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PREPARATION PROCESS FOR BLOOD, TISSUES AND CELLS

Technical annex 2 to overall guidance: assessing the quality and safety of donor testing, pathogen reduction and sterilisation steps as part of Preparation Process Authorisation (PPA)

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Introduction

Blood and blood components, and tissues and cells for clinical use, bear the risk of carrying a number of infectious agents. If present, an infectious agent may then be unintentionally transmitted through transfusion/transplantation, which could then lead to disease and even death in recipients. Over the years there have been numerous reports of infectious disease transmissions through blood, tissues and cells (BTC). Today, regulations, standards, improved donor selection procedures and testing are in place, all helping to minimising the risk of infectious disease transmission. However, cases of viral, bacterial, parasitical and fungal infections from BTC still occur. Moreover, new threats affecting donations or recipients have also been identified, for example prions (e.g. in variant Creutzfeldt-Jakob disease; vCJD), emerging viruses (e.g. West Nile virus, dengue virus, Chikungunya virus, Zika virus), parasites (e.g. *Plasmodium spp.* in malaria, *Trypanosoma cruzi* in Chagas disease, *Babesia* in babesiosis) and multidrug-resistant bacteria. Emergence of novel pathogens is rather unpredictable, however mathematical models suggest that every 5 years a new transfusion-transmissible infectious agent could emerge (Gallagher *et al*. 2013). The severe acute respiratory syndrome (SARS) coronavirus in 2002/2003, the Middle East respiratory syndrome coronavirus (MERS-CoV) in 2012, the Zika virus in 2015/2016, and the new severe acute respiratory syndrome coronavirus (SARS-CoV-2) from 2019 onwards are good examples of how unexpectedly pathogens can emerge and spread (Kuiken *et al*. 2003; Qi *et al*. 2013; Talero-Gutiérrez *et al.* 2018; Wang *et al*. 2020). Therefore, the risk of transmission of an infectious agent through BTC remains a rare but ongoing concern.

Since the large number of cases of transfusion-transmitted human immunodeficiency virus (HIV) in the 1980s, the probability of BTC transmitted HIV, hepatitis B virus (HBV) and hepatitis C virus (HCV) infections has markedly decreased through the introduction of risk mitigation strategies: for example revised donor selection criteria, post-donation information management, improved and expanded testing strategies, including nucleic acid amplification technique (NAT) testing of donors/donations for an increasing range of infectious agents. Indeed, appropriate and reliable laboratory testing of each donation and/or donor, control of reagents, pathogen reduction, as well as, where appropriate, post-processing microbiological testing of BTC, can substantially reduce the risk of transmission, and improve the overall safety of BTC.

In this guidance, use of the word '*must*' indicates mandatory compliance in alignment with applicable EU legislation, whereas the use of the word '*should*' indicates recommended compliance in accordance with commonly accepted relevant guidance.

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Aims

This annex provides high level requirements and criteria for verifying that the microbiological safety of blood, tissues and cells is in accordance with current European Blood, Tissues and Cells Directives (EUBTCDs; Directives 2002/98/EC, 2004/33/EC, 2005/61/EC, 2005/62/EC, 2011/38/EU, 2014/110/EU, 2016/1214/EU, 2004/23/EC, 2006/17/EC, 2006/86/EC, 2012/39/EU, 2015/565/EU, 2015/566/EU) and other standards and guidelines that ensure the quality and safety of BTC.

In more detail, this guidance describes aspects which the Competent Authorities (CA) of Member States (MS) should take into account when assessing:

- competence of laboratories performing donor/donation infectious disease testing and microbiological testing of BTC
- reliability of the donor/donation infectious disease marker test kits
- effectiveness of pathogen reduction during BTC processing
- effectiveness of sterilisation methods during BTC processing
- microbiological status of final BTC products

Microbiological safety will be assessed in relation to the potential presence of bacteria, viruses, fungi, parasites and prions in BTC (as defined by GAPP).

Scope

The content of this document only applies to BTC and their applications as regulated in EUBTCDs, and all novel BTC that are not currently covered by other regulations.

BTC 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 Advanced Therapy Medicinal Product (ATMP) Regulation 1394/2007/EC), BTC products classified as Medical Devices and other Medicinal products (such as plasma-derived medicinal products), are not part of the scope of the GAPP Joint Action. Donation, procurement and testing of BTC intended for ATMP manufacturing do fall under the scope of the GAPP Joint Action.

The procedures of BTC donor and product monitoring and quality control testing themselves are not in the scope of this guidance.

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Furthermore, this guidance does not extend to the assessment of activities such as aseptic working methods, cleanroom maintenance or environmental monitoring, which are assessed by CAs during the Blood Establishment/Tissue Establishment (BE/TE) inspections. Health and safety issues for staff are also out of the scope of this guidance.

1. General validation requirements

Performance validation is required for donor/donation infectious disease marker test kits, pathogen reduction and sterilisation, and thus, the general validation requirements described here apply to chapters 3, 4 and 5, respectively.

Validation is usually split into two components: qualification and process or method validation. Each part of the process, and individual items (including facilities, equipment, computer systems, materials and staff), should be qualified before they are first used in a process, and then re-qualified at predetermined intervals, or when significant changes are made. Process or method validation should only be performed once all the items used have been qualified and before a new process or method is used routinely. (Adapted from EDQM T&C 2.16.1.) Retrospective validation is no longer an acceptable approach (Directive (EU) 2016/1214 Art. 1: Good Practice Guidelines/GPG Blood 4.4.1.2). Process validation of new BTC should cover all intended processes and sites of preparation. A scientific and risk-based validation approach could be justified for new blood components based on extensive process knowledge from the development stage in conjunction with the appropriate ongoing statistical process control (SPC) (GPG Blood 4.4.1.3), and for new tissues and cells, if applicable.

The key elements of the site qualification and validation programme should be clearly defined and documented in a validation master plan or equivalent document (GPG Blood 4.3.2.3-4).

The process or method to be used, as well as acceptance criteria should be documented in a validation plan and approved by suitably qualified and competent organisation management before qualification or process/method validation begins. The results of the validation are compared with the acceptance criteria, and any deviation from the expected results or from the validation plan should be recorded and fully investigated during the validation and documented in the validation report. Following validation, the acceptance or rejection of the process by designated organisation management should be documented. (Adapted from EDQM T&C 2.16.1.) Equipment, facilities, systems and processes should be evaluated at an appropriate frequency to ensure that they are still operating appropriately (GPG Blood 4.4.1.6).

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If processes are outsourced to external service providers it is required that responsibilities between BE/TE and service provider are clearly defined, and specifications for the whole process are produced. External service providers should meet the requirements of EUBTCD (Directive 2005/62/EC Annex, paragraph 8; Directive 2004/23/EC Art. 24). Data supporting qualification and/or validation studies obtained from sources outside of the establishment may be used provided that this approach has been justified and there is adequate assurance that controls were in place throughout the acquisition of such data (GPG Blood 4.3.1.4).

In general, the process or method validation needs to be performed once by each organisation. If the process or method has been successfully validated by any organisation, it may be transferred between organisations. In this case the receiving organisation should repeat the validation to a reduced extent, guided by the sending organisation, if in accordance with any relevant national regulations. This on-site validation should focus on “worst case” conditions. (Adapted from WHO guidelines on transfer of technology in pharmaceutical manufacturing, Annex 7.)

Details concerning specificity of validation of donor/donation testing, pathogen reduction and sterilisation will be described in the chapters 3, 4 and 5.

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2. Requirements and criteria for laboratories performing donor/donation infectious disease testing and microbiological testing of BTC

BE/TE can perform infectious disease testing of BTC donors themselves or they could outsource this work to an appropriately qualified and competent external laboratory selected by the BE/TE (Directive 2002/98/EC Art. 3; Directive 2004/23/EC Art. 3; Directive 2006/17/EC Annex; Directive 2005/62/EC Annex 8; GPG T&C ch. 8). Such a laboratory may be part of a hospital or private clinic, but may also be an independent enterprise offering the appropriate testing services. In addition to donor testing, the laboratory can examine preparations of BTC to determine, measure or otherwise describe the presence or absence of various micro-organisms (see chapter 6) (EDQM T&C ch. 10). Whether the laboratory performing these activities is part of the BE/TE or a third party offering its services to BE/TE, it must meet requirements laid down in the EUBTCD (Directive 2002/98/EC Art. 2; Directive 2004/23/EC Art. 24.2).

2.1. Testing and screening of donors/donations

In this document, the word 'testing' is used to refer to the investigations performed on either donor or donation sample to determine any infectious disease risk associated with the donation. Although some MS may use the word 'screening' to describe this activity, 'testing' has been used because of the wide range of practices in MS, including the different establishments/laboratories involved in provision of blood, tissue and cells, for broad clarity, and for consistency with the wording in the relevant EU directives (Directive 2006/17/EC; Directive 2002/98/EC). The testing performed is to look for the presence of specific markers of infection for a range of infectious agents.

This document is intended to cover the testing activities performed to identify evidence of the presence of infectious agents which may be present in blood, tissue and cell donations. The basic testing process and procedures are the same for blood, tissues and cells, although there may be some differences in the specific testing requirements. Most donations are collected from selected, low risk donors, the expectation being that the majority have no evidence of infectious disease risk (except for e.g. partner donations in MAR or autologous donors). The one key difference between the testing activities is that for blood donation, it is a sample from the donation itself that is tested, whereas for tissue and cell donation the sample to be tested is taken from the donor (the exception being cord blood donation, when a sample of the cord blood may be tested as well as the maternal sample).

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2.2. Quality system

Based on the EUBTCDs, any laboratory undertaking the testing of donors/donations must have a well-managed quality system (Directive 2002/98/EC Art. 11; Directive 2004/23/EC Art. 16). As any structure or body, that is responsible for any aspect of the testing of human blood or blood components is determined to be a Blood Establishment (except hospital blood banks) (Directive 2002/98/EC Art. 3), laboratories testing blood donations and performing microbiological testing of blood components must develop and maintain a quality system that is based on EU Good Manufacturing Practices (GMP) (Directive 2003/94/EC), and meet the requirements identified in Directive 2005/62/EC (Art. 2), as amended by Directive (EU) 2016/1214 and the Good Practice Guidelines (GPG Blood). Similarly, the quality system of tissue and cell donor and microbiological testing laboratories must meet the requirements laid down in the Directive 2004/23/EC (Art. 16 and 24).

