Procedure for Rhino horn **DNA** Sampling





environmental affairs Department: **Environmental Affairs**



Procedure for taking DNA samples from seized whole Rhino horn, pieces of horn or horn shavings/powder in line with forensic requirements

What is Chain of Custody ?

One of the cornerstones of the judicial process is the presentation of evidence in a court of law. Evidence is anything that tends logically to prove or disprove a fact at issue in a judicial case. Evidence essentially consists of oral evidence, documentary evidence and real evidence (forensic evidence in the form of deoxyribonucleic acid (DNA)). The value of evidence is indisputable; it can either prove or disprove the fact that a crime has been committed, it supports the testimony of witnesses and links a suspect to the crime in question.

The foundation of any forensic evidence and its use in legal proceedings is the Chain of Custody (CoC) which must always be maintained for every piece of evidence gathered. CoC is the documented process of the collection, movement and preservation of evidence items that are intended to be used in a court of law. This must form an unbroken chain from collection to its transportation to the laboratory, through to its processing and final reporting, with sealing and identification of each evidence item and identification of each person handling the items of evidential value underpinning the process. It is crucial to be able to demonstrate every single step undertaken to ensure traceability and continuity of the evidence from the crime scene to the courtroom.

Factors that can affect the CoC include the method used to collect samples at the crime scene, mixing of samples, contamination of samples, incorrect sealing of samples and mislabelling. Correct CoC procedures rule out tampering with the samples and substitution of samples. Substantial alteration of the sample as a result of incorrect handling and storage also constitutes a break in the CoC, since the samples are changed from the original sample collected. In summary, Chain of Custody refers to the documented process over time from when the evidence was collected and subsequently handled, thus every person involved needs to be accounted for.

Other items of evidential value

Please remember that during any seizure, there is additional evidence that should be collected in order to assist in further investigation. This includes, but is not limited to, items such as packaging material (newspaper/cloth), finger prints on packaging material, hand writing, or information on accompanying documents, such as the waybill or other cargo documents. The inside of the parcel or container is also extremely valuable as it is unlikely to have been touched by others after the suspect wrapped or packaged the horns. Accordingly, it is essential to wear surgical gloves in order to ensure that any potential evidence is not lost and/or contaminated. All such items should thus be collected and handled as part of the collection of evidence process.



Examples of possible existence of evidential material





Equipment required

- Camera;
- Forensic tamper-evident evidence sealing bags (A bag with the ability to be sealed and that will show signs should someone tamper or attempt to tamper with the seal or the contents of the bag. The bags ensure that the samples/evidence are handled and transported in line with the chain of custody principles);
- Small uncontaminated plastic containers with lids for storing of the samples;
- Marker with indelible ink or labels to mark sampling container with reference number;
- Electric drill and drill bit/s (4.5 mm) or scalpel;
- Surgical gloves;
- Bleach sterilizing solution;
- Soft measuring tape (to measure horn dimensions);
- Calibrated scale, for weighing of horn;
- Data capture board and white-board pen to record horn details in photograph;
- Sterile disposable sheet and
- Microchip reader.

Initial field identification methods for Rhino horn

- Prior to conducting the formal sample collecting process for Rhino horn, the following initial identification methods can be used to determine if the horn is indeed real or fake.
- The following **points** provide quick and simple methods that can be applied in the field for initial identification purposes





1. Sample burning: Smell

Where possible, remove a small piece of the horn and apply an open flame. The smell that emanates from the "real" Rhino horn is very similar to that of burning hair. Fake horn made from plastics, wood etc. would have a distinctively different smell. Alternatively, one can simply burn some of the hair-like fibres which are located at the base of a Rhino horn which will provide a similar result.



2. Scratch / Scrape: Smell

Another identification method using your sense of smell is to scrape the horn with the sharp edge of a knife; and in so doing clear a fresh surface. If one smells the scraped areas, fake horns made from any artificial substance such as resin, plastic etc. will have a distinctive chemical smell, whereas a more natural or earthy smell emanates from real Rhino horn.



3. Light test: Sight

Real Rhino horn has transluscent characteristics. In other words, it will allow a certain amount of light to shine through when a light source of sufficient brightness, such as a torch, is applied. This method can be effectively used on a full horn and/or just a part thereof. Place the torch on any of the sides of the horn, and a soft glow will be visible. Fake horns made of wood and plastic tend to be opaque and will not allow light to shine through them.



