UMWELTPROBENBANK
DES BUNDES

Guideline for Sampling and Sample Processing

Whole Blood and Blood Plasma

Dominik Lermen, Martina Bartel-Steinbach, Nicole Jost

Fraunhofer Institute for Biomedical Engineering IBMT
Josef-von-Fraunhofer. Weg 1, 66280 Sulzbach, Germany

Umwelt Bundesamt

Contents

1 German Environmental Specimen Bank 2
2 Objective of this Guideline 2
3 Function of the Sample 2
4 Group of Participants 2
5 Sampling Period and Frequency 2
6 Devices, Reagents and Materials 3
7 Preparations for Sampling 3
7.1 Infrastructure 3
7.2 Contamination Risks 4
7.3 Cleaning of Sample Containers 4
7.4 Labeling of Sample Containers 4
8 Conduct of Sampling 4
8.1 Taking Blood 4
8.2 Collecting Blood Plasma 5
9 Sample Aliquoting 5
10 Cryopreservation of Archive Samples 5
11 Removal of Samples for Retrospective Analyses 6
12 Literature 6

Appendix A: Excerpt from the Sampling Protocol – Whole Blood
Appendix B: Excerpt from the Sampling Protocol – Blood Plasma

Guidelines for Sampling, Transport, Storage and Chemical Characterization of Environmental and Human Samples

Status: September 2015, V 3.0

1 German Environmental Specimen Bank

The German Environmental Specimen Bank (ESB) is an instrument for environmental monitoring of the Federal Ministry for the Environment, Nature Conservation, Building and Nuclear Safety (BMUB) subject to specialist and administrative coordination by the Federal Environment Agency (UBA). The ESB collects ecologically representative environmental and human samples, stores them and investigates them for environmentally-relevant substances (BMUB 2008).

The long-term storage is carried out under conditions which, as far as possible, exclude a change in state or a loss of chemical characteristics over a period of several decades. The archive therefore provides samples for retrospective investigation of substances for which the potential risk for the environment or human health is not yet known.

Comprehensive information on the ESB is available at www.umweltprobenbank.de.

2 Objective of this Guideline

This guideline defines all necessary work steps for standardized collection of whole blood and plasma samples from young adults. It describes precautions and measures in order to reduce external contamination of the samples to a minimum and to ensure the chemical information content of each individual sample even during storage for an indefinite period. In 2014, a quality management system according to DIN EN ISO/IEC 17025 (Lermen et al. 2014) was established for the division of the ESB operated by the Fraunhofer Institute for Biomedical Engineering (IBMT), which includes the collection, storage and initial characterization of human samples. This guideline represents a non-controlled excerpt of this QM system in relation to the sampling and sample processing for whole blood and blood plasma samples. In this form, it is not an integrated component of the QM system.

3 Function of the Sample

In human biomonitoring and environmental medicine, whole blood and blood plasma are basic, well-examined matrices. In whole blood and blood plasma samples it is possible to analyze a number of hydrophilic and lipophilic environmental pollutants and their metabolites. Depending on the substance and its distribution between blood plasma and the cellular components of the whole blood, a decision must be made about which matrix is more suitable for the analysis. Whole blood samples can easily be collected under routine conditions that are reasonable for the subjects.

4 Group of Participants

According to the conceptual design of the ESB, every year 120–150 young adults aged 20 to 29 with as even a gender distribution as possible are tested at each of the four sites (Münster, Halle/Saale, Greifswald, Ulm). The selection of these groups is intended to ensure that samples are collected from individuals not specifically exposed to pollutants and therefore is intended to represent the average background exposure of young adults in Germany (BMUB 2008).

5 Sampling Period and Frequency

In order to ensure comparability of the subject groups at the individual sampling sites, the individual samplings are carried out at defined times every year (Tab. 1).

