MILJØPRØVEBANKEN
- Nasjonal prøvebank for miljøgifter

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Procedure 004:
Collection and sampling of mammals

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1. Aim of procedure

This procedure describes all the different steps in collection and sampling of mammals for the Environmental Specimen Bank (ESB). This procedure describes every step of the sampling and handling of samples to ensure that the procedure can be used in a clear way. The procedure should cover all aspects of reproducibility, quality and registration of data for the sampling material.

2. Field sampling

2.1. Trapping methods and handling of the animals

Collection and handling of samples and material should be carried out in order to avoid contamination of potential environmental pollutants and protect the samples from any kind of impact that will affect their usages as research and reference materials. During sampling in field, transportation and lab work, the sampled tissues should not be in contact with any kind of potential contaminated areas such as benches or compounds. Gloves should be used as long as it is feasible.

If tissue samples are prepared in the field, they should be wrapped in aluminum foil and put in a clean bag of the same type as the ones used for freezing and storage in the environmental specimen bank (MAGIC VAC®; polyethylene with an external reinforcement by a nylon membrane). If not available, clean bags made by non-dyed polyethylene (PE) can be used. A label with sample identification should be put into the bag and the bag is sealed/closed.

2.2. Registration of field data

For each sample, a field data sheet which explains the location, time, species, organ, and the person responsible for collection of the samples. For otter and reindeer that has been shot in the field, age and gender of the animal should be noted as well as the kind of ammunition used (lead or other kind of material). Any deviations from normal hunting procedures should be noted. If tissue samples are taken in the field (reindeer), a protocol should be made on forehand to standardise and make a quality assurance on the sampling procedure. As a consequence of this, packing material, scalpels, nitrile gloves, aluminum foil and bags should be sent to local hunters before the hunting begins.

The coordinates for the position of the animal should be written in UTM as stated in the national maps M711 and preferably as UTM/EUREF89 (Universal Transverse Mercator) with the zone 33N (EPSG projection 32633), although as an alternative, WGS84 (World Geodetic System 1984) in decimal degrees can be used (Statens kartverk, 2009). The WGS84 system is the reference coordinate system used by GPS.

2.3. Storage before shipping

The samples should be frozen as soon as possible after the hunting and stored frozen until they can be sent to NINA.

3. Transport of samples

3.1 Packing

The samples should be transported frozen and packed as specified above. The way of packing and the material should be chosen to minimise thawing of samples during transport. Freezing elements should be placed together with the samples to ensure this. The parcels should be marked clearly with sender's name and address, sent to NINA Trondheim and addressed to a contact person there. The parcel should be marked with a text which states that the parcel contains biological material that needs to be kept cool.

3.2 Transport routines

The samples should be transported as quickly as possible to NINA. A contact person at NINA should be informed on forehand regarding time of delivery to ensure that the parcel is received in a proper way. If post or shipping companies are used, the parcel must be sent in a traceable manner.

4. Sampling within the lab

4.1 Equipment and cleaning procedures

Before sampling, the staff in the lab should prepare the necessary clean equipment, a working space covered with clean aluminum foil and a registration sheet. The staff should use clean gloves (nitrile-gloves).

The following equipment is needed for sampling of organs:

- Petri dishes
- Scalpel and extra scalpel blades
- Scales (precision: 0.01 g)
- Paper tissues (free from chlorine)
- Clean aluminum foil
- Solvents (acetone, cyclo-hexane)
- Glass for washing of equipment
- Nitrile-gloves

All tools used for sampling should be of either stainless steel or of glass, quarts or other in-organic ceramic materials. The tools should be cleaned in accordance with the procedure below.

Cleaning of equipment:
Washing with in-organic (base) soap (Neodisher UW). The equipment is thereafter rinsed three times with distilled water or ion-changed MilliQ-water and then washed with solvents (HPLC grade). First, 5 minutes in acetone and then 5 minutes in cyclo-hexane. This rinsing is done twice. Cleaned equipment can be stored wrapped in clean aluminum foil. It should be stored in a place with good ventilation (e.g. fume hood).

The bags with samples should be marked with labels (that can withstand freezing) with a unique sampling number (P_ID) that has been generated from the database of the Environmental Specimen Bank.

4.2 Individual data

A unique number is given to the sample (identification number) and is generated from the database. In addition, the sample should be labelled with the lab-number from NPI. The following individual data should be registered: species, location, date, gender, age, type of organ, weight of animal and trapping method (hunting, traffic accident, drowning etc). It is important that the lab-number from NINA also is registered on the MPB sampling sheet to be able to gather other data from NINA regarding the individual animal.

4.3 Preparation of sample

The sample should not be touched by anything else than clean equipment. Hands (with or without gloves) shall not touch the sample. The sampling should preferably be made in a clean room. All equipment should be cleaned between sampling of different organs. Dry it carefully with paper tissues and distilled water before cleaning with solvents (rinsing twice with acetone and cyclo-hexane). Scalpel blades are changed between each sample. Note down any visual abnormalities in the sampling log. About 100g should be taken of each organ type (muscle, liver). Avoid damaged tissues (wounds, shooting wounds, blood cloths, necrosis etc). The sample is divided in 5 fairly equal parts, which are wrapped individually in aluminum foil and thereafter collected together in a plastic bag. Only 1 ID-number should be given (specific for the individual animal) and the bag should be labelled with this number and the lab-number from NINA.

When the samples are put in the bag and it is sealed, the samples will be frozen. Put the samples in the freezing storage of NINA in a box labelled with content and the name of the scientific responsible person.

5. Registration of data, marking and freezing samples

Data from the field and sample schemes are transferred to electronic format at the Environmental Specimen Bank by the scientific responsible person at NINA after the data has been through quality assurance. Every sample has a unique identification number (lab-number) and are also give a unique sample number (P_ID). In the data base, information about where the samples are stored will be included. This information should include which rack and section of it, which shelf and box where the sample is stored.

6. Transport to the Environmental Specimen Bank

The frozen samples should be packed in polystyrene-insulated boxes, which are sent as freezing goods or shipped to ESB with agreed means of transportation. Prior to transportation, a contact person at the ESB must be informed of the delivery date to ensure that the shipment is received in due time. If post or shipping companies are used, the parcel must be sent in a traceable manner. After arrival, personnel at ESB will register the samples and store them frozen at -25 ° C in the freezer of the Environmental Specimen Bank.

7. References

Statens kartverk. 2009. Koordinatbaserte referansesystemer. versjon 2.1 - Desember 2009. 48 s. URL: http://www.statkart.no/filestore/Standardisering/docs/koo_referansesyst.pdf. (04.01.2013)