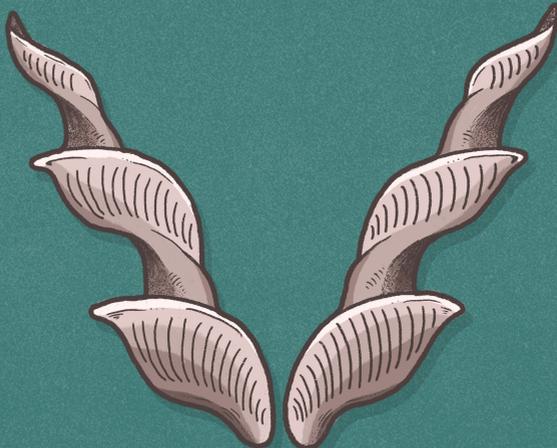


BIOLOGICAL SAMPLE COLLECTION FOR WILDLIFE FORENSICS



BIOLOGICAL SAMPLE COLLECTION FOR WILDLIFE FORENSICS

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<https://india.wcs.org/>



Wildlife Conservation Society-India's (WCS-India) Counter Wildlife Trafficking (CWT) programme aims to support government agencies by building and improving their access to information, skills, technology, and expert support to tackle wildlife crime in India.

The purpose of this guide is to provide a reference for Law Enforcement officials on best practices and procedure to be followed while collecting and preserving different types of samples of wildlife biological evidence found in a wildlife crime scene, along with guidelines on sending them for forensic analysis.

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Special thanks to:

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References and further reading:

1. Thakur M., Joshi B. D. and Chandra K. (2020). Wildlife Forensics: A reference Guide and Standard Operating Procedures in the Investigation of Wildlife Crime. Pp. 49
2. Gaur, A. and P. A. Reddy. 2017. DNA Techniques in Wildlife Forensics (Animals): Standard Operating Procedures (SOP). CSIR Centre for Cellular and Molecular Biology, Hyderabad, 37 p. <http://www.ccmr.res.in/lacones/>
3. Guidelines on Methods and Procedures for Ivory Sampling and Laboratory Analysis. Prepared by the United Nations Office on Drugs and Crime Laboratory and Scientific Section (LSS) and the Global Programme for Combating Wildlife and Forest Crime, Sustainable Livelihoods Unit (GPWLFC, SLU). United Nations, New York, 2014.
4. Wildlife Crime Investigation: A Handbook for Investigation Officers. Wildlife Crime Control Bureau, Ministry of Environment and Forests, Government of India. 2013. 1st Edition. pg 68.



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1. INTRODUCTION

SAMPLE COLLECTION TOOLS



Sterile gloves

To avoid contamination, and for personal safety while collecting samples



Surgical mask



Hand sanitizer



Safety goggles

To protect eyes while using equipment such as hacksaws



Sterilized collection spoon/spatula

To collect samples such as soil, scat



Forceps

To collect samples such as feathers, hair, meat, etc.

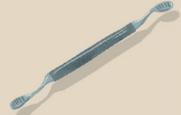


Scissors



Scalpel/Blade

To cut or scrape samples



Filer



Zip lock pouches
(of different sizes)



Vials/Containers

To store and secure samples such as scat, feathers, cured skin samples



Petri dishes



50 ml plastic centrifuge tubes



Cotton swabs

To be dabbed in ethanol/bleach solution for sterilizing sample collection equipment



10% bleach solution

To sterilize equipment such as hacksaws and filers



70% ethanol

To store samples and sterilize sample collection equipment



90-95% ethanol



0.9% NaCl

To store samples such as meat



Silica gel



Filter paper

Used as an absorbent, and separator while storing samples such as scat in silica gel



Circular saw/Hacksaw

To cut samples such as part of a large bone or ivory



Aluminium foil



White cotton cloth



Butter paper

To wrap or store samples such as bones, horns

SAMPLE STORAGE

To identify a sample by DNA or other analyses, it must be ideally preserved to keep it in the best condition possible, till it reaches the laboratory.

These are some commonly used preservatives - they help prevent the sample from degrading due to moisture and microbial contamination.



Silica gel



70% ethanol



90-95% ethanol

(absolute alcohol)



0.9% NaCl

9 grams of NaCl (salt)
per 1000 ml sterile water

STERILIZING INSTRUMENTS

FOR SAMPLE COLLECTION

It important to make sure that the instruments and containers to be used for sample collection are sterilized prior to the collection of samples.