4. X-ray: Sight

The images depicted below display the difference between a fake horn made of plastic (left) and a real horn (right) when an x-ray machine is applied. The real Rhino horn, being uniformly composed of organic material, displays a consistent coloration throughout the x-ray image, whereas the colouring of fake horn is normally inconsistent; and has lighter and darker areas. This characteristic could indicate signs of inorganic material.



Left: x-ray image fake horn *Second from left:* x-ray image real horn (The dark stripe visible is the microchip inside the horn)

Right: photograph of real horn that was x-rayed **Second from right:** photograph of fake horn that was x-rayed

5. Visual inspection/observation: Profile and Composition of the Base

Other visual inspections are also advisable when dealing with Rhino Horn.

- When examining the base of the horn, one should pay attention to the shape. In many instances fake Rhino horn would have a more flat shape surface, whereas a deep concave profile is noticeable in a real Rhino horn.
- In addition, many types of fake horn show signs of bone at the base, due to the use of the top part of other animal skulls in its manufacture. In real Rhino horn, the base would have a similar look and feel as the rest of the horn.







Above: Base of fake Rhino Horn

Above: Base of real Rhino Horn

6. Visual inspection / observation: Texture of the base

One of the last features that indicate a real Rhino horn is the presence of loose "hair like" fibres at the outer base section of the horn. This characteristic is very difficult to fake, and in most instances, the surface of the outer base section of a fake horn would be smooth.



It is however important to note that these tests and observations should only be seen as guidelines, as they differ in effectiveness depending on the form of which the suspected horn and/or part thereof is found. When in doubt, the best suggestion would be to rather collect a sample for analysis.

Protective measures are necessary to avoid contamination between different samples

- Use a seperate sterile diposable sheet as a work surface for each item.
- Treat each horn or piece of horn separately and use separate sample bottles for each sample.
- Wear gloves at all times while handling horns.
- Use only sterilized, uncontaminated sampling equipment.
- Ensure to sterilize any equipment to be re-used, for example, drill bits, by using a bleaching solution.

Step-By-Step process for taking DNA samples from whole Rhino horns, pieces of horn or horn shavings/powder



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Record the following information on the data capture board as well as on the sample data collection form (As per the Annex to this guideline):

- Case or reference number;
- Place/Port of seizure;
- Date of seizure;
- Country of origin;
- Date of sampling;
- DNA sample number;
- Outside length of horn (Convex side);
- Inside length of horn (Concave side);
- Circumference of base of horn;
- Microchip number if present;
- External number/markings on the horn; and
- Weight

Note: Ensure to clearly state the unit form that is used for measurements and weight. For example, lbs./kg, cm/inch





 Photograph the horn with the data board included, from all angles (as well as the base of the horn) against a light coloured background to show the profile of the horn;

In the event that Rhino horn <u>pieces</u>, <u>shavings</u> or <u>powder</u> were confiscated, note the following:

- Photograph the horn pieces, shavings or powder together with applicable information displayed on the data board;
- Use the sample bottle to collect some of the shavings or powder, to be submitted for DNA analysis; and
- Close the sample bottle and follow Step 5 for sealing of the sample.

Case / Reference Number: CAS 121 /01/2016 Place / Port of Selzure: South Africa OR Tambo Int. ate of Seizure: 31/01/2016 Country of Origin: Unknown Date of Sampling: 03/02/2016 DNA Sample Number: RHS 121 3664 Length Outside: 26.5 cm Length Inside: 23 cm Base Circumference: 62.5 cm Microchip Number: None External Numbers / Markings: None Weight: 2.235 Kg





Case / Reference Number: Place of seizure: Date of seizure: Port / country of origin: Date of sampling: DNA sample number: Length side 1: Length side 2: Length side 2: Length side 3: Length side 4: Weight kilogram/grams: Microchip number if any: External numbers if any:

Example of piece of horn



Example of horn shavings



Take a photo of the item of evidence, (the Rhino horn, pieces of horn or horn shavings/powder), the sterile, unused container and the unused tamper-evident evidence sealing bag.





