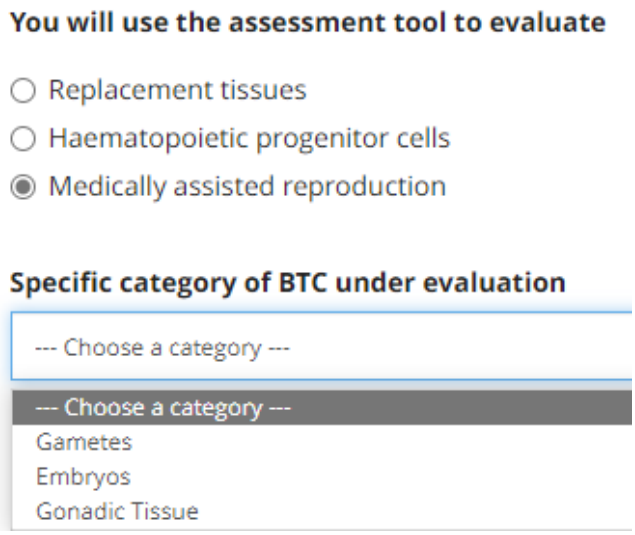

Figure 6.1. **IAT screenshot: different options for MAR**

6.1. Evaluation of novelty (Step 1)

Before any risk analysis can be performed, it has to be determined if the process change under evaluation consists of a novelty or not. If not, then no further action is needed in addition to the regular follow-up of established protocols. If the change in process is indeed a novelty, the risk assessment needs to be performed (Step 2) and the tool will determine the specifics of the follow-up needed.

The exercise in Step 1 consists of a set of questions to determine if the users are facing a novelty. Novelty is present whenever the user answers "no" to at least one of the seven questions.

If all answers are positive (yes), users are not dealing with any novelty. For this standard/established BTC, the regular, internal validations and follow-up procedures should be put in place/maintained.

One example is used in Table 6.1: vitrification of sperm procured via testicular extraction (TESE), where the standard protocol in your TE was slow freezing.

Table 6.1. **Exercise for assessing novelty**

		Yes	No	Na
A	Has this type of BTC previously been collected, processed/prepared and issued for clinical use by your establishment?			

Explanation
Consider if your TE has previous experience working with the BTC or not.

Examples
You want to implement vitrification of sperm in your TE, thus the answer to question A would be YES: you have previously prepared sperm and issued it for clinical use.

		Yes	No	Na
B	Will the starting material used to prepare this BTC be obtained from the same donor population previously used by your establishment for this type of BTC?			

Explanation
Consider if the starting material is from the same donor population or not.

Examples
You want to implement vitrification of sperm in your TE, thus the answer to question B would be YES: the starting material is from the same donor population.

		Yes	No	Na
C	Will the starting material for this BTC be procured/collected using a procedure used previously by your establishment for this type of BTC?			

Explanation
Consider the starting material and how it is procured or collected and if this changes in the novel protocol or therapy.

Examples
You want to implement vitrification of sperm in your TE, thus the answer to question C would be YES: the starting material is procured using the same procedure. There is no change in TESE protocol, only the cryopreservation method is different.

		Yes	No	Na
D	Will this BTC be prepared by a procedure (processing, decontamination and preservation) used previously in your establishment for this type of BTC?			

Explanation
Consider the complete processing procedure of the product. If changes occur in the new protocol or therapy, answer the question accordingly.

Examples
You want to implement vitrification of sperm in your TE, thus the answer to question D would be NO: there are indeed changes in processing and preservation of the sperm when vitrification will be introduced in comparison to the standard slow freezing protocol currently used.

		Yes	No	Na
E	Will this BTC be packaged, stored and distributed using a protocol and materials used previously in your establishment for this type of BTC?			

Explanation
Consider if there are changes to the packaging and storage and if you have experience with these items in your TE for the specific cell or tissue product where the novelty is introduced.

Examples
You want to implement vitrification of sperm in your TE, thus the answer to question E would be NO if there are changes in the type of packaging where straws will be used for the vitrified sperm instead of vials.

		Yes	No	Na
F	Will this type of BTC provided by your establishment be applied clinically using an implantation/application method used previously?			

Explanation
Consider if the product or therapy has been clinically applied previously and answer accordingly.

Examples
You want to implement vitrification of sperm in your TE, thus the answer to question F would be YES: there is no difference in clinical application for the sperm.

		Yes	No	Na
G	Has your establishment provided this type of BTC for the same clinical indication or for application/transfusion/infusion into the same anatomical site?			

Explanation
Consider the clinical application of the product and answer the question concerning the intended anatomical site of implantation or transplantation.

Examples/Explanations
The clinical application of vitrified sperm is the same as slow frozen sperm, so the answer would be YES in this example. In another example this question will be answered 'NO', e.g. if heterotopic transplantation of ovarian tissue strips is a new protocol, where previously only orthotopic transplantations were performed in your clinic.

If you answered 'NO' to one of these questions, the level of risk must be determined in Step 2.

6.2. Level risk analysis (Step 2)

The second step of the exercise aims to determine the risk associated with the novelties identified in the process being evaluated.

Every modification in the processes associated with the donation, procurement, testing, processing, storage and distribution of cells and tissues may have potential consequences for the quality of these products and the safety of recipients and the corresponding offspring in MAR.

Moreover, different levels of novelty represent different risks and have a distinct impact on the quality and safety of the tissue and cell products. The evaluation of such risks could be performed using the methodology proposed in the current rationale.

Step 2A: Identification of risk factors

First, select the risk factors associated with the changes in the process. There are eight risk factors that could be applicable to changes in processes concerning gametes and embryos or MAR treatments and nine risk factors that could apply to MAR where gonadic tissues are concerned (loss of viability and/or functionality is not considered). Definitions for the correct interpretation of risk factors and examples can be found in Table 6.2.

Step 2B: Identification of risk consequences

When a risk factor is applicable, potential risk consequences must be considered and the probability scored. The potential risk consequences must be considered in comparison with the BTC prior to the implementation of novelty. When selecting the potential risk consequence, it is important to think of the potential harm that the novelty may cause to the recipients, the resulting offspring and/or the impact on the availability and accessibility of treatment. It is important to note that the risk consequences are not about the viability of the embryo. For example, if the viability of a blastocyst could be harmed because of a novel biopsy procedure, then the risk factor loss of viability and/or functionality should be chosen. However the quantification of the potential risk consequences should be assessed bearing the patient and thus the recipient in mind. So, the question to be asked is: would there be unexpected immunogenicity in the recipient when this damaged embryo was transferred? Would there be implant failure or pregnancy loss? Would there be a risk of disease transmission in this patient? Some examples are given to explain the risks.

Potential risks associated with the clinical use of MAR tissue and cell products are:

- Unexpected immunogenicity: this is only applicable for gonadic tissue and this option will only appear if gonadic tissues are selected at the start of the risk assessment.
- Implant failure and/or pregnancy loss: for MAR, this risk is self-explanatory. Additionally, the loss of a batch of gametes or embryos requiring an

extra treatment for the patient should also be considered under this risk.

- Disease transmission (including infection): consider if the novelty in the BTC has a potential risk of introducing disease transmission or infection in the recipient.
- Toxicity/Carcinogenicity: consider if the novelty in the BTC can introduce toxicity reactions in the recipient or if there is a risk for carcinogenicity.
- Other: consider other risks associated with the changes to the BTC and score them accordingly. It is very important to make use of this category as many of the above-stated risks may seem not to be attributable to MAR recipients since gametes and embryos are clinically applied in a very specific way and have different risks than tissues and cells being transplanted into recipients. As an example: the risk of complication in the recipient such as pelvic inflammatory disease could be a potential risk when certain novelties are introduced in MAR.

In order to have a complete overview of the combination of risk factors and risk consequences, examples are given in Table 6.2. It is important to note that not all risk factors apply to changes to protocols and procedures, likewise, not all risk consequences apply to a risk factor. For the ease of interpretation, the explanation of the risks is based on the example.

Table 6.2. **Combined table of the identification of the risk factors and the associated risks**

	Risk factor	Examples and Explanation	Risks	Examples and Explanation
Donation	Donor characteristics	Consider if the novelty in your process or procedure changes donor characteristics and if these changes could impart a risk to the recipient. Examples: • Change in collecting sperm from peripubertal boys (aged 12-14) to collecting sperm from pubertal boys (aged > 14) • Change from autologous to allogeneic donors: if the BTC is sourced from an allogeneic donor, there may be risks that immunogenicity could impact the clinical performance of the BTC, and there is a risk of disease transmission.	Unexpected immunogenicity	Not applicable for this example, only for gonadic tissue.
			Implant failure/ pregnancy loss	Consider and quantify the risk that sperm collected from peripubertal boys may lead to pregnancy loss when used in MAR.
			Disease transmission	Although highly unlikely, consider and quantify the risk that sperm collected from peripubertal boys may lead to disease transmission in the recipient.
			Toxicity/ Carcinogenicity	It is highly unlikely that this change in donor characteristics would have a risk for toxicity in the recipient. In the case of gonadic tissue that came from a donor with oncological disease, this risk must be taken into account.
			Other	Consider other risks if applicable.
Procure	Recovery/Procurement process and environment	Consider where and how the BTC is collected, procured or recovered, and if this process could have an influence on the BTC. How long does the process take, how complex is it and what is quality of the environment? Examples: • Change from semen production in the clinic to collection of sperm at the home of the patient and transporting it to the TE. • Change to a new type of sterile semen container.	Unexpected immunogenicity	Not applicable for this example, only for gonadic tissue.
			Implant failure/ pregnancy loss	It would be highly unlikely that the use of a new semen container during collection would impact implant failure.
			Disease transmission	It could be possible that if this new container is non-sterile it may influence disease transmission, although the risk would be rare.
			Toxicity/ Carcinogenicity	Consider the risk that using a new semen container would have on the toxicity or carcinogenicity in the recipient.
			Other	Consider other risks if applicable.

	Risk factor	Examples and Explanation	Risks	Examples and Explanation
Processing/storing/transport	Processing and environment	Consider where and how the BTC is prepared. How long does processing take and how complex is it? This may impact the risk of contamination, or suggest that it may not be prepared to consistent specifications and quality. Also consider the quality of the processing environment, which may also affect the risk of contamination. Examples: • Change from laser-assisted hatching on day 3 to day 5 for trophectoderm biopsy. • Change from performing ICSI inside a laminar flow hood to outside.	Unexpected immunogenicity	Not applicable for this example, only for gonadic tissue.
			Implant failure/ pregnancy loss	Changing from performing ICSI inside a laminar flow hood to outside of the hood will probably rarely effect pregnancy loss.
			Disease transmission	If this procedure took place in a different environment where the risk for environmental contamination was higher, then a risk for disease transmission in the recipient may be impacted.
			Toxicity/ Carcinogenicity	It is highly unlikely that the change from day 3 to day 5 laser-assisted hatching would introduce toxic compounds in the recipient.
			Other	Consider other risks if applicable.
Processing/storing/transport	Reagents	Consider any reagents used during recovery, processing, preparation, decontamination and storage of the BTC. Could they damage the BTC in any way, or could residual traces of reagent remain in the BTC that could cause toxic or immunogenic effects in recipients? Example: • Change to a new cryopreservation medium. • Change to a new anaesthetic during oocyte collection.	Unexpected immunogenicity	Not applicable in this example, only for gonadic tissue.
			Implant failure/ pregnancy loss	The change in reagents is unlikely to impact the risk of pregnancy loss.
			Disease transmission	If this new reagent contains, for example, albumin from a source that is doubtful, then there is a risk of disease transmission to the recipient.
			Toxicity/Carcinogenicity	If the new medium contains different types of antibiotics, this may have an impact on toxicity reactions in the recipient.
			Other	Consider other risks if applicable

	Risk factor	Examples and Explanation	Risks	Examples and Explanation
Processing/storing/transport	Storage conditions	Consider any potential risks arising from how the starting material and BTC are stored, not only after processing and before clinical application, but also in intermediate steps: e.g. between procurement and processing, during processing, and between processing steps. Examples: • Change from storage of stimulation medication at room temperature to a refrigerated storage at 4 °C. • Change from sperm being stored in liquid nitrogen to storage in the vapour phase.	Unexpected immunogenicity	Not applicable in this example, only for gonadic tissue.
			Implant failure/ pregnancy loss	The change in storage conditions may have a direct impact on implant failure when this preserved sperm is used for insemination.
			Disease transmission	The change in storage may theoretically have an impact on disease transmission, although the risk is very rare.
			Toxicity/ Carcinogenicity	The impact of the novel storage conditions will, in this example, have very little or even no impact on the introduction of toxic compounds.
			Other	Consider other risks in the patient if applicable.
	Transport conditions	Consider any potential risks arising from how the starting material and BTC are transported, for example between the sites of procurement and processing, and between the sites of storage and clinical application. Examples: • Change to a new type of dry-shipper for the distribution of frozen sperm to clinical sites. • Change from only MAR treatments from own patients to IVF for satellite patients where oocytes are collected in another clinic.	Unexpected immunogenicity	Not applicable in this example, only for gonadic tissue.
			Implant failure/pregnancy loss	New transport conditions may have an impact on pregnancy loss if not adequately controlled.
			Disease transmission	Disease transmission is rarely impacted if only transport conditions are changed.
			Toxicity/ Carcinogenicity	Toxicity could be impacted if not only transport conditions are changed, but there are also differences, e.g. medium used, between the satellite centre and the current TE.
			Other	Consider other risks if applicable.
	Reliability of microbiology testing (in the case of gonadic tissue)	Consider the risk that the testing methodology and/or presence of residual processing reagents such as antibiotics in the finished BTC may impact the accuracy of any microbiology testing of the BTC. This risk factor is not about blood tests on the donor. Example: • Change to a new ovarian tissue processing medium that could mask the current microbiology testing because of the presence of antibiotics.	Unexpected immunogenicity	This change could have an impact on unexpected immunogenicity when the tissue is transplanted in the recipient.
			Implant failure/pregnancy loss	This change could lead to implant failure due to residual microbiological load that has an impact on the graft viability.
			Disease transmission	If this change is solely to the processing medium, but it is still autologous use of the tissue, the risk of disease transmission will probably not change in comparison with the former procedure.
			Toxicity/Carcinogenicity	There may be a risk of introducing toxic compounds.
			Other	Consider other risks if applicable.
Product	Loss of viability and or functionality	Consider the risk that the changes in procedures or processes can have on the viability or functionality of the gametes or embryos Example: • Change from a 2-step cryopreservation protocol to a 5-step protocol. • Change from a blastomere biopsy programme to a trophectoderm biopsy programme.	Unexpected immunogenicity	Not applicable
			Implant failure/pregnancy loss	This novelty can have a direct impact on implant failure and pregnancy loss when the blastocysts are harmed because of inadequate technical expertise.
			Disease transmission	The impact on disease transmission due to harming of the embryo because of the new biopsy technique is highly unlikely.
			Toxicity/Carcinogenicity	The loss of viability will probably only have a rare impact on the introduction of toxicity or carcinogenicity in the recipient.
			Other	Consider other risks if applicable.

	Risk factor	Examples and Explanation	Risks	Examples and Explanation
Product	Presence of unwanted cellular material and/or graft vascularity(in the case of gonadic tissue)	This risk must be considered in light of the fact that the presence of intact vital cells is desirable for some BTCs, but that this may affect tumour formation, immunogenicity and disease transmission risks. Vascular tissues may be more at risk of infiltration by pathogens or malignant cells than avascular tissues Example: • When autologous ovarian tissue transplantation is performed in patients with a history of blood cancer at the time of tissue procurement. The risk of transmission of malignant cells should be considered.	Unexpected immunogenicity	Consider the risk that presence of cellular material/graft vascularity could have on unexpected immunogenicity in the recipient.
			Implant failure/pregnancy loss	There could be a risk of implant failure when malignant cells are present in the graft.
			Disease transmission	If malignant cells are transplanted together with the graft, there is a risk of transmission of oncological disease.
			Toxicity/Carcinogenicity	Presence of cells may impact the risk of carcinogenicity.
			Other	Consider other risks if applicable.
Clinical application procedure	Complexity of the pre-implantation preparation and/or application method	Consider how complex the method of clinical application will be for this BTC. How long will it take, and could this introduce risks? What is the scope for errors to be made, and what could the consequences of these errors be? Low feasibility of application standardisation may influence the risks of implant failure and disease transmission, at minimum. Example: • Change to a new transfer catheter for clinical application.	Unexpected immunogenicity	Not applicable in this example, only for gonadic tissue.
			Implant failure/pregnancy loss	There may be an impact on the risk of implant failure when a new transfer catheter is introduced.
			Disease transmission	It is highly unlikely that the risk for disease transmission would be impacted when only a new transfer catheter is implemented.
			Toxicity/Carcinogenicity	A new transfer catheter may introduce toxicity to the recipient, although this is unlikely.
			Other	Consider other risks if applicable.

Step 2C: Quantification of risk consequences

When the risk factors are selected and the potential risk consequences are identified, the potential impact of this risk analysis needs to be determined according the definitions present in Section 3.3 and summarised in Annex IV.

Step 2D: Assessment of risk reduction

Having calculated probability, severity and detectability, and thus an overall risk based on 'internal' knowledge and data, it may be possible to adjust this score by taking into account other external sources of information. These external data are not used to specifically reduce probability, severity or detectability, but to calculate a general reduction in the overall risk score.

Data that should be taken into account when calculating risk reduction may include:

- Published data in peer-reviewed literature on specific changes to procedures or protocols could be helpful. Additionally, guidelines from national and international scientific societies could be a source of information.
- Unpublished data from external sources: it could be interesting to obtain information from other MAR centres who have experience with the changes that you would like to implement in your processes or procedures.
- Advice and information from external experts: it could be interesting to contact ESHRE special interest groups to obtain expert opinions on certain novelties.
- Technical improvements from formal internal validation studies: it is possible that you have your own data from previous validation studies that can be used as retrospective validation data.
- Clinical outcome data from external sources (e.g. registries): national registries may be of interest, and for global European data, the European IVF Monitoring (EIM) consortium of ESHRE could be contacted (www.eshre.eu/eim).

When calculating the risk reduction factor, it

is important that the quality and reliability of the data are considered. For systematic reviews and evidence-based guidelines or recommendations that are based on a solid methodology, the risk reduction can be considered high. For other information, it is important to consider a fair reduction factor and this could be subjective.

6.3. Interpretation of the outcomes of the risk analysis and definition of extent of studies needed based on the risks quantified (Step 3)

Using the tool you will be able to perform a risk analysis, determine the risk profile and the level of risk associated with the novel process or procedure. As a result, the tool will provide the *Final Risk Score* and the respective classification as a level of risk. It is important to state that MAR centres should be prepared to stop certain treatments when they prove problematic (in terms of safety and effectiveness), even when a novelty of negligible risk was implemented. Therefore MAR centres should always collect data and register follow-up data in a systematic way. Data should be made available to the scientific community regardless of the success of the treatment: not withholding results that point to a negative outcome or that turn out to be inconclusive.[24] It is important in all processes, regardless of the level of risk, to monitor and register SARE.

Table 6.3 (adjusted according to Provoost V. *et al.* 2014[24]) gives guidance on the clinical evaluation/follow-up studies needed in accordance with the level of risk.

Table 6.3. **Generic review of extent of studies needed**

Level of Risk	Extent of studies needed
Negligible	***Step3A: Risk reduction strategies*** A change in process could have a negligible level of risk because it is part of a therapy or procedure that is considered as established or standard. In this case multi-centre studies (ideally RCTs) are published in peer-reviewed journals and the procedures are performed according to a validated and standard protocol. Minimal process validation is needed. The technical performance of staff should be monitored and comparable with other TEs or published studies; therefore, standard key performance indicators (KPIs) should be monitored on the technical quality of the staff performing the procedures. Dropping KPIs indicating protocol drift must lead to investigation of both the procedural steps and/or the possibility to retrain staff.
	Step 3B: Extent of clinical evaluation A routine/safety follow-up programme is enough as good practice states. Follow-up procedures should be focused on assessing efficacy, comparing the clinical follow-up with the results obtained before the implementation of the change in the process. Long-term (ideally transgenerational) health effects, including aspects such as fertility, oncology and mental health, should be monitored.
Low	***Step3A: Risk reduction strategies*** IImplementing a standard procedure or treatment in an MAR centre that has never performed it requires an intensive validation. Training of staff is necessary in order to reach the outcomes published in scientific literature. Having a mentor/mentee relationship with a MAR centre with experience is highly recommended. Performance specifications should be determined and when these limits are met by training on spare tissues and cells, staff can be authorised to perform the procedure. A learning curve may be expected and should be part of the validation report. When implementing the procedure, additional quality controls must be performed to monitor critical process parameters (CPPs) and critical quality attributes (CQAs). For example, when a TE is switching from IVF to ICSI (which they have never performed before), fertilisation rates and damage rates, etc. of embryos should be carefully monitored in relation to the staff performing the procedure.
	Step 3B: Extent of clinical evaluation A safety follow-up programme is necessary. Follow-up procedures should be focused on assessing efficacy, comparing the clinical follow-up with the results obtained before the implementation of the change in the process and in relation to the results published in scientific literature. The procedure or treatment encompasses an established or standard technique. The expected learning curve should be kept as short as possible and put in relation to the follow-up programme. Likewise, established techniques are subject to long-term (ideally transgenerational) follow-up of the health effects. TEs or ORHAs implementing an established technique shall perform long-term follow-up and could base their follow-up items on the mentor facility. This way of working could lead to periodic evaluation of performance in the mentor/mentee relationship.

Level of Risk	Extent of studies needed
Moderate	***Step3A: Risk reduction strategies*** Novel procedures or treatments that exert a moderate risk and are considered innovative. The treatment has shown proof of principle and there are reassuring data in literature in terms of both safety and effectiveness, at least in animal studies, and preclinical data show normal embryology development. The studies that have published these data should have a sound methodology and be published in peer-reviewed journals. In order to implement an innovative treatment, an enhanced validation is necessary including a range of additional quality controls performed to monitor critical process parameters (CPPs), critical quality attributes (CQAs) and the impact of the implemented changes on gametes, embryos and gonadic tissue in the preclinical studies. Since reassuring non-clinical data of this innovative treatment should at least be already available, a more specific monitoring of the published critical parameters can be performed instead of a registration of all critical parameters.
	Step 3B: Extent of clinical evaluation Clinical evaluation and follow-up programmes should be implemented to assess mid-term safety (3 months up to 5 years post-delivery, including data on psychological wellbeing) and these studies should refer to patients undergoing the procedure as well as the children born from it.
High	***Step3A: Risk reduction strategies*** A new procedure can be offered to patients in an experimental design aimed at showing proof of principle, A new procedure can be offered to patients with an experimental design aimed at showing proof of principle, short-term safety and/or effectiveness. An extensive validation and a range of additional quality controls performed to monitor critical process parameters (CPPs), critical quality attributes (CQAs), and the impact of the implemented changes is required. This extensive validation should include: Non-clinical studies: there should preferably be studies showing the experimental procedure is safe in animals. Preclinical Studies: when experimental treatments encompass a laboratory IVF phase, then at minimum the structural integrity of the gametes, embryos or gonadic tissue should be looked at in detail, monitored and registered. Clinical embryology data should indicate a normal cleavage embryo morphology and blastocyst formation.
	Step 3B: Extent of clinical evaluation Follow-up programme: experimental treatments should only be offered to a selected and limited patient cohort and these patients should be clearly informed of the experimental status and should receive information about (the lack of knowledge about) possible risks, alternative treatments, etc. ORHAs should only offer experimental treatments or treatments based on experimental procedures after approval by a commission of medical ethics.

The purpose of Step 3 is to provide users with guidance to evaluate and mitigate the risks through the application of specific tests. This section is purely informative and far from complete.

Process validation

Process validation studies can be very helpful in tackling risks when novelties are addressed in procedures. Additional quality controls and monitoring of certain process indicators is critical. There are some reports in the literature containing MAR process indicators: the alpha consensus report on indicators concerning cryopreservation processes[25] and the Vienna consensus report on MAR laboratory performance indicators[26].

When performing process validation studies, it is important to set out specific parameters that should be monitored and results that should be met. There is a vast variety of tests that can be carried out when process validation studies are performed. The novelty being introduced in the process and the risk factors and risk consequences identified will determine which test to be used. These include: fertilisation rates, embryo cleavage patterns, blastocyst formation rates, packaging sealing tests when novel containers are introduced, cryopreservation survival rates when new steps in cryopreservation programmes are introduced.

Preclinical *in vitro* studies

When novelties are introduced in MAR, a variety of *in vitro* tests can be performed: microscopic observations can be helpful in determining the morphological integrity of the gametes and embryos, the cell viability can be assessed by live/dead assays, DNA fragmentation assays, immunohistochemical testing of e.g. markers for apoptosis or proliferation can be informative in certain studies or analysing certain secreted factors in *in vitro* cultures. Depending on the changes and novelties introduced, it is important to perform certain preclinical *in vitro* studies.

Preclinical *in vivo* studies

If possible, animal models should be used to verify safety of highly novel BTCs in MAR. Although animal models can be helpful, it is known that the results cannot always be translated to the human.

7. Tissue & Cell Database

The Tissue and Cells (T&C) database is intended to be a compendium of tissue/cell preparations, preparation processes, applications and therapies.

7.1. Purpose of the BTC database (the compendium)

The purpose of the European *BTC Compendium* is to promote the safe and effective use of BTCs by providing data related to their specifications and authorisation.

The structure and contents of the Compendium were defined in order to ensure its consistency, harmonise the characterisation of BTCs, and support the collection of efficacy and quality data associated with the clinical use of SoHO at European level.

The Compendium was designed to be appropriate for the needs of:

- TEs and those engaged in the quality control and design of preclinical studies and clinical evaluation of BTC;
- End users/ORHAs;
- CAs.

The distribution of BTCs between European member states is a common practice, and the exchange of scientific and clinical information promotes the assessment of safety and efficacy of novel and traditional SoHO therapies.

The aim of this tool is to provide structured and systematic information regarding BTCs implemented by the TEs, and include an overview regarding new BTCs, information on clinical application and references to available efficacy and safety data.

The *T&C Database* is intended to:

- collate references and evidence relating to safety and efficacy data;
- encourage stakeholders/CAs to accept the validity of data generated for products in other countries (harmonisation of practices);
- promote collaboration amongst TEs, encouraging multicentre collaborations for the development of novel BTCs;
- promote accessibility for patients by promoting knowledge among clinicians regarding the availability of BTCs.

The data included in the *T&C Database* were voluntarily shared by TEs, with the intention of contributing to the knowledge base associated with novel and well-established BTCs within Europe.

These data should be periodically reviewed and updated by experts nominated by the European scientific associations that collaborated with the EuroGTP II project: EBMT, EATB, EEBA and ESHRE. This review is intended to ensure that the data are trustworthy and up to date, and to avoid redundant entries.

7.2. General Principles

The is a registry of BTC consisting of information provided by European TEs.

TEs are encouraged to register information associated with clinical evaluation studies performed to determine the safety and efficacy of the BTCs distributed for therapeutic treatment.

Furthermore, this information promotes dialogue between the European CAs and TEs seeking collaborations and sharing of expertise and information.

In order to assure consistency and scientific reliability, the EuroGTP II project has defined the principles and procedures required for the correct inclusion and interpretation of data submitted to the Compendium.

The technical guidelines (instructions and definitions) to correctly complete, submit and review data are part of the current document and are intended to assist users and contributors.

The principles applied should be periodically revised by experts in the future: strategies for this purpose will be defined in co-operation with the scientific associations.

TE contributors should provide sufficient information to ensure that the data included are robust, comprehensive and evidence-based. Data relating to authorisation status should also be provided.

While the products entered by TEs are already listed in the EU Coding Platform, the *T&C Database* provides additional information related to processing and clinical use and novel products/therapies that are not part of the EU Platform. Each record includes a summary description and information about the current status of the BTC with regard to clinical uses, risks assessed and authorisation status.

Some BTC entries may include the number of recipients already treated on an annual basis, the number of patients defined for the clinical evaluation studies, and cross references to the Notify Library (optional information).

7.3. Accessing the T&C Database

The T&C Database is publicly accessible (http://db.goodtissuepractices.site/).

Three different levels of access have been defined in order to achieve an appropriate security level, and allow the correct management of database contents (Table 7.1).

Table 7.1. **Users profiles of the T&C database**

Level of Access	Credentials holders	Functionalities
Administrators	Hosts of *T&C Database*	Can view, add, edit and delete content in the database
User	Members of the Experts' Committees defined by the Scientific Associations	Can view, add, and edit content in the database
Guest	General public – free access	Can view content in the database

7.4. Introduction of data

The data will be entered by the TEs and supervised/peer-reviewed by experts nominated by the scientific associations (more details will be defined in the *GTP Management Model*) that will promote the use of this database.

7.5. Description of Contents

Please refer to Tables 7.2, 7.3, 7.4.

M – Mandatory field | OP – Optional field

Table 7.2. **Contents used to describe TEs in the** *T&C Database*

	Field Name	Description	Observations
M	EU TE Code + TE Name	(2 letters 6 Numbers) + Full Name of TE	Data imported from EU Coding Platform

	Field Name	Description	Observations
M	Country	Name of Country + ISO code (2 letter code of ME)	Data imported from EU Coding Platform
M	City	Name of city	Data imported from EU Coding Platform
OP	Website	Link	–

During the design and implementation, the information was directly imported from the EU Coding Platform, an accredited source of information provided by the CAs of the different member states. New TEs, or organisations authorised after the implementation of the *T&C Database*, will be added manually by the database's administrator, after confirming the authorisation status in the *EU Coding Platform*.

Information related to a TE's authorisation status requires confirmation with the EU Coding Platform, as there may be a delay with information related to authorisations that have been revoked.

Table 7.3. **Contents used to describe preparations and processes in the *T&C Database***

	Field Name	Description
M	Product ID	EUTC Code and Name (Primary Key (PK))
M	SoHO Class	Tissue/Cells/ART (Select Option)
M	Product Type	Amniotic Membrane/Cardiovascular/Ocular/Other Membranes/Mature cells/MSK/Progenitor Cells/ Skin/ Embryo /Oocyte /Ovarian Tissue/ Sperm /Testicular Tissue (Select Option)
OP	Product Sub classification	Adipose / Cardiovascular, Valves / Cardiovascular, Vessels / Mature Cell, Hepatocyte / Mature Cell, Keratinocyte / Mature Cell, Pancreatic Islet Cells/ Mature Cell, T Cell (DLI) / Mature Cells, MNC (DLI) / Membrane, Amniotic/ Membrane, Dura Mater/ Membrane, Fascia Lata / Membrane, Fascia Rectus / Membrane, Pericardium/ Musculoskeletal, Bone / Musculoskeletal, Cartilage / Musculoskeletal, Tendon & Ligament / Neuronal / Ocular / Other / Parathyroid / Progenitor Cell, Hematopoietic, Bone Marrow / Progenitor Cell, Hematopoietic, Cord Blood / Progenitor Cell, Hematopoietic, PBSC / Progenitor Cell, Hematopoietic, Unspecified / Reproductive, Embryos/Zygotes / Reproductive, Oocytes / Reproductive, Ovarian / Reproductive, Sperm / Reproductive, Testicular / Skin / Umbilical Cord (Tissue)
M	Product Name	Open text – (Product name given by the TE)
M	Product Characteristics	Open text – (Main characteristics/specifications of the product, defined by the TE)
OP	Donor/Recipient Relationship	Allogeneic (postmortem donors)/ Allogeneic (living donors)/ related / unrelated / Autologous
OP	Specific Donor Criteria	Open text (Optional) (Donor selection criteria applied, over and above EUTCD requirements)
OP	Collection/Recovery Method	Default Ejaculated Extracted (optional only for ART)
OP	Additive Solution	Describes additives introduced during the processing of the product. Text (optional)
OP	Pathogen Reduction	No pathogen reduction / Not specified / Antibiotics / Combined process / ETO / No pathogen reduction / Peracetic acid / Radiation sterilization/ Other (optional)
OP	Storage Solution	Open text (Optional)
OP	Preservation	Not specified/default / Cryopreserved / Dehydrated / Freeze dried / Frozen / Glycerol (high conc) / Refrigerated / Solvent dehydrated (optional)
OP	Other Info: (Storage Temperature; Storage requirements after issue and/or Shelf life from donation/after issue)	Open text (Optional)

OP	Update (innovation and changes)	Open text (Optional)
M	Date of authorization of process and/or product	Open text (Optional)

Table 7.4. **Contents used to describe clinical indications and associated information in the T&C Database**

	Field Name	Description
M	Classification of Diseases	ECode (1 letter + 2 digits) –Optional (https://icd.who.int/browse10/2019/en)
OP	Supplementary information – Clinical Indications	Open text – details of clinical indication Users may choose to follow ICD10 detailed classification: Optional (https://icd.who.int/browse10/2019/en)
M	Level of Risk – IAT Level	AResult given by EuroGTP II IAT – Evaluation made by TEs Select from: Negligible/low; moderate; high; Not performed (authorised prior to EuroGTP II)
M	Risk Assessment Date	When was the risk assessment performed – DD/MM/ YYYY
OP	Bibliographic References	Open text, allows to add links or/and references
OP	Notify references	Relevant Codes of Notify Library or Links

7.6. Codes used

- EU TE ID codes and Product ID Code –SEC Platform
- Classification of Diseases – http://apps.who.int/classifications/icd10/browse/2016/en
- Notify Library

7.7. Structure of data

Please refer to figure 7.1.

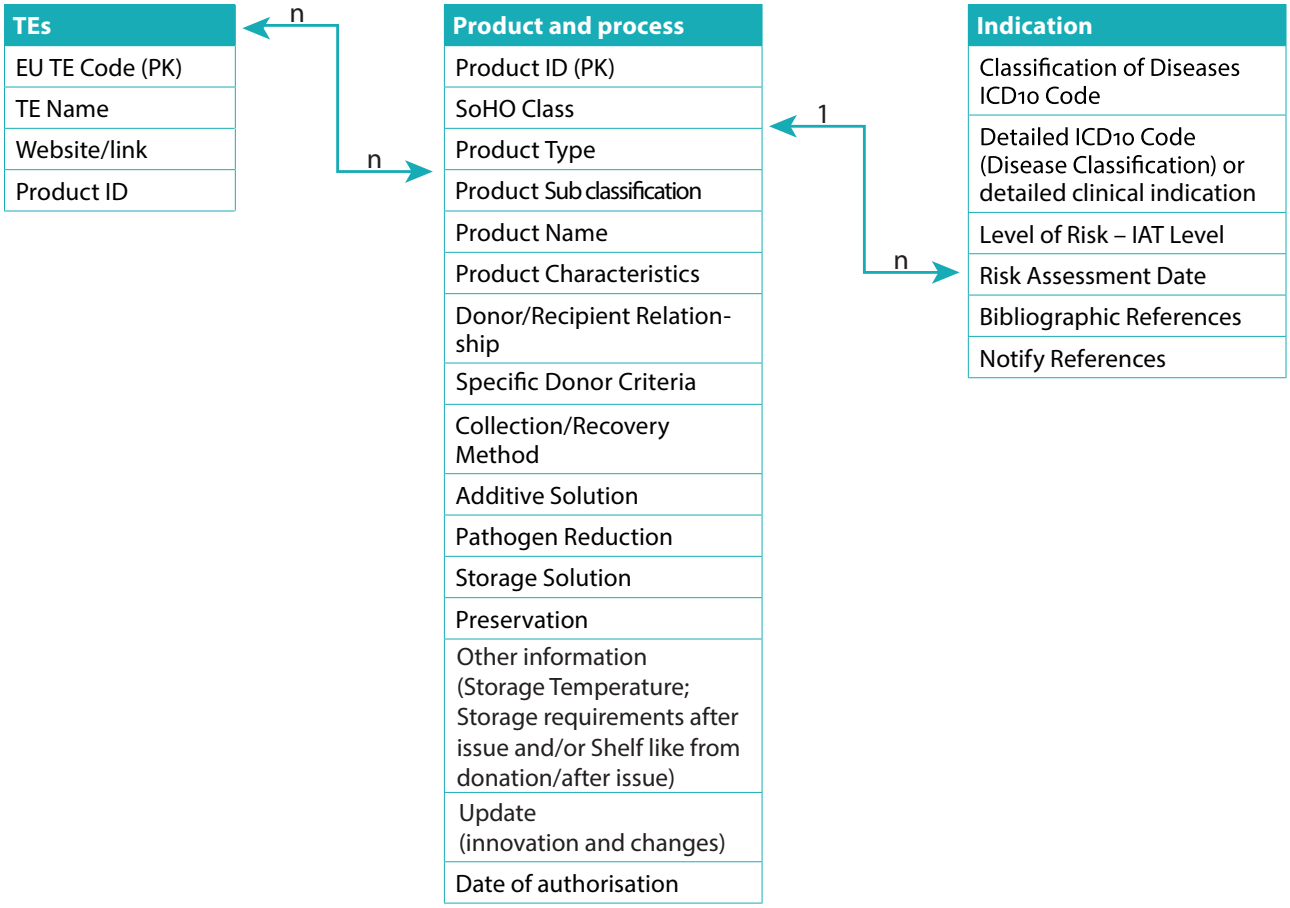

Figure 7.1. **Database scheme/entity relationship diagram**

7.8. Searches and practical use of the database

As mentioned above, the database may be used to search for products and therapies made available by different TEs in Europe.

