Sampling Methodology for Forensic Evidence in Wildlife Crime Cases

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https://forensics.natur.cuni.cz/odber-vzorku/

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Contents

Examination of female grouse (photo: Zdeněk Novák)

Sampling in wildlife crime cases

The basic prerequisite for the use of forensic methods in the investigation of wildlife crime cases is the provision of the necessary traces and their proper documentation and preservation, including the correct collection of samples for forensic examination. For different forensic methods, various types of samples are needed. They may differ not only in the type of biological material but also in the required size, method of fixation, storage requirements, etc. During sampling and subsequent handling, it is necessary to prevent deterioration of the samples, e.g. by biological decomposition or contamination. Maintaining the chain of custody is also of key importance.

A distinction can be made between the two types of sampling for forensic analysis:

- Live animal sampling, which requires adequate procedures. In certain situations (invasive interventions, e.g. blood sampling), the procedure must be carried out by a competent person, e.g. a veterinarian.
- Sampling from inanimate specimens, the performance of which depends on the type of specimen and the desired method of the examination.

Sampling equipment

Equipment and tools are required for sampling (see recommendations below). The size and shape of the sampling containers should be chosen, according to the type and quantity of material to be sampled, the forensic method chosen and the further use of the sample. Plastic containers are preferable (glass can break).

Overview of sampling equipment:

- ✓ Scalpel, surgical scissors
- ✓ Anatomical tweezers, needle
- ✓ Spatula for faeces
- ✓ Electric drilling machine (drill bits of different diameters)
- ✓ Miniature grinder, pliers
- ✓ Nitrile gloves
- ✓ Cotton pads
- ✓ FLOQSwabs-type tampons
- ✓ Tape for removing DNA from surfaces
- ✓ Blood smear slides
- ✓ 96% ethanol
- ✓ DNA Removal solution
- ✓ DNA/RNA Shield-type solution
- ✓ Filter paper Whatman, FTA cards
- ✓ Disinfectant wipes

Material for packaging:

- ✓ Sampling tubes of various sizes
- ✓ Larger plastic containers
- ✓ Plastic bags
- ✓ Bags with zip closure
- ✓ Paper envelopes, bags, boxes
- ✓ Stericlin-type bags
- ✓ Bubble wrap
- ✓ Solid closable container for transportation of bags with biological material
- ✓ Container for sharp objects
- ✓ Egg cartons
- ✓ Entomological boxes
- ✓ Sealing tapes, seals
- ✓ Aluminium foil
- ✓ Cooler bag/box

Sampling containers must be marked with a **unique identifying description or unique number**. It is not appropriate to simply number them with simple serial or trace numbers (when sampling at multiple locations or from multiple sources, the same numbers may be used, leading to problems and possible confusion).

Wear **gloves and sterile instruments** during sampling! Clean/sterilise instruments or use disposable instruments before collection. Sterilisation can be done:

- by rinsing with 96% ethanol and DNA Removal solution or
- by wiping with disinfectant wipes with ethanol or sodium hypochlorite, e.g. Mikrozid AF or
- by spraying with disinfectant and rinsing with distilled water (allow to dry) or
- in emergency conditions (in the field) objects can be rinsed with 96% ethanol and burned over a flame (matches, lighter).

In the case of a large number of samples or samples from several different individuals, it is necessary to sterilise the instruments between samples (to eliminate cross-contamination) or to have several sets of instruments available.
Special kits are available for certain types of samples and sample collections (e.g. FLOQSwabs, etc.).

Packaging and transport

The method of packaging must be appropriate to the type of trace or sample obtained (see later chapters).

Moist items (including residual moisture) should generally not be packed in leak-proof packaging (plastic) unless they are immediately transferred to the laboratory or frozen. Moisture causes rapid degradation of biological traces (moulds, bacteria). Pack samples and traces of this type in breathable or semi-permeable packaging to allow them to dry out gradually (paper bags, envelopes, Stericlin sterilisation bags permeable by vapour and air, etc.). Double-wrap traces to dry - e.g. FLOQSwabs are placed in a plastic case/test tube into which a hole is made with a sterile instrument before inserting the swab to ensure sufficient air supply to dry the swab. The test tube is then placed in a breathable container (paper envelope).
Another option is to freeze the wet evidence/samples or store them in a collection container with 96% ethanol or DNA/RNA Shield-type solution, etc.

Pack dry traces/samples in paper envelopes, boxes, security bags, containers with sealing lids, etc. If plastic bags are used (larger items), the bags should

not be filled more than 3/4 full so that they can be easily tied or sealed with tape.

Use thermo boxes, ice packs, etc. for transporting items/samples requiring refrigeration; in case of emergency, a thermo mug with ice can be used for smaller samples. Larger items in the vehicle should be secured against movement and bags should be placed in plastic barrels, buckets or boxes with lids.

Seized tanned lynx fur ready for packing (photo: Pavla Říhová)

Chain of custody

The chain of custody is the continuous record of all changes that relate to the evidence secured (samples taken are part of the evidence) and constitutes a record of the persons who were in physical possession of the item and the activities that were carried out with the item/sample.

Any handling of evidence, transfer, handing over for expert opinion or examination, taking of a sample from the object, etc., must be properly documented to avoid any doubt of tampering or confusion.

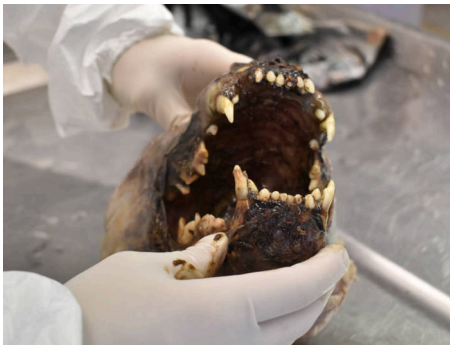
Each handover must be accompanied by **a signed handover protocol**, the evidence must be stored securely and activities performed (including the processes involved in examining it) must be recorded and documented. For evidence to be admissible in court, it must be handled appropriately, including documentation of the chronological history of the evidence and the unquestionability of the chain of custody.

Morphology

Morphology is the most commonly used method for species identification and evaluation of individual specimens of animals and plants. It deals with the shape and external structure of organisms. Macroscopic features (shape, colour, texture, specific characteristics) can be examined, but microscopic features can be used s well. Morphologically, the complete specimen or all available parts should always be examined. For this reason, **samples are not taken** for morphological examination (except for the determination of wood).

What can be examined morphologically

- **Animals** - whole individuals (live, dead, frozen, preserved e.g. in alcohol, smoked, mounted etc.), individual parts of their bodies (bones, cadavers, skins, hides, furs, teeth, ivory, claws, horns…).
- **Products from animal bodies** - morphological examination depends on whether key features are preserved in a recognisable form (e.g. ivory can usually be distinguished quite clearly from other materials and from teeth of other groups of animals).
- **Hair, wool** - microscopic examination (trichology see below).
- **Feathers** - bird species can be distinguished mainly by their plumage (i.e. tectrices, remiges and rectrices); inner down feathers are unsuitable for morphological determination.
- **Eggs** - identification is possible in birds with colour and shape-specific eggs; it is not possible for species with plain white eggs.
- **Collections of invertebrates** - butterflies, beetles, molluscs, etc.
- **Plants** - whole plants (live, frozen, preserved in alcohol, dried, pressed herbarium items), plant fragments, seeds, fruits, etc.
- **Wood** - morphological determination is usually possible at the genus level (rarely at the species level) and requires sampling. The examination is carried out macroscopically or microscopically according to the wood structure on the different cut surfaces.



A tiger skull, the animal has not yet fully erupted permanent teeth (photo: Zdeněk Novák)

Examining the limbs of a wolf (photo: Pavla Říhová)

Packaging

The variability of the objects submitted for morphological examination is high and the method of packaging depends on the nature of the item. The expert invited to the house search should be consulted on the packaging. The condition of the seized specimen must be taken into account to avoid damage during transport or degradation by decomposition.

Cool and freeze animal cadavers, parts and other decomposable items as soon as possible. Pack small objects (bone fragments, teeth, claws, etc.) in separate containers to prevent their loss during handling. Wrap fragile or sharp objects (beaks, horns, broken bones, etc.) with multiple layers of packaging material.

Some specimens require special packaging, a specimen of a perched brown bear wrapped in bubble wrap here (photo: Customs Administration)

Photography

If it is not possible to physically hand over the specimen for examination, it is possible in certain circumstances to assess it using photographs or video. This may be the case when an expert is not present during the house search and it is necessary to decide whether it may be a protected species.

- Photograph the object in an **overall view from all sides and from different angles** (one photograph is not sufficient). Photographs should also show the position of the object/trace before handling (overview photographs).
- Photograph **semi-details and details** that may be important for the determination or evaluation of the specimen. If possible, consult with the expert who will be examining the specimen (should specify how to photograph a particular specimen and which details may be relevant). Any additional information related to the find (location, condition, circumstances) may contribute to successful identification.
- Photographs must be sharp, with details clearly visible. It is important to hold the scale close to the subject to determine its size. Any object that can be measured afterwards (pencil, etc.) can be used temporarily instead of the scale. Keep the resolution of photographs as high as possible; do not edit or compress them.
- Video recordings can complement the taking of photographs. In certain circumstances, e.g. when an object needs to be rotated (reading a ring on a bird's leg) or to document a feature that cannot be captured in a single photograph, video recording is indispensable.

