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European Platform for Neurodegenerative Diseases

WP3 – SOP development

D3.2 – Best practices for biobanking

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Abstract

The European Platform for Neurodegenerative Diseases (EPND) project is aiming for the acceleration of the discovery of diagnostics and treatment for neurodegenerative diseases by removing barriers to data and sample sharing and fostering collaboration to change the future for patients and their caregivers. The way to do this is to build an accessible, secure, scalable, and self-sustaining platform which will facilitate access to neurodegenerative disease data and clinical samples from across Europe. For the success of the EPND project one essential aspect is that the prospective biospecimen and associated data collection will be harmonised and standardised to ensure the highest possible quality. The use of uniform standard operating procedures in the consortium as well as the awareness of and adherence to existing standards, guidelines and best practices are crucial for this. For this reason, a survey was first conducted among some of the EPND partner cohorts to determine the status quo of their current biobanking practices. Based on the survey results, the necessary topic areas for this best practice guideline were identified. Topics in which the surveyed partner cohorts still showed potential for improvement are dealt with in more detail.

Contents

1. Introduction

The European Platform for Neurodegenerative Diseases (EPND) project[1] is aiming for the acceleration of the discovery of diagnostics and treatment for neurodegenerative diseases, which will create a huge estimated economic burden in the years and decades to come, by removing barriers to data and sample

[1] Further information on the topic is accessible on the EPND project website: https://epnd.org/ (last accessed: 2022-10-14)

sharing and fostering collaboration to change the future for patients and their caregivers. The way to do this is to build an accessible, secure, scalable, and self-sustaining platform which will facilitate access to neurodegenerative disease data and clinical samples from across Europe.
For the success of the EPND project one essential aspect is that the prospective biospecimen and associated data collection will be harmonised and standardised to ensure the highest possible quality. The use of uniform standard operating procedures (SOPs) in the consortium as well as the awareness of and adherence to existing standards, guidelines and best practices are crucial for this. Creating a common basis in this regard within the EPND project was task 3.1 and resulted in this deliverable 3.2: Best practices for biobanking.
To be able to write a best practice guideline specific to the EPND project, it was first necessary to determine the status quo regarding the current biobanking practices of some of the partner cohorts involved. For this purpose, a survey was prepared with questions on the following main topics: Quality Management System (QMS), biospecimen collection and transfer, biospecimen processing, biospecimen storage, quality control (QC) and method validation/verification, biospecimen and data distribution. For the development of the survey, the requirements of the biobanking standard ISO 20387:2018[2] and feedback from Work Package (WP) 3 members were considered. The online survey set up in Microsoft Forms was circulated to the contact persons of the seven selected partner cohorts by dedicated responsible persons from EPND WP4 (WP is responsible for Cohort Interaction). The response rate was 100%. The following is a summary of the results of the survey in relation to the main topics.

QMS:

- Four out of seven partner cohorts implemented and maintain a QMS in their cohort/biobank.
- Three of these four partner cohorts established a QMS according to the ISO 9001 standard[3], one partner cohort according to the ISO 15189 standard[4] and one partner cohort in addition to the ISO 9001 standard also according to the ISO 17025[5] and ISO 20387 standards as well as Good Clinical Practice (GCP) and Good Clinical Laboratory Practice (GCLP) guidelines.
- Most of the respondents to the questionnaire are familiar with one or two standards of the selection. Main standards mentioned were ISO 20387, ISO 15189, and ISO 9001.
- All partner cohorts described their relevant biobanking processes in the form of SOPs or similar instructions.
- The partner cohorts are mainly associated to an institute. In addition, some of them are also associated to a department, a clinic, or a biobank.
- In five out of seven partner cohorts the associated institute/clinic/biobank is certified and/or accredited. Three partner cohorts are certified according to the ISO 9001 standard, two partner cohorts are accredited according to the ISO 15189 standard and one partner cohort is in addition to being ISO 9001 certified also ISO 17025 accredited.
- The personnel in all seven partner cohorts are competent to perform their assigned duties and activities. Six cohorts maintain documented information regarding professional competence and education and training.

Biospecimen collection and transfer:

[2] ISO 20387:2018 Biotechnology – Biobanking – General requirements for biobanking
[3] ISO 9001:2015 Quality management systems – Requirements
[4] ISO 15189:2012 Medical laboratories — Requirements for quality and competence
[5] ISO/IEC 17025:2017 General requirements for the competence of testing and calibration laboratories

- Biospecimen collection mainly takes place in the clinic and/or study centre based on own SOPs. In one cohort the specifications of the clinic are additionally considered. One cohort takes published pre-analytical standards in addition to its own SOPs into account.
- Information related to the collection of biospecimens (e.g., place, date, time, procedure) is documented across all partner cohorts.
- In six out of the seven partner cohorts' procedures for receiving or acquiring biological material and associated data (e.g., internal transfers or external shipments/transfers) are in place. The shipping manifests include in all partner cohorts the sample identifier (ID) in the form of a, for example, text identifier and/or barcode and in two cohorts additionally the subject ID from the collection process.

Biospecimen processing:

- Biospecimen processing takes place in laboratories in the clinic, in the study centre, in their own department/institute and/or in the biobank, mainly based on own SOPs. One of the cohorts considered instead published pre-analytical standards and harmonised protocols originating from a neurodegenerative disease research project and a collaborative network of University Medical Centres.
- Traceability of biospecimens and associated data is ensured along the whole biobanking process chain in all partner cohorts.

Biospecimen storage:

- Biospecimens are (long-term) stored either in a biobank and/or the respective department. The biobanking facilities (storage space) are in some cases located within the clinic.
- All have indicated that the facilities/dedicated areas and their environmental conditions which are monitored are suitable for biospecimen storage.
- All partner cohorts have -80°C freezers. Two of them have indicated that they also use -20°C freezers. Two other partner cohorts also have nitrogen tanks available, one of which also has -150°C freezers. The storage conditions (e.g., temperature in the storage devices) are measured, monitored, controlled, and recorded by all partner cohorts.
- In four out of the seven partner cohorts an inventory or tracking system allows for the annotation and query of relevant information associated with the whole biobanking process chain from collection to distribution. In these cases, it is primarily ensured using a Laboratory Information Management System (LIMS), in two cases supplemented by Microsoft Access or an in-house software and reporting system. In the other three partner cohorts this is partially the case as individual process steps (e.g., transport, distribution) are not covered in their inventory or tracking system. These partner cohorts currently use either a Biobank Information Management System (BIMS), Microsoft Excel and/or paper-based documentation.
- In six out of the seven partner cohorts the storage records contain data fields for sample IDs. If this is the case, the sample ID and, in one case, also the subject ID are documented.

QC and method validation/verification:

- Procedures specifying QC activities throughout the biobanking processes are established, implemented, and documented in four out of the seven partner cohorts.
- Validated or verified methods (if already validated) are applied in four partner cohorts.

Biospecimen and data distribution:

- All partner cohorts have application and approval procedures in place to ensure that the biospecimens and associated data are only distributed according to the consented purpose from the informed consent (IC) form.

Based on the defined main topics of the survey and the corresponding results presented before, the chapters for this best practice guideline were determined. Since only some of the partner cohorts have already implemented a QMS, a LIMS and/or BIMS as inventory/tracking system and use validated and/or verified methods, these topics are dealt with more comprehensively below (see chapters 2, 7 and 8.2).

Apart from the respective referenced sources (see footnotes) information from the following already existing best practice documents were used for the preparation of this document:

- Campbell *et al*. The 2018 Revision of the ISBER Best Practices: Summary of Changes and the Editorial Team's Development Process. Biopreserv Biobank. 2018;16(1):3-6. DOI: 10.1089/bio.2018.0001 (download of ISBER Best Practices: Recommendations for Repositories 4th Edition for free from the ISBER website at https://www.isber.org/page/BPR)
- National Cancer Institute (NCI) Best Practices for Biospecimen Resources 2016 (free download from the NCI website at https://biospecimens.cancer.gov/bestpractices/index.asp)
- 2012 best practices for repositories collection, storage, retrieval, and distribution of biological materials for research. International society for biological and environmental repositories. Biopreserv Biobank. 2012;10(2):79-161. DOI: 10.1089/bio.2012.1022

2. Quality Management System

2.1. General

If human biospecimens and associated data are collected for research, for example in the context of a clinical trial or a cohort, it is necessary that legal and regulatory requirements are adhered to.

Furthermore, it is of enormous importance that the biospecimen collection and all subsequent process steps are carried out in a highly standardized way to fulfil pre-defined requirements and to satisfy or even exceed end user expectations. To ensure this and thus provide high-quality biospecimens and associated data for research, it is essential that the respective study/cohort and/or the responsible biobank has all its processes clearly defined and under control. Only in this way it is possible to meet all the requirements and to sustainably maintain operations in the long term. This is best achieved if the study/cohort and/or biobank commits to and implements a QMS. According to the ISO 9000 standard a QMS, among other things, "comprises activities by which the organization identifies its objectives and determines the processes and resources required to achieve desired results"[6]. It is described as a dynamic system that is continuously improved and thus evolves. Quite often quality management (QM) activities are already taking place in an organisation, but they may not be subject to a formal system. To develop such a system, there are several international standards that can assist. The most relevant ones for biobanking are described in the following subchapter 2.2.

2.2. Standards for the development and implementation of a QMS

2.2.1. ISO 9001:2015

Generic guidance for the development of a basic QMS for any type of organisation is provided by the ISO 9001 standard which employs the process approach, considering the Plan-Do-Check-Act (PDCA) cycle and the risk-based thinking approach. This enables the organisation to plan its processes and their interactions, to adequately resource and manage its processes, and to identify and implement opportunities for improvement. It can also identify factors that could lead to process deviations and enable the organisation to take preventive measures[7].