In principle, one TE can register several different BTCs, and the same BTCs can be prepared and distributed by several different TEs in Europe.

Different users may find it useful to perform different searches depending on their interests and goals. Examples:

- TEs may want to know who in Europe is preparing a particular BTC in order to establish a collaboration or gather scientific references;
- When CAs intend to search for references for BTCs previously authorised in other member states, but implemented for the first time by national TEs;
- Surgeons may search for new BTC options to treat specific pathologies; these examples were used to validate the functionality of the *T&C Database*.

Definitions*

***Unless stated otherwise, the definitions in this guide follow the definitions of EUTCD[2, 4-7] or proposed in the 5th Edition of the EDQM/Council of Europe Guide to the Quality and Safety of Tissues and Cells for Human Application[12], prior EU funded projects[1, 10, 11], or are new definitions proposed by the EuroGTP II project.**

ADVERSE EVENT: any untoward occurrence associated with the procurement, 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.)

ALLOGENEIC: refers to cells and tissues donated by one person for clinical application to another person.

ALLOGRAFT: tissues or cells transplanted between two genetically different individuals of the same species.

APHERESIS: medical technique in which peripheral blood from a donor or patient is passed through an apparatus that separates out one particular constituent.

ASSISTED REPRODUCTIVE TECHNOLOGIES (ART) OR MEDICALLY ASSISTED REPRODUCTION (MAR): all treatments or procedures that include the *in vitro* handling of human oocytes, spermatozoa or embryos for establishing a pregnancy.

AUTOLOGOUS: cells or tissues removed from and applied in the same person. In ART/MAR, the terms 'autologous donors' and 'autologous use' apply to cases of preservation of fertility.

BEST PRACTICE: a method or technique that has consistently shown results superior to those achieved with other means, and that is used as a benchmark.

BTC PREPARATION: a particular type of BTC that:

a. has been subjected to one or more BTC activities, including processing, in accordance with defined quality and safety parameters;
b. meets a predefined specification; and
c. is intended for application to a recipient for a specific clinical indication or is intended for distribution for manufacture of a product regulated by other European Union legislation, or as the starting and raw material thereof.

CELLS: individual human cells or a collection of human cells when not bound by any form of connective tissue.

CLINICAL AUDIT: *a process for monitoring standards of clinical care to see if it is being carried out in the best way possible (known as 'best practice'). Clinical audit can be described as a systematic 'cycle'. It involves measuring care against specific criteria, taking action to improve it if necessary, and monitoring the process to sustain improvement.*[27] (In the context of

this guide, clinical audit refers to retrospective or prospective evaluation of routinely collected clinical data.)

CLINICAL DATA: information concerning safety or performance that is generated from the use of a T&C product and is sourced from the following: clinical investigation(s) of the T&C product concerned, clinical investigation(s) or other studies reported in scientific literature of a T&C product for which equivalence to the T&C product in question can be demonstrated, reports published in peer-reviewed scientific literature on other clinical experience of either the T&C product in question or a T&C product for which equivalence to the T&C product in question can be demonstrated, clinically *relevant information from post-application surveillance, in particular the clinical follow-up (definition adapted from Regulation (EU) 2017/745 27).*

CLINICAL EVALUATION/FOLLOW-UP STUDY: for the purposes of this document, this term refers to *monitoring predefined clinical outcome indicators to evaluate quality, safety and efficacy/effectiveness of the BTC for a predefined number of patients.*

CLINICAL EVIDENCE: *clinical data and clinical evaluation results pertaining to a device of a sufficient amount and quality to allow a qualified assessment of whether the device is safe and achieves the intended clinical benefit(s), when used as intended by the manufacturer*[27].

COMPETENT AUTHORITY (CA): organisation designated by an EU member state as responsible for implementing the requirements of Directive 2004/23/EC.

CONTAMINATION: accidental inclusion or growth of harmful micro-organisms, such as bacteria, yeast, mould, fungi, viruses, prions, protozoa or their toxins and by-products. Contamination is different from colonisation, which is the natural, biological presence of micro-organisms.

CORD BLOOD BANK: a specific type of TE where HPCs collected from the placental and umbilical cord blood vessels are processed, cryopreserved and/or stored. It may also be responsible for procurement, testing or distribution.

CRITICAL: potentially having an effect on the quality and/or safety of or having direct contact with the cells and tissues.

CROSS CONTAMINATION: transfer of micro-organisms from one material to another.

CRYOPRESERVATION: preservation and storage of viable tissues and cells (including gametes and embryos) to preserve viability, either by freezing or vitrification, or alternatively (to extend their viable life) by low-temperature storage.

CRYOPROTECTANT: a chemical compound that is able to protect cells and tissues against freezing injury. Also used as a compatible solute tolerated in high concentrations by cells and tissues for cryopreservation by vitrification.

DECEASED DONOR: a person declared to be dead according to established medical criteria and from whom cells, tissues or organs have been recovered for the purpose of human application.

DECONTAMINATION: the process of removing or neutralising contaminants.

DISTRIBUTION: transportation and delivery of tissues or cells intended for human application.

DONATION: donating human tissues or cells intended for human applications.

DONOR: every human source, whether living or deceased, of human cells or tissues.

EFFICACY/EFFECTIVENESS: presence of desired (clinical) effects depending on the mode of action of the product.

EMBRYO: pre-implantation reproductive tissue resulting from the combination of oocyte and sperm.

END USER: a healthcare practitioner who undertakes human application procedures.

ETHICS COMMITTEE: "an independent body established in a Member State in accordance with the law of that Member State and empowered to give opinions for the purposes of this Regulation, taking into account the views of laypersons, in particular patients or patients' organisations."[27]

FINAL PRODUCT: any tissue or cell preparation intended to be transplanted or administered after the final release step.

FOLLOW-UP: subsequent examinations of a patient, living donor or recipient, for the purpose of monitoring the results of the donation or transplantation, care maintenance and initiating post-donation or post-transplantation interventions.

GAMETE: mature human germ cell, whether oocyte or sperm.

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.

HAEMATOPOIETIC PROGENITOR CELLS (HPC): 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.

HUMAN APPLICATION: the use of tissues or cells on or in a human recipient and extracorporeal applications.

IMPLANTATION/GRAFTING: the process of inserting a piece of tissue or cells into a recipient.

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.

NON-PARTNER DONATION: means that the donor is another person apart from the couple (third party donation).

NOVELTY: any change that could significantly affect the quality and/or safety of the BTC and/or the safety of recipients.

ORGANISATION RESPONSIBLE FOR HUMAN APPLICATION (ORHA): a healthcare establishment or unit of a hospital or another body that carries out human application of human tissues or cells.

PACKAGING: containers or coverings, including primary and secondary packaging, used to protect tissues and cells and to present them to the operator (starting or in-process packaging) or to the clinical user (final packaging) in a suitable manner.

PARTNER DONATION: the donation of reproductive cells between a man and a woman who declare that they have an intimate physical relationship.

PATIENT: in MAR, relates to individuals or couples seeking treatment.

PRESERVATION: the use of chemical agents, alterations in environmental conditions or other means during processing to prevent or retard biological or physical deterioration of cells or tissues.

PROCESS: a series of related actions to achieve a defined outcome.

PROCESSING: all operations involved in the preparation, manipulation, preservation and packaging of tissues or cells intended for human applications.

PROCUREMENT ORGANISATION: a healthcare establishment or unit of a hospital or another body that undertakes the procurement of human tissues and cells and that may not be accredited, designated, authorised or licensed as a TE.

PROCUREMENT: a process by which tissues or cells are made available.

QUALIFICATION: according to EU GMP, the action of proving that any equipment works correctly and actually leads to the expected results. More generally, qualification is applied to the inputs to a process, i.e. equipment, facilities, materials and software (and their suppliers), as well as operators and the relevant written procedures.

QUALITY: totality of characteristics of an entity that bear on its ability to satisfy stated and implied needs. Consistent and reliable performance of services or products in conformity with specified standards.

RANDOMISED CONTROL TRIAL (RCT): 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.

RECIPIENT: person to whom human tissues, cells or embryos are applied.

RECOVERY OR RETRIEVAL: the procedure of removing cells, tissues or organs from a donor for the purpose of transplantation or assisted reproduction.

REPRODUCTIVE CELLS: all tissues and cells intended to be used for the purpose of assisted reproduction.

RISK ASSESSMENT: identification of potential hazards with an estimation of the likelihood that they will cause harm and of the severity of the harm should it occur.

SAFETY: relative risk; proportional difference from a suggested baseline value.

SERIOUS ADVERSE EVENT (SAE): any untoward occurrence associated with the procurement, testing, processing, storage and distribution of tissues and cells that may lead to the transmission of a communicable disease, to death or life-threatening, disabling or incapacitating conditions for the patient or which may result in, or prolong, hospitalisation or morbidity. In addition, the definition of SAE includes the

total loss of germinal tissues, gametes or embryos for one cycle and any mix-up of gametes or embryos.

SERIOUS ADVERSE REACTION (SAR): 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. The definition of SAR should be extended to the offspring in the case of non-partner donation, only for cases of transmission of genetic diseases.

SEVERITY: Directive 2006/86/EC defines serious as: fatal, life-threatening, disabling, incapacitating or which results in, or prolongs, hospitalisation or morbidity. EuroGTP II project follows the grading system for severity that has been agreed and is presented in the SoHO V&S project[10].

SINGLE ARM STUDY/TRIAL: sample of individuals with the targeted medical condition is given the experimental therapy and then followed over time to observe their response [28].

STORAGE: maintaining the tissues and cells under appropriate controlled conditions until distribution.

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.

T&C SUPPLY CHAIN: *The sequence of processes and activities involved in the donation, procurement/retrieval, processing, testing, transport, preservation, storage, distribution and application of T&C.*

TISSUE ESTABLISHMENT (TE): a tissue bank or a unit of a hospital or another body where activities of processing, preservation, storage or distribution of human tissues and cells are undertaken. It may also be responsible for procurement or testing of tissues and cells. In the field of MAR, TE applies to establishments performing MAR activities: MAR centres, MAR laboratories, sperm banks, etc.

TISSUE: all constituent parts of the human body formed by cells; an aggregate of cells joined together by, for example, connective structures which perform the same particular function, e.g. ovarian tissue.

TOXICITY: degree to which a substance can damage an organism.

TRANSPLANTATION: the transfer (engraftment) of human cells, tissues or organs from a donor to a recipient with the aim of restoring function(s) in the body.

TRANSPORT: to transfer or convey tissues and cells from one place to another.

VALIDATION: establishing documented evidence that provides a high degree of assurance that a specific process, piece of equipment or environment will consistently produce a product meeting its predetermined specifications and quality attributes; a process is validated to evaluate the performance of a system with regard to its effectiveness based on intended use.

VIGILANCE: an alertness or awareness of serious adverse events, serious adverse reactions or complications related to donation and clinical application of cells, tissues and organs involving an established process at a local, regional, national or international level for reporting.

Bibliography

1. [VISTART] [676969]; Vigilance and Inspection for the Safety of Transfusion, A. R. and T. Principles for Competent Authorities for the evaluation and approval of clinical follow up protocols for blood, tissues and cells prepared with newly developed and validated processing methodologies. 2018 [available at https://www.ec.europa.eu/research/participants/documents/downloadPublic?documentIds=080166e5bb34e80a&appId=PPGMS, accessed 15 Sep 2023].
2. Directive 2004/23/EC of the European Parliament and of the Council of 31 March 2004 on setting standards of quality and safety for the donation, procurement, testing, processing, preservation, storage and distribution of human tissues and cells. 2004.
3. Commission Directive 2012/39/EU of 26 November 2012 amending Directive 2006/17/EC as regards certain technical requirements for the testing of human tissues and cells. 2012.
4. Commission Directive 2006/86/EC of 24 October 2006, implementing Directive 2004/23/EC of the European Parliament and of the Council as regards traceability requirements, notification of serious adverse reactions and events and certain technical requirements for the coding, processing, preservation, storage and distribution of human tissues and cells. 2006.
5. Commission Directive 2006/17/EC implementing Directive 2004/23/EC of the European Parliament and of the Council as regards certain technical requirements for the donation, procurement and testing of human tissues and cells. 2006.
6. Commission Directive (EU) 2015/566 of 8 April 2015 implementing Directive 2004/23/EC as regards the procedures for verifying the equivalent standards of quality and safety of imported tissues and cells. 2015.
7. Commission Directive (EU) 2015/565 of 8 April 2015 amending Directive 2006/86/EC as regards certain technical requirements for the coding of human tissues and cells. 2015.
8. EuroGTP Project, Euro GTP Guidance (Project 2007207). 2011.
9. ARTHIQS -Assisted Reproductive Technologies and Haematopoietic stem cells Improvements for Quality and Safety throughout Europe, Deliverable 9 "Guide of Recommendations for Cord Blood Banking".
10. Vigilance and Surveillance of Substances of Human Origin (SOHO V&S), (Project Number: 20091110), SOHO V&S Guidance for Competent Authorities: Communication and investigation of serious adverse events and reactions associated with human tissues and cells. 2013 [available at https://www.notifylibrary.org/sites/default/files/SOHO%20V%26S%20Communication%20and%20Investigation%20Guidance.pdf, accessed 15 Sep 2023].
11. European Union Standards and Training for the Inspection of Tissues Establishments (Eustite) - Project 2005204. 2011 [available at https://www.notifylibrary.org/content/european-union-standards-and-training-inspection-tissue-establishments-project-eustite, accessed 15 Sep 2023].
12. EDQM, Council of Europe. Guide to the Quality and Safety of Tissues and Cells for Human Application. 5th Edition. Strasbourg, France: EDQM, Council of Europe; 2022.
13. FACT-JACIE, International Standards for Hematopoietic Cellular Therapy Product Collection, Processing, and Administration. 2015.
14. ESHRE. Guideline of the European Society of Human Reproduction and Embryology - Revised guidelines for good practice in IVF laboratories. 2015 [available at

https://www.eshre.eu/Guidelines-and-Legal/Guidelines/Revised-guidelines-for-good-practice-in-IVF-laboratories-(2015), accessed 15 Sep 2023].
15. ICH E6: Good Clinical Practice: Consolidated guideline. Good Clinical Practice. *Good Clin Pract* 50 (1997).
16. World Medical Association. WMA Declaration of Helsinki – Ethical Principles for Scientific Requirements and Research Protocols. 29–32. 2013 [available at https://www.wma.net/policies-post/wma-declaration-of-helsinki-ethical-principles-for-medical-research-involving-human-subjects/, accessed 15 Sep 2023].
17. (NICE). Developing NICE guidelines: the Manual. 2014 [available at https://www.nice.org.uk/media/default/about/what-we-do/our-programmes/developing-nice-guidelines-the-manual.pdf, accessed 15 Sep 2023].
18. Shea BJ, Hamel C, Wells GA *et al.* AMSTAR is a reliable and valid measurement tool to assess the methodological quality of systematic reviews. *J Clin Epidemiol* 2009;62:1013-20.
19. Sterne JA, Hernán MA, Reeves BC *et al.* ROBINS-I: A tool for assessing risk of bias in non-randomised studies of interventions. *BMJ* 2016;355:i4919.
20. Cochrane Handbook for Systematic Reviews of Interventions. 2011
21. GRADE Working Group. GRADE Handbook. 2013 [available at https://gdt.gradepro.org/app/handbook/handbook.html, accessed 15 Sep 2023].
22. Yepes-Nunez JJ, Morgan RL, Mbuagbaw L *et al.* Two alternatives versus the standard Grading of Recommendations Assessment, Development and Evaluation (GRADE) summary of findings (Of) tables to improve understanding in the presentation of systematic review results: a three-arm, randomised, controlled, non-inferiority trial. *BMJ Open* 2018;8:e015623.
23. European Society for Blood and Marrow Transplantation. The EBMT Patient Registry [available at: https://www.ebmt.org/ebmt-patient-registry, accessed: 7 Jan 2019].
24. Provoost V, Tilleman K, D'Angelo A *et al.* Beyond the dichotomy: a tool for distinguishing between experimental, innovative and established treatment. *Hum Reprod* 2014;29:413-17.
25. Alpha Scientists in Reproductive Medicine. The Alpha consensus meeting on cryopreservation key performance indicators and benchmarks: proceedings of an expert meeting. *Reprod Biomed Online* 2012;25:146-67.
26. ESHRE Special Interest Group of Embryology and Alpha Scientists in Reproductive Medicine. The Vienna consensus: report of an expert meeting on the development of ART laboratory performance indicators. *Reprod Biomed Online* 2017;35:494-510.
27. European Parliament & Council of the European Union. Regulation (EU) 2017/745 of the European Parliament and of the Council of 5 April 2017 on medical devices. *Off J Eur Union* 2017;60:1-175.
28. Evans SR. Clinical trial structures. *J Exp Stroke Transl Med* 2010;3:8-18.

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Annex II. Template form: characterisation of BTCs

Name of BTC	
Brief summary	Highlight the significant proprieties of the BTC and/or clinical applications under study and the main differences from related SoHO products. Justification for the implementation of change, including the key benefits of the innovation.
BTC characterisation	Main characteristics/critical attributes of the BTC. Example: How is the BTC processed? What, if any, changes have been made to the established preparation or treatment protocol? (e.g. number of cells, structural characteristics). What is the origin of the BTC (autologous/allogeneic)? What excipients or other reagents or residues could be transplanted with the BTC (such as carriers or preservatives). Add a description of novelty (if applicable).
Clinical application	In what format is the BTC presented for clinical application? (e.g. need to add solutions, cut.) What, if any, excipients or other reagents or residues could be transferred through the clinical application with the BTC (such as carriers or preservatives)?
Identification of risks	Description of the possible risks and adverse reactions anticipated based on prior experience and risk assessment.
Clinical indication(s) of the BTC	
Minimal follow-up data required to assess safety and efficacy	
SARE monitoring and report	Communication procedures with the TEs
Information on prior preclinical evaluations	Brief description of tests and results obtained in validation studies and quality controls, performed before issuing the novel BTC for clinical application.
Date	

Annex III. Template forms: methodologies for assessing the risks associated with novel BTCs

Co-funded by the European Union
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Co-funded and implemented by the Council of Europe

Methodologies for Assessing the Risks
associated to novel Blood, Tissue and Cell Component (BTCs).
-Replacement Tissues Template-

Please follow the EuroGTP II Guide in order to correctly evaluate your BTCs

BTC characterisation

The evaluation of the level of novelty and the risks associated, should start with a characterisation of the novel process or BTC.

General category: Replacement Tissues

Specific category:

- ☐ Musculoskeletal
- ☐ Cardiovascular
- ☐ Amniotic Membrane
- ☐ Ocular
- ☐ Skin
- ☐ Other

Name of the product, therapy or process under evaluation:

Description of BTC under evaluation:
(Describe the relevant aspects of the BTC, detailing the modifications/novelties associated with **donation**, **processing** and **clinical application** under evaluation)

Step 1 – Evaluation of novelty

The purpose of this exercise is to evaluate our proposed methodology for determining if a BTC, therapy or process is novel or not.
Please answer the following questions in order to determine if the product or process is novel. This process represents the first stage of the overall procedure for evaluating novelty and risk.

	Yes	No	Not Applicable/ Not Relevant
A. Has this type of BTC previously been collected, processed/ prepared and issued for clinical use by your establishment?			
Comment (optional):			
B. Will the starting material used to prepare this BTC be obtained from the same donor population previously used by your establishment for this type of BTC?			
Comment (optional):			
C. Will the starting material for this BTC be procured/collected using a procedure used previously by your establishment for this type of BTC?			
Comment (optional):			
D. Will this BTC be prepared by a procedure (processing/preparation, decontamination/pathogen reduction and preservation) used previously in your establishment for this type of BTC?			
Comment (optional):			
E. Will this BTC be packaged, stored, and distributed using a protocol and materials used previously in your establishment for this type of BTC?			
Comment (optional):			
F. Will this type of BTC provided by your establishment be applied/infused clinically using an application/transfusion/infusion method used previously?			
Comment (optional):			
G. Has your establishment provided this type of BTC for the same clinical indication or for application/transfusion/infusion into the same anatomical site?			
Comment (optional):			

Step 2 – Level of risk analysis

Novelties represent different risks with distinct impact in the quality and safety of the products.

Select the specific risks that apply to this risk Factor (note that some risk factors may not apply to your product/therapy).

Risk Factor: Donor characteristics

Consider whether the donor population you intend to obtain the BTC from could impart any risk, for example if the BTC is sourced from an allogeneic donor, there may be risks that immunogenicity could impact on the clinical performance of the BTC, and risks of disease transmission.

Applicable Yes ☐ No ☐

Comments and/or references (optional):

Specific Risks					
Unexpected immunogenicity				**Applicable** ☐	**NA** ☐
Probability	1- Rare ☐	2- Unlikely ☐	3- Possible ☐	4- Likely ☐	5- Almost certain ☐
Severity		1- Non Serious ☐	2- Serious ☐	3- Life-Threatening ☐	4- Fatal ☐
Detectability	1- Very High ☐	2- Moderately high ☐	3- Low ☐	4- Very Low ☐	5- Cannot be detected ☐
Risk Reduction	None (0%) ☐	Limited (25%) ☐	Moderate (50%) ☐	Substantial (75%) ☐	Extensive (95%) ☐
Implant failure				**Applicable** ☐	**NA** ☐
Probability	1- Rare ☐	2- Unlikely ☐	3- Possible ☐	4- Likely ☐	5- Almost certain ☐
Severity		1- Non Serious ☐	2- Serious ☐	3- Life-Threatening ☐	4- Fatal ☐
Detectability	1- Very High ☐	2- Moderately high ☐	3- Low ☐	4- Very Low ☐	5- Cannot be detected ☐
Risk Reduction	None (0%) ☐	Limited (25%) ☐	Moderate (50%) ☐	Substantial (75%) ☐	Extensive (95%) ☐
Disease transmission				**Applicable** ☐	**NA** ☐
Probability	1- Rare ☐	2- Unlikely ☐	3- Possible ☐	4- Likely ☐	5- Almost certain ☐
Severity		1- Non Serious ☐	2- Serious ☐	3- Life-Threatening ☐	4- Fatal ☐
Detectability	1- Very High ☐	2- Moderately high ☐	3- Low ☐	4- Very Low ☐	5- Cannot be detected ☐
Risk Reduction	None (0%) ☐	Limited (25%) ☐	Moderate (50%) ☐	Substantial (75%) ☐	Extensive (95%) ☐
Toxicity / Carcinogenicity				**Applicable** ☐	**NA** ☐
Probability	1- Rare ☐	2- Unlikely ☐	3- Possible ☐	4- Likely ☐	5- Almost certain ☐
Severity		1- Non Serious ☐	2- Serious ☐	3- Life-Threatening ☐	4- Fatal ☐
Detectability	1- Very High ☐	2- Moderately high ☐	3- Low ☐	4- Very Low ☐	5- Cannot be detected ☐
Risk Reduction	None (0%) ☐	Limited (25%) ☐	Moderate (50%) ☐	Substantial (75%) ☐	Extensive (95%) ☐
Other (_____)				**Applicable** ☐	**NA** ☐
Probability	1- Rare ☐	2- Unlikely ☐	3- Possible ☐	4- Likely ☐	5- Almost certain ☐
Severity		1- Non Serious ☐	2- Serious ☐	3- Life-Threatening ☐	4- Death ☐
Detectability	1- Very High ☐	2- Moderately high ☐	3- Low ☐	4- Very Low ☐	5- Cannot be detected ☐
Risk Reduction	None (0%) ☐	Limited (25%) ☐	Moderate (50%) ☐	Substantial (75%) ☐	Extensive (95%) ☐

-Replacement Tissues Template-

Step 2

Novelties represent different risks with distinct impact in the quality and safety of the products.

Select the specific risks that apply to this risk Factor (note that some risk factors may not apply to your product/therapy).

Risk Factor: Procurement/collection process and environment

Consider where and how the BTC is collected, procured or recovered, and if this process could have an influence on the BTC. How long does the process take, how complex is it, and what is the quality of the environment - for example, these factors may impact on the probability that the BTC becomes contaminated, or damaged during recovery

Applicable Yes ☐ No ☐

Comments and/or references (optional):

Risks					
Unexpected immunogenicity				**Applicable** ☐	**NA** ☐
Probability	1- Rare ☐	2- Unlikely ☐	3- Possible ☐	4- Likely ☐	5- Almost certain ☐
Severity		1- Non Serious ☐	2- Serious ☐	3- Life-Threatening ☐	4- Fatal ☐
Detectability	1- Very High ☐	2- Moderately high ☐	3- Low ☐	4- Very Low ☐	5- Cannot be detected ☐
Risk Reduction	None (0%) ☐	Limited (25%) ☐	Moderate (50%) ☐	Substantial (75%) ☐	Extensive (95%) ☐
Implant failure				**Applicable** ☐	**NA** ☐
Probability	1- Rare ☐	2- Unlikely ☐	3- Possible ☐	4- Likely ☐	5- Almost certain ☐
Severity		1- Non Serious ☐	2- Serious ☐	3- Life-Threatening ☐	4- Fatal ☐
Detectability	1- Very High ☐	2- Moderately high ☐	3- Low ☐	4- Very Low ☐	5- Cannot be detected ☐
Risk Reduction	None (0%) ☐	Limited (25%) ☐	Moderate (50%) ☐	Substantial (75%) ☐	Extensive (95%) ☐
Disease transmission				**Applicable** ☐	**NA** ☐
Probability	1- Rare ☐	2- Unlikely ☐	3- Possible ☐	4- Likely ☐	5- Almost certain ☐
Severity		1- Non Serious ☐	2- Serious ☐	3- Life-Threatening ☐	4- Fatal ☐
Detectability	1- Very High ☐	2- Moderately high ☐	3- Low ☐	4- Very Low ☐	5- Cannot be detected ☐
Risk Reduction	None (0%) ☐	Limited (25%) ☐	Moderate (50%) ☐	Substantial (75%) ☐	Extensive (95%) ☐
Toxicity / Carcinogenicity				**Applicable** ☐	**NA** ☐
Probability	1- Rare ☐	2- Unlikely ☐	3- Possible ☐	4- Likely ☐	5- Almost certain ☐
Severity		1- Non Serious ☐	2- Serious ☐	3- Life-Threatening ☐	4- Fatal ☐
Detectability	1- Very High ☐	2- Moderately high ☐	3- Low ☐	4- Very Low ☐	5- Cannot be detected ☐
Risk Reduction	None (0%) ☐	Limited (25%) ☐	Moderate (50%) ☐	Substantial (75%) ☐	Extensive (95%) ☐
Other (_____)				**Applicable** ☐	**NA** ☐
Probability	1- Rare ☐	2- Unlikely ☐	3- Possible ☐	4- Likely ☐	5- Almost certain ☐
Severity		1- Non Serious ☐	2- Serious ☐	3- Life-Threatening ☐	4- Fatal ☐
Detectability	1- Very High ☐	2- Moderately high ☐	3- Low ☐	4- Very Low ☐	5- Cannot be detected ☐
Risk Reduction	None (0%) ☐	Limited (25%) ☐	Moderate (50%) ☐	Substantial (75%) ☐	Extensive (95%) ☐

Step 2

Novelties represent different risks with distinct impact in the quality and safety of the products.

Select the specific risks that apply to this risk Factor (note that some risk factors may not apply to your product/therapy).

Risk Factor: Processing and environment

Consider where and how the BTC is prepared. How long does the preparation process take and how complex is it – this may impact on the risk of contamination, or that it may not be prepared to consistent specifications and quality. Also consider the quality of the preparation environment, which may also affect the risk of contamination.

Applicable **Yes** ☐ **No** ☐

Comments and/or references (optional):

Specific Risks					
Unexpected immunogenicity				**Applicable** ☐	**NA** ☐
Probability	1- Rare ☐	2- Unlikely ☐	3- Possible ☐	4- Likely ☐	5- Almost certain ☐
Severity		1- Non Serious ☐	2- Serious ☐	3- Life-Threatening ☐	4- Fatal ☐
Detectability	1- Very High ☐	2- Moderately high ☐	3- Low ☐	4- Very Low ☐	5- Cannot be detected ☐
Risk Reduction	None (0%) ☐	Limited (25%) ☐	Moderate (50%) ☐	Substantial (75%) ☐	Extensive (95%) ☐
Implant failure				**Applicable** ☐	**NA** ☐
Probability	1- Rare ☐	2- Unlikely ☐	3- Possible ☐	4- Likely ☐	5- Almost certain ☐
Severity		1- Non Serious ☐	2- Serious ☐	3- Life-Threatening ☐	4- Fatal ☐
Detectability	1- Very High ☐	2- Moderately high ☐	3- Low ☐	4- Very Low ☐	5- Cannot be detected ☐
Risk Reduction	None (0%) ☐	Limited (25%) ☐	Moderate (50%) ☐	Substantial (75%) ☐	Extensive (95%) ☐
Disease transmission				**Applicable** ☐	**NA** ☐
Probability	1- Rare ☐	2- Unlikely ☐	3- Possible ☐	4- Likely ☐	5- Almost certain ☐
Severity		1- Non Serious ☐	2- Serious ☐	3- Life-Threatening ☐	4- Fatal ☐
Detectability	1- Very High ☐	2- Moderately high ☐	3- Low ☐	4- Very Low ☐	5- Cannot be detected ☐
Risk Reduction	None (0%) ☐	Limited (25%) ☐	Moderate (50%) ☐	Substantial (75%) ☐	Extensive (95%) ☐
Toxicity / Carcinogenicity				**Applicable** ☐	**NA** ☐
Probability	1- Rare ☐	2- Unlikely ☐	3- Possible ☐	4- Likely ☐	5- Almost certain ☐
Severity		1- Non Serious ☐	2- Serious ☐	3- Life-Threatening ☐	4- Fatal ☐
Detectability	1- Very High ☐	2- Moderately high ☐	3- Low ☐	4- Very Low ☐	5- Cannot be detected ☐
Risk Reduction	None (0%) ☐	Limited (25%) ☐	Moderate (50%) ☐	Substantial (75%) ☐	Extensive (95%) ☐
Other (_____)				**Applicable** ☐	**NA** ☐
Probability	1- Rare ☐	2- Unlikely ☐	3- Possible ☐	4- Likely ☐	5- Almost certain ☐
Severity		1- Non Serious ☐	2- Serious ☐	3- Life-Threatening ☐	4- Fatal ☐
Detectability	1- Very High ☐	2- Moderately high ☐	3- Low ☐	4- Very Low ☐	5- Cannot be detected ☐
Risk Reduction	None (0%) ☐	Limited (25%) ☐	Moderate (50%) ☐	Substantial (75%) ☐	Extensive (95%) ☐

-Replacement Tissues Template-

Step 2

Novelties represent different risks with distinct impact in the quality and safety of the products.

Select the specific risks that apply to this risk Factor (note that some risk factors may not apply to your product/therapy).

Risk Factor: Reagents/added components

Consider any reagents used during preparation, decontamination, preservation, storage and transport of the BTC. Could they damage the BTC in any way, or could residual traces of reagent remain in the BTC that could cause toxic or immunogenic effects in recipients?

Applicable **Yes** ☐ **No** ☐

Comments and/or references (optional):

Risks					
Unexpected immunogenicity				**Applicable** ☐	**NA** ☐
Probability	1- Rare ☐	2- Unlikely ☐	3- Possible ☐	4- Likely ☐	5- Almost certain ☐
Severity		1- Non Serious ☐	2- Serious ☐	3- Life-Threatening ☐	4- Fatal ☐
Detectability	1- Very High ☐	2- Moderately high ☐	3- Low ☐	4- Very Low ☐	5- Cannot be detected ☐
Risk Reduction	None (0%) ☐	Limited (25%) ☐	Moderate (50%) ☐	Substantial (75%) ☐	Extensive (95%) ☐
Implant failure				**Applicable** ☐	**NA** ☐
Probability	1- Rare ☐	2- Unlikely ☐	3- Possible ☐	4- Likely ☐	5- Almost certain ☐
Severity		1- Non Serious ☐	2- Serious ☐	3- Life-Threatening ☐	4- Fatal ☐
Detectability	1- Very High ☐	2- Moderately high ☐	3- Low ☐	4- Very Low ☐	5- Cannot be detected ☐
Risk Reduction	None (0%) ☐	Limited (25%) ☐	Moderate (50%) ☐	Substantial (75%) ☐	Extensive (95%) ☐
Disease transmission				**Applicable** ☐	**NA** ☐
Probability	1- Rare ☐	2- Unlikely ☐	3- Possible ☐	4- Likely ☐	5- Almost certain ☐
Severity		1- Non Serious ☐	2- Serious ☐	3- Life-Threatening ☐	4- Fatal ☐
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Risk Reduction	None (0%) ☐	Limited (25%) ☐	Moderate (50%) ☐	Substantial (75%) ☐	Extensive (95%) ☐
Toxicity / Carcinogenicity				**Applicable** ☐	**NA** ☐
Probability	1- Rare ☐	2- Unlikely ☐	3- Possible ☐	4- Likely ☐	5- Almost certain ☐
Severity		1- Non Serious ☐	2- Serious ☐	3- Life-Threatening ☐	4- Fatal ☐
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Other (_____)				**Applicable** ☐	**NA** ☐
Probability	1- Rare ☐	2- Unlikely ☐	3- Possible ☐	4- Likely ☐	5- Almost certain ☐
Severity		1- Non Serious ☐	2- Serious ☐	3- Life-Threatening ☐	4- Fatal ☐
Detectability	1- Very High ☐	2- Moderately high ☐	3- Low ☐	4- Very Low ☐	5- Cannot be detected ☐
Risk Reduction	None (0%) ☐	Limited (25%) ☐	Moderate (50%) ☐	Substantial (75%) ☐	Extensive (95%) ☐

Step 2

Novelties represent different risks with distinct impact in the quality and safety of the products.

Select the specific risks that apply to this risk Factor (note that some risk factors may not apply to your product/therapy).

Risk Factor: Reliability of Microbiology Testing

Consider the risk that the nature of the BTC, the testing methodology and/or the presence of residual processing reagents such as antibiotics in the finished BTC may impact the accuracy of any microbiology tests. Note, this refers specifically to bacteriology/mycology testing of the BTC, not any blood tests performed on the donor.