If the same instrument is used to collect multiple samples, the instrument should be sterilized before each sample collection to avoid contamination.

Instruments need to be sterilized by cleaning with a fresh cotton swab dipped in ethanol or 10% bleach.

Containers or vials need to be sterilized with ethanol or 10% bleach.



70% ethanol



90-95% ethanol

(absolute alcohol)



10% bleach solution

To make 100 ml of 10% bleach solution:
Add 10 ml bleach to 90 ml sterile water

PRECAUTIONS: DO's AND DON'Ts



DO's

- **Wash your hands thoroughly with soap** (for at least 30 seconds), before and after sample collection.
- For your safety, **always wear protective gear**, like masks and gloves, when collecting biological evidence. When examining fresh carcasses, wear fully covered gear such as a PPE kit.
- **Prepare and keep a forensic kit** for collection of evidence during a wildlife seizure.

- **Always use sterilized equipment** (scalpels, blades, scissors) and sterilized containers (vials, centrifuge tubes) when collecting and storing samples.

To avoid contamination when taking samples from different specimens, ensure you sterilize equipment between each use.

- Try to take **multiple samples from each specimen** as a backup, and label accordingly.

- **Always label the sample** (containers) to avoid the mixing up of samples from different specimens.

--- / --- / ---

- To avoid degradation of the samples, ensure you store them under the **right conditions and at the right temperature**.

- **Work cautiously when using sharp equipment** such as scalpels and blades.

- **If you have any doubt** in collecting or storing biological evidence, **seek expert advise**.

- To prevent accidents, make sure to **label buffer solution bottles**, such as ethanol and bleach.



DON'Ts



- **DO NOT** touch any biological evidence, such as meat, scat, or fluid, with your bare hands.

- **DO NOT** inhale, taste or ingest any suspicious sample, such as a liquid or crystallised liquid.



- **DO NOT** use formalin to preserve samples that have to be sent for DNA analysis.

- **DO NOT** store samples from different specimens in the same container.

- **DO NOT** reuse single-use equipment such as razor blades or syringes.

DO NOT reuse single-use containers such as vials, petri dish, zip lock pouch, and aluminium foil.

DO NOT reuse single-use protective gear such as latex gloves and masks.



- **DO NOT** use drinking water bottles or soda bottles to store buffer solutions.

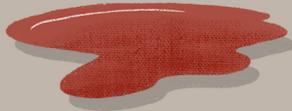
- **DO NOT** use equipment in the forensic kit for anything other than collection of samples.



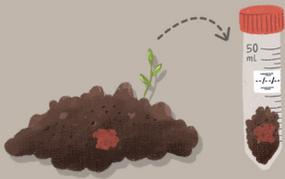
2. SAMPLE COLLECTION

SAMPLE COLLECTION

BLOOD STAINS

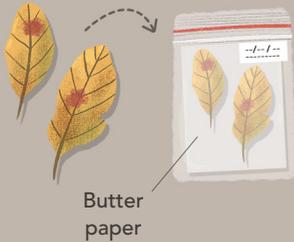


INSTRUCTIONS FOR COLLECTION:



FROM SOIL:

Using a spatula, collect some blood-stained soil matter in a clean, dry **50 ml centrifuge tube**.



FROM PLANT PARTS:

Wrap blood-stained plant parts in **butter paper** or **aluminium foil**, to safeguard the blood stain. Store in a clean **zip lock pouch**.



FROM CLOTH:

Wrap blood-stained cloth in a clean **zip lock pouch**.



FROM WEAPONS:

With a sterilized scalpel/blade, gently scrape dried blood stains from knives/axes. Collect the scrapings in a clean, dry **50 ml centrifuge tube**.

REQUIREMENTS:



Surgical mask



Zip lock pouches



Sterile gloves



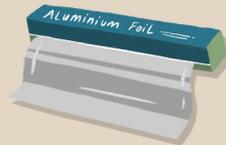
Butter paper



Sterile collection spatula



50 ml centrifuge tubes



Aluminium foil



Scalpel/ Blade

If the weapon is small:

Wrap the whole weapon in aluminium foil so as not to contaminate the sample.

SAMPLE COLLECTION

FECES (SCAT)



INSTRUCTIONS FOR COLLECTION:



Collect scat using a **sterilized, dry spoon** or **spatula**. Use any **one** of the methods below for preservation of the scat sample:

1



Clean, dry **zip lock pouch** filled (at least 1/10th) with **silica gel**

(DRY PRESERVATION)

3



50 ml centrifuge tube.