2.2.2. ISO 20387:2018

More specifically geared to the general requirements for competence, impartiality and consistent operation of biobanks including QC requirements and the requirements for the respective QMS is the ISO 20387 standard. It is "applicable to all organisations performing biobanking, including biobanking of biological material from multicellular organisms (e.g., human, animal, fungus and plant) and microorganisms for research and development"[8].

The following sections include a short description of the requirements of the respective chapters of the ISO 20387 standard:

Introduction (chapter 1):
It describes the scope of the ISO 20387 standard specifying the general requirements for the competence, impartiality and consistent operation of biobanks including QC requirements to ensure that the biological material and data collections are of appropriate quality. Furthermore, it outlines for which organisations/purposes the document is applicable and that it can be used to confirm or recognize the competence of biobanks.

General requirements (chapter 4):
The biobank shall have procedures in place for every biobanking process step for each type of biospecimen and the associated data. It is recommended to define the purpose of the respective biobank

[6] ISO 9000:2015 Quality management systems – Fundamentals and vocabulary
[7] ISO 9001:2015 Quality management systems – Requirements
[8] ISO 20387:2018 Biotechnology – Biobanking – General requirements for biobanking

(e.g., mission and vision of the biobank). Furthermore, this chapter describes the concept of impartiality and confidentiality and how it can be applicable to the biobanking area according to the requirements of the standard. A biobank, whatever its purpose and whatever the nature of the samples it processes, should observe these principles in a manner appropriate to its specific characteristics.

Structural requirements (chapter 5):
The biobank shall define the specifications of organisational, financial, governance and other structural components and has to explain them in more detail by creating a documented QMS accordingly. The scope of conformity with the standard, a risk-based approach and the strategic direction of the biobank are just a few elements within the QMS which need to be discussed and established to meet the requirements of the ISO 20387 standard.

Resource requirements (chapter 6):
This chapter and its respective subchapters 6.1-6.5 deal with the general aspects of the necessary resources of the biobank, the personnel, facilities/dedicated areas and environmental conditions, externally provided processes, products and services and equipment. The biobank shall address its financial viability and safeguard it for its continuous activities. The personnel have to act according to the confidentiality arrangements, they have to be competent and further personnel management activities have to be carried out. Considerations related to processes of safeguarding facilities and environmental conditions have to be made (e.g., chemical safety, biosafety/biosecurity, physical safety, inventory protection, facility management, information/data security, fire safety etc., if applicable). All the externally provided processes, products, and services, which are being supplied by a legal entity (or part thereof) that is not the biobank, need to be reviewed for compliance with the biobank's requirements. The biobank shall have all the necessary equipment at its disposal. It shall take care of it and use it in a controlled way based on instructions.

Process requirements (chapter 7):
All the relevant processes that occur during the life cycle of the biological material and its associated data in the biobank, including e.g., collection/acquisition, processing, storage, access, and distribution, have to be defined and described.
Collection, reception and distribution, transport (internal/external), traceability, preparation and preservation, storage, retrieval, and QC of the biospecimens and associated data are key elements in the entire handling process. As these steps can take place at different clinical sites and/or laboratories, a description of the implemented standardised workflow is essential in order to guarantee the appropriate quality of samples for further analysis and/or for the intended use.
According to the standard validated and/or verified methods shall be used for critical activities, which need to be defined by the biobank, at all stages of the biospecimen life cycle to ensure fitness for the intended purpose.
The subchapter 7.10 on management of information and data explains the requirements regarding the procedures for implementation, modification and use of computer systems, software, hardware, and database(s) when applicable.
The subchapters 7.11 and 7.13 on nonconforming output and complaints relate to each other and define that procedures need to be in place for the management, the initiation of appropriate countermeasures and the required documented information in case the output doesn't comply with the predefined requirements of the biobank and/or agreement with the recipient/user, and/or agreement with the provider and received complaints.
The subchapter 7.12 on report requirements describes the minimum information which needs to be included in the report that the biobank has to provide for its stakeholders and/or users.

Quality management system requirements (chapter 8):
According to the standard it is required that a biobank establishes, documents, implements, and maintains a QMS which is capable of consistently achieving the requirements described in the standard and assure the quality of biobanking. For this purpose, either the requirements of chapters 8.2 to 8.9 (option A) must be fulfilled, or the biobank must already have established and maintains a QMS according to the ISO 9001 standard (see chapter 2.2.1) and fulfils the requirements of the chapters 4-7 (option B).

The minimum a biobank QMS shall address is:

- Documented information for the QMS
- Control of QMS documents
- Control of records
- Actions to address risks and opportunities
- Improvement
- Corrective action for nonconforming outputs
- Internal audits
- Quality management reviews

A supportive document in the implementation of the quality management, management, and technical requirements of the ISO 20387 biobanking standard is the ISO/TR 22758:2020[9]. "It expands on aspects of ISO 20387 and provides examples for illustration purposes. The aim of this document is to assist biobanks to address competency of personnel and appropriate quality of biological material and data collections. This technical report is equally applicable to newly established and existing biobanks"[10].

As required by the ISO 20387 standard all the relevant biobanking processes have to be defined and described. Such a detailed description of the operational process steps is referred to as standard operating procedure (SOP). Biobank personnel should be trained on these SOPs and carry out the processes as described. Of course, the SOPs must correspond to the processes and procedures carried out on site, but templates can be a useful support for the initial creation of the documents. Some examples of template collections in the field of biobanking are:

- German Biobank Node: Handbook for quality management in biobanking. Version 3 in English. DOI: 10.5281/zenodo.3403067 (free download from Zenodo at https://zenodo.org/record/3403067#.Y1fdaeRBxPY, recent versions 4 to 6 available in German only)
- SOPs, forms, and templates from the Swiss Biobanking Platform (free download from the Swiss Biobanking Platform website at https://swissbiobanking.ch/documents/)

The compliance of the biobank processes carried out compared to the valid SOPs can be determined, for example, through audits. In the EPND project, these are planned as part of task 4.3.2 in WP4. For this purpose, the relevant cohorts first complete a Self-Assessment Survey (SAS), and remote audits are then carried out by BBMRI-ERIC auditors based on the results of the SAS. The audit results will be evaluated, and conclusions and recommendations will be shared with the auditees. At a later stage of the project, the SAS will be repeated, and it will be determined whether suggestions for improvement have been implemented.

2.3. Competence of personnel and training

A focus topic of the ISO 20387 standard is the competence of the personnel who are to be available to the biobank. The specific requirements are described in chapter 6.2 of the standard. Besides

[9] ISO/TR 22758:2020 Biotechnology — Biobanking — Implementation guide for ISO 20387
[10] Quote from abstract published on https://www.iso.org/standard/73829.html (last accessed: 2022-10-11)

communicating duties, responsibilities, and authorities of the personnel (e.g., in the form of job descriptions) it is essential to define and document the competence that is required for personnel who are involved in biobank activities.
The duties should only be carried out by competent personnel. Therefore, it is important to initially determine the competence of the personnel based on previous education, training, demonstrated skills and/or experience and to identify the need for further education and training based on this. It is the responsibility of the biobank to make the required internal or external training available to personnel and to allow the necessary time for participation. Staff members who are in training or further education shall perform the respective tasks under supervision until the competence is confirmed by the biobank and the tasks can be performed independently.
To ensure that the activities of the biobank are always state of the art, it is essential to determine the necessary competence of the staff at regular intervals and, if necessary, to provide training to maintain and/or expand competence. Documented information that provides evidence of competence and education/training shall be available.

Training courses/resources on specific topics in biobanking are available at (without guarantee of completeness, partly with access restrictions, websites last accessed on 2022-10-12):

- BBMRI-ERIC: Training series (recordings) about the biobanking standard ISO 20387:2018 (further information: https://www.bbmri-eric.eu/services/bbmriqm-webcon-series/)
- BBMRI-ERIC: Training series (recordings) about molecular in vitro diagnostic examinations – specifications for pre-examination processes (frozen tissue, formalin-fixed and paraffin-embedded tissue, venous whole blood) (further information: https://www.bbmri-eric.eu/services/bbmri-qm-training-education/)
- BBMRI-ERIC Academy: Webinar series (recordings) on pre-analytical phases of biobanking (phlebotomy, sample fractionation, science behind the pre-analytical phase/quality assurance schemes in biobanking) (further information: https://www.bbmri-eric.eu/services/e-learning/)
- BBMRI-ERIC Academy: Webinar (recording) on Frozen Tissue Collection and Biobanking (further information: https://www.bbmri-eric.eu/services/e-learning/)
- BBMRI-ERIC: Ethical, Legal and Societal Issues (ELSI) Training (further information on webinars: https://www.bbmri-eric.eu/category/news-events/webinars/, further information on training courses: https://www.bbmri-eric.eu/elsi/external-training/, further information on the knowledge base: https://www.bbmri-eric.eu/elsi-knowledge-base/)
- University of Luxembourg: Principles of Biobanking (further information: https://wwwfr.uni.lu/formations/fstm/certificate_principles_of_biobanking)
- European, Middle Eastern & African Society for Biopreservation and Biobanking (ESBB): Education & Training overview (webinars, courses, mentoring, exchange programs) (further information: https://esbb.org/page/Education)
- International Society for Biological and Environmental Repositories (ISBER): list of available courses and webinar resources (further information: https://www.isber.org/page/BiobankEduOpp/Biobanking-Education-Opportunities.htm)
- International Agency for Research on Cancer (IARC): Learning Platform Biobanking (further information: https://learning.iarc.fr/biobanking/)

3. Biospecimen collection

3.1. General

The collection of biospecimens should only take place if the donor has consented to it based on an informed decision. The document in which this is recorded is the IC. This defines, for example, the

allowed uses for the donated biospecimens which must be adhered to in later use. At all times, the IC must comply with applicable laws, policies, and regulations.
Each biospecimen should retain the biology that was present prior to its removal from the donor as much as possible. To ensure the collection of high-quality biospecimens, personnel should be appropriately trained (see chapter 2.3) and adhere to predefined specific SOPs. Suitable handling procedures should be in place to minimize any effect the environment and other factors might have on the biospecimen as, ultimately, they might undermine its molecular integrity and physical characteristics, thus biasing any subsequent downstream analysis.
Numerous protocols describing proper collection procedures are nowadays available for the majority of biospecimen types normally encompassed in research and clinical studies. The chosen protocol(s) should suit study needs, the kind of biospecimens meant to be collected and the planned downstream analysis.
This section will focus primarily on the procedures for the prospective collection of the various biospecimens in the context of the EPND project.