Genetics

Always use **gloves and a face mask** when sampling!

The appropriate preservation procedure for most types of samples is **drying**, which is done by placing them in breathable or semi-permeable containers (paper bags, envelopes, Stericlin-type bags, etc.) in which drying occurs spontaneously. **Double wrap** traces to dry (e.g. place swabs in a test tube into which you have previously made a hole with a sterile instrument to ensure sufficient air supply for drying; then place the test tube in a paper envelope). Dried samples are fixed and stabilised for months to years.

Moist/wet samples (soft tissue, faeces, etc.) can be collected to well-sealed plastic containers or test tubes and **frozen** as soon as possible to prevent DNA degradation. These samples may also be **stabilised with 96% ethanol before freezing** (do not use denatured ethanol!). In this case, the sample must be fully immersed (volume ratio: 1/3 sample volume, 2/3 ethanol). It is always best to consult the laboratory that will process the genetic analysis in advance (laboratories may have different requirements).

Do not wrap moist items (even with residual moisture) in leak-proof packaging (plastic) unless they are immediately transferred to the laboratory or frozen. Moisture causes rapid degradation of biological traces (moulds, bacteria).

Soft tissue

- Place soft tissue and muscle (including cooked meat, fresh meat or skin) in resealable test tubes.
- Using a sterile scalpel and tweezers, remove approximately **1-2 cm^3** (due to possible contamination, sample from the inner layers of the material, not from the surface). In complete cadaver, it is possible to take a sample by cutting off a peripheral part (a piece of an ear, tail).

Sampling of tiger skin (photo: Vít Lukáš)

- Place in a test tube and freeze at -20 °C. Store in the freezer, transport in a thermobox. The sample can be fixed with 96% ethanol before freezing.
- Dried tissue residues can be scraped off the surface of objects or smooth surfaces. To scrape, use a sterile scalpel, scraping the material onto a piece of weighing paper. Then place it in a breathable or semi-

permeable container (paper bags, sterilisation rolls and Stericlin-type bags, etc.).
- The dried meat can be stored in resealable bags or test tubes with silica gel at room temperature.

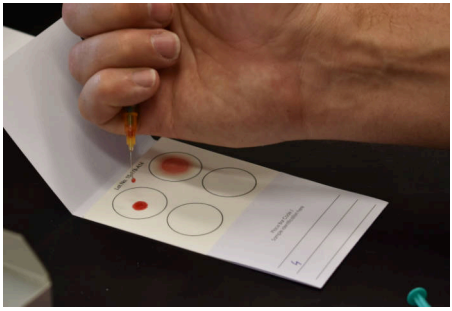
Blood

Blood sampling in live animals

- Blood from live animals is taken by a competent person. Collection is carried out with a sterile needle in a test tube. If anticoagulants (EDTA) are added, consult the laboratory that will process the sample beforehand.
- Test tubes with blood can be stored in the refrigerator at 2-6 °C for short periods of time. For long-term storage, freeze the test tubes.

Blood sampling on filter paper

- Blood from live animals, fresh cadavers and fresh blood traces can be collected on filter paper. Take a small amount of blood (even clotted) to make a small (<1 cm) stain on the filter paper. It is best to use special **FTA cards** or **Whatman filter paper**.
- After drying, place the sample in an envelope and store it at room temperature in a dry place, out of sunlight.



Blood samples on filter paper (photo: Dominika Formanová)

Blood traces

- Primarily secure the blood traces together with the object on which the blood was deposited (knife, trap, etc).
- If it is not possible to provide a blood trace with its carrier, take the sample by wiping it with an **absorbent swab** (head made of cotton, wool), which is marked “DNA free”.
- Wipe off fresh (still damp) stains with a dry swab.
- For dried blood, moisten the swab head with a drop of distilled water first (it is better to use water marked as DNA free). Use a dropper. CAUTION - the swab must not be over-wetted, otherwise its absorbency is lost and the trace may be too diluted! Wipe the moistened swab over the dried blood. A drop of distilled water can also be dropped directly on the dried blood and wiped off with a dry swab after a few moments of soaking.

- **Store the swabs as follows**:
  - Use sterile scissors to make a hole in the cap of the test tube (part of the swab set) to allow air to enter (necessary to dry the swab).
  - Insert a swab into the test tube, break off its longer part, and cap the test tube with the head of the swab inside.
  - Place in an envelope or paper bag. Store at room temperature.
  - When using special self-drying swabs with silica gel, it is not necessary to cut the test tube or make a hole in the cap.
- At the same time as sampling, we recommend performing a **control smear** to test for the possible presence of contaminating DNA on the swabs or in distilled water. Moisten the tip of the one swab with distilled water, place it in the test tube (see procedure above), close and mark it as a control smear.
- Collect samples from larger dried blood spots by **scraping** them into paper or other semi-permeable packaging (e.g. Stericlin, Quick Clean, Eurosteril bags). They can also be scraped into a microtube (make a hole in the lid to allow residual moisture to dry and store in a paper envelope).
- Wipe off blood stains on ice or snow with an absorbent sampling swab or transfer to filter paper and allow to dry (see above for procedures). Another option is to collect in a test tube with 96% ethanol (take as little snow as possible into the sample). The ratio should be 1/3 sample and 2/3 ethanol. If there is only a little blood, it is better to just freeze the collected snow/ice (do not add ethanol). Store in a freezer at -20° C.

Blood of a shot lynx on the snow (photo: Josefa Volfová)

- Collect the blood soaked in the sand or soil including the material in which it is soaked (collect as little material as possible so that the trace is not too "diluted"). Allow to dry (breathable container) or place in a plastic container and freeze.

Faeces

- Take the faeces fresh, 3-5 days old at most. In the field, it can also be collected from snow.
- Sampling can be done by swabbing (intestinal epithelial cells adhere to the surface of the faeces) or by taking an adequate portion of the faeces.

- **Wiping with a swab:**
  - Use the FLOQSwab to wipe the entire surface of the faeces. Place the swab into a test tube with a pre-made hole in the cap (use sterile scissors to make the hole). Cap and place the test tube in a paper envelope/bag.
- **Sampling of faeces:**
  - Take the sample with a spoon, or spatula, or in the field with a piece of wood, etc. If you are taking multiple samples (from different animals), use a new instrument for each new sample or sterilise it.
  - Take a sample about the **size of a hazelnut** in a plastic sealing container. The target DNA (intestinal epithelial cells) is in the surface layer, so take material from the outer surface of the faeces, or you can take the whole "slimy" end of the faeces.
  - Store collected faecal samples in the freezer at -20 °C. You can also fix the sample with 96% ethanol before storing it in the freezer. The sample should be completely submerged, a suitable ratio is 1/3 sample to 2/3 ethanol. The sample can also be stored in ethanol for short periods at room temperature or in a cool dark place.
  - Fresh faeces can also be fixed using silica gel (volume ratio - 1/3 faeces and 2/3 silica gel), this procedure is usually chosen in conditions where rapid evaporation of ethanol occurs, e.g. when collecting in tropical areas.
  - Another option is to store the sample in a **DNA/RNA Shield-type solution** (produced by Zymo Research, Biomatrica...), which is a special solution for preserving nucleic acids and inactivating pathogens. Samples fixed in this way can be stored at room temperature (guaranteed for up to 30 days). However, this solution is quite expensive.

Urine

- Collect fresh, moist urine with an **absorbent swab**. For dried urine, moisten the swab with a drop of distilled water (DNA-free) first. After collection, place the swab in a swab tube in which you have previously made a hole in the cap with sterile scissors (necessary for the swab to dry). Place the swab tube in a paper envelope and store it at room temperature.
- Remove the urine from the snow from the most concentrated part of the patch (the deepest yellow spot). Take about a teaspoon of snow with urine into a test tube. Store samples in the freezer at -20° C.

Saliva

- Saliva can be taken from a torn prey or from a wound caused by an animal.
- Use a **cotton swab**. Use the swab to wipe the area around the wound to

collect the predator's saliva. Try to avoid the places bloodstained from the bitten animal. Then makuse a second swab to directly wipe the wound. You can also cut the hair from around the wound (place the cut hair in a paper envelope).

- Place the swab in a swab tube in which you have previously made a hole in the cap with sterile scissors (air access is important for the sample to dry). Place the swab tube in a paper envelope, seal and store at room temperature.
- Another option is to insert the swab with the sample into the **DNA/RNA Shield-type solution** (produced by Zymo Research, Biomatrica...), which is a special solution for preserving nucleic acids and inactivating pathogens. Samples fixed in this way can be stored at room temperature (guaranteed for at least 30 days). However, this solution is quite expensive.