Furthermore, standards and specifications of quality systems for laboratories testing blood donations and performing microbiological testing of blood components are defined in GPG Blood (ch. 1.2), the application of which are mandatory in MS. Even though no similar legal requirement exists for tissue and cell donor testing laboratories and microbiological testing laboratories, it is recommended that these laboratories follow the general requirements regarding quality systems and quality management as described in Good Practice Guidelines for TEs (GPG T&C ch. 2).

2.3. Standard

The EUBTCD are the minimum standards for laboratories performing donor infectious disease testing and microbiological testing of BTC. The EDQM Guide to the preparation, use and quality assurance of blood components is an additional standard for blood donor testing laboratories, and the EDQM Guide to the Quality and Safety of Tissues and Cells for Human Application could be used for tissues and cells, respectively. In addition to the EUBTCD, laboratories must meet the relevant national legislation and national standards which apply to those specific activities.

Many medical laboratories in EU follow EN ISO standards, either voluntarily or if required by national legislation. EN ISO 15189, the international standard for medical laboratories specifying requirements for quality and competence is internationally and within the EU the most used (Zima 2017; Boursier *et al.* 2016; Buchta *et al.* 2018). In some EU MS, other national or international standards which have adopted essential contents of EN ISO 15189 are used, whereas medical laboratories in some EU MS use EN ISO 17025, which outlines general requirements for the competence of testing and calibration laboratories, as an alternative or additional standard (Zima 2017). Furthermore, a laboratory using an in-house test for BTC donor infectious disease marker testing must be compliant with standard EN ISO 15189 (Regulation (EU)

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(2017/746 Art. 5). If a laboratory follows ISO standard(s), it must ensure that in addition the relevant requirements within the EUBTCD are met.

2.4. Accreditation, designation, authorisation or licensing of laboratory by Competent Authorities

Based on the EUBTCDs, any laboratory undertaking donor, blood component, tissue or cell testing must be accredited, designated, authorised or licensed by a relevant CA (Directive 2002/98 Art. 5, Directive 2006/17 Annex II 2.1). Designation, authorisation and licencing by CA mean that the laboratory has been identified and given official permission to perform testing. Accreditation means an attestation by a National Accreditation Body - officially recognised by their national government - when a laboratory meets the requirements set by harmonised standards and, where applicable, any additional requirements including those set out in relevant sectoral schemes, to carry out a specific activity (Regulation (EC) No 765/2008; https://european-accreditation.org/).

Where the national legislation and fundamentals of permission vary between MS, accreditation is based on harmonised standards. Accreditation according to standards is an effective way to prove competence of the laboratory, and it further facilitates accurate and reliable outcomes and reduces errors in the laboratory processes (Allen 2013). Furthermore, accreditation increases harmonisation and transparency (https://european-accreditation.org/). Whether laboratories are accredited, designated, licensed or authorised by the CA, this information should be shared, in addition to the other requirements set out above for laboratories, to increase mutual trust, especially when BTC are distributed to other EU MS.

2.5. Additional requirements for testing laboratories

If BE/TE outsources the donor/donation testing or microbiological testing of BTC, it must establish a written contract with the laboratory performing testing (Directive 2004/23/EC Art. 24; Directive 2005/62/EC Annex 8). Any contract between BE/TE and an external testing laboratory should describe the roles and responsibilities of all parties, and specify detailed procedures (Directive 2004/23/EC Art. 24; GPG Blood 8.1.2). Good Practice Guidelines describe the general principles regarding a written contract (GPG T&C 3.1; GPG Blood 8.1), as well as requirements for contract giver, the service/product provider and the contract itself (GPG T&C 3.2.-3.4; GPG Blood 8.2.-8.4).

Donor testing laboratories should use appropriate algorithms to ensure that their testing procedures have maximum sensitivity without loss of specificity (EDQM T&C 5.4). The algorithms

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should be defined in writing (i.e. standard operating procedures) to deal with initially reactive specimens, and to resolve discrepancies in results after retesting (GPG T&C 9.14; GPG Blood 6.4.7). It is recommended that donor testing algorithms would be defined nationally taking into account the epidemiology of infectious agents in the national donor population, and enabling the appropriate and consistent investigation and resolution of test reactivity (EDQM Blood ch. 9). As such algorithms are often specific to the individual MS, no model algorithm has been included in this guidance. An example of a widely used algorithm for infectious disease marker primary testing and confirmatory testing is presented in the EDQM Blood Guide (ch. 9).

3. Requirements for selection, validation and performance of donor/donation infectious disease marker test kits

Blood transfusion, as well as tissue and cell transplantation, and reproductive cell transfer, may result in transmission of infectious diseases. In order to prevent such transmission and to ensure an equivalent level of safety for all donation types, each donor/donation must be tested in accordance with the requirements laid down in EUBTCDs (Directive 2002/98/EC Annex IV; Directive 2006/17/EC Annex II and III).

As a minimum requirement, all donors/donations must be tested for HIV, HBV and HCV (as summarised in Table 1). In addition to the minimum requirements, testing of donors/donations for additional infectious agents or infectious markers may be required for specific blood components, tissues or cells. Additionally, differences in endemicity of infectious agents in different MS or regions, together with the emergence and spread of transmissible infectious agents, may, in some MS, require testing for a number of other infectious agents (Directive 2002/98/EC Annex IV; Directive 2006/17/EC Annex II 1 and Annex III 2-3; EDQM T&C 5.5). Furthermore, national legislation may result in additional variation between the EU MS.

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Table 1. Summary of infectious disease markers required to be tested, as a minimum, for BTC donors/donations.

Donor type	Mandatory tests within the EU	Directive	Additional tests
Blood donors	HIV 1/2 (anti-HIV 1/2) Hepatitis B (HBs-Ag) Hepatitis C (anti-HCV)	2002/98/EC Annex IV	Based on e.g. the donor's history, the characteristics of the BTC donated, national epidemiological situation, requirements in national legislation, guidance by European Centre for Disease Prevention and Control (ECDC) and recommendations by WHO, additional testing may be required e.g. *Treponema pallidum,* HTLV-1, Cytomegalovirus (CMV), malaria, toxoplasma, Epstein-Barr virus (EBV), *Trypanosoma cruzi*, Hepatitis E virus.
Tissue and cell donors	HIV 1/2 (anti-HIV-1,2) Hepatitis B (HBsAg, anti-HBc) Hepatitis C (anti-HCV-Ab) *Treponema pallidum*	2006/17/EC Annex II	
Reproductive cell donors	HIV 1/2 (anti-HIV-1/2) Hepatitis B (HBsAg, anti-HBc) Hepatitis C (anti-HCV-Ab) *Treponema pallidum* (non-partner donors) Chlamydia (non-partner sperm donors; urine sample NAT testing)	2006/17/EC Annex III	

Effective testing for the detection of transmissible infectious agents can reduce the risk of transmission to a very low level (EDQM Blood 2.3.3.; WHO TTI guidelines 2009). In addition to effective laboratory testing of donors/donations for a range of markers of specific infectious agents, the choice of test kits or platforms together with the quality management systems in place are crucial to maximise the microbiological safety of transfusion and transplantation. Requirements for selection, validation, and performance of infectious disease marker test kits for the testing of BTC donors/donations are summarised below.

3.1. Selection of infectious marker test kits

The selection of appropriate test kits is a critical part of the donor/donation testing. Numerous commercial infectious marker test kits are available. These are based on various types of assays which detect antibodies, antigens or the nucleic acid of the infectious agent (EDQM T&C 5.5;

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WHO TTI guidelines 2009 3.1-3.2). Different types of assays include (WHO TTI guidelines 2009 3.1.):

- Immunoassays
    - Enzyme immunoassays (EIAs);
    - Chemiluminescent immunoassays (CLIAs);
    - Haemagglutination (HA)/particle agglutination assays (PAs);
    - Rapid/simple single-use assays (rapid tests);
- NAT assays.

However, not all test kits are suitable in all situations and each testing system may have specific advantages and/or limitations that should be taken into consideration when selecting infectious marker test kits (WHO TTI guidelines 2009). As a minimum requirement the following factors should be considered in selecting the most appropriate test kits.

Test kits used *in vitro* for the testing of BTC donors/donations are considered as *in vitro* diagnostic medical devices which must be *Conformité Européenne* (CE) -marked before placing on the market within the EU (Regulation (EU) 2017/746 Art. 2). Therefore, infectious disease marker testing of BTC donors/donations should be carried out using CE-marked test kits, where appropriate.

Test kits should be suitable for the detection of the required markers in the sample types being tested (EDQM T&C5 5.3; JPAC 2013 9.2). Typically infectious-disease marker test kits specifically intended for the testing of donors/donations are designed to be used with samples from a living or deceased heart-beating donors (i.e. donor after brain death) (EDQM T&C 5.3.3). If there is a need for collection of post-mortem samples (from deceased non-heart beating donor), the test kit should have been validated for this purpose, either by the manufacturer, or by the user (EDQM T&C Appendix 19). Additionally, ideally only test kits specifically designed and validated for donor/donation testing should be selected. Test kits and systems specifically intended for use for diagnostic purposes could be selected after the appropriate validation for use for testing purposes (EDQM T&C 5.4; WHO guidelines 2011 7.4).

In selecting a specific test kit for the testing of BTC donors/donations, both sensitivity and specificity should be as high as possible (WHO TTI guidelines 2009 3.3; EDQM T&C 5.1). High sensitivity ensures identification of infection and high specificity decreases rates of non-specific reactivity, which could result in the wastage of donations and unnecessary deferral of donors (WHO TTI guidelines 2009).

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3.2. Validation of infectious marker test kits

All infectious marker test kits must be validated for their intended use in accordance with current scientific knowledge (Directive 2006/17/EC Annex II 2.1). Furthermore, all testing procedures related to blood donor/donation testing must be validated before use (Directive 2005/62/EC Annex 6.3.1).

Chapter 1 (General validation requirements) applies also to this chapter 3.

All CE-marked donor/donation infectious disease marker test kits have undergone performance evaluation (see section 3.3) by manufacturers (Regulation (EU) 2017/746 Art. 56). The IVD manufacturer is responsible for the performance evaluation of the CE-marked donor/donation infectious disease marker test kit. This implies an assessment and analysis of the data used to establish or verify the scientific validity, the analytical and, where applicable, the clinical performance of a device (Regulation (EU) 2017/746, Directive 98/79/EC. Note: 2017/746 will be fully applied from 26.5.2022 onwards).

All IVD devices are further required to undergo conformity assessment, the process of demonstrating whether the requirements relating to a device have been fulfilled. For high risk devices (e.g. mandatory donor/donation infectious disease marker test kits), conformity assessment always requires assessment by a Notified Body. After full application of the IVD regulation in 2022, all IVD devices for the detection of infectious disease markers will require assessment by a Notified Body. Before 26.5.2022, based on the IVD Directive (98/79/EC), some infectious disease markers may fall out of the scope of Notified Body assessment, and conformity assessment is the responsibility of the manufacturer.