3.2. Determining which biospecimen to collect

The type of biospecimen to be collected should be feasible for the laboratory/clinical setting and 'fit-for-purpose' for the planned downstream applications. Prior to launching any collection initiative, it is crucial to perform an assessment of each biospecimen type's sensitivity to collection, processing, storage, and distribution procedures. Logistic planning should also be in place to consider the distance from the collection point (e.g., clinic, study centre) to the processing laboratory and/or to the biobank itself. Already tested and validated SOPs, describing biospecimen stabilisation and/or preservation methods during transportation, should also be in place.

3.3. Pre-analytical variables

Many and diverse factors may negatively affect biospecimen integrity and/or quality and, ultimately research results. These factors are normally referred to as 'pre-analytical variables' (see

Figure 1). Pre-analytical variables can be divided into three general areas: (1) donor intrinsic physiological factors, (2) SOPs in place for biospecimen collection (e.g., type of collection tube, timing, and intermediate storage temperature), and (3) further sample handling prior its inclusion in any downstream applications.

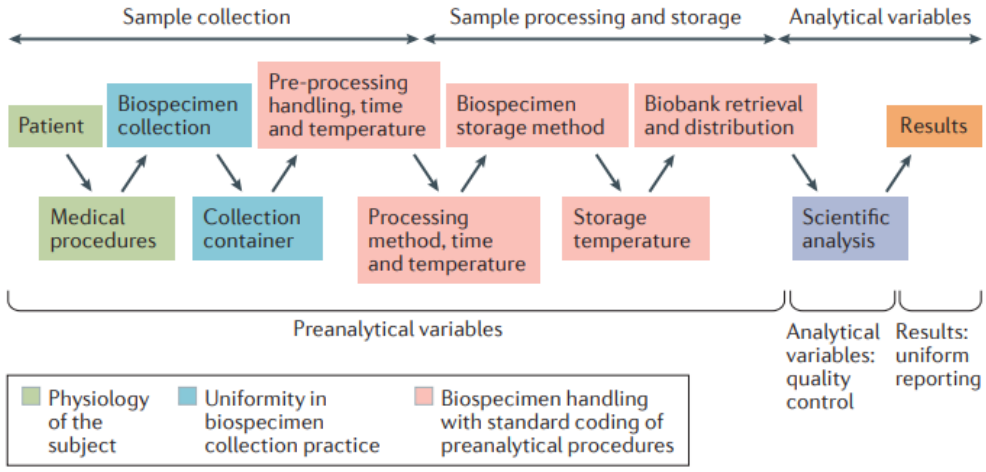

Figure 1: Biospecimen handling pipeline (modified from Yeung *et al*.[11]): pre-analytical variables can affect various steps of the pipeline, from biospecimen collection to downstream analysis

The documentation of a code (e.g., SPREC[12] annotation) is therefore strongly recommended to note and track the most critical pre-analytical variables in a standardized fashion.
The collection step should always include the recording of date and time (preferably according to ISO 8601 standard), collection site, collection method and all other deviations and anomalies (if applicable) to help identifying potential sources of pre-analytical variations later on.
In 2017, the Standardisation of generic Pre-analytical procedures for In-vitro DIAgnostics for Personalized Medicine[13,14] (SPIDIA4P) project was launched to conduct targeted research to address and categorize the major pre-analytical factors jeopardising biospecimen original quality and integrity. In light of this constant biospecimen research effort, newly developed evidence-based practices (

[11] Yeung *et al*. Enhancing translational research in paediatric rheumatology through standardization. Nat. Rev. Rheumatol. 2016;12:684-690. DOI: 10.1038/nrrheum.2016.156
[12] Betsou *et al*. Standard PREanalytical Code Version 3.0. Biopreserv Biobank. 2018;16:9-12. DOI: 10.1089/bio.2017.0109
[13] Riegman *et al*. How standardization of the pre-analytical phase of both research and diagnostic biomaterials can increase reproducibility of biomedical research and diagnostics. N Biotechnol. 2019;53:35-40. DOI: 10.1016/j.nbt.2019.06.007
[14] https://www.spidia.eu/ (last accessed: 2022-12-05)

Table 1) have been deployed with the aim to guide new biospecimen collection initiatives, and to mitigate the pre-analytical impact when collecting and utilizing (long-term) stored samples.

Table 1: ISO and Comité Européen de Normalisation (CEN) standards for pre-examination processes for biospecimens relevant for the EPND project

Biospecimen	Isolated RNA	Isolated DNA	Others
Venous whole blood	**ISO 20186-1:2019** - Specifications for pre-examination processes for venous whole blood – Part 1: Isolated cellular RNA	**ISO 20186-2:2019** - Specifications for pre-examination processes for venous whole blood – Part 2: Isolated genomic DNA	
	CEN/TS 17742:2022 - Specifications for pre-examination processes for venous whole blood - Isolated circulating cell free RNA from plasma	**ISO 20186-3:2019** - Specifications for pre-examination processes for venous whole blood – Part 3: Isolated circulating cell free DNA from plasma	
Urine		**CEN/TS 17811:2022** - Specifications for pre-examination processes for urine and other body fluids - Isolated cell free DNA	**ISO 23118:2021** - Specifications for pre-examination processes in metabolomics in urine, venous blood serum and plasma
Serum			
Plasma			
Saliva		**ISO 4307:2021** - Specifications for pre-examination processes for saliva - Isolated human DNA	

Biospecimen	Isolated RNA	Isolated DNA	Others
Stool		**CEN/TS 17626:2021** - Specifications for pre-examination processes for human specimen - Isolated microbiome DNA	

3.4. EPND SOPs for biospecimen collection

As already emphasised several times, it is essential that biospecimen collection for scientific studies is standardised and comprehensively documented. For this reason, the following chapters contain specific SOPs for the prospective biospecimen collection in the EPND project.

3.5. Cerebrospinal fluid

3.5.1. General

Lumbar puncture is the medical procedure that involves the draw of cerebrospinal fluid (CSF) via a hollow spinal needle inserted into the lumbar subarachnoid space. CSF can be used for both diagnostic and therapeutic purposes. A careful documentation of the procedure is recommended to facilitate the identification of non-conformities but also to show adherence to study specific aims. On the day intended for CSF withdrawal, the donor should not undergo any cognitive testing, as the procedure could strongly affect the results.

The process steps presented below are based on the following sources: Deisenhammer *et al*.[15], Engelborghs *et al*.[16] and Teunissen *et al*.[17].

3.5.2. Preparation to lumbar puncture

Prior lumbar puncture, the physician will:

1. Explain to the donor the whole procedure and the risks including the possible complications (e.g., post-lumbar puncture headache). The physician shall ask the donor if they plan to drive a vehicle directly after CSF withdrawal. In the case of a positive answer, the physician should check if the donor is fit for driving after the post-procedure one hour resting time.
2. Verify that the IC has been signed by the donor or an authorised representative.
3. Check for all supplies which should be available in sufficient quantities and appropriate for their intended use in the CSF collection process.

Sharps disposal containers should be within arm's length.

3.5.3. Time of the day for CSF withdrawal

Circadian rhythms are known to influence many circulating biomarkers. As it is very difficult to standardise 'time' in clinical practice, it is highly recommended to record CSF withdrawal date and time to later select samples for analysis, where the effect of this variable will be minimal.

[15] Deisenhammer *et al*. EFNS Task Force. Guidelines on routine cerebrospinal fluid analysis. Report from an EFNS task force. Eur J Neurol. 2006;13:913-922. DOI: 10.1111/j.1468-1331.2006.01493.x
[16] Engelborghs *et al*. Consensus guidelines for lumbar puncture in patients with neurological diseases. Alzheimers Dement (Amst). 2017;18:111-126. DOI: 10.1016/j.dadm.2017.04.007
[17] Teunissen *et al*. A consensus protocol for the standardization of cerebrospinal fluid collection and biobanking. Neurology 2009;73:1914-1922. DOI: 10.1212/WNL.0b013e3181c47cc2

3.5.4. CSF withdrawal procedure

The physician in charge of CSF withdrawal procedure:

- Wears a facial mask and gloves from the very beginning of the procedure.
- Helps the patient settle in the most comfortable position, either sitting or in lateral recumbent position.
- Disinfects (e.g., with alcoholic chlorhexidine 0.5% or equivalent) the area of the skin where the procedure will take place to remove grease/proteins. The disinfectant must be left to air dry completely.
- Performs the lumbar puncture in the inter-space between L3/L5, via atraumatic 20-22-gauge needle and drip method, or a syringe. The use of atraumatic needles is highly recommended.
- Removes the stylet of the spinal needle to observe the CSF free flow.
- Performs a standard collection of at least 12 ml. The collected volume, the site of withdrawal as well as date and time must be recorded. Normally, the first 2 mL are discarded or used for basic CSF analysis.
- Applies sterile compresses to the puncture site to avoid any bleeding.
- Asks the donor to lie down or remain lying down on their back for at least 1 h after the procedure and recommends drinking more liquids (from 1 L up to 2 L) for the first 12-24 h following the lumbar puncture to diminish the risk of headache. Provides information to the donor regarding relevant signs of complications. Instructions and contact information are provided in case side-effects will occur.

3.5.5. Removal of bloody CSF samples

Blood-contaminated CSF can lead to false positive results for biomarkers that have high serum concentrations (e.g., coagulation factors) or compromise proteomics analysis. Determining and recording erythrocyte count helps screening samples fit for downstream analysis. It is therefore strongly recommended, even for samples collected for research purposes only. In general, a count higher than 500 erythrocytes/µL CSF is indicative of samples that are not fit for biomarker studies.