Buccal and nasal smears

- For buccal or nasal smears from live animals, use **cotton swabs** or **nylon swabs such as FLOQSwabs**. Collection should be performed by a person experienced in animal handling.
- It is more appropriate to take a **sample from the nostrils** than from the mouth. Nostril sampling does not risk DNA contamination from possible food or from multiple individuals licking each other.

Buccal smear of a tiger (photo: Dominika Formanová)

- Gently insert the swab into the nostril and rub thoroughly against the walls of the nasal cavity. In the case of the collection from the mouth, simply insert the head of the swab brush under the lip (immediately before collection, the animal should not ingest food or lick another animal).
- Place the swab in the test tube in which you have made a hole in the cap with sterile scissors (important for drying the sample). Place the swab tube in a paper envelope, seal and store at room temperature.

Human DNA samples

The scene of a wildlife crime should also be examined for human DNA as a standard:

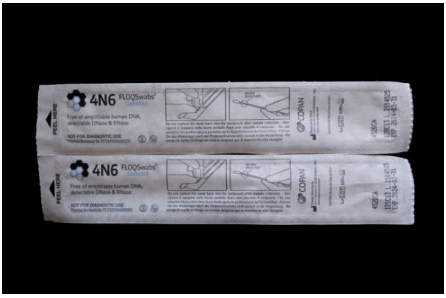
- ✓ discarded cigarette butts, gums, and drink cans;
- ✓ items that have been handled such as tools, knives, gloves, tubes from egg blowing kits, etc;
- ✓ blood stains caused by injuries while using tools, climbing trees or

handling animals;
- ✓ hand contact marks on eggs used as poisoned bait;
- ✓ hand contact marks on traps;
- ✓ interior surfaces of contraband shipments; etc.

Wear **gloves and a face mask** at all times during sampling! This testing procedure is aimed at detecting human DNA and, if not handled properly, contamination of the sample by the person taking the sample can easily occur. Control buccal swab should be performed on persons who have handled the traces and they will be designated as "house persons". DNA profiles from these swabs are not entered into police database, they are only used to exclude the profile from examination if contamination is suspected.

- Secure portable items as a whole, place them separately in two breathable containers (paper bags, envelopes, Stericlin-type bags, etc.) and send them to the laboratory.
- If it is not possible to secure the entire object, make a **surface swab** of the area most likely to be touched by the suspect (loose epithelial cells remain on the surface at the point of contact).
- Unfortunately, it is unrealistic to collect touch DNA samples from a larger area, so it is necessary to try to **specify as much as possible the area where the touch DNA** of the suspect could be. Touch **DNA can also be collected from mammalian hair or feathers of birds**. In the case of animal cadavers that have been carried or otherwise handled, the most likely area of grip is the terminal part of the limbs (ankle, wrist), the end or root of the tail, or the neck. Eggs used as bait are usually held at the widest part, etc.
- Carry out the swabbing as soon as possible before any handling of the objects by persons present at the crime scene.
- Collect the touch DNA with a **FLOQSwab** (e.g. FLOQSwabs from Copan, Prionics set of Forensix) or a **forensic adhesive tape** (e.g. Forensic DNA Grade).

- **Sampling by FLOQSwab:**
  - Moisten the swab pad with 1-2 drops of distilled water (DNA-free water). Use a dropper to moisten the swab.
  - Use the moistened swab to thoroughly wipe the target area where you expect the suspect to have touched. It is necessary to wipe the target area several times under some pressure, rotating the swab as you move it so that its full surface area can be used. It is recommended to take two swabs (possible repeat extraction).



FLOQSwabs (photo: Zdeněk Novák)

- After use, place the swab in a collection tube in which you have previously made a hole with **sterile scissors** (air access for the swab to dry), break off the stick and close the collection tube. Place it in a paper envelope or bag and store at room temperature.
- Swabs with an active desiccant can also be used, which do not need a hole in the collection tube for air access.

Procedure of placing the collected sample in a test tube (photo: Zdeněk Novák)

- **Sampling by adhesive tape:**
  - Remove the protective film from the adhesive part of the tape. Repeatedly press the strip of tape over the target area (until the tape stops sticking). Next, place the strip in the collection tube included in the set, close and store it at room temperature.

Hair

- Sample hair by pulling it out (for animals unused to handling, this should be done as quickly as possible). Plucking with surgical gloves is best, using tweezers is not as effective (tweezers often do not hold the hair). Pull out about **10-15 hairs** with their roots (the hair bulb/follicle contains most of the DNA). Handle the hairs by the tip, not the root.
- It is also possible to collect hair that has fallen out (hair caught in scratching places, in passages, on fences, in snow, etc.), but the yield of

Tiger hair sampling (photo: Dominika Formanová)

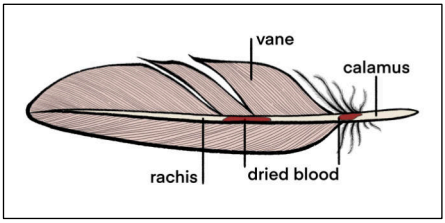
DNA analysis is low, depending on the age of the hair. In these cases, collect as many hairs as possible. For carnivores, take care not to collect hairs from consumed animals (there are many in the enclosures).

- Keep the collected hairs dry at room temperature in paper envelopes (great care is needed when removing the hairs from the envelope, if necessary, shining with a flashlight inside the envelope may help not to miss the hairs in the corners of the envelope). Completely dried material can be stored in plastic bags.
- Another option is to place the hairs in plastic microtubes (1.5 or 2 ml test tubes) filled with 96% ethanol. Store them in a freezer at -20 °C. The advantage of the test tube is the visibility of the hairs (in a paper envelope they may be overlooked or spilled).

Feathers

- The most suitable for DNA extraction are contour feathers with a strong calamus.
- Collect feathers by plucking 3-4 pcs from the breast area of the bird. The feathers should be plucked (not cut) to preserve the tip of the quill with the tissue or blood.
- DNA can also be extracted from moulted (shed) feathers (lower DNA yield). Do not collect feathers contaminated with faeces. The dried blood clot, located inside the rachis about where the feather vane begins, can also be used for analysis. Feathers that are several years old can also be analysed in this way.
- Store removed material at room temperature in sealable paper bags.

Feather collection of Hyacinth Macaw (photo: Zdeněk Novák)



Blood clots inside a feather quill (drawing: David Říha)

Eggs

- Genetic analysis can be carried out from egg shells after hatching (the membrane on the inside of the shell is analysed) or from the lifeless egg content. It is best to send the shell or egg to the laboratory as a whole.
- Store shells in a breathable container to allow them to dry. Another option is to use silica gel or fix it with 96% ethanol.
- Whole eggs can be stored in a refrigerator or freezer at -20 °C.

Caviar

- If the eggs appear homogeneous, take ideally 5-10 eggs.
- If the eggs appear heterogeneous, e.g. different in colour or size (perhaps from more fish species), take at least 5 eggs from each part or package showing heterogeneity.
- Place the samples in 96% ethanol (the ratio should be 1/3 sample to 2/3 ethanol) and store in a freezer at -20 °C.

Teeth, ivory

- Secure teeth or items made from them as a **whole**. Place in a breathable container (in case of residual moisture) or plastic bag (completely dried items).
- Samples from these items are **not taken on site**, but only afterwards at the storage site or the laboratory.
- The sample for DNA analysis is obtained from the larger teeth/tusks by drilling, and smaller teeth are ground whole. The dental material is very hard, so suitable tools must be used.
- When drilling teeth, pulp material from **the inside of the tooth** has a higher yield of DNA, therefore it is necessary to drill mainly in the area around the pulp cavity and obtain approx. 1-1.5 ml of powder.
- Cutting off the tooth root of the tooth or part of it is also an option (used, for example, when taking samples from cleaned skulls; the cut tooth can be glued back into the skull after collection and the intervention is not visible). For sampling, you can use, for example, a Dremel mini milling machine. It is advisable to wear a face shield when cutting, as pieces can fly off. If the tooth is to be returned to the skull after extraction, do not cut the roots through the thickest point (the tooth would fall through the hole and not hold in place).
- In ivory, it depends on whether the whole tusk or the carved object is sampled.
  - For tusks, the sample is cut from the **base of the tusk** (the part connected to the skull), where the tusk grows from and where the DNA is also most concentrated. The sample should measure approximately 3 cm x 3 cm and be 1 cm thick. If the base of the tusk is very thin, it is better to start cutting a few centimetres from the base so that the final sample is at least 5 mm thick. Use an electric grinder with a disc or a circular saw, etc.

Samples of ivory
(photo: Zdeněk Novák)

for cutting. After cutting each sample, the saw blade must be cleaned with a 10% bleach solution (100 ml of bleach per 900 ml of distilled water) or 96% ethanol.
    - In carved objects, it depends on how much the object can be damaged. For a successful DNA analysis, approximately 1.5 g of material needs to be drilled. It is more convenient to drill from the base of the object, where the subsequent hole is less visible.
- Store the collected material in sealable bags or test tubes at room temperature.