Applicable **Yes** ☐ **No** ☐

Comments and/or references (optional):

Risks					
Unexpected immunogenicity				**Applicable** ☐	**NA** ☐
Probability	1- Rare ☐	2- Unlikely ☐	3- Possible ☐	4- Likely ☐	5- Almost certain ☐
Severity		1- Non Serious ☐	2- Serious ☐	3- Life-Threatening ☐	4- Fatal ☐
Detectability	1- Very High ☐	2- Moderately high ☐	3- Low ☐	4- Very Low ☐	5- Cannot be detected ☐
Risk Reduction	None (0%) ☐	Limited (25%) ☐	Moderate (50%) ☐	Substantial (75%) ☐	Extensive (95%) ☐
Implant failure				**Applicable** ☐	**NA** ☐
Probability	1- Rare ☐	2- Unlikely ☐	3- Possible ☐	4- Likely ☐	5- Almost certain ☐
Severity		1- Non Serious ☐	2- Serious ☐	3- Life-Threatening ☐	4- Fatal ☐
Detectability	1- Very High ☐	2- Moderately high ☐	3- Low ☐	4- Very Low ☐	5- Cannot be detected ☐
Risk Reduction	None (0%) ☐	Limited (25%) ☐	Moderate (50%) ☐	Substantial (75%) ☐	Extensive (95%) ☐
Disease transmission				**Applicable** ☐	**NA** ☐
Probability	1- Rare ☐	2- Unlikely ☐	3- Possible ☐	4- Likely ☐	5- Almost certain ☐
Severity		1- Non Serious ☐	2- Serious ☐	3- Life-Threatening ☐	4- Fatal ☐
Detectability	1- Very High ☐	2- Moderately high ☐	3- Low ☐	4- Very Low ☐	5- Cannot be detected ☐
Risk Reduction	None (0%) ☐	Limited (25%) ☐	Moderate (50%) ☐	Substantial (75%) ☐	Extensive (95%) ☐
Toxicity / Carcinogenicity				**Applicable** ☐	**NA** ☐
Probability	1- Rare ☐	2- Unlikely ☐	3- Possible ☐	4- Likely ☐	5- Almost certain ☐
Severity		1- Non Serious ☐	2- Serious ☐	3- Life-Threatening ☐	4- Fatal ☐
Detectability	1- Very High ☐	2- Moderately high ☐	3- Low ☐	4- Very Low ☐	5- Cannot be detected ☐
Risk Reduction	None (0%) ☐	Limited (25%) ☐	Moderate (50%) ☐	Substantial (75%) ☐	Extensive (95%) ☐
Other (_____)				**Applicable** ☐	**NA** ☐
Probability	1- Rare ☐	2- Unlikely ☐	3- Possible ☐	4- Likely ☐	5- Almost certain ☐
Severity		1- Non Serious ☐	2- Serious ☐	3- Life-Threatening ☐	4- Fatal ☐
Detectability	1- Very High ☐	2- Moderately high ☐	3- Low ☐	4- Very Low ☐	5- Cannot be detected ☐
Risk Reduction	None (0%) ☐	Limited (25%) ☐	Moderate (50%) ☐	Substantial (75%) ☐	Extensive (95%) ☐

Step 2

Novelties represent different risks with distinct impact in the quality and safety of the products.

Select the specific risks that apply to this risk Factor (note that some risk factors may not apply to your product/therapy).

Risk Factor: Storage Conditions

Consider any potential risks arising from how the starting material and BTC are stored, between procurement and processing, during processing, and between processing and clinical application.

Applicable **Yes** ☐ **No** ☐

Comments and/or references (optional):

Risks					
Unexpected immunogenicity				**Applicable** ☐	**NA** ☐
Probability	1- Rare ☐	2- Unlikely ☐	3- Possible ☐	4- Likely ☐	5- Almost certain ☐
Severity		1- Non Serious ☐	2- Serious ☐	3- Life-Threatening ☐	4- Fatal ☐
Detectability	1- Very High ☐	2- Moderately high ☐	3- Low ☐	4- Very Low ☐	5- Cannot be detected ☐
Risk Reduction	None (0%) ☐	Limited (25%) ☐	Moderate (50%) ☐	Substantial (75%) ☐	Extensive (95%) ☐
Implant failure				**Applicable** ☐	**NA** ☐
Probability	1- Rare ☐	2- Unlikely ☐	3- Possible ☐	4- Likely ☐	5- Almost certain ☐
Severity		1- Non Serious ☐	2- Serious ☐	3- Life-Threatening ☐	4- Fatal ☐
Detectability	1- Very High ☐	2- Moderately high ☐	3- Low ☐	4- Very Low ☐	5- Cannot be detected ☐
Risk Reduction	None (0%) ☐	Limited (25%) ☐	Moderate (50%) ☐	Substantial (75%) ☐	Extensive (95%) ☐
Disease transmission				**Applicable** ☐	**NA** ☐
Probability	1- Rare ☐	2- Unlikely ☐	3- Possible ☐	4- Likely ☐	5- Almost certain ☐
Severity		1- Non Serious ☐	2- Serious ☐	3- Life-Threatening ☐	4- Fatal ☐
Detectability	1- Very High ☐	2- Moderately high ☐	3- Low ☐	4- Very Low ☐	5- Cannot be detected ☐
Risk Reduction	None (0%) ☐	Limited (25%) ☐	Moderate (50%) ☐	Substantial (75%) ☐	Extensive (95%) ☐
Toxicity / Carcinogenicity				**Applicable** ☐	**NA** ☐
Probability	1- Rare ☐	2- Unlikely ☐	3- Possible ☐	4- Likely ☐	5- Almost certain ☐
Severity		1- Non Serious ☐	2- Serious ☐	3- Life-Threatening ☐	4- Fatal ☐
Detectability	1- Very High ☐	2- Moderately high ☐	3- Low ☐	4- Very Low ☐	5- Cannot be detected ☐
Risk Reduction	None (0%) ☐	Limited (25%) ☐	Moderate (50%) ☐	Substantial (75%) ☐	Extensive (95%) ☐
Other (_____)				**Applicable** ☐	**NA** ☐
Probability	1- Rare ☐	2- Unlikely ☐	3- Possible ☐	4- Likely ☐	5- Almost certain ☐
Severity		1- Non Serious ☐	2- Serious ☐	3- Life-Threatening ☐	4- Fatal ☐
Detectability	1- Very High ☐	2- Moderately high ☐	3- Low ☐	4- Very Low ☐	5- Cannot be detected ☐
Risk Reduction	None (0%) ☐	Limited (25%) ☐	Moderate (50%) ☐	Substantial (75%) ☐	Extensive (95%) ☐

Step 2

Novelties represent different risks with distinct impact in the quality and safety of the products.

Select the specific risks that apply to this risk Factor (note that some risk factors may not apply to your product/therapy).

Risk Factor: Transport Conditions

Consider any potential risks arising from how the starting material and BTC are transported, for example between the sites procurement and processing, and between the sites of storage and clinical application.

Applicable Yes ☐ No ☐

Comments and/or references (optional):

Risks					
Unexpected immunogenicity				**Applicable** ☐	**NA** ☐
Probability	1- Rare ☐	2- Unlikely ☐	3- Possible ☐	4- Likely ☐	5- Almost certain ☐
Severity		1- Non Serious ☐	2- Serious ☐	3- Life-Threatening ☐	4- Fatal ☐
Detectability	1- Very High ☐	2- Moderately high ☐	3- Low ☐	4- Very Low ☐	5- Cannot be detected ☐
Risk Reduction	None (0%) ☐	Limited (25%) ☐	Moderate (50%) ☐	Substantial (75%) ☐	Extensive (95%) ☐
Implant failure				**Applicable** ☐	**NA** ☐
Probability	1- Rare ☐	2- Unlikely ☐	3- Possible ☐	4- Likely ☐	5- Almost certain ☐
Severity		1- Non Serious ☐	2- Serious ☐	3- Life-Threatening ☐	4- Fatal ☐
Detectability	1- Very High ☐	2- Moderately high ☐	3- Low ☐	4- Very Low ☐	5- Cannot be detected ☐
Risk Reduction	None (0%) ☐	Limited (25%) ☐	Moderate (50%) ☐	Substantial (75%) ☐	Extensive (95%) ☐
Disease transmission				**Applicable** ☐	**NA** ☐
Probability	1- Rare ☐	2- Unlikely ☐	3- Possible ☐	4- Likely ☐	5- Almost certain ☐
Severity		1- Non Serious ☐	2- Serious ☐	3- Life-Threatening ☐	4- Fatal ☐
Detectability	1- Very High ☐	2- Moderately high ☐	3- Low ☐	4- Very Low ☐	5- Cannot be detected ☐
Risk Reduction	None (0%) ☐	Limited (25%) ☐	Moderate (50%) ☐	Substantial (75%) ☐	Extensive (95%) ☐
Toxicity / Carcinogenicity				**Applicable** ☐	**NA** ☐
Probability	1- Rare ☐	2- Unlikely ☐	3- Possible ☐	4- Likely ☐	5- Almost certain ☐
Severity		1- Non Serious ☐	2- Serious ☐	3- Life-Threatening ☐	4- Fatal ☐
Detectability	1- Very High ☐	2- Moderately high ☐	3- Low ☐	4- Very Low ☐	5- Cannot be detected ☐
Risk Reduction	None (0%) ☐	Limited (25%) ☐	Moderate (50%) ☐	Substantial (75%) ☐	Extensive (95%) ☐
Other (_____)				**Applicable** ☐	**NA** ☐
Probability	1- Rare ☐	2- Unlikely ☐	3- Possible ☐	4- Likely ☐	5- Almost certain ☐
Severity		1- Non Serious ☐	2- Serious ☐	3- Life-Threatening ☐	4- Fatal ☐
Detectability	1- Very High ☐	2- Moderately high ☐	3- Low ☐	4- Very Low ☐	5- Cannot be detected ☐
Risk Reduction	None (0%) ☐	Limited (25%) ☐	Moderate (50%) ☐	Substantial (75%) ☐	Extensive (95%) ☐

-Replacement Tissues Template-

Step 2

Novelties represent different risks with distinct impact in the quality and safety of the products.

Select the specific risks that apply to this risk Factor (note that some risk factors may not apply to your product/therapy).

Risk Factor: Presence of unwanted cellular material and/or graft vascularity

This risk must be considered from the perspective that for some BTCs, the presence of intact vital cells is desirable, although it may also increase risks of, for example, immunogenicity or disease transmission.
This presence might affect to tumour formation, immunogenicity and disease transmission risks.
Vascular tissues may be more at risk to infiltration by pathogens or malignant cells than avascular tissues.

Applicable **Yes** ☐ **No** ☐

Comments and/or references (optional):

Risks					
Unexpected immunogenicity				**Applicable** ☐	**NA** ☐
Probability	1- Rare ☐	2- Unlikely ☐	3- Possible ☐	4- Likely ☐	5- Almost certain ☐
Severity		1- Non Serious ☐	2- Serious ☐	3- Life-Threatening ☐	4- Fatal ☐
Detectability	1- Very High ☐	2- Moderately high ☐	3- Low ☐	4- Very Low ☐	5- Cannot be detected ☐
Risk Reduction	None (0%) ☐	Limited (25%) ☐	Moderate (50%) ☐	Substantial (75%) ☐	Extensive (95%) ☐
Implant failure				**Applicable** ☐	**NA** ☐
Probability	1- Rare ☐	2- Unlikely ☐	3- Possible ☐	4- Likely ☐	5- Almost certain ☐
Severity		1- Non Serious ☐	2- Serious ☐	3- Life-Threatening ☐	4- Fatal ☐
Detectability	1- Very High ☐	2- Moderately high ☐	3- Low ☐	4- Very Low ☐	5- Cannot be detected ☐
Risk Reduction	None (0%) ☐	Limited (25%) ☐	Moderate (50%) ☐	Substantial (75%) ☐	Extensive (95%) ☐
Disease transmission				**Applicable** ☐	**NA** ☐
Probability	1- Rare ☐	2- Unlikely ☐	3- Possible ☐	4- Likely ☐	5- Almost certain ☐
Severity		1- Non Serious ☐	2- Serious ☐	3- Life-Threatening ☐	4- Fatal ☐
Detectability	1- Very High ☐	2- Moderately high ☐	3- Low ☐	4- Very Low ☐	5- Cannot be detected ☐
Risk Reduction	None (0%) ☐	Limited (25%) ☐	Moderate (50%) ☐	Substantial (75%) ☐	Extensive (95%) ☐
Toxicity / Carcinogenicity				**Applicable** ☐	**NA** ☐
Probability	1- Rare ☐	2- Unlikely ☐	3- Possible ☐	4- Likely ☐	5- Almost certain ☐
Severity		1- Non Serious ☐	2- Serious ☐	3- Life-Threatening ☐	4- Fatal ☐
Detectability	1- Very High ☐	2- Moderately high ☐	3- Low ☐	4- Very Low ☐	5- Cannot be detected ☐
Risk Reduction	None (0%) ☐	Limited (25%) ☐	Moderate (50%) ☐	Substantial (75%) ☐	Extensive (95%) ☐
Other (_____)				**Applicable** ☐	**NA** ☐
Probability	1- Rare ☐	2- Unlikely ☐	3- Possible ☐	4- Likely ☐	5- Almost certain ☐
Severity		1- Non Serious ☐	2- Serious ☐	3- Life-Threatening ☐	4- Fatal ☐
Detectability	1- Very High ☐	2- Moderately high ☐	3- Low ☐	4- Very Low ☐	5- Cannot be detected ☐
Risk Reduction	None (0%) ☐	Limited (25%) ☐	Moderate (50%) ☐	Substantial (75%) ☐	Extensive (95%) ☐

Step 2

Novelties represent different risks with distinct impact in the quality and safety of the products.

Select the specific risks that apply to this risk Factor (note that some risk factors may not apply to your product/therapy).

Risk Factor: Complexity of the immediate pre-implantation preparation and/or application method

Consider how complex the method of clinical application will be for this BTC. How long will it take, and could this introduce risks? What is the scope for errors to be made, and what could the consequences of these errors be?
Low feasibility of application standardization might have influence in the risks of implant failure and disease transmission at least.

Applicable **Yes** ☐ **No** ☐

Comments and/or references (optional):

Risks					
Unexpected immunogenicity				**Applicable** ☐	**NA** ☐
Probability	1- Rare ☐	2- Unlikely ☐	3- Possible ☐	4- Likely ☐	5- Almost certain ☐
Severity		1- Non Serious ☐	2- Serious ☐	3- Life-Threatening ☐	4- Fatal ☐
Detectability	1- Very High ☐	2- Moderately high ☐	3- Low ☐	4- Very Low ☐	5- Cannot be detected ☐
Risk Reduction	None (0%) ☐	Limited (25%) ☐	Moderate (50%) ☐	Substantial (75%) ☐	Extensive (95%) ☐
Implant failure				**Applicable** ☐	**NA** ☐
Probability	1- Rare ☐	2- Unlikely ☐	3- Possible ☐	4- Likely ☐	5- Almost certain ☐
Severity		1- Non Serious ☐	2- Serious ☐	3- Life-Threatening ☐	4- Fatal ☐
Detectability	1- Very High ☐	2- Moderately high ☐	3- Low ☐	4- Very Low ☐	5- Cannot be detected ☐
Risk Reduction	None (0%) ☐	Limited (25%) ☐	Moderate (50%) ☐	Substantial (75%) ☐	Extensive (95%) ☐
Disease transmission				**Applicable** ☐	**NA** ☐
Probability	1- Rare ☐	2- Unlikely ☐	3- Possible ☐	4- Likely ☐	5- Almost certain ☐
Severity		1- Non Serious ☐	2- Serious ☐	3- Life-Threatening ☐	4- Fatal ☐
Detectability	1- Very High ☐	2- Moderately high ☐	3- Low ☐	4- Very Low ☐	5- Cannot be detected ☐
Risk Reduction	None (0%) ☐	Limited (25%) ☐	Moderate (50%) ☐	Substantial (75%) ☐	Extensive (95%) ☐
Toxicity / Carcinogenicity				**Applicable** ☐	**NA** ☐
Probability	1- Rare ☐	2- Unlikely ☐	3- Possible ☐	4- Likely ☐	5- Almost certain ☐
Severity		1- Non Serious ☐	2- Serious ☐	3- Life-Threatening ☐	4- Fatal ☐
Detectability	1- Very High ☐	2- Moderately high ☐	3- Low ☐	4- Very Low ☐	5- Cannot be detected ☐
Risk Reduction	None (0%) ☐	Limited (25%) ☐	Moderate (50%) ☐	Substantial (75%) ☐	Extensive (95%) ☐
Other (_____)				**Applicable** ☐	**NA** ☐
Probability	1- Rare ☐	2- Unlikely ☐	3- Possible ☐	4- Likely ☐	5- Almost certain ☐
Severity		1- Non Serious ☐	2- Serious ☐	3- Life-Threatening ☐	4- Fatal ☐
Detectability	1- Very High ☐	2- Moderately high ☐	3- Low ☐	4- Very Low ☐	5- Cannot be detected ☐
Risk Reduction	None (0%) ☐	Limited (25%) ☐	Moderate (50%) ☐	Substantial (75%) ☐	Extensive (95%) ☐

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Methodologies for Assessing the Risks
associated to novel Blood, Tissue and Cell Component (BTCs).
-HPC Template-

Please follow the EuroGTP II Guide in order to correctly evaluate your BTCs

BTC characterisation
The evaluation of the level of novelty and the risks associated, should start with a characterisation of the novel process or BTC.

General category: Haematopoietic progenitor cells

Specific category:

- ☐ Bone Marrow
- ☐ Peripheral Blood
- ☐ Cord Blood
- ☐ Other

Name of the product, therapy or process under evaluation:

Description of BTC under evaluation:
(Describe the relevant aspects of the BTC, detailing the modifications/novelties associated with **donation**, **processing** and **clinical application** under evaluation)

Step 1 – Evaluation of novelty

The purpose of this exercise is to evaluate our proposed methodology for determining if a BTC, therapy or process is novel or not.
Please answer the following questions in order to determine if the product or process is novel. This process represents the first stage of the overall procedure for evaluating novelty and risk.

	Yes	No	Not Applicable/ Not Relevant
A. Has this type of BTC previously been collected, processed/ prepared and issued for clinical use by your establishment?			
Comment (optional):			
B. Will the starting material used to prepare this BTC be obtained from the same donor population previously used by your establishment for this type of BTC?			
Comment (optional):			
C. Will the starting material for this BTC be procured/collected using a procedure used previously by your establishment for this type of BTC?			
Comment (optional):			
D. Will this BTC be prepared by a procedure (processing/preparation, decontamination/pathogen reduction and preservation) used previously in your establishment for this type of BTC?			
Comment (optional):			
E. Will this BTC be packaged, stored, and distributed using a protocol and materials used previously in your establishment for this type of BTC?			
Comment (optional):			
F. Will this type of BTC provided by your establishment be applied/infused clinically using an application/transfusion/infusion implantation method used previously?			
Comment (optional):			
G. Has your establishment provided this type of BTC for the same clinical indication or for application/transfusion/infusion into a same anatomical site?			
Comment (optional):			

Step 2 – Level of risk analysis

Novelties represent different risks with distinct impact in the quality and safety of the products.

Select the specific risks that apply to this risk Factor (note that some risk factors may not apply to your product/therapy).

Risk Factor: Donor Characteristics

Consider whether the novelty in your process has an impact at the moment of the donation. This factor requires that you consider whether the donor population you intend to obtain the BTC from, could cause any risk for the recipient.

Applicable Yes ☐ No ☐

Comments and/or references (optional):

Specific Risks					
Unexpected immunogenicity				**Applicable** ☐	**NA** ☐
Probability	1- Rare ☐	2- Unlikely ☐	3- Possible ☐	4- Likely ☐	5- Almost certain ☐
Severity		1- Non Serious ☐	2- Serious ☐	3- Life-Threatening ☐	4- Fatal ☐
Detectability	1- Very High ☐	2- Moderately high ☐	3- Low ☐	4- Very Low ☐	5- Cannot be detected ☐
Risk Reduction	None (0%) ☐	Limited (25%) ☐	Moderate (50%) ☐	Substantial (75%) ☐	Extensive (95%) ☐
Engraftment failure				**Applicable** ☐	**NA** ☐
Probability	1- Rare ☐	2- Unlikely ☐	3- Possible ☐	4- Likely ☐	5- Almost certain ☐
Severity		1- Non Serious ☐	2- Serious ☐	3- Life-Threatening ☐	4- Fatal ☐
Detectability	1- Very High ☐	2- Moderately high ☐	3- Low ☐	4- Very Low ☐	5- Cannot be detected ☐
Risk Reduction	None (0%) ☐	Limited (25%) ☐	Moderate (50%) ☐	Substantial (75%) ☐	Extensive (95%) ☐
Disease transmission				**Applicable** ☐	**NA** ☐
Probability	1- Rare ☐	2- Unlikely ☐	3- Possible ☐	4- Likely ☐	5- Almost certain ☐
Severity		1- Non Serious ☐	2- Serious ☐	3- Life-Threatening ☐	4- Fatal ☐
Detectability	1- Very High ☐	2- Moderately high ☐	3- Low ☐	4- Very Low ☐	5- Cannot be detected ☐
Risk Reduction	None (0%) ☐	Limited (25%) ☐	Moderate (50%) ☐	Substantial (75%) ☐	Extensive (95%) ☐
Toxicity / Carcinogenicity				**Applicable** ☐	**NA** ☐
Probability	1- Rare ☐	2- Unlikely ☐	3- Possible ☐	4- Likely ☐	5- Almost certain ☐
Severity		1- Non Serious ☐	2- Serious ☐	3- Life-Threatening ☐	4- Fatal ☐
Detectability	1- Very High ☐	2- Moderately high ☐	3- Low ☐	4- Very Low ☐	5- Cannot be detected ☐
Risk Reduction	None (0%) ☐	Limited (25%) ☐	Moderate (50%) ☐	Substantial (75%) ☐	Extensive (95%) ☐
Other (_____)				**Applicable** ☐	**NA** ☐
Probability	1- Rare ☐	2- Unlikely ☐	3- Possible ☐	4- Likely ☐	5- Almost certain ☐
Severity		1- Non Serious ☐	2- Serious ☐	3- Life-Threatening ☐	4- Death ☐
Detectability	1- Very High ☐	2- Moderately high ☐	3- Low ☐	4- Very Low ☐	5- Cannot be detected ☐
Risk Reduction	None (0%) ☐	Limited (25%) ☐	Moderate (50%) ☐	Substantial (75%) ☐	Extensive (95%) ☐

Step 2

Novelties represent different risks with distinct impact in the quality and safety of the products.

Select the specific risks that apply to this risk Factor (note that some risk factors may not apply to your product/therapy).

Risk Factor: Procurement/collection process and environment

Consider where and how the BTC is recovered/collected currently and whether the changes proposed with the novel method change recovery time, complexity, quality of the environment?
For example, how long does the process take, how complex is it, and how does the procurement devices affect the quality of the HPC?

Applicable Yes ☐ No ☐

Comments and/or references (optional):

Risks					
Unexpected immunogenicity				**Applicable** ☐	**NA** ☐
Probability	1- Rare ☐	2- Unlikely ☐	3- Possible ☐	4- Likely ☐	5- Almost certain ☐
Severity		1- Non Serious ☐	2- Serious ☐	3- Life-Threatening ☐	4- Fatal ☐
Detectability	1- Very High ☐	2- Moderately high ☐	3- Low ☐	4- Very Low ☐	5- Cannot be detected ☐
Risk Reduction	None (0%) ☐	Limited (25%) ☐	Moderate (50%) ☐	Substantial (75%) ☐	Extensive (95%) ☐
Engraftment failure				**Applicable** ☐	**NA** ☐
Probability	1- Rare ☐	2- Unlikely ☐	3- Possible ☐	4- Likely ☐	5- Almost certain ☐
Severity		1- Non Serious ☐	2- Serious ☐	3- Life-Threatening ☐	4- Fatal ☐
Detectability	1- Very High ☐	2- Moderately high ☐	3- Low ☐	4- Very Low ☐	5- Cannot be detected ☐
Risk Reduction	None (0%) ☐	Limited (25%) ☐	Moderate (50%) ☐	Substantial (75%) ☐	Extensive (95%) ☐
Disease transmission				**Applicable** ☐	**NA** ☐
Probability	1- Rare ☐	2- Unlikely ☐	3- Possible ☐	4- Likely ☐	5- Almost certain ☐
Severity		1- Non Serious ☐	2- Serious ☐	3- Life-Threatening ☐	4- Fatal ☐
Detectability	1- Very High ☐	2- Moderately high ☐	3- Low ☐	4- Very Low ☐	5- Cannot be detected ☐
Risk Reduction	None (0%) ☐	Limited (25%) ☐	Moderate (50%) ☐	Substantial (75%) ☐	Extensive (95%) ☐
Toxicity / Carcinogenicity				**Applicable** ☐	**NA** ☐
Probability	1- Rare ☐	2- Unlikely ☐	3- Possible ☐	4- Likely ☐	5- Almost certain ☐
Severity		1- Non Serious ☐	2- Serious ☐	3- Life-Threatening ☐	4- Fatal ☐
Detectability	1- Very High ☐	2- Moderately high ☐	3- Low ☐	4- Very Low ☐	5- Cannot be detected ☐
Risk Reduction	None (0%) ☐	Limited (25%) ☐	Moderate (50%) ☐	Substantial (75%) ☐	Extensive (95%) ☐
Other (_____)				**Applicable** ☐	**NA** ☐
Probability	1- Rare ☐	2- Unlikely ☐	3- Possible ☐	4- Likely ☐	5- Almost certain ☐
Severity		1- Non Serious ☐	2- Serious ☐	3- Life-Threatening ☐	4- Fatal ☐
Detectability	1- Very High ☐	2- Moderately high ☐	3- Low ☐	4- Very Low ☐	5- Cannot be detected ☐
Risk Reduction	None (0%) ☐	Limited (25%) ☐	Moderate (50%) ☐	Substantial (75%) ☐	Extensive (95%) ☐

Step 2

Novelties represent different risks with distinct impact in the quality and safety of the products.

Select the specific risks that apply to this risk Factor (note that some risk factors may not apply to your product/therapy).

Risk Factor: Processing and environment

Consider the current processing method for the BTC how the novelty in processing can affect the product. How long does the novel preparation process take and how complex is it – this may have an impact on the risk of contamination, or cells characteristics that may not be consistent with product specifications. Also consider the quality of the preparation environment, which may also affect the risk of contamination.

Applicable **Yes** ☐ **No** ☐

Comments and/or references (optional):

Specific Risks					
Unexpected immunogenicity				**Applicable** ☐	**NA** ☐
Probability	1- Rare ☐	2- Unlikely ☐	3- Possible ☐	4- Likely ☐	5- Almost certain ☐
Severity		1- Non Serious ☐	2- Serious ☐	3- Life-Threatening ☐	4- Fatal ☐
Detectability	1- Very High ☐	2- Moderately high ☐	3- Low ☐	4- Very Low ☐	5- Cannot be detected ☐
Risk Reduction	None (0%) ☐	Limited (25%) ☐	Moderate (50%) ☐	Substantial (75%) ☐	Extensive (95%) ☐
Engrafment failure				**Applicable** ☐	**NA** ☐
Probability	1- Rare ☐	2- Unlikely ☐	3- Possible ☐	4- Likely ☐	5- Almost certain ☐
Severity		1- Non Serious ☐	2- Serious ☐	3- Life-Threatening ☐	4- Fatal ☐
Detectability	1- Very High ☐	2- Moderately high ☐	3- Low ☐	4- Very Low ☐	5- Cannot be detected ☐
Risk Reduction	None (0%) ☐	Limited (25%) ☐	Moderate (50%) ☐	Substantial (75%) ☐	Extensive (95%) ☐
Disease transmission				**Applicable** ☐	**NA** ☐
Probability	1- Rare ☐	2- Unlikely ☐	3- Possible ☐	4- Likely ☐	5- Almost certain ☐
Severity		1- Non Serious ☐	2- Serious ☐	3- Life-Threatening ☐	4- Fatal ☐
Detectability	1- Very High ☐	2- Moderately high ☐	3- Low ☐	4- Very Low ☐	5- Cannot be detected ☐
Risk Reduction	None (0%) ☐	Limited (25%) ☐	Moderate (50%) ☐	Substantial (75%) ☐	Extensive (95%) ☐
Toxicity / Carcinogenicity				**Applicable** ☐	**NA** ☐
Probability	1- Rare ☐	2- Unlikely ☐	3- Possible ☐	4- Likely ☐	5- Almost certain ☐
Severity		1- Non Serious ☐	2- Serious ☐	3- Life-Threatening ☐	4- Fatal ☐
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Risk Reduction	None (0%) ☐	Limited (25%) ☐	Moderate (50%) ☐	Substantial (75%) ☐	Extensive (95%) ☐
Other (_____)				**Applicable** ☐	**NA** ☐
Probability	1- Rare ☐	2- Unlikely ☐	3- Possible ☐	4- Likely ☐	5- Almost certain ☐
Severity		1- Non Serious ☐	2- Serious ☐	3- Life-Threatening ☐	4- Fatal ☐
Detectability	1- Very High ☐	2- Moderately high ☐	3- Low ☐	4- Very Low ☐	5- Cannot be detected ☐
Risk Reduction	None (0%) ☐	Limited (25%) ☐	Moderate (50%) ☐	Substantial (75%) ☐	Extensive (95%) ☐

Step 2

Novelties represent different risks with distinct impact in the quality and safety of the products.

Select the specific risks that apply to this risk Factor (note that some risk factors may not apply to your product/therapy).

Risk Factor: Reliability of Microbiology Testing

Consider the risk that the testing methodology and/or presence of residual processing reagents such as antibiotics in the finished BTC may impact the accuracy of any microbiology/mycology testing of the BTC. This risk factor is not about blood tests on the donor.

Applicable Yes ☐ No ☐

Comments and/or references (optional):

Risks					
Unexpected immunogenicity				**Applicable** ☐	**NA** ☐
Probability	1- Rare ☐	2- Unlikely ☐	3- Possible ☐	4- Likely ☐	5- Almost certain ☐
Severity		1- Non Serious ☐	2- Serious ☐	3- Life-Threatening ☐	4- Fatal ☐
Detectability	1- Very High ☐	2- Moderately high ☐	3- Low ☐	4- Very Low ☐	5- Cannot be detected ☐
Risk Reduction	None (0%) ☐	Limited (25%) ☐	Moderate (50%) ☐	Substantial (75%) ☐	Extensive (95%) ☐
Engrafment failure				**Applicable** ☐	**NA** ☐
Probability	1- Rare ☐	2- Unlikely ☐	3- Possible ☐	4- Likely ☐	5- Almost certain ☐
Severity		1- Non Serious ☐	2- Serious ☐	3- Life-Threatening ☐	4- Fatal ☐
Detectability	1- Very High ☐	2- Moderately high ☐	3- Low ☐	4- Very Low ☐	5- Cannot be detected ☐
Risk Reduction	None (0%) ☐	Limited (25%) ☐	Moderate (50%) ☐	Substantial (75%) ☐	Extensive (95%) ☐
Disease transmission				**Applicable** ☐	**NA** ☐
Probability	1- Rare ☐	2- Unlikely ☐	3- Possible ☐	4- Likely ☐	5- Almost certain ☐
Severity		1- Non Serious ☐	2- Serious ☐	3- Life-Threatening ☐	4- Fatal ☐
Detectability	1- Very High ☐	2- Moderately high ☐	3- Low ☐	4- Very Low ☐	5- Cannot be detected ☐
Risk Reduction	None (0%) ☐	Limited (25%) ☐	Moderate (50%) ☐	Substantial (75%) ☐	Extensive (95%) ☐
Toxicity / Carcinogenicity				**Applicable** ☐	**NA** ☐
Probability	1- Rare ☐	2- Unlikely ☐	3- Possible ☐	4- Likely ☐	5- Almost certain ☐
Severity		1- Non Serious ☐	2- Serious ☐	3- Life-Threatening ☐	4- Fatal ☐
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Risk Reduction	None (0%) ☐	Limited (25%) ☐	Moderate (50%) ☐	Substantial (75%) ☐	Extensive (95%) ☐
Other (_____)				**Applicable** ☐	**NA** ☐
Probability	1- Rare ☐	2- Unlikely ☐	3- Possible ☐	4- Likely ☐	5- Almost certain ☐
Severity		1- Non Serious ☐	2- Serious ☐	3- Life-Threatening ☐	4- Fatal ☐
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Risk Reduction	None (0%) ☐	Limited (25%) ☐	Moderate (50%) ☐	Substantial (75%) ☐	Extensive (95%) ☐

Step 2

Novelties represent different risks with distinct impact in the quality and safety of the products.

Select the specific risks that apply to this risk Factor (note that some risk factors may not apply to your product/therapy).

Risk Factor: Storage Conditions

Consider any potential risks arising from how the starting material and BTC are stored, between procurement and processing, during processing, and between processing and implantation.

Applicable Yes ☐ No ☐

Comments and/or references (optional):

Risks					
Unexpected immunogenicity				**Applicable** ☐	**NA** ☐
Probability	1- Rare ☐	2- Unlikely ☐	3- Possible ☐	4- Likely ☐	5- Almost certain ☐
Severity		1- Non Serious ☐	2- Serious ☐	3- Life-Threatening ☐	4- Fatal ☐
Detectability	1- Very High ☐	2- Moderately high ☐	3- Low ☐	4- Very Low ☐	5- Cannot be detected ☐
Risk Reduction	None (0%) ☐	Limited (25%) ☐	Moderate (50%) ☐	Substantial (75%) ☐	Extensive (95%) ☐
Engrafment failure				**Applicable** ☐	**NA** ☐
Probability	1- Rare ☐	2- Unlikely ☐	3- Possible ☐	4- Likely ☐	5- Almost certain ☐
Severity		1- Non Serious ☐	2- Serious ☐	3- Life-Threatening ☐	4- Fatal ☐
Detectability	1- Very High ☐	2- Moderately high ☐	3- Low ☐	4- Very Low ☐	5- Cannot be detected ☐
Risk Reduction	None (0%) ☐	Limited (25%) ☐	Moderate (50%) ☐	Substantial (75%) ☐	Extensive (95%) ☐
Disease transmission				**Applicable** ☐	**NA** ☐
Probability	1- Rare ☐	2- Unlikely ☐	3- Possible ☐	4- Likely ☐	5- Almost certain ☐
Severity		1- Non Serious ☐	2- Serious ☐	3- Life-Threatening ☐	4- Fatal ☐
Detectability	1- Very High ☐	2- Moderately high ☐	3- Low ☐	4- Very Low ☐	5- Cannot be detected ☐
Risk Reduction	None (0%) ☐	Limited (25%) ☐	Moderate (50%) ☐	Substantial (75%) ☐	Extensive (95%) ☐
Toxicity / Carcinogenicity				**Applicable** ☐	**NA** ☐
Probability	1- Rare ☐	2- Unlikely ☐	3- Possible ☐	4- Likely ☐	5- Almost certain ☐
Severity		1- Non Serious ☐	2- Serious ☐	3- Life-Threatening ☐	4- Fatal ☐
Detectability	1- Very High ☐	2- Moderately high ☐	3- Low ☐	4- Very Low ☐	5- Cannot be detected ☐
Risk Reduction	None (0%) ☐	Limited (25%) ☐	Moderate (50%) ☐	Substantial (75%) ☐	Extensive (95%) ☐
Other (_____)				**Applicable** ☐	**NA** ☐
Probability	1- Rare ☐	2- Unlikely ☐	3- Possible ☐	4- Likely ☐	5- Almost certain ☐
Severity		1- Non Serious ☐	2- Serious ☐	3- Life-Threatening ☐	4- Fatal ☐
Detectability	1- Very High ☐	2- Moderately high ☐	3- Low ☐	4- Very Low ☐	5- Cannot be detected ☐
Risk Reduction	None (0%) ☐	Limited (25%) ☐	Moderate (50%) ☐	Substantial (75%) ☐	Extensive (95%) ☐

Step 2

Novelties represent different risks with distinct impact in the quality and safety of the products.

Select the specific risks that apply to this risk Factor (note that some risk factors may not apply to your product/therapy).

Risk Factor: Transport Conditions

Consider any potential risks arising from how the starting material and BTC are transported, for example between the sites of procurement and processing, and between the sites of storage and implantation.