3.5.6. Collection tubes

Polypropylene tubes, in the absence of any additive, have a low protein binding potential and therefore should be the tube of choice for CSF collection. Due to laboratory personnel safety concerns, glass tubes must be avoided.

3.5.7. CSF transportation to the processing site (where applicable)

If biospecimens will not be processed at the collection site, they should be dispatched to the processing laboratory via a dedicated courier at room temperature (RT) as soon as possible, to allow for processing within 2 h from collection. If CSF cannot be processed in less than 2 h post-collection, place it at 2-8°C until centrifugation. Record temporary storage and shipment temperatures and time periods.

3.6. Blood

3.6.1. General

When collecting blood, some general aspects need to be considered[18]. One is the fasting status of the donor. As non-fasting can influence biomarkers the fasting state should be defined accordingly. If metabolomics analyses are intended a minimum of 8 h fasting should be observed[19]. In general, 12 h fasting is recommended. Where applicable, the phlebotomist should verify donor fasting status before blood is drawn. If blood collection is performed in the non-fasting state, or a patient has not been properly prepared, the phlebotomist should document the fact to allow correct interpretation of test results.
In addition, circadian rhythm can also have an influence on biomarkers. This must be determined individually to be able to design the study protocols accordingly. Therefore, it is recommended that the blood samples are always taken in the same period (e.g., between 7:00-9:00 am). Furthermore, the following should be noted: Water consumption is allowed but alcohol intake is forbidden. Cigarette smoking prior to blood draw is not permitted. Caffeine-containing beverages (coffee, energy drinks and tea) and chewing gum should not be consumed before blood sampling. Unless vital for the donors, medications should be avoided. Finally, donors should not perform intense physical activity 24 h prior to blood draw.
Since a change in donor body position from supine to upright and vice versa can significantly influence laboratory parameters, this should be avoided within 15 minutes before blood collection. If this is unavoidable, it should be documented. A short walk from the waiting area to the blood collection site is acceptable if the donor has already rested for 15 minutes in the waiting area.
The process steps presented below are based on the following sources: CLSI H3-A6 guidelines[20] and Simundic *et al*.[18].

3.6.2. Standard precautions

Considering that it is often impossible to know which biospecimen might be infectious, all patient-derived samples shall be treated as potentially infectious and handled according to ‘Standard Precautions’.
‘Standard and Universal Precaution Guidelines’ are available from the US Centers for Disease Control and Prevention. Universal precaution guidelines apply only to transmission of blood-borne pathogens. Conversely, standard precautions are comprehensive guidelines as they cover the transmission of all infectious agents.
When drawing and handling blood, the phlebotomist must:

1. Wear well-fitting disposable gloves when collecting and processing specimens. To reduce prolonged blood stasis, gloves can be put on before applying the tourniquet (if needed).
2. Store needles in a locked cabinet when the collection centre is closed.
3. Clean up any spills of blood and/or derivatives with 0.1% sodium hypochlorite (household bleach). The same solution will be used to clean up all laboratory work surfaces at the completion of work activities.
4. Safely dispose all needles and tubing in puncture-resistant containers for safe disposal.
5. Avoid formation of potentially infectious aerosol by careful pipetting and centrifugation procedures.
6. Place in biohazard bags all used blood collection tubes and blood products for safe disposal.

Should the phlebotomist accidentally sustain an injury from a contaminated needle-stick, the wound should be thoroughly cleansed with soap and water. A local physician should be notified to evaluate whether donor's blood will be analysed for possible infections.

3.6.3. Preparation for venepuncture

[18] Simundic *et al*. Joint EFLM-COLABIOCLI Recommendation for venous blood sampling: v 1.1, June 2018 Clinical Chemistry and Laboratory Medicine 2018;56:2015-2038. DOI: 10.1515/cclm-2018-0602
[19] ISO 23118:2021 Molecular in vitro diagnostic examinations — Specifications for pre-examination processes in metabolomics in urine, venous blood serum and plasma
[20] CLSI H3-A6 guidelines Procedures for the collection of diagnostic blood specimens by venepuncture; Approved Standard-Sixth Edition. (Publication Date: 10/31/2007)

1. Blood collection must be performed by personnel properly qualified by training to draw blood.
2. All equipment and supplies should be available in sufficient quantities and appropriate for their intended use in the venous blood collection process. The workplace should be arranged in a way that the phlebotomist can reach all the necessary supplies without leaving his/her place. Sharps disposal containers should be within arm's length.
3. The phlebotomist should verify collection kits' expiry date, as well as their integrity. Storing collection tubes in conditions not consistent with the manufacturer's recommendations might affect blood draw volume, as well as the additives and the stability of gels.
4. All the required materials must be assembled before blood draw.
5. Following donor identification, all blood draw documents are filled in accurately. The phlebotomist checks that the IC has been obtained.
6. All donor-derived biospecimens must be correctly identified at collection time. Each blood collection tube must display a firmly attached label.
7. Any communication is aimed at making the donor more comfortable with the procedure and should show that donor confidentiality is highly respected. Being mindful and attentive to donor's concerns helps with reducing anxiety episodes and identifying individuals at risk of vasovagal reaction (syncope).

In preparation of the venepuncture, the phlebotomist performs hand hygiene before biospecimen collection. This procedure encompasses washing hands with soap and water, followed by drying with a single-use towel. Finally, the phlebotomist should put on well-fitting disposable gloves so that the venepuncture procedure can take place.

3.6.4. Venepuncture

1. The phlebotomist ensures that the donor is in a comfortable position, either sitting in a chair or lying down, with the arm hyperextended and stretched downwards.
2. The phlebotomist selects the appropriate site for venepuncture:
   i. The most prominent veins in the cubital fossa (e.g., cephalic, basilica, median cubital and median antebrachial veins) are the more frequently used sites, if available (see
   ii. Figure **2**). The cubital vein is usually the most prominent. It does not roll under the skin and can be localised in the same area in the majority of donors. While hand and wrist veins are normally acceptable, it is highly recommended to draw blood from an antecubital vein. Areas presenting excessive scarring and/or hematomas should be avoided. At all times, the risk of arterial puncturing and nerve damage must be minimized.
   iii. The vein should be visible without the need to apply a tourniquet. Palpation of the vein could also help in the assessment of the appropriate venepuncture site.
   iv. Locating the vein will help to determine the correct size of the needle (21G or 23G). The use of butterfly needles with flash visualization may be helpful in the case of donors with difficult veins.
3. Prior to sampling, the selected venepuncture site is cleaned with 70% ethyl alcohol or any other appropriate disinfectant to avoid skin contamination by pathogens. A single wipe is moved beginning at the site and then outward from it. In any case, the necessary exposure time of the disinfectant must be observed, usually according to the manufacturer's instructions. The site should not be touched after the cleaning and should be left to completely air dry.
4. If needed, the tourniquet should be applied 4-5 finger widths (7-8 cm) above the identified venepuncture site. It should be tight enough to stop venous but not arterial blood flow. Following

tourniquet application, the donor should not clench or pump the fist to avoid altering biochemistry and haematology parameters. The tourniquet should never be applied for periods longer than 1 minute, to avoid changes in blood composition.

5. The vein is anchored by holding the donor's arm and placing a thumb below the venepuncture site. Keeping the bevel up will cause less trauma to the vein and minimizes the pain.
6. The needle is inserted swiftly at a 5-30 degree angle, depending on the vein's depth. At least 0.5 cm of the needle should be inserted into the vein. Excessive probing and trauma to the site must be avoided.
7. Once the blood flows freely the tourniquet (if applied) is then removed.
8. The phlebotomist will hold the collection tube steady. The phlebotomist's hand is supported against the donor's arm.
9. Blood can be drawn using the vacuum (a) or the (b) aspiration technique. In (a), the pre-evacuated collection tube is inserted in the holder so that the cap is perforated, and the blood is drawn. In (b), the plunger of the collection tube is slowly withdrawn.
10. A blood drawing sequence (see Table 2) must be followed to avoid cross-contamination of additives between collection tubes.
11. Tubes must be fully filled, up to the level indicated on each collection tube by the manufacturer, to ensure the correct mixing ratio between blood and additive.
12. Collection tubes must be gently inverted according to the manufacturer's instructions, with the non-dominant hand, immediately after blood draw. Evidence has shown that any delay could compromise sample quality and integrity[21]. The term 'inversion' refers to turning the tube vertically for 180° and putting it back to the starting position. Collection tubes are then placed in racks in an upright position.
13. Following the last tube disconnection, a gauze pad is applied without exerting pressure on the collection area. The needle is then removed gently, and the pad is pressed on the puncture site to avoid excessive bleeding.
14. All needles and supplies should be disposed of in a safe manner. Needles must never be recapped.
15. A period ranging from 2 minutes (routine draws) to 10 minutes (for donors under treatment with anticoagulants) might be required for bleeding to stop. Once the bleeding has stopped, the wound is treated by applying a patch or a bandage by placing an adhesive tape over a dry pad/gauze square. To avoid hematoma formation or excessive bleeding, the phlebotomist will ask the donor not to bend their arm. The phlebotomist removes the first glove, turning it inside out and then encloses it by rolling the second glove over it. Gloves are discarded.
16. Hand hygiene is performed once again (see chapter 3.6.3).
17. Any nonconformity (e.g., blood collection unsuccessful, change of drawing site, collection tube not fully filled, etc.) must be duly noted and tracked.
18. Collection tubes are finally ready to be processed on-site, or to be shipped to the processing site according to established SOPs. Specific SOPs for shipping temperature range and logistics must have been put in place beforehand, to minimize the risk of time to centrifugation (TTC) delays.