Bones

- Secure the bones as a whole and send them to the laboratory. NOTE on the sequence of the examination: if the bones are to be assessed morphologically, this must be done before the genetic examination (the morphologist must receive the bones complete and intact, i.e. before sampling for genetic analyses).
- Store wet bones in plastic bags and freeze at -20°C. Transport them in thermoboxes. Pack the dried bones in breathable or semi-permeable packaging (paper packaging, Stericlin-type bags, etc.). Dissected and completely dried bones can be packed in plastic bags.
- Bone samples are **not taken on site**, but usually in the laboratory or at the storage place (geneticists can take the samples themselves).
- Smaller bones are used whole (ground) for genetic analysis. Samples are drilled from larger bones. When drilling, targeting places where the bone is not hollow or where blood vessels lead is recommended. If there are remains of tissues, ligaments or tendons on the bone, or if the bone marrow is preserved, these tissues are a more suitable source of DNA (they have a higher yield than bone tissue).

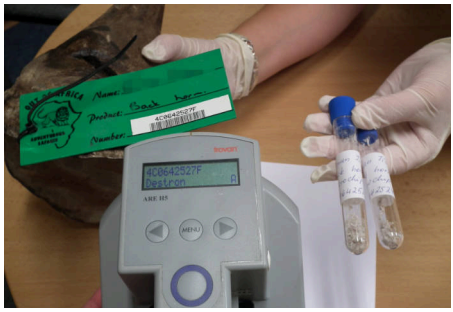
Horns, antlers

- Secure complete the horns or antlers and send them to the laboratory. NOTE on the sequence of examination: if the horns/antlers are to be assessed morphologically, this must be done before the genetic examination (the morphologist must receive the objects complete and intact, i.e. before sampling for genetic analyses).
- Sampling of horns or antlers is **not carried out on site**, but usually in the laboratory. Samples are cut off with a saw or file, or drilled.
- A special procedure is used for the sampling of **rhinoceros horns**:
    - Use an electric drill and drills with a diameter of 4-5 mm or similar for sampling.
    - Drill in the centre of the horn base or from its side.
    - When drilling, keep the speed very low to avoid excessive heating of the drill bit. Drilling too fast will destroy DNA. If you can smell burnt keratin (the smell of burnt hair) you are drilling too fast.
    - Drill out several spiral shavings. Place them in a sealable test tube

and store them at room temperature. Samples must be stored out of direct sunlight at all times.

- For each additional sample, use a new drill or sterilise the used one.
- If the horns are already in the form of chips, shavings or powder, there is no need to drill; only take a sample.

Sampling of rhinoceros horn (photo: Dominika Formanová)



Rhinoceros horn samples and microchip identification (photo: Dominika Formanová)

Claws, scales and other keratin derivatives

- Secure claws, scales, etc. as a whole and send them to the laboratory. NOTE on the sequence of examination: if claws, scales, etc. are to be assessed morphologically, this must be done before the genetic examination (the morphologist must receive the objects complete and intact, i.e. before sampling for genetic analyses).
- Sampling of claws, scales, etc. is not carried out on site, but usually in the laboratory (geneticists can take the samples themselves).
- Samples are taken by drilling, breaking off or cutting off a piece of claw or scale. Also, a thin layer of keratin can be removed from the part of the claw that is closer to the finger. Approximately 50-250 mg of material is usually required for one DNA isolation.
- When drilling, keep the speed low (see rhinoceros horns). Take a few drilled spiral shavings. Regarding scales, collect 1-2 pcs (more in smaller animals). Preferably, take the scales on which the skin remains have adhered (higher DNA yield). If there are several packages (e.g. shipments of pangolin scales), take one scale from each package at a minimum.
- Store dry samples in breathable containers at room temperature. If samples are wet, store frozen at -20 °C.

Pangolin scales (photo: Dominika Formanová)

Leather, furs, mounted and stuffed taxidermy

DNA analyses are more effective with raw, salted or dried skins. Completely tanned skins and furs contain many chemical inhibitors, of which chromium salts in particular damage DNA. The success rate of DNA analyses for this type of specimen is very low. When analysing samples from stuffed animals, the possibility of so-called **fake taxidermy** must be taken into account. Taxidermists often supplement missing or damaged animal body parts with remains from other individuals. A taxidermy can thus be composed of several animals. If necessary, samples must be taken from several parts of the specimen.

- Secure complete skins, furs or taxidermy. Pack dry items in plastic or paper bags, and wrap larger exhibits in bubble wrap. Freeze wet items.
- Sampling of skins, furs, stuffed or mounted animals is **not carried out on site**, but usually at the storage, or in the laboratory (geneticists can take the samples themselves). Small items can be delivered to the laboratory directly. For larger items, it is more appropriate to arrange a sampling with the geneticist at the place of storage place of the seized items.
- Use sterile scissors (cut of approx. 5 cm^2) to take fur samples. Take 200 mg of material at a minimum for one DNA isolation. The success rate of DNA analysis is significantly higher for fresh or dried untanned skins than for tanned ones.
- If paws (Carnivora) are preserved on the taxidermy, you can try to take a sample by drilling foot pads (there may be dried material inside, which may not be so disturbed by chemical agents).
- If the fur cannot be damaged, it is possible to try pulling out the hairs with hair bulbs (do not cut). However, the probability of success of the analysis with hairs from tanned furs is low.
- Store dry samples at room temperature in breathable containers. Store fresh or wet samples in a freezer at -20 °C.

Taking a sample from the paw of a lynx cub taxidermy (photo: Pavla Říhová)

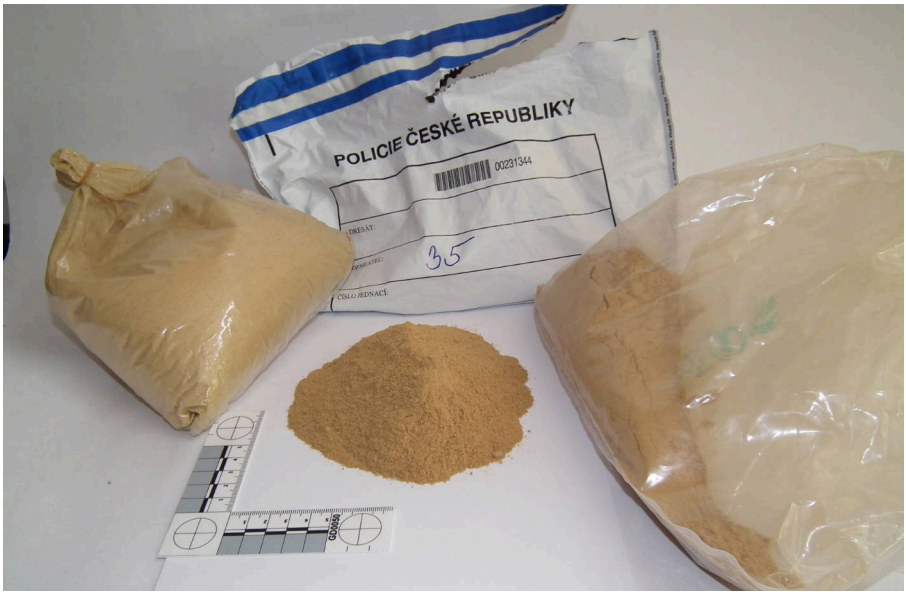
Traditional medicine

Products of traditional medicine can be in the form of tablets, ointments, plasters, drops, infusions, dried mixtures, parts of tissues, loose powders, etc.

- Secure the material in its entirety and its original packaging.
- Samples from traditional medicine are **not taken on site**, but usually at the storage place or in the laboratory (geneticists will take the samples themselves). Consult an expert or laboratory in advance about the

possibility of analysis and the volume of material required.

- When considering the assignment of genetic analysis, take into account the **definition of a specimen** according to Council Regulation (EC) No. 338/97 (Article 2, letter t), which states that a specimen of the species listed in Annexes A-D shall mean also any goods which appear from an accompanying document, the packaging or a mark or label, to be or to contain parts or derivatives of these species.
- Collect samples of powders, dry preparations, extracts, infusions or creams in plastic tubes or resealable containers. Store at room temperature (extracts or infusions may be stored in the refrigerator depending on their condition). Regarding the quantity, we recommend taking a larger volume (at least 100 ml) for a possible repetition of the analysis.
- In the case of the bile, take at least 1-2 ml of bile or crystals into a test tube. Store it at room temperature. If the entire gallbladder is seized, submit it to the laboratory as a whole. If the gallbladder is wet, store it in the freezer at - 20 °C (it can be stored in a refrigerator for a short time). Store the dried gallbladder in a breathable container (drying) at room temperature.



Tiger powder used as traditional medicine in the Vietnamese community in the Czech Republic (photo: Dominika Formanová)

Pathology

Veterinary pathological examination is a key part of investigations into animal cadavers. The goal is to determine whether the animal died from natural or unnatural causes. A pathologist can determine not only the cause of death, the manner of death (e.g. blunt trauma, shooting) and the reason (e.g. collision with a vehicle), but can also determine whether the animal was abused or define the causes of poor health. For pathology purposes, samples are **not taken directly on the location;** cadavers or their remains are secured as a whole. The veterinary pathologist takes the necessary samples during the examination.