Submerge sample in **70% ethanol**

2



Feces

Filter paper/tissue paper/absorbent paper

Silica gel

50 ml sample container

filled with **silica gel**

(DRY PRESERVATION)

REQUIREMENTS:



Sterile gloves



Surgical mask



Filter paper



Zip lock pouches



Sterile collection spatula



50 ml centrifuge tubes



Sample containers (50 ml)



70% ethanol



Silica gel

PRECAUTIONS:



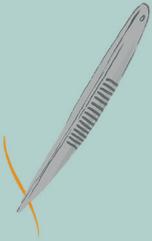
The feces should not be older than 72 hours.

SAMPLE COLLECTION

HAIR/FEATHERS



INSTRUCTIONS FOR COLLECTION:



Pluck hair or feathers with forceps. Ensure that the root of the hair shaft or calamus of the feather stays intact.

Wrap with **butter paper** or sterile **aluminium foil** to ensure the integrity of the root and hair follicle. Store in either:



A clean and dry **zip lock pouch**

OR

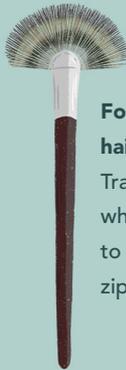


Butter paper

A **petri dish**



For a bundle of feathers:
Pick feathers that have blood at the base.



For mongoose hair brushes:
Transfer the whole brush to a clean, dry ziplock pouch.

REQUIREMENTS:



Zip lock pouches



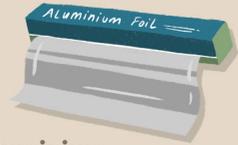
Petri dish



Forceps



Butter paper



Aluminium foil

PRECAUTIONS:



DO NOT CUT hair or feather samples. Pluck them with the root intact.

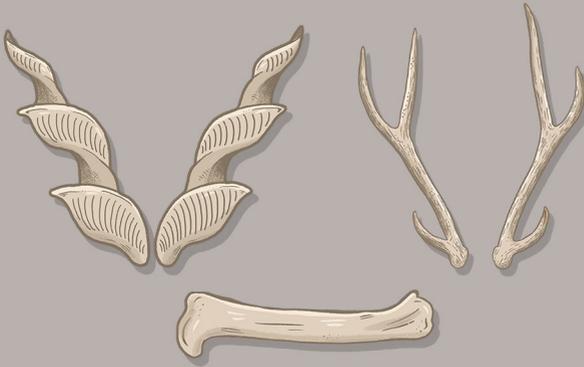


DO NOT TOUCH the basal part of hair or feathers.



SAMPLE COLLECTION

HORNS/BONES/ANTLERS



INSTRUCTIONS FOR COLLECTION:

- Wrap the whole horn / bone / antler in paper, white cotton cloth or muslin cloth.



If the horn / bone / antler seized is too large for transportation:

- Wipe the horn / bone / antler with a dry cloth to remove any external impurities.
- Use a sterilized filer to cut small pieces of the hard tissue. Place the pieces obtained in a zip lock pouch.



Storage: Bones with soft tissue attached should be stored at 4°C (refrigerator temperature). Bones without soft tissue can be stored at room temperature.

REQUIREMENTS:



Sterile gloves



White cotton cloth/
muslin cloth



Zip lock
pouches



Filer

PRECAUTIONS:

Clean the filer with **absolute alcohol** or **10% bleach solution**, to sterilize it before use.



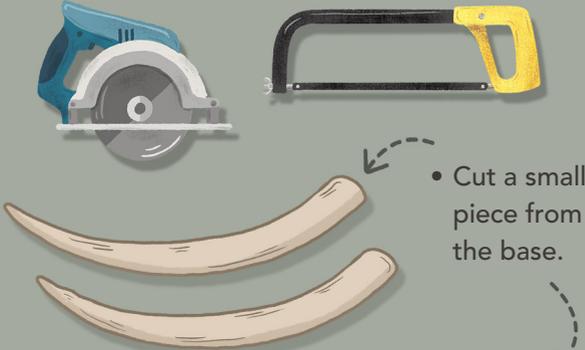
In case of multiple samples, clean the filer thoroughly between samples.



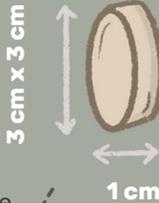
SAMPLE COLLECTION

IVORY

If the ivory seized is too large for transportation:



- Cut a small piece from the base.



- The piece should measure approximately **3 cm x 3 cm** and **1 cm** thick.