Applicable **Yes** ☐ **No** ☐

Comments and/or references (optional):

Risks					
Unexpected immunogenicity				**Applicable** ☐	**NA** ☐
Probability	1- Rare ☐	2- Unlikely ☐	3- Possible ☐	4- Likely ☐	5- Almost certain ☐
Severity		1- Non Serious ☐	2- Serious ☐	3- Life-Threatening ☐	4- Fatal ☐
Detectability	1- Very High ☐	2- Moderately high ☐	3- Low ☐	4- Very Low ☐	5- Cannot be detected ☐
Risk Reduction	None (0%) ☐	Limited (25%) ☐	Moderate (50%) ☐	Substantial (75%) ☐	Extensive (95%) ☐
Engraftment failure				**Applicable** ☐	**NA** ☐
Probability	1- Rare ☐	2- Unlikely ☐	3- Possible ☐	4- Likely ☐	5- Almost certain ☐
Severity		1- Non Serious ☐	2- Serious ☐	3- Life-Threatening ☐	4- Fatal ☐
Detectability	1- Very High ☐	2- Moderately high ☐	3- Low ☐	4- Very Low ☐	5- Cannot be detected ☐
Risk Reduction	None (0%) ☐	Limited (25%) ☐	Moderate (50%) ☐	Substantial (75%) ☐	Extensive (95%) ☐
Disease transmission				**Applicable** ☐	**NA** ☐
Probability	1- Rare ☐	2- Unlikely ☐	3- Possible ☐	4- Likely ☐	5- Almost certain ☐
Severity		1- Non Serious ☐	2- Serious ☐	3- Life-Threatening ☐	4- Fatal ☐
Detectability	1- Very High ☐	2- Moderately high ☐	3- Low ☐	4- Very Low ☐	5- Cannot be detected ☐
Risk Reduction	None (0%) ☐	Limited (25%) ☐	Moderate (50%) ☐	Substantial (75%) ☐	Extensive (95%) ☐
Toxicity / Carcinogenicity				**Applicable** ☐	**NA** ☐
Probability	1- Rare ☐	2- Unlikely ☐	3- Possible ☐	4- Likely ☐	5- Almost certain ☐
Severity		1- Non Serious ☐	2- Serious ☐	3- Life-Threatening ☐	4- Fatal ☐
Detectability	1- Very High ☐	2- Moderately high ☐	3- Low ☐	4- Very Low ☐	5- Cannot be detected ☐
Risk Reduction	None (0%) ☐	Limited (25%) ☐	Moderate (50%) ☐	Substantial (75%) ☐	Extensive (95%) ☐
Other (_____)				**Applicable** ☐	**NA** ☐
Probability	1- Rare ☐	2- Unlikely ☐	3- Possible ☐	4- Likely ☐	5- Almost certain ☐
Severity		1- Non Serious ☐	2- Serious ☐	3- Life-Threatening ☐	4- Fatal ☐
Detectability	1- Very High ☐	2- Moderately high ☐	3- Low ☐	4- Very Low ☐	5- Cannot be detected ☐
Risk Reduction	None (0%) ☐	Limited (25%) ☐	Moderate (50%) ☐	Substantial (75%) ☐	Extensive (95%) ☐

Step 2

Novelties represent different risks with distinct impact in the quality and safety of the products.

Select the specific risks that apply to this risk Factor (note that some risk factors may not apply to your product/therapy).

Risk Factor: Presence of unwanted cellular material

Consider the risk of the presence of inactivated cells, debris or cell components, which may cause immunogenicity or disease transmission.

Applicable Yes ☐ No ☐

Comments and/or references (optional):

Risks					
Unexpected immunogenicity				**Applicable** ☐	**NA** ☐
Probability	1- Rare ☐	2- Unlikely ☐	3- Possible ☐	4- Likely ☐	5- Almost certain ☐
Severity		1- Non Serious ☐	2- Serious ☐	3- Life-Threatening ☐	4- Fatal ☐
Detectability	1- Very High ☐	2- Moderately high ☐	3- Low ☐	4- Very Low ☐	5- Cannot be detected ☐
Risk Reduction	None (0%) ☐	Limited (25%) ☐	Moderate (50%) ☐	Substantial (75%) ☐	Extensive (95%) ☐
Engraftment failure				**Applicable** ☐	**NA** ☐
Probability	1- Rare ☐	2- Unlikely ☐	3- Possible ☐	4- Likely ☐	5- Almost certain ☐
Severity		1- Non Serious ☐	2- Serious ☐	3- Life-Threatening ☐	4- Fatal ☐
Detectability	1- Very High ☐	2- Moderately high ☐	3- Low ☐	4- Very Low ☐	5- Cannot be detected ☐
Risk Reduction	None (0%) ☐	Limited (25%) ☐	Moderate (50%) ☐	Substantial (75%) ☐	Extensive (95%) ☐
Disease transmission				**Applicable** ☐	**NA** ☐
Probability	1- Rare ☐	2- Unlikely ☐	3- Possible ☐	4- Likely ☐	5- Almost certain ☐
Severity		1- Non Serious ☐	2- Serious ☐	3- Life-Threatening ☐	4- Fatal ☐
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Risk Reduction	None (0%) ☐	Limited (25%) ☐	Moderate (50%) ☐	Substantial (75%) ☐	Extensive (95%) ☐
Toxicity / Carcinogenicity				**Applicable** ☐	**NA** ☐
Probability	1- Rare ☐	2- Unlikely ☐	3- Possible ☐	4- Likely ☐	5- Almost certain ☐
Severity		1- Non Serious ☐	2- Serious ☐	3- Life-Threatening ☐	4- Fatal ☐
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Risk Reduction	None (0%) ☐	Limited (25%) ☐	Moderate (50%) ☐	Substantial (75%) ☐	Extensive (95%) ☐
Other (_____)				**Applicable** ☐	**NA** ☐
Probability	1- Rare ☐	2- Unlikely ☐	3- Possible ☐	4- Likely ☐	5- Almost certain ☐
Severity		1- Non Serious ☐	2- Serious ☐	3- Life-Threatening ☐	4- Fatal ☐
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Risk Reduction	None (0%) ☐	Limited (25%) ☐	Moderate (50%) ☐	Substantial (75%) ☐	Extensive (95%) ☐

Step 2

Novelties represent different risks with distinct impact in the quality and safety of the products.

Select the specific risks that apply to this risk Factor (note that some risk factors may not apply to your product/therapy).

Risk Factor: Complexity of the pre-implantation preparation and/or application method

Consider how complex the method of implantation will be for this BTC. How long will it take, and could this introduce risks? What is the scope for errors to be made, and what could the consequences of these errors be?

Applicable **Yes** ☐ **No** ☐

Comments and/or references (optional):

Risks					
Unexpected immunogenicity				**Applicable** ☐	**NA** ☐
Probability	1- Rare ☐	2- Unlikely ☐	3- Possible ☐	4- Likely ☐	5- Almost certain ☐
Severity		1- Non Serious ☐	2- Serious ☐	3- Life-Threatening ☐	4- Fatal ☐
Detectability	1- Very High ☐	2- Moderately high ☐	3- Low ☐	4- Very Low ☐	5- Cannot be detected ☐
Risk Reduction	None (0%) ☐	Limited (25%) ☐	Moderate (50%) ☐	Substantial (75%) ☐	Extensive (95%) ☐
Engraftment failure				**Applicable** ☐	**NA** ☐
Probability	1- Rare ☐	2- Unlikely ☐	3- Possible ☐	4- Likely ☐	5- Almost certain ☐
Severity		1- Non Serious ☐	2- Serious ☐	3- Life-Threatening ☐	4- Fatal ☐
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Risk Reduction	None (0%) ☐	Limited (25%) ☐	Moderate (50%) ☐	Substantial (75%) ☐	Extensive (95%) ☐
Disease transmission				**Applicable** ☐	**NA** ☐
Probability	1- Rare ☐	2- Unlikely ☐	3- Possible ☐	4- Likely ☐	5- Almost certain ☐
Severity		1- Non Serious ☐	2- Serious ☐	3- Life-Threatening ☐	4- Fatal ☐
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Risk Reduction	None (0%) ☐	Limited (25%) ☐	Moderate (50%) ☐	Substantial (75%) ☐	Extensive (95%) ☐
Toxicity / Carcinogenicity				**Applicable** ☐	**NA** ☐
Probability	1- Rare ☐	2- Unlikely ☐	3- Possible ☐	4- Likely ☐	5- Almost certain ☐
Severity		1- Non Serious ☐	2- Serious ☐	3- Life-Threatening ☐	4- Fatal ☐
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Other (_____)				**Applicable** ☐	**NA** ☐
Probability	1- Rare ☐	2- Unlikely ☐	3- Possible ☐	4- Likely ☐	5- Almost certain ☐
Severity		1- Non Serious ☐	2- Serious ☐	3- Life-Threatening ☐	4- Fatal ☐
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Risk Reduction	None (0%) ☐	Limited (25%) ☐	Moderate (50%) ☐	Substantial (75%) ☐	Extensive (95%) ☐

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Methodologies for Assessing the Risks
associated to novel Blood, Tissue and Cell Component (BTCs).
-MAR (Gametes and Embryos) Template-

Please follow the EuroGTP II Guide in order to correctly evaluate your BTCs

BTC characterisation

The evaluation of the level of novelty and the risks associated, should start with a characterisation of the novel process or BTC.

General category: Medically assisted reproduction

Specific category:

- ☐ Gametes
- ☐ Embryos

Name of the product, therapy or process under evaluation:

Description of BTC under evaluation:
(Describe the relevant aspects of the BTC, detailing the modifications/novelties associated with **donation**, **processing** and **clinical application** under evaluation)

Step 1 – Evaluation of novelty

The purpose of this exercise is to evaluate our proposed methodology for determining if a BTC, therapy or process is novel or not.
Please answer the following questions in order to determine if the product or process is novel. This process represents the first stage of the overall procedure for evaluating novelty and risk.

	Yes	No	Not Applicable/ Not Relevant
A. Has this type of BTC previously been collected, processed/ prepared and issued for clinical use by your establishment?			
Comment (optional):			
B. Will the starting material used to prepare this BTC be obtained from the same donor population previously used by your establishment for this type of BTC?			
Comment (optional):			
C. Will the starting material for this BTC be procured/collected using a procedure used previously by your establishment for this type of BTC?			
Comment (optional):			
D. Will this BTC be prepared by a procedure (processing, decontamination and preservation) used previously in your establishment for this type of BTC?			
Comment (optional):			
E. Will this BTC be packaged, stored, and distributed using a protocol and materials used previously in your establishment for this type of BTC?			
Comment (optional):			
F. Will this type of BTC provided by your establishment be applied/infused clinically using an application/transfusion/infusion method used previously?			
Comment (optional):			
G. Has your establishment provided this type of BTC for the same clinical indication or for application/transfusion/infusion into a same anatomical site?			
Comment (optional):			

Step 2 – Level of risk analysis

Novelties represent different risks with distinct impact in the quality and safety of the products.

Select the specific risks that apply to this risk Factor (note that some risk factors may not apply to your product/therapy).

Risk Factor: Donor Characteristics

Consider whether the novelty in your process or procedures change donor characteristics and if these changes could impart a risk to the recipient.

Applicable **Yes** ☐ **No** ☐

Comments and/or references (optional):

Specific Risks					
Implant failure/ Pregnancy loss				**Applicable** ☐	**NA** ☐
Probability	1- Rare ☐	2- Unlikely ☐	3- Possible ☐	4- Likely ☐	5- Almost certain ☐
Severity		1- Non Serious ☐	2- Serious ☐	3- Life-Threatening ☐	4- Fatal ☐
Detectability	1- Very High ☐	2- Moderately high ☐	3- Low ☐	4- Very Low ☐	5- Cannot be detected ☐
Risk Reduction	None (0%) ☐	Limited (25%) ☐	Moderate (50%) ☐	Substantial (75%) ☐	Extensive (95%) ☐
Disease transmission				**Applicable** ☐	**NA** ☐
Probability	1- Rare ☐	2- Unlikely ☐	3- Possible ☐	4- Likely ☐	5- Almost certain ☐
Severity		1- Non Serious ☐	2- Serious ☐	3- Life-Threatening ☐	4- Fatal ☐
Detectability	1- Very High ☐	2- Moderately high ☐	3- Low ☐	4- Very Low ☐	5- Cannot be detected ☐
Risk Reduction	None (0%) ☐	Limited (25%) ☐	Moderate (50%) ☐	Substantial (75%) ☐	Extensive (95%) ☐
Toxicity / Carcinogenicity				**Applicable** ☐	**NA** ☐
Probability	1- Rare ☐	2- Unlikely ☐	3- Possible ☐	4- Likely ☐	5- Almost certain ☐
Severity		1- Non Serious ☐	2- Serious ☐	3- Life-Threatening ☐	4- Fatal ☐
Detectability	1- Very High ☐	2- Moderately high ☐	3- Low ☐	4- Very Low ☐	5- Cannot be detected ☐
Risk Reduction	None (0%) ☐	Limited (25%) ☐	Moderate (50%) ☐	Substantial (75%) ☐	Extensive (95%) ☐
Other (_____)				**Applicable** ☐	**NA** ☐
Probability	1- Rare ☐	2- Unlikely ☐	3- Possible ☐	4- Likely ☐	5- Almost certain ☐
Severity		1- Non Serious ☐	2- Serious ☐	3- Life-Threatening ☐	4- Fatal ☐
Detectability	1- Very High ☐	2- Moderately high ☐	3- Low ☐	4- Very Low ☐	5- Cannot be detected ☐
Risk Reduction	None (0%) ☐	Limited (25%) ☐	Moderate (50%) ☐	Substantial (75%) ☐	Extensive (95%) ☐

Step 2 – Level of risk analysis

Novelties represent different risks with distinct impact in the quality and safety of the products.

Select the specific risks that apply to this risk Factor (note that some risk factors may not apply to your product/therapy).

Risk Factor: Procurement process and environment

Consider where and how the BTC is recovered now and how this would happen when the novelty is taken into account. Is there a change in recovery time, complexity, quality of the environment

Applicable **Yes** ☐ **No** ☐

Comments and/or references (optional):

Specific Risks					
Implant failure/ Pregnancy loss				**Applicable** ☐	**NA** ☐
Probability	1- Rare ☐	2- Unlikely ☐	3- Possible ☐	4- Likely ☐	5- Almost certain ☐
Severity		1- Non Serious ☐	2- Serious ☐	3- Life-Threatening ☐	4- Fatal ☐
Detectability	1- Very High ☐	2- Moderately high ☐	3- Low ☐	4- Very Low ☐	5- Cannot be detected ☐
Risk Reduction	None (0%) ☐	Limited (25%) ☐	Moderate (50%) ☐	Substantial (75%) ☐	Extensive (95%) ☐
Disease transmission				**Applicable** ☐	**NA** ☐
Probability	1- Rare ☐	2- Unlikely ☐	3- Possible ☐	4- Likely ☐	5- Almost certain ☐
Severity		1- Non Serious ☐	2- Serious ☐	3- Life-Threatening ☐	4- Fatal ☐
Detectability	1- Very High ☐	2- Moderately high ☐	3- Low ☐	4- Very Low ☐	5- Cannot be detected ☐
Risk Reduction	None (0%) ☐	Limited (25%) ☐	Moderate (50%) ☐	Substantial (75%) ☐	Extensive (95%) ☐
Toxicity / Carcinogenicity				**Applicable** ☐	**NA** ☐
Probability	1- Rare ☐	2- Unlikely ☐	3- Possible ☐	4- Likely ☐	5- Almost certain ☐
Severity		1- Non Serious ☐	2- Serious ☐	3- Life-Threatening ☐	4- Fatal ☐
Detectability	1- Very High ☐	2- Moderately high ☐	3- Low ☐	4- Very Low ☐	5- Cannot be detected ☐
Risk Reduction	None (0%) ☐	Limited (25%) ☐	Moderate (50%) ☐	Substantial (75%) ☐	Extensive (95%) ☐
Other (_____)				**Applicable** ☐	**NA** ☐
Probability	1- Rare ☐	2- Unlikely ☐	3- Possible ☐	4- Likely ☐	5- Almost certain ☐
Severity		1- Non Serious ☐	2- Serious ☐	3- Life-Threatening ☐	4- Fatal ☐
Detectability	1- Very High ☐	2- Moderately high ☐	3- Low ☐	4- Very Low ☐	5- Cannot be detected ☐
Risk Reduction	None (0%) ☐	Limited (25%) ☐	Moderate (50%) ☐	Substantial (75%) ☐	Extensive (95%) ☐

Step 2 – Level of risk analysis

Novelties represent different risks with distinct impact in the quality and safety of the products.

Select the specific risks that apply to this risk Factor (note that some risk factors may not apply to your product/therapy).

Risk Factor: Processing and environment

Consider where and how the BTC is prepared at the moment and how the novelty in the process can affect this step. How long does the novel preparation process take and how complex it is – this may impact the risk of contamination, or it may not be prepared to consistent specifications and quality. Also consider the quality of the preparation environment, which may also affect the risk of contamination.

Applicable Yes ☐ No ☐

Comments and/or references (optional):

Specific Risks					
Implant failure/ Pregnancy loss				**Applicable** ☐	**NA** ☐
Probability	1- Rare ☐	2- Unlikely ☐	3- Possible ☐	4- Likely ☐	5- Almost certain ☐
Severity		1- Non Serious ☐	2- Serious ☐	3- Life-Threatening ☐	4- Fatal ☐
Detectability	1- Very High ☐	2- Moderately high ☐	3- Low ☐	4- Very Low ☐	5- Cannot be detected ☐
Risk Reduction	None (0%) ☐	Limited (25%) ☐	Moderate (50%) ☐	Substantial (75%) ☐	Extensive (95%) ☐
Disease transmission				**Applicable** ☐	**NA** ☐
Probability	1- Rare ☐	2- Unlikely ☐	3- Possible ☐	4- Likely ☐	5- Almost certain ☐
Severity		1- Non Serious ☐	2- Serious ☐	3- Life-Threatening ☐	4- Fatal ☐
Detectability	1- Very High ☐	2- Moderately high ☐	3- Low ☐	4- Very Low ☐	5- Cannot be detected ☐
Risk Reduction	None (0%) ☐	Limited (25%) ☐	Moderate (50%) ☐	Substantial (75%) ☐	Extensive (95%) ☐
Toxicity / Carcinogenicity				**Applicable** ☐	**NA** ☐
Probability	1- Rare ☐	2- Unlikely ☐	3- Possible ☐	4- Likely ☐	5- Almost certain ☐
Severity		1- Non Serious ☐	2- Serious ☐	3- Life-Threatening ☐	4- Fatal ☐
Detectability	1- Very High ☐	2- Moderately high ☐	3- Low ☐	4- Very Low ☐	5- Cannot be detected ☐
Risk Reduction	None (0%) ☐	Limited (25%) ☐	Moderate (50%) ☐	Substantial (75%) ☐	Extensive (95%) ☐
Other (_____)				**Applicable** ☐	**NA** ☐
Probability	1- Rare ☐	2- Unlikely ☐	3- Possible ☐	4- Likely ☐	5- Almost certain ☐
Severity		1- Non Serious ☐	2- Serious ☐	3- Life-Threatening ☐	4- Fatal ☐
Detectability	1- Very High ☐	2- Moderately high ☐	3- Low ☐	4- Very Low ☐	5- Cannot be detected ☐
Risk Reduction	None (0%) ☐	Limited (25%) ☐	Moderate (50%) ☐	Substantial (75%) ☐	Extensive (95%) ☐

Step 2 – Level of risk analysis

Novelties represent different risks with distinct impact in the quality and safety of the products.

Select the specific risks that apply to this risk Factor (note that some risk factors may not apply to your product/therapy).

Risk Factor: Reagents

Consider any reagents used during recovery, processing, preparation, decontamination and storage of the BTC. Could they damage the BTC in any way, or could residual traces of reagent remain in the BTC that could cause toxic or immunogenic effects in recipients?

Applicable **Yes** ☐ **No** ☐

Comments and/or references (optional):

Specific Risks					
Implant failure/ Pregnancy loss				**Applicable** ☐	**NA** ☐
Probability	1- Rare ☐	2- Unlikely ☐	3- Possible ☐	4- Likely ☐	5- Almost certain ☐
Severity		1- Non Serious ☐	2- Serious ☐	3- Life-Threatening ☐	4- Fatal ☐
Detectability	1- Very High ☐	2- Moderately high ☐	3- Low ☐	4- Very Low ☐	5- Cannot be detected ☐
Risk Reduction	None (0%) ☐	Limited (25%) ☐	Moderate (50%) ☐	Substantial (75%) ☐	Extensive (95%) ☐
Disease transmission				**Applicable** ☐	**NA** ☐
Probability	1- Rare ☐	2- Unlikely ☐	3- Possible ☐	4- Likely ☐	5- Almost certain ☐
Severity		1- Non Serious ☐	2- Serious ☐	3- Life-Threatening ☐	4- Fatal ☐
Detectability	1- Very High ☐	2- Moderately high ☐	3- Low ☐	4- Very Low ☐	5- Cannot be detected ☐
Risk Reduction	None (0%) ☐	Limited (25%) ☐	Moderate (50%) ☐	Substantial (75%) ☐	Extensive (95%) ☐
Toxicity / Carcinogenicity				**Applicable** ☐	**NA** ☐
Probability	1- Rare ☐	2- Unlikely ☐	3- Possible ☐	4- Likely ☐	5- Almost certain ☐
Severity		1- Non Serious ☐	2- Serious ☐	3- Life-Threatening ☐	4- Fatal ☐
Detectability	1- Very High ☐	2- Moderately high ☐	3- Low ☐	4- Very Low ☐	5- Cannot be detected ☐
Risk Reduction	None (0%) ☐	Limited (25%) ☐	Moderate (50%) ☐	Substantial (75%) ☐	Extensive (95%) ☐
Other (_____)				**Applicable** ☐	**NA** ☐
Probability	1- Rare ☐	2- Unlikely ☐	3- Possible ☐	4- Likely ☐	5- Almost certain ☐
Severity		1- Non Serious ☐	2- Serious ☐	3- Life-Threatening ☐	4- Fatal ☐
Detectability	1- Very High ☐	2- Moderately high ☐	3- Low ☐	4- Very Low ☐	5- Cannot be detected ☐
Risk Reduction	None (0%) ☐	Limited (25%) ☐	Moderate (50%) ☐	Substantial (75%) ☐	Extensive (95%) ☐

Step 2 – Level of risk analysis

Novelties represent different risks with distinct impact in the quality and safety of the products.

Select the specific risks that apply to this risk Factor (note that some risk factors may not apply to your product/therapy).

Risk Factor: Storage conditions

Consider any potential risks arising from how the starting material and BTC are stored, not only after processing and before clinical application, but also in intermediate steps: e.g. between procurement and processing, during processing, and between processing steps.

Applicable **Yes** ☐ **No** ☐

Comments and/or references (optional):

Specific Risks					
Unexpected immunogenicity				**Applicable** ☐	**NA** ☐
Probability	1- Rare ☐	2- Unlikely ☐	3- Possible ☐	4- Likely ☐	5- Almost certain ☐
Severity		1- Non Serious ☐	2- Serious ☐	3- Life-Threatening ☐	4- Fatal ☐
Detectability	1- Very High ☐	2- Moderately high ☐	3- Low ☐	4- Very Low ☐	5- Cannot be detected ☐
Risk Reduction	None (0%) ☐	Limited (25%) ☐	Moderate (50%) ☐	Substantial (75%) ☐	Extensive (95%) ☐
Implant failure/Pregnancy loss				**Applicable** ☐	**NA** ☐
Probability	1- Rare ☐	2- Unlikely ☐	3- Possible ☐	4- Likely ☐	5- Almost certain ☐
Severity		1- Non Serious ☐	2- Serious ☐	3- Life-Threatening ☐	4- Fatal ☐
Detectability	1- Very High ☐	2- Moderately high ☐	3- Low ☐	4- Very Low ☐	5- Cannot be detected ☐
Risk Reduction	None (0%) ☐	Limited (25%) ☐	Moderate (50%) ☐	Substantial (75%) ☐	Extensive (95%) ☐
Disease transmission				**Applicable** ☐	**NA** ☐
Probability	1- Rare ☐	2- Unlikely ☐	3- Possible ☐	4- Likely ☐	5- Almost certain ☐
Severity		1- Non Serious ☐	2- Serious ☐	3- Life-Threatening ☐	4- Fatal ☐
Detectability	1- Very High ☐	2- Moderately high ☐	3- Low ☐	4- Very Low ☐	5- Cannot be detected ☐
Risk Reduction	None (0%) ☐	Limited (25%) ☐	Moderate (50%) ☐	Substantial (75%) ☐	Extensive (95%) ☐
Toxicity / Carcinogenicity				**Applicable** ☐	**NA** ☐
Probability	1- Rare ☐	2- Unlikely ☐	3- Possible ☐	4- Likely ☐	5- Almost certain ☐
Severity		1- Non Serious ☐	2- Serious ☐	3- Life-Threatening ☐	4- Fatal ☐
Detectability	1- Very High ☐	2- Moderately high ☐	3- Low ☐	4- Very Low ☐	5- Cannot be detected ☐
Risk Reduction	None (0%) ☐	Limited (25%) ☐	Moderate (50%) ☐	Substantial (75%) ☐	Extensive (95%) ☐
Other (_____)				**Applicable** ☐	**NA** ☐
Probability	1- Rare ☐	2- Unlikely ☐	3- Possible ☐	4- Likely ☐	5- Almost certain ☐
Severity		1- Non Serious ☐	2- Serious ☐	3- Life-Threatening ☐	4- Death ☐
Detectability	1- Very High ☐	2- Moderately high ☐	3- Low ☐	4- Very Low ☐	5- Cannot be detected ☐
Risk Reduction	None (0%) ☐	Limited (25%) ☐	Moderate (50%) ☐	Substantial (75%) ☐	Extensive (95%) ☐

Step 2 – Level of risk analysis

Novelties represent different risks with distinct impact in the quality and safety of the products.

Select the specific risks that apply to this risk Factor (note that some risk factors may not apply to your product/therapy).

Risk Factor: Transport Conditions

Consider any potential risks arising from how the starting material and BTC are transported, for example between the sites of procurement and processing, and between the sites of storage and clinical use.

Applicable **Yes** ☐ **No** ☐

Comments and/or references (optional):

Specific Risks					
Implant failure/ Pregnancy loss				**Applicable** ☐	**NA** ☐
Probability	1- Rare ☐	2- Unlikely ☐	3- Possible ☐	4- Likely ☐	5- Almost certain ☐
Severity		1- Non Serious ☐	2- Serious ☐	3- Life-Threatening ☐	4- Fatal ☐
Detectability	1- Very High ☐	2- Moderately high ☐	3- Low ☐	4- Very Low ☐	5- Cannot be detected ☐
Risk Reduction	None (0%) ☐	Limited (25%) ☐	Moderate (50%) ☐	Substantial (75%) ☐	Extensive (95%) ☐
Disease transmission				**Applicable** ☐	**NA** ☐
Probability	1- Rare ☐	2- Unlikely ☐	3- Possible ☐	4- Likely ☐	5- Almost certain ☐
Severity		1- Non Serious ☐	2- Serious ☐	3- Life-Threatening ☐	4- Fatal ☐
Detectability	1- Very High ☐	2- Moderately high ☐	3- Low ☐	4- Very Low ☐	5- Cannot be detected ☐
Risk Reduction	None (0%) ☐	Limited (25%) ☐	Moderate (50%) ☐	Substantial (75%) ☐	Extensive (95%) ☐
Toxicity / Carcinogenicity				**Applicable** ☐	**NA** ☐
Probability	1- Rare ☐	2- Unlikely ☐	3- Possible ☐	4- Likely ☐	5- Almost certain ☐
Severity		1- Non Serious ☐	2- Serious ☐	3- Life-Threatening ☐	4- Fatal ☐
Detectability	1- Very High ☐	2- Moderately high ☐	3- Low ☐	4- Very Low ☐	5- Cannot be detected ☐
Risk Reduction	None (0%) ☐	Limited (25%) ☐	Moderate (50%) ☐	Substantial (75%) ☐	Extensive (95%) ☐
Other (_____)				**Applicable** ☐	**NA** ☐
Probability	1- Rare ☐	2- Unlikely ☐	3- Possible ☐	4- Likely ☐	5- Almost certain ☐
Severity		1- Non Serious ☐	2- Serious ☐	3- Life-Threatening ☐	4- Fatal ☐
Detectability	1- Very High ☐	2- Moderately high ☐	3- Low ☐	4- Very Low ☐	5- Cannot be detected ☐
Risk Reduction	None (0%) ☐	Limited (25%) ☐	Moderate (50%) ☐	Substantial (75%) ☐	Extensive (95%) ☐

Step 2 – Level of risk analysis

Novelties represent different risks with distinct impact in the quality and safety of the products.

Select the specific risks that apply to this risk Factor (note that some risk factors may not apply to your product/therapy).

Risk Factor: Loss of viability and/or functionality

Consider the risk that the changes in procedures of processes can have on the viability or functionality of the BTC.

Applicable **Yes** ☐ **No** ☐

Comments and/or references (optional):

Specific Risks					
Implant failure/ Pregnancy loss				**Applicable** ☐	**NA** ☐
Probability	1- Rare ☐	2- Unlikely ☐	3- Possible ☐	4- Likely ☐	5- Almost certain ☐
Severity		1- Non Serious ☐	2- Serious ☐	3- Life-Threatening ☐	4- Fatal ☐
Detectability	1- Very High ☐	2- Moderately high ☐	3- Low ☐	4- Very Low ☐	5- Cannot be detected ☐
Risk Reduction	None (0%) ☐	Limited (25%) ☐	Moderate (50%) ☐	Substantial (75%) ☐	Extensive (95%) ☐
Disease transmission				**Applicable** ☐	**NA** ☐
Probability	1- Rare ☐	2- Unlikely ☐	3- Possible ☐	4- Likely ☐	5- Almost certain ☐
Severity		1- Non Serious ☐	2- Serious ☐	3- Life-Threatening ☐	4- Fatal ☐
Detectability	1- Very High ☐	2- Moderately high ☐	3- Low ☐	4- Very Low ☐	5- Cannot be detected ☐
Risk Reduction	None (0%) ☐	Limited (25%) ☐	Moderate (50%) ☐	Substantial (75%) ☐	Extensive (95%) ☐
Toxicity / Carcinogenicity				**Applicable** ☐	**NA** ☐
Probability	1- Rare ☐	2- Unlikely ☐	3- Possible ☐	4- Likely ☐	5- Almost certain ☐
Severity		1- Non Serious ☐	2- Serious ☐	3- Life-Threatening ☐	4- Fatal ☐
Detectability	1- Very High ☐	2- Moderately high ☐	3- Low ☐	4- Very Low ☐	5- Cannot be detected ☐
Risk Reduction	None (0%) ☐	Limited (25%) ☐	Moderate (50%) ☐	Substantial (75%) ☐	Extensive (95%) ☐
Other (_____)				**Applicable** ☐	**NA** ☐
Probability	1- Rare ☐	2- Unlikely ☐	3- Possible ☐	4- Likely ☐	5- Almost certain ☐
Severity		1- Non Serious ☐	2- Serious ☐	3- Life-Threatening ☐	4- Fatal ☐
Detectability	1- Very High ☐	2- Moderately high ☐	3- Low ☐	4- Very Low ☐	5- Cannot be detected ☐
Risk Reduction	None (0%) ☐	Limited (25%) ☐	Moderate (50%) ☐	Substantial (75%) ☐	Extensive (95%) ☐

Step 2 – Level of risk analysis

Novelties represent different risks with distinct impact in the quality and safety of the products.

Select the specific risks that apply to this risk Factor (note that some risk factors may not apply to your product/therapy).

Risk Factor: Complexity of the pre-implantation preparation and/or application method

Consider how complex the method of clinical use will be for this BTC. Are there any novelties in this step? How long will it take, and could this introduce risks? What is the scope for errors to be made, and what could the consequences of these errors be? Low feasibility of application standardization might have influence in the risks of implant failure and disease transmission at least.

Applicable **Yes** ☐ **No** ☐

Comments and/or references (optional):

Specific Risks					
Implant failure/ Pregnancy loss				**Applicable** ☐	**NA** ☐
Probability	1- Rare ☐	2- Unlikely ☐	3- Possible ☐	4- Likely ☐	5- Almost certain ☐
Severity		1- Non Serious ☐	2- Serious ☐	3- Life-Threatening ☐	4- Fatal ☐
Detectability	1- Very High ☐	2- Moderately high ☐	3- Low ☐	4- Very Low ☐	5- Cannot be detected ☐
Risk Reduction	None (0%) ☐	Limited (25%) ☐	Moderate (50%) ☐	Substantial (75%) ☐	Extensive (95%) ☐
Disease transmission				**Applicable** ☐	**NA** ☐
Probability	1- Rare ☐	2- Unlikely ☐	3- Possible ☐	4- Likely ☐	5- Almost certain ☐
Severity		1- Non Serious ☐	2- Serious ☐	3- Life-Threatening ☐	4- Fatal ☐
Detectability	1- Very High ☐	2- Moderately high ☐	3- Low ☐	4- Very Low ☐	5- Cannot be detected ☐
Risk Reduction	None (0%) ☐	Limited (25%) ☐	Moderate (50%) ☐	Substantial (75%) ☐	Extensive (95%) ☐
Toxicity / Carcinogenicity				**Applicable** ☐	**NA** ☐
Probability	1- Rare ☐	2- Unlikely ☐	3- Possible ☐	4- Likely ☐	5- Almost certain ☐
Severity		1- Non Serious ☐	2- Serious ☐	3- Life-Threatening ☐	4- Fatal ☐
Detectability	1- Very High ☐	2- Moderately high ☐	3- Low ☐	4- Very Low ☐	5- Cannot be detected ☐
Risk Reduction	None (0%) ☐	Limited (25%) ☐	Moderate (50%) ☐	Substantial (75%) ☐	Extensive (95%) ☐
Other (_____)				**Applicable** ☐	**NA** ☐
Probability	1- Rare ☐	2- Unlikely ☐	3- Possible ☐	4- Likely ☐	5- Almost certain ☐
Severity		1- Non Serious ☐	2- Serious ☐	3- Life-Threatening ☐	4- Fatal ☐
Detectability	1- Very High ☐	2- Moderately high ☐	3- Low ☐	4- Very Low ☐	5- Cannot be detected ☐
Risk Reduction	None (0%) ☐	Limited (25%) ☐	Moderate (50%) ☐	Substantial (75%) ☐	Extensive (95%) ☐

Methodologies for Assessing the Risks
associated to novel Blood, Tissue and Cell Component (BTCs).
-MAR (Gonadic Tissue) Template-

Please follow the EuroGTP II Guide in order to correctly evaluate your BTCs

BTC characterisation

The evaluation of the level of novelty and the risks associated, should start with a characterisation of the novel process or BTC.

General category: Medically assisted reproduction

Specific category:

☐ Gonadic Tissue

Name of the product, therapy or process under evaluation:

Description of BTC under evaluation:
(Describe the relevant aspects of the BTC, detailing the modifications/novelties associated with **donation**, **processing** and **clinical application** under evaluation)

Step 1 – Evaluation of novelty

The purpose of this exercise is to evaluate our proposed methodology for determining if a BTC, therapy or process is novel or not.
Please answer the following questions in order to determine if the product or process is novel. This process represents the first stage of the overall procedure for evaluating novelty and risk.

	Yes	No	Not Applicable/ Not Relevant
A. Has this type of BTC previously been collected, processed/ prepared and issued for clinical use by your establishment?			
Comment (optional):			
B. Will the starting material used to prepare this BTC be obtained from the same donor population previously used by your establishment for this type of BTC?			
Comment (optional):			
C. Will the starting material for this BTC be procured/collected using a procedure used previously by your establishment for this type of BTC?			
Comment (optional):			
D. Will this BTC be prepared by a procedure (processing, decontamination and preservation) used previously in your establishment for this type of BTC?			
Comment (optional):			
E. Will this BTC be packaged, stored, and distributed using a protocol and materials used previously in your establishment for this type of BTC?			
Comment (optional):			
F. Will this type of BTC provided by your establishment be applied/infused clinically using an application/transfusion/infusion method used previously?			
Comment (optional):			
G. Has your establishment provided this type of BTC for the same clinical indication or for application/transfusion/infusion into a same anatomical site?			
Comment (optional):			

- MAR (Gonadic Tissue) Template-

Step 2 – Level of risk analysis

Novelties represent different risks with distinct impact in the quality and safety of the products.

Select the specific risks that apply to this risk Factor (note that some risk factors may not apply to your product/therapy).