[21] Karlsson *et al*. Delayed mixing of vacuum tubes clearly affects platelet counts but not haemoglobin concentration and prothrombin time (INR) results. Int J Lab Hematol. 2013;35(6):e15-7. DOI: 10.1111/ijlh.12096.

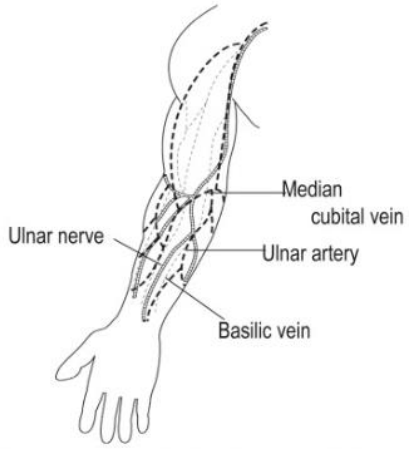

Figure 2: Site selection for venepuncture[22]

Table 2: Revised, simplified recommended order of draw for vacuum tubes or syringe and needle, based on United States National Committee Clinical Laboratory Standards consensus (2003)[23]**.**
EDTA, ethylenediaminetetraacetic acid; ACD, acid-citrate-dextrose; CTAD, citrate, theophylline, adenosine, dipyridamole.

Order of use	Type of tube
1	Blood culture bottle
2	Non-additive tube
3	Sodium citrate
4	Clot activator (silica)
5	Serum separator
6	Heparin
7	Heparin and gel separator
8	EDTA

[22] Figure adapted from World Health Organization. WHO Guidelines on Drawing Blood: Best Practices in Phlebotomy. World Health Organization press 2010; Geneva.
[23] Table adapted from World Health Organization. WHO Guidelines on Drawing Blood: Best Practices in Phlebotomy. World Health Organization press 2010; Geneva.

9	ACD
10	CTAD
11	Oxalate/fluoride

3.6.5. Blood transportation to processing site (where applicable)

Whole blood samples should be transported at RT. Filled blood collection tubes must not be exposed to temperatures below 0°C or above 25°C. Due to high acceleration/deceleration forces, which can influence the biospecimen quality, the use of a pneumatic tube transport system should be validated. If samples are dispatched from a location far from the processing centre, collection tubes should be shipped in a suitable transport container via a dedicated courier.

3.7. Urine

3.7.1. Preparation for urine collection

Prior to urine collection, the collection site should advise the donor not to drink extra fluids and not to ingest food and drinks that are diuretic (e.g., alcoholic beverages, coffee, tea, energy drinks and soft drinks). For metabolomics analysis, the first midstream urine of the morning should be collected after a minimum of 8 h fasting[24].

3.7.2. Urine collection

1. Personnel collects the IC or verifies that it has already been given.
2. A sterile pre-labelled urine collection container is handed over to the donor.
3. The donor is instructed to:
   a. Disinfect the urethral opening with a wipe.
   b. Open the urine collection container without touching the inside of the container or the closing lid.
   c. Discard the first part of the voided urine into the toilette as only midstream urine should be collected. Collect, without disturbing the flow, the defined sufficient urine volume into the pre-labelled urine collection container and close it with the lid.
   d. Clean the outside of the urine collection container if necessary. Wash your hands with soap and hand the urine collection container over to the personnel.
4. Personnel duly fills in the biospecimen collection form once the donor returns the urine collection container with the urine sample. In case urine cannot be immediately processed it should be stored at 2-8°C until processing.

Time stamps for urine collection must be recorded as well as temporary storage temperatures and duration.

Any nonconformity (e.g., non-adequate sample volume, urine cloudiness, urine discoloration, etc.) should be noted and tracked.

3.7.3. Urine transportation to processing site (where applicable)

[24] ISO 23118:2021 Molecular in vitro diagnostic examinations — Specifications for pre-examination processes in metabolomics in urine, venous blood serum and plasma

In case urine cannot be immediately processed and shall be used for metabolomics, it should be stored between 2-8°C[24]. Samples must not be frozen or exposed to ambient temperatures higher than 25°C. If samples are dispatched from a location far from the processing centre, biospecimens should be shipped in a suitable transport container via a dedicated courier. Samples for metabolomics should be kept cool at 2-8°C during transport and a period of 2 h between urine collection and delivery at the processing site should not be exceeded[24]. Shipment temperature and duration should be recorded.

3.8. Stool

3.8.1. Preparation for stool collection

Stool collection by means of the OMNIgene·GUT OM-200 tube (DNA Genotek) which is an all-in-one system for easy self-collection (also possible at donors' home) and stabilisation of microbial DNA from stool for gut microbiome profiling.
Verify that the IC has been signed by the donor or an authorised representative. Prior to collection the donor must have been instructed about the procedure and given details regarding the amount of stool to be transferred to the collection tube.

3.8.2. Collection, transport, and storage

The procedure for stool collection is described below following the manufacturer's instructions[25].
The donor will:

1. Empty the bladder before beginning the collection. Collect fecal sample free of urine and toilet water. Toilet paper or tissues may be required.
2. Unscrew, while holding the yellow tube top, only the purple cap from the kit and set aside for later use. Important: the donor shall not
   (1) remove the yellow tube top
   (2) spill the stabilising liquid in the tube.
3. Use the spatula to collect a small amount of fecal sample.
4. Transfer the fecal sample into the yellow tube top. Repeat until the sample fills the yellow tube top. Important: the donor shall not push the sample into the tube.
5. Scrape horizontally across the tube top to level the sample and remove any excess. The donor will then wipe the exterior of the tube and top with toilet paper or tissue as needed.
6. Pick up the purple cap with the solid end facing down and screw onto the yellow tube top until tightly closed.
7. Shake the sealed tube as hard and as fast possible in a back and forth motion for a minimum of 30 seconds. The fecal sample will be mixed with the stabilising liquid in the tube; not all particles will dissolve. Important: the donor shall continue shaking if large particles remain.
8. Place spatula in original packaging or wrap in toilet paper and discard in garbage. Important: the donor shall send sample for processing following the delivery instructions supplied separately by the kit provider.

Stool samples can be stored in the original collection tube at RT (15-25°C) for up to 60 days. Storage at 4°C is not recommended by the manufacturer. Transfer to -20°C or -80°C within 60 days of collection allows storage of up to one year in the original collection tube. If a longer storage period is planned, then the stool samples should be vigorously mixed, aliquoted into cryotubes and stored at -80°C[26].

[25] https://www.dnagenotek.com/row/products/collection-microbiome/omnigene-gut/OM-200.html (last accessed: 2022-12-13)
[26] https://www.dnagenotek.com/row/pdf/PD-PR-01036.pdf (last accessed: 2022-12-13)

3.9. Saliva

3.9.1. Preparation for saliva collection

Before the visit, donors should be instructed not to:

1. Brush their teeth or use oral hygiene products
2. Smoke
3. Eat (including chewing gum)
4. Drink (only water is allowed)

3.9.2. Collection, transport, and storage

Verify that the IC has been signed by the donor or an authorised representative. For saliva collection, bubble formation should be avoided. The collection tube's opening must never be touched with bare hands. All tubes and collection devices must always be placed on a clean and dry surface.
Depending on downstream application, up to three different kinds of collection tubes can be used. The following process steps for the individual kits correspond to the manufacturer's instructions[27,28,29].

Microbiome

1. The donor is handed over a DNA Genotek OMNIgene·ORAL saliva collection kit (cat. No. OME-505) and will:
   a. spit into the funnel until the amount of liquid (not bubbles) reaches the fill line.
   b. hold the tube upright with one hand. Close the funnel lid with the other hand by firmly pushing the lid until a loud click is heard (make sure it is closed tightly). The liquid in the lid will be released into the tube to mix with the sample.
   c. hold the tube upright and unscrew the funnel from the tube.
   d. use the small cap to close the tube tightly.
   e. shake the capped tube for five seconds.
   f. discard or recycle the funnel.
2. Personnel should place the tube in a suitable container for RT transport (never freezing conditions or T>25°C).

Delivery should take no longer than 7 days after biospecimen collection. Record temporary storage and shipment time periods. At the processing site, biospecimens can be stored at RT until processing. Storage at 4°C is not recommended by the manufacturer.
Saliva samples should be incubated at 50°C for 1 hour in a water bath or 2 hours in a dry incubator, mixed well by inversion, aliquoted into cryotubes and stored at -20°C or -80°C within 21 days of collection[30]. If long-term storage doesn't take place at the processing site, the frozen saliva biospecimen must be shipped to the biobank on dry ice.

Metabolomics

[27] https://www.dnagenotek.com/ROW/support/collection-instructions/omnigene-oral/OME-505.html (last accessed: 2022-11-24)
[28] CS-5016.04, Rev. 3 (2021-09-09), see https://salimetrics.com/wp-content/uploads/2018/02/passive-drool-saliva-collection-instructions.pdf (last accessed: 2022-11-24)
[29] https://filgen.jp/Product/Bioscience4/Oasis/RPSAL-701.pdf (last accessed: 2022-11-24)
[30] https://www.dnagenotek.com/row/pdf/PD-PR-01036.pdf (last accessed: 2022-12-13)

1. The donor is handed over a Salimetrics Saliva collection kit (cat. No. 5016.04) and a pre-labelled collection vial and will:
   a. open the pouch and remove the Saliva Collection Aid (SCA), if the saliva collection kit is still packaged
   b. place the ribbed-end of the SCA securely into a pre-labelled collection vial
   c. allow saliva to pool in the mouth. Then, with the head tilted forward, **gently** guide the saliva through the SCA into the vial and fill it to the required volume. Reserve a small amount of air space in the vial to accommodate liquid expansion during freezing.
   d. remove and discard the SCA. Attach the cap to the collection vial and tighten it.
   e. hand over the collection vial to the responsible personnel.
2. Personnel should freeze the sample immediately at or below -20°C. If this is not possible refrigerate the sample immediately at 4°C and maintain at this temperature for no longer than necessary (ideally less than 2 hours) before freezing at or below -20°C.