For the highest risk class (D), the Notified Body assessing the device/test kit may request the European Union reference laboratory(ies) designated by the Commission to verify the performance claims and compliance with common specifications, where they exist (Regulation (EU) 2017/746 Art. 48). Infectious disease test kits intended for testing of BTC donations/donors will fall into class D according to the IVD regulation (for respective classification rule, see Regulation (EU) 2017/746 Annex VIII rule 1, as well as Medical Device Coordination Group Guidance on classification of IVD devices, under development in 2020).

However, depending on the intended use of the test kit, because of variation in the performance of CE-marked test kits, and differences in populations and the background disease prevalences in different EU MS, additional laboratory evaluation and validation work may be required by the individual MS.

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An on-site validation of the CE-marked donor/donation infectious disease marker test kit should be required prior to its routine use in each laboratory. On-site validation should demonstrate, in addition to qualification, that the basic performance specifications of the assay established by the kit manufacturer are met in the laboratory (WHO guidelines 2011 7.4; GPG Blood 6.3.3).

Additionally, donor/donation testing laboratories are required, by their quality system and/or regulation, to demonstrate that in routine use, the performance specifications of the test kits/assays are constantly maintained (WHO guidelines 2011 7.4). The means by which this could be demonstrated are a combination of, for example:

- appropriate reactivity with manufacturers' and any internal and external quality control materials with every series of tests (WHO guidelines 2011 7.4; JPAC 2013 9.1);
- statistically monitoring trends in control measurements on defined control material (WHO guidelines 2011 7.4; JPAC 2013 9.1);
- successful participation in external quality assessment schemes (proficiency testing) by all qualified members of staff (WHO guidelines 2011 7.4; Directive 2005/62 Annex, 6.3.5).

When the testing laboratory intends to use in-house tests instead of CE-marked kits, the performance of each in-house test must be validated by the laboratory itself before being brought into routine use. This means that the laboratory must demonstrate conformity with the relevant general safety and performance requirements set out in Annex I of Regulation (EU) 2017/746 which apply to it, taking into account its intended purpose. In addition to these, the conditions listed in point 5 of Article 5 of Regulation (EU) 2017/746 must be met (e.g. manufacture and use of the test under appropriate quality management systems, laboratory compliance with standard EN ISO 15189, a justification of their manufacturing, modification and use etc.). Before application of the IVD Regulation (EU) 2017/746 in 2022, laboratories must follow respective national legislation.

User validation of CE-marked test kits and in-house tests for use with post-mortem samples should be undertaken in accordance with any EDQM guidance (e.g. Example of validation of screening: infectious disease assays of blood from deceased donors in EDQM T&C Appendix 19).

3.3. Performance of infectious marker test kits

The performance of an infectious marker test kit means the assessment of its ability to achieve its intended purpose as claimed by the manufacturer. This consists of the analytical and the clinical performance (Regulation (EU) 2017/746 Art. 2), as well as scientific validity. The analytical performance means the ability of a donor/donation test kit to correctly detect a particular

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analyte (adapted from Regulation (EU) 2017/746 Art. 2). Characteristics describing the analytical performance include (Regulation (EU) 2017/746 Annex I 9.1.a; WHO guidelines 2011 7.3):

- analytical sensitivity
- analytical specificity
- trueness (bias)
- precision
    - repeatability (replicates of series)
    - reproducibility, variation by operator, by day or by lot of reagents
- accuracy (degree of closeness of measurements to the true value, resulting from trueness and precision)
- lower and upper limits of detection (serial dilution) and quantitation
- measuring range, linearity, cut off
- determination of appropriate criteria for specimen collection and handling
- control of known relevant endogenous and exogenous interference (e.g. haemolytic sera, lipemic sera)

The clinical performance means the ability of a test kit to yield results that are correlated with a particular pathological state in accordance with the target population (Regulation (EU) 2017/746 Art. 2). Specifically, characteristics of the clinical performance include (diagnostic) sensitivity, (diagnostic) specificity, positive predictive value, negative predictive value, likelihood ratio, and expected values in normal and affected populations (Regulation (EU2017/746 Annex I 9.1.b).

CE-marked donor/donation infectious marker test kits (which have undergone a performance evaluation by a manufacturer and demonstrated conformity) should meet these above mentioned general performance requirements (Regulation (EU) 2017/746 ch. II Art. 5). These general performance requirements also apply to in-house donor/donation tests and therefore these tests should also meet these requirements (Regulation (EU) 2017/746 Art. 5.5).

In addition to general performance requirements, CE-marked donor/donation test kits are compliant with the common technical specifications for the detection, confirmation and quantification in human specimens of markers of HIV infection (HIV 1 and 2), HTLV 1 and 2, and hepatitis B, C, D (Commission Decision 2009/886/EC Annex, 3). Specifically the requirements for sensitivity and specificity of these test kits are set out in Table 1 of the Commission decision 2009/886/EC. It is recommended that the minimum evaluated (diagnostic) sensitivity and (diagnostic) specificity levels of all donor/donation infectious disease marker test kits should be as high as possible and preferably not less than 95 - 99.5% (Commission Decision 2009/886/EC).

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3.4. Donor/donation testing for emerging infectious agents

New and emerging infectious agents, or those that have moved to infect a new geographical area can pose a significant risk of transmission via transfusion/transplantation (EDQM Blood 2.3.3; EDQM T&C 16.4.1.1). Even though transmission of infectious agents can be minimised by donor deferral, there are situations where donor/donation testing is the main tool to reduce the risk of transmission. Donor/donation testing becomes especially important when donor deferral may reduce BTC supply e.g. in the newly affected area. In addition, a possibility of asymptomatic infection or existence of a carrier state may increase need for donor/donation testing (EDQM Blood 2.3.3). Thus, reliable donor/donation infectious disease marker testing may be vitally important to maintain the safety and sustainability of BTC supply.

As with mandatory donor/donation infectious disease marker testing, CE-marked kits should be used, if available. However, in the presence of an unexpected outbreak caused by a new agent, new test kits may become available in the market without following the standard procedures for CE mark (self-certification of the producer). Therefore it is recommended that the guidance of European Commission and ECDC on BTC donor/donation infectious disease marker testing is followed (EDQM T&C 16.4.1.1). It is also important to define, in accordance with the professionals involved, the minimum acceptable specifications of the new test kits based on the scientific information available at that time.

In-house tests developed for the detection of rare or new emergent diseases can be used when commercial CE-marked test kits are not available on the market in the EU. However, they must meet the general performance and validation requirements as set in Regulation (EU) 2017/746 and summarised above.

4. Criteria for validation of pathogen reduction steps

Even with sensitive and specific testing, there remains a residual risk of transmission of infectious agents during the window period, when the pathogen is present but undetectable by the test in use. It is also possible that a pathogen could mutate in a way that makes it undetectable by the NAT-based testing, or, in the case of newly identified threats such as emerging viruses and prions, there may not be a suitable test available. BTC can also be contaminated by bacteria and fungi during procurement or processing, and those stored at or close to room temperature are more likely associated with bacterial or fungal growth (for example platelets).

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One way to address these concerns and further enhance the safety of BTC is to introduce pathogen reduction technologies (PRT), if possible. PRT have been demonstrated, through validation studies, to inactivate pathogens or decrease their number, using physical and/or chemical methods, without significantly compromising the safety of the BTC (see chapter 4.4). Currently available systems can inactivate or decrease the number of a wide range of viruses, bacteria and parasites but they do not reduce infectivity associated with prion proteins such as the causative agent of vCJD (EDQM Blood 4.4.4). PRT could also represent a more generalised approach against emerging pathogens.

This section relates to the MS CA assessment of validation packages that demonstrate the performance of PRT.

Chapters 1 (General validation requirements) and 6.3 (Methods for microbiological control) also apply to chapter 4.

4.1. Validation requirements depend on the type of PRT

According to the Blood Directive 2005/62/EC (Annex, 6.4), the processing of blood components must be carried out using appropriate and validated procedures including measures to prevent contamination and microbial growth in the final blood products. Also according to the Tissues and Cells Directive 2004/23/EC (Art. 20), all processes that affect quality and safety of tissues and cells need to be validated and carried out under controlled conditions. Thus, PRT need to be validated before they are introduced into the processing procedures for BTC, to provide evidence that a chosen PRT process can reliably inactivate or decrease the number of pathogens in a given BTC without compromising the quality, safety and effectiveness of final BTC products.

A range of PRT are already established and in widespread use (see Table 2). Others are under development or were developed as in-house PRT systems, meaning that each method was developed and used only in the BE/TE/laboratory which developed it. For blood components, established PRT are in many cases commercially available products authorised by the CA. At the time of writing this guidance, an entire portfolio of PRTs suitable for all blood components was not available, but the sector has been steadily progressing: PRT systems for red cells and whole blood were in development but not currently in use in Europe (EDQM Blood 4.4.4). For tissues, established PRT are likely to be established protocols rather than commercially available products. Validation requirements in these two cases are different and described below:

- **On-site validation of established PRT systems.** A reduced validation strategy is usually sufficient when using a PRT system/device that has already been authorised by a relevant CA and recognised in the EU (CE-marked devices for e.g. platelets or

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routinely used systems for e.g. plasma components). Validation of PRT systems in accordance with published methods, or following long-established practices using the same materials and equipment, may rely on ongoing quality control and periodic reviews to confirm that the method has the intended outcome (EDQM T&C 2.16.1). For example, the performance of spiking studies (see later) is not mandatory. Both data from the PRT supplier as well as relevant literature can be referred to. A comprehensive assessment of the relevance of these data by the BE/TE is required to ensure it is directly applicable to the treatment process to be used and the operational conditions at the site. However, possible changes in sample processing procedures, instruments and equipment or the BTC itself should be partially validated according to a risk-based approach.

- **Validation of novel or in-house PRT systems.** The use of an in-house PRT requires an extensive validation, covering parameters of a primary validation study (*Ph. Eur* 5.1.1; EMA/CHMP/CVMP/QWP/850374/2015). Elements such as the degree of pathogen reduction, capacity, specificity and robustness should be addressed. The PRT systems should be validated using "worst case" scenarios. This will usually involve spiking the material with a larger-than normal level of the target pathogens or suitable model organisms, and demonstrating their effective removal, or reduction to acceptable levels, by the process. See Section 4.2.

Table 2. Examples of existing PRT.