Risk Factor: Donor Characteristics

Consider whether the novelty in your process or procedures change donor characteristics and if these changes could impart a risk to the recipient.

Applicable Yes ☐ No ☐

Comments and/or references (optional):

Specific Risks					
Unexpected immunogenicity				**Applicable** ☐	**NA** ☐
Probability	1- Rare ☐	2- Unlikely ☐	3- Possible ☐	4- Likely ☐	5- Almost certain ☐
Severity		1- Non Serious ☐	2- Serious ☐	3- Life-Threatening ☐	4- Fatal ☐
Detectability	1- Very High ☐	2- Moderately high ☐	3- Low ☐	4- Very Low ☐	5- Cannot be detected ☐
Risk Reduction	None (0%) ☐	Limited (25%) ☐	Moderate (50%) ☐	Substantial (75%) ☐	Extensive (95%) ☐
Implant failure/Pregnancy loss				**Applicable** ☐	**NA** ☐
Probability	1- Rare ☐	2- Unlikely ☐	3- Possible ☐	4- Likely ☐	5- Almost certain ☐
Severity		1- Non Serious ☐	2- Serious ☐	3- Life-Threatening ☐	4- Fatal ☐
Detectability	1- Very High ☐	2- Moderately high ☐	3- Low ☐	4- Very Low ☐	5- Cannot be detected ☐
Risk Reduction	None (0%) ☐	Limited (25%) ☐	Moderate (50%) ☐	Substantial (75%) ☐	Extensive (95%) ☐
Disease transmission				**Applicable** ☐	**NA** ☐
Probability	1- Rare ☐	2- Unlikely ☐	3- Possible ☐	4- Likely ☐	5- Almost certain ☐
Severity		1- Non Serious ☐	2- Serious ☐	3- Life-Threatening ☐	4- Fatal ☐
Detectability	1- Very High ☐	2- Moderately high ☐	3- Low ☐	4- Very Low ☐	5- Cannot be detected ☐
Risk Reduction	None (0%) ☐	Limited (25%) ☐	Moderate (50%) ☐	Substantial (75%) ☐	Extensive (95%) ☐
Toxicity / Carcinogenicity				**Applicable** ☐	**NA** ☐
Probability	1- Rare ☐	2- Unlikely ☐	3- Possible ☐	4- Likely ☐	5- Almost certain ☐
Severity		1- Non Serious ☐	2- Serious ☐	3- Life-Threatening ☐	4- Fatal ☐
Detectability	1- Very High ☐	2- Moderately high ☐	3- Low ☐	4- Very Low ☐	5- Cannot be detected ☐
Risk Reduction	None (0%) ☐	Limited (25%) ☐	Moderate (50%) ☐	Substantial (75%) ☐	Extensive (95%) ☐
Other (_____)				**Applicable** ☐	**NA** ☐
Probability	1- Rare ☐	2- Unlikely ☐	3- Possible ☐	4- Likely ☐	5- Almost certain ☐
Severity		1- Non Serious ☐	2- Serious ☐	3- Life-Threatening ☐	4- Death ☐
Detectability	1- Very High ☐	2- Moderately high ☐	3- Low ☐	4- Very Low ☐	5- Cannot be detected ☐
Risk Reduction	None (0%) ☐	Limited (25%) ☐	Moderate (50%) ☐	Substantial (75%) ☐	Extensive (95%) ☐

Step 2 – Level of risk analysis

Novelties represent different risks with distinct impact in the quality and safety of the products.

Select the specific risks that apply to this risk Factor (note that some risk factors may not apply to your product/therapy).

Risk Factor: Procurement process and environment

Consider where and how the BTC is recovered now and how this would happen when the novelty is taken into account. Is there a change in recovery time, complexity, quality of the environment

Applicable **Yes** ☐ **No** ☐

Comments and/or references (optional):

Specific Risks					
Unexpected immunogenicity				**Applicable** ☐	**NA** ☐
Probability	1- Rare ☐	2- Unlikely ☐	3- Possible ☐	4- Likely ☐	5- Almost certain ☐
Severity		1- Non Serious ☐	2- Serious ☐	3- Life-Threatening ☐	4- Fatal ☐
Detectability	1- Very High ☐	2- Moderately high ☐	3- Low ☐	4- Very Low ☐	5- Cannot be detected ☐
Risk Reduction	None (0%) ☐	Limited (25%) ☐	Moderate (50%) ☐	Substantial (75%) ☐	Extensive (95%) ☐
Implant failure/Pregnancy loss				**Applicable** ☐	**NA** ☐
Probability	1- Rare ☐	2- Unlikely ☐	3- Possible ☐	4- Likely ☐	5- Almost certain ☐
Severity		1- Non Serious ☐	2- Serious ☐	3- Life-Threatening ☐	4- Fatal ☐
Detectability	1- Very High ☐	2- Moderately high ☐	3- Low ☐	4- Very Low ☐	5- Cannot be detected ☐
Risk Reduction	None (0%) ☐	Limited (25%) ☐	Moderate (50%) ☐	Substantial (75%) ☐	Extensive (95%) ☐
Disease transmission				**Applicable** ☐	**NA** ☐
Probability	1- Rare ☐	2- Unlikely ☐	3- Possible ☐	4- Likely ☐	5- Almost certain ☐
Severity		1- Non Serious ☐	2- Serious ☐	3- Life-Threatening ☐	4- Fatal ☐
Detectability	1- Very High ☐	2- Moderately high ☐	3- Low ☐	4- Very Low ☐	5- Cannot be detected ☐
Risk Reduction	None (0%) ☐	Limited (25%) ☐	Moderate (50%) ☐	Substantial (75%) ☐	Extensive (95%) ☐
Toxicity / Carcinogenicity				**Applicable** ☐	**NA** ☐
Probability	1- Rare ☐	2- Unlikely ☐	3- Possible ☐	4- Likely ☐	5- Almost certain ☐
Severity		1- Non Serious ☐	2- Serious ☐	3- Life-Threatening ☐	4- Fatal ☐
Detectability	1- Very High ☐	2- Moderately high ☐	3- Low ☐	4- Very Low ☐	5- Cannot be detected ☐
Risk Reduction	None (0%) ☐	Limited (25%) ☐	Moderate (50%) ☐	Substantial (75%) ☐	Extensive (95%) ☐
Other (_____)				**Applicable** ☐	**NA** ☐
Probability	1- Rare ☐	2- Unlikely ☐	3- Possible ☐	4- Likely ☐	5- Almost certain ☐
Severity		1- Non Serious ☐	2- Serious ☐	3- Life-Threatening ☐	4- Death ☐
Detectability	1- Very High ☐	2- Moderately high ☐	3- Low ☐	4- Very Low ☐	5- Cannot be detected ☐
Risk Reduction	None (0%) ☐	Limited (25%) ☐	Moderate (50%) ☐	Substantial (75%) ☐	Extensive (95%) ☐

Step 2 – Level of risk analysis

Novelties represent different risks with distinct impact in the quality and safety of the products.

Select the specific risks that apply to this risk Factor (note that some risk factors may not apply to your product/therapy).

Risk Factor: Processing and environment

Consider where and how the BTC is prepared at the moment and how the novelty in the process can affect this step. How long does the novel preparation process take and how complex it is – this may impact the risk of contamination, or it may not be prepared to consistent specifications and quality. Also consider the quality of the preparation environment, which may also affect the risk of contamination.

Applicable **Yes** ☐ **No** ☐

Comments and/or references (optional):

Specific Risks					
Unexpected immunogenicity				**Applicable** ☐	**NA** ☐
Probability	1- Rare ☐	2- Unlikely ☐	3- Possible ☐	4- Likely ☐	5- Almost certain ☐
Severity		1- Non Serious ☐	2- Serious ☐	3- Life-Threatening ☐	4- Fatal ☐
Detectability	1- Very High ☐	2- Moderately high ☐	3- Low ☐	4- Very Low ☐	5- Cannot be detected ☐
Risk Reduction	None (0%) ☐	Limited (25%) ☐	Moderate (50%) ☐	Substantial (75%) ☐	Extensive (95%) ☐
Implant failure/Pregnancy loss				**Applicable** ☐	**NA** ☐
Probability	1- Rare ☐	2- Unlikely ☐	3- Possible ☐	4- Likely ☐	5- Almost certain ☐
Severity		1- Non Serious ☐	2- Serious ☐	3- Life-Threatening ☐	4- Fatal ☐
Detectability	1- Very High ☐	2- Moderately high ☐	3- Low ☐	4- Very Low ☐	5- Cannot be detected ☐
Risk Reduction	None (0%) ☐	Limited (25%) ☐	Moderate (50%) ☐	Substantial (75%) ☐	Extensive (95%) ☐
Disease transmission				**Applicable** ☐	**NA** ☐
Probability	1- Rare ☐	2- Unlikely ☐	3- Possible ☐	4- Likely ☐	5- Almost certain ☐
Severity		1- Non Serious ☐	2- Serious ☐	3- Life-Threatening ☐	4- Fatal ☐
Detectability	1- Very High ☐	2- Moderately high ☐	3- Low ☐	4- Very Low ☐	5- Cannot be detected ☐
Risk Reduction	None (0%) ☐	Limited (25%) ☐	Moderate (50%) ☐	Substantial (75%) ☐	Extensive (95%) ☐
Toxicity / Carcinogenicity				**Applicable** ☐	**NA** ☐
Probability	1- Rare ☐	2- Unlikely ☐	3- Possible ☐	4- Likely ☐	5- Almost certain ☐
Severity		1- Non Serious ☐	2- Serious ☐	3- Life-Threatening ☐	4- Fatal ☐
Detectability	1- Very High ☐	2- Moderately high ☐	3- Low ☐	4- Very Low ☐	5- Cannot be detected ☐
Risk Reduction	None (0%) ☐	Limited (25%) ☐	Moderate (50%) ☐	Substantial (75%) ☐	Extensive (95%) ☐
Other (_____)				**Applicable** ☐	**NA** ☐
Probability	1- Rare ☐	2- Unlikely ☐	3- Possible ☐	4- Likely ☐	5- Almost certain ☐
Severity		1- Non Serious ☐	2- Serious ☐	3- Life-Threatening ☐	4- Death ☐
Detectability	1- Very High ☐	2- Moderately high ☐	3- Low ☐	4- Very Low ☐	5- Cannot be detected ☐
Risk Reduction	None (0%) ☐	Limited (25%) ☐	Moderate (50%) ☐	Substantial (75%) ☐	Extensive (95%) ☐

Step 2 – Level of risk analysis

Novelties represent different risks with distinct impact in the quality and safety of the products.

Select the specific risks that apply to this risk Factor (note that some risk factors may not apply to your product/therapy).

Risk Factor: Reagents

Consider any reagents used during recovery, processing, preparation, decontamination and storage of the BTC. Could they damage the BTC in any way, or could residual traces of reagent remain in the BTC that could cause toxic or immunogenic effects in recipients?

Applicable **Yes** ☐ **No** ☐

Comments and/or references (optional):

Specific Risks					
Unexpected immunogenicity				**Applicable** ☐	**NA** ☐
Probability	1- Rare ☐	2- Unlikely ☐	3- Possible ☐	4- Likely ☐	5- Almost certain ☐
Severity		1- Non Serious ☐	2- Serious ☐	3- Life-Threatening ☐	4- Fatal ☐
Detectability	1- Very High ☐	2- Moderately high ☐	3- Low ☐	4- Very Low ☐	5- Cannot be detected ☐
Risk Reduction	None (0%) ☐	Limited (25%) ☐	Moderate (50%) ☐	Substantial (75%) ☐	Extensive (95%) ☐
Implant failure/Pregnancy loss				**Applicable** ☐	**NA** ☐
Probability	1- Rare ☐	2- Unlikely ☐	3- Possible ☐	4- Likely ☐	5- Almost certain ☐
Severity		1- Non Serious ☐	2- Serious ☐	3- Life-Threatening ☐	4- Fatal ☐
Detectability	1- Very High ☐	2- Moderately high ☐	3- Low ☐	4- Very Low ☐	5- Cannot be detected ☐
Risk Reduction	None (0%) ☐	Limited (25%) ☐	Moderate (50%) ☐	Substantial (75%) ☐	Extensive (95%) ☐
Disease transmission				**Applicable** ☐	**NA** ☐
Probability	1- Rare ☐	2- Unlikely ☐	3- Possible ☐	4- Likely ☐	5- Almost certain ☐
Severity		1- Non Serious ☐	2- Serious ☐	3- Life-Threatening ☐	4- Fatal ☐
Detectability	1- Very High ☐	2- Moderately high ☐	3- Low ☐	4- Very Low ☐	5- Cannot be detected ☐
Risk Reduction	None (0%) ☐	Limited (25%) ☐	Moderate (50%) ☐	Substantial (75%) ☐	Extensive (95%) ☐
Toxicity / Carcinogenicity				**Applicable** ☐	**NA** ☐
Probability	1- Rare ☐	2- Unlikely ☐	3- Possible ☐	4- Likely ☐	5- Almost certain ☐
Severity		1- Non Serious ☐	2- Serious ☐	3- Life-Threatening ☐	4- Fatal ☐
Detectability	1- Very High ☐	2- Moderately high ☐	3- Low ☐	4- Very Low ☐	5- Cannot be detected ☐
Risk Reduction	None (0%) ☐	Limited (25%) ☐	Moderate (50%) ☐	Substantial (75%) ☐	Extensive (95%) ☐
Other (_____)				**Applicable** ☐	**NA** ☐
Probability	1- Rare ☐	2- Unlikely ☐	3- Possible ☐	4- Likely ☐	5- Almost certain ☐
Severity		1- Non Serious ☐	2- Serious ☐	3- Life-Threatening ☐	4- Death ☐
Detectability	1- Very High ☐	2- Moderately high ☐	3- Low ☐	4- Very Low ☐	5- Cannot be detected ☐
Risk Reduction	None (0%) ☐	Limited (25%) ☐	Moderate (50%) ☐	Substantial (75%) ☐	Extensive (95%) ☐

Step 2 – Level of risk analysis

Novelties represent different risks with distinct impact in the quality and safety of the products.

Select the specific risks that apply to this risk Factor (note that some risk factors may not apply to your product/therapy).

Risk Factor: Storage conditions

Consider any potential risks arising from how the starting material and BTC are stored, not only after processing and before clinical application, but also in intermediate steps: e.g. between procurement and processing, during processing, and between processing steps.

Applicable **Yes** ☐ **No** ☐

Comments and/or references (optional):

Specific Risks					
Unexpected immunogenicity				**Applicable** ☐	**NA** ☐
Probability	1- Rare ☐	2- Unlikely ☐	3- Possible ☐	4- Likely ☐	5- Almost certain ☐
Severity		1- Non Serious ☐	2- Serious ☐	3- Life-Threatening ☐	4- Fatal ☐
Detectability	1- Very High ☐	2- Moderately high ☐	3- Low ☐	4- Very Low ☐	5- Cannot be detected ☐
Risk Reduction	None (0%) ☐	Limited (25%) ☐	Moderate (50%) ☐	Substantial (75%) ☐	Extensive (95%) ☐
Implant failure/Pregnancy loss				**Applicable** ☐	**NA** ☐
Probability	1- Rare ☐	2- Unlikely ☐	3- Possible ☐	4- Likely ☐	5- Almost certain ☐
Severity		1- Non Serious ☐	2- Serious ☐	3- Life-Threatening ☐	4- Fatal ☐
Detectability	1- Very High ☐	2- Moderately high ☐	3- Low ☐	4- Very Low ☐	5- Cannot be detected ☐
Risk Reduction	None (0%) ☐	Limited (25%) ☐	Moderate (50%) ☐	Substantial (75%) ☐	Extensive (95%) ☐
Disease transmission				**Applicable** ☐	**NA** ☐
Probability	1- Rare ☐	2- Unlikely ☐	3- Possible ☐	4- Likely ☐	5- Almost certain ☐
Severity		1- Non Serious ☐	2- Serious ☐	3- Life-Threatening ☐	4- Fatal ☐
Detectability	1- Very High ☐	2- Moderately high ☐	3- Low ☐	4- Very Low ☐	5- Cannot be detected ☐
Risk Reduction	None (0%) ☐	Limited (25%) ☐	Moderate (50%) ☐	Substantial (75%) ☐	Extensive (95%) ☐
Toxicity / Carcinogenicity				**Applicable** ☐	**NA** ☐
Probability	1- Rare ☐	2- Unlikely ☐	3- Possible ☐	4- Likely ☐	5- Almost certain ☐
Severity		1- Non Serious ☐	2- Serious ☐	3- Life-Threatening ☐	4- Fatal ☐
Detectability	1- Very High ☐	2- Moderately high ☐	3- Low ☐	4- Very Low ☐	5- Cannot be detected ☐
Risk Reduction	None (0%) ☐	Limited (25%) ☐	Moderate (50%) ☐	Substantial (75%) ☐	Extensive (95%) ☐
Other (_____)				**Applicable** ☐	**NA** ☐
Probability	1- Rare ☐	2- Unlikely ☐	3- Possible ☐	4- Likely ☐	5- Almost certain ☐
Severity		1- Non Serious ☐	2- Serious ☐	3- Life-Threatening ☐	4- Death ☐
Detectability	1- Very High ☐	2- Moderately high ☐	3- Low ☐	4- Very Low ☐	5- Cannot be detected ☐
Risk Reduction	None (0%) ☐	Limited (25%) ☐	Moderate (50%) ☐	Substantial (75%) ☐	Extensive (95%) ☐

Step 2 – Level of risk analysis

Novelties represent different risks with distinct impact in the quality and safety of the products.

Select the specific risks that apply to this risk Factor (note that some risk factors may not apply to your product/therapy).

Risk Factor: Transport conditions

Consider any potential risks arising from how the starting material and BTC are transported, for example between the sites of procurement and processing, and between the sites of storage and clinical use.

Applicable **Yes** ☐ **No** ☐

Comments and/or references (optional):

Specific Risks					
Unexpected immunogenicity				**Applicable** ☐	**NA** ☐
Probability	1- Rare ☐	2- Unlikely ☐	3- Possible ☐	4- Likely ☐	5- Almost certain ☐
Severity		1- Non Serious ☐	2- Serious ☐	3- Life-Threatening ☐	4- Fatal ☐
Detectability	1- Very High ☐	2- Moderately high ☐	3- Low ☐	4- Very Low ☐	5- Cannot be detected ☐
Risk Reduction	None (0%) ☐	Limited (25%) ☐	Moderate (50%) ☐	Substantial (75%) ☐	Extensive (95%) ☐
Implant failure/Pregnancy loss				**Applicable** ☐	**NA** ☐
Probability	1- Rare ☐	2- Unlikely ☐	3- Possible ☐	4- Likely ☐	5- Almost certain ☐
Severity		1- Non Serious ☐	2- Serious ☐	3- Life-Threatening ☐	4- Fatal ☐
Detectability	1- Very High ☐	2- Moderately high ☐	3- Low ☐	4- Very Low ☐	5- Cannot be detected ☐
Risk Reduction	None (0%) ☐	Limited (25%) ☐	Moderate (50%) ☐	Substantial (75%) ☐	Extensive (95%) ☐
Disease transmission				**Applicable** ☐	**NA** ☐
Probability	1- Rare ☐	2- Unlikely ☐	3- Possible ☐	4- Likely ☐	5- Almost certain ☐
Severity		1- Non Serious ☐	2- Serious ☐	3- Life-Threatening ☐	4- Fatal ☐
Detectability	1- Very High ☐	2- Moderately high ☐	3- Low ☐	4- Very Low ☐	5- Cannot be detected ☐
Risk Reduction	None (0%) ☐	Limited (25%) ☐	Moderate (50%) ☐	Substantial (75%) ☐	Extensive (95%) ☐
Toxicity / Carcinogenicity				**Applicable** ☐	**NA** ☐
Probability	1- Rare ☐	2- Unlikely ☐	3- Possible ☐	4- Likely ☐	5- Almost certain ☐
Severity		1- Non Serious ☐	2- Serious ☐	3- Life-Threatening ☐	4- Fatal ☐
Detectability	1- Very High ☐	2- Moderately high ☐	3- Low ☐	4- Very Low ☐	5- Cannot be detected ☐
Risk Reduction	None (0%) ☐	Limited (25%) ☐	Moderate (50%) ☐	Substantial (75%) ☐	Extensive (95%) ☐
Other (_____)				**Applicable** ☐	**NA** ☐
Probability	1- Rare ☐	2- Unlikely ☐	3- Possible ☐	4- Likely ☐	5- Almost certain ☐
Severity		1- Non Serious ☐	2- Serious ☐	3- Life-Threatening ☐	4- Death ☐
Detectability	1- Very High ☐	2- Moderately high ☐	3- Low ☐	4- Very Low ☐	5- Cannot be detected ☐
Risk Reduction	None (0%) ☐	Limited (25%) ☐	Moderate (50%) ☐	Substantial (75%) ☐	Extensive (95%) ☐

Step 2 – Level of risk analysis

Novelties represent different risks with distinct impact in the quality and safety of the products.

Select the specific risks that apply to this risk Factor (note that some risk factors may not apply to your product/therapy).

Risk Factor: Reliability of Microbiology Testing

Consider the risk that the testing methodology and/or presence of residual processing reagents such as antibiotics in the finished BTC may impact the accuracy of any microbiology/mycology testing of the BTC. This risk factor is not about blood tests on the donor.

Applicable **Yes** ☐ **No** ☐

Comments and/or references (optional):

Specific Risks					
Unexpected immunogenicity				**Applicable** ☐	**NA** ☐
Probability	1- Rare ☐	2- Unlikely ☐	3- Possible ☐	4- Likely ☐	5- Almost certain ☐
Severity		1- Non Serious ☐	2- Serious ☐	3- Life-Threatening ☐	4- Fatal ☐
Detectability	1- Very High ☐	2- Moderately high ☐	3- Low ☐	4- Very Low ☐	5- Cannot be detected ☐
Risk Reduction	None (0%) ☐	Limited (25%) ☐	Moderate (50%) ☐	Substantial (75%) ☐	Extensive (95%) ☐
Implant failure/Pregnancy loss				**Applicable** ☐	**NA** ☐
Probability	1- Rare ☐	2- Unlikely ☐	3- Possible ☐	4- Likely ☐	5- Almost certain ☐
Severity		1- Non Serious ☐	2- Serious ☐	3- Life-Threatening ☐	4- Fatal ☐
Detectability	1- Very High ☐	2- Moderately high ☐	3- Low ☐	4- Very Low ☐	5- Cannot be detected ☐
Risk Reduction	None (0%) ☐	Limited (25%) ☐	Moderate (50%) ☐	Substantial (75%) ☐	Extensive (95%) ☐
Disease transmission				**Applicable** ☐	**NA** ☐
Probability	1- Rare ☐	2- Unlikely ☐	3- Possible ☐	4- Likely ☐	5- Almost certain ☐
Severity		1- Non Serious ☐	2- Serious ☐	3- Life-Threatening ☐	4- Fatal ☐
Detectability	1- Very High ☐	2- Moderately high ☐	3- Low ☐	4- Very Low ☐	5- Cannot be detected ☐
Risk Reduction	None (0%) ☐	Limited (25%) ☐	Moderate (50%) ☐	Substantial (75%) ☐	Extensive (95%) ☐
Toxicity / Carcinogenicity				**Applicable** ☐	**NA** ☐
Probability	1- Rare ☐	2- Unlikely ☐	3- Possible ☐	4- Likely ☐	5- Almost certain ☐
Severity		1- Non Serious ☐	2- Serious ☐	3- Life-Threatening ☐	4- Fatal ☐
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Risk Reduction	None (0%) ☐	Limited (25%) ☐	Moderate (50%) ☐	Substantial (75%) ☐	Extensive (95%) ☐
Other (_____)				**Applicable** ☐	**NA** ☐
Probability	1- Rare ☐	2- Unlikely ☐	3- Possible ☐	4- Likely ☐	5- Almost certain ☐
Severity		1- Non Serious ☐	2- Serious ☐	3- Life-Threatening ☐	4- Death ☐
Detectability	1- Very High ☐	2- Moderately high ☐	3- Low ☐	4- Very Low ☐	5- Cannot be detected ☐
Risk Reduction	None (0%) ☐	Limited (25%) ☐	Moderate (50%) ☐	Substantial (75%) ☐	Extensive (95%) ☐

- MAR (Gonadic Tissue) Template-

Step 2 – Level of risk analysis

Novelties represent different risks with distinct impact in the quality and safety of the products.

Select the specific risks that apply to this risk Factor (note that some risk factors may not apply to your product/therapy).

Risk Factor: Presence of unwanted cellular material and/or graft vascularity

This risk must be considered from the perspective that for some BTCs, the presence of intact vital cells is desirable, although it may also increase risks of, for example, immunogenicity or disease transmission. This presence might affect to tumor formation, immunogenicity and disease transmission risks. Vascular tissues may be more at risk to infiltration by pathogens or malignant cells than avascular tissues.

Applicable Yes ☐ No ☐

Comments and/or references (optional):

Specific Risks					
Unexpected immunogenicity				**Applicable** ☐	**NA** ☐
Probability	1- Rare ☐	2- Unlikely ☐	3- Possible ☐	4- Likely ☐	5- Almost certain ☐
Severity		1- Non Serious ☐	2- Serious ☐	3- Life-Threatening ☐	4- Fatal ☐
Detectability	1- Very High ☐	2- Moderately high ☐	3- Low ☐	4- Very Low ☐	5- Cannot be detected ☐
Risk Reduction	None (0%) ☐	Limited (25%) ☐	Moderate (50%) ☐	Substantial (75%) ☐	Extensive (95%) ☐
Implant failure/Pregnancy loss				**Applicable** ☐	**NA** ☐
Probability	1- Rare ☐	2- Unlikely ☐	3- Possible ☐	4- Likely ☐	5- Almost certain ☐
Severity		1- Non Serious ☐	2- Serious ☐	3- Life-Threatening ☐	4- Fatal ☐
Detectability	1- Very High ☐	2- Moderately high ☐	3- Low ☐	4- Very Low ☐	5- Cannot be detected ☐
Risk Reduction	None (0%) ☐	Limited (25%) ☐	Moderate (50%) ☐	Substantial (75%) ☐	Extensive (95%) ☐
Disease transmission				**Applicable** ☐	**NA** ☐
Probability	1- Rare ☐	2- Unlikely ☐	3- Possible ☐	4- Likely ☐	5- Almost certain ☐
Severity		1- Non Serious ☐	2- Serious ☐	3- Life-Threatening ☐	4- Fatal ☐
Detectability	1- Very High ☐	2- Moderately high ☐	3- Low ☐	4- Very Low ☐	5- Cannot be detected ☐
Risk Reduction	None (0%) ☐	Limited (25%) ☐	Moderate (50%) ☐	Substantial (75%) ☐	Extensive (95%) ☐
Toxicity / Carcinogenicity				**Applicable** ☐	**NA** ☐
Probability	1- Rare ☐	2- Unlikely ☐	3- Possible ☐	4- Likely ☐	5- Almost certain ☐
Severity		1- Non Serious ☐	2- Serious ☐	3- Life-Threatening ☐	4- Fatal ☐
Detectability	1- Very High ☐	2- Moderately high ☐	3- Low ☐	4- Very Low ☐	5- Cannot be detected ☐
Risk Reduction	None (0%) ☐	Limited (25%) ☐	Moderate (50%) ☐	Substantial (75%) ☐	Extensive (95%) ☐
Other (_____)				**Applicable** ☐	**NA** ☐
Probability	1- Rare ☐	2- Unlikely ☐	3- Possible ☐	4- Likely ☐	5- Almost certain ☐
Severity		1- Non Serious ☐	2- Serious ☐	3- Life-Threatening ☐	4- Death ☐
Detectability	1- Very High ☐	2- Moderately high ☐	3- Low ☐	4- Very Low ☐	5- Cannot be detected ☐
Risk Reduction	None (0%) ☐	Limited (25%) ☐	Moderate (50%) ☐	Substantial (75%) ☐	Extensive (95%) ☐

Step 2 – Level of risk analysis

Novelties represent different risks with distinct impact in the quality and safety of the products.

Select the specific risks that apply to this risk Factor (note that some risk factors may not apply to your product/therapy).

Risk Factor: Complexity of the pre-implantation preparation and/or application method

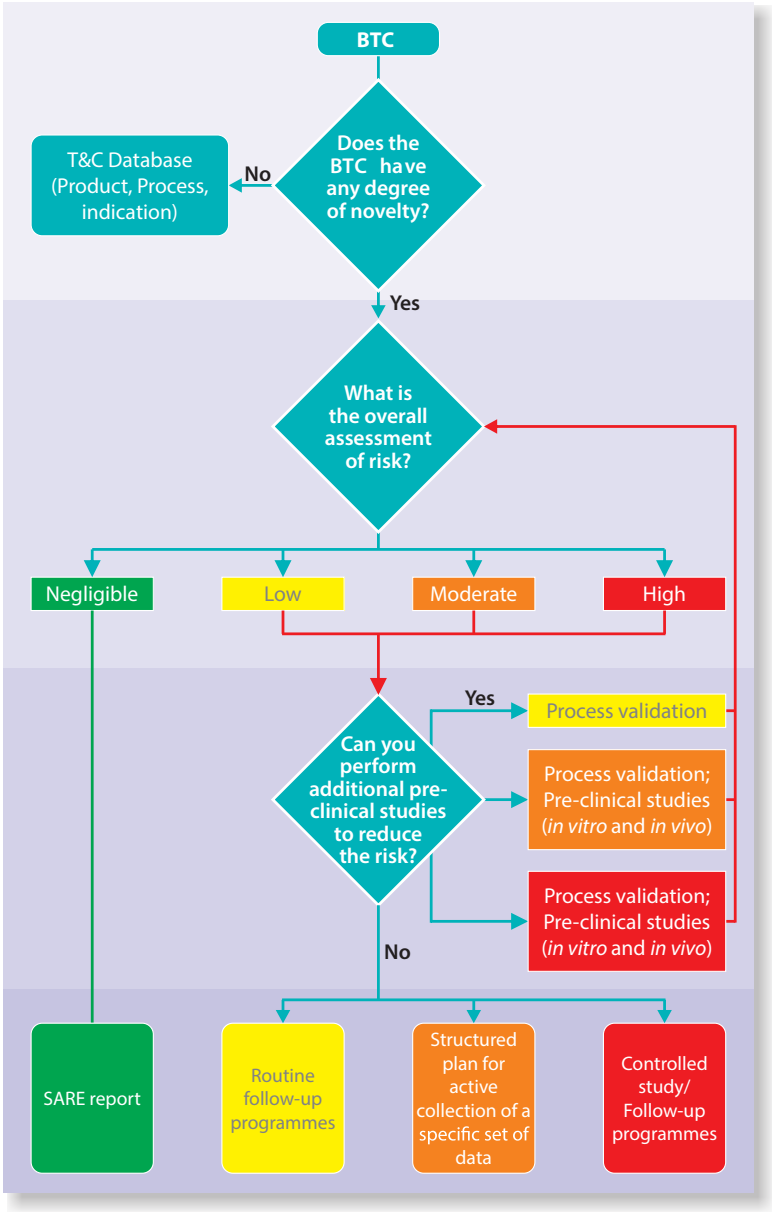
Consider the method of clinical use for this BTC. Are there any novelties in this step? How long will it take, and could this introduce risks? What is the scope for errors to be made, and what could the consequences of these errors be? Low feasibility of application standardization might have influence in the risks of implant failure and disease transmission at least.

Applicable **Yes** ☐ **No** ☐

Comments and/or references (optional):

Specific Risks					
Unexpected immunogenicity				**Applicable** ☐	**NA** ☐
Probability	1- Rare ☐	2- Unlikely ☐	3- Possible ☐	4- Likely ☐	5- Almost certain ☐
Severity		1- Non Serious ☐	2- Serious ☐	3- Life-Threatening ☐	4- Fatal ☐
Detectability	1- Very High ☐	2- Moderately high ☐	3- Low ☐	4- Very Low ☐	5- Cannot be detected ☐
Risk Reduction	None (0%) ☐	Limited (25%) ☐	Moderate (50%) ☐	Substantial (75%) ☐	Extensive (95%) ☐
Implant failure/Pregnancy loss				**Applicable** ☐	**NA** ☐
Probability	1- Rare ☐	2- Unlikely ☐	3- Possible ☐	4- Likely ☐	5- Almost certain ☐
Severity		1- Non Serious ☐	2- Serious ☐	3- Life-Threatening ☐	4- Fatal ☐
Detectability	1- Very High ☐	2- Moderately high ☐	3- Low ☐	4- Very Low ☐	5- Cannot be detected ☐
Risk Reduction	None (0%) ☐	Limited (25%) ☐	Moderate (50%) ☐	Substantial (75%) ☐	Extensive (95%) ☐
Disease transmission				**Applicable** ☐	**NA** ☐
Probability	1- Rare ☐	2- Unlikely ☐	3- Possible ☐	4- Likely ☐	5- Almost certain ☐
Severity		1- Non Serious ☐	2- Serious ☐	3- Life-Threatening ☐	4- Fatal ☐
Detectability	1- Very High ☐	2- Moderately high ☐	3- Low ☐	4- Very Low ☐	5- Cannot be detected ☐
Risk Reduction	None (0%) ☐	Limited (25%) ☐	Moderate (50%) ☐	Substantial (75%) ☐	Extensive (95%) ☐
Toxicity / Carcinogenicity				**Applicable** ☐	**NA** ☐
Probability	1- Rare ☐	2- Unlikely ☐	3- Possible ☐	4- Likely ☐	5- Almost certain ☐
Severity		1- Non Serious ☐	2- Serious ☐	3- Life-Threatening ☐	4- Fatal ☐
Detectability	1- Very High ☐	2- Moderately high ☐	3- Low ☐	4- Very Low ☐	5- Cannot be detected ☐
Risk Reduction	None (0%) ☐	Limited (25%) ☐	Moderate (50%) ☐	Substantial (75%) ☐	Extensive (95%) ☐
Other (_____)				**Applicable** ☐	**NA** ☐
Probability	1- Rare ☐	2- Unlikely ☐	3- Possible ☐	4- Likely ☐	5- Almost certain ☐
Severity		1- Non Serious ☐	2- Serious ☐	3- Life-Threatening ☐	4- Death ☐
Detectability	1- Very High ☐	2- Moderately high ☐	3- Low ☐	4- Very Low ☐	5- Cannot be detected ☐
Risk Reduction	None (0%) ☐	Limited (25%) ☐	Moderate (50%) ☐	Substantial (75%) ☐	Extensive (95%) ☐

Annex IV. Methodologies Wall Chart



Probability levels (definitions from V&S SoHO Project)

Level of probability	Definition
1 – Rare	Difficult to believe it could happen
2 – Unlikely	Not expected to happen but possible
3 – Possible	May occur occasionally
4 – Likely	Probable but not persistent
5 – Almost certain	Likely to occur on many occasions

Severity levels (definitions from V&S SoHO Project)

Level of severity	Definition
1 – Non-serious	Mild clinical or psychological consequences for the recipient, however with no hospitalisation, or anticipated long term consequences/disability
2 – Serious	Hospitalisation and/or: Persistent/significant disability or incapacity Intervention to preclude permanent damage Evidence of a serious transmitted infection Significant decrease in the expected treatment success Birth of a child with an infectious or genetic disease following MAR with donor gametes or embryos
3 – Life-threatening	Major intervention necessary to prevent death Evidence of a life threatening transmissible infection Birth of a child with life threatening genetic disease following MAR with donor gametes of embryos
4 – Fatal	Death of the patient

Detectability levels

Level of detectability	Definition
1 – Very high	The potential defect will almost certainly be detected before clinical application in the recipient.
2 – Moderately high	There is a reasonable chance that the potential defect will be detected before clinical application in the recipient.
3 – Low	There is a low chance that the potential defect will be detected before clinical application in the recipient.
4 – Very low	It is unlikely that the potential defect will be detected before clinical application in the recipient.
5 – Cannot be detected	The potential defect will be detected only after clinical application in the recipient.