Not all dead animals found have been killed illegally; many deaths occur in nature as a result of disease, age, intra-species fights, predation or natural accidents. Animals can also die due to collisions with vehicles or trains. It is often impossible to distinguish between these situations in the field - not all animals that are hit by a car die immediately on the spot (larger species in particular may travel some distance from the road or track injured before bleeding to death due to internal bleeding or ruptured organ(s). Poachers can also place a shot animal next to the road or track to make it look like it has been knocked down (cases of this type have been recorded multiple times in the Czech Republic with poached wolves). On the surface of the body, a gunshot wound can look like the result of a collision with a vehicle and vice versa. Finding an animal in a field or forest does not rule out a collision with a vehicle, and vice versa finding an animal near a road or track does not rule out poaching!

A lynx hit by a car in the Beskydy Mountain (photo: Michal Bojda)

Even other causes of death can be easily confused without a more detailed pathological examination - the entry of a tooth when bitten by the beast can resemble a gunshot wound, a bird struck by electricity can be in a position similar to carbofuran poisoning, etc. Animals caught in meshes or traps can be subsequently shot or beaten to death, so the death may not be directly caused by the trap (marks of strangulation, blows or shooting may remain on their body).

Imprint of the body of a poached lynx in the snow (photo: Josefa Volfová)

A specific situation is the poaching of lynx or wolves. The cadaver is usually taken by the hunter as

a trophy by the hunter and not left in place. Even so, under certain circumstances, it is possible to find traces in the field - blood or remains of animal hair, the imprint of the body in the snow or the soil, footprints of the perpetrator, tyre prints, etc.

Pathological examination is performed macroscopically (**necropsy**) and microscopically (**histological examination of organs and tissues**). Histopathological examination of tissues can prove whether it is an old or a recent injury (this is important, for example, in cases of abuse). Samples are usually taken during the pathological examination for further necessary analyses, e.g. toxicology.

The most complete information about the cause of death can be obtained from fresh, unfrozen cadavers. The freezing process damages the cell (after thawing, the cells rupture and haemoglobin spills out of them), the tissue situation imitates congestion and the histological examination does not give a realistic picture (some tissues, e.g. skeletal muscle, are less susceptible to this type of damage). Even a bacteriological examination cannot be performed on frozen cadavers (also bacteria are damaged by temperatures below zero). It is therefore important, if the situation allows, **not to freeze** the cadaver before dissection, but **only to cool** it and deliver it to the relevant veterinary pathology department as soon as possible.

However, the post-mortem processes take place, even in the cold, albeit slowly, therefore cooling should last **a maximum of 24 hours**. If the necropsy does not take place by this time, it is better to freeze the cadaver immediately. It is always **better to consult a pathologist** about the real situation and procedure (cooling × freezing).

Various circumstances influence the speed of postmortem processes, which occur almost immediately after death. If a fresh cadaver is placed in the refrigerator, its cooling rate also depends on its size and the cause of death. If the animal was fat, died as a result of convulsions, fever, or has a thick fleece (sheep), the body cools much more slowly despite refrigeration, and internal autolysis can occur quickly despite cooling.

A poached wolf in an advanced stage of decomposition, Broumovsko region (photo: Jan Koranda)

Dead animals in wildlife crime cases are often found in advanced

stages of decomposition, in which case it is necessary to **freeze the cadaver as soon as possible** (histological examination will not show much).

On the spot

- Use protective equipment when handling cadavers (see the Protective equipment, personal safety chapter).
- Handle animal cadavers at the crime scene judiciously and sparingly to prevent the loss of possible evidence such as projectile fragments, etc. Fibres from the suspect's clothing can stick to the feathers or fur of the animal and the touch DNA can also stick to certain parts of the cadaver if someone has manipulated it.
- If the animal was also disembowelled at the place of killing, the entrails should also be secured and examined (possible presence of bullets).
- If traps, snares or baits are in place, they may contain fingerprints or touch DNA. Perpetrators check traps (especially hawk baskets with live bait), so there may be other traces in the area.

A poached lynx with intestines bulged out,Vimperk region (photo: Luděk Bufka)

- To find bullets in the field, you can use a hand-held metal detector, which can also be used to preliminary check the presence of a bullet inside an animal, try to find a bullet that passed through the body, etc. Smaller metal particles may not be detected this way. Therefore subsequently, an X-ray of the cadaver should be performed before the necropsy.

Cadaver packing and transport

- Handle the cadavers with gloves and wrap them tightly in multiple plastic bags to prevent leakage of fluids and odours. Cover sharp edges (beaks, claws, teeth, broken bones...). For cadavers in a more advanced stage of decomposition, if it is suspected that the animal was shot, it is also possible to dig up and secure the ground under the body where a bullet or its fragments could have fallen (for this reason, pathologists often

Head of a beaver: Such specimen must be packed in an impermeable package to prevent leakage of fluids (photo: Dominika Formanová)

dissect shot animals on absorbent pads to capture anything that falls out).

- Cool/freeze the remains and **transport them to necropsy** as soon as possible after agreement with the veterinary pathology department. Regarding temporary cooling (max. 24 hours) before performing the necropsy, the remains should be placed in a cool place or a refrigerator. Refrigerators or freezers used for cadavers storage must not be used for storing food or feed.
- If the necropsy cannot be performed immediately (this also depends on the possibilities of the veterinary workplace), freeze the cadaver. Cadavers that are in a more advanced stage of decomposition should be frozen immediately. If poisoning is suspected, also baits can be stored frozen.
- In cases of **mass fish mortality**, fish cadavers are kept refrigerated (not frozen). In a multi-species fish stock, take 3-5 specimens of each species (sorted by gender and weight), in a single-species fish stock, take 5-20 specimens. If possible, also secure live fish with signs of injury/damage (transport them for analysis in the water they were originally). At the same time, take a sample of 2-4 litres of water (preferably in a glass sample box) and approx. 2 kg of sediment from the bottom. Take water and sediment samples upstream of the site of death (in areas of suspected contamination), at the site of the fish mortality, and downstream from the site of death. Refrigerate the samples and transport them to the toxicology laboratory as soon as possible. If the transport takes longer, freeze the samples.
- When transporting by car, place the wrapped cadavers in an impermeable tub/box or on a waterproof mat. You can place an absorbent pad on the bottom in case of fluid leakage. If they are available, it is advisable to use cooling systems even during transport (e.g. thermobox and ice packs usage).

Cadavers of birds of prey found in the suspect's freezer were packed separately and transported for necropsy (photo: Pavla Řihová)

Submission for necropsy

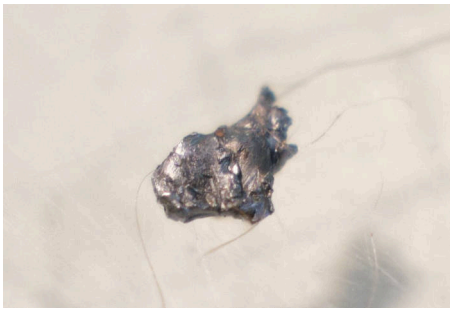
- Give the veterinary pathologist all the necessary information (it is also appropriate to provide photographs), such as the location and position of the cadaver, a description of the locality and the context of the entire site (forest, meadow, proximity to water, position in the sun, in the shade, ambient temperature, etc.).
- All cadavers suspected of being shot must be **x-rayed BEFORE**

necropsy! An X-rays can be used to rule out or confirm gunshot wounds, locate shotgun pellets (including those that have "healed") and metal particles in the body, discover fractures caused by polytrauma, etc. External examination before the necropsy alone is not sufficient, as the appearance of an injury can be misleading (an animal hit by a car can look like it has been shot and vice versa), bullet entrances in fur or feathers may not be visible on the body surface, etc.

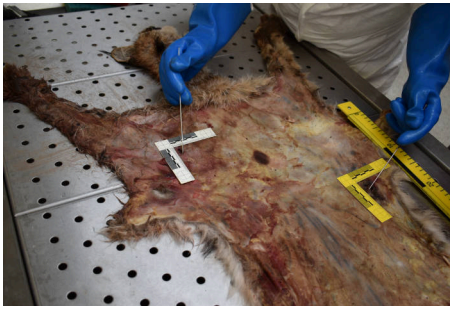
External examination of a killed otter (photo: Dominika Formanová)

- After removing the cadaver from the package, it is necessary to carefully examine the package to see if any evidence has fallen out of the body during transportation (bullet fragments).
- During the necropsy, samples are taken as required for toxicological, bacteriological, virological examination, etc.

