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Guideline for Sampling and Sample Processing

24h-Sampling Urine

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

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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 sampling of 24h-sampling urine samples. 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 comprehensive 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. The following guideline represents a non-controlled excerpt of this QM system in relation to the sampling and sample processing for 24h-sampling urine. In this form, it is not an integrated component of this QM system.

3 Function of the Sample

As the excretion of the kidneys, urine provides a lot of information about metabolism and state of health. A person's urine collected over 24 hours also provides important information about the daily resorbed inorganic and organic xenobiotics and allows for the calculation of total excretion. 24h-sampling urine is easily accessible as a biological material for environmental medical matters and can be reasonably collected under routine conditions 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 student 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 for solutions (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 CH_3OH 99.8% p.a.
- Nitric acid HNO_3 65% p.a., ISO diluted
- Nitric acid, diluted (2%) from 65% p.a. with ultrapure water (> 18.2 MΩ*cm)
- 3 l PE urine collection bottle
- 13.5 ml and 30 ml PP reagent and centrifuge tubes (sample containers)
- Stands for sample containers

For the sampling
- Surface disinfectant
- Hand disinfectant
- Stands for 30 ml and 13.5 ml sample containers
- Pre-cleaned 30 ml and 13.5 ml sample containers
- 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 recording clinical physical parameters
- Balance with a weighing range of 0 to 6200 g
- Empty 3 l PE urine collection bottle for taring the balance
- Hydrometer with a measuring range of 1.000 to 1.060 g/ml
- Conductivity meter, measurement in mS/cm
- Conductivity standard buffer 12.88 mS/cm
- 1000 µl pipettes 250 ml PE measuring cylinder
- Stands for 30 ml sample containers

For freezing the samples
- Mobile cryo transport container
- 300 l liquid nitrogen storage tank
- Liquid nitrogen
- Storage system for cryo transport 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.

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 em-

ployees 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 sample 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 stands in a safety cabinet (class 2). The sample containers must then be sealed with a lid in the safety cabinet.

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

The sampling of the 24h-sampling urine is carried out by the subjects themselves. Before the sampling, when they are given the declaration of consent each subject is also sent a general information letter about the sampling from the ESB, a detailed description of the conduct of the 24h-sampling urine and a 3 l collection vessel.

Before the sampling, the subjects receive the following regulation for the collection of the urine:

Over a period of 24 hours, all urine is collected in the 3 l collection container provided. This must only include one instance of morning urine, i.e. if collection is started with the first morning urine then it must be completed with the last urine at night.
In order to avoid contaminations from your clothing, please only open the collection container shortly before passing urine (i.e. when you have already opened/removed your clothing accordingly). Please ensure that you put the lid of the container at some distance with the opening upwards.

8.1 Determination of Clinical Physical Parameters

Clinical physical parameters, such as volume, density and conductivity are required to characterize the degree of dilution of the urine, in which case density and conductivity are inversely proportional to the 24 h total volume, i.e. the greater the volume, the lower the density and conductivity. The total volume of the 24h-sampling urine is also required in order to determine the daily exposure to external substances with a very low dwell time in the organism (low half-life) via the renal excretion.

Caution: After each measurement, the measuring cylinder must be rinsed with the urine of the next subject.

Measuring Weight
Before the start of measuring, the balance must be calibrated using standard weights for internal quality assurance and then tared with an empty 24h-sampling urine container. The test must be documented. Once the subject's urine sample is received in the laboratory, it must be weighed and the weight in grams to one decimal place must be transferred to the sampling protocol – urine form (see Appendix). Any discrepancies (e.g. only 20h urine collection, several collection containers etc.) must be recorded in the "Comments" column.

Then the urine collection container must be rotated in large circular movements in order to ensure even mixing.

Measuring Density

In order to measure the density, approx. 230 ml urine must be poured into a 250 ml PP measuring cylinder and then the areometer must be submerged. It must be ensured that the areometer floats and does not touch the base of the measuring cylinder. The density (g/ml) must be read from the areometer and entered in the sampling protocol – urine form (see Appendix) to three decimal places. Any discrepancies must be recorded in the "Comments" column. After reading the result, the urine must be discarded into a provided collection container (waste). For internal quality assurance, in each measurement series the first three urine samples that are received are measured with two calibrated areometer.

Measuring Conductivity

The conductivity meter must be calibrated once per working day before starting work using the relevant standard, and the calibration must be documented. For internal quality assurance for the recorded measured values, a quality control measurement must also be carried out with another standard solution before and after each measurement series. The results of these quality control measurements must also be documented. In order to measure conductivity, an approx. 100 ml container (standard cylinder or beaker) must be filled to 3/4 with the urine sample, then the conductivity meter probe must be submerged and the measurement started. The result (mS/cm) must be recorded on the sampling protocol – urine form (see Appendix) to two decimal places. Any discrepancies must be noted in the "Comments" column.

9 Sample Aliquoting

Once the initial parameters have been measured, the collection container must again be rotated in large circular movements and the measuring cylinder must be filled with the corresponding urine sample. Then the measuring cylinder must be used to fill the prepared sample containers (9 x 13.5 ml and 3 x 30 ml tubes), each to the 3/4-level. The filled sample containers must be collected on appropriate stands (e.g. cryo tank storage system). The sample containers are sorted according to size. The tubes for elemental analysis must be placed on a separate stand.

10 Cryopreservation of Archive Samples

The urine samples must be frozen in a mobile cryo transport container cooled with liquid nitrogen. For this purpose, immediately after aliquoting, the individual urine aliquots must be systematically and successively sorted into the storage racks for the individual volumes at room temperature. If a storage rack is full of urine samples, then it must be immediately placed in the designated space in the cryo transport container. Once the storage racks have been inserted, the cryo transport container must be closed. 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 urine samples must be placed in the tank so that they 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 24h-sampling urine 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

DIN EN ISO/IEC 17025:2005 (2005): General requirements for the competence of testing and calibration laboratories

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: Excerpt from the sampling protocol – urine

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Fraunhofer IBMT

Sampling protocol - urin: RTM xxx / location / dd. – dd. month 201x

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

Subject ID	Weight (g)	Density (g/ml)	Conductivity (mS/cm)	Comment
001				
002				
003				
004				
005				
006				
007				

Miscellaneous:_____

_____

_____