DZHK-SOP-B-02

Biosample processing

(DZHK Clinical Study Units)

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Note: Printouts are not subject to the update process!

Change note: Changes to wording
Editorial changes

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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
SOP	Standard operating procedure
DZHK	German centre for cardiovascular research
EDTA	Ethylene-diamine-tetra-acetic acid

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.

Study biobanking[3] to answer the research question

[1] Former „DZHK-Basic-Biobanking“
[2] Former „DZHK-Basic-Set “
[3] Former „Study specific biobanking“

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.

DZHK-LIMS

The "CentraXX" software is used as the central DZHK LIMS. It is used for process control and documentation of sample collection, processing, storage and retrieval. A defined biosample data set is stored for each biosample. This is then used to automatically determine the quality level of the individual biosamples for subsequent use in further research projects.

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:

- Access to the DZHK-LIMS with user role MTLA
- Completed documentation of sample collection (workflow 1 in the DZHK-LIMS)
- Hand scanner for scanning the identification codes (sample IDs)
- Centrifuge (refrigerated or non-refrigerated)
- Calibrated pipettes
- Freezer -80°C with connection to monitoring system and fail-safe concept
- Filled primary vessels from DZHK set and/or study set, if applicable
- Aliquot tubes on 96-well rack, which are shown in the following illustrations for the DZHK set. When ordering the aliquot tubes, it is essential to note the **working volume** specified by the manufacturer, which must be at least 300 µl, as aliquoting is always carried out with a fixed

[4] Former „Study-specific set “

pipetting volume of 300 µl and the liquid also requires sufficient space to expand during freezing. Support for ordering can be found in the slide set "Information for DZHK clinical study units" (in German).

Fig. 1: Example aliquot tubes from Azenta (formerly Brooks or FluidX).

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

Abb. 2: Schematic representation of the standard positions of the aliquot tubes on the rack with sample types and pipetting volumes (*The buffy coat is intended for later DNA extraction. Removal of the buffy coat is not necessary if the corresponding primary vessel is frozen after removal of the plasma supernatant, see also section 6.3)

4 Sample processing and documentation in the DZHK-LIMS

4.1 Specifications

Biosample processing must be carried out by trained personnel in accordance with this SOP. The following requirements must also be recognized:

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

4.2 Flow-Chart

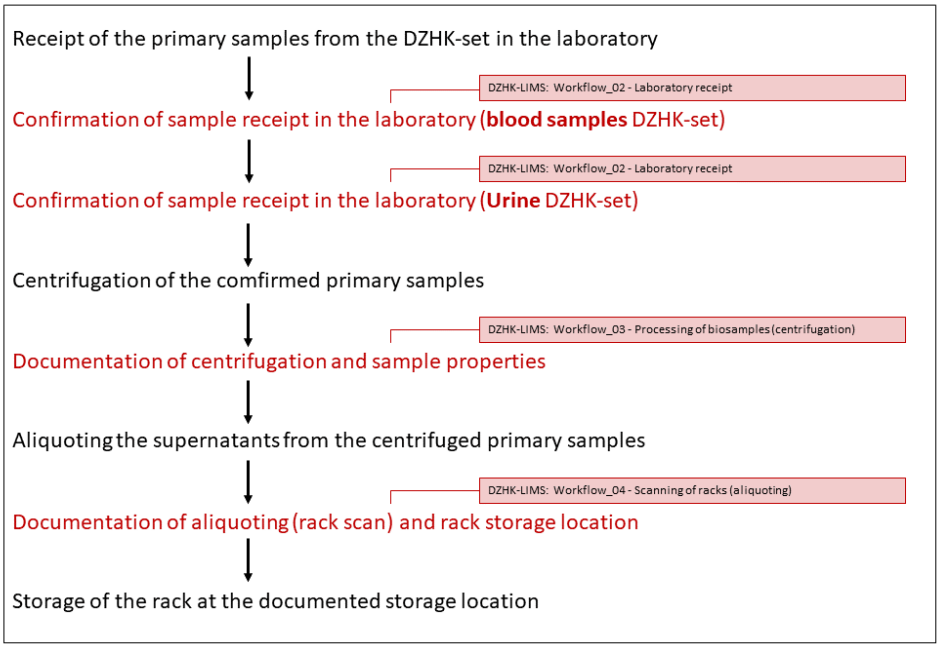

Fig.4: Processing of biosamples at a glance.

4.3 Sample processing procedure and documentation in detail

After **sample receipt in the laboratory**, confirm the laboratory receipt in the DZHK-LIMS:

- To do this, scan the identification codes of the primary vessels with the hand scanner (NO manual ID entry).
- Matching the number of primary vessels: If more primary samples of the set are listed than are physically present, these primary samples must be deleted from the DZHK-LIMS. This step should already have been carried out by the study nurse.

After the sample receipt has been confirmed in the laboratory, **all primary tubes of the DZHK set are centrifuged**:

- The primary vessels must be processed within 60 minutes of sample collection.
- 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 in the DZHK-LIMS.

Aliquoting the supernatants after centrifugation:

- Aliquoting of 300 µl samples into appropriate aliquot tubes.
- The buffy coat is obtained from the EDTA plasma primary 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, after pipetting the supernatant (plasma aliquot), it is possible to freeze the primary vessel with the remaining volume and use the material for subsequent DNA isolation. This procedure requires documentation in the DZHK-LIMS that deviates from the standard process, which can be found in the guideline LF-B-02 (in German). In exceptional cases, citrated blood can also be used instead of the EDTA plasma primary tube. This must be noted in the corresponding documentation of the aliquots in the DZHK-LIMS.

- Seal the aliquot tubes.
- If necessary, remove empty aliquot tubes from the rack.

Scan the rack with the filled aliquots using the rack scanner and document in the DZHK-LIMS:

- Scan the primary vessel and assign it to the aliquot tube on the rack.
- Document the storage location and time of storage.

Local **storage of the sample aliquots** at -80°C at the documented storage location.

4.4 Processing times and storage - sample qualities

Quality levels are assigned on the basis of the documented times from extraction to storage and the storage conditions.

DZHK Quality level 1

- The primary vessel was not processed within 60 min of sample collection.
- The sample aliquot was temporarily stored at -20°C for 6h or longer.
- The total duration of biomaterial collection, processing and storage at -80°C was longer than 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. 120 min.

Note: The aliquots of the DZHK-sets can be summarized on a rack following the recording of 2-3 patients. The guideline LF-B-02 (in German) can be used for this purpose. If the samples are not stored in automated storage facilities, only DZHK-sets are to be compacted on a rack and **NOT mixed with study sets**.

Attention: The freezer opening times must always be kept to a minimum. Condensation on the tubes and, in particular, large accumulations of water lead to considerable restrictions in the retrieval processes of individual tubes.

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 existing DZHK-SOPs

The latest available version is valid.

SOP-ID	Title
DZHK-SOP-B-01	Collecting biosamples (DZHK Clinical Study Units)

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

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