Tab. 1: Overview of the specified sampling periods

Sampling site	Sampling period
Münster	January/February
Halle/Saale	February/March
Greifswald	March/April
Ulm	April/May

6 Devices, Reagents and Materials

For the cleaning of the sample containers

- Dispensettes (5–50 ml/2.5–25 ml)
- Multipette plus with Combitips 10 ml
- Variable pipettes (1000 µl/200 µl) with corresponding pipette tips
- DURAN® glass bottles (5 l, 3.5 l, 2 l)
- Laboratory balances with a range of 0 to 6000 g and 0 to 16000 g with printer
- Safety cabinet (class 2)
- Fume cupboard with solvent cabinet
- Acid and alkali cabinet
- Ultrapure water (> 18.2 MΩ*cm)
- Methanol CH3OH 99.8% p.a.
- Nitric acid HNO3 65% p.a., ISO diluted
- Heparin sodium 25,000 I.U./5 ml
- 8 ml, 13.5 ml and 30 ml PP reagent and centrifuge tubes (sample containers)
- Stands for sample containers

For the sampling

- Surface disinfectant
- Hand disinfectant
- Pressure cuff
- Winged needle infusion set (Sarstedt, Safety-Multifly-Set 20G, order no.: 851.637.235)
- 20 ml disposable syringe (BD, Discardit II, order no.: 300.296)
- Sterile alcohol disposable swabs
- Adhesive bandage
- Cannula waste box
- Cellulose swab
- Leukosilk
- Stands for 30 ml sample containers
- Pre-cleaned 30 ml and 13.5 ml sample containers
- Centrifuge
- Safety cabinet (class 2)
- 5 ml pipettes
- Stands for 8 ml and 30 ml sample containers
- Prepared 8 ml sample containers for blood plasma
- 60 l rectangular WIWA container, BAM (Federal Institute for Materials Research and Testing) tested and without biohazard labeling AVV (Waste Classification Ordinance) code sticker
- Disposable gloves
- Absorbent disposable cloths

For freezing the samples

- Mobile cryo transport container
- 300 l liquid nitrogen storage tank
- Liquid nitrogen
- Storage system for cryotransport container
- Oxygen deficiency warning system
- Power supply
- Cryo gloves
- Protective visor
- Cryo apron

7 Preparations for Sampling

In order to carry out sampling on humans, approval from an ethics committee must generally be obtained in advance.
The subjects must be informed in writing of the scope, objectives and purpose of the sampling. In addition, it must be ensured that there is consent from each subject and therefore transfer of the ownership rights to the sample from the subject to the UBA by signing a declaration of consent.

7.1 Infrastructure

In preparation for the sampling it must be ensured that there is laboratory infrastructure which meets the requirements of the Labor Protection Act (ArbSchG) and the Biological Agents Regulation (BioStV) in relation to the work to be carried out. Appropriate hygiene measures must be established and stipulated in a hygiene plan. In addition, a user manual must be produced for the laboratory where the work is carried out, which summarizes the potential hazards and will be used by the head of the laboratory to instruct the staff about the potential risks and safe handling of biological materials on an annual basis.

Biological materials like whole blood and blood plasma are generally deemed to be potentially infectious and therefore may only be processed by qualified personnel in laboratories authorized for biological safety level 2. Employees are trained for this work. Briefings, instruction, training and further education must be documented and archived.

7.2 Contamination Risks

In order to ensure comparable samples for sensitive residue analysis investigations in human biomonitoring, it is of fundamental importance that human samples are collected and prepared properly in the pre-analytical phase. In this case, the priority is to avoid potential contaminations. In addition to contaminations, which may occur during sampling, e.g. due to sample containers being left open, production-related contaminations of the sample containers due to the production process are also of particular significance. In relation to the first aspect, it is important to provide the employees with workplace-specific instruction before sampling and in particular to point out the contamination risks.
The cleaning described below is intended to avoid contaminations of the sample container due to the production process.