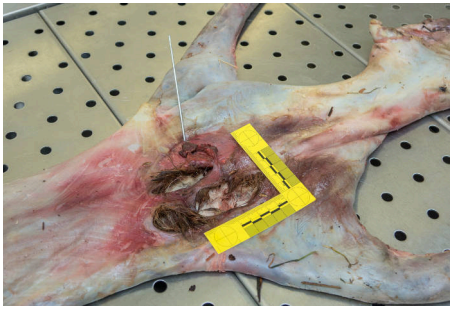
Shot lynx (photo: Customs Administration)



Bullet fragment taken from the body of a poached beaver (photo: Pavla Říhová)



Marking of the entry and exit gunshot wound on the lynx skin (photo: Pavla Říhová)



The exit wound is usually significantly larger than the entry wound, as seen here in the lynx skin (photo: Pavla Říhová)

Toxicology

In cases of animal poisoning, animal cadavers and any baits are seized as a whole. Samples for toxicological analysis are not taken at the site of the discovery in the field; these are taken by a veterinarian during the animal's necropsy.

In cases of suspected carbofuran poisoning, you can use special **rapid tests**, which can be used to roughly verify the presence of carbofuran residues, directly at the site of discovery. The price of one test is approx. EUR 6. Our team successfully tested **Smark!T CBF tests** from Nankai Biotech Co., Ltd. (www.nkbiotech.com). **Warning: tests must not be stored in the freezer**.

How to use the rapid test:

- Use a cotton swab to wipe the mouth, beak or throat of a dead animal or wipe the bait.
- Place the swab in the test tube. It is also possible to put an entire smaller sample (approx. 2 g, ideally multiple small pieces) into the test tube.
- Add 6 ml of solution "A" (A Solution) and shake vigorously for 30 seconds.
- Let the solution with the sample stand undisturbed.
- Draw 1.5 ml of the upper layer of the solution with a pipette and mix it with 3 ml of PBST buffer (dilution 1:2) in another test tube).
- Take 0.1 ml of the mixed liquid with a pipette and apply to the tester (section "S").
- You can read the test result after 3-5 minutes. If carbofuran is present, darker red colour line appears at section "C".

Use of rapid tests in the field (photo: Klára Hlubocká/CSO)

Packaging of cadavers of poisoned animals

- Pack the cadavers in solid plastic bags or into bags in two layers. Put each individual find in a special bag, mark it with a number, tie and seal, and record the exact location. In some cases (e.g. multiple smaller birds of the same species) multiple cadavers can be packed into a small bag.
- Collect smaller baits, tissue remnants or vomit in smaller plastic bags.
- Pick up any **dead insects** lying on, near or under the cadaver. Place the insect in a test tube.

- Safest procedure for **packing a poisoned animal cadaver**:
  - Prepare two plastic bags. Put on two pairs of gloves, one on top of the other.
  - Roll up the first bag, place the cadaver on the bottom and roll the bag back up (you can also grab the cadaver over the bag and pull it over the body). Be careful not to contaminate the outside of the bag with body fluids.

Poisoned eggs used as bait, showing clear punctures after the application of poison (photo: Klára Hlubocká/CSO)

  - It is not advisable to throw the cadaver into the bag - the impact with the bottom raises aerosol and dust that can be inhaled. Hold the opening of the bag away from you as you close the bag and squeeze the air out of it. It is advisable to use a mask when handling.
  - After closing the first bag, take off the top soiled pair of gloves and throw them into the second bag, into which you also put the first bag with the body. Handle the second bag with the second, clean pair of gloves (this ensures that there is no contamination from the cadaver on the outer surface of the package). Take off the second pair of gloves before closing the second bag and throw them inside. Mark the outside of the bag well so that it is obvious that it contains hazardous material.

Cadaver transportation

- In the transport area of the car, place the wrapped cadavers in an impermeable tub/box or on a waterproof mat. You can place an absorbent pad on the bottom in case of fluid leakage. It is appropriate to use cooling systems during transport, if available (e.g. placing in a thermobox and using ice packs).

A poisoned white-tailed eagle (photo: Klára Hlubocká/CSO)

- If it is necessary to temporarily store cadavers prior to the necropsy, place them in a cool place or in the refrigerator (freezing will change the histological findings); baits can be stored frozen. Refrigerators or freezers used for these purposes must not be used for storing food for people or animals.

Sampling for toxicological examination

Samples for toxicological analyses are not taken at the site of the discovery, but are taken by a veterinarian during the necropsy.

- The most important sample is the content of the **animal's stomach (in birds, also the content of the crop), vomit and bait**, in which the poison has not yet been metabolised. Furthermore, samples are usually taken from the **liver**, where the toxins have already been metabolised. Samples of dead insects can be important and can also be analysed for toxins.
- **Recommended sample types** when suspected:
  - **carbofuran** - stomach content, vomit, presumed bait, liver, insects
  - **rodenticides** - stomach content and the stomach itself, liver, kidney, intestinal content including intestine
  - **Stutox II** - liver, kidney (tissues must be collected as soon as possible and stored in an airtight, unbreakable container, as phosphane gas is highly volatile)
  - **heavy metals** - liver, kidney, in some cases blood
  - **pharmaceutical** - liver, kidney, blood and urine.

Carbofuran
(photo: Dominika Formanová)

- Samples must be collected in plastic sealing containers, which are then packed in plastic bags (double packaging principle). They should be stored and transported frozen at -20 °C. The accurate labelling of containers, compliance with the chain of custody and use of appropriate protective equipment are essential, as many substances can be hazardous to humans.
- In cases of **mass fish mortality**, fish cadavers are kept refrigerated (not frozen). In a multi-species fish stock, take 3-5 specimens of each species (sorted by gender and weight), in a single-species fish stock, take 5-20 specimens. If possible, also secure live fish with signs of injury/damage (transport them for analysis in the water they were originally). At the same time, take a sample of 2-4 litres of water (preferably in a glass sample box) and approx. 2 kg of sediment from the bottom. Take water and sediment samples upstream of the site of death (in areas of suspected contamination), at the site of the fish mortality, and downstream from the site of death. Refrigerate the samples and transport them to the toxicology laboratory as soon as possible. If the transport takes longer, freeze the samples.

Radiocarbon dating

Samples for radiocarbon dating are **not taken directly on the spot**, but subsequently at the storage place of the objects or in the laboratory. Samples are collected in resealable plastic bags, squares of aluminium foil or test tubes/vials. They **MUST NOT be placed in paper envelopes** due to contamination of the sample with carbon from the paper fibres.

Solid specimens (bones, ivory, horn …)

- When collecting, place the object on a surface covered with aluminium foil, do not place it on paper.
- Take the sample by drilling (clean the drill bit with 96% ethanol) or by cutting off a smaller piece with a mini-grinder. Drill very slowly to avoid overheating of the material and possible temperature degradation. Place a small square of foil under the drill hole to catch the drilled material.
- The minimum amount of hard tissue removed is **100 mg** of material. If possible, take more material (200 mg). Weigh the quantity continuously on appropriate scale.
- Collect the drilled material in a plastic or glass test tube.
- For ivory objects, take the sample from the **bottom of the base** (the hole will be less visible).
- Sample tusks or larger objects longitudinally in two places.
- For rhinoceros horns, take the sample at the base of the horn, and approximately in the middle.

Sampling of ivory for radiocarbon dating (photo: Zdeněk Novák)

Sampling of rhino horn for radiocarbon dating (photo: Pavla Říhová)

Hairs

- The minimum quantity of hair to be taken is **7 mg**, but it is preferable to take a larger sample.
- Remove the longest hairs (for easier handling). It does not matter where on the body the hairs are taken from, but beware of so-called fake taxidermy (making a mount from multiple individuals).
- Place the sample in a folded square of aluminium foil, place it in a plastic bag. Do not put hairs directly into plastic bags due to static electricity.
- If possible, take samples of hard tissue (bone, tooth) and hair from the same individual. This combination will greatly refine the dating.

Chemistry

There is a wide range of chemical methods, some of which are also applicable in wildlife crime cases. It is best to consult with the relevant specialised department in advance for specific options and procedures. Some methods (e.g. spectrometer measurements) do not require sampling, but others do. Sampling may need to be carried out on site (e.g. bloodstain analysis using proteins), while in some cases (e.g. wood analysis) samples are taken afterwards at the storage site or in the laboratory.

Use of spectrometers

There are various types of spectrometers (including mobile types) that only need to be attached to the object under examination in order to make the analysis. The surface of the object to be measured must be properly cleaned so that the measurement is not affected by contaminants. Spectrometers identify the substances or elements of which an object is composed, but are **not able to identify a particular species of animal or plant**. They can be used to distinguish between imitations made from other materials, e.g. to distinguish between real and fake ivory (they can recognise the artificial material but as for the real one, they cannot distinguish whether it is elephant, mammoth or walrus ivory, etc., since the composition of dental material is more or less the same in all mammals).

X-ray fluorescence spectrometry determines the elemental composition of an object. The instruments used are Olympus ED-XRF DELTA, XRF VANTA, ProSpector 3, etc. In wildlife crime cases, these spectrometers can be used to measure, for example, the presence of arsenic in taxidermy mounts. Arsenic was used in animal mounts as a preservative until about

Measurement of ivory using a Delta X-ray fluorescence spectrometer (photo: Pavla Říhová)

1990 (in the Czech Republic), and therefore high arsenic concentrations are specific to old mounts.