PRT mechanism	BTC for which is used	Specific considerations when assessing a validation study
Blood		
Amotosalen + UVA light (320-400 nm)	Platelets (whole blood or apheresis derived) Plasma (whole blood or apheresis derived)	CE-marked. Evaluation of platelet concentration loss (< 10%), *in vitro* platelet function (swirling, pH etc.), and *in vivo* post-transfusion platelet recovery (post-transfusion platelet count increment). Include effectiveness of removal of the active agent. For pathogen inactivation effectiveness, see Schlenke (2014), Tables 3 and 6.

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Riboflavin + UVB light (280-360 nm)	Platelets (whole blood or apheresis derived) Plasma (whole blood or apheresis derived)	CE-marked. Does not require removal of the active agent. For pathogen inactivation effectiveness, see Schlenke (2014), Tables 3 and 6.
UVC light Filtration + Methylene Blue + visible light (400-700 nm)	Platelets Fresh frozen plasma	CE-marked. No toxicological assessment necessary. For pathogen inactivation effectiveness, see Schlenke (2014), Table 3.
Solvent/Detergent	Large-pool of plasma (whole blood or apheresis derived)	At the time of writing this guidance, authorised in several European countries (e.g. AT, BE, BG, CZ, DE, DK, EE, ES, FI, FR, HR, HU, IE, IT, LT, LU, LV, MT, NL, NO, PL, PT, RO, SE, SI, SK, UK). (EMA List of nationally authorised medicinal products)
Solvent/Detergent	Single donation or mini-pool of plasma (whole blood or apheresis derived)	CE-marked.
Tissues		
Antibiotic/anti-mycotic treatments	Amniotic membrane tissue, musculoskeletal tissue, adipose tissue, cardiovascular tissues, ocular tissues, skin tissue	Allowed residual concentration, or removal of antibiotics should be described.
High concentrate glycerol	Skin tissue, amniotic membrane tissue, ocular tissue	Glycerol solutions used should be sterile and of high quality (e.g. see *Ph.Eur.* monograph 0497 – Glycerol 85 %)
Decellularisation	Skin tissue, cardiovascular tissues, amniotic membrane tissue	All solutions used for decellularisation should be prepared from sterile and high quality products

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		whenever possible; or sterility filtered (<0.22µm).
Chemical decontamination (e.g. peracetic acid, iodophors, ethanol)	Musculoskeletal tissue	Possible residue issues should be justified.
Low dose irradiation (e.g. 15 kGy)	E.g. musculoskeletal tissue, amniotic membrane	E.g. when using a combination of PRTs.
Supercritical carbon dioxide treatment	Musculoskeletal tissue, pericardium tissue	Does not require removal of the active agent.

(EDQM T&C monographs)

4.2. Aspects of PRT validation

The application should describe all relevant information that the CA requires to undertake its review:

- **Starting material**. The effectiveness of a PRT should be shown in the BTC preparation itself and not only in an aqueous solution (EDQM T&C 8.8.2).
- **Specification of reduction capacity.** Prior to the PRT validation it is necessary to assess the bioburden usually present in the BTC material as well as defining worst case scenarios. The latter can be critical for a successful pathogen reduction and should be also addressed in the study.
- **Target organisms**. Appropriate model organisms for the spiking studies include typical contaminants likely to be found in the BTC material as well as micro-organisms that might represent a challenge for the PRT. In addition, model organisms should be stable in the presence of the matrix. Ideally, known and well characterised reference organisms should be used (Spindler-Raffel et al. 2017: WHO Bacterial reference strains; EDQM T&C 2.16.9 and 8.6.1.2; *Ph. Eur* 2.6.1; CPMP/BWP/268/95). The applicant should justify the choice of micro-organism in accordance with the aims of the validation study.
    - Suitable spike stocks. To demonstrate high magnitude reduction ability, BE/TE or contracted testing laboratory should source representative high titre stocks of pathogens (JPAC Validation on Plasma and Platelet Pathogen Inactivation). A panel with relevant characteristics should be included. Where WHO bacteria reference strains are available they should be used. The quantitation range of the assay should cover the bioburden concentration range expected in the BTC.

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  - Key bacteria against which PRT should demonstrate effective reduction: see Table 1 in the JPAC guidance "Validation of Plasma and Platelet Pathogen Inactivation".
  - Strains of micro-organism that are known to be resistant to antimicrobial treatment, e.g. spore-forming, heat-resistant bacteria, may be used for spiking (EDQM T&C 2.16.9).
  - Viruses that may contaminate BTC vary considerably in their size, physical properties and genomic material. In addition, the pathogenicity of a virus may depend on the patient group and on the BTC being administered. To demonstrate effectiveness against known viruses and emerging risks, PRT validation data should demonstrate removal or inactivation of a wide range of enveloped and non-enveloped viruses, including viruses of concern and/or established models. Typically, validation studies involve several virus types. Guidance is available for the selection and assay of model viruses (CPMP/BWP/268/95 Table 1).

- **Interfering factors.** Factors which might have an effect on the reduction capacity have to be considered in the validation study (e.g. hemoglobin concentration in UV inactivated platelet concentrates). Monitoring the levels of these factors should be in place to ensure levels are within an acceptable and valid range.
- **Critical process parameters (CPP).** CPPs are used to measure the performance of the PRT treatment unit, and relate to the reduction performance of the target pathogen (PRT treatment effectiveness). Continuous monitoring of CPPs provides assurance that the system is under control and alerts operators and control systems if PRT treatment effectiveness is reduced to an unacceptable level.
- **Quantitative assays for each model pathogen.** To determine the reduction capacity accurately, validated quantitative assays for each model pathogen have to be in place at the BE/TE or contracted testing laboratory. These should detect live pathogen. NAT testing will not differentiate between live and inactivated pathogens but can be used in the validation of removal processes.
- **Model process.** If a scaled-down model of the PRT process is used during validation (e.g. to conserve material, virus stocks, or protect the usual processing environment), the validation documentation should verify the PRT scale model and its comparability with the proposed/current preparation process.
- **Controls.** Suitable sample controls should be collected during the validation to demonstrate the mechanism of pathogen reduction.

Additional aspects for the CA to consider:

- Critical reagents and materials must be CE-marked, when applicable (Directive 2006/86/EC, Annex I, C.6; GPG Blood 4.1.9).

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- PRT should be carried out at appropriate interval after BTC donation (in many cases, as short as possible). If commercial PRT kit is used, manufacturer gives instructions on the maximum interval and these should be followed. If PRT is carried out after the maximum recommended interval, any bacteria present may have multiplied, and the level of bacteria may be significantly higher. Additionally, growth of bacteria may lead to the formation of pyrogenic agents and endotoxin whose immunological activity is not diminished by the PRT. (JPAC Validation of Plasma and Platelet Pathogen Inactivation).
- The product matrix and its components might have a significant effect on the model organisms and their behavior (e.g. complement killing of bacteria). Bacteria can start to grow in the product after spiking thereby altering the initial spiking concentration. A non-inactivated control should be performed in parallel.
- The PRT validation process requires BTC to be deliberately spiked with known and defined micro-organisms, so that the reduction achieved by the PRT can then be demonstrated. Certain requirements (e.g. EU GMP Guidelines chapter 5.18) might restrict the deliberate use of potential contaminants in the production facilities. For validation purposes, the sharing of equipment and facilities that would be used in BTC processing should be avoided due to the risk of cross-contamination. Exemptions can be made in cases in which validation procedures are performed in closed systems.
- Re-validation should be performed in case of change of facility, change of process or any relevant new knowledge.

4.3. Validation criteria

The methodology for the statistical assessment of PRT assays and limitations of such studies have been described previously (CPMP/BWP/268/95; CPMP/ICH/295/95).

In summary, the validation will result in a set of data for each pathogen or representative model used in the spiking study.

The titre of the spiked test material before undertaking the scale model PRT can be compared to the titre of the sample(s) collected from the test material when the scale model process has been completed, in order to determine the overall pathogen reduction achieved. Sample titres and reduction figures achieved are normally reported on a logarithmic scale. The reduction achieved for each pathogen or appropriate model should be reported and include the 95% confidence intervals wherever possible.

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Although it is considered that the level of bacterial contamination in blood donation which may result in clinically significant levels of bacteria in stored platelet components is below 100 cfu/unit, a higher minimum proven level of pathogen reduction should be demonstrated: PRT should reduce any bacterial contamination by the amount specified i.e. 10^4 (4 $\log_{10}$) to ensure maximum effectiveness (JPAC Validation of Plasma and Platelet Pathogen Inactivation; Murphy *et al.* 2008; Pearce *et al.* 2011). However, this also depends on the time when the PRT takes place: the later in the process the PRT takes place, the higher the necessary effectiveness. PRT should also be able to remove or inactivate substantial amounts of virus, typically 4 $\log_{10}$ or more, although, the log number reduction should not be used as the single, absolute measure of the effectiveness of PRT (CPMP/BWP/268/95 ch. 6.1).

Validation reports should include a discussion of the suitability of the scale model system, taking into consideration the results from appropriate assay control samples, and the degree to which these support the proposed mechanism of pathogen reduction.

4.4. Effect of PRT on BTC properties

PRT should not change the properties of the BTC in such a way to make it unacceptable for the clinical use. However, depending on the PRT method the functionality and quality of the resulting BTC can become reduced. The selection of recipients in whom treatment with these BTC might be relevant should therefore be taken into account for safety reasons. The benefits of PRT in reducing microbiological risk should be balanced against any loss of potency or effectiveness of the BTC and this should be assessed as part of the validation (JPAC Validation of Plasma and Platelet Pathogen Inactivation). A framework to assess this will be provided in the GAPP *Technical Annex 1 to overall guidance: authorisation of changes in donation, procurement and collection, processing, preservation, storage and distribution*.

5. Criteria for validation of sterilisation methods

Essential step for microbiological and viral safety of BTC is the confirmation of validated processes for pathogen reduction (see chapter 4 of this guidance) or sterilisation, where applicable. The sterilisation methods and criteria described here apply primarily for bacteria and fungi. If a risk assessment points out a viral contamination possibility, it is necessary to demonstrate the process capability of removing/inactivating relevant viruses during the process.

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Sterilisation is defined as a process that results in the state of complete absence of all cell-based micro-organisms capable of replication (sterility) (*Ph. Eur* 5.1.1; EDQM T&C 8.6.1). According to Directive 2006/17/EC (Annex IV, 1.3), sterile, wherever possible CE marked, instruments and devices must be used for tissue and cell procurement. Where possible, single-use instruments for procurement are recommended. When re-usable instruments are used, a validated cleaning, disinfection, packaging and sterilisation process for removal of infectious agents has to be in place (Directive 2006/17/EC Annex IV 1.3.9).