7.3 Cleaning of Sample Containers

In order to avoid contaminations of the sample containers used resulting from the production process, standardized cleaning of the sample containers must be carried out. For this purpose, all sample containers (8, 13.5 and 30 ml) must be rinsed with methanol to remove organic contaminants, 2% nitric acid to remove inorganic contaminants, and ultrapure water (> 18.2 MΩ*cm). The sample containers must be half-filled with methanol and then completely filled with nitric acid and ultrapure water. After each filling, the containers must be shaken for a minute. The methanol and ultrapure water must be disposed of after shaking. The nitric acid is left in the containers overnight at room temperature to remove inorganic contaminants and is only disposed of on the following day. After each rinsing process, the sample containers must be left to dry in appropriate tube stands in a safety cabinet (class 2). The sample containers must then be sealed with a lid in the safety cabinet.
Before sealing of the sample containers intended for whole blood collection, the anticoagulant heparin sodium (25,000 I.U./5 ml) must be added (200 µl in 30 ml sample container and 100 µl in 13.5 ml sample container). This must be carried out in a safety cabinet (class 2).

7.4 Labeling of Sample Containers

After successful cleaning, each individual sample container must be equipped with a label with an appropriate bar code. In addition, each sample container must be labeled with the information of the bar code using a waterproof marker. In order to avoid mixing the samples containers up during sampling they have to be sorted according to the subject ID.

8 Conduct of Sampling

8.1 Taking Blood

Before taking blood, it must be ensured that there is a declaration of consent from the subject. Blood is taken under constant medical supervision by two teams in parallel. These are made up of a medical doctor or an assistant (medical-technical laboratory assistant, nurse, doctor's assistant) for taking the blood and a trained specialist who transfers the blood from the syringes to the sample containers provided.

Before starting taking blood, the work stations must be prepared so that there are a sufficient number of disposable gloves, alcohol swabs, winged needle infusion sets, 20 ml syringes, adhesive bandages, cotton wool pads, Leukosilk and sample containers for all intended subjects on a sampling day.

The following is required for each subject:
- 1 x alcohol disposable swabs
- 1–2 x winged needle infusion set
- 7 x 20 ml disposable syringes
- 1 x plaster or alternatively cellulose swab and Leukosilk
- 1 x disposable gloves
- 1 x pre-cleaned and labeled 13.5 ml sample container for the metal analysis
- 7 x 30 ml pre-cleaned and labeled sample containers for whole blood
- 10 x 8 ml pre-cleaned and labeled sample containers for blood plasma

Before starting to take blood, the sample containers labeled with the corresponding number for the

relevant subject must be pre-sorted into an appropriate sample container stand and placed in the safety cabinet.
The blood must be taken by pulling the syringe plunger in a controlled manner. In this case the following order of filling must be observed:

- 3 x 30 ml sample containers with labeling "Centr"
- 1 x 13.5 ml sample container with labeling "Analysis"
- 4 x 30 ml sample containers with the labeling "1-4, 2-4, 3-4 and 4-4".

Once a syringe is full, the collection of blood must be stopped and the syringe must be handed directly to the second person so that the blood can be transferred into the 30 ml sample container. The empty syringe must be disposed of immediately in the waste container provided. After transferring the blood into the sample container, the container must be quickly and carefully sealed with the appropriate lid and gently and carefully rotated to ensure even mixing of the blood with the previously provided sodium heparin.
Discrepancies during blood collection, e.g. a reduced filling volume of the tubes, must be documented on the sampling protocol – full blood form (see Appendix).

8.2 Collecting Blood Plasma

For each subject, 3 x 30 ml sample containers filled with 20 ml whole blood are centrifuged in the centrifuge for 10 minutes at 3500 revolutions per minute and 4°C. When doing so, it must be ensured that the lids of the sample containers are screwed on correctly and that they are not cracked. It must also be ensured that the sample containers are not contaminated with blood residues on the outside. If necessary, these must be carefully removed using a cloth soaked in disinfectant, and when doing so it must be ensured that the labeling remains in place. The blood plasma is carefully removed with a 5 ml pipette in the safety cabinet.