If biospecimens will not be processed or long-term stored at the collection site, they should be dispatched to the processing laboratory or biobank in a suitable transport container via a dedicated courier. Refrigerated samples should be kept cool at 2-8°C during transport and delivery should take no longer than 2 hours after biospecimen collection. Frozen samples must be shipped to the biobank on dry ice. Record temporary storage and shipment temperatures and time periods. If the samples are to be stored for more than 4 months, they should be stored at -80°C.

RNA and protein

1. The donor is handed over a RNAPro•SAL™ tube (cat. no. #RPSAL-701) and will:
   a. place the contents on a clean and dry surface. In preparation for saliva collection, saliva must be pooled in the mouth.
   b. attach the two Collection Tubes provided to the base of the Splitting Unit.
   c. attach the Compression Tube into the top end of the Splitting Unit firmly. At this stage, saliva collection can begin.
   d. place the tip of the pad of the Collector where saliva has pooled. The Collector may be removed from the mouth periodically to read the Sample Volume Adequacy Indicator (SVAI), but collection should be resumed immediately afterward.
   e. collect until the appearance of the SVAI changes completely to red. The collection procedure should last approximately one to five minutes.
   f. remove the Collector from the mouth and place the white absorbent pad end into the Compression Tube, holding the Collector in an upright position, and firmly push the plunger downwards to transfer the saliva from the absorbent pad into the Collection Tubes. Push and hold for thirty seconds.
2. Personnel will:
   a. remove gently the Eppendorf tubes from the end of the Splitting Unit.
   b. add immediately, if required, a protein stabilising agent or an RNA stabilising agent according to the planned analysis (stabilising agents are not included in the kit).
   c. close tightly the lids of both Eppendorf tubes
   d. invert both tubes 2-3x each
   e. personnel should process the samples immediately or refrigerate at 4°C.

If biospecimens will not be processed or long-term stored at the collection site, they should be dispatched to the processing laboratory or biobank in a suitable transport container via a dedicated courier. Samples

should be kept cool at 2-8°C during transport and delivery should take no longer than 2 hours after biospecimen collection. Record temporary storage and shipment temperatures and time periods. Following aliquoting biospecimens must be transferred to -80°C storage. If long-term storage doesn't take place at the processing site, the frozen samples must be shipped to the biobank on dry ice.

3.10. Biospecimen reception

If biospecimen collection is not performed at the processing site biospecimen transport is necessary. It must be ensured that the biospecimen transport is carried out in accordance with the applicable regulations and that biospecimen integrity is maintained (see chapter 6.3). For specific information on transport of certain biospecimens, please refer to the collection SOPs.
Biobank personnel should follow SOPs for receiving biospecimens. Acceptance criteria defined by the biobank shall be checked for compliance upon arrival of the biospecimens. For example, personnel should check for packaging integrity and for any potential cold chain breaches during transportation (data logger check and/or coolant conditions). A notification shall be sent to the sender confirming that received samples match those listed in the shipping manifest. Any discrepancy should be timely communicated to the sender and should be clarified. A record of the receipt and all relevant documents should be maintained in the biobank archive.

4. Biospecimen processing

To ensure proper biospecimen processing, personnel should be appropriately trained (see chapter 2.3) and adhere to predefined, specific SOPs. Biobanks should establish internal processing SOPs or follow previously validated ones. Many online resources are available and can be used as a starting point for new protocol draft creation. As SOPs for CSF, blood, urine, stool, and saliva processing were compiled in the deliverable 3.3 (SOP body materials collection) of the EPND project, they are not part of this document. Refer to D3.3 for the respective information.

General safety precautions should be taken during biospecimen processing. All processing procedures should maintain biospecimen integrity (i.e., stability, sterility, temperature). For this, it is recommended that the biospecimen processing is performed as soon as possible after biospecimen collection. In addition, the traceability of biospecimens must be ensured (e.g., by unique labelling).
Complete sterile conditions might not be a strict requirement during the processing phase. However, adequate consideration should be given to cleanliness of instruments, surfaces and equipment used for specimen processing and handling.
Processing equipment should be qualified prior its first use and at defined time points (e.g., following maintenance or repair). A system for preventative maintenance, repair and calibration of all processing instruments/equipment should be in place.

To ensure that processing methods are fit for purpose, methods or assays need to be validated (see chapter 8.2) for the intended end use(s). Scientific and technical literature, feedback from the end user and QC results (see chapter 8.1) represent some of the factors contributing to method validation and method re-validation according to specific circumstances (e.g., personnel/instrument change, new lot number of reagent(s), change in biospecimen type, etc.).
Any nonconformity observed and noted during biospecimen processing (e.g., insufficient volume in blood collection tube, haemolytic blood sample) should be documented in conjunction with the subsequent actions taken.

5. Biospecimen storage

5.1. General

To ensure proper storage of different types of biospecimens, trained personnel should be well qualified (see chapter 2.3) and shall follow appropriate SOPs.
Such SOPs should be evidence-based and specific to the biospecimen type and the biomolecules (RNA, DNA, proteins, etc.) that are planned to be analysed.
Storage conditions and any SOP deviation (e.g., freezer failure, unintentional thaw/refreeze episodes, etc.) should be documented. At all times, sample quality and integrity must be preserved to avoid introduction of bias in future studies aimed at sample downstream analysis.
While it is true that in the context of a biobank the majority of samples is long-term stored at very low temperatures (-80°C or below), it might be appropriate for some biospecimen types or derivatives to be stored at dry or ambient (controlled) temperature.
In recent times, biological storage matrices that allow for long-term preservation at RT of certain specimens (e.g., nucleic acids) have been identified. It is expected that in the near future more matrices will be made available for a wider variety of biospecimen types or derivatives. They could represent an interesting alternative when appropriate storage equipment is either not available or when back-up resources are needed.

5.2. Biospecimen stabilization and stability studies

All biospecimens should be stored in a stabilized state. To avoid multiple thawing/refreezing of frozen biospecimens, or derived samples, which may influence the sample composition and/or quality, an appropriate size for aliquots should be determined in advance depending on the planned downstream applications (e.g., several hundred µl for plasma or serum).
Biospecimen stability might be affected not only by temperature, but also by parameters pertaining to its collection (e.g., anticoagulants), changes in pH due to dead-air volume in the storage container, exposure to UV rays, etc. Long- and short-term studies, based on validated protocols, might be periodically planned to thaw, and analyse samples to evaluate marker(s) of interest stability over time, when the storage temperature remains unchanged.

5.3. Selection of the appropriate storage container

Storage containers (e.g., cryotubes) should be stable under the planned storage conditions (e.g., -80°C). Containers should be chosen keeping in mind long-term use and analytical downstream applications (e.g., platforms, automation, protocol standardisation, etc.).
Storage containers should be tested to exclude contamination or chemical leaching into the biospecimen. Frequently, the selection of optimal volume and type of container may prevent sample loss and thereby minimizes the costs of daily biobank operations.
If labelling is carried out manually, biospecimen labels must be firmly attached to the container. The labelling must endure excursions in and out of cold conditions, exposure to high humidity, ambient temperatures, and long-term storage periods. For this reason, it is recommended to use cryotubes which have already been provided with a 1D barcode on side and/or 2D code on base by the manufacturer and which are also machine-readable in addition to the previously mentioned necessary properties.

According to the ISBER Best Practices:

- Screw cap cryotubes might be used for long-term storage and low temperatures (see chapter 5.4)
- Amber-vials or amber-coated bags should be used to store light sensitive materials

5.4. Selection of the appropriate storage temperature

When choosing the proper temperature for sample storage, the following items should be considered: (1) biospecimen type, (2) planned length of storage, and (3) future sample analysis. Other considerations should be made on a case-by-case basis in relation to the biospecimen(s) and study/collection scope scenario.
Even a short exposure of the biospecimen to a warm storage environment can lead to physiological stress and macromolecular degradation and can thus massively influence the subsequent analyses.

According to ISBER Best Practices:

- Serum and plasma, blood derivatives, must be separated before cryopreservation, while cryopreserved whole blood, previously collected in proper collection tubes, is an efficient and cost-effective option to preserve viable cells and stabilized nucleic acids until further use.
- Viable cells (e.g., Peripheral Blood Mononuclear Cells (PBMCs)) should be stored at low temperatures, in the presence of a cryoprotectant and by means of controlled-rate (slow programmable) cooling. Viable cells might also be stored at low temperatures in the presence of higher concentrations of cryoprotectants (vitrification process).

5.5. Storage equipment, alarm system and security

All biospecimens should be stored in a secure location that meets the relevant safety requirements and whose access is limited only to authorised personnel. Every access to the storage location(s) should be documented.
Storage equipment should be qualified prior to its first use and at defined time points (e.g., following a freezer unit defrosting, clean up and after repair). System for preventative maintenance, repair and calibration of all storage equipment should be in place.
Automated systems should continuously monitor storage equipment functionality and display features to warn predesignated “on call” personnel about equipment failure (e.g., enormous temperature increase in a freezer). It is highly recommended that critical systems (e.g., temperature monitoring, oxygen sensors, ventilation systems, controllers for liquid nitrogen freezers) are protected by an uninterruptable power supply functioning as a backup in case of power failure or abnormalities. Motor generators are mainly used as a backup power system. Depending on the facility, a risk-based decision must be made as to whether it is better to have one large generator or several small generators. It must be always ensured that sufficient fuel is available in an emergency situation or can be procured very quickly. In some cases, biobanks also have the option of connecting to an existing emergency system, e.g., of the hospital or university, which is recommended. In all cases, the backup power system should be subjected to regular routine tests to ensure its functionality.
Appropriate number of back-up freezer units should be operational in case of possible main equipment failure, thus requiring biospecimen transfer from one freezer unit to the other. For this purpose, trained personnel must be available on demand (24/7) to be able to react as quickly as possible to an alarm and to avoid damage to or loss of the biospecimens. A SOP or checklist that can be followed by the respective personnel in such a case is essential.

