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DES BUNDES

Guidelines for Sampling and Sample Processing

Transporting Environmental Samples under Cryogenic Conditions

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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 of environmental monitoring for the Federal Ministry for the Environment, Nature Conservation and Nuclear Safety (BMU) underlying specialized and administrative coordination of the Federal Environmental Agency (Umweltbundesamt, UBA). The ESB collects ecologically representative environmental specimens in addition to human samples, maintains and analyses them with respect to relevant environmental substances. The operation of the ESB is based on specific operating procedures (UMWELTBUNDESAMT) and the conceptual design of the UBA (BMU 2008).

Long term storage is accomplished under conditions, which minimize a change of state or loss of chemical characteristics, over a period of several decades. Thus, the archive is able to provide samples for the retrospective examination of substances whose potential risk to the environment and human health is as yet unknown.

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

2 Objective of the Guideline

This guideline applies to environmental biota samples such as bream muscle, spruce shoots, and beech leaves as well as to abiotic samples like soil and suspended particulate matter. For storage in the ESB most samples are deep-frozen immediately after sampling.

Exceptions are, e.g., livers of roe deer, eggs of pigeon and herring gull and earthworms. Specific transport conditions for these samples are described in the respective guidelines for sampling and sample processing. In any case it is important that the cold chain, once begun, must be maintained. Any further transport, as well as storage, grinding, and homogenisation of the samples is performed under cryogenic conditions (i.e. temperatures below -130°C) in accordance with specific safety provisions.

The particularly high demands on quality assurance arise from the importance of the samples as archive material. Representativeness and reproducibility of the samples are prerequisites for the comparability of test results in time and space.

The objective of this guideline is to provide information on the safe transport of temperature sensitive material under cryogenic conditions in compliance with occupational safety regulations and without loss of sample quality.

The guideline applies only to road transport and not to the shipping of samples with parcel services or freight companies.

3 Procedure for Transport

3.1 Cryogenic conditions

Cryogenic conditions are achieved at temperatures below about -130°C (approximately < 140 K). The temperature is below the glass transition temperature of water (BURDEN 1999) so that no recrystallisation of ice and growth of ice crystals should occur. This ensures that chemical processes in the samples are minimized and morphological changes, e.g. due to further growth of ice crystals is prevented. Moreover, when stored in the gas phase above liquid nitrogen the evaporating nitrogen generates an inert gas atmosphere which largely prevents alterations of the samples caused by oxygen from the ambient air (oxidation processes).

3.2 Safety instructions for handling liquid nitrogen

Liquid nitrogen is cryogenic. As it evaporates it can act asphyxiant causing suffocation. Contact with living tissue causes rapid freezing and severe damages.

When handling liquid nitrogen attention must be paid to the information in the safety data sheet according to Article 31 of the REACH directive (REACH 2006).

Handling of liquid nitrogen in buildings requires proper ventilation. When transporting liquid nitrogen in vehicles, monitoring with an oxygen sensor is essential next adequate ventilation (see also section 3.3).

3.3 Legal basis

The legal basis for the transportation of liquid nitrogen in vehicles for cooling purposes is set by Section 5.5.3 of the „European Agreement concerning the International Carriage of Dangerous Goods by Road (ADR 2009)“. Vehicles with substances that are used for cooling and conditioning purposes during transport are subject to the provisions of this section only. These provisions are mandatory.

3.4 Preparatory work

The transport container (vacuum-insulated stainless steel container with lid of insulation material) has to be cooled down before the transport.

In the precooled transport container the samples are stored in suitable containers or racks and placed securely on a platform to prevent falling and direct contact with the liquid nitrogen.

According to the ADR (2009) the containers holding the samples must be designed in such a manner that a release of gas is possible in order to prevent pressure built-up which could rupture the packaging.

Before carrying out the transport the staff must be instructed about the requirements of ADR (2009).

3.5 Material and Equipment

For transport under cryogenic conditions the following equipment is required:

- Insulated shipping container, appropriate for cooling with liquid nitrogen, with suitable storage racks for the samples. Labelling: UN No. 1977 nitrogen, refrigerated, liquid; label no. 2.2 (gas cylinder on green background).
- Reservoir tank with liquid nitrogen for cooling (during transport in a closed state, approval as pressure vessel for road transport is necessary), dangerous good identification: UN No. 1977 nitrogen, refrigerated, liquid; label no. 2.2 (gas cylinder on green background).
- Dip stick for measuring the fill level of the liquid nitrogen.
- Suitable thermometer/thermocouple to check the temperature.
- Fire extinguisher: dry powder extinguishers for fire classes A, B, C, with a valid inspection sticker; 2 kg for vehicles up to 3.5 tons maximum gross mass or 4 kg in vehicles of more than 3.5 t to 7.5 t permissible gross mass.

Transport containers containing liquid nitrogen as a coolant, may be transported only in well-ventilated vehicles (e.g., extra ceiling ventilation in the separated cargo space).

Each access to the transport vehicle has to be marked with a warning sign (nitrogen, refrigerated, liquid, as a coolant; with visual indication of the risk of oxygen deficiency).

The following sample containers are used:

- Vials (e.g., 20 mL scintillation vials from Perkin Elmer, Rodgau-Jügesheim, Germany); made of borosilicate glass with the following standard dimensions: height 60 mm, diameter 25 mm; screw caps made of plastic, the inside coated with a metal foil. The vials are not closed tight to prevent explosion in case liquid nitrogen that may be contained in the vials evaporates at rising temperatures.
- Glass bottles, 100 mL volume of borosilicate glass, with plastic screw caps.
- Stainless steel containers with stainless steel lids and stainless steel clips for transport of not yet homogenized samples.
- Cryo-vials (e.g., to be used for blood plasma samples); in case of exclusive storage in the gas phase lids may be closed tightly.

3.6 Performance of transport

During sample transport cryogenic conditions must be maintained. The temperature is controlled, for example, using portable or stationary temperature probes and a thermologger with digital display. This allows a continuous documentation of the temperature conditions during the entire transport.

The freezing of fresh, warm samples consumes a relatively large amount of liquid nitrogen, which demands frequent temperature monitoring and may require a refill.

3.7 Storage at destination

At destination, the samples are transferred into appropriate pre-cooled cryo-storage containers.

3.8 Cleaning instructions

If the cryo-transport containers are warmed to room temperature after transport, they should be washed out. Cleaning is intended to prevent health hazards caused by microbial contamination. After thorough drying of the container, the lids are fitted. To prevent mould growth by condensation of water, the lid should not be sealed.

4 Documentation

Each transport requires a list with the sample data and a sample accompanying document (respectively a handover protocol) which are archived afterwards. The form and content of the accompanying documents may vary from case to case. For the transport between different institutions a handover protocol is mandatory. The document must contain at least the following information:

- Delivering institution (contact details).
- Recipient of the samples (contact details).
- List of samples including description and ESB codes.
- Date and place of delivery.
- Signatures of recipients and representatives of the delivering institution.

5 Literature

ADR (2009): European Agreement concerning the International Carriage of Dangerous Goods by Road. www.unece.org/trans/danger/publi/adr/adr2013/13contentse.html

BMU (Bundesministerium für Umwelt, Naturschutz und Reaktorsicherheit, Eds.) (2008): The German Environmental Specimen Bank - Concept (Status: October 2008). www.umweltprobenbank.de/en

BURDEN, D. W. (1999): Issues in Contamination and Temperature Variation in the Cryopreservation of Animal Cells and Tissues. Revco Technologies, Asheville, NC 28806 (USA), Application Note 99-08.

REACH (2006): Regulation concerning the Registration, Evaluation, Authorisation and Restriction of Chemicals (REACH), establishing a European Chemicals Agency, amending Directive 1999/45/EC and repealing Council Regulation (EEC) No 793/93 and Commission Regulation (EC) No 1488/94 as well as Council Directive 76/769/EEC and Commission Directives 91/155/EEC, 93/67/EEC, 93/105/EC and 2000/21/EC.

UMWELTBUNDESAMT: German Environmental Specimen Bank - Standard Operating Procedures. Editor: Umweltbundesamt. http://www.umweltprobenbank.de/en/documents/10022.