The sample containers are sorted according to subject and, in order to avoid confusion, assigned to the relevant prepared 10 x 8 ml sample containers for blood plasma samples and 1 x sample tube of the Cobas c111 (the labeling/labels must also be checked!).
Approx. 2.5 to 3 ml blood plasma is pipetted into each 8 ml sample container.
Discrepancies that may occur during pipetting, e.g. too little blood plasma or different characterization of the blood plasma (lipaemic, haemolytic), must be documented in the sampling protocol .– blood plasma.
The 30 ml sample containers with the remaining blood cells (sediment) are disposed of in the proper manner.

9 Sample Aliquoting

Whole blood
After whole blood samples are received in the laboratory, they must be checked for completeness (7 x 30 ml, 1 x 13.5 ml) and sorted according to subject numbers (subject ID). Aliquots 1 to 4 and the 13.5 ml whole blood container with the additional information "Analysis" must be checked for correct labeling and frozen immediately.

Blood plasma
The 8 ml sample containers for blood plasma (7 items) with the aliquot information (e.g. 1-7, 2-7, 3-7, 4-7, 5-7, 6-7, 7-7) and the 8 ml sample container for blood plasma with the additional information "Analysis" are checked for correct labeling and frozen.

10 Cryopreservation of Archive Samples

Immediately after sampling, the whole blood and blood plasma samples are frozen in a mobile cryo transport container cooled with liquid nitrogen. The individual aliquots are systematically and successively sorted into the pre-chilled storage racks provided for the individual volumes in the cryo transport container. Once the storage racks have been inserted, the cryo transport container is sealed. Protective clothing must be worn when freezing the samples.

Caution: In order to avoid damage to the sample containers due to large differences in temperature, the storage racks must be placed in the tank

so that the samples are far away from the inlet hose for liquid nitrogen, and under no circumstances must they come into contact with this tube or released LIN.
The samples remain in this cryo transport container until they are transferred into the cryo storage container at the ESB cryo storage facility in Münster/Wolbeck. Nitrogen is supplied automatically during transport. In order to monitor the temperature of the samples, there is a thermograph integrated into the tank with a recording interval of 15 minutes.

Before storage of the samples, lists are generated with defined locations on the basis of the actual number of whole blood and blood plasma samples collected and on the basis of the storage structure available in the storage database. These lists are used to sort the samples into the storage racks consecutively to the previously stored samples. During transfer, it must be ensured that the temperature of the frozen samples remains stable and that the cold chain is not interrupted. Protective clothing must be worn when storing the samples.

11 Removal of Samples for Retrospective Analyses

Consent from the UBA is required in order to remove archived samples of the ESB.

12 Literature

BMUB (German Ministry for the Environment, Nature Conservation, Building and Nuclear Safety, Ed.) (2008): German Environmental Specimen Bank – conceptual design (Status: October 2008); www.umweltprobenbank.de

Lermen D, Schmitt D, Bartel-Steinbach M, Schröter-Kermani C, Kolossa-Gehring M, von Briesen H, Zimmermann H (2014). A New Approach to Standardize Multicenter Studies: Mobile Lab Technology for the German Environmental Specimen Bank. PloS one, 9(8), e105401.

Appendix A: Excerpt from the Sampling Protocol – Whole Blood

UMWELTPROBENBANK DES BUNDES

Fraunhofer IBMT

Sampling protocol – whole blood: RTM xxx / Location / dd.- dd. Month 201x

Edited by:_____ Edited on:_____
(Name) (Date)

Subject ID	Edited (✓)	Comment
001		
002		
003		
004		
005		
006		
007		

Miscellaneous:_____
_____
_____

Appendix B: Excerpt from the Sampling Protocol – Blood Plasma

UMWELTPROBENBANK DES BUNDES

Fraunhofer IBMT

Sampling protocol – blood plasma: RTM xxx / Location / dd.- dd. Month 201x

Edited by:_____ (Name) Edited on:_____ (Date)

Subject ID	Edited (✓)	Comment
001		
002		
003		
004		
005		
006		
007		

Miscellaneous:_____

_____

_____