Step 4

- Take the DNA sample in the following way using a drill:
- Select the slowest speed setting on the electric drill;
- If there is a burning smell while drilling, then it might be that your drill speed is too fast;
- A high drill speed will cause excessive heat which will destroy the DNA;
- Slowly drill a hole towards the core of the horn ;
- The sample extracted from drilling, should be in the form of a spiral;
- Drop the sample directly into the sample bottle to avoid contamination; and
- Close the sample bottle with the sample inside





- Place the sample bottle (with sample inside), together with the completed part C of the sample data collection form (As per the Annex to this guideline) inside the tamper-evident evidence sealing bag;
- Seal the tamper-evident evidence sealing bag; and
- Take a photograph depicting the sealed bag, the item, the data board and the removed paper strip of the tamper-evident evidence sealing bag, to show that the bag was properly sealed.



Obtaining of Necessary Permits in respect of samples from seized Rhino horn

- Ensure that you have CITES permits both export/re-export and import permits.
- Apply for the export or re-export permit from the CITES Management Authority in the country where the seizure took place. Information can be obtained from the CITES Website at www.cites.org under National contacts and information.
- If the samples are being sent to South Africa for analysis, in order to obtain the CITES import permit in South Africa, contact Brigadier Sonja de Klerk at the Forensic Science Laboratory of the South African Police Service who will apply on your behalf, e-mail address: deklerks@saps.gov.za
- Veterinary import permits for DNA samples will also be needed.
- Please contact the Department of Agriculture, Forestry and Fisheries on VetPermits@daff.gov.za or visit their website on www.daff.gov.za for more information relating to these permits.
- If you need assistance or information on any of the above, please contact Ms Sonja Meintjes at the Department of Environmental Affairs on landline +27 12 399 9597, mobile +27 82 655 4711 or e-mail: smeintjes@environment.gov.za
- The DNA samples have to be sent with the original CITES permits (import and export/re-export) to the address below. This address should also be used as the address of the importer on the CITES export/re-export permit:

Forensic Science Laboratory Materials Analysis South African Police Service 270 Pretoria Road Silverton, Pretoria, 0001 South Africa Tel: +27 12 845 5619 Mobile: +27 82 778 9651 Fax: +27 86 630 5615



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Example of CITES permit



With thanks to the following organisations for their contribution:

- Forensic Science Laboratory (FSL) of the South African Police Service.
- Veterinary Genetics Laboratory (VGL); and
- Secretariat of the Convention on International Trade in Endangered Species of Wild Fauna and Flora (CITES)



Notes



Annexure:

SAMPLE DATA COLLECTION FORM

Record (case, file) number (as also provided in Part A): _

• Please complete a separate copy of this page for each whole raw rhinoceros horn seized.

• For worked horn, pieces of horn, skin, powdered horn or other specimens, kindly include as an Annex to Part C, detailed information about what was seized and as applicable, information on the weight, length, breadth and height of each seized item.

• Please provide information about any marks, microchip number(s), or any other relevant information about the seized specimens, where available.

• Include photographs of all the seized rhinoceros horn specimens.

Whole raw horn Front horn or back horn? A - Outer length (cm) B- Circumference base(cm) C- Inner length (cm) Weight (kg) Microchip number(s) Photograph taken (Yes / No)



Inspect the rhinoceros horn for any numbering that might be punched or written on it, andfor any other relevant markings. Record these in detail.

Have or will samples be collected from any of the seized rhinoceros horn specimens for forensic analyses?

Yes 🗆 🛛 No 🗆

If your answer above is "Yes", please complete Part C.2 below. After completion, please make a copy of Parts C.1 and C.2 of this form. These copies should be sent together with the collected samples, to the to the laboratory where the analyses will be conducted.

Record (case, file) number (as also provided in Part A): ______

Please provide the following:

a) Name of the laboratory to which the samples will be sent:_____

b) Date of sampling: _



c) The following information about the officer investigating the case: Name: _____

Department and designation: _____

Telephone number: ______ Email address:

d) The following information about the person that collected the samples:

Name:

Department and designation: _____

Telephone number: _____

Email address:

e)DNA sample reference number:_____

f)Evidence bag number: _____

g) Signature of the person that collected the samples: _____

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