Consistently, Directive 2005/62/EC sets the standards for using sterile CE-marked blood bag systems for the collection and processing of blood and blood components (Directive 2005/62/EC Annex 6.2.2).

The conventional test for sterility is described in *Ph. Eur* 2.6.1. Additionally, use of a validated automated culture system may be advantageous if available (*Ph. Eur* 2.6.27). If sterility test is not feasible, sterility needs to be assured by the use of suitably designed, validated and controlled processes.

Chapters 1 (General validation requirements) and 6.3 (Methods for microbiological control) apply also to this chapter 5.

5.1. Uses of sterilisation

Tissues (e.g. bone and amniotic membrane) can in some cases be subject to sterilisation methods (EDQM T&C 1.3). Sterilisation should be applied to tissue grafts in their final packaging without subsequent exposure (JPAC General guidelines for tissue processing, 21.5.3.2). Sterilisation is not applicable to cells, blood components and most tissues. Wherever possible, sterilisation methods should be applied to instruments, procurement devices and materials (e.g. raw materials, reagents, excipients, single-use components, containers, gowning and cloth) which are in contact with starting materials, process intermediates or final products (Directive 2006/17/EC Art. 2, section 7 and Annex IV 1.3.8).

5.2. Sterilisation methods

The sterilisation method used should be shown to be suitable to remove or destroy the type and number of contaminants in the source material. Whenever possible, sterilisation should be done using methods described in the European Pharmacopoeia (5.1.1), the main points are also presented in European Medicines Agency guideline (EMA/CHMP/CVMP/QWP/850374/2015). These methods are based on moist heat (steam), dry heat, gas, irradiation or membrane filtration.

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Selection of the sterilisation method should be based on the characteristics of the object of the sterilisation and its associated bioburden and justified (see Table 3). Modifications or combinations of the described methods may be used, provided that the procedure(s) is validated.

Table 3. Sterilisation methods according to *Ph. Eur* 5.1.1 "Methods of preparation of sterile products"

Sterilisation Method	Application examples	Reference conditions
Steam	Instruments, materials, cloth and media	Terminal steam sterilisation at ≥121 °C for 15 min
Dry Heat	Glass and metal instruments/ tools	Terminal dry heat sterilisation at ≥160 °C for ≥2 h
Ionisation radiation (irradiation)	Musculoskeletal tissues (EDQM T&C 21.4.3), skin (EDQM T&C 19.4.3), amniotic membrane (EDQM T&C 18.4.3) (Singh *et al.* 2016). Containers, equipment and gowns	Terminal ionising radiation of absorbed dose ≥ 25 kGy (IAEA 2007)
Gas (chemical agent) (acceptable only if no other sterilisation methods are feasible; EMA/CHMP/CVMP/QWP/850374/2015)	Containers and equipment	Depends on chemical, no general conditions predefined
Membrane filtration	Fluid or gas products that are not amenable to other sterilisation methods	Nominal pore size ≤0.22 µm

5.3. General validation requirements for sterilisation

Validation should be performed in order to demonstrate the consistent effectiveness of the method chosen and to provide the assurance of sterility. Whenever a sterilisation step is introduced, the following general validation requirements need to be addressed (*Ph. Eur* 5.1.1; JPAC General guidelines for tissue processing, 21.5.3).

5.3.1. Sterility Assurance Level (SAL)

For sterilisation processes with a well-defined dose/kill relationship, a very high level of sterility assurance can be achieved (EDQM T&C 8.6.1). This is quantified by the SAL value which is an

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experimentally-derived number expressing the likelihood of a contaminant to survive the process. In order to determine the SAL, the bioburden of the respective matrix should be known. Frequently, sterilisation processes are validated to assure the SAL ≤ 10^{-6} for sterile products or equipment. To validate the sterilisation technique, SAL of 10^{-6} should be achieved for the most resistant micro-organism (often bacterial spores). This is a "worst-case" validation and will guarantee a significant overkill for more sensitive microbes. SAL means that the likelihood of non-sterile item is 1 in 1 million. The SAL 10^{-6} cannot be applied to membrane filtration method or to quantify the effectiveness of virus inactivation/removal. (EDQM T&C 2.16.9; *Ph. Eur* 5.1.1; JPAC General guidelines for tissue processing, 21.5.3; EDQM T&C 8.6.1.2, 10.3.6).

5.3.2. Biological indicators

Biological indicators are test systems (e.g. inoculated carriers) containing viable micro-organisms (usually spores of bacteria, e.g. *Bacillus* or *Clostridium* sp.) that provide a defined worst case challenge to verify the required effectiveness of a specified sterilisation process. Commercially available biological indicators intended for specific sterilisation processes are recommended, but if suitable ones are not available, custom-made may be used. (EDQM T&C 2.16.9; *Ph. Eur* 5.1.2)

Bioburden (and where relevant, bacterial endotoxins) should be specified prior to sterilisation. Bioburden is usually expressed as a measure of the numbers and identification of the species of micro-organisms in the material to be sterilised (EDQM T&C 10.3.6, 10.4.3; *Ph. Eur* 2.6.12 and 2.6.13). Validation of sterilisation potency requires that the maximum predicted level of microbiological contamination can be eliminated by determining the elimination capacity as the number of log scale reductions of the spiked micro-organism. The micro-organisms should verify the required effectiveness of the selected sterilisation method by covering all relevant micro-organisms commonly found on the object including, for example, vegetative Gram positive and negative bacteria, vegetative fungi, fungal and bacterial spores, and viruses, if applicable (EDQM T&C 10.3.6; *Ph. Eur* 5.1.2; EMA/CHMP/CVMP/QWP/850374/2015; CPMP/BWP/268/95).

Viral indicators should be chosen to resemble viruses which may contaminate the donation. Further detailed recommendations of viral safety (*Ph. Eur* 5.1.7) as well as examples of the used virus indicators are listed in CPMP/BWP/268/95 *Virus validation studies: the design, contribution and interpretation of studies validating the inactivation and removal of viruses.*

5.4. Specific validation criteria for sterilisation methods

Depending on the sterilisation method in question, more specific data on the effectiveness of the method may need to be evaluated. As a main principle, validation of the effectiveness of the method should be undertaken using a combination of physical indicators (e.g. thermo-couples in

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moist heat sterilisation) and biological indicators, which should be placed at the locations where sterilising conditions are most difficult to achieve (e.g. cold spots when using heat, difficult to penetrate areas when using gas, minimum/maximum load) (*Ph. Eur* 5.1.2). (This principle is not applicable to membrane filtration). Parameters to achieve the required SAL and examples of the most widely accepted biological indicators are described under the relevant sterilisation methods below.

Conditions of the sterilisation methods should be developed and validated in compliance with *Ph. Eur* 5.1.1 and 5.1.2. In addition, guidelines for validation of sterilisation methods are explained e.g. in the publication by European Medicines Agency (EMA/CHMP/CVMP/QWP/850374/2015).

5.4.1. Steam sterilisation (Autoclaving)

Steam (moist heat) sterilisation is performed in saturated steam under pressure in autoclaves (Directive 2014/68/EU for pressure equipment) and the critical parameters are pressure, time and temperature. When using the method, equal distribution and adequate penetration of steam should be verified. The reference cycle for steam sterilisation is 15 min at 121 °C. Depending on the product and load, another combination of time and temperature may be adopted based on cycle validation, with a minimum acceptable temperature of 110 °C. The sterilisation effectiveness may be calculated by F0 concept. F0 is the time in minutes for the specified temperature that causes the same lethality as one minute at 121 °C, with minimum F0 not less than 8 min. (Ph. Eur 5.1.5)

- Suitable test micro-organism: *Geobacillus stearothermophilus* (e.g. strains ATCC 7953, NCTC 10007, CIP 52.81, NCIMB 8157, ATCC 12980)
- Additional information can be found in: ISO 17665-1:2006*: Sterilization of health care products* - Moist heat - Part 1: Requirements for the development, validation and routine control of a sterilization process for medical devices.

5.4.2. Dry heat

For dry heat, the critical parameters are time and temperature. Reference conditions are minimum of 160 °C for at least 2 h. Other combinations may be used if validated and SAL ≤ 10^{-6} is demonstrated. Validation should be done using a combination of temperature mapping and biological indicator.

- Suitable test micro-organism: *Bacillus atrophaeus* (e.g. strains ATCC 9372, NCIMB 8058, NRRL B-4418, CIP 77.18), at temperatures between 160 °C and 180 °C.

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- Additional information can be found in: ISO 20857:2010 *Sterilization of health care products* — Dry heat — Requirements for the development, validation and routine control of a sterilization process for medical devices

5.4.3. Irradiation

Sterilisation by irradiation is achieved by gamma rays, accelerated electron beams or x-rays. The reference absorbed dose is 25 kGy (*Ph. Eur* 5.1.1; IAEA 2007). In practice, to maintain the properties of the tissues, some TE prefer low irradiation dose (e.g. 15 kGy) and generally the given dose varies ranging from 15 kGy to 35 kGy (Nguyen et al. 2013). The irradiation dose is selected depending on the bioburden and it should result in SAL of $\leq 10^{-6}$. Depending on bioburden, ≥ 25 kGy irradiation dose may be required for sterilisation of bacteria and fungi. Quite often ≥ 34 kGy may be required for virus inactivation, since many viruses are resistant to irradiation. Viral inactivation data should be supported by appropriate marker viruses (EDQM T&C 8.6.2.1). Validation is usually performed by using dosimeters placed throughout the load.

- Suitable test micro-organism: *Bacillus pumilus* (e.g. strains ATCC 27142, NCTC 10327, NCIMB 10692, CIP 77.25). For this method, biological indicators are not always necessary, but may be required for the validation of irradiation sterilisation of tissues (*Ph. Eur* 5.1.2).
- Additional information can be found in: ISO 11137-2: *Sterilization of health care products* -- Radiation -- Part 2: Establishing the sterilization dose
  ISO 11737-2: *Sterilization of medical devices* -- Microbiological methods -- Part 2: Tests of sterility performed in the definition, validation and maintenance of a sterilization process

5.4.4. Gas sterilisation

Multiple gas sterilisation processes are currently used and they are divided in two categories: alkylating agents (e.g. ethylene oxide) and oxidising agents (e.g. hydrogen peroxide and peracetic acid). With all options, sufficient gas and moisture penetration is essential and thus gas concentration, exposure time, temperature and humidity are the parameters to follow. It is the responsibility of the user to define the suitability of the biological indicator for reactive chemical in question. It should be noted that the levels of residual toxic substances after sterilisation should be minimised (e.g. residual ethylene oxide in the product should not exceed a limit of 1 ppm, CPMP/QWP/159/01).