Cutting a piece this size will ensure that the sample contains enough DNA to conduct the analyses.

- Immediately place the cut sample in its correspondingly labelled vial. Screw on its lid.

If multiple pieces were taken from the **same ivory sample**, they can all be placed in the **same vial**.

If multiple pieces were taken from **different ivory samples**, they should each be placed in a **different vial**.

Note: Take a clean cloth and wipe the saw blade used to cut the ivory sample with **absolute alcohol** or **10% bleach solution** before cutting the next sample.

REQUIREMENTS:



Sterile gloves



Vials



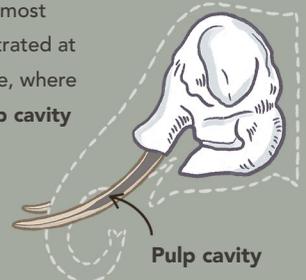
Circular saw/Hacksaw



Safety goggles

When cutting a sample for DNA analysis, it is important to cut a piece from the **base of the tusk** (the end that was connected to the skull), if possible.

DNA is most concentrated at the base, where the **pulp cavity** exists.



Pulp cavity
(contains blood vessels and tissues)



SAMPLE COLLECTION

MEAT/TISSUE

Cooked,
partially cooked
or raw meat



INSTRUCTIONS FOR COLLECTION:



Cut a slice of the meat with a **sterilized blade**.



Using **sterilized forceps**, place the slice in a container with either of the following preservatives:



90-95% ethanol,
(absolute alcohol)

OR



0.9% NaCl

REQUIREMENTS:



Sterile gloves



Sample containers



90-95% ethanol
(absolute alcohol)



Scalpel/ Blade



0.9% NaCl



Forceps

PRECAUTIONS:



Sterilize the blade and forceps with ethanol before and after use.



Sterilize the blade and forceps thoroughly between samples.

SAMPLE COLLECTION

SKIN/HIDE



INSTRUCTIONS FOR COLLECTION:

Wrap the whole skin/hide in paper or white cotton cloth

If the skin/hide seized is too large for transportation:



Using a **sterilized blade or scissors**, cut an approximately **5 cm square** of the skin or hide



Place the skin piece in a clean and dry **zip lock pouch** or **petri dish**

REQUIREMENTS:



Scissors



Blade



Sterile gloves



Zip lock pouches



Petri dish



White cotton cloth/
muslin cloth

PRECAUTIONS:



Sterilize the blade or scissors with ethanol

SAMPLE COLLECTION

OTHER SAMPLES

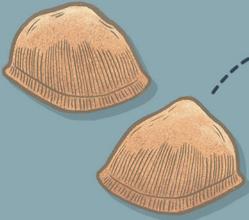
VENOM:

Snake venom is mostly traded in dried form (in containers like glass jars)



Send the whole container of dried venom for analysis (seal securely shut)

PANGOLIN SCALES:



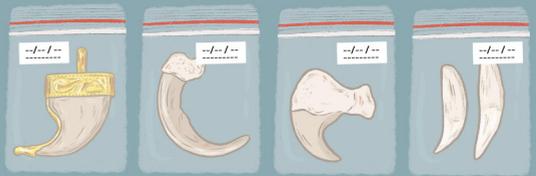
Place in a clean, dry zip lock pouch

PORCUPINE QUILLS:



Wrap quills in aluminium foil and place in a clean, dry zip lock pouch

TEETH/CLAWS/TALONS:



Place in a clean, dry zip lock pouch.

Note: Use separate zip lock pouches for samples with different origins

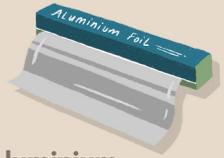
REQUIREMENTS:



Sterile gloves



Zip lock pouches



Aluminium foil

3. POST SAMPLE COLLECTION

EXAMPLE FORMAT: LABELLING BIOLOGICAL SAMPLES AFTER SEIZURE

Label

Label should be placed on the outside of the container/pouch which has the sample to be sent for analysis.



Pointers to include on the label:

- 1 Name of the exhibit**
(A / B / C / D, etc.)
- 2 Date of Collection**
- 3 Name of the item**
Along with a brief description -- including quantity seized, colour of the sample, dimensions (length, width, height) of the sample, etc.
- 4 Signatures**
(of the witnesses, the accused and the seizing officer)

Example:

Dated: 10/11/2021

'A'

12 (twelve) Pangolin scales

Brown in colour

Individual scales - Length: 6.5 cm (approx.)

