CONVENTION ON INTERNATIONAL TRADE IN ENDANGERED SPECIES
OF WILD FAUNA AND FLORA

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Eighteenth meeting of the Conference of the Parties
Geneva (Switzerland), 17-28 August 2019

# TIGRIS ID: GUIDELINES AND SAMPLING PROTOCOLS

This document has been submitted by the Czech Republic in relation to agenda item 71.*

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# Tigris ID: Guidelines and Sampling Protocols


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## Background:

This document builds on the internationally distributed material representing the Czech project Tigris ID which aim is to **develop novel tools for DNA based identification** of biological material of *Panthera tigris*. Major components of the projects are:

- ✓ to develop and validate methods for species identification of *Panthera tigris* in heavy-processed material as broths, tiger paste, wine, boiled bones etc.
- ✓ to develop STR kit(s) for the individual identification of tigers (multiplex for simultaneous amplification of informative tetranucleotide STR loci)
- ✓ to create a database for the storage and comparison of DNA profiles from reference, inspection and unknown forensic samples of *Panthera tigris*.

The key point of the project is an **accurate population study** needed for kinship calculations and selection of informative STR markers and thus it is necessary to analyse the genetic material from as many unrelated tigers as possible. **The collection of any genetic samples of tigers and their shipment to the Czech Republic** is therefore very welcome. Although the research will be carried out by the Czech Republic, it would nevertheless be more effective if the information, experience and results will be shared with other countries. The aim of this project is collaboration with tiger range and consuming countries as well as with countries where tigers are held in captivity. **Mutual agreements about collaboration, sharing tiger DNA samples** and subsequently sharing the **results of genetic research** can be signed by authorities of concerned countries. The database of tiger DNA profiles can be easily extended to an international database in future.

## Types of samples:

Any samples from tigers in captivity, wild tigers, suspiciously seized products potentially containing biological material of *Panthera tigris* (e.g. broth, paste) etc., are welcome.
Requested samples from *Panthera tigris* specimens can include just one or a combination of the following materials:

- **blood**
- **saliva**
- **hair**
- **soft tissue**
- **hard tissue (tooth, bone)**
- **faecal samples (droppings).**

From live animals, **samples of blood, saliva, tissue or hair plugged with bulbs** are preferred. If the collection of these samples is not possible, samples of hair found in the environment or animal droppings can be collected. In the case of collection from the bodies of dead animals, it is possible to collect all types of the above-mentioned samples. Depending on the degree of body decomposition, it is preferable to collect blood or tissue samples as well as tooth samples in the case of skeletal remains.

## Collection and marking of samples:

Since the material is to be used for genetic analysis, it should be minimally contaminated by the collector during collection. The collection of any sample should be carried out with disposable **rubber gloves**. The gloves should be changed before each new collection of samples.

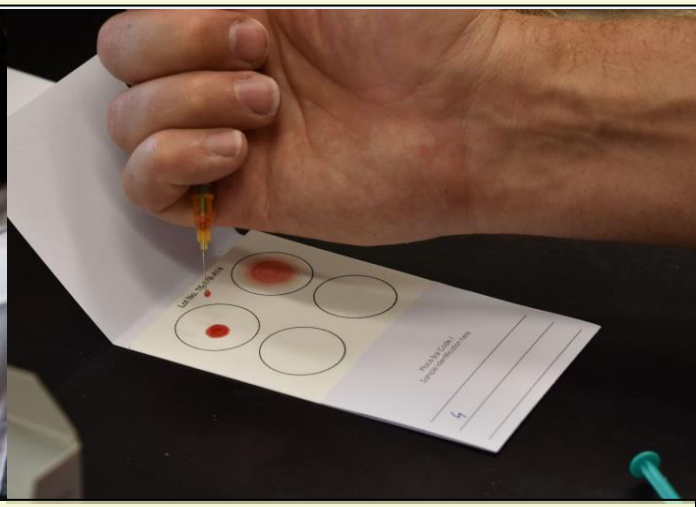
All samples collected must be **properly marked** and accompanied with a **detailed information sheet**. In the case of animals kept in captivity, it is appropriate to indicate at the minimum the country of collection, breeding facility and the collection day. Ideally, also the sex of the animal and its identification number (microchip), if you are confident that the sample comes from that specific animal. In the case of a non-invasive collection of hair or faeces in enclosures with multiple animals, all of the animals present should be mentioned. It is also appropriate to **indicate any possible family relations** between the animals whose samples you are sending. A simple solution is to mark the sample with a number and print a breeder's card with all of the available data. For wild animals, at a minimum the country of origin, the coordinates of the place where the specimen was collected, and the sampling date must be recorded. The more information you provide us about the sample, the better.