6. Biospecimen distribution

6.1. General

Biospecimen distribution is a process that includes the receipt of a sample request, sample selection and inspection, in conjunction with subsequent sample shipment and delivery to another place (e.g., laboratory, biobank). A defined process, including responsibilities, should be in place.

At first the sample requestor prepares a sample request. For this purpose, a biobank often provides specific forms to the sample requestor so that all necessary information is available for the further process. Upon reception, the sample request must be checked for feasibility (e.g., in terms of allowed uses according to IC, biospecimen volume/amount, etc.) and, if so, needs to be approved by a designated person or committee. Once approved, the sample request is passed on to the biobank personnel for sample picking and subsequent shipment.

Another document, the Material Transfer Agreement (MTA), is completed by the sample requestor to accompany the original sample request. The MTA should be in place before the biospecimen transfer occurs. The scope of the MTA is to govern: (1) the rights, obligations, and restrictions of both biospecimen provider and recipient with respect to the ‘material’ (e.g., in terms of data privacy), in agreement with

applicable laws, policies and regulations, (2) the purpose and terms of biospecimen transfer. It is recommended that biobanks create such MTA template which can be directly used or adapted if necessary.

6.2. Biospecimen retrieval and necessary documentation

Biospecimens should be retrieved from their storage location following SOPs that safeguard their quality and integrity. Biobank personnel should adhere to protocols for proper biospecimen inventory and tracking, respecting safety standards when working with any kind of storage equipment.
Biospecimens should be located and pulled from the storage unit as documented on specimen requisition forms. Operator speed is key when biospecimens are frozen or vitrified. During the whole retrieval process, biospecimens should be kept at the storage temperature. This is possible, for example, by temporary storage on dry ice or transfer to a workbench with a cryogenic workspace for handling frozen biospecimens. A cross check should be performed to ensure that all biospecimens listed in the request form have been retrieved. For biospecimens in storage containers with machine-readable labelling (see chapter 5.3) depending on the coding (1D and/or 2D) the use of a handheld barcode scanner, single tube reader and/or rack scanner is highly recommended for this purpose.

6.3. Packaging and shipping

6.3.1. Regulatory requirements

All applicable regulations for packaging and shipping of biospecimens should be followed. In case of air shipments, they should conform to the International Air Transportation Association Dangerous Goods Regulations (IATA DGR)[31]. Biobanking personnel should be trained regularly according to the main standards relevant to biological material shipping and transportation (IATA DGR, Agreement concerning the International Carriage of Dangerous Goods by Road (ADR), etc.). To proper classify biospecimens for shipment, the shipping institution should refer to specialist staff (e.g., dangerous goods officers).
When shipping biospecimens to (but also from) an international location, the shipper should:

- Consider variations in national and regional standards regarding biospecimen transportation. Special permits concerning the import/export of certain biospecimens might be required. ISBER guidelines recommend including an official letter documenting package content, handling requirements and copies of all import permits and sanitary certificates, when and where applicable.
- Prepare a custom clearance note to be clearly displayed outside the package if needed. A customs broker, or the same courier, could provide their valuable expertise. In case of custom clearance delays, the courier might even replenish refrigerant for temperature sensitive material.

6.3.2. Temperature requirements

Biobank personnel should be trained and understand the conditions required for shipping different types of biospecimens.
To maintain biospecimen quality and integrity, shipment temperature should be tracked (e.g., by temperature loggers) and documented at all times. Temperature fluctuations should be minimized or avoided. Therefore, factors including, but not limited to, shipping time, distance, season and means of transportation, should be taken into account.
Cold or frozen biospecimens should be shipped with a proper amount of coolant to maintain the temperature throughout the shipping cycle, allowing for at least a 24 h-delay in arrival time.
As per ISBER Best Practices, below are some typical temperature conditions required for transport of biospecimens, including the insulation/coolant helpful to maintain the desired temperature, detailed:

[31] https://www.iata.org/en/publications/dgr/ (last accessed: 2022-10-27)

- Refrigerated (2°C to 8°C): wet ice or gel packs that have been previously conditioned at -15°C, designed for refrigerated temperatures, or phase change material rated for refrigerated transport.
- Frozen (-20°C): gel packs designed for frozen temperatures, conditioned at or below -20°C.
- Frozen (-70°C): dry ice pellets, blocks, or sheets. Appropriate labelling for storage containers should be used as dry ice (solid CO_2) is considered a hazardous substance.
- Frozen (at or below -150°C): liquid nitrogen (LN2) dry shipper (vapour shipper). Dry nitrogen shippers are insulated containers equipped with porous material that fully absorbs LN2, making it non-dangerous and, therefore, if properly filled, not subjected to IATA regulations. In some cases, biospecimen transport instead takes place on dry ice as described in the previous bullet point.

6.3.3. Packaging preparation, shipment, and arrival

Biobank personnel are responsible for choosing the appropriate packaging for biospecimens to be shipped (also based on applicable regulations).
A courier, providing real time tracking capability and with a proven reputation of successful on-time delivery, should be chosen as a key element in keeping the 'cold chain' unbroken. Prior the actual shipment of very valuable biospecimens, a dry run with test material will allow verifying packaging configuration and coolant adequacy. A device recording temperature during transportation should be added to ensure that temperature requirements have not been surpassed, during both trial time and actual shipment scenarios.
The number of biospecimens per package will affect the type of packaging and the amount of coolant required to maintain the appropriate temperature for all biospecimens in the shipment. After biospecimens and refrigerant are allocated inside the shipping container, any empty space should be filled with padded paper or Styrofoam to prevent any movement of the biospecimens during transportation. Multiple, smaller shipments are preferable to shipment involving a large number of biospecimens. When planning a shipment, the time required to process the shipment should be considered as well. Shipments should be initiated when at least two working days are left in the week after the estimated delivery date in case they do not arrive on the day scheduled for delivery. Shipping with a forecasted arrival time during the weekend or holiday breaks is not recommended. The biobank personnel should electronically send a shipment manifest to the receiver. The manifest is a list of sample identification numbers and descriptions of samples. A paper copy should be included with the shipment itself. The biobank should notify the recipient about shipment arrival on a specific date. The receiver should confirm that he is able to receive the shipment and that there is storage capability for the incoming biospecimens. Shipment tracking number should be immediately shared with the receiver as soon as it will be made available by the courier.
Following package arrival, personnel (biobank staff, researchers, clinicians, etc.) should verify for packaging integrity and any potential disruption of the cold chain that might have happened during transportation. Biospecimen labels should be verified, against the manifest, for consistency and correctness. Any discrepancy should be immediately resolved. For quality management purposes, a questionnaire requesting feedback about the integrity of the samples received (e.g., were the samples still frozen?) might be enclosed in each shipment.

7. Biobank Information Management System (BIMS)

7.1. General

Sample collection, processing, storage, shipment, and redistribution are daily biobanking core activities that could quickly turn into a logistic nightmare in the absence of a proper biospecimen inventory and a BIMS.
According to the biobanking standard ISO 20387 and best practice guidelines (e.g., ISBER Best Practices), the implementation of a configurable/customisable and regulatory-compliant informatics system, might help biobanks overcoming not only logistical challenges, but also those linked to: (1) sample quality and integrity, (2) regulatory requirements (e.g., General Data Protection Regulation), (3) personnel safety and (4) staff training and competency records.

7.2. BIMS selection

The biobank manager, the quality manager, and all relevant stakeholders (IT, biobank operators, etc.) should be involved in the software selection process to identify necessary system features, functionality, and technical requirements.
Ideally, a BIMS should be user-friendly, easy to access while secure, and display all the necessary features to comply with regulatory guidelines.

Both financial and strategic decisions will dictate which system to purchase. A pre-defined, out-of-the-box informatics system relies on the existing technology and is readily available at reduced costs and timelines. Nevertheless, it might not meet the actual biobanking operational requirements.
A customised system, while more demanding in terms of human resources and periodic financial inputs, will provide the envisioned interface to meet the daily biobanking workflow and will handle rapid changes to workloads and user demands.

7.3. Key characteristics of the BIMS

A computer-based inventory system should track the location and pertinent annotation of every biospecimen in the biobank.
The informatics system:

- Assigns to each biospecimen a unique ID that could also be read via instrument (e.g., scanner). The use of barcodes/codes (e.g., 1D, 2D) is therefore highly recommended.
- Links the uniquely identified biospecimen, and its container, to already pre-defined downstream processing workflows and any other biospecimen information that will be subsequently added (e.g., processing activities-related time stamps, operator initials, freeze/thaw cycles, potential nonconformities affecting the biospecimen or its aliquots, etc.)
- Allows real-time mapping of biospecimens within the biobanking infrastructure. It identifies each biospecimen position according to the pre-defined storage unit configuration (e.g., position in the box, box number, rack number, etc.)
- Keeps the original biospecimen lineage by assigning new identifiers to newly generated aliquots ('child' samples)
- Updates automatically biospecimen/aliquots position, following a change from the initial storage position, and keeps tracking further position changes
- Monitors sample inventory at all times while tracking any significant event that characterises the biospecimen life cycle from collection, internal processing and QC activities, aliquot freeze and thaw cycles to partial sample usage, distribution, destruction, etc.
- Displays full query capability to be interrogated about total biospecimen number, stored versus distributed aliquots, number of different biospecimen types, single aliquot allocation, etc.
- Displays full audit trail capability to track all changes to biospecimen data, and other data types, where applicable, generating reports detailing original data and new data, time and date stamps, user identification, reason for the change(s), etc. The audit trail should be automatically recorded and available for read-only access. Record changes should not obscure previously recorded information in the audit trail.
- Must have the ability to produce and save reports
- Should be a system ready for integration and interoperability

7.4. Security

The BIMS must keep all data secure by means of data encryption and controlled data access. User authentication information should always be encrypted.
Access to the BIMS should be tightly controlled. Users should be assigned different privileged access according to their institutional role. Following a predefined period of inactivity (idle time), users should be logged off from the system. Similarly, the system should lock out any users following a failed number of login attempts.