Breadth: 8.5 cm (approx.)

Total weight: 60 grams (approx.)

WITNESSES:

[Name of Witness 1] 

[Name of Witness 2] 

SEIZED FROM:

[Name of Accused] 

SEIZED BY:

[Name and designation of the seizing officer]





STORAGE AND TRANSPORT OF SAMPLES

Summary of methods for short-term preservation and storage:

Type of tissue	Preservation	Storage
Blood stains		Room temperature
Feces (scat)	Silica gel/ethanol	Room temperature
Hair/Feathers		Room temperature
Horns/Bones/Antlers		Room temperature
Ivory		Room temperature
Meat/Tissue	0.9% NaCl/ethanol	Room temperature
Skin		Room temperature

Summary of methods for long-term preservation and storage:

Type of tissue	Preservation	Storage
Blood stains		Room temperature
Feces (scat)	Silica gel/ethanol	4°C
Hair/Feathers		Room temperature
Horns/Bones/Antlers		Room temperature
Ivory		Room temperature
Meat/Tissue	0.9% NaCl/ethanol	Room temperature
Skin		4°C

References:

1. Thakur M., Joshi B. D. and Chandra K. (2020). Wildlife Forensics: A reference Guide and Standard Operating Procedures in the Investigation of Wildlife Crime. Pp. 492.
2. Gaur, A. and P. A. Reddy. 2017. DNA Techniques in Wildlife Forensics (Animals): Standard Operating Procedures (SOP). CSIR Centre for Cellular and Molecular Biology, Hyderabad, 37 p. <http://www.ccmcb.res.in/lacones/>

EXAMPLE FORMAT: REQUEST LETTER TO THE CONCERNED INSTITUTE FOR FORENSIC ANALYSIS



Seek permission from the Magistrate before sending any sample for forensic analysis.



Sample/specimen must be sealed with the government stamp

(Forest Department, Police) or Magistrate seal or stamp.



Include a request letter

(written on the official letterhead of the forwarding officer).

If sending the sample in person, the request letter should mention the name of the carrier.

REQUEST LETTER TO THE FORENSIC SCIENCE LABORATORY/INSTITUTE:

Case No./POR:

Range/Division:

District:

State:

Sections of law:

1. Brief facts of the case:
2. List of exhibits/samples enclosed for examination:
3. Nature of examinations to be made:

Forwarded to:

Name and designation of the forwarding Officer:

Name of the forwarding court, if applicable:

WHERE TO SEND SAMPLES FOR ANALYSIS



FOR SPECIMEN IDENTIFICATION

(INCLUDING DNA / FORENSIC ANALYSIS):

1. Zoological Survey of India (ZSI)

To,
The Director
Zoological Survey of India
M-Block, New Alipore,
Kolkata - 700053
West Bengal, India
Tel: +91 33 - 24008595

2. Centre for Cellular & Molecular Biology (CCMB)

To,
The Director
Centre for Cellular & Molecular Biology
Habsiguda, Uppal Road
Hyderabad - 500 007
Telangana, India
Telephone: +91 40 27160222-31,
27160232-41
Fax: +91 40 27160591, 27160311
Email: director@ccmb.res.in

3. Wildlife Institute of India (WII)

To,
The Director
Wildlife Institute of India
Post Box #18, Chandrabani
Dehradun - 248001
Uttarakhand, India
E-mail: wii@wii.gov.in
Telephone: +91 135 2640114-15, 2646100
Fax: +91 135 2640117



FOR VENOM SAMPLE ANALYSIS:

1. Haffkine Institute for Training, Research and Testing

To,
Haffkine Institute for Training,
Research and Testing
Acharya Donde Marg, Parel,
Mumbai - 400012, Maharashtra
Telephone - +9122-24160947,
24160961, 24160962
Fax - +91 22-24161787
Timings – 9.00 a.m to 5.30 p.m

2. Centre for Cellular & Molecular Biology (CCMB)

To,
The Director
Centre for Cellular & Molecular Biology
Habsiguda, Uppal Road
Hyderabad - 500 007
Telangana, India
Telephone: +91 40 27160222-31, 27160232-41
Fax: +91 40 27160591, 27160311
Email: director@ccmb.res.in



FOR TOXICOLOGY TESTS:

Respective state forensic labs



Advisory: It is advised to communicate with the concerned institution over phone/email before sending samples.

For any further assistance, please contact the
Counter Wildlife Trafficking (CWT) Helpline:

+91 - 99575 67525