### 1. Blood collection

Blood collection may only be performed by a veterinarian or a person authorized to do so. Blood samples for genetic purposes can be processed in both liquid and dry states. However, **dry samples are more suitable for long haul transport**. After the animal's vein has been pierced and blood drawn, the blood drops are transferred on the paper storage cards. The diameter of the blood stain should be **at least 2 cm**. Prior to storing the paper storage card with the sample in a paper envelope, please wait until the blood sample has **completely dried**. If this procedure is not possible, put the blood into the EDTA collection tube.

- **Tools - injection needles, filter paper for blood collection, paper envelope, microEDTA collection tube**

Photo: WWF



### 2. Saliva collection

Some tigers (especially those who have a good relationship with the zoo keeper and are not afraid to come to the grid) can be offered a cotton/synthetic swab through the bars which the tiger will take to the mouth. This collection can only be carried out by the tiger's zoo keeper. Samples of saliva can also be taken from an anesthetized animal. The swabs should then be placed in a paper box (sampling set).

- **Tools: sampling set - cotton/synthetic swab, paper box (a prepared box that can be folded on site)**


## 3. Tissue collection from a dead animal

In the case of a dead animal, tissue sampling is very simple and can be done by anyone. A sterile knife/scalpel is sufficient to collect a part of the soft tissue from the animal (for example, a part of the ear 0.5 $cm^2$) and put it into a sterile, sealable tube filled with 96% alcohol.
The collection of sample can also be made from a taxidermized or prepared-to-be-taxidermized part of the tiger body that is available (skin, tooth, claw, bone). If some chemical reagents have already been applied, it is advisable to make a note of it on the sample. The packaging of such samples is described in the chapter on storage.

- **Tools: rubber gloves, sterile knife/scalpel, sterile sealable tube, 96% ethanol or isopropanol**

## 4. Tissue collection from the living animal

A live animal tissue sample can be collected in two ways, either when the animal is fully conscious or during immobilization. However, in both cases, the collected sample should only be carried out by an experienced veterinarian or a person authorized to do so.
**Collection when the animal is fully conscious** is possible using **biopsy darts** both in captive animals and in wild animals. These darts are similar to those used with immobilized animals from a distance. It is necessary to always use a sterilized dart. More information is available here: http://www.globalsupplies.co.za/index.php/pneu-darts/biopsy. The dart is fired at the animal and when it penetrates the skin, it collects a sample but then drops off the tiger's body. Due to the distinctive colour of the darts rear end, it is easy to track it. It is then possible to unscrew the part of the dart covering the needle with the sample of the skin. The sample should be removed with sterile tweezers and inserted into a sterile, sealable micro tube filled with alcohol.

- **Tools: biopsy darts, dart gun, sterile tweezers, sterile sealable micro tube, 96% alcohol**

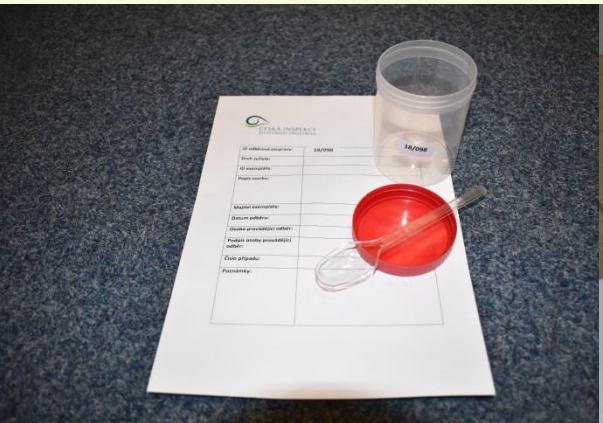
**If the animal is anesthetized,** it is possible to collect a sample using biopsy punches. Their use is the same as darts. However, the difference is that the sample is collected by being manually pushed into skin. More information is available here: https://www.wpiinc.com/product-listers/biopsy-punches/.

