UMWELTPROBENBANK
DES BUNDES

Guideline for Sampling and Sample Processing

24-h Urine Collection

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

Status: December 2021, V 4.0

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, and Nuclear Safety (BMU) and is coordinated by the German Environment Agency (Umweltbundesamt, UBA). The ESB collects ecologically representative environmental and human samples, stores them and investigates them for environmentally relevant substances.

Specific operating procedures as well as the conception of the ESB are the basis of the program (Umweltbundesamt 2008, 2014).

The long-term storage is carried out under conditions that, as much 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 standardised sampling of 24-h urine samples and is an update of the Lermen et al. (2015) version. It describes precautions and measures in order to reduce external contamination of the samples to a minimum and to ensure sample integrity pertaining to its chemical information 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. This guideline represents an excerpt of this QM system pertaining to the sampling and sample processing of 24-h urine.

3 Function of the Specimen Type

As a product excreted by the kidneys, urine provides a lot of information about metabolism and health. In human biomonitoring (HBM), the use of 24-h urine is the gold standard for determining the total daily exposure. Compared to spot or morning urine samples, 24-h urine can provide a more complete picture of the daily pollutant excretion and, as a biological material for environmental medical issues, can be obtained non-invasively and reasonably for those affected (Scher et al., 2007, Triebig et al., 2012). Incorrect 24-h urine collection mostly results from too short or too long collection time (Barr et al., 2005; Suwazono et al., 2005; Ye et al., 2011). Therefore, standardised sampling, standardised pre-analytical processes and standardised cryopreservation are of decisive importance for the integrity of the samples and the securing of the complete information content. The provision of standardised and high-quality samples is a prerequisite for the comparability of analysis results and thus forms the basis for a correct risk assessment. For comparability with other studies, in addition to the total urine volume, urine creatinine, density and conductivity are routinely recorded within the scope of the ESB, which can be used to normalize the measured analyte concentrations. However, since these parameters can vary over time and between individuals, it is advisable to normalize the measured analyte concentration using the total urine volume, especially when investigating trends in the daily total exposure (Lermen et al. 2019).

4 Group of Participants

According to the conceptual design of the ESB, every year 120-150 young adults aged 20 to 29 in a balanced sex ratio are recruited at each of the four sites (Muenster, Halle/Saale, Greifswald, Ulm). The focus only on this group is intended to ensure that samples are taken from participants who are not specifically exposed to pollutants in order to reflect the average background exposure of young adults in Germany (UBA 2008).

5 Sampling Period and Frequency

In order to ensure comparability of participant groups at the individual sampling sites, the individual samplings are carried out at defined time slots 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, Materials and Reagents

For cleaning of sample containers

- personal protective clothing (lab coat, safety glasses, disposable gloves, acid-resistant gloves)
- safety cabinet (class 2)
- acid and alkali cabinet
- laboratory fume cupboard with solvent cabinet
- laboratory balance with a measuring range from 0 to 6000 g
- dispensettes (5-50 mL/2.5-25 mL)
- multipette plus with combitips 10 mL
- variable pipettes (1000 µL/200 µL) with associated pipette tips
- DURAN® glass bottles (5 L, 3.5 L, 2 L)
- DURAN® beakers (250 mL, 400 mL, 1000 mL)
- 3 L urine collection containers made of PE (Sarstedt)
- 4 mL sample tubes (Greiner Cryo.s ™ with external thread)
- cryoboxes in SBS format for 4 mL sample tubes
- cap carriers
- stands for sample tubes
- methanol $CH_3OH \geq = 99.8$ % p.a.
- ultrapure water (18.2 MΩ*cm)
- nitric acid HNO_3 65% p.a. ISO thinned

For sampling

- personal protective clothing (lab coat, safety glasses, disposable gloves)
- conductivity meter
- hydrometer
- laboratory balance with a measuring range from 0 to 6000 g
- automatic hand held decapper
- tweezers/pliers for removing individual tubes
- multipette E3, single-channel and combitips advanced
- pipettes (1 mL)
- empty urine collection containers for taring the balance
- calibration weights for functional testing of the balance
- measuring cylinder 100 mL made of glass
- measuring cylinder 250 mL made of PP
- 60 L waste container for the disposal of potentially infectious materials
- rinsed 3 L urine collection containers made of PE (Sarstedt)
- rinsed sample tubes (Greiner Cryo.s ™ with external thread) in cryoboxes (SBS format)
- holder for cryoboxes and cap carriers
- cap carrier
- hand disinfectants
- surface disinfectants
- absorbent paper towels
- conductivity standards 12.88 mS/cm and 1413 µS/cm

For freezing samples

- personal protective clothing (cryogenic gloves, protective visor, cryo apron)
- mobile cryogenic transport container
- liquid nitrogen storage container (capacity at least 300 L)
- liquid nitrogen (LIN)
- storage systems for cryogenic transport containers
- oxygen depletion warning system
- power supply

7 Preparations for Sampling

In order to carry out sampling on humans, approval from an ethics committee must be generally obtained in advance.