Percentage risk reduction definitions

Percentage risk reduction	Definition
0 None	There are no relevant data available to support reducing the calculated risk score.
25 Limited	There is a moderate amount of relevant data available to support reducing the calculated risk score, based predominantly on unpublished data.
50 Moderate	There is a moderate amount of good quality relevant data available to support reducing the calculated risk score, including published and unpublished data from external sources, and some data, which have been through an independent peer review process.
75 Substantial	There are high-quality relevant data to support reducing the calculated risk score, including data that have been peer-reviewed and published.
95 Extensive	There is an extensive amount of high quality relevant data, including multiple peer-reviewed publications, that demonstrate that the probability of the risk occurring, having a significant impact and/or being undetected is negligible.

Annex V. EuroGTP II Algorithm for the calculation of Final Risk Score

EuroGTP II Algorithm for the calculation of Final Risk Score

Estimate the Preliminary Score associated with the BTC

$$\text{Preliminary Score} = \Sigma \text{ risks} =$$

$$= \Sigma ((S \times P \times D) - ((S \times P \times D) \times (\%\text{risk reduction}))$$

P = *Probability*
S = *Severity*
D = *Detectability*

The combined risk is determined using the following steps:

$$\text{Combined Risk Value} = \frac{\text{Preliminary Score} \times \text{Highest Possible Score}}{(\text{Max S} \times \text{Max P} \times \text{Max D} \times \text{Number of Applicable Risks Consequences})}$$

Max P = 5
Max S = 4
Max D = 5
Applicable Number of Risk Consequences = Range from: 0 to 45 for tissues (including gonadic tissues) and HPC; 0 to 32 for MAR (see details in the specific chapters: 4 – Replacement tissues, 5 – HPC and 6 – MAR)
Highest Possible Risk Score = (Max S × Max P × Max D × Number of Risks) × Risk Factors = 4500 for Replacement tissues and HPC, and 3200 for MAR

$$\text{Final Risk Score} = \frac{\text{Combined Risk Value} \times 100}{\text{Highest Possible Score}}$$

Two ancillary rules have been implemented in the algorithm to ensure that individual highly scored risks are not masked by adding various low risk scores. Thus, independently of the determined *Final Risk Score*,

individual risks with scores higher than 30 result in "moderate risks" and individual risks with scores higher than 50 result in "high risks".

(For demonstrations of the algorithm with practical examples – see Annex VII, Annex VIII and Annex IX.)

The *Preliminary* and *Combined Risk Scores* resulting from the risk assessment do not have a direct correspondence with the *Final Risk Score*.
The calculation of the *Final Risk Score* must be proportional to the number of risk consequences evaluated in the assessment of the BTC.

Table 2.1. Levels of risk based on the Final Risk Value determined by the algorithm

0 – 2	Negligible Risk
>2 – 6	Low Risk
> 6 – 22*	Moderate Risk
>22*	High Risk

* Lower values may result in moderate and high risk scores due to the application of the ancillary rules (described in the algorithm).

Annex VI. Risk reduction strategies and definition of clinical evaluation for tissues

Contents

Abbreviations

AFM	atomic force microscopy
A	acetoxymethyl
ATP	adenosine triphosphate
BOP	bovine corneal opacity permeability
CT	computed tomography
DAPI	4',6-diamidino-2-phenylin-dole
DMMB	dimethylmethylene blue assay
ECD	endothelial cell density
ECM	extracellular matrix
ELISA	enzyme-linked immunosorbent assay
EM	electron microscopy
EthD-1	ethidium homodimer-1
GAG	glycosaminoglycan
GC	gas chromatography
GuHCl	guanidine hydrochloride
H&E	haematoxylin and eosin
HPLC	high-performance liquid chromatography
ICC	immunocytochemistry
ICE	isolated chicken eye
ICRS	International Cartilage Regeneration & Joint Preservation Society (formerly International Cartilage Repair Society)
IHC	immunohistochemistry
IKDC	International Knee Documentation Committee
KOOS	Knee Injury and Osteoarthritis Outcome Score
LDI	laser Doppler imaging
MRI	magnetic resonance imaging
MS	mass spectrometry
MTT	3-(4,5-dimethylthiazol-2-yl)-2,5-diphen-yltetrazolium bromide
MVP	moisture vapour permeability
NRS	numeric rating scale
OCT	optical coherence tomography
PAS	periodic acid–Schiff
PCR	polymerase chain reaction pERG pattern electroretinography PFA paraformaldehyde
PG	proteoglycan
PROM	patient-reported outcome measure
QIRC	quality of life impact of refractive correction
QoL	quality of life
RCM	reflectance confocal microscopy
RNA	ribonucleic acid
RT-PCR	reverse transcription polymerase chain reaction
SAGE	serial analysis of gene expression
SARE	serious adverse reaction and/or event
SEM	scanning electron microscopy
TEM	transmission electron microscopy
TER	transepithelial resistance
TEWL	transepidermal water loss
TUNEL	terminal deoxynucleotidyl transferase deoxyuridine triphosphate nick-end labelling assay
VAS	visual analogue scale
VEP	visual evoked potential
WOMAC	Western Ontario and McMaster Universities Osteoarthritis Index
WOMET	Western Ontario Meniscal Evaluation Tool
WVTR	water vapour transmission rate

Definitions

DONOR CELL FUNCTIONALITY: the ability of donor cells to perform their required function; assays of donor cell functionality may address, for example, manufacture of specific ECM components or secretion of specific growth factors.

DONOR CELL VIABILITY: the ability of donor cells to survive; assays of donor cell viability measure generalised aspects of the health of cells, such as membrane integrity or mitochondrial activity.

Tests listed in the matrices are for guidance only and are not intended to be an exhaustive list of mandatory tests.
The references provided in this document aim to describe the generic assays/tests suggested as pre-clinical and clinical evaluations. These references do not describe the specific tests applicable to the different type of tissues.

Corneas

Step 3A: Risk reduction strategies

Preclinical evaluation – Examples of *in vitro* tests to assist in potentially reducing the risk consequences identified (blue cells represent the tests that may be used to address the respective risk consequences) - Tissue: Corneas.

Criteria	Specific test	Immunogenicity			Graft failure			Toxicity/ Carcinogenicity			Disease transmission		
		Systemic immune response	Localised immune response	Anaphylaxis	Failure to integrate with host tissue	Gradual mechanical failure	Sudden mechanical failure	Localised cytotoxicity	Systemic cytotoxicity	Carcinogenicity	Teratogenicity	Presence of donor-derived infectious agents	Infections acquired during procurement or processing
Process validation	Validation of the efficacy of the de-contamination process												
	Validation of the efficacy of the de-cellularisation process (if the graft has been decellularised)												
	Validation of the reliability of microbiology analytical methods												
	Aseptic handling (media fill) validation												
	Validation of packaging integrity following simulated use (including sealing tests)												
	Validation of the transport methodologies												
	Validation of the stability of the BTC during storage ('shelf life')												
In vitro cytotoxicity	TER												
	Staining with trypan blue												
	Cell apoptosis by detection of specific markers (e.g. caspase 3).												
	Microculture viability assays (e.g. mitochondrial dehydrogenase performance (MTT test[1]))												
Donor cell viability	Glucose uptake												
	ECD[2,3] using trypan blue												
	Measurement of ATP levels												
	Hoechst/ethidium/calcein (HEC)[3,4] staining - endothelial cell triple staining viability assay (Hoechst, EthD-1 and calcein acetoxymethyl (AM))												
	Mitochondrial activity (e.g. MTT)												

		Immunogenicity			Graft failure			Toxicity/ Carcinogenicity			Disease transmission		
Criteria	Specific test	Systemic immune response	Localised immune response	Anaphylaxis	Failure to integrate with host tissue	Gradual mechanical failure	Sudden mechanical failure	Localised cytotoxicity	Systemic cytotoxicity	Carcinogenicity	Teratogenicity	Presence of donor-derived infectious agents	Infections acquired during procurement or processing
Donor cell functionality	Immunostaining to determine the expression of different proteins and/ or markers (ZO-1; Na+/K+ ATPase, p63, K12, αSMA, etc.)												
	Transparency												
	Central corneal thickness												
	Tomography and microscopyy												
	Measurement of expression of specific markers/proteins using molecular assays, IHC and/or ELISA												
Histological evaluation of the ECM	Histological analysis to determine the presence of each layer (PFA[4] fixing and PAS[5] staining)												
	Biophysical investigations of ECM structure, collagen fibril orientation and distribution of GAGs in the collagen matrix: X-ray diffraction and TEM												
	PAS staining[5]												
	H&E[1] staining												
	IHC												
	Tomography and microscopy												
	ELISA												
Biochemical evaluation of the ECM	Quantification of ECM contents: collagen, GAGs, mucopolysaccharides, etc.												
Histological evaluation of cell content	Morphology: intercellular borders, polymorphism, dystrophy, degeneration												
	Staining with alizarin red S[6]												
	Presence of tight junctions, hemidesmosomes, etc.												
	H&E staining[1]												
	PAS staining[5]												
	SEM/TEM												
	Staining with alizarin red S[6]												
Residual DNA content	DAPI and Hoechst staining[7]												
	In situ hybridisation												
	PCR												

Criteria	Specific test	Immunogenicity			Graft failure			Toxicity/ Carcinogenicity			Disease transmission		
		Systemic immune response	Localised immune response	Anaphylaxis	Failure to integrate with host tissue	Gradual mechanical failure	Sudden mechanical failure	Localised cytotoxicity	Systemic cytotoxicity	Carcinogenicity	Teratogenicity	Presence of donor-derived infectious agents	Infections acquired during procurement or processing
Biomechanical properties	Use of AFM[8]												
In vitro functionality	Cell biology and metabolic assays												
	Physiological measures of EC function (e.g. perfusion and modulation of bicarbonate concentrations to turn off the endothelial pump and switch back on – measure rates of swelling and thinning)												
Residual processing & preservation reagents	Chemical and biochemical tests												
	Immuno-based assays (IHC, ICC, ELISA, etc.)												
	Direct detection and quantification methodologies (e.g. HPLC-MS; GC-MS; reagent-specific assays)												

Preclinical evaluation – Examples of *in vivo* tests to assist in potentially reducing the risk consequences identified (green cells represent the tests that may be used to address the respective risk consequences) - Tissue: Corneas

Criteria	Specific test	Immunogenicity			Graft failure			Toxicity/ Carcinogenicity			Disease transmission		
		Systemic immune response	Localised immune response	Anaphylaxis	Failure to integrate with host tissue	Gradual mechanical failure	Sudden mechanical failure	Localised cytotoxicity	Systemic cytotoxicity	Carcinoge nicity	Teratogenicity	Presence of donor-derived infectious agents	Infections acquired during procurement or processing
Biocompatibility	Ocular staining assays to evaluate defects: fluorescein test, rose bengal test, lissamine green test[9]												
	Presence of palpebral signs (meibomitis), conjunctivitis, corneal perforation, corneal ulceration, blood in the anterior chamber, neovascularisation												

Criteria	Specific test	Immunogenicity			Graft failure			Toxicity/ Carcinogenicity			Disease transmission		
		Systemic immune response	Localised immune response	Anaphylaxis	Failure to integrate with host tissue	Gradual mechanical failure	Sudden mechanical failure	Localised cytotoxicity	Systemic cytotoxicity	Carcinoge nicity	Teratogenicity	Presence of donor-derived infectious agents	Infections acquired during procurement or processing
Immunological response	Histology sections to investigate signs of inflammation (vessels, neo-vascularisation, etc.), the presence of proinflammatory agents, such as cytokines, or the presence of infiltrates (monocytes, macrophages, etc.).												
	Gross examination of eye and cornea; transparency												
	Use of specific (transgenic, knockout, etc.) animal models. Careful consideration should be given to the choice of strain.												
Ocular Functionality	Imaging (e.g. OCT)												
	Histology sections for IHC-based assays (e.g. evaluation of the expression of specific proteins important for cellular function)												
	In vivo functional assessment: a) pERG; b) VEP[10]; c) Evaluation of the light reflex (Iridal response)												
	Morphological assessment (histology, IHC, EM, etc.)												
Irritation/Corrosion/Toxic effect	Isolated rabbit eye test (ex vivo)												
	ICE test (ex vivo)												
	BOP test (ex vivo)												
Health	General condition/wellbeing after implantation (alive and well, sick, deceased)												
	Presence of ocular infections												
	Growth/weight increase												
	Unexplained fever (due to immune-induced reaction and/or toxicity)												
	Visual acuity evaluation: use of animal maze												

Step 3B: Definition of clinical studies

Clinical evaluation and follow up plans - Tissues: Corneas

Test category	Detailed investigational options
Physical investigation (functional)	1. Assessment of visual acuity 2. Eye movements 3. Visual field 4. Measurement of intraocular pressure
Physical investigation (anatomy)*	1. Observation of external structures (cornea, eye lid, sclera, conjunctiva, pupil and iris, etc.) 2. Assessment of pupils 3. Analysis of the fundus 4. Presence of defects, pathologies, inflammation, etc. 5. Topography 6. Pachymetry 7. Endothelial cell density 8. Optical Coherence Tomography for cornea/retina
Overall clinical outcome measures†	1. Graft transparency 2. Endothelial cell density and loss 3. Severe Adverse Reactions and Events 4. Best corrected visual acuity 5. Topography 6. Graft rejection 7. Infection 8. Optical Coherence Tomography 9. Angio Optical Coherence Tomography 10. Fluoro angiography 11. Schirmer test 12. Measurement of mechanical sensation (esthesiometry - Cochet Bonnet anaesthesiometer)
PROMs‡	Note: It is important to use only QoL and visual disability instruments that have been validated by Rasch analysis, which takes into account both difficulty of task and an individual's ability. Users should consider if the PROMs they propose to use meet this criteria. EQ-5D QoL - https://euroqol.org/) 1. Proceedings of PROMs which are more specific for ophthalmology treatments and that are available in the UK at https://onlineproms.co.uk/, such as: 2. Patient-reported outcomes are measured using questionnaires (CatQuest) QIRC VAS satisfaction NRS to assess pain 12-Item Short Form Health Survey (SF-12) or 36-Item Short Form Health Survey (SF-36) 3. Ocular surface disease index
Procedure or graft failure	1. Graft failure. Slit lamp examination can reveal clinical signs of graft rejection including: ◆ corneal edema ◆ keratic precipitates on the corneal graft, but not on the peripheral recipient cornea ◆ corneal vascularisation ◆ stromal infiltrates ◆ Khodadoust line ◆ an epithelial rejection line ◆ subepithelial infiltrates 2. Corneal endothelial cell density (where possible) 3. Confocal microscopy 4. High intra ocular pressure 5. Infection 6. Optical Coherence Tomography 7. Angio Optical Coherence Tomography 8. Fluoro angiography 9. Examination of the fundus

Test category	Detailed investigational options
Post operative complications§	1. Slit lamp and fundus examination to evaluate ◆ Post-op infection - (corneal scraping) ◆ Suture problems ◆ Corneal vascularisation ◆ Epithelial defects ◆ Haemorrhage ◆ Graft detachment ◆ Graft rejection ◆ Inflammation ◆ Eyelid disorders (blepharitis, ptosis, trichiasis) ◆ Symblepharon and conjunctival disorder ◆ Corneal melting/perforation ◆ Cataract ◆ Retinal detachment 2. Ocular hypertension (after tonometry) 3. Pain/photophobia/burning (patient reported symptoms) 4. Re-bubbling rate 5. Re-grafting rate 6. Systemic disease transmission

* Depends on the type of patient and the procedure; Select the appropriate combination and schedule of tests according to the risk category of the patients (low/medium/high).
† These tests will be done pre and post operatively so that improvement can be evaluated.
‡ Other PROMS are available.
§ The clinician will determine which examinations are relevant. It is important to distinguish failure due to non-graft-related reasons from graft-related failure. Routine follow-up for systemic infection/disease is not needed; however, if a recipient develops a post-operative systemic infection, investigation is needed and reported as a SARE.

Sclera

Step 3A: Risk reduction strategies

Preclinical evaluation – Examples of *in vitro* tests to assist in potentially reducing the risk consequences identified (blue cells represent the tests that may be used to address the respective risk consequences) - Tissue: Sclera

		Immunogenicity			Graft failure			Toxicity/ Carcinogenicity			Disease transmission		
Criteria	Specific test	Systemic immune response	Localised immune response	Anaphylaxis	Failure to integrate with host tissue	Gradual mechanical failure	Sudden mechanical failure	Localised cytotoxicity	Systemic cytotoxicity	Carcinogenicity	Teratogenicity	Presence of donor-derived infectious agents	Infections acquired during procurement or processing
Process validation	Validation of the efficacy of the de-contamination process												
	Validation of the efficacy of the de-cellularisation process (if the graft has been decellularised)												
	Validation of the reliability of microbiology analytical methods												
	Aseptic handling (media fill) validation												
	Validation of packaging integrity following simulated use (including sealing tests)												
	Validation of the transport methodologies												
	Validation of the stability of the BTC during storage ('shelf life')												
Histological evaluation of the ECM	Histological analysis to determine the presence of each layer (e.g. PFA fixing and PAS staining)												
	H&E staining[1]												
	Assessment of morphology (microscopy)												
Biochemical evaluation of the ECM	Quantification of collagen, GAGs, mucopolysaccharides, etc.												
Residual processing & preservation reagents	Chemical and biochemical tests appropriate to the specific reagent												
	Direct detection and quantification methodologies (e.g. HPLC-MS; GC-MS; reagent-specific assays)												

Preclinical evaluation – Examples of *in vivo* tests to assist in potentially reducing the risk consequences identified (green cells represent the tests that may be used to address the respective risk consequences) - Tissue: Sclera

		Immunogenicity			Graft failure			Toxicity/ Carcinogenicity			Disease transmission		
Criteria	Specific test	Systemic immune response	Localised immune response	Anaphylaxis	Failure to integrate with host tissue	Gradual mechanical failure	Sudden mechanical failure	Localised cytotoxicity	Systemic cytotoxicity	Carcinoge nicity	Teratogenicity	Presence of donor-derived infectious agents	Infections acquired during procurement or processing
Biocompatibility	Ocular staining assays to evaluate defects: fluorescein test, rose bengal test, lissamine green test[1]												
Biocompatibility	Presence of palpebral signs (meibomitis), conjunctivitis, corneal perforation, corneal ulceration, blood in the anterior chamber, neovascularisation												
Ocular Functionality	Imaging (e.g. OCT)												
Ocular Functionality	Histology sections for IHC-based assays (e.g. evaluation of the expression of specific proteins important for cellular function)												
Ocular Functionality	Morphological assessment (histology, IHC, EM, etc.)												
Health	General condition/wellbeing after implantation (alive and well, sick, deceased)												
Health	Presence of ocular infections												
Health	Growth/weight increase												
Health	Unexplained fever (due to immune-induced reaction and/or toxicity)												

Step 3B: Definition of clinical studies

Clinical evaluation and follow-up plans - Tissue: Sclera

Test category	Detailed investigational options
Physical investigation (functional)	1. Eye movements 2. Measurement of intraocular pressure
Physical investigation (anatomy)	1. Observation of external structures (cornea, eyelid, sclera, conjunctiva, pupil and iris, etc.) 2. Presence of defects, pathologies, inflammation, etc. 3. Topography 4. Pachymetry 5. OCT for cornea/retina
Overall clinical outcome measures	1. Severe adverse reactions and events 2. Topography 3. Infection 4. OCT

Test category	Detailed investigational options
PROMs	1. EQ-5D (QoL - https://euroqol.org/) 2. Proceedings of PROMs which are more specific for ophthalmology treatments and that are available in the UK at https://onlineproms.co.uk/, such as: ◆ Patient-reported outcomes are measured using questionnaires (CatQuest) ◆ QIRC ◆ VAS satisfaction ◆ NRS to assess pain ◆ 12-Item Short Form Health Survey (SF-12) or 36- Item Short Form Health Survey (SF-36) 3. Ocular surface disease index
Procedure or graft failure	1. Graft failure. Slit lamp examination 2. Confocal microscopy 3. High intraocular pressure 4. Infection 5. OCT
Post-operative complications	1. Slit lamp ◆ Post-op infection ◆ Suture problems ◆ Haemorrhage ◆ Graft detachment ◆ Inflammation ◆ Eyelid disorders (blepharitis, ptosis, trichiasis) ◆ Symblepharon and conjunctival disorder ◆ Corneal melting/perforation ◆ Cataract ◆ Retinal detachment 2. Ocular hypertension (after tonometry) 3. Pain/photophobia/burning (patient-reported symptoms) 4. Re-grafting rate 5. Systemic disease transmission

Amniotic membrane

Step 3A: Risk reduction strategies

Preclinical evaluation – Examples of *in vitro* tests to assist in potentially reducing the risk consequences identified (blue cells represent the tests that may be used to address the respective risk consequences) - Tissue: Amniotic membrane

		Immunogenicity			Graft failure			Toxicity/ Carcinogenicity			Disease transmission		
Criteria	Specific test	Systemic immune response	Localised immune response	Anaphylaxis	Failure to integrate with host tissue	Gradual mechanical failure	Sudden mechanical failure	Localised cytotoxicity	Systemic cytotoxicity	Carcinogenicity	Teratogenicity	Presence of donor-derived infectious agents	Infections acquired during procurement or processing
Process validation	Validation of the efficacy of the decontamination process												
Process validation	Validation of the efficacy of the decellularisation process (if the graft has been decellularised)												
Process validation	Validation of the reliability of microbiology analytical methods												
Process validation	Aseptic handling (media fill) validation												
Process validation	Validation of packaging integrity following simulated use (including sealing tests)												
Process validation	Validation of the transport methodologies												
Process validation	Validation of the stability of the BTC during storage ('shelf life')												
In vitro cytotoxicity	Thermal gravimetric analysis												
In vitro cytotoxicity	Cell proliferation												
In vitro cytotoxicity	Microculture viability assays (e.g. MTT).												
In vitro cytotoxicity	Direct contact method[11]												
Donor cell viability	MTT test (mitochondrial activity of cells)												
Donor cell viability	Fluorescence microscopy (live/dead staining)												
Donor cell viability	Proliferation test												
Donor cell functionality	Differentiation potential												
Donor cell functionality	RT-PCR, real-time PCR (expression levels of molecules related to the properties of the amniotic membrane, e.g. cytokines)												
Donor cell functionality	ELISA, western blot (content of specific protein)												
Donor cell functionality	Water absorption												
Donor cell functionality	Trypan blue staining												
Donor cell functionality	Assessment of the membrane architecture (e.g. IHC analysis, Immunophenotypical characterisation)												
Donor cell functionality	Flow cytometry												
Histological evaluation of the ECM	H&E Staining[1], Mallory's trichrome[12]												
Histological evaluation of the ECM	PAS staining[5]												
Histological evaluation of the ECM	SEM												
Histological evaluation of the ECM	TEM												

		Immunogenicity			Graft failure			Toxicity/ Carcinogenicity			Disease transmission		
Criteria	Specific test	Systemic immune response	Localised immune response	Anaphylaxis	Failure to integrate with host tissue	Gradual mechanical failure	Sudden mechanical failure	Localised cytotoxicity	Systemic cytotoxicity	Carcinogenicity	Teratogenicity	Presence of donor-derived infectious agents	Infections acquired during procurement or processing
Histological evaluation of cell content	Light microscopy (e.g. H&E staining)												
	SEM												
	TEM												
Absence of donor cells, cell remnants & nucleic acids	DAPI staining												
	Spectrophotometric analysis												
Biochemical evaluation of ECM quality	IHC												
	Infrared spectrometry analysis (degradation of the tissue)												
Biomechanical properties	Tensile testing												

		Immunogenicity			Graft failure			Toxicity/ Carcinogenicity			Disease transmission		
Criteria	Specific test	Systemic immune response	Localised immune response	Anaphylaxis	Failure to integrate with host tissue	Gradual mechanical failure	Sudden mechanical failure	Localised cytotoxicity	Systemic cytotoxicity	Carcinogenicity	Teratogenicity	Presence of donor-derived infectious agents	Infections acquired during procurement or processing
In vitro functionality	Cell count and proliferation assay												
	Microbial permeability												
	Oxygen permeability												
	WVTR[13]												
	MVP[14]												
	Flow cytometry for cell viability (e.g. propidium iodide) and apoptosis (e.g. annexin V, caspase 3/7)												
	Differentiation of mesenchymal stem cells isolated from the tissue and cultured under specific conditions												
	Immunofluorescence detection of intracellular molecules												
	Flow cytometry for antigen expression pattern analysis												
	ICC												
	RT-PCR, real-time PCR (e.g. expression of regulatory proteins related to the undifferentiated state)												
	ELISA, western blot (content of specific protein)												
	SAGE, microarray (gene expression analysis)*												
Residual processing & preservation reagents	Direct detection and quantification methodologies, (e.g. HPLC-MS; GC-MS; reagent-specific assays)												

* Relevant for clinical applications where the intended effect is to actively promote healing

Preclinical evaluation – Examples of *in vivo* tests to assist in potentially reducing the risk consequences identified (green cells represent the tests that may be used to address the respective risk consequences) - Tissue: Amniotic membrane

		Immunogenicity			Graft failure			Toxicity/ Carcinogenicity			Disease transmission		
Criteria	Specific test	Systemic immune response	Localised immune response	Anaphylaxis	Failure to integrate with host tissue	Gradual mechanical failure	Sudden mechanical failure	Localised cytotoxicity	Systemic cytotoxicity	Carcinoge nicity	Teratogenicity	Presence of donor-derived infectious agents	Infections acquired during procurement or processing
Biocompatibility	Histology and staining of cellular infiltrates												
	Measurement of serum/wound fluid - cytokines, chemokines (ELISA, flow cytometry, etc.)												
	Blood testing – HLA (donor antigens)												
Functionality	Number of adhesions												
	Mean wound size reduction												
	Barrier												
	Scar reduction												
Health	General condition/wellbeing after implantation (alive and well, sick, deceased)												
	Local infections												
	Growth/weight increase												
	Unexplained fever (due to immune-induced reaction and/or toxicity)												

Step 3B: Definition of clinical studies

Clinical evaluation and follow-up plans - Tissue: Amniotic membrane

	Clinical Indication*		
	Tissue patch, barrier or wrap	Surface wound healing	Ocular surface healing
Test category	**Detailed investigational options**		
Physical investigation (functional)	1. Mechanical performance		1. Assessment of visual acuity 2. Eye movements 3. Visual field 4. Measurement of intraocular pressure
Physical investigation (anatomy)	1. Absence of calcification 2. MRI 3. Cereb ospinal fluid leaks 4. Integration with native tissue (biopsy)	1. Bleeding/seroma formation (visual assessment) 2. Size of wound 3. Revascularisation 4. Scar retraction	1. Observation of external structures (cornea, eyelid, sclera, conjunctiva, pupil and iris, etc.) 2. Assessment of pupils 3. Analysis of the fundus 4. Presence of defects, pathologies, inflammation, etc. 5. Topography 6. Pachymetry 7. Endothelial cell count 8. OCT for cornea/retina

	Clinical Indication*		
	Tissue patch, barrier or wrap	**Surface wound healing**	**Ocular surface healing**
Test category	**Detailed investigational options**		
Overall clinical outcome measures	1. Alloimmunisation 2. Prevention of adhesions 3. Urodynamics		1. Graft transparency 2. ECD and loss 3. Severe adverse reactions and events 4. Best corrected visual acuity 5. Topography 6. Graft rejection 7. Infection 8. OCT 9. Angio-OCT 10. Fluoro angiography 11. Schirmer's test 12. Measurement of mechanical sensation (aesthesiometry – Cochet-Bonnet aesthesiometer)
PROMs		1. Pain score	1. EQ-5D (QoL - https://euro- qol.org/) 2. Proceedings of PROMs which are more specific for ophthalmology treatments and that are available in the UK at https://onlineproms.co.uk/, such as: ◆ Patient-reported outcomes are measured using questionnaires (CatQuest) ◆ QIRC ◆ VAS satisfaction ◆ NRS to assess pain ◆ 12-Item Short Form Health Survey (SF-12) or 36-Item Short Form Health Survey (SF-36) 3. Ocular surface disease index
Procedure or graft failure			1. Graft failure. Slit lamp examination can reveal clinical signs of graft rejection including: ◆ corneal oedema keratic precipitates on the corneal graft, but not on the peripheral recipient cornea ◆ corneal vascularisation ◆ stromal infiltrates ◆ a Khodadoust line ◆ an epithelial rejection line ◆ subepithelial infiltrates 2. Corneal endothelial cell count (where possible) 3. Confocal microscopy 4. High intraocular pressure 5. Infection 6. OCT 7. Angio-OCT 8. Fluoro angiography 9. Examination of the fundus

	Clinical Indication*		
	Tissue patch, barrier or wrap	Surface wound healing	Ocular surface healing
Test category	**Detailed investigational options**		
Post-operative complications	1. Infection 2. Haemorrhage	1. Infection 2. Inflammation	1. Slit lamp and fundus examination to evaluate: ◆ Post-op infection - (corneal scraping) ◆ Suture problems ◆ Corneal vascularisation ◆ Epithelial defects ◆ Haemorrhage ◆ Graft detachment ◆ Graft rejection ◆ Inflammation ◆ Eyelid disorders (blepharitis, ptosis, trichiasis) ◆ Symblepharon and conjunctival disorders ◆ Corneal melting/perforation ◆ Cataract ◆ Retinal detachment 2. Ocular hypertension (after tonometry) 3. Pain/photophobia/burning (patient-reported symptoms) 4. Re-bubbling rate 5. Re-grafting rate 6. Systemic disease transmission
Examples (of Clinical Applications)	1. Cardiac surgery - device wrapping to prevent adhesions 2. Neurosurgery - malformation of the newborn spinal cord 3. Neurosurgery - Dural reconstruction	1. Plastic surgery - wound healing 2. Plastic surgery - bi-oregeneration 3. Plastic surgery -- skin graft donor site healing 4. Burn surgery - treat-ment of burn wounds 5. Burn surgery - post stomal ulcer	1. Ophthalmology - promote healing of the ocular surface

* In situations where the amniotic membrane is used for induction of tissue regeneration (e.g. maxillofacial surgery - osteone-crosis of the jaw; orthopaedic surgery - tendinopathy treatment; orthopaedics - treatment of osteoarthritis) please consider tests appropriate to the tissue being treated.

Skin

Step 3A: Risk reduction strategies

Preclinical evaluation – Examples of *in vitro* tests to assist in potentially reducing the risk consequences identified (blue cells represent the tests that may be used to address the respective risk consequences) - Tissue: Skin as a biological dressing on (burn) wounds

		Immunogenicity			Graft failure			Toxicity/ Carcinogenicity			Disease transmission		
Criteria	Specific test	Systemic immune response	Localised immune response	Anaphylaxis	Failure to integrate with host tissue	Gradual mechanical failure	Sudden mechanical failure	Localised cytotoxicity	Systemic cytotoxicity	Carcinogenicity	Teratogenicity	Presence of donor-derived infectious agents	Infections acquired during procurement or processing
Process validation	Validation of the efficacy of the de-contamination process												
	Validation of the efficacy of the de-cellularisation process (if the graft has been decellularised)												
	Validation of the reliability of microbiology analytical methods												
	Aseptic handling (media fill) validation												
	Validation of packaging integrity following simulated use (including sealing tests)												
	Validation of the transport methodologies												
In vitro cytotoxicity	Microculture cytotoxicity assays (co-culture with keratinocytes or fibroblasts)												
Donor cell viability	Trypan blue exclusion of cells (in suspension)												
	Microculture viability assays (e.g. MTT)												
Donor cell functionality	Growth factor production (e.g. ELISA)												
Histological evaluation of the ECM	H&E staining												
	Collagen (Mason Trichrome)												
	Thickness												
Histological evaluation of cell content	H&E staining												
Biomechanical properties	Pliability, stiffness												
	Epidermal-dermal attachment												

		Immunogenicity			Graft failure			Toxicity/ Carcinogenicity			Disease transmission		
Criteria	Specific test	Systemic immune response	Localised immune response	Anaphylaxis	Failure to integrate with host tissue	Gradual mechanical failure	Sudden mechanical failure	Localised cytotoxicity	Systemic cytotoxicity	Carcinogenicity	Teratogenicity	Presence of donor-derived infectious agents	Infections acquired during procurement or processing
In vitro functionality	Tears upon handling (preparation after storage)												
Residual processing & preservation reagents	Concentration measurement in wash out fluids												
	Cytotoxicity test of washout fluid												
	Direct detection and quantification methodologies, (e.g. HPLC-MS; GC-MS; reagent-specific assays)												
	pH of washing fluid												

Preclinical evaluation – Examples of *in vivo* tests to assist in potentially reducing the risk consequences identified (green cells represent the tests that may be used to address the respective risk consequences) - Tissue: Skin

Note: since this use of skin is as a temporary biological dressing, the risk may never be so high that the results of *in vitro* tests are not sufficient to decide whether the new method for this type of skin is suitable or not for clinical use.

		Immunogenicity			Graft failure			Toxicity/ Carcinogenicity			Disease transmission		
Criteria	Specific test*	Systemic immune response	Localised immune response	Anaphylaxis	Failure to integrate with host tissue	Gradual mechanical failure	Sudden mechanical failure	Localised cytotoxicity	Systemic cytotoxicity	Carcinoge nicity	Teratogenicity	Presence of donor-derived infectious agents	Infections acquired during procurement or processing
Immunological response	Biopsies during healing time												
	Staining for inflammatory cells												
	Adherence to wound												
	Wound healing time (closure)												
	Wound contraction, scar quality												
	Granulation tissue formation												

		Immunogenicity			Graft failure			Toxicity/ Carcinogenicity			Disease transmission		
Criteria	Specific test*	Systemic immune response	Localised immune response	Anaphylaxis	Failure to integrate with host tissue	Gradual mechanical failure	Sudden mechanical failure	Localised cytotoxicity	Systemic cytotoxicity	Carcinoge nicity	Teratogenicity	Presence of donor-derived infectious agents	Infections acquired during procurement or processing
Health	General condition/wellbeing after implantation (alive and well, sick, deceased)												
	Wound infection												
	Growth/weight increase												
	Unexplained fever (due to immune-induced reaction and/or toxicity)												

* Specific tests using porcine wound model; comparative.

Step 3B: Definition of clinical studies

Clinical evaluation and follow-up plans - Tissue: Skin

Test category	Detailed investigational options
Physical investigation (functional)	1. Elasticity, using a cutometer 2. Adherence of graft to wound bed
Physical investigation (anatomical)	1. Non-invasive imaging (e.g. LDI) 2. Histological evaluation of tissue biopsies (H&E staining) 3. Stimulation of granulation tissue
Overall clinical outcome measures	1. Wound closure. Evaluate by: ◆ Visual assessment ◆ Quantitative evaluation using a grid system ◆ Computerised image analysis of wound photographs ◆ Inference from treatment records, e.g. stopping use of ointments or dressings 2. Quality of healing. Objective assessment, e.g. Vancouver Scar Scale
PROMs	1. QoL evaluated by using a questionnaire for the patient (pain, itching, scarring, pigmentation/vascularity, surface texture, surface area, scar height, sensitivity, psychological aspects, etc.)
Procedure or graft failure	1. Detachment of graft during dressing change (e.g. due to poor fixation/adherence to the wound bed)
Post-operative complications (causing difficulties in moving the graft material)	1. Infection 2. Formation of seroma or haematoma between the graft and wound bed 3. Adherence of donor skin to the wound bed
Clinical indications	1. Applied following excision of necrotic tissue to: ◆ Prepare the wound for autografting ◆ Protect the wound from infection ◆ Reduce fluid/heat loss 2. Coverage of meshed autografts
General notes	1. The type of wound will determine the appropriate tests 2. Burn wounds should be followed up for a minimum of 2 years. Longer follow-up is advised. 3. Consider resource requirements. 4. The quality of the wound bed preparation prior to graft application is critical to success of the graft.