- **Tools: biopsy punches, sterile tweezers, sterile sealable micro tube, 96% ethanol or isopropanol**

## 5. Droppings collection

The sampling can be done by keepers during cage cleaning. The collected sample should be a size of walnut. For DNA analysis **intestinal epithelial cells** are important, which are **on the surface of the droppings (not inside).** Therefore, it is necessary to collect the sample as much as possible from the surface of the droppings (not from the inside) or collect the whole dropping with a removable plastic bag turned inside out (as it is done when picking up after dogs). When using a tool, the collection must always be done with a clean tool - for example, with a disposable plastic spoon that is discarded after use to avoid contamination of the DNA sample. Place the droppings sample into a clean, sealable plastic cup, box or a heavy-duty plastic bag.

- **Tools: plastic bag/collecting container, collecting tool – e.g. disposable plastic catering supplies or gloves**

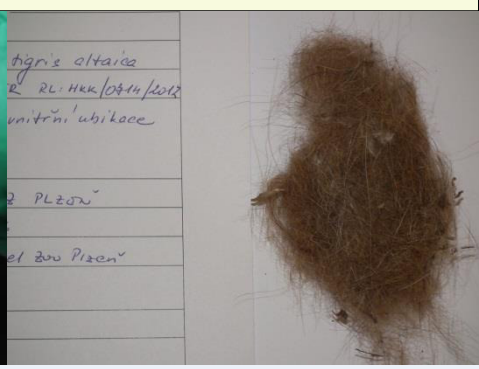


## 6. Hair collection

The collection of the hair sample can be done invasively as well as non-invasively. However, in both cases, it is necessary for the hair to contain bulbs of hair. In case of invasive collection, the hair samples are pulled straight out of the animal with, for example, tweezers or a rubber gloved hand through the bars. In all cases, it is up to the zoo keeper to determine from which animal the collection of a sample is possible and perform the collection himself. This is usually possible for tigers trained for the circus, tigers raised by people like pet animals and tiger cubs taken from their mother. This collection is of course also possible for immobilized animals. The advantage of invasive collection is the certainty that the sample comes from specific animal and is freshly collected. Non-invasive sampling is done through collection of hair samples in places where the tigers scratch or rub. The hair is often trapped on bunks or bars and can be collected and put into a paper envelope (not in a plastic bag where DNA can degrade). The disposable gloves (gloves must be replaced between each collection of samples) or tweezers (between each collection of samples it is necessary to remove all hair or sterilize it with alcohol or fire which is even better) may be used.

- **Tools: tweezers/rubber gloves, paper envelope**





### The storing and shipping of the samples:

- **Blood samples**

As already mentioned in the section on collecting of blood samples, wait until the sample of blood has dried completely before placing the filter paper with the sample in the paper envelope and shipping. It is always advisable to keep these samples in a dry place to prevent humidity. If samples are left in a liquid state, it is advisable to keep the blood collected in the micro-tubes frozen at -20 °C. Blood in a liquid state can be sent only in the special transport medium (DNA shield) – please contact Forensic DNA service before such shipping.

- **Saliva samples**

The sample of saliva on the cotton swabs should be stored in a paper box pervious to air (sampling set) - humidity in a plastic or closed plastic box must be prevented. The paper box with the swab can be sent in a paper envelope.

- **Tissue samples**

Small tissue samples placed in sealable microtubes and treated with alcohol are suitable to store frozen at -20° C until shipping. Larger samples collected from dead animals can be treated with salt and stored frozen at -20° C. Samples of taxidermized body parts can be stored in paper envelopes, plastic boxes or frozen if taxidermy has not been completed (prevent the formation of humidity in the plastic bags containing samples).

- **Droppings sample**

If a fresh, warm dropping is collected, it should be allowed to naturally cool. It is then necessary to freeze it as soon as possible and it is the best to keep it cool until placing it in a freezer. It is advisable to prepare in advance frozen cooling inserts or ice-bags placed into thermobags or thermoboxes together with droppings packed in plastic bags/collection containers. Avoid the smudging or wetting of paper labels with the description. It is advisable to mark the samples with permanent markers or use an ordinary pencil to write on paper labels. Samples should be stored at -20° C until shipping. Please contact Forensic DNA service before shipping.

- **Hair samples**

Unlike droppings, keep hair dry in a paper envelope (not in a plastic bag) and at room temperature (do not freeze, moisture leads to DNA degradation). Send them in a paper envelope.

The sharing of DNA samples of tigers between countries can be **limited by certain restrictions**, e.g. CITES or national rules. The sending within EU should be without problems**, CITES permits** are necessary for the sending from non-EU countries. Please contact us before shipping any samples you have collected.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