DZHK
Deutsches Zentrum für
Herz-Kreislauf-Forschung

DZHK-SOP-B-04

Biosample processing

(Study centre without DZHK Clinical Study Unit)

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Note: Printouts are not subject to the update process!

Change note: Changes to wording
Editorial changes

	Scientific author	Scientific review	Approval WGCR spokesperson	Approval DZHK
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Date				
Signature	**This SOP is a translation from the original German SOP and valid without signatures.**			

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1 Introduction

1.1 List of abbreviations

Abbreviation	Full form
DZHK	German centre for cardiovascular research
EDTA	Ethylene-diamine-tetra-acetic acid
eCRF	Electronic case report form
SOP	Standard operating procedure

1.2 Objectives

This standard operating procedure describes the processing and storage of biosamples obtained within the framework of DZHK projects (usually a clinical study, a registry or a cohort) for the establishment of a biosample resource under standardized conditions.

Core elements are highlighted in grey in this SOP.

1.3 Background

In scientific studies, various approaches are used to examine human biosamples, such as the determination of (circulating) biomarkers, determination of standard laboratory values, OMICs procedures, DNA/RNA extraction or "biomonitoring", for which a high sample quality is the basis. In order to minimize pre-analytical factors, which have a significant influence on sample quality, the steps of collection, processing and storage must be standardized according to DZHK SOPs. This standardization not only ensures that as many of the above-mentioned techniques as possible can still be carried out after long-term storage of the biosamples, but also enables the comparability of the analytical data obtained from the biosamples processed and stored at the different study centres at a later stage.

1.4 Terms and definitions

Biosamples *are e.g. blood, urine, stool and tissue as well as the materials obtained after processing (e.g. plasma, serum, extracted DNA, RNA, stem cells).*

DZHK-Biobanking[1] for the DZHK Heart Bank

The DZHK Heart Bank combines valuable resources of biosamples, associated clinical data, image data and genomic data, which are collected within the framework of DZHK projects and other projects. The aim is to make a sustainable contribution to research and the improvement of health. The open resource is available for research projects worldwide. DZHK biobanking is carried out with a firmly defined ***DZHK-set[2]***. The DZHK Heart Bank contains all the DZHK sets gained.

[1] Former „DZHK-Basic-Biobanking“

[2] Former „DZHK-Basic-Set “

Study biobanking[3] to answer the research question

In addition to the DZHK set, a study-specific sample collection according to this SOP may be necessary. The composition of these ***study-sets[4]*** as well as the determination of the collection times is up to the responsible Principle Investigator of the respective DZHK project and will be communicated to the participating study centres and scheduled at the initiation event at the latest.

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In addition to documenting the clinical items, the clinical data management system is also used to record a defined biosample data set (via eCRF). Once the data entry is complete, the correctness, completeness and plausibility are confirmed by assigning a Review A and the biosample data of the eCRF is released for transfer to the central laboratory information and management system of the DZHK (DZHK-LIMS).

2 DZHK-set

A standardized DZHK-set is taken from each participant of a DZHK project **once at the baseline visit (before intervention)**, provided that the participant has consented. The composition of the DZHK-set is listed below in Table 1. According to the established clinical routines, either BD or Sarstedt tubes can be used.

Table 1 Type, volume and number of primary vessels

	Primary vessel	BD		Sarstedt	
		Volume	number	Volume	number
DZHK-set	serum	10,0 ml	1	7,5 ml	1
	EDTA	10,0 ml	1	7,5 ml	1
	citrate	2,7 ml	1	3,0 ml	1
	urine	11,0 ml	1	10,0 ml	1

3 Requirements for biosample collection and documentation

The following requirements are needed to carry out sample processing and storage for the DZHK-set and, if applicable, the study-set:

- Centrifuge (refrigerated or non-refrigerated)
- Calibrated pipettes
- Freezer -80°C with connection to monitoring system and fail-safe concept
- Completed biosample consignment bill for sample collection
- Collected biosamples in labelled primary sample containers (DZHK set/study set)
- Aliquot tubes labelled and placed on a 96-well rack (see Fig. 1)

[3] Former „Study specific biobanking“

[4] Former „Study-specific set “

Fig. 1: Example of lasered 500 µl aliquot tubes from LVL-Technologies.