Acellular dermis

Step 3A: Risk reduction strategies

Preclinical evaluation – Examples of *in vitro* tests to assist in potentially reducing the risk consequences identified (blue cells represent the tests that may be used to address the respective risk consequences) - Tissue: Acellular dermis

		Immunogenicity			Graft failure			Toxicity/ Carcinogenicity			Disease transmission		
Criteria	Specific test	Systemic immune response	Localised immune response	Anaphylaxis	Failure to integrate with host tissue	Gradual mechanical failure	Sudden mechanical failure	Localised cytotoxicity	Systemic cytotoxicity	Carcinogenicity	Teratogenicity	Presence of donor-derived infectious agents	Infections acquired during procurement or processing
Process validation	Validation of the efficacy of the de-contamination process												
	Validation of the efficacy of the de-cellularisation process (if the graft has been decellularised)												
	Validation of the reliability of microbiology analytical methods												
	Aseptic handling (media fill) validation												
	Validation of packaging integrity following simulated use (including sealing tests)												
	Validation of the transport methodologies												
	Validation of the stability of the BTC during storage ('shelf life')												
In vitro biocompatibility	Cell adhesion (histological analysis)												
	Cell proliferation												
	Non-invasive analysis (e.g. OCT)												
In vitro cytotoxicity	Microculture cytotoxicity assay (e.g. MTT, trypan blue)												
Presence of donor cells, cell remnants & nucleic acids	Histological analysis (H&E staining)												
	Quantitative analysis of DNA												
	Qualitative analysis of DNA (e.g. DAPI stain)												

Criteria	Specific test	Immunogenicity			Graft failure			Toxicity/ Carcinogenicity			Disease transmission		
		Systemic immune response	Localised immune response	Anaphylaxis	Failure to integrate with host tissue	Gradual mechanical failure	Sudden mechanical failure	Localised cytotoxicity	Systemic cytotoxicity	Carcinogenicity	Teratogenicity	Presence of donor-derived infectious agents	Infections acquired during procurement or processing
Histological evaluation of the ECM	H&E staining												
	Elastin (Verhoeff-van Gieson[15], orcein[16])												
	Collagen IV immunostaining*												
	Non-invasive imaging techniques to evaluate 3D structure and vasculature of the ECM (e.g. OCT, RCM)												
	ECM inter-fibre spacing (e.g. OCT)												
Biochemical evaluation of ECM quality	Quantification of ECM contents: collagen, GAGs, mucopolysaccharides, etc.												
	Assessment of collagen nativity (chymotrypsin assay[17])												
	Quantification of ECM contents (e.g. collagen and elastin)												
Biomechanical properties	Mechanical tensile testing (ultimate tensile stress, ultimate tensile strain, stiffness)												
	Suture pullout resistance†												
In vitro functionality	Tears upon handling (preparation after storage)												
Residual processing & preservation reagents	Direct detection and quantification methodologies, (e.g. HPLC-MS; GC-MS; reagent-specific assays)												
	pH of washout fluid												

* Only relevant if basement membrane is important.
† As an indicator of ease of suturing.

Preclinical evaluation – Examples of *in vivo* tests to assist in potentially reducing the risk consequences identified (green cells represent the tests that may be used to address the respective risk consequences) - Tissue: Acellular dermis

		Immunogenicity			Graft failure			Toxicity/ Carcinogenicity			Disease transmission		
Criteria	Specific test	Systemic immune response	Localised immune response	Anaphylaxis	Failure to integrate with host tissue	Gradual mechanical failure	Sudden mechanical failure	Localised cytotoxicity	Systemic cytotoxicity	Carcinoge nicity	Teratogenicity	Presence of donor-derived infectious agents	Infections acquired during procurement or processing
Biocom-patibility	Implantation subcutaneous model, in growth of host cells												
Immunologi-cal response	Porcine wound model; comparative												
	Biopsies during healing time												
	Staining for inflammatory cells												
Functionality	Porcine full thickness wound model												
	Incorporation in wound bed												
	Take of autograft on product, wound healing time (closure)												
	Wound contraction, scar quality												
Health	General condition/wellbeing after implantation (alive and well, sick, deceased)												
	Wound infection												
	Growth/weight increase												
	Unexplained fever (due to im-mune-induced reaction and/or toxicity)												
Other, func-tional test	Implantation in abdominal wall (rat/ porcine), adhesion*												
	Occurrence of calcification or early breakdown (bulging)												

* Specific for hernia repair indication. Specific for hernia repair indication.

Step 3B: Definition of clinical studies

Clinical evaluation and follow-up plans - Tissue: Acellular dermis (used for treating burns*)

Test category*	Detailed investigational options
Physical investigation (func-tional)	1. Elasticity, using cutometer 2. Range of motion during articulation (can be assessed by physiotherapy) 3. Permeability of wound (TEWL evaluation, e.g. by using a Tewameter) 4. Skin hydration/surface evaporation, using corneometer 5. Pigmentation and colouration (Mexameter) 6. pH (compared to healthy skin from the same patient; normal range is 5.5 - 6.0) 7. Dermal scan, compared to healthy skin from the same anatomical area using commercially available apparatus (OCT, LDI, etc.)

* Other clinical indications exist but were not considered in this guide: plastics (e.g. hypospadia correction and oculoplasty); wound healing (e.g. chronic vascular/diabetic ulcers, following excision of dermal malignancies); Tendon/ligament repair (e.g. re-enforcement of tendon/ligament repair & improvement of tissue regeneration); Biological patch/barrier material (e.g. breast reconstruction, abdominal wall repair).

Test category*	Detailed investigational options
Physical investigation (anatomical)	1. Wound contraction (e.g. evaluated by using planimetry)
Overall clinical outcome measures	1. Wound closure. Evaluate by: 1.1 Visual assessment 1.2 Quantitative evaluation using a grid system 1.3 Computerised image analysis of wound photographs 1.4 By inference from treatment records (e.g. stopping use of ointments or dressings) 2. Quality of healing. Objective assessment (e.g. Vancouver Scar Scale)
PROMs	1. QoL evaluated by using a questionnaire for the patient (pain, itching, scarring, pigmentation/vascularity, surface texture, surface area, scar height, psychological aspects, etc.) 2. Sensitivity (touch)
Procedure or graft failure	1. Non-integration with wound bed‡ 2. Seroma/haematoma formation
Post-operative complications	1. Infection
Examples	1. To regain mechanical function of damaged skin

* General remark: as technologies evolve, the suggested apparatus should be adapted to the new available technologies, accordingly.
† May be due to either infection, poor wound bed preparation or patient factors (e.g. use of drugs that reduce peripheral blood flow - the key measure is lack of vascularisation).
‡ May be due to either infection, poor wound bed preparation or patient factors (e.g. use of drugs that reduce peripheral blood flow - the key measure is lack of vascularisation).

Cardiovascular tissues – heart valves and vascular grafts

Step 3A: Risk reduction strategies

Preclinical evaluation – Examples of *in vitro* tests to assist in potentially reducing the risk consequences identified (blue cells represent the tests that may be used to address the respective risk consequences) - Tissue: Cardiovascular

		Immunogenicity			Graft failure			Toxicity/ Carcinogenicity			Disease transmission		
Criteria	Specific test	Systemic immune response	Localised immune response	Anaphylaxis	Failure to integrate with host tissue	Gradual mechanical failure	Sudden mechanical failure	Localised cytotoxicity	Systemic cytotoxicity	Carcinogenicity	Teratogenicity	Presence of donor-derived infectious agents	Infections acquired during procurement or processing
Process validation tests	Validation of the efficacy of the decontamination process												
	Validation of the efficacy of the decellularisation process (if the graft has been decellularised)												
	Validation of the reliability of microbiology analytical methods												
	Aseptic handling (media fill) validation												
	Validation of packaging integrity following simulated use (including sealing tests)												
	Validation of the transport methodologies												
	Validation of the stability of the BTC during storage ('shelf life')												

		Immunogenicity			Graft failure			Toxicity/ Carcinogenicity			Disease transmission		
Criteria	Specific test	Systemic immune response	Localised immune response	Anaphylaxis	Failure to integrate with host tissue	Gradual mechanical failure	Sudden mechanical failure	Localised cytotoxicity	Systemic cytotoxicity	Carcinogenicity	Teratogenicity	Presence of donor-derived infectious agents	Infections acquired during procurement or processing
In vitro cytotoxicity	Extract cytotoxicity[11]												
	Contact cytotoxicity[11]												
Donor cell viability	Microculture viability assays (e.g. MTT, fibroblast culture)												
	Expression of cell surface markers												
Physical/morphological properties	Evaluation of the morphology/anatomy of processed tissue (leaflet morphology, fenestrations, coaptation of leaflets, calcification, atheromatosis)												
	Hydrodynamic properties: competency test under pressure and pulsatile flow testing												
Biomechanical properties	Uniaxial/biaxial tensile strength testing assays												
	Cyclic testing												
	Suture pullout												
Histological evaluation of the ECM	Safranin O[18] (proteoglycans (PGs) & GAGs)												
	Alizarin red S or von Kossa19 (calcium)												
	van Gieson[15] (collagen)												
	Masson´s trichrome staining[20]												
	Protein quantification (e.g. collagen and elastin)												
Histological evaluation of cell content	H&E stain												
	DAPI staining												
Presence of donor cells, cell remnants & nucleic acids	DNA quantification												
	Qualitative testing (DAPI)												

		Immunogenicity			Graft failure			Toxicity/ Carcinogenicity			Disease transmission		
Criteria	Specific test	Systemic immune response	Localised immune response	Anaphylaxis	Failure to integrate with host tissue	Gradual mechanical failure	Sudden mechanical failure	Localised cytotoxicity	Systemic cytotoxicity	Carcinogenicity	Teratogenicity	Presence of donor-derived infectious agents	Infections acquired during procurement or processing
Biochemical evaluation of ECM quality	Quantification of ECM contents, e.g. collagen and elastin												
	Collagenase resistance												
	Collagen nativity												
Residual processing reagents	Direct detection and quantification methodologies, (e.g. HPLC-MS; GC-MS; reagent-specific assays)												
	IHC												

Preclinical evaluation – Examples of *in vivo* tests to assist in potentially reducing the risk consequences identified (green cells represent the tests that may be used to address the respective risk consequences) - Tissue: Cardiovascular

		Immunogenicity			Graft failure			Toxicity/ Carcinogenicity			Disease transmission		
Criteria	Specific test	Systemic immune response	Localised immune response	Anaphylaxis	Failure to integrate with host tissue	Gradual mechanical failure	Sudden mechanical failure	Localised cytotoxicity	Systemic cytotoxicity	Carcinogenicity	Teratogenicity	Presence of donor-derived infectious agents	Infections acquired during procurement or processing
Biocompatibility	IHC staining (post-explantation: cell infiltration)												
	HLA matching												
	Calcification												
	Doppler echo/echocardiography/CT scan/MRI												
Functionality in vivo (stenosis or regurgitation)	Echocardiography for regurgitation and stenosis evaluation; bleeding, thrombosis, infection												
	Regurgitation grade												
	Tissue regeneration												
	Bleeding events												
	Rupture of the graft												
	Thrombosis/thromboembolic event												

		Immunogenicity			Graft failure			Toxicity/ Carcinogenicity			Disease transmission		
Criteria	Specific test	Systemic immune response	Localised immune response	Anaphylaxis	Failure to integrate with host tissue	Gradual mechanical failure	Sudden mechanical failure	Localised cytotoxicity	Systemic cytotoxicity	Carcinogenicity	Teratogenicity	Presence of donor-derived infectious agents	Infections acquired during procurement or processing
Health	General condition/wellbeing after implantation (alive and well, sick, deceased)												
	Infection/endocarditis												
	Growth/weight increase												
	Unexplained fever (due to immune-induced reaction and/or toxicity)												
Valve functionality/ integrity	Post-explantation histological analysis												
	Thrombogenicity												
	Morphological evaluation post-explantation structural integrity, fibrosis, calcification												
	Radiograph analysis												

Step 3B: Definition of clinical studies

Clinical evaluation and follow-up plans - Tissues: Heart valves and Vascular grafts

	Clinical Indication	
	Heart valves	Vascular grafts
Test category	**Detailed investigational options**	
Graft failure (during procedure/immediately after implantation)	1. Perioperative (surgical) graft failure (transoesophageal echocardiography)	1. Perioperative (surgical) graft failure (Doppler echo)
Post-operative complications	1. Unexplained fever (due to immune-induced reaction and/or toxicity) 2. Bleeding events 3. Rupture of the graft 4. Thrombosis/thromboembolic event 5. Infection/endocarditis	1. Unexplained fever (due to immune-induced reaction and/or toxicity) 2. Bleeding events 3. Rupture of the graft 4. Thrombosis/thromboembolic event 5. Infection/endocarditis
Patient-reported symptoms and outcome	1. Fatigue 2. Loss of physical capacity 3. Dyspnoea	1. Pain in the operated limb 2. Colour and temperature changes in the skin distal to the graft 3. Decreased functional capacity of the operated limb

	Clinical Indication	
	Heart valves	Vascular grafts
Test category	**Detailed investigational options**	
Physical investigation (discrete outcome measures with quantifiable results) and Overall clinical outcome measures	1. Graft-related mortality 2. Graft normal function (auscultation/echocardiogram/MRI) 3. Abnormal function (increased peak pressure gradient) due to mismatch, calcific degeneration with/without stenosis – (auscultation/echocardiogram and CT scan) 4. Abnormal function - annular dilation (by echocardiogram or CT scan) 5. Regurgitation (by echo- cardiogram or MRI) 6. Graft-related re-operation (due to graft survival)	1. Lack of pulsation 2. Graft-related mortality 3. Graft normal function (pulse palpation/auscultation/Doppler echo) 4. Abnormal function (increase pressure gradient) due to mismatch, calcific degeneration with/without stenosis – (auscultation/Doppler echo/CT scan) 5. Abnormal function - graft dilation (aneurism formation) by Doppler echo, angiography or CT scan) 6. Graft-related re-operation (due to graft survival)

Bone

Step 3A: Risk reduction strategies

Preclinical evaluation – Examples of *in vitro* tests to assist in potentially reducing the risk consequences identified (blue cells represent the tests that may be used to address the respective risk consequences) - Tissue: Bone

		Immunogenicity			Graft failure			Toxicity/ Carcinogenicity			Disease transmission		
Criteria	Specific test	Systemic immune response	Localised immune response	Anaphylaxis	Failure to integrate with host tissue	Gradual mechanical failure	Sudden mechanical failure	Localised cytotoxicity	Systemic cytotoxicity	Carcinogenicity	Teratogenicity	Presence of donor-derived infectious agents	Infections acquired during procurement or processing
Process validation tests	Validation of the efficacy of the decontamination process												
	Validation of the efficacy of the decellularisation process (if the graft has been decellularised)												
	Validation of the efficacy of the demineralisation process												
	Validation of the reliability of microbiology analytical methods												
	Aseptic handling (media fill) validation												
	Validation of packaging integrity following simulated use (including sealing tests)												
	Validation of the transport methodologies												
	Validation of the stability of the BTC during storage ('shelf life')												

		Immunogenicity			Graft failure			Toxicity/ Carcinogenicity				Disease transmission	
Criteria	Specific test	Systemic immune response	Localised immune response	Anaphylaxis	Failure to integrate with host tissue	Gradual mechanical failure	Sudden mechanical failure	Localised cytotoxicity	Systemic cytotoxicity	Carcinogenicity	Teratogenicity	Presence of donor-derived infectious agents	Infections acquired during procurement or processing
In vitro Immunogenicity	Mixed lymphocyte reaction												
In vitro cytotoxicity	Co-culture of cells with graft (toxicity/ proliferation)												
In vitro biocompatibility	Microculture toxicity assays												
	Contact toxicity testing												
Presence of donor cells, cell remnants & nucleic acids	DAPI staining												
	Safranin O (lipids)												
	Lipid content (solvent extraction)												
	Cell-specific markers												
Biomechanical properties	Ultimate tensile stress (load at failure)												
	Ultimate compressive stress (load at failure)												
	Presence of microfractures after stress												
	Elastic modulus												
	Shear testing												
	Three point pending												
Residual processing reagents	Direct detection and quantification methodologies (e.g. HPLC-MS; GC-MS; reagent-specific assays)												
Histological evaluation of the ECM	von Kossa staining												
	van Gieson[15] staining												
	H&E staining												

		Immunogenicity			Graft failure			Toxicity/ Carcinogenicity			Disease transmission		
Criteria	Specific test	Systemic immune response	Localised immune response	Anaphylaxis	Failure to integrate with host tissue	Gradual mechanical failure	Sudden mechanical failure	Localised cytotoxicity	Systemic cytotoxicity	Carcinogenicity	Teratogenicity	Presence of donor-derived infectious agents	Infections acquired during procurement or processing
In vitro functionality	*In vitro* osteoinduction												
	BMP content												
Biochemical evaluation of the ECM	Collagen denaturation												

Preclinical evaluation –Examples of *in vivo* tests to assist in potentially reducing the risk consequences identified (green cells represent the tests that may be used to address the respective risk consequences) - Tissue: Bone

		Immunogenicity			Graft failure			Toxicity/ Carcinogenicity			Disease transmission		
Criteria	Specific test	Systemic immune response	Localised immune response	Anaphylaxis	Failure to integrate with host tissue	Gradual mechanical failure	Sudden mechanical failure	Localised cytotoxicity	Systemic cytotoxicity	Carcinoge nicity	Teratogenicity	Presence of donor-derived infectious agents	Infections acquired during procurement or processing
Biocompatibility	Histology and staining of cellular infiltrates												
Immunological response	Analysis of HLA (alloimmunisation)												
Functionality	Osteogenesis in extraskeletal sites												
	Bone induction chamber												
	Healing of a critical size defect												
	Fusion												

		Immunogenicity			Graft failure			Toxicity/ Carcinogenicity			Disease transmission		
Criteria	Specific test	Systemic immune response	Localised immune response	Anaphylaxis	Failure to integrate with host tissue	Gradual mechanical failure	Sudden mechanical failure	Localised cytotoxicity	Systemic cytotoxicity	Carcinoge nicity	Teratogenicity	Presence of donor-derived infectious agents	Infections acquired during procurement or processing
Health	General condition/wellbeing after implantation (alive and well, sick, deceased)												
	Infection												
	Growth/weight increase												
	Unexplained fever (due to im-mune-induced reaction and/or toxicity)												

Step 3B: Definition of clinical studies

Clinical evaluation and follow-up plans - Tissue: Bone

	Clinical Indication			
	Joint revision	Spinal surgery	Fracture repair	Replacement of lost bone mass
Test category	**Detailed investigational options**			
Physical investigation (functional)	1. Prosthesis survival rate	1. Spinal curve correction 2. Length of hospital stay	1. Full weight bearing	
Physical investigation (anatomical)	1. Stem subsidence 2. Cortical repair (radi-ography) 3. Graft incorporation (radiography, CT scan) 4. Trabecular remodel-ling (radiography, CT scan)	1. Bone graft mass (radi-ography) 2. Graft in- corporation (union with host bone) 3. Bone bridging (fusion) between vertebral bodies - arthrodesis	1. Radio- graphic assessment of union, callus formation	1. Radiographic assess-ment of bone fill 2. Bone biopsy
PROMs	1. Harris Hip Score (pain and function) 2. WOMAC21 test	1. Pain scores 2. MacNab score 3. Oswestry Disability Index 4. SF-36 score 5. Neck disability index	1. Numeric pain scale (0-10) 2. Numeric satisfac-tion score (0-5)	
Procedure or graft failure	1. Dislocation (e.g. pros-thesis dislocation) 2. Periprosthetic fracture 3. Need for revision 4. Aseptic loosening	1. Pseudarthrosis (non-union) rate 2. Loss of correction	1. Non-union or de-layed union	
Post-operative com-plications	1. Infection 2. Alloimmunisation 3. Pain	1. Dural tear 2. Neurologic Injury 3. Haematoma 4. Infection 5. Adjacent segment degeneration 6. Dysphagia 7. Alloimmunisation 8. Pain	1. Infection 2. Alloimmunisation 3. Pain	
Examples	1. Hip replacement/ revision	1. Scoliosis surgery 2. Spinal fusion		

Tendons

Step 3A: Risk reduction strategies

Preclinical evaluation – Examples of *in vitro* tests to assist in potentially reducing the risk consequences identified (blue cells represent the tests that may be used to address the respective risk consequences) - Tissue: Tendons

		Immunogenicity			Graft failure			Toxicity/ Carcinogenicity				Disease transmission	
Criteria	Specific test	Systemic immune response	Localised immune response	Anaphylaxis	Failure to integrate with host tissue	Gradual mechanical failure	Sudden mechanical failure	Localised cytotoxicity	Systemic cytotoxicity	Carcinogenicity	Teratogenicity	Presence of donor-derived infectious agents	Infections acquired during procurement or processing
Process validation tests	Validation of the efficacy of the de-contamination process												
	Validation of the efficacy of the de-cellularisation process (if the graft has been decellularised)												
	Validation of the reliability of microbiology analytical methods												
	Aseptic handling (media fill) validation												
	Validation of packaging integrity following simulated use (including sealing tests)												
	Validation of the transport methodologies												
	Validation of the stability of the BTC during storage ('shelf life')												
In vitro Immunogenicity	Mixed lymphocyte reaction												
In vitro cytotoxicity	Microculture toxicity assays												
	Contact toxicity testing												
	Co-culture of cells with graft (toxicity, proliferation)												
In vitro biocompatibility	Proinflammatory response												
	Co-culture of cells with graft (maintenance of phenotype)												

		Immunogenicity			Graft failure			Toxicity/ Carcinogenicity			Disease transmission		
Criteria	Specific test	Systemic immune response	Localised immune response	Anaphylaxis	Failure to integrate with host tissue	Gradual mechanical failure	Sudden mechanical failure	Localised cytotoxicity	Systemic cytotoxicity	Carcinogenicity	Teratogenicity	Presence of donor-derived infectious agents	Infections acquired during procurement or processing
Presence of donor cells, cell remnants & nucleic acid	DAPI staining												
	Quantitative DNA analysis (total DNA content)												
	H&E staining												
	Cell-specific markers												
Biomechanical properties	Ultimate tensile stress (load at failure)												
	Ultimate tensile strain (extension at failure)												
	Displacement under constant load (creep)												
	Elastic modulus/stiffness												
Residual processing reagents	Direct detection and quantification methodologies, (e.g. HPLC-MS; GC-MS; reagent-specific assays)												
Histological evaluation of the ECM	van Gieson[22] stain												
	Inter-fibre space												
Biochemical evaluation of the ECM	Collagen denaturation												
	Collagenase resistance												

Preclinical evaluation – Examples of *in vivo* tests to assist in potentially reducing the risk consequences identified (green cells represent the tests that may be used to address the respective risk consequences) - Tissue: Tendons

		Immunogenicity			Graft failure			Toxicity/ Carcinogenicity			Disease transmission		
Criteria	Specific test	Systemic immune response	Localised immune response	Anaphylaxis	Failure to integrate with host tissue	Gradual mechanical failure	Sudden mechanical failure	Localised cytotoxicity	Systemic cytotoxicity	Carcinoge nicity	Teratogenicity	Presence of donor-derived infectious agents	Infections acquired during procurement or processing
Biocompati-bility	Histology and staining of cellular infiltrates												
	Macrophage type identification[23]												
	Histological assessment of graft/ donor tissue interface												
Immunologi-cal response	Analysis of HLA (alloimmunisation)												
Functionality	Radiography/CT scan/MRI to estab-lish bony fusion												
	Force plate analysis												
	Tetracycline labelling for new bone formation												
	Joint stability												
Health	General condition/well- being after implantation (alive and well, sick, deceased)												
	Infection												
	Growth/weight increase												
	Unexplained fever (due to im-mune-induced reaction and/or toxicity)												
	Quality of gait												

Step 3B: Definition of clinical studies

Clinical evaluation and follow-up plans - Tissue: Tendons

Test category	Detailed investigational options
Physical investigation (func-tional)	1. Laxity (KT-1000, Lachman test) 2. Range of motion (ROM) assessment
Physical investigation (ana-tomical)	1. Graft biopsy to evaluate cellularity, collagen structure, necrosis, inflammatory cell infiltrate 2. Bone resorption 3. Tunnel enlargement 4. MRI 5. Graft (bone) incorporation
Overall clinical outcome measures	1. Patellofemoral crepitus 2. Hop and jump tests
PROMs	1. IKDC Subjective Knee Form (IKDC score)[24] 2. Lysholm score[25] 3. Cincinnati score 4. KOOS[24] 5. Tegner score[25,26] 6. Frequency/level of sporting participation

Test category	Detailed investigational options
Procedure or graft failure	*Note some of the clinical outcome measures (e.g. excess laxity) may denote graft failure.* 1. Graft rupture 2. Requirement for revision
Post-operative complications	1. Effusion 2. Cyst formation 3. Post-op infection 4. Pain 5. Elevated temperature
Examples	1. ACL Revision/Repair

Meniscus

Step 3A: Risk reduction strategies

Preclinical evaluation – Examples of *in vitro* tests to assist in potentially reducing the risk consequences identified (blue cells represent the tests that may be used to address the respective risk consequences) - Tissue: Meniscus

		Immunogenicity			Graft failure			Toxicity/ Carcinogenicity				Disease transmission	
Criteria	Specific test	Systemic immune response	Localised immune response	Anaphylaxis	Failure to integrate with host tissue	Gradual mechanical failure	Sudden mechanical failure	Localised cytotoxicity	Systemic cytotoxicity	Carcinogenicity	Teratogenicity	Presence of donor-derived infectious agents	Infections acquired during procurement or processing
Process validation tests	Validation of the efficacy of the de-contamination process												
	Validation of the efficacy of the de-cellularisation process (if the graft has been decellularised)												
	Validation of the reliability of microbiology analytical methods												
	Aseptic handling (media fill) validation												
	Validation of packaging integrity following simulated use (including sealing tests)												
	Validation of the transport methodologies												
	Validation of the stability of the BTC during storage ('shelf life')												
In vitro cytotoxicity	Extract cytotoxicity assays[11]												
	Contact cytotoxicity assays[11]												
	Co-culture of cells with allograft (toxicity/proliferation)												
In vitro immunogenicity	Mixed lymphocyte reaction												

		Immunogenicity			Graft failure			Toxicity/ Carcinogenicity			Disease transmission		
Criteria	Specific test	Systemic immune response	Localised immune response	Anaphylaxis	Failure to integrate with host tissue	Gradual mechanical failure	Sudden mechanical failure	Localised cytotoxicity	Systemic cytotoxicity	Carcinogenicity	Teratogenicity	Presence of donor-derived infectious agents	Infections acquired during procurement or processing
Donor cell functionality	Evaluation of donor cell phenotype - quantification of secreted/produced biomolecules (PGs, GAGs, proteins)												
	Donor cell viability (e.g. trypan blue, live/dead staining, flow cytometry, confocal microscopy)												
Histological evaluation of the ECM	H&E staining												
	Safranin O, Alcian blue - PGs												
	IHC to evaluate type II collagen												
Presence of donor cells, cell remnants & nucleic acids	DAPI staining												
	Residual nucleic acid quantification												
	H&E staining												
Residual processing reagents	Direct detection and quantification methodologies, (e.g. HPLC-MS; GC-MS; reagent-specific assays)												
Biochemical properties of the ECM	Collagen denaturation												
	Collagenase susceptibility												
	Evaluation of proteoglycan quality – GuHCl extraction												
	Composition of the ECM - Water (gravimetric/aW assessment) - Collagen (hydroxyproline) - GAGs (DMMB assay)												
Biomechanical properties	Static tensile modulus												
	Dynamic tensile modulus												
	Indentation test												

		Immunogenicity			Graft failure			Toxicity/ Carcinogenicity			Disease transmission		
Criteria	Specific test	Systemic immune response	Localised immune response	Anaphylaxis	Failure to integrate with host tissue	Gradual mechanical failure	Sudden mechanical failure	Localised cytotoxicity	Systemic cytotoxicity	Carcinogenicity	Teratogenicity	Presence of donor-derived infectious agents	Infections acquired during procurement or processing
Morphological/ physical properties	Microscopic surface examination												

Preclinical evaluation – Examples of *in vivo* tests to assist in potentially reducing the risk consequences identified (green cells represent the tests that may be used to address the respective risk consequences) - Tissue: Meniscus

		Immunogenicity			Graft failure			Toxicity/ Carcinogenicity			Disease transmission		
Criteria	Specific test	Systemic immune response	Localised immune response	Anaphylaxis	Failure to integrate with host tissue	Gradual mechanical failure	Sudden mechanical failure	Localised cytotoxicity	Systemic cytotoxicity	Carcinoge nicity	Teratogenicity	Presence of donor-derived infectious agents	Infections acquired during procurement or processing
Biocompatibility/integration with recipient tissue	Characterisation of recipient cell infiltrates												
	Evaluation of donor cell content												
	Histological assessment of graft/ donor tissue interface												
	Evaluation of graft ascularity												
	Biomechanical evaluation of graft insertion												
Immunological response	Analysis of HLA (alloimmunisation)												
Graft quality/ remodelling	Composition of the ECM - Water (gravimetric assessment) - Collagen (hydroxyproline) - GAGs (DMMB)												

Criteria	Specific test	Immunogenicity: Systemic immune response	Immunogenicity: Localised immune response	Immunogenicity: Anaphylaxis	Graft failure: Failure to integrate with host tissue	Graft failure: Gradual mechanical failure	Graft failure: Sudden mechanical failure	Toxicity/ Carcinogenicity: Localised cytotoxicity	Toxicity/ Carcinogenicity: Systemic cytotoxicity	Toxicity/ Carcinogenicity: Carcinoge nicity	Disease transmission: Teratogenicity	Disease transmission: Presence of donor-derived infectious agents	Disease transmission: Infections acquired during procurement or processing
Functionality	Radiography/CT/MRI to establish bony fusion												
Functionality	Tetracycline labelling for new bone formation												
Functionality	Evaluation of recipient knee articular cartilage quality												
Health	General condition/well-being after implantation (alive and well, sick, deceased)												
Health	Infection												
Health	Growth/weight increase												
Health	Unexplained fever (due to im-mune-induced reaction and/or toxicity)												
Health	Quality of gait												

Step 3B: Definition of clinical studies

Clinical evaluation and follow-up plans - Tissue: Meniscus

Test category	Detailed investigational options
Physical investigation (functional)	*May intersect with PROMs below*
Physical investigation (anatomical)	1. Post-operative MRI/X-ray or arthroscopy, to investigate position, integration and degeneration of graft 2. Graft degeneration investigated by arthroscopy/arthrotomy (ICRS grading score27 can be used to grade cartilage degeneration) 3. Biopsy to investigate ECM structure, donor cell phenotype and IHC, matrix remod-elling, localised immunogenicity 4. Alloimmunisation
Overall clinical outcome measures	1. Standard knee functionality scales
PROMs	1. Lysholm Knee Score[26] 2. Activity level 3. IKDC Score[24] 4. SF-36 5. Functional Knee score 6. Tegner score 7. Cincinnati Knee Rating[28]
Procedure or graft failure	1. Graft survival
Post-operative complications	1. Swelling 2. Pain 3. Effusion 4. Synovitis
Examples	Meniscal transplantation

Fresh cartilage

Step 3A: Risk reduction strategies

Preclinical evaluation – Examples of *in vitro* tests to assist in potentially reducing the risk consequences identified (blue cells represent the tests that may be used to address the respective risk consequences) - Tissue: Fresh cartilage

		Immunogenicity			Graft failure			Toxicity/ Carcinogenicity			Disease transmission		
Criteria	Specific test	Systemic immune response	Localised immune response	Anaphylaxis	Failure to integrate with host tissue	Gradual mechanical failure	Sudden mechanical failure	Localised cytotoxicity	Systemic cytotoxicity	Carcinogenicity	Teratogenicity	Presence of donor-derived infectious agents	Infections acquired during procurement or processing
Process validation tests	Validation of the efficacy of the de-contamination process												
	Validation of the efficacy of the de-cellularisation process (if the graft has been decellularised)												
	Validation of the reliability of microbiology analytical methods												
	Aseptic handling (media fill) validation												
	Validation of packaging integrity following simulated use (including sealing tests)												
	Validation of the transport methodologies												
	Validation of the stability of the BTC during storage ('shelf life')												
In vitro cytotoxicity	Extract cytotoxicity assays[11]												
	Contact cytotoxicity assays[11]												
	Co-culture of cells with allograft (toxicity/proliferation)												
In vitro immunogenicity	Mixed lymphocyte reaction												
Evaluation of donor cell functionality	Evaluation of donor cell phenotype - quantification of secreted/produced biomolecules (PGs, GAGs, proteins)												
	Donor cell viability (trypan blue, live/dead staining)												
Histological evaluation of the ECM	H&E staining												
	Safranin O, Alcian Blue - PGsI												
	HC to evaluate type II collagen												

		Immunogenicity			Graft failure			Toxicity/ Carcinogenicity			Disease transmission		
Criteria	Specific test	Systemic immune response	Localised immune response	Anaphylaxis	Failure to integrate with host tissue	Gradual mechanical failure	Sudden mechanical failure	Localised cytotoxicity	Systemic cytotoxicity	Carcinogenicity	Teratogenicity	Presence of donor-derived infectious agents	Infections acquired during procurement or processing
Residual processing reagents	Direct detection and quantification methodologies, (e.g. HPLC-MS; GC-MS; reagent-specific assays)												
Biochemical properties of the ECM	Collagen denaturation												
	Collagenase susceptibility												
	Evaluation of proteoglycan quality – GuHCl extraction[29]												
	Composition of the ECM - Water (gravimetric/aW assessment) - Collagen (hydroxyproline) - GAGs (DMMB)												
Physical/morphological properties	Macroscopic surface examination												

Preclinical evaluation - Examples of *in vivo* tests to assist in potentially reducing the risk consequences identified (green cells represent the tests that may be used to address the respective risk consequences) - Tissue: Fresh cartilage

		Immunogenicity			Graft failure			Toxicity/ Carcinogenicity			Disease transmission		
Criteria	Specific test	Systemic immune response	Localised immune response	Anaphylaxis	Failure to integrate with host tissue	Gradual mechanical failure	Sudden mechanical failure	Localised cytotoxicity	Systemic cytotoxicity	Carcinoge nicity	Teratogenicity	Presence of donor-derived infectious agents	Infections acquired during procurement or processing
Biocompatibility/integration with recipient tissue	Characterisation of recipient cell infiltrates												
	Evaluation of donor cell viability												
	Histological assessment of graft/ donor tissue interface												
	Evaluation of graft ascularity												
	Biomechanical evaluation of graft insertion												

		Immunogenicity			Graft failure			Toxicity/ Carcinogenicity			Disease transmission		
Criteria	Specific test	Systemic immune response	Localised immune response	Anaphylaxis	Failure to integrate with host tissue	Gradual mechanical failure	Sudden mechanical failure	Localised cytotoxicity	Systemic cytotoxicity	Carcinoge nicity	Teratogenicity	Presence of donor-derived infectious agents	Infections acquired during procurement or processing
Immunological response	Analysis of HLA (alloimmunisation)												
Graft quality/ remodelling	Composition of the ECM - Water (gravimetric assessment) - Collagen (hydroxyproline) - GAGs (DMMB)												
Functionality	Radiography/CT/MRI to establish bony fusion												
	Tetracycline labelling for new bone formation												
	Evaluation of recipient knee articular cartilage quality												
Health	General condition/well-being after implantation (alive and well, sick, deceased)												
	Infection												
	Growth/weight increase												
	Unexplained fever (due to immune-induced reaction and/or toxicity)												
	Quality of gait												

Step 3B: Definition of clinical studies

Clinical evaluation and follow-up plans - Tissue: Fresh cartilage

Test category	Detailed investigational options
Physical investigation (functional)	1. Range of motion 2. Daily living activities functionality
Physical investigation (anatomical)	1. Post-operative MRI/CT scan 2. Arthroscopy, to investigate position, integration and degeneration of graft 3. Radiography to evaluate mechanical axis 4. Alloimmunisation
Overall clinical outcome measures	1. Standard knee functionality scales

Test category	Detailed investigational options
PROMs	1. Lysholm Knee Score[26] 2. Activity level 3. IKDC Score24 4. SF-36 5. WOMET[30] 6. Tegner score 7. Cincinnati Knee Rating[28] 8. Kujala score[31,32] 9. KOOS[24] 10. WOMAC[21] 11. VAS for pain
Procedure or graft failure	1. Graft rupture/resorption 2. Requirement for revision
Post-operative complications	1. Infection 2. Immune reaction 3. Repetitive effusion
Examples of application	Large focal osteochondral injury of the patella

References for Annex VI

1. Li M, Feng C, Gu X *et al.* Effect of cryopreservation on proliferation and differentiation of periodontal ligament stem cell sheets. *Stem Cell Res Ther* 2017;8:1-10.
2. Tran KD, Dye PK, Odell K *et al.* Evaluation and quality assessment of prestripped, preloaded descemet membrane endothelial keratoplasty grafts. *Cornea* 2017;36:484-90.
3. Pipparelli A Thuret G, Toubeau D *et al.* Pan-corneal endothelial viability assessment: Application to endothelial grafts predissected by eye banks. *Investig Ophthalmol Vis Sci* 2011;52:6018-25.
4. Romano V, Parekh M, Ruzza A *et al.* Comparison of preservation and transportation protocols for preloaded Descemet membrane endothelial keratoplasty. *Br J Ophthalmol* 2018;102:549-55.
5. Moon I Kang HG, Yeo A *et al.* Comparison of ocular surface mucin expression after topical ophthalmic drug administration in dry eye-induced mouse model. *J Ocul Pharmacol Ther* 2018;34:1-9.
6. Koh SM, Coll T, Gloria D *et al.* Corneal endothelial cell integrity in precut human donor corneas enhanced by autocrine vasoactive intestinal peptide. *Cornea* 2017;36:476-83.
7. Amano S, Shimomura N, Yokoo S *et al.* Decellularizing corneal stroma using N_2 gas. *Mol Vis.* 2008;14:878-82.
8. Di Mundo R, Recchia G, Parekh M *et al.* Sensing inhomogeneous mechanical properties of human corneal Descemet's membrane with AFM nano-indentation. *J Mech Behav Biomed Mater* 2017;74:21-7.
9. Korb DR, Herman JP, Finnemore VM, *et al.* An evaluation of the efficacy of fluorescein, rose bengal, lissamine green, and a new dye mixture for ocular surface staining. *Eye Contact Lens* 2008;34:61-4.
10. Sponsel WE, Johnson SL, Trevino R *et al.* Pattern electroretinography and visual evoked potentials provide clinical evidence of CNS modulation of high- and low-contrast VEP latency in glaucoma. 2017;6:6.
11. Li W, Zhou J, Xu Y. Study of the *in vitro* cytotoxicity testing of medical devices. *Biomed Rep* 2015;3:617-20. doi:10.3892/br.2015.481.
12. Morton WR. Observations on the full-term foetal membranes of three members of the Camelidae (*Camelus dromedarius L., Camelus bactrianus L.* and *Lama glama L.*). *J Anat* 1961;95:200-9.
13. Zahari NK, Sheikh Ab Hamid S, Yusof N. Effects of different doses of gamma irradiation on oxygen and water vapour transmission rate of preserved human amniotic membrane. *Cell Tissue Bank.* 2015;16:55-63.
14. van Versen-Hoeynck, F, Steinfeld AP, Becker J. Sterilization and preservation influence the biophysical properties of human amnion grafts. *Biologicals* 2008;36:248-55.
15. Kazlouskaya V, Malhotra S, Lambe J *et al.* The utility of elastic Verhoeff-Van Gieson staining in dermatopathology. *J Cutan Pathol* 2013;40:211-25.
16. Gudienė D, Baltrušaitis K, Rackauskas M. Features of elastic tissue staining and its arrangement in the wall of human basilar artery. *Medicina (Kaunas)* 2003;39:946-50.
17. Dean DD, Woessner JF Jr. A sensitive, specific assay for tissue collagenase using telopeptide-free [3H] acetylated collagen. *Anal Biochem* 1985;148:174-81.