7.5. Validation and operation of the BIMS

7.5.1. Operation access

The operational infrastructure should support access 24/7 a week (system dependability).

7.5.2. System downtime and disaster recovery

The BIMS should have defined and running processes to cope with system downtimes and disaster recovery. Planned testing time should be dedicated to ensuring the quality of backups and restore process. Backups should be regularly validated to ensure data can be accurately recovered.

7.5.3. Inventory

It is highly recommended to perform periodic and random checks on the BIMS. The aim of this procedure is to confirm that the system outputted inventory matches the actual physical location of the stored biospecimens in the biobank.

7.5.4. Quality Assurance

The electronic inventory system should comply with industry-applicable current Good Practices (cGP guidelines). An established Quality Assurance program for the informatics system should be aimed at preventing and detecting nonconformities and implementing corrective actions and processes. It is best practice to regularly perform an audit of the database to ensure data accuracy, completeness, and consistency.

8. Quality control and method validation and verification

8.1. Quality control

Biospecimen and associated data quality are of utmost importance and can have a great influence on the subsequent analyses. Especially in the pre-analytical phase (biospecimen collection to storage), there are many factors/variables that can significantly reduce the biospecimen quality. It is therefore essential to carry out QCs.
According to chapter 7.8 of the ISO 20387 standard every critical biobanking activity with an impact on the quality of the biospecimens and associated data shall be identified, and appropriate, regularly updated QC procedures must be established, implemented, and documented to provide biospecimens and associated data fit for purpose. It is important to note that specific QC can often only take place if the end-use analysis for the biospecimens is known (not always the case). QC activities should take place at planned intervals based on defined QC criteria. The QC data generated should be analysed to identify when pre-defined QC criteria have not been met. It is key to communicate these limitations to the user of the biospecimens and associated data so that they can decide whether they are suitable for the desired analyses. Furthermore, it is highly recommended to regularly analyse the results of QC procedures (e.g., via control charts) to identify trends and initiate actions when needed.

General support for the selection of suitable assays to qualify specific biospecimen types is provided, for example, by the following free online tool designed by the Integrated Biobank of Luxembourg (IBBL): https://www.findmyassay.com/

Based on the biospecimens to be prospectively collected in the EPND context, only QC considerations for fluid, cell and nucleic acid specimens are discussed below.
With respect to liquid biospecimens (e.g., blood derivatives like plasma or serum, CSF, urine, saliva), biobank procedures may affect the structure and/or function of molecular components. Pre-analytical deviations are particularly relevant here (see chapter 3.3). Several molecular markers have already been identified to assess specific pre-analytical variables:

- Haemoglobin content to assess haemolysis in blood derivatives[32,33] and CSF samples[34]
- Determination of LacaScore or measuring concentration of Interleukin (IL) 16 in EDTA plasma to identify time to centrifugation delays[35]
- sCD40L concentration to assess prolonged exposure of EDTA plasma or serum to RT after centrifugation[32]
- Taurine levels[36], transferrin receptor concentration[33] or IL8[33] levels as quality indicators for time to centrifugation delays in serum
- Measuring levels of transthyretin isoforms or cystatin C truncation in CSF samples to identify suboptimal storage conditions[34]
- Measuring alkaline phosphatase activity in urine samples to identify suboptimal storage conditions[37]

For cell biospecimens (e.g., PBMCs) the cell viability can be assessed. To do this, the total number, and the number of living cells in a defined volume (aliquot) must be determined, e.g., using staining methods. In addition to the number of viable cells contained in the sample after thawing that are available for analysis, it is also interesting to compare how many viable cells were present before and after freezing to estimate how high the loss due to the freezing process is. In addition, functional assays can be performed (e.g., cell proliferation) or cell purity can be determined.
If nucleic acids (DNA, RNA) are isolated from biospecimens regular quality checks should be performed. These can include tests for purity, integrity and/or for the presence of interfering substances. Detailed requirements for isolated RNA or DNA from venous whole blood are included in the ISO standards 20186-1[38] and 20186-2[39] respectively and for isolated human DNA from saliva in ISO 4307[40].
To demonstrate that the biospecimen quality is comparable the biobank shall regularly participate in external quality assessment (EQA), proficiency testing and/or interlaboratory comparison programs. A large provider of proficiency testing in the field of biobanking is the IBBL. In 2022, it offers 20 different

[32] Betsou *et al*. Assays for qualification and quality stratification of clinical biospecimens used in research: A technical report from the ISBER Biospecimen Science Working Group. Biopreserv Biobank, 2016;14:398-409. DOI: 10.1089/bio.2016.0018
[33] Information from https://www.findmyassay.com/ - Query: Fluid > Derivative > Fluid derivative from blood > Serum (last accessed: 2022-11-04)
[34] Information from https://www.findmyassay.com/ - Query: Fluid > Primary > CSF (last accessed: 2022-11-04)
[35] Information from https://www.findmyassay.com/ - Query: Fluid > Derivative > Fluid derivative from blood > EDTA plasma (last accessed: 2022-11-04)
[36] Schwarz *et al*. Quality Assessment of the Preanalytical Workflow in Liquid Biobanking: Taurine as a Serum-Specific Quality Indicator for Preanalytical Process Variations. Biopreserv Biobank, 2019;17:458-467. DOI: 10.1089/bio.2019.0004
[37] Information from https://www.findmyassay.com/ - Query: Fluid > Primary > Urine (last accessed: 2022-11-04)
[38] ISO 20186-1:2019 Molecular in vitro diagnostic examinations — Specifications for pre-examination processes for venous whole blood — Part 1: Isolated cellular RNA
[39] ISO 20186-2:2019 Molecular in vitro diagnostic examinations — Specifications for pre-examination processes for venous whole blood — Part 2: Isolated genomic DNA
[40] ISO 4307:2021 Molecular in vitro diagnostic examinations — Specifications for pre-examination processes for saliva — Isolated human DNA

processing and testing schemes for several biospecimen types[41]. It is also possible that the biobank develops its own approaches based on certified/regularly in EQA programs tested reference/control materials or previously examined or shared samples. The results are to be reviewed and corrective actions taken if necessary to ensure compliance with the pre-defined QC criteria.

8.2. Method validation and verification

To ensure high quality, comparable and fit for purpose biospecimens and/or derivatives and associated data, only validated and/or verified methods shall be used for critical process steps in biobanking (e.g., biospecimen processing).

8.2.1. Method validation

According to the ISO Standard 9000:2015[42], a validation is a "confirmation, through the provision of objective evidence, that the requirements for a specific intended use or application have been fulfilled. Note 1 to entry: The objective evidence needed for a validation is the result of a test or other form of determination such as performing alternative calculations or reviewing documents.". A validation must always be carried out if a new processing method is to be introduced that is not an unmodified standard processing method. In this case, a standard processing method is understood to be, according to the ISO 21899:2020[43] standard, a "method officially accepted and recognized described in unambiguous details and validated for a stated purpose. Note 1 to entry: Standard processing methods include e.g., methods published by a standardization body or approved by regulatory authorities or published in peer-reviewed scientific literature, dedicated, at least partly, to the validation (3.25) of the method.".
For method validation trained and competent personnel is crucial (see chapter 2.3) to perform the validation according to a documented and approved validation plan including the specific validation conditions and acceptance criteria for the respective method. The following are described in more detail as possible targets for processing method validation in the corresponding ISO 21899 standard chapter 8.5: fitness for purpose, reproducibility, robustness, homogeneity, and stability. The validation results shall be compiled in a validation report which shall be reviewed and approved by the quality manager of the biobank. Depending on the result, the method can then either be designated as validated or adjustments must be made to the current version of the SOP to subsequently restart the validation. Alternatively, the current version of the SOP can be retained and only the scope of application restricted. It shall be defined by the biobank in which cases a new validation of an already validated method has to be performed (e.g., changes in critical reagents and/or equipment, type of processed biospecimen).

8.2.2. Method verification

If unmodified standard processing methods shall be introduced into routine use a verification is necessary in advance. According to the ISO Standard 9000:2015, a verification is a "confirmation, through the provision of objective evidence, that specified requirements have been fulfilled. Note 1 to entry: The objective evidence needed for a verification can be the result of an inspection or of other forms of determination such as performing alternative calculations or reviewing documents.". Such specified requirements can, for example, be based on manufacturer specifications regarding yield, concentration, or purity of the method output.
Analogous to validation verification shall be performed by trained and competent personnel according to a documented and approved verification plan including the method scope and requirements, the

[41] According to the IBBL website: https://biospecimenpt.ibbl.lu/ (last accessed: 2022-10-13)
[42] ISO 9000:2015 Quality management systems — Fundamentals and vocabulary
[43] ISO 21899:2020 Biotechnology — Biobanking — General requirements for the validation and verification of processing methods for biological material in biobanks

externally provided performance characteristics and how they shall be verified as well as critical parameters. Depending on the verification result, either the compliance of the method to the specified requirements can be stated or actions need to be taken to achieve compliance. Verification procedure and obtained results have to be documented. Detailed information on the topic of method verification is included in the ISO 21899 standard.

8.2.3. Monitoring of a processing method

As kind of a QC procedure (cf. chapter 8.1) the output of processing methods shall be internally assessed regarding specified parameters (e.g., qualitative or quantitative properties of biospecimens and/or derivatives like growth, yield, purity) over a period of time according to the ISO 21899 standard. For each processing method the chosen monitoring approach shall be documented in a monitoring plan including:

- Applied assay(s) for monitoring
- Biospecimen(s) or derivative(s) to be tested
- Responsible person for testing
- Timespan the processing method was used

Procedures for systematic or periodic monitoring are described in more detail in the ISO 21899 standard. Furthermore, it is recommended to participate in the applied processing methods corresponding EQA and/or interlaboratory comparison programs for complementary external monitoring.