		1	2	3	4	5	6	7	8	9	10	11	12
Serum (10 x 300µl)	**A**	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐		
	B												
EDTA-Plasma (10 x 300µl)	**C**	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐		
Buffy Coat* (2 x bis 300µl)	**D**	☐	☐										
	E												
Citrate (4 x 300µl)	**F**	☐	☐	☐	☐								
	G												
Urine (8 x 300µl)	**H**	☐	☐	☐	☐	☐	☐	☐	☐				

Fig. 2: Schematic representation of the positions of the aliquot tubes on the rack with sample type -pipetting scheme.

4 Sample processing and documentation

4.1 Specifications

Biosample processing and documentation must be carried out by trained staff in accordance with the locally applicable specifications in accordance with this SOP. Deviations from the following requirements are documented as a deviation on the biospecimen consignment bill and in SecuTrial® (see section 6.4).

The following must also be noted:

- Regular disinfection of work surfaces
- Compliance with current hygiene regulations

4.2 Flow Chart

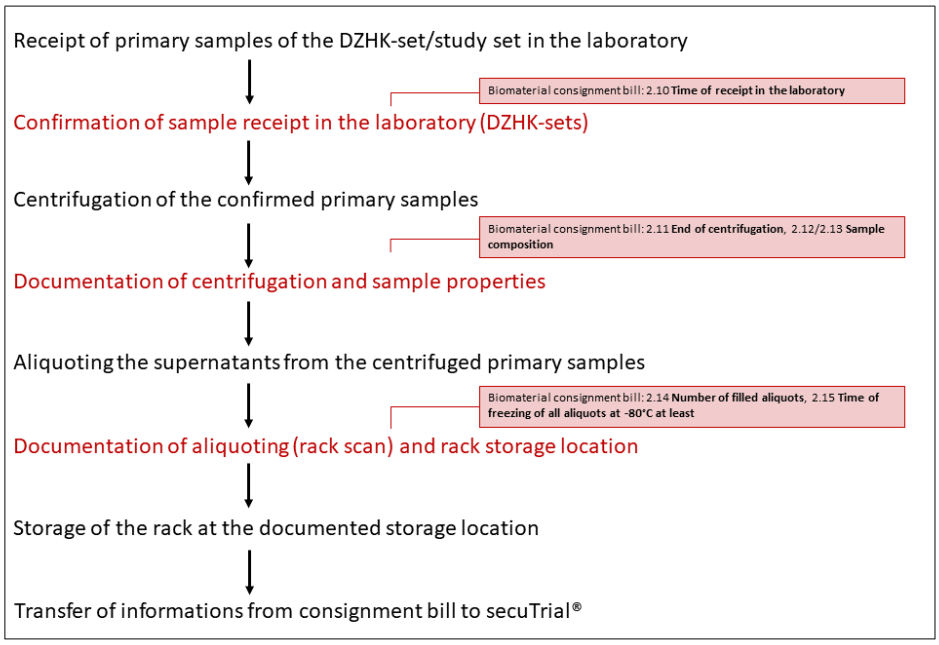


Fig.4: Overview of the processing of biosamples and documentation.

4.3 Sample processing procedure and documentation in detail

Confirm **receipt of the sample in the laboratory** promptly on the biosample consignment bill.

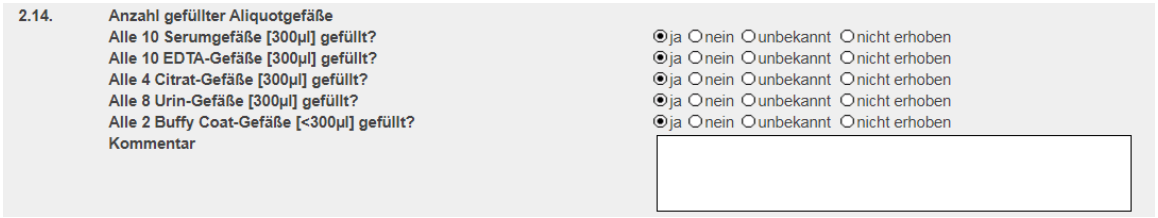
Centrifugation of all primary samples takes place after the sample receipt has been confirmed in the laboratory:

- One of the following two options must be used for centrifugation of the primary samples: 2,000 g for 10 minutes OR 3,000 g for 5 minutes.
- The centrifugations can be carried out in refrigerated centrifuges: temperature setting at 18°C OR non-refrigerated centrifuges at room temperature (RT): Allow the centrifuge to cool down after each run.
- The type of centrifugation, the time of centrifugation (end of centrifugation) and the condition of each centrifuged primary sample (blood: unremarkable, haemolytic, icteric, lipemic and urine: unremarkable, cloudy, bloody) are documented on the biosample consignment bill.

Aliquoting the supernatants after centrifugation:

- Aliquot 300 µl portions into appropriate aliquot tubes.
- The Buffy Coat is obtained from the primary EDTA plasma vessel. If not enough material is available, the aliquot tube can also be filled with less than 300 µl of buffy coat.

In exceptional cases, citrated blood can be used instead of the primary EDTA plasma vessel. In this case, additional documentation is mandatory on the biosample consignment bill in SecuTrial® in field 2.14 under "Comment". Enter the following under "Comment": **"Citrated blood used".**



- Seal the aliquot tubes.
- Remove empty aliquot tubes.
- Documentation of the aliquot and the time of storage on the biosample consignment bill.

4.4 Storage of biosamples

- Local documentation of the r**ack storage location**, **rack ID and LIMSPSN**.
- Store the rack at the documented storage location.
- **Do not compact any sample aliquots on the racks.** If compaction is necessary, this should only be carried out after consultation with the DZHK support team (biobanking@dzhk.de).

4.5 Biosample data in SecuTrial®

- Digitalization of the documented data from the biosample consignment bill **in the eCRF in SecuTrial®**.

4.6 Sending of biosamples (DZHK-set/Stuy-set)

If you do not have the required storage capacity (-80°C) at your study centre, you can send the **already processed biosamples, i.e. the aliquots**, to the **main study centre**. The shipment will only be processed after consultation with the main study centre. The main study centre is responsible for handling the shipping process, including the necessary documentation steps.

4.7 Processing times and storage - sample qualities

The quality levels are assigned on the basis of the documented times from extraction to storage and the storage conditions. If at least one of the following conditions is met (*documentation required, see section 6.8), the sample is classified in the respective quality level:

DZHK quality level 1

- The primary vessel was not processed within 60 min after sample collection. *
- The sample aliquot was temporarily stored for 6h or longer at -20°C.*
- The total duration of biomaterial collection, processing and storage at -80°C was over 240min.

DZHK quality level 2

- The sample aliquot was temporarily stored at -20°C for a maximum of 6 hours. *
- The total duration of biomaterial extraction, processing and storage at -80°C was max. 240 min.

DZHK quality level 3

- The total duration of biomaterial extraction, processing and storage at -80°C was max. 120min.

4.8 In case of deviations

Any deviations from those described in sections 4.1-4.6 must be documented in SecuTrial® under **"3. Special features"** on the biosample consignment sheet.

Fig.5: Examples of SOP deviations and their documentation under "3. Special features".

5 Literature

Guideline of the German Medical Association for the quality assurance of laboratory medical examinations (in German)**.** Deutsches Ärzteblatt | DOI: 10.3238 / arztebl.2019.rili_baek_QS_Labor20192312

6 References to other DZHK-SOPs

The latest available version is valid.

SOP-ID	**Title**
DZHK-SOP-P-06	Capture-IDAT-Informed Consent
DZHK-SOP-B-01	Biosample collection (Study centres without DZHK clinical study units)
DZHK-SOP-B-05	Sending of biosamples

7 Changes

Changes compared to the last version.

Section	Description of the change compared to the previous version.
Whole document	Wording former biomaterial becomes biosamples former DZHK basic biobanking becomes DZHK-biobanking former DZHK Basic set becomes DZHK-set former study specific biobanking becomes study biobanking former study specific sample set becomes study-set
Whole document	Editorial changes

8 Participating persons

Name	**Role**	**Contribution**
Prof. Dr. Tanja Zeller	First author	Draft
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