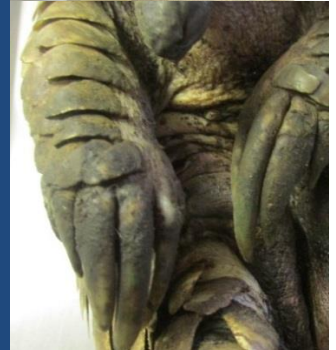


Pangolins

Guidelines and Sampling Protocols

For

Confiscations

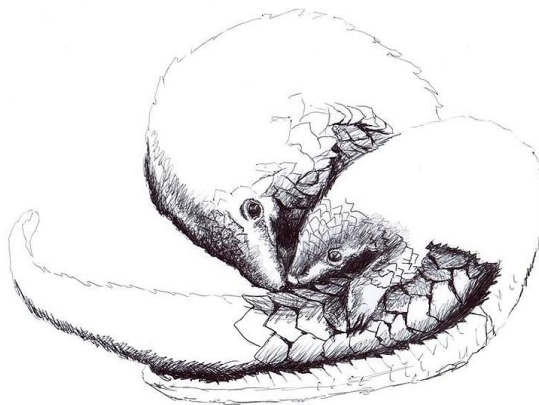


Prepared by

National Zoological Gardens of South Africa (NZG)



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ABBREVIATIONS

APWG	African Pangolin Working Group
CITES	Convention on International Trade in Endangered Species of Wild Fauna and Flora
COC	Chain of custody
IUCN	International Union of Conservation of Nature
IWT	Illegal Wildlife Trade
NZG	National Zoological Gardens of South Africa

DEFINITIONS

Chain of custody:

The step by step documentation or paper trail procedure, done with crime scene photographs, that shows the recording (detailed log), collecting, packaging (custody and control), transfer, analysis, and disposition of evidence samples or exhibits.

Package:

The container/bag/holder holding units or contents recovered from a seizure.

Sample:

An item(s) or unit(s) selected from a seizure.

Sampling protocol:

A sampling procedure used to select samples from seizures, particularly larger seizure to reduce practicability implications for forensic analysis.

Scale morph or type:

Refers to the different scales of pangolin the identified by shape or colour characteristics.

Seizure (or confiscation):

The total items seized or confiscated from illegal wildlife trade.

Unit:

A single item from a set of distinct items usually grouped together for analysis.

1. OVERVIEW

Illegal wildlife trade (IWT) is an extensive global network of organised crime worth billions of US Dollars annually. This trade is heavily reliant on illegally poaching of wildlife from their natural habitats and selling them through international markets for use in traditional medicine, for breeding, as pets and as well as food preparations. Pangolins are severely impacted by this illegal wildlife trade and they are considered the most illegally trafficked mammal globally. They are primarily targeted for their hard keratinous scales that is used as a ground up powder as an ingredient in traditional Asian medicine. For example, the number of pangolins seized between 2007 and 2013 amounted to at least 107 060 individuals¹. The amount of pangolin scales intercepted leaving Africa in 2017 has already reached 34.7 tonnes which likely only represents 10% of the actual trade.

There are currently eight known species of pangolins, four in Africa (*Phataginus tricuspis*, *P. tetradactyla*, *Smutsia gigantea* and *S. temminckii*) and four in Asia (*Manis pentadactyla*, *M. javanica*, *M. culionensis*, and *M. crassicaudata*). All known pangolin species are listed on the IUCN Red List of threatened species, categorised as vulnerable (all African pangolin species), endangered (*M. crassicaudata* and *M. culionensis*), or critically endangered (*M. javanica* and *M. pentadactyla*). The Convention on International Trade in Endangered Species of Wild Fauna and Flora (CITES) has recently uplisted all eight species of pangolins to Appendix I, meaning that all commercial international trade in pangolins or their products is prohibited. This important move was made to help with the