Raman spectrometry is used to identify substances/mixtures. Devices used include, for example, TacticID or Progeny Resq. These spectrometers can also measure at a distance and through transparent packaging (e.g. liquids in a bottle); they are mainly used for the detection of explosives, drugs and chemicals. A laser beam is used for the measurement, as the sample may be damaged if higher wavelengths are used.

Measuring of the false rhinoceros horn using a Raman spectrometer (photo: Dominika Formanová)

Chemical analysis

In the Czech Republic, chemical analyses are most often used to identify wood (Customs Laboratory). A sample must be taken from the object for analysis. It is advisable to leave the sampling to the laboratory, it is usually sufficient to take a relatively small amount of shavings. Mass spectrometers based on liquid or gas chromatography are used for analysis.

Wood samples for comparative analyses (photo: Zdeněk Novák)

Different types of chromatography can also be used to identify animal and plant species in traditional Chinese medicinal products (e.g. determining the presence of bear bile). However, there is still no laboratory in the Czech Republic capable of carrying out these analyses.

Protein analyses

Protein analyses can be used to identify animal species, e.g. from blood stains, flesh (even cooked), etc. Specific immunoassay tests exist to distinguish particular species. The only known test for wildlife so far, is the Bear Detection Kit (test for bear proteins), which was developed in the United Kingdom and is used for the rapid verification of traditional medicine products.

Parasitology

Ectoparasite sampling

- Remove samples with fine tweezers, by cutting off feathers, hairs, scraping with a scalpel...
- In cadavers, do not remove insects that attack the body post-mortem (flies, worms, beetles), and collect only obvious external parasites (ticks, lice, fleas, etc.).
- Place collected parasites in tightly sealed plastic containers with 96% ethanol (not denatured). The volume of ethanol should be at least 5 times and ideally 10 times the volume of material collected.
- Store samples in a refrigerator (approx. temperature +4 °C) or freezer.

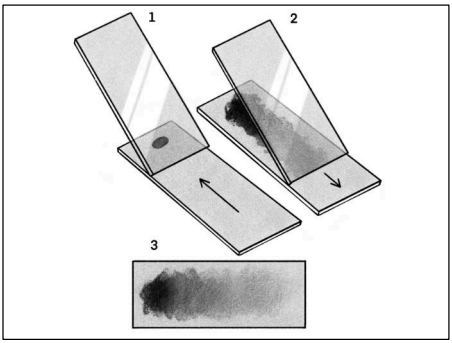
Adult tick Hyalomma aegyptium on a Greek tortoise (photo: Jana Bulantová)

Collection of faeces

- Collect fresh faeces using a sterile tool in a plastic zip-lock bag or in a closable plastic container. The sample must be matched to the relevant examined individual (e.g. if there are multiple animals in the enclosure or shipment).
- It is sufficient to take a sample about the size of a hazelnut, for fragmented faeces (droppings) do a mixed sample (from a single identified individual) from several pieces.
- Store the samples frozen, storage in 96% ethanol is less suitable.

Blood collection

- Blood samples are taken by a veterinarian, usually during the examination of seized animals. Blood is drawn into a plastic container.
- Keep fresh and clotted blood samples frozen or in 96% ethanol.
- If the blood is fresh and uncoagulated, it is advisable to take a "blood smear" - spread a drop of blood on a microscope slide and allow to dry.



Procedure for blood smear on slide (drawing: David Říha)

Sampling of organs, tissues, etc.

- Take the sample using a sterile scalpel or tweezers.
- Place samples in plastic zip-lock bags or test tubes (depending on the type of sample).
- Store in 96% ethanol or in a freezer at -20 °C. CAUTION - fixation of tissues in 10% formalin (= 4% formaldehyde) used for possible histological examination of tissues will prevent the determination of parasites by molecular biological procedures.


Bird lice in the feathers of common blackbird (photo: Jana Bulantová)


Aponoma latum tick on a royal python (photo: Jana Bulantová)

Forensic entomology

On the spot

- Use the entomological sampling kits supplied by the Institute of Criminalistics to secure insect specimens and their life stages.
- Securing entomological traces at the site of a cadaver find is a non-repeatable task. Secure the maximum amount of insects of all shapes and sizes and from various locations on and around the cadaver.
- Collect samples using tweezers, a plastic spoon or spatula. Measure the temperature at the point of examination and subsequently when storing the traces.
- The primary sites of insects on a cadaver are the eyes, mouth, nostrils, ears, rectal area, skin folds, and wound sites. In the later stages of decomposition, insects are found anywhere on the body.
- Entomological traces are primarily submitted for expert examination alive. Take **live insect samples in containers with perforated lids** (live insects need air). Place different looking larvae and eggs separately in multiple containers. Ensure a minimum of 200 eggs, 200 larvae and possibly 200 pupae in separate containers. Fill the containers to a maximum of 1/4 or 1/3 full.
- For each sample, record the date, time and location from which the sample was collected (head, wound, etc.).

Larvae of II. and III. instar of flies of the family Calliphoridae on an animal cadaver (photo: Hana Šuláková)

- Collect a secondary sample of the killed insects. Pour the killing and preservative solution (use 96% ethanol in the absence of a special solution) into a container and place the insects, i.e. all adults and larvae with limbs, e.g. flies, beetle larvae, mites, add a representative sample of eggs, larvae and fly pupae.
- Always take samples also from the place where the cadaver is lying (under the body) and from its surroundings (the larvae migrate out of the body, it is difficult to tell whether they have left the body or not). Take soil samples (to a maximum depth of 5-10 cm), including vegetation and insects. Take 4-6 samples (approx. 250 ml each, optimally 2-5 kg in total) from under the cadaver. Take samples from the area around the cadaver in a ray pattern to a distance of 1-2 m; take 10-15 samples (approx. 250 ml each, optimally 3-5 kg in total). It is not necessary to separate entomological material from the soil.
- Do not fill the bags with soil samples to the top and do not tie them just

above the soil. Leave an empty space above the soil and tie firmly.
- For buried cadaver, secure the vegetation and soil taken during the uncovering of the cadaver (above and around the body), optimally a 5-10 kg sample. Entomological traces documented at necropsy may also be secured.
- If the cadaver is found in an aquatic environment (body floating on the surface), secure separately the invertebrates from the body parts above and below the water surface. Kill aquatic species (crustaceans, molluscs, etc.) and place them in a suitable container.

Photographic documentation

- Document the environment around where the cadaver was found.
- Before any handling, document the position and location of the cadaver in the space - overview photos, semi-details and details.
- Document any factors affecting insect development.

Transport

- Use containers with holes in the lid or large enough shipping containers with an air pocket to transport live insect specimens.
- Place the killed insects separately in a bag (do not put them in containers or bags together with live specimens - live insects may be killed unintentionally by the vapours of the lethal solution). Close bags or containers properly.
- Label soil and insect samples from around the cadaver with a detailed description of where the sample was taken.
- If the secured traces are not immediately transported to the expert laboratory, place the samples in a refrigerator or a cold room as soon as possible, optimally at a temperature of 2-6 °C. Samples can be stored in this way for a maximum of 3 days. **Never freeze these samples!**
- Significant temperature changes (exposure to frost or heat in a car) must be avoided when transporting samples. It is advisable to use a thermobox.

Flies of the genus Lucilia laying eggs in open wounds (photo: Hana Šuláková)

Larvae of the Necrodes littoralis on animal cadaver (photo: Hana Šuláková)

Stable isotope analysis

Samples for stable isotope analysis are **not taken directly on the spot**, but subsequently at the storage place of the objects or in the laboratory. It is more appropriate if the samples are taken directly by an expert for the isotope analysis.

- Use sterile instruments and containers for sampling to avoid contamination.
- Use a sterile scalpel or scissors to remove tissue.
- Collect blood in sterile test tubes with a suitable preservative.
- Cut **ivory samples** from tusks using a small hand saw or break them off with pliers (for processed ivory, a mini grinder or drill is preferable). Samples should be cut from the base of the tusk as close to the skull as possible. The base is the youngest part of the tusk, so the isotopic signal reflects the environment in which the animal lived before death. Samples taken from the edge of the pulp cavity at the base of the tusk will provide geographic information for the final 6 to 12 months of the elephant's life. The weight of the ivory sample should be at least **30 mg** (fingernail size). Take a sample at least from two different spots.

Ivory sampling for stable isotope analysis (photo: Jitka Kufnerová)

- For **tortoise shell** sampling use a pair of pliers or a small saw to cut off samples. For **snake shed skins** remove skin shed preferably as a whole, or cut off a piece of adequate size.
- The required sample weight generally depends on which isotope is to be analysed, and may also vary depending on the specific analytical method and sample type. It is recommended to consult the sample weight requirements with the laboratory that will make the analysis. The greatest quantity of material is required for strontium analysis; approximately 0.2 g of sample should be taken (however, if there is a sufficient concentration of Sr in the material, a smaller sample will be enough). For carbon, nitrogen and sulphur isotopes, a sample of 500 µg - 1 mg should be sufficient, and for hydrogen and oxygen isotopes, 150 µg.
- Place the samples in sterile bags or containers.
- Label the samples, including the place and date of collection. Make a detailed record of each sample, including GPS coordinates of the collection site, for any field collections.
- Keep samples cool (e.g. in a refrigerator or portable cooler) and transport them to the laboratory as soon as possible.