ESB fully respects the rights of the study participants and accurately informs study participants about:

- purpose, methods, risks, and benefits of the study
- inclusion and exclusion criteria
- informed consent procedures in which individuals confirm that they have understood all information, and make a voluntary decision about whether to participate; it informs also about the right to leave the study whenever they like
- sampling procedures and related risks
- sample collection, preparation, storage, and shipment
- data management, -processing & -storage
- data sharing in a public database (e.g., ISUPB).

7.1 Infrastructure

In preparation for the sampling, it must be ensured that there is a laboratory infrastructure that meets the requirements of the Occupational Safety and Health Act (ArbSchG) and the Ordinance on Safety and Health Protection at Workplaces with Biological Agents (Biological Agents Ordinance- BioStoffV) with regard to the work to be carried out. Appropriate hygiene measures must be summarised in a hygiene plan. Potential risks and instructions for safe handling of biological materials must be summarised in an operating instruction. According to this operation instruction, the head of the laboratory must instruct and train staff members on an annual basis. Briefings, instruction, training and further education must be documented and archived.

As defined in the Technical Rule for Biological Agents 100 (TRBA 100), human body fluids, including whole blood and blood plasma from donors without defined infection status, are classified as potentially infectious. Therefore, such materials may only be processed by qualified stuff in laboratories authorized for biological safety level 2.

7.2 Contamination Risks

In order to ensure comparable samples for sensitive analysis in human biomonitoring, it is of utmost importance that human samples are collected and processed properly in the pre-analytical phase to avoid potential contaminations. In addition to contaminations, which may occur during sampling, e.g. due to sample containers being left open, 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 stuff members with workplace-specific instructions 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 and Sample Tubes

Urine collection container
The urine collection containers have to be filled with ultrapure water up to half of their volume, closed and shaken for one minute. Then ultrapure water must be disposed of. The lid and the open urine collection container must be set up to dry in a safety cabinet (class 2). The urine collection container must be tightly closed with the lid in the safety cabinet.

Sample tubes
The sample tubes have to be rinsed with methanol to remove organic contaminants, 2% nitric acid to remove inorganic contaminants and finally rinsed with 18.2 MΩ*cm of ultrapure water. For this purpose, the sample tubes have to be filled to half of their volume with methanol and completely with 2% nitric acid and ultrapure water. Shake the tubes for one minute after each filling. The 2% nitric acid remains in the tubes for 12 hours at room temperature to remove inorganic contaminants and is discarded of on the following day. Methanol and ultrapure water have to be discarded after shaking. After the rinsing process has been completed, the sample tubes must be placed in a safety workbench (class 2) to dry in appropriate cryoboxes and then tightly closed with the lid. Fraunhofer IBMT uses an automated decontamination unit for standardised decontamination of the sample tubes.

Identification of the sample tubes
The sample tubes used are coded by the manufacturer with a linear barcode (type 128) on their side including a human-readable representation of the

code content. In addition, this barcode information is contained redundantly in a data matrix code (ECC 200) at the bottom of the tube.

8 Sampling

8.1 Collection of 24-h urine

Participants are asked to collect their 24-h urine sample. Prior to sampling, each participant is sent two copies of the declaration of consent, a general information letter about the sampling procedures of the German ESB, a detailed description how to conduct the 24-h urine collection, and a 3 L collection container. The participants receive the following instruction on how to collect the 24-h urine:

Guideline for the collection of 24-h urine:
Please collect all of your urine over a period of 24 hours in the 3L collection container provided. Morning urine (= the urine immediately after getting up) may only be contained once. After getting up, please empty your bladder into the toilet first and note the time of voiding the bladder on the enclosed label that should be put on the 24-h urine collection container when sampling is completed. Start collecting the 24-h urine with the next urge to urinate and collect each urine until the following morning. The next morning, you empty the bladder for the last time at the time noted on the label for the previous day. The 24-h urine collection is now complete.
To avoid contamination through your clothing, please open the collection container just before you begin to urinate (i.e. when you have already opened/removed your clothing accordingly). Please ensure that you put down the lid of the container with the opening facing upwards at a certain distance. Please store your urine sample at best at 4-8° C until the sampling date, but in such a way that it is not exposed to direct sunlight or excessive heat.

8.2 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 and required for normalization of analyte concentrations. 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 24-h urine collection 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:
Before measuring the conductivity and the density, the urine collection container must be swivelled in large circular movements for even mixing. After each measurement, the measuring cylinder used must be rinsed with the urine of the next participant! If a participant's 24-h urine collection has been collected in two urine collection containers provided, the collected 24-h urine must be transferred from the individual containers to a larger PE container that has been cleaned in the same way as the urine collection containers and to swivel it in large circular movements for even mixing. Further processing is carried out analogously to the procedure described below.

Measuring Weight
Before starting the measuring, the balance must be calibrated using standard weights for internal quality assurance. The result must be documented. Before each weighing, the balance must be tared with an empty 24-h urine collection container. Once a participant's urine sample arrives in the laboratory, it must be weighed and the weight in grams with one decimal place must be documented on the urine sampling protocol – 24-h urine (see Appendix). Deviations (e.g. several collection containers etc.) must be recorded in the "comments" column. When using several collection container, these must be weighed individually.

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 hydrometer must be submerged. It must be ensured that the hydrometer floats and does not touch the base of the measuring cylinder. The density (g/ml) must be read from the hydrometer and documented in the sampling protocol – 24-h urine (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, the first and last three incoming urine samples of the measuring series are measured with a second calibrated hydrometer to check the functioning of the hydrometer used and to control the collected data. The results of these quality control measurements must be documented.

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. Subsequently, the conductivity meter probe must be submerged and the measurement started. The latency period with regard to the temperature adjustment of the measuring probe to the temperature of the urine sample must be taken into account. The result (mS/cm) must be documented on the sampling protocol – 24-h urine (see Appendix) to two decimal places. Any discrepancies must be noted in the "comments" column.

Measurement of urine creatinine

The measurement of urine creatinine is described in detail in the ESB Guideline for Sampling and Sample Processing - Analysis of Clinical-Chemical Parameters

8.3 Sample Aliquoting

For each participant 48 aliquots of the 24-h urine sample are generated for long-term storage in the environmental specimen bank and two aliquots for real-time monitoring. After the measurement of the above-mentioned parameters has been completed, the collection container must be swivelled in large circular movements. The 24-h urine collection samples are then to be taken directly from the 24-hour urine collection vessel using a multipette and 4 mL of urine collection is to be aliquoted into the cleaned sample tubes. Deviations such as additional collection vessels, missing aliquots or insufficient quantities must be documented on the sampling protocol - 24-hour urine collection.

Before storage, the barcodes of the cryobox and all individual sample tubes are scanned to document the storage position of each aliquot in the cryobox.

8.4 Cryopreservation of Samples

Protective clothing must be worn when samples are frozen or stored. Urine samples must be frozen in the gas phase of a mobile cryogenic transport container cooled with LIN. The filled cryoboxes are sorted into storage racks provided for this purpose. Once the storage racks have been inserted, the cryogenic transport container must be closed.

The samples remain in this cryogenic transport container until they are transferred into the cryo storage container at the ESB repository. During the transport, LIN supply is realized automatically via the control and monitoring unit of the cryogenic transport container. A thermograph integrated in the control and monitoring unit of the cryogenic transport container with a recording interval of 15 minutes is used to monitor the temperature of the samples. The German ESB uses a Biosafe 420 that has been upgraded for the transport of samples, including the respective control and monitoring unit from Cryotherm for this purpose.

When the samples are transferred from the cryogenic transport container to the cryogenic storage container, the barcodes of the individual cryoboxes are scanned again and the position of the cryobox in the storage rack and the number of the cryogenic storage container are recorded. 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.

9 Removal of Samples for Retrospective Analyses

Consent from the UBA is required in order to remove archived samples of the ESB. For shipment, samples are removed from the ESB without interrupting the cold chain and packaged in accordance with the PI650 packaging guideline (UN3373).

Samples are shipped on dry ice at approx. -80 °C. The recipient must confirm the integrity of the samples by signing a sample transfer protocol attached to the shipment.

10 Literature

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Appendix: Excerpt from the sampling protocol - 24-h urine

GERMAN ENVIRONMENTAL SPECIMEN BANK

Sampling Protocol

24-h Urine

Identification:

__ __ __ __ / X / __ __ __ __ / __ __ __ __ __ / __

- Specimen Type
- Specimen Condition
- Collection Date (MM/JJ)
- Sampling Site
- Additional Information

Sampling Site (plaintext) _____ **RTM:** _____

from: __ __ . __ __ . __ __ __ __	Sampling Date	to: __ __ . __ __ . __ __ __ __
from: __ __ : __ __	Time	to: __ __ : __ __

Participant		Processed	
from	**to**	**by**	**date**

Participant No.	Weight	Density	Conductivity	Remarks
001	_ _ _ _ , _ g	_ , _ _ _ g/mL	_ _ , _ _ mS/cm	
002	_ _ _ _ , _ g	_ , _ _ _ g/mL	_ _ , _ _ mS/cm	
003	_ _ _ _ , _ g	_ , _ _ _ g/mL	_ _ , _ _ mS/cm	
004	_ _ _ _ , _ g	_ , _ _ _ g/mL	_ _ , _ _ mS/cm	