18. Tran D, Golick M, Rabinovitz H *et al.* Hematoxylin and safranin O staining of frozen sections. *Dermatol Surg* 2000;26:197-99.
19. Wang YH, Liu Y, Maye P *et al.* Examination of mineralized nodule formation in living osteoblastic cultures using fluorescent dyes. *Biotechnol Prog* 2006;22:1697-701.
20. Tan H, Junor L, Price RL *et al.* Expression and deposition of fibrous extracellular matrix proteins in cardiac valves during chick development. *Microsc Microanal* 2011;17:91–100.
21. Western Ontario and McMaster Universities Arthritis Index (WOMAC) [available at http://www.womac.org/, accessed 23 Oct 2018].
22. Mallouris A, Kakagia D, Yiacoumettis A *et al.* Histological comparison of the human trunk skin creases: the role of the elastic fiber component. *Eplasty* 2016;16:e15.
23. Sridharan R, Cameron AR, Kelly DJ *et al.* Biomaterial based modulation of macrophage polarization: a review and suggested design principles. *Mater Today* 2015;18:313-25.
24. Huang C-C, Chen W-S, Tsai M-W *et al.* Comparing the Chinese versions of two knee-specific questionnaires (IKDC and KOOS): reliability, validity, and responsiveness. *Health Qual Life Outcomes* 2017;15:238.
25. Briggs KK, Lysholm J, Tegner Y, *et al.* The reliability, validity, and responsiveness of the Lysholm score and Tegner activity scale for anterior cruciate ligament injuries of the knee: 25 years later. *Am J Sports Med* 2009;37:890-7.
26. Collins NJ, Misra D, Felson DT *et al.* Measures of knee function. *Arthritis Care Res (Hoboken).* 2011:63:S208-28.
27. Hoemann C, Kandel R, Roberts S *et al.* International Cartilage Repair Society (ICRS) recommended guidelines for histological endpoints for cartilage repair studies in animal models and clinical trials. *Cartilage* 2011;2:153-72 (2011).
28. Modified Cincinnati Rating System Questionnaire [available at: http://www. orthopaedicscore.com/scorepages/cincinnati.html, accessed 23 Oct 2018].
29. Hoemann CD, Sun J, Chrzanowski V *et al.* A multivalent assay to detect glycosaminoglycan, protein, collagen, RNA, and DNA content in milligram samples of cartilage or hydrogel-based repair cartilage. *Anal Biochem* 2002;300:1-10.
30. Sgroi M, Däxle M, Kocak S, *et al.* Translation, validation, and cross-cultural adaption of the Western Ontario Meniscal Evaluation Tool (WOMET) into German. *Knee Surg Sports Traumatol Arthrosc* 2018;26:2332-7.
31. Dammerer D, Liebensteiner MC, Kujala UM *et al.* Validation of the German version of the Kujala score in patients with patellofemoral instability: a prospective multi-centre study. *Arch Orthop Trauma Surg.* 2018;138:527-35.
32. Orthotoolkit. Free Online Kujala (Anterior Knee Pain Scale) Score Calculator [available at http://orthotoolkit.com/kujala/, accessed 5 Oct 2018]

Annex VII. Worked example of risk assessment: replacement tissues

SOHO GUIDES REPORT **EuroGTP II tool v1.0.0**

DECONTAMINATION OF HEART VALVES

Submitted on 27/10/2023

GENERAL INFORMATION

First name	Last name
Test	User

Tissue Establishment

Test Lab

General category of soho under evaluation

Replacement tissues

Specific category of btc under evaluation

Cardiovascular

Name of the product, therapy or process under evaluation:

Decontamination of heart valves

Description

Heart valves allografts are currently decontaminated with an antibiotic solution prior to being cryopreserved. TE is proposing to change the formulation of our antibiotic solution.

European Directorate for the Quality of Medicines & HealthCare | Direction européenne de la qualité du médicament & soins de santé
COUNCIL OF EUROPE CONSEIL DE L'EUROPE

EVALUATION OF NOVELTY

A. Has this type of BTC previously been collected, processed/prepared and issued for clinical use by your establishment?

Yes

TE already provides heart valves

B. Will the starting material used to prepare this BTC be obtained from the same donor population previously used by your establishment for this type of BTC?

Yes

Donor selection criteria are not changing

C. Will the starting material for this BTC be procured/collected using a procedure used previously by your establishment for this type of BTC?

Yes

Procurement procedure is not changing

D. Will this BTC be prepared by a procedure (processing/preparation, decontamination/pathogen reduction and preservation) used previously in your establishment for this type of BTC?

No

The composition of a critical processing reagent is changing

E. Will this BTC be packaged, stored and distributed using a protocol and materials used previously in your establishment for this type of BTC?

Yes

Storage and packaging are not changing

F. Will this type of BTC provided by your establishment be applied/infused clinically using an application/transfusion/infusion method used previously?

Yes

Clinical application is not changing

G. Has your establishment provided this type of BTC for the same clinical indication or for application/transfusion/infusion into the same anatomical site?

Yes

Clinical application is not changing

LEVEL OF RISK ANALYSIS

Donor characteristics

No

No comment has been provided

Procurement/collection process and environment

No

No comment has been provided

Processing and environment

No

No comment has been provided

Reagents/added components

Yes

Our current antibiotic solution contains five antibiotics. The manufacturing of one antibiotic has been discontinued. We are replacing this with another antibiotic.

Risk of immunogenicity: we know that traces of antibiotic can be retained in the tissue. There is a risk that the new antibiotic may cause allergies. Manufacturer guidance suggests that 1/10 000 patients may have allergic reactions. (Probability: we considered this is possible because there is evidence the antibiotic can cause an allergic response in a small number of patients; therefore, we selected a score of 3; Severity: despite the nature of this graft, it is unlikely to be life-threatening; Detectability: there is no way to implement a routine quality control test to ensure the absence of traces in the graft. We have no evidence to suggest whether or not the antibiotic remains in the graft after treatment, which justifies a high score for detectability; Risk Reduction: there is no evidence of risk reduction at this stage because we have no viable data or literature regarding this issue).

Implant failure: as this is a new chemical that has not previously been applied to cardiac tissue, the risk that it may damage the tissue needs to be considered. Following a literature search, we identified evidence suggesting that this antibiotic does not damage heart valve allografts. (Probability: is unlikely because we have some evidence that shows the antibiotic does not damage the graft in any detectable way; Severity: a mechanical/sudden failure of the graft would have severe consequences for the recipient; Detectability: we cannot test the properties of the graft during routine quality control; Risk Reduction: we do have some documented evidence to suggest that interaction of the antibiotic with the graft is safe).

Disease transmission: the purpose of the antibiotic solution is to reduce or eliminate bioburden in the heart valve If it does not do this, the valve could transmit disease, which in the valve recipient could be very serious. We have received advice from a microbiology expert that our new antibiotic is highly active and is an effective substitute for the former one. However, we do not know if the efficacy of the antibiotic would be compromised in combination with our solution. (Probability: we have not done any validation tests with this antibiotic; Severity: an infection could have severe consequences for the recipient; Detectability: we routinely evaluate individual grafts; however, we know there are limitations in the reliability of the testing process; Risk Reduction: we have evidence provided by a microbiologist advising of the efficacy of this antibiotic).

Toxicity/carcinogenicity: we know that traces of antibiotic can be retained in the tissue. This may cause toxic/carcinogenic effects in the recipient due to the new chemical itself or the interaction with other reagents in the antibiotic solution. The information from the manufacturer suggests there is no evidence of toxic or carcinogenic effects (Probability: difficult to believe that an interaction between the antibiotics could happen; Severity: the potential quantities that would be transferred to the recipient are very low; Detectability: there is no routine test; Risk Reduction: the information from the manufacturer suggests there is no evidence of a toxic or carcinogenic effect).

Risk consequences	Probability	Severity	Detectability	Potential risk	Risk reduction	Risk
Unexpected immunogenicity	3	2	4	24	0 %	24
Implant failure	2	3	4	24	50 %	12
Disease transmission	3	3	2	18	50 %	9
Toxicity / Carcinogenicity	1	2	4	8	75 %	2

Reliability of microbiology testing

Yes

It is possible that residual quantities of the antibiotic could compromise decontamination.

Disease Transmission: (Probability: there is evidence that it could potentially lead to a false negative result; Severity: an infection could have severe consequences for the recipient; Detectability: there is no routine test in place which considers the presence of this antibiotic; Risk Reduction: currently we have no evidence).

Risk consequences	Probability	Severity	Detectability	Potential risk	Risk reduction	Risk
Disease transmission	3	3	4	36	0 %	36

Storage conditions

No

No comment has been provided

Transport conditions

No

No comment has been provided

Presence of unexpected cellular material and/or graft vascularity

No

No comment has been provided

Complexity of the immediate pre-implantation preparation and/or application method

No

No comment has been provided

Preliminary score: **83**
Number of applicable risks consequences: **5**
Number of risk consequences: **5**
Max individual Risk value = **36**

Highest possible risk score = 5 * 4 * 5 * 5 * 9 = **4500**
Applicable risk score = 5 * 4 * 5 * 5 = **500**

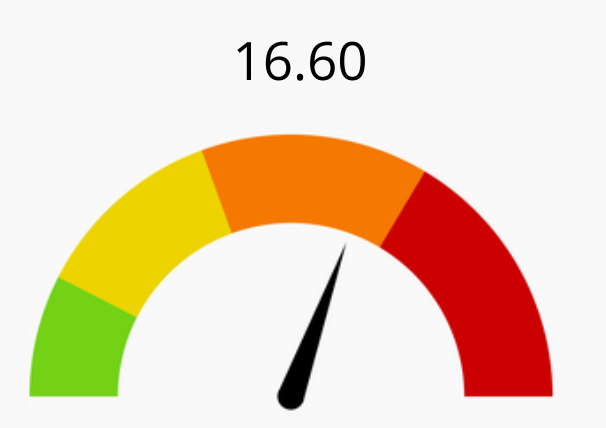
Combined risk value = (Risk value * Highest possible risk score) / Number of applicable risks = (83*4500) / 500 = **747**
Final risk score = (Final risk score * 100) / Highest possible risk score = (747*100) / 4500 = **16.60**

Your assessment has final risk score of: **16.60**

This suggests that your BTC falls into the level of risk: **Moderate**

ADDITIONAL INFORMATION

RESULTS AND EXTENT OF STUDIES



Level of risk

Moderate

Extent of studies needed

Step3A: Risk reduction strategies

- The assessment indicates that more evidence is needed to support safe and effective use of this BTC and mitigate risk. Process validation should be performed; however, if the nature of the risk is not related to the process itself, the requirement for validation may not apply, for example where the novelty is in the method of clinical application.
- Pre-clinical in vitro evaluation, specific to the identified risks, should be performed if not already done.
- Pre-clinical in vivo evaluation, specific to the identified risks, using an animal model should be done if applicable (and if not already completed).

Please refer to Annex VI of the EuroGTP II tool Guide for additional details.

Step 3B: Extent of clinical evaluation

- A structured plan for active collection of a specific set of data relating to the safety and efficacy of the BTC should be put in place, in addition to routine clinical follow-up. Ethical approval may be required and the principles of Good Clinical Practice (GCP) adhered to.
- Consideration should be given to restricting provision of the BTC to a limited number of patients and/or centres until the risks have been adequately mitigated.

Please refer to Annex VI of the EuroGTP II tool Guide for additional details.

Please refer to EuroGTP II Guide and Guide to the quality and safety of tissues and cells for human application for additional details.

Annex VIII. Worked example of risk assessment: HPC

SOHO GUIDES REPORT **EuroGTP II tool v1.0.0**

PLERIXAFOR
Submitted on 27/10/2023

GENERAL INFORMATION

First name	Last name
Test	User

Tissue Establishment

Test Lab

General category of soho under evaluation

Haematopoietic progenitor cells

Specific category of btc under evaluation

Peripheral Blood

Name of the product, therapy or process under evaluation:

Plerixafor

Description

Mobilization of healthy haplo identical donors with plerixafor

EVALUATION OF NOVELTY

A. Has this type of BTC previously been collected, processed/prepared and issued for clinical use by your establishment?

No

No comment has been provided

B. Will the starting material used to prepare this BTC be obtained from the same donor population previously used by your establishment for this type of BTC?

No

No comment has been provided

C. Will the starting material for this BTC be procured/collected using a procedure used previously by your establishment for this type of BTC?

Yes

No comment has been provided

D. Will this BTC be prepared by a procedure (processing/preparation, decontamination/pathogen reduction and preservation) used previously in your establishment for this type of BTC?

Yes

No comment has been provided

E. Will this BTC be packaged, stored and distributed using a protocol and materials used previously in your establishment for this type of BTC?

Yes

No comment has been provided

F. Will this type of BTC provided by your establishment be applied/infused clinically using an application/transfusion/infusion method used previously?

Yes

No comment has been provided

G. Has your establishment provided this type of BTC for the same clinical indication or for application/transfusion/infusion into the same anatomical site?

No

No comment has been provided

LEVEL OF RISK ANALYSIS

Donor characteristics

Yes

Use of a new mobilization agent

Risk consequences	Probability	Severity	Detectability	Potential risk	Risk reduction	Risk
Unexpected immunogenicity	2	1	2	4	25 %	3
Engraftment failure	2	1	3	6	25 %	4.5

Procurement/collection process and environment

No

No comment has been provided

Processing and environment

No

No comment has been provided

Reagents

Yes

proinflammatory 6-sulfo-LacNac+ detected

Risk consequences	Probability	Severity	Detectability	Potential risk	Risk reduction	Risk
Unexpected immunogenicity	2	1	1	2	0 %	2
Engraftment failure	2	1	1	2	0 %	2

Reliability of microbiology testing

No

No comment has been provided

Storage conditions

No

No comment has been provided

Transport conditions

No

No comment has been provided

Presence of unexpected cellular material

No

No comment has been provided

Complexity of the pre-implantation preparation and/or application method

Yes

No comment has been provided

Risk consequences	Probability	Severity	Detectability	Potential risk	Risk reduction	Risk
Unexpected immunogenicity	2	1	1	2	0 %	2

Preliminary score: **13.5**
Number of applicable risks consequences: **5**
Number of risk consequences: **5**
Max individual risk value = **4.5**

Highest possible risk score = 5 * 4 * 5 * 5 * 9 = **4500**
Applicable risk score = 5 * 4 * 5 * 5 = **500**

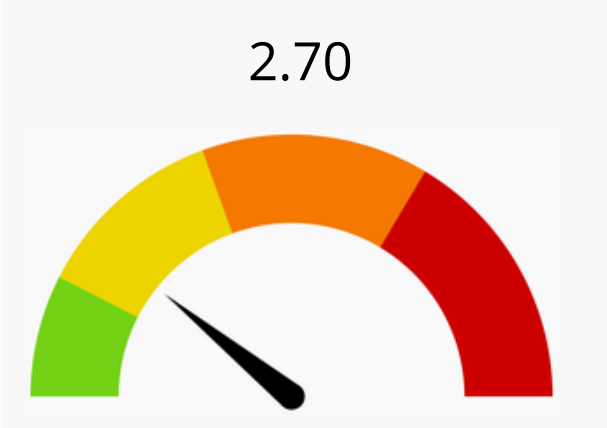
Combined risk value = (Risk value * Highest Possible Risk Score) / Number of applicable risks = (13.5 * 4500) / 500 = **121.5**
Final risk score = (Final risk score * 100) / Highest possible risk score = (121.5 * 100) / 4500 = **2.70**

Your assessment has final risk score of: **2.70**

This suggests that your BTC falls into the level of risk: **Low**

ADDITIONAL INFORMATION

RESULTS AND EXTENT OF STUDIES



Level of risk

Low

Extent of studies needed

Step 3A: Risk reduction strategies

Implementing a standard procedure or treatment in an HPC centre that has never performed it requires an **intensive validation**. Training of staff (as required by Joint Accreditation Committee ISCT-Europe & EBMT (JACIE)) is necessary in order to reach the outcomes published in scientific literature.

A learning curve may be expected and should be part of the validation report. When implementing the procedure, additional quality controls must be performed to **monitor critical process parameters (CPPs) and critical quality attributes (CQAs)**. For example, when a TE is switching from T-cell depletion (TCD) to CD34+ selection (which they have never performed before), engraftment rate, and graft-versus-host reactions should be carefully monitored.

Step 3B: Extent of clinical evaluation

A **safety follow-up programme** is necessary. Follow-up procedures (conforming to EBMT Med-A, Med-B or Med-A cellular) should focus on assessing efficacy, comparing the clinical follow-up with the results obtained before the implementation of the change in the process and in relation to the results published in scientific literature. The expected learning curve should be kept as short as possible and put in relation to the follow-up programme. Likewise, established techniques are subject to long-term (ideally transgenerational) follow-up of the health effects, as established by EBMT.

Please refer to EuroGTP II Guide and Guide to the quality and safety of tissues and cells for human application for additional details.

Annex IX. Worked examples of risk assessment: MAR

SOHO GUIDES REPORT **EuroGTP II tool v1.0.0**

OOCYTE
Submitted on 27/10/2023

GENERAL INFORMATION

First name	Last name
Test	User

Tissue Establishment

Test Lab

General category of soho under evaluation

Medically assisted reproduction

Specific category of btc under evaluation

Gametes

Name of the product, therapy or process under evaluation:

Oocyte

Description

Usage of a new aspiration pump for oocyte recovery: to change the manual aspiration into aspiration with pump

EVALUATION OF NOVELTY

A. Has this type of BTC previously been collected, processed/prepared and issued for clinical use by your establishment?

Yes

No comment has been provided

B. Will the starting material used to prepare this BTC be obtained from the same donor population previously used by your establishment for this type of BTC?

Yes

No comment has been provided

C. Will the starting material for this BTC be procured/collected using a procedure used previously by your establishment for this type of BTC?

No

Previously only an aspiration pump or hand aspiration has been used, but not an aspiration/follicle irrigation system.

D. Will this BTC be prepared by a procedure (processing, decontamination and preservation) used previously in your establishment for this type of BTC?

Yes

No comment has been provided

E. Will this BTC be packaged, stored and distributed using a protocol and materials used previously in your establishment for this type of BTC?

Yes

No comment has been provided

F. Will this type of BTC provided by your establishment be applied/infused clinically using an application/infusion method used previously?

Yes

No comment has been provided

G. Has your establishment provided this type of BTC for the same clinical indication or for application/transfusion/infusion into the same anatomical site?

Yes

No comment has been provided

LEVEL OF RISK ANALYSIS

Donor characteristics

No

In this case the donor population is the same.

Procurement process and environment

Yes

With a new aspiration/irrigation system the process of oocyte retrieval is different and may affect the quality of the oocytes/embryos (e.g. affect the incidence of aneuploidy or oocyte/embryo degeneration); however, this pump has been used by other centres and therefore a substantial risk reduction can be applied.

Risk consequences	Probability	Severity	Detectability	Potential risk	Risk reduction	Risk
Implant failure / Pregnancy loss	1	2	2	4	75 %	1
Toxicity / Carcinogenicity	1	1	1	1	75 %	0.25

Processing and environment

No

In this case the processing and environment is the same; however, it may be different if the system requires special containers that can change the environment surrounding the eggs (temperature, pH, etc.)

Reagents

No

The processing in this case should be the same and with the same medium.

Storage conditions

No

Should be the same.

Transport conditions

No

Should be the same.

Loss of viability and or functionality

Yes

In this case, there may be loss of viability due to pressure, temperature, pH, or other factors that may result in a higher aneuploidy rate or higher degeneration rate.
If there were data from literature or from other centres using this pump that showed good results, then there could be a substantial risk reduction.

Risk consequences	Probability	Severity	Detectability	Potential risk	Risk reduction	Risk
Implant failure / Pregnancy loss	1	2	2	4	75 %	1

Complexity of the pre-implantation preparation and/or application method

No

Clinical application has not changed in this example.

Preliminary score: **2.25**
Number of applicable risks consequences: **3**
Number of risk consequences: **4**
Max individual risk value = **1**

Highest possible risk score = 5 * 4 * 5 * 4 * 8 = **3200**
Applicable risk score = 5 * 4 * 5 * 3 = **300**

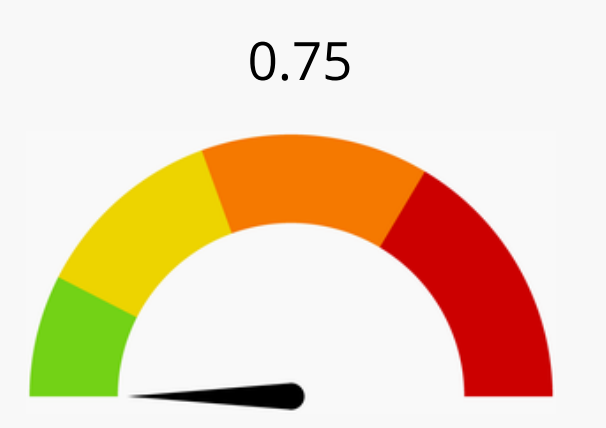
Combined risk value = (Risk value * Highest possible risk score) / Number of applicable risks = (2.25*3200) / 300 = **24**
Final Risk Score = (Final risk score * 100) / Highest possible risk score = (24*100) / 3200 = **0.75**

Your assessment has final risk score of: **0.75**

This suggests that your BTC falls into the level of risk: **Negligible**

ADDITIONAL INFORMATION

RESULTS AND EXTENT OF STUDIES



Level of risk

Negligible

Extent of studies needed

Step 3A: Risk reduction strategies

A change in process could have a negligible level of risk because it is part of a therapy or procedure that is considered as established or standard. In this case multi-centre studies (ideally RCTs) are published in peer-reviewed journals and the procedures are performed according to a validated and standard protocol. **Minimal process validation is needed**. The technical performance of staff should be monitored and comparable with other TEs or published studies; therefore, standard key performance indicators (KPIs) should be monitored on the technical quality of the staff performing the procedures. Dropping KPIs indicating protocol drift must lead to investigation of the procedural steps and/or the possibility to retrain staff.

Step 3B: Extent of clinical evaluation

A **routine/safety follow-up programme** is enough as good practice states. Follow-up procedures should be focused on assessing efficacy, comparing the clinical follow-up with the results obtained before the implementation of the change in the process. Long-term (ideally transgenerational) health effects, including aspects such as fertility, oncology and mental health, should be monitored.

Please refer to EuroGTP II Guide and Guide to the quality and safety of tissues and cells for human application for additional details.

SOHO GUIDES REPORT **EuroGTP II tool v1.0.0**

SPERM
Submitted on 27/10/2023

GENERAL INFORMATION

First name	Last name
Test	User

Tissue Establishment

Test Lab

General category of soho under evaluation

Medically assisted reproduction

Specific category of btc under evaluation

Gametes

Name of the product, therapy or process under evaluation:

Sperm

Description

Change from slow freezing of ejaculated sperm to lyophilisation of ejactulated sperm.

EVALUATION OF NOVELTY

A. Has this type of BTC previously been collected, processed/prepared and issued for clinical use by your establishment?

Yes

No comment has been provided

B. Will the starting material used to prepare this BTC be obtained from the same donor population previously used by your establishment for this type of BTC?

Yes

There is no change in donor population

C. Will the starting material for this BTC be procured/collected using a procedure used previously by your establishment for this type of BTC?

Yes

Collection is the same in the slow protocol as the lyophilisation protocol

D. Will this BTC be prepared by a procedure (processing, decontamination and preservation) used previously in your establishment for this type of BTC?

No

The procedure is completely different and in the exercise your TE has no experience with lyophilisation of sperm

E. Will this BTC be packaged, stored and distributed using a protocol and materials used previously in your establishment for this type of BTC?

Yes

If the same containers can be used for cryopreserved sperm, then the answer is 'yes', if the exercise would have an additional change: from vial to straw e.g., then this answer should be 'no'

F. Will this type of BTC provided by your establishment be applied/infused clinically using an application/infusion method used previously?

Yes

No comment has been provided

G. Has your establishment provided this type of BTC for the same clinical indication or for application/transfusion/infusion into the same anatomical site?

Yes

No comment has been provided

LEVEL OF RISK ANALYSIS

Donor characteristics

No

There are no changes in the donor characteristics.

Procurement process and environment

No

Changes in the cryopreservation protocol have no effect on the procurement. There are no extra risks that need to be evaluated during this procurement.

Processing and environment

Yes

Changing to lyophilisation definitely has an effect and risk needs to be considered.
For this example, it is likely that due to the lyophilisation, there will be no implantation when using this sperm because of loss of functionality or viability after thawing. The severity is serious in this example: if the sperm is not viable after lyophilisation and thawing then there is a significant decrease in the expected treatment success, and thus score.
You may be tempted to use 'life-threatening' here because there will not be a pregnancy or the gametes may be destroyed. We would like to point out that 'fatal' is only used if there is a risk of death of the patient and not the embryo or the foetus.
This assessment is on the risks for the recipient, not the embryo. If the sperm was not vital after using this novel cryopreservation method, we would most certainly detect this. In this example, you could consider risk reduction based on animal studies; however, there are no data in a human setting. So at this stage a risk reduction is not possible. It is important to only take into account the processing steps when evaluating this risk: during the processing steps, DNA fragmentation can be introduced in the sperm. Literature shows that the preparation for lyophilisation is quite easy and quick, so a shift from slow freezing to lyophilisation may not increase the complexity of the method, and therefore there is no risk of introducing contaminants because of a very complex procedure. So the risk of disease transmission would be unlikely. If this did occur, it could be serious as hospitalisation could be necessary. The presence of viruses could be detected: for several viruses, PCR can be performed. If a sperm bank is interested in using lyophilisation, validation could be performed using PCR testing after thawing and hydration of the sample. A risk reduction can be applied when, for example, a validated post-thawing wash is performed which is known to remove HIV and HCV. Moreover, this post-thaw wash is referenced in many peer-reviewed data. As an example you might end up with a risk reduction of 75%. There is no evidence that the preparation process would introduce toxic substances or carcinogenicity into the sperm. This risk is not applicable and therefore should not be addressed.
It is always possible to include risk factors other than those cited in the tool.

Risk consequences	Probability	Severity	Detectability	Potential risk	Risk reduction	Risk
Implant failure / Pregnancy loss	5	2	1	10	0 %	10
Disease transmission	2	2	1	4	75 %	1

Reagents

Yes

When different types of reagents are used in the lyophilisation protocol, a potential risk needs to be considered. When considering the reagents needed in the lyophilisation procedure, it is important to not take the processing steps into account, otherwise you could end up with the same result as before. Only consider the new reagents. For example: the reagents used for lyophilisation would be TE buffer (1mM tris, 1 mM EDTA, pH 8.0).
Would they have an impact on the implant failure, including pregnancy loss? Probably not, because most of the reagents are not toxic for gametes. However, if they turn out to have an impact, the result would be fatal. Would it be possible to detect this: yes, it is possible to look at the morphological changes to the sperm and/or perform vitality staining. There are data that suggest that this TE buffer has no effect on sperm; however, these data may not include combination with a lyophilisation procedure.

Risk consequences	Probability	Severity	Detectability	Potential risk	Risk reduction	Risk
Implant failure / Pregnancy loss	1	2	1	2	25 %	1.5

Storage conditions

Yes

It may be that the fact that lyophilisation is going to be performed means that additional care has to be taken with regard to the storage conditions during the preparation steps, e.g. the sample needs to be placed on ice after procurement and before lyophilisation. Cryostorage after lyophilisation is at 4°C, so no liquid nitrogen would be necessary. Consider the risks with this change in the protocol.

Risk consequences	Probability	Severity	Detectability	Potential risk	Risk reduction	Risk
Implant failure / Pregnancy loss	4	2	1	8	0 %	8

Transport conditions

No

In this example we expect no differences in transport conditions.

Loss of viability and or functionality

Yes

Risks need to be considered. It is known from the literature that sperm (from animals) becomes immotile after lyophilisation. This could impact the success of the treatment.

Risk consequences	Probability	Severity	Detectability	Potential risk	Risk reduction	Risk
Implant failure / Pregnancy loss	5	2	1	10	0 %	10

Complexity of the pre-implantation preparation and/or application method

No

We expect no changes in the method of application in this example. However, it could be the case that the manipulation after storage is very different and would have an impact on the outcome. Hypothetically, if lyophilised sperm need to be put in a very different insemination catheter, this could take much more time to load. In this case, you will have to consider this risk. So it is only the complexity of the application method or the preparation for clinical application.

Preliminary score: **30.5**
Number of applicable risks consequences: **5**
Number of risk consequences: **4**
Max individual risk value = **10**

Highest possible risk score = 5 * 4 * 5 * 4 * 8 = **3200**
Applicable risk score = 5 * 4 * 5 * 5 = **500**

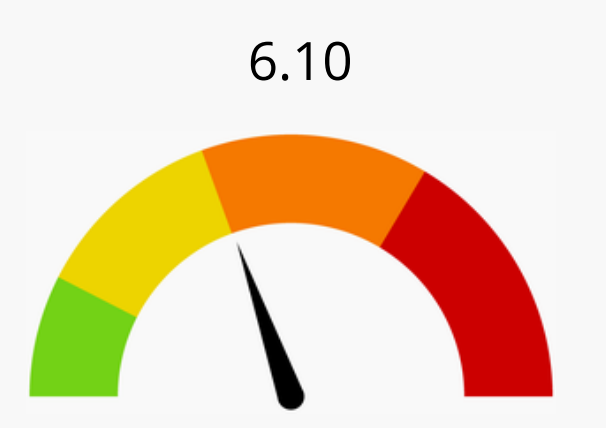
Combined risk value = (Risk value * Highest possible risk score) / Number of applicable risks = (30.5*3200) / 500 = **195.2**
Final risk score = (Final risk score * 100) / Highest possible risk score = (195.2*100) / 3200 = **6.10**

Your assessment has final risk score of: **6.10**

This suggests that your BTC falls into the level of risk: **Moderate**

ADDITIONAL INFORMATION

RESULTS AND EXTENT OF STUDIES



Level of risk

Moderate

Extent of studies needed

Step 3A: Risk reduction strategies

Novel procedures or treatments that exert a moderate risk and are considered innovative. The treatment has shown proof of principle and there are reassuring data in literature in terms of both safety and effectiveness, at least in animal studies, and preclinical data show normal embryology development. The studies that have published these data should have a sound methodology and be published in peer-reviewed journals. In order to implement an innovative treatment, an **enhanced validation** is necessary, including a range of **additional quality controls** performed to monitor critical process parameters (CPPs), critical quality attributes (CQAs) and the impact of the implemented changes on gametes, embryos and gonadic tissue in the preclinical studies. Since reassuring non-clinical data of this innovative treatment should at least be already available, a more specific monitoring of the published critical parameters can be performed instead of a registration of all critical parameters.

Step 3B: Extent of clinical evaluation

Clinical evaluation and follow-up programmes should be implemented to assess mid-term safety (3 months up to 5 years post-delivery, including data on psychological wellbeing) and these studies should refer to patients undergoing the procedure as well as the children born from it.

Please refer to EuroGTP II Guide and Guide to the quality and safety of tissues and cells for human application for additional details.

Annex X. Classification of tissues and cells in the interactive assessment tool

General category	Specific category	Category per tissue and cell
Replacement tissues	Musculoskeletal	Whole or part of structural/supporting bone
		Tendon (including with bony attachments) ligaments/ fascia
		Osteochondral grafts
		Bone filling material (excluding femoral heads)
		Femoral heads
		Demineralised bone matrix (including combined with a carrier)
		Meniscus
		Other musculoskeletal (e.g. ear ossicles, cranial bone, cartilage)
	Cardiovascular	HV, aortic
		HV, pulmonary
		HV, aortic decellularised
		HV, pulmonary decellularised
		Non-valved patches and conduits
		Pericardium
		Other heart tissues
		Vessels, arteries
		Vessels, veins
	Amniotic membrane	Amniotic membrane
		Amniotic membrane eye drops
		Other placenta
	Ocular	Cornea full thickness (high endothelial cell density)
		Cornea full thickness (low endothelial cell density)
		Cornea for endothelial keratoplasty (pre-cut/peeled in the tissue establishment)
		Sclera
		Other ocular
	Skin	Skin
		Acellular dermal matrix
		Keratinocytes/melanocytes
		Other cutaneous tissues
	Other	Neuronal tissue (nerves)
		Adipose tissue
		Hepatocytes
		Pancreatic islets
		Parathyroid tissue
		Other than above
Haematopoietic progenitor cells	Bone Marrow	-
	Peripheral blood	-
	Cord blood	-
	Other	-
Medically assisted reproduction	Gametes	-
	Embryos	-
	Gonadic tissue	-

ENG

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