- Suitable test micro-organisms:
  Ethyleneoxide: *Bacillus atrophaeus* (e.g. strains ATCC 9372, NCIMB 8058, NRRL B-4418, CIP 77.18); Hydrogen peroxide: *Geobacillus stearothermophilus*

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- Additional information can be found in: ISO 11135:2014: *Sterilization of health care products* - Ethylene oxide - Requirements for the development, validation and routine control of a sterilization process for medical devices

5.4.5. Filtration

In contrast to other methods, the principle of membrane filtration is not inactivation but removal/reduction of micro-organisms. If filtration is used as a sterilisation method, the nominal pore size of the microporous membrane should not be greater than 0.22 µm. The sterilisation capacity of single use filters are usually validated by the manufacturers. Before filtration, filter capacity should be evaluated by the user and the method should retain microbial challenge of at least 10^7 cfu/cm^2 on filter surface using suitable micro-organism (*Ph. Eur* 5.1.2). It should be noted that filtration is not suitable sterilisation method for viruses and mycoplasma.

- Suitable test micro-organism: *Brevundimonas diminuta* as single cells suspension (for filters with nominal pore size ≤0.22 µm) (ATCC 19146, NCIMB 11091, CIP 103020). In addition, if possible, a suspension of vegetative bacterial cells representing the natural flora in question.
- Additional information can be found in: GMP Guide, Annex 1

5.5. Information on sterilisation validation

Validation should be planned and reported. Plan/report should include the following relevant information:

- The object(s) to be sterilised.
- The sterilisation method used, together with the justification for selection of the particular method. Method selection should be based on the properties of the object(s) to be sterilised.
- Selected biological indicator with which SAL is determined.
- Validation procedure to be followed, appropriate to ensure the sterility of the object(s) to be sterilised, and including e.g. maximum/minimum loads, package instructions for the sterilisation process and effect of it, special requirements of the objects, worst case approach and risk assessment/evaluation.

5.6. Effect of sterilisation on tissue properties

It should be noted that, to ensure the required SAL is achieved, the sterilisation method used may have an effect on the mechanical and biological properties of the tissues. Sterilisation should not render tissues clinically ineffective or harmful to the recipient nor should it adversely affect

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the essential properties. (JPAC General guidelines for tissue processing 21.5.3.2; see also GAPP *Technical Annex 1 to overall guidance: authorisation of changes in donation, procurement and collection, processing, preservation, storage and distribution*)

6. Requirements for assessing microbiological safety of the final BTC product

The microbiological safety of BTC is based on donor selection, donor/donation testing and minimisation of initial contamination, with protocols to control and monitor contamination during procurement and processing (adapted from GPG T&C 13.2.1). Even if BTC collection and processing procedures are intended to produce non-infectious final BTC products for the recipients, in some cases microbiological contamination may still occur. The risk of microbiological contamination of BTC depends on for example the origin, collection and procurement methods and processing steps of the BTC (EDQM Blood 4.1.8). As an example, causes of bacterial contamination in blood products include occult bacteraemia in the donor, inadequate or contaminated skin preparation at the phlebotomy site, coring of a skin plug by the phlebotomy needle and breaches of the closed system from equipment defects or mishandling.

Release is the act of certifying compliance of specific BTC or batch of BTC with the predefined requirements and specifications. Before any BTC are released for clinical use, all relevant records (including donor records, test results, processing and storage records, and BTC post-processing quality-control test results) should have been reviewed, approved and documented as acceptable by an authorised and trained person according to the relevant standard operating procedure (SOP) and/or national regulations. Microbiological quality criteria form part of final release criteria for some, but not all, BTC.

Requirements and criteria for microbiological safety of the final BTC product differ according to the level of microbiological quality achievable, by any method:

- For some tissues (e.g. musculoskeletal tissues and amniotic membrane), terminal sterilisation can be applied and the aim is to reach sterile tissue grafts. Parametric release with acceptance criteria for the control of identified process parameters can replace microbiological testing of the tissue grafts. Validated procedures for all critical production steps (procurement of tissues, transportation, all processing steps, packaging and

storage) and a fully validated sterilisation method should be applied (see chapter 5) (EDQM T&C 10.3.5.2).

- Some BTC will not require and do not tolerate further disinfection or sterilisation after procurement and/or processing (e.g. reproductive cells, embryos, other cells).
- Many BTC cannot be sterilised and in those cases, aspects of microbiological testing of BTC need to be considered in order to ensure the microbiological quality of the final BTC product. Those aspects are described in chapters below.

BTC collection/procurement and processing should be performed using aseptic techniques and in aseptic environment. Requirements for these and for environmental microbiological monitoring are not in scope of this guidance (additional information can be found e.g. in EU GMP Annex I; JPAC General guidelines for tissue processing, 21.5.1).

6.1. Common requirements

Differences in BTC make it difficult to establish a general rule for microbiological testing requirements (see Table 4). Therefore, for each procedure, risk assessment should be applied to determine the quality control strategy to be followed through the whole process and to identify critical steps to reduce the possibility of contamination and cross-contamination.

Microbiological safety of BTC can be demonstrated by microbiological testing using validated methods of known sensitivity and specificity. Currently such methods are primarily compendial microbiological methods of the European Pharmacopoeia (*Ph. Eur*). Microbiological testing of final BTC products should be performed by an authorised testing laboratory (see chapter 2) and in compliance with *Ph. Eur* requirements (see chapter 6.3).

In general, testing, when applicable, is recommended to be performed for both pre-processing samples of the procured BTC and on post-processing samples of final BTC products. Based on a risk analysis, some steps of the process can be identified as critical for the quality and safety of the final BTC product and samples may need to be tested. Sampling should be conducted immediately before packaging or as late as possible during the procurement or processing. (EDQM T&C 10.3.1).

Wherever possible, representative samples of BTC should be removed and tested for bacterial and fungal contamination using validated protocols . Swabs, contact solutions or other validated non-destructive sampling methods should be used where it is impossible to remove samples without damaging the BTC graft. (EDQM T&C 10.3.1, JPAC General guidelines for tissue processing, 21.5.2). Aseptic techniques to obtain samples are required in order to minimise the risk of false positive cultures due to contamination at the time of sampling or upon inoculation in culture.

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For some BTC, pathogen reduction can be applied (see chapter 4). Validated PRT may offer alternative approaches to assuring the bacterial safety of BTC (EDQM Blood 4.4.2). Further processing after the PRT should be conducted without antimicrobial agents. Methods for testing of final BTC products should be evaluated carefully with respect to possible inhibition of microbiological growth due to decontaminating agents or their residues (EDQM T&C 10.3.5.3).

BTC with a short shelf-life may be released based on negative-to-date results. In this context, implementation and documentation of sufficient assurance of the microbiological quality of the final BTC product when released is essential. This will include in-process microbiological tests that have been established on the basis of risk analysis, usually including sterility testing of the starting material and/or of samples from the intermediate product at critical steps, if applicable, in combination with final results of in-process controls (EDQM T&C 13.2.2.10). Final testing is still ongoing after the BTC is released and will be completed. Procedures for handling positive results after release should be in place, including potential recall, notifying the clinician caring for the recipients and identification of the microbial species and resistogram (EDQM T&C 10.4.2.1).

Whenever the analysis indicates data that is outside of specified control limits, an investigation into potential causes of contamination should be undertaken and, where appropriate, the strain should be identified and collection and processing procedures should be revalidated (adapted from EDQM Blood 4.4.2). In this case the final BTC product should not be used for clinical application unless a risk–benefit analysis indicates that it is the best option for the recipient.

Table 4. Factors affecting microbiological safety that should be considered when determining the microbiological testing protocol (sample types, sampling times, analyses) (modified from EDQM T&C Table 7.2).

Phase	Risk factors	Examples/information
Procurement	BTC type	BTC type specific micro-organisms should be taken into account when validating the microbiological analyses.
	Procurement environment	Funeral home, operating theatre etc. Skin disinfection prior to venepuncture.
Processing	Contamination during processing; open *vs* closed processing	Closed processes are less prone to contamination during processing than processes where BTC are exposed to the environment. Tissues that are minimally processed, cellularised, or contain blood, blood vessels and lipids are more likely to support

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		microbiological contaminants than those that are blood- and cell-depleted.
	Effectiveness of the PRT to remove contaminants	Some BTC can be treated with PRT which reduces the risks of transferring any microbiological contamination (see chapter 4).
Sampling	Suboptimal detection of contaminants due to the sampling method	If the only option for final microbiological sampling is swabbing or testing of unrepresentative samples, the risk that contaminants will be undetected is higher than in processes where 5-10 % destructive testing of final BTC products can be performed.
	Sampling of preservation method	Validation of storage using a sterile barrier test: samples from preservation media can be tested to validate storage method (materials).
	Use of antibiotic/antimycotic agents during processing	Culture media for some BTC contains antibiotics/antimycotics which, if not inactivated properly in samples, might inhibit microbial growth during testing, leading to possible false-negative results.
	Number of samples	The amount of samples to be tested depends on batch size, e.g. *Ph. Eur* 2.6.1, table 2.6.1-2. Not based on statistical process control approach.
Storage	Storage method of the final BTC product	Room temperature *vs* cooling.
	Packaging	Appropriate packaging for BTC in question should be used. If tissue is sterilised, it should be sterilised in its final packaging and its packaging should be compatible with sterilisation method used.
	Shelf-life of the final BTC product	Limited time for testing; Preparations of BTC with a short shelf-life may be released based on an intermediate readout of the test before the test period is completed (negative-to-date result).
Application/ transfusion	Transfer of contaminants at application/transfusion	Method of application/transfusion (e.g. permanent *vs* temporary) and application site both affect the risk of transfer of contaminants.

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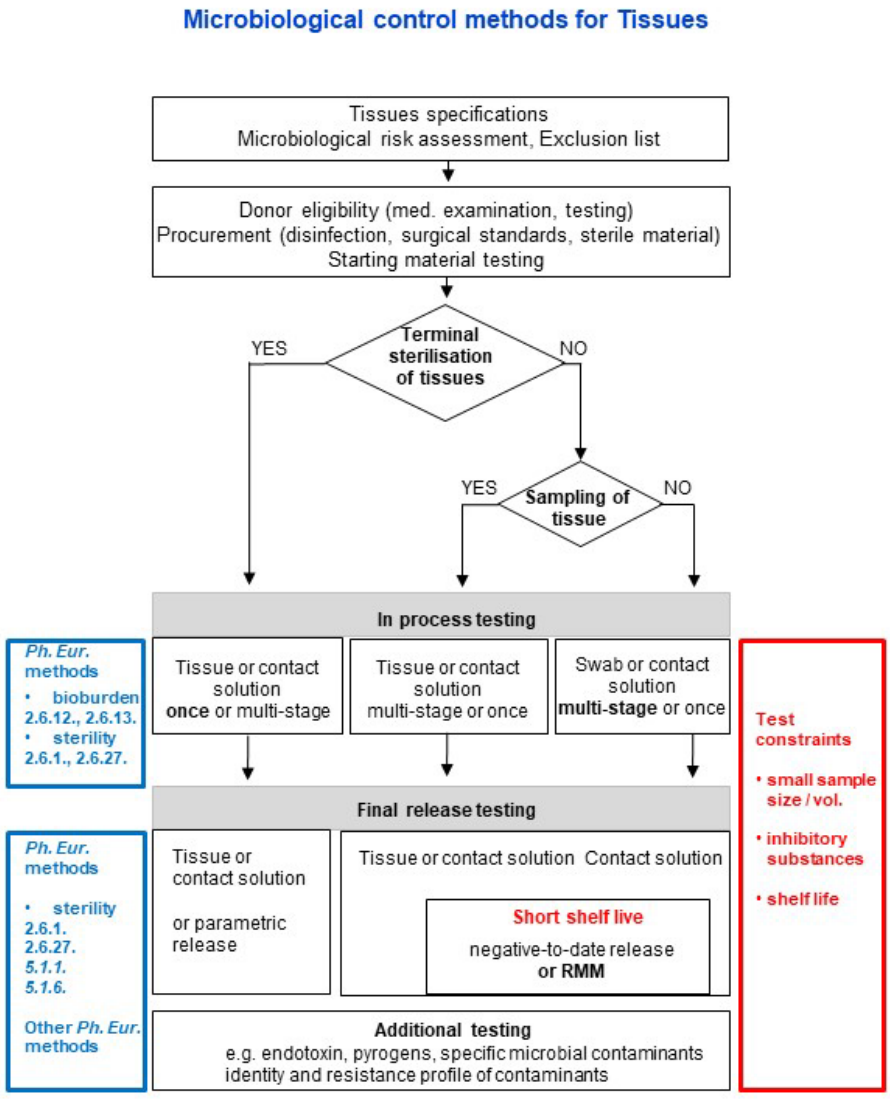
The microbiological control methods are not identical for all BTC. Whereas blood and haematopoietic progenitor cells are collected and processed in closed systems, most tissues and for example reproductive cells are collected in open systems. PRT may be applied to some BTC, but not all. Whilst some tissues can be sterilised, most BTC cannot be. As an example, microbiological control methods for tissues are schematically presented below (Figure 1).

Figure 1. Microbiological control methods for tissues.



Ph. Eur. 2.6.1. Sterility
Ph. Eur. 2.6.12. Microbiological examination of non-sterile products: microbial enumeration tests
Ph. Eur. 2.6.13. Microbiological examination of non-sterile products: test for specified micro-organisms
Ph. Eur. 2.6.27. Microbiological examination of cell-based preparations
Ph. Eur. 5.1.1. Methods of preparation of sterile products
Ph. Eur. 5.1.6. Alternative methods for control of microbiological quality

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6.2. Specific requirements depending on the type of BTC processing

Different requirements for testing of microbiological safety of BTC apply for cases where BTC has been procured and processed in closed or open systems. BTC procurement systems can either be closed, with equipment designed and operated in such way that the BTC are not exposed to the environment, or can be open, exposing the BTC to the environment.

Chapter 6.3 Methods for microbiological control apply to both cases.

6.2.1. BTC with processing in closed systems

Use of closed systems is strongly recommended for all steps in blood component processing. Open systems may exceptionally be necessary due to local constraints and should be undertaken in an environment specifically designed to minimise the risk of contamination (GPG Blood 6.6.3). Processing in closed systems are generally used also for haematopoietic progenitor cells and mononuclear cells procured by apheresis (EDQM T&C 22.3).

For BTC which are processed in closed systems, repeated testing steps do not yield additional information on the microbiological status of the BTC and are thus not required. In such cases, a reduced testing strategy that relies on single testing of samples taken at an appropriate time point may be applicable. (EDQM T&C 10.3.5.1)

According to the Directive 2004/33/EC (Annex V, 2.2), appropriate microbiological control of the collection and processing of blood products must be performed. Bacterial cultures of platelet components provide the best indication of the overall rate of contamination of whole blood donations, provided that the samples for culture are obtained in a suitable volume and at a suitable time after collection. Data on routine bacterial monitoring should be analysed using statistical process control techniques to ensure that the process remains in control. (EDQM Blood 4.4.2).

6.2.2. BTC with processing in open systems

Most tissues and cells, including those for which PRT has been applied to, are exposed to the environment at certain processing stages between procurement and packaging. If terminal sterilisation cannot be used, the contamination risk during open processing should be avoided to the greatest possible extent. The requirements for microbiological sampling and testing are expected to be most stringent in these situations (EDQM T&C 10.3.5.4).

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Sampling and microbiological assessment should include the starting material, the transport solution, any solutions used to wash BTC (EDQM T&C 10.3.5.4) and critical steps identified on a risk-based analysis, if applicable.

Microbiological testing of tissues and cells should be performed according to the tissue-specific requirements in Part B of EDQM T&C Guide and general criteria described in chapter 10.3. Tissue-specific requirements describe the minimum standards to control microbiological safety of each BTC type and microbial contaminants that should result in BTC discard, if applicable.

6.3. Methods for microbiological control

The conventional method for control of microbiological quality in relation to the absence of micro-organisms in BTC is described in *Ph. Eur* 2.6.1. For cell-based preparations, compendial method 2.6.27 can be applied. For quantification of microbiological contamination (bioburden testing) of starting material or of preparations during processing before sterilisation/ decontamination, the appropriate method is described in *Ph. Eur* 2.6.12 whenever bioburden limits need to be ensured. The method 2.6.12 should be used together with 2.6.13 if the risk assessment applied to the BTC requires the absence of specific highly pathogenic micro-organisms.

The samples for sterility testing should be representative of all types of the components, but if this is not possible, surrogate testing may be performed (EDQM T&C 10.4). This testing may require use of validated methods employing special media and/or conditions to enable growth of such micro-organisms and their detection.

Highly virulent micro-organisms should be predefined in order to exclude BTC if these micro-organisms are detected at any stage of processing.

Several BTC derived preparations are short lived and of small quantity. Conventional compendial methods, e.g. growth based microbiological methods (*Ph. Eur* 2.6.1, 2.6.27), are now increasingly outperformed by alternative rapid microbiological methods (RMM) in terms of sensitivity, speed and width of information. Use of RMM may mean that final test results are available much faster, allowing a timely and often more substantiated final BTC product release. (EDQM T&C 10.4)

The use of RMM for testing of BTC preparations is still limited. One reason is the considerable effort for the control laboratory to validate new methods with respect to method performance in comparison to the compendial reference method. *Ph. Eur* 5.1.6 “Alternative methods for control of microbiological quality” and 2.6.27 “Microbiological examination of cell-based preparation” provide the current EU framework for RMM validation. EDQM provides an online resource (see Bibliography) in which information on exemplary RMM validation procedures for a

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particular application are made available to control laboratories. This resource is currently regarded as a starting point for users although not peer-reviewed and not exhaustive.

Based on risk assessment, tests for absence of mycoplasma (*Ph. Eur* 2.6.7) and bacterial endotoxins (*Ph. Eur* 2.6.14 and *Ph. Eur* 5.1.10) should also be performed, where required.

Any deviations from the *Ph. Eur* standards should be justified, and alternative test methods should be validated in accordance with *Ph. Eur* 5.1.6.

Different practices are in place among BE/TEs in the MS in terms of percentage of testing, methods, sample volumes, time of sampling, shelf life and residual risk. Microbiological testing is performed using culture-based or rapid detection devices. In order to support harmonisation of the microbiological strategies among EU MS, recommendations for microbiological testing should be taken into account, for example:

- detection of a broad range of (transfusion/transplantation-relevant) bacteria,
- applicability of test procedure with a late sampling time point,
- quality controlled testing procedure.

7. Final considerations

Most of the recommendations described in this guidance apply to all types of BTC: blood, tissues and cells. They share same requirements and recommendations for laboratories performing donor/donation infectious disease testing and microbiological testing of BTC, donor/donation infectious disease marker test kits, and validation of pathogen reduction technologies and sterilisation. In this respect, harmonisation of the blood, tissue and cells sectors is already taking place.

Throughout GAPP, in particular within the technical annexes to the Guidance to CA, extensive reference has been made to existing requirements and recommendations, the applicable European Union legislation and the publications of the Council of Europe: the EDQM T&C Guide, the EDQM Blood Guide and the European Pharmacopeia. The authors are aware that the guidance provided at the time of publication will require regular updating as the practices in science and medicine change, to take into account the evolution of research and of available therapeutics and technologies. This can be observed by the constant evolution of the EDQM guides themselves.

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It is also of note that the means of making information available are being revolutionised by the profound changes that information technology has brought about and will continue to bring, to the ease of access to information and the ability to target it. Thus, rarely published and quickly out of date reference books can be succeeded by knowledge bases, such as that proposed by the European Pharmacopoeia, which provide up to date information, customised to the profile of the user.

The work carried out in GAPP WP9 developing the concept of an information system may bring solutions to how to keep the GAPP guidance up to date, provide the users with the particular guidance they require, and wherever needed, make specific national regulations easily accessible and transparent.

EUDAMED, the database under development by the EC to implement Regulation (EU) 2017/745 on medical devices and Regulation (EU) 2017/746 on *in vitro* diagnosis medical devices, will improve transparency and coordination of information (e.g. device registration, clinical investigations and performance studies) regarding CE-marked infectious disease marker test kits available on the EU market (EC website concerning EUDAMED). When IVD device data will become available in EUDAMED in 2022, it will help both BEs/TEs and CAs to ensure the appropriate test kits for donor infectious disease marker testing will be selected.

Some topics and part of guidance in this Annex 2 cover borderline activities, also falling under other regulatory frameworks (e.g. medicinal products, medical devices). For example, issues related to authorisation, accreditation, designation, licensing of laboratories, infectious disease marker test kits and sterilisation may be under a mandate of some other authorities than BTC CAs. Relevant authorities should take into account regulatory requirements of BTC oversight. Therefore, in addition to the effective communication between authorities of different sectors within MS, clarity and transparency across regulatory borderlines should be achieved through a revision of the European Union legislation.

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Acronyms

Ab	antibody
Ag	antigen
ATMP	Advanced Therapy Medicinal Product
BE	Blood Establishment
BTC	Blood, Tissues and Cells
CA	Competent Authority
CE	*Conformité Européenne*
cfu	colony-forming unit
CLIA	chemiluminescent immunoassay
CPP	Critical Process Parameter
CMV	cytomegalovirus
EBV	Epstein-Barr virus
EC	European Commission
ECDC	European Centre for Disease Prevention and Control
EIA	enzyme immunoassay
EMA	European Medicines Agency
EUBTCD	European Blood, Tissues and Cells Directives
GAPP	Facilitating the Authorisation of Preparation Process for blood, tissues and cells
GMP	Good Manufacturing Practices
GPG	Good Practice Guidelines
HA	haemagglutination
HBV	hepatitis B virus
HCV	hepatitis C virus
HIV	human immunodeficiency virus
HTLV	human T-cell leukaemia virus
IAEA	International Atomic Energy Agency
IVD	*in vitro* diagnostic
JA	Joint Action
JPAC	Joint United Kingdom Blood Transfusion and Tissue Transplantation Services Professional Advisory Committee
MERS	Middle East Respiratory Syndrome
MS	Member State

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NAT	nucleic acid amplification technique
PA	particle agglutination
Ph. Eur.	European Pharmacopeia
PPA	Preparation Process Authorisation
PRT	Pathogen Reduction Technology
RMM	rapid microbiological methods
SAL	Sterility Assurance Level
SARS	severe acute respiratory syndrome
SOP	standard operating procedure
SPC	statistical process control
TE	Tissue Establishment
UV	ultraviolet
vCJD	variant Creutzfeldt-Jakob disease
WHO	World Health Organization
WP	Work Package

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Definitions

Accreditation An attestation by a national accreditation body that a conformity assessment body meets the requirements set by harmonised standards and, where applicable, any additional requirements including those set out in relevant sectoral schemes, to carry out a specific conformity assessment activity (Regulation (EC) No 765/2008)

Analytical sensitivity The limit of detection, i.e. the smallest amount of the target marker that can be precisely detected (Official Journal of the European Union L 318/ Commission decision 2009/886/EC)

Analytical specificity The ability of the method to determine solely the target marker (Official Journal of the European Union L 318 /Commission decision 2009/886/EC)

Bioburden Total number of viable micro-organisms or total microbial count present, on or in BTC or in the environment, usually measured before the application of a decontamination or sterilisation process (adapted from EDQM T&C)

Biological indicators Test systems containing viable micro-organisms (usually spores of bacteria) that provide a defined challenge to verify the required effectiveness of a specified sterilisation process (Ph.Eur. 5.1.2.)

CE-marked kit Test kit marked by a manufacturer to indicate that the test kit is in conformity with the applicable requirements set out in Regulation (EU) 2017/746 on *in vitro* medical devices and other applicable Union harmonisation legislation providing for its affixing (modified from Regulation (EU) 2017/746).

Closed system A procurement/processing system with equipment designed and operated such that the cells are not exposed to the environment (adapted from EDQM T&C)

Conformity assessment The process demonstrating whether the requirements of the Regulation (EU) 2017/746 relating to a test kit have been fulfilled (modified from Regulation (EU) 2017/746).

Critical process parameter (CPP) A process parameter whose variability has an impact on a critical quality attribute and which therefore should be monitored and controlled to ensure the process produces the desired quality (Directive (EU) 2016/1214 Art. 1, GPG Blood)

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Deceased donor	A person declared to be dead according to established medical criteria and from whom cells, tissues and organs have been recovered for the purpose of human application (EuroGTP II Guide)
Diagnostic specificity	The probability that the test kit gives a negative result in the absence of the target marker (adapted from Official Journal of the European Union L 318/ Commission decision 2009/886/EC)
Diagnostic sensitivity	The probability that the test kit gives a positive result in the presence of the target marker (adapted from Official Journal of the European Union L 318/ Commission decision 2009/886/EC)
Donation (the process of)	Donating human blood, tissues or cells intended for human applications (adapted from Directive 2004/23/EC)
Donation (types of biological material)	The blood, tissues, and cells collected from the donors
Donor	A living or deceased human being, who donates BTC for another human being or for him/herself
Effectiveness	Presence of desired functionality proven by *in vitro* analytics (adapted from the EDQM T&C)
Evaluation	See 'Validation'
F_0	The time in minute for the specified temperature that causes the same lethality as one minute at 121 °C (*Ph.Eur* 5.1.5)
In-house	Manufactured and used within an organisation (for example BE/TE/ laboratory) and not distributed outside facility/organisation (adapted from Regulation (EU) 2017/746)
In-process control	Checks undertaken during processing to monitor and, if necessary, to adjust the process to ensure that a product conforms to its specification. Control of the environment or equipment may also be regarded as a part of in-process control. (EDQM T&C)
***In vitro* diagnostic medical device**	Any medical device which is a reagent, reagent product, calibrator, control material, kit, instrument, apparatus, piece of equipment, software or system, whether used alone or in combination, intended by the manufacturer to be used *in vitro* for the examination of specimens, including blood and tissue donations, derived from the human body, solely or principally for the purpose of providing information on one or more of the following: (a) concerning a physiological or pathological process or state; (b) concerning congenital physical or mental

	impairments; (c) concerning the predisposition to a medical condition or a disease; (d) to determine the safety and compatibility with potential recipients. (Regulation (EU) 2017/746)
Kit	A set of components that are packaged together and intended to be used to perform a specific *in vitro* diagnostic, or a part thereof (Regulation (EU) 2017/746)
Likelihood ratio	The likelihood of a given result arising in an individual with the target clinical condition or physiological state compared to the likelihood of the same result arising in an individual without that clinical condition or physiological state (Regulation (EU) 2017/746)
Microbiological quality	Fulfilment of a specific set of microbiological standards, characteristics and criteria. Microbiological quality may also be seen as an indicator of the microbiological safety of the BTC. (adapted from EDQM T&C)
Microbiological safety	Approach to minimise the risk of contamination by viable micro-organisms or micro-organism derived toxic substances. Microbiological safety of BTC results from the management of donor selection, procurement of BTC, testing and the preparation processes. (adapted from EDQM T&C)
Musculoskeletal	Tissues that are part of the skeleton and muscular system, including muscles, bones, cartilage, tendons and ligaments, which function in the support and movement of the body (EDQM T&C)
National accreditation body	The sole body in a Member State that performs accreditation with authority derived from the State (Regulation (EC) No 765/2008)
Negative-to-date release	The release of BTC for clinical use before completion of testing for bacterial or fungal cultures. The cultures are negative at the time of release. (adapted from EDQM T&C)
Negative predictive value	The ability of a donor test kit/test to separate true negative results from false negative results for a given attribute in a given population (adapted from Regulation (EU) 2017/746)
Open system	A procurement/processing system that exposes the BTC to the environment (adapted from EDQM T&C)

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Partner donation The donation of reproductive cells between a man and a woman who declare that they have an intimate physical relationship (Directive 2006/17/EC)

Pathogen reduction technologies Procedures that irreversibly impede proliferation of pathogens in BTC, either by removal or inactivation with physical and/or chemical methods (EDQM Blood)

Performance evaluation An assessment and analysis of data to establish or verify the scientific validity, the analytical and, where applicable, the clinical performance of a donor testing kit/assay (adapted from Regulation (EU) 2017/746)

Performance study A study undertaken to establish or confirm the analytical or clinical performance of a donor test kit/test (modified from Regulation (EU) 2017/746)

Positive predictive value The ability of a donor testing kit/assay to separate true positive results from false positive results for a given attribute in a given population (adapted from Regulation (EU) 2017/746)

Predictive value The probability that a person with a positive test result has a given condition under investigation, or that a person with a negative test result does not have a given condition (adapted from Regulation (EU) 2017/746)

Proficiency testing The evaluation of participant performance against pre-established criteria by means of external quality assessment scheme, inter-laboratory comparisons by use of externally sourced samples or panels (EDQM Blood)

Qualification As part of validation, means the action of verifying that any personnel, premises, equipment or material works correctly and delivers the expected results (Directive 2005/62/EC)

Quality system The organisational structure, defined responsibilities, procedures, processes, and resources for implementing quality management and includes all activities which contribute to quality, directly or indirectly (Directives 2005/62/EC, 2006/17/EC).

Rapid test Qualitative or semi-quantitative *in vitro* diagnostic medical devices, used singly or in a small series, which involve non-automated procedures and have been designed to give a fast result (Official Journal of the European Union L 318/25)

Reproductive cells All tissues and cells intended to be used for the purpose of medically assisted reproduction (adapted from Directive 2006/17/EC)

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Spiking	The addition of a known amount of a mixture to a standard, sample or placebo, typically for the purpose of confirming the performance of an analytical procedure (adapted from WHO guidelines on transfer of technology in pharmaceutical manufacturing, Annex 7)
Standard	The requirements that serve as the basis for comparison (Directive 2005/62/EC)
Sterilisation	Any process that eliminates or inactivates transmissible infectious agents (pathogens) containing nucleic acids, e.g. vegetative and spore forms of bacteria and fungi, parasites or viruses, present on a surface, in a fluid, in medication or in a compound such as biological culture media. Sterilisation can be achieved by applying the proper combinations or conditions of heat, chemicals, irradiation, high pressure and filtration. (EDQM T&C)
Sterility	The absence of viable microorganisms, as defined by a sterility assurance level (SAL) equal to or less than 10^{-6} (*Ph. Eur* 5.1.1.)
Sterility assurance level (SAL)	Represents the expected probability of a micro-organism surviving on an individual unit of product after exposure to a sterilisation process. SAL 10^{-6} has been established as the standard for allografts and indicates a probability of one chance in a million that one unit of product will be contaminated with a single organism after a sterilisation process, and grafts are then considered sterile. (EDQM T&C)
Terminal sterilisation	A process in which the product is sterilised in its final container (*Ph.Eur* 5.1.1)
Validation	Establishing documented evidence that provides a high degree of assurance that a specific process 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. This evidence may include laboratory assessment of test kit performance. In the context of this document, the term 'evaluation' of test or method performance, can be considered to be part/all of any 'validation'. (modified from Directive 2006/17/EC)
Validation plan	A document describing the activities to be performed in a validation, including the acceptance criteria for the approval of a process or method for routine use (adapted from WHO guidelines on transfer of technology in pharmaceutical manufacturing, Annex 7)

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Validation report	A document in which the records, results and evaluation of a completed validation program are assembled and summarised (adapted from WHO guidelines on transfer of technology in pharmaceutical manufacturing, Annex 7)

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