Other possible traces or samples

Some forensic methods and procedures that are used in common criminalistic practice (especially in violent crimes) can also provide important information and evidence in wildlife crime cases. However, they are still used less frequently in the clarification of wildlife crime.

Fingerprints (dactyloscopy)

In cases of wildlife crime, fingerprints can be taken, for example:

- ✓ from inside smuggled shipments
- ✓ from set traps and live traps
- ✓ from jars with stored poison
- ✓ from eggs used as bait…

Dactyloscopic traces can also be analysed using genetic methods (touch DNA analysis).

In cases of animal poisoning where **poisoned eggs are used, fingerprints can be taken from the egg shells**. When injecting poison, the poisoner usually holds the egg around its widest part and seals the hole with duct tape or wax after application. It can be assumed that when handling the poison he uses gloves, but when handling eggs, taping or placing eggs in the field he may no longer wear gloves. Fingerprints are relatively greasy and should endure for some time, even in an outdoor environment. They can be taken with ordinary magnetic powder or flexible tape used to take fingerprints from light bulbs.

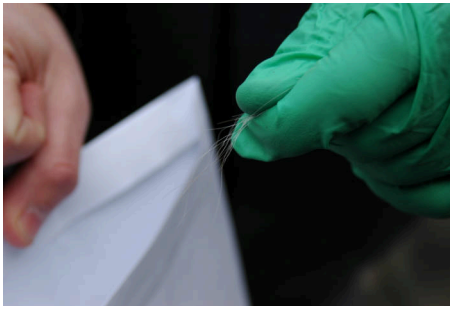
Trichology (fibres, hairs)

Examples of possible use in wildlife crime cases:

- ✓ fibres from clothing or rope caught on tree bark, rock, etc. during the illegal picking of nests of birds of prey
- ✓ hairs in the trunk of a suspected poacher's car
- ✓ hairs on the place where the cadaver was processed or on the tools used
- ✓ hairs from used traps…

- Take photos of the location where the trichological material was found before it was secured and handled - overview photos, semi-details, details. Take photos of precisely marked collection sites.
- Use sterile tweezers to collect the trichological material. Do not use adhesive tapes or gelatine films. Those hairs that are firmly stuck to the object should be secured together with this object if possible. In the case of fur products, seize the products as a whole.
- Store samples in breathable containers (paper envelopes) for drying. Trichological material must never be cleaned or rinsed. Fully dried material can be stored in resealable plastic containers.

- Comparative hair samples are taken to capture the overall variability of the animal's coat, both in terms of hair type (underfur, guard hair, vibrissae), as well as length, colour, waviness, etc. Samples are taken from different parts of the body, typically the forehead, the area between the shoulders, hind leg thighs, chest, abdomen, tail and upper side of foot.



Store the trichological material primarily in paper envelopes (photo: Zdeněk Novák)

Footprints, tire marks and tools prints

Examples of possible use in wildlife crime cases:

- ✓ footwear marks, tire marks at the site of poisoning or killing of wildlife
- ✓ marks from climbing equipment on trees
- ✓ prints of pliers used to set or modify traps
- ✓ examination of bird identification rings that may have been damaged/ modified
- ✓ marks from knives or tools used to kill or injure the animal (especially if they came into contact with bones)…

Soil, plant debris, paint residues, metal fragments etc.

Examples of possible use in wildlife crime cases:

- ✓ remains of soil on tools used to bury cadavers
- ✓ soil or plant residues in tire or shoe tread patterns
- ✓ bark and lichens on clothing or climbing equipment used to climb trees while robbing bird nests
- ✓ soil residue on ropes or climbing equipment used to steal eggs from the cliff-ledge nests of birds of prey
- ✓ glass fragments, debris from tools, vehicles, transport boxes or other material that may be related to illegal activity…

- Store fresh plant material in breathable containers (paper bags, envelopes, sacks, Stericlin-type bags) in a dry place at room temperature. Plastic packaging can be used for dried material. Plant microtraces provide primarily with a carrier (e.g. clothing, bark) or take a sample by scraping into a test tube or by taping onto transparent gelatine film.
- Use sterile cotton swabs to take pollen samples. Place the swab in the capsule/test tube in which you have previously made a hole in the cap with sterile scissors (air access). Place the tube in a breathable container (paper envelope).
- For timber, provide a sample of at least 1 cm^3.
- Pack soil samples in breathable material (drying).

Protective equipment, personal safety

The health of people may be at risk during the handling of living and non-living specimens of animals or plants and the collection of samples. In addition to possible injuries caused by live animals (handling of live animals is not the subject of this methodology), there is also a risk of transmission of zoonotic pathogens, especially of viral and bacterial origin. Pathogens of primates and carnivores pose the greatest risk to humans. Places where birds, rodents and bats defecate and where faeces accumulate also carry some risk. The transmission of zoonosis occurs as a result of injuries (sharp beaks, claws, bones...), insufficient hygiene at work or inhalation of spores from an aerosol when handling cadavers, dust contaminated with faeces, etc.

Therefore, **use protective equipment**, preferably disposable, at all times when securing biological traces and collecting samples!

When handling animal cadavers, disposable nitrile gloves (double ones are also used in some cases), a protective suit and protective overshoes (or rubber boots) are essential. Protective glasses (or a shield), mask or respirator prevent the inhalation of contaminated aerosol or dust.

Recommended protective equipment:
- ✓ disposable nitrile gloves
- ✓ Tyvek protective suit
- ✓ rubber shoes (or disposable overshoes)
- ✓ mask, respirator
- ✓ safety glasses
- ✓ paper towels
- ✓ disinfectant spray
- ✓ disinfectant wipes (e.g. Mikrozid AF)
- ✓ antibacterial soap
- ✓ basic first aid kit

Hygiene

Do not eat, drink, smoke or touch your face when handling biological material and samples. Tie back long hair or hide under a disposable head covering.

After handling, wash your hands thoroughly with antibacterial soap for at least 20 seconds. Dry your hands completely. Any scratches, cuts, punctures, splashes of liquid, etc. should be rinsed thoroughly with water and washed with antibacterial soap. Inform your supervisor of the risk of exposure.

All equipment that is not disposable must be thoroughly cleaned and disinfected when work has been completed. Use ethanol-based disinfectants (70% minimum) or chlorine-based disinfectants (10% bleach) to clean work surfaces or soiled containers.

Place disposable protective equipment in resealable bags after use and

dispose of them appropriately. In case of high risk, use biohazard waste bags or containers and disposal adequate for this type of material. Containers for infectious waste should be securely fastened in the cargo area of the vehicle when transported; do not transport them in the passenger space. Repeatedly disinfect bags and containers externally by spraying with disinfectant.

Securing of biological material in the facility used for preparing tiger bone broth during the Operation Trophy (photo: Customs Administration)

Poisoned red kite (photo: Klára Hlubocká/CSO)

The principles of forensic packaging

Think contamination
Especially when dealing with items for DNA analysis

Wear barrier clothing
- two pairs of gloves (change the top pair before handling another item)
- protective clothing
- a mask (avoid coughing or sneezing over the item)

Health and safety
Protect yourself and others

Eliminate the risk of disease transmission
- maintain hygiene, do not eat, drink or touch your face while working

Package items appropriately
- wrap sharp items (bones, claws, knives, antlers ...)
- prevent leakage of liquids and odours during transport (use suitable containers)

Integrity
Ensuring that the item and any trace evidence on it remains in the same state as when found

Ensure evidence is protected
- do not put wet items in airtight containers (unless you freeze them immediately)
- beware of fingerprint wiping

Record the details accurately on the exhibit label
- description
- location, date and time
- other relevant information

Make adequate photo documentation

Security
Demonstrating that any attempt to tamper with the item would be evident

Ensure packaging is sealed
- use tamper evident bags
- use tape and security seals

Continuity
Demonstrating that everybody who has been in contact with the item is recorded

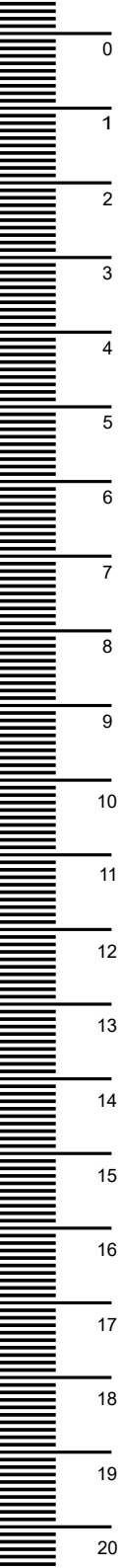
Each transfer of an item must be documented
- sign and date the bag/exhibit label in the appropriate places

Storage
Ensure your items are stored correctly to reduce degradation of evidence

Depending on the type of item, store in a dry, fridge or freezer

If in doubt, consult the investigator or the relevant laboratory

based on the material from the City of London Police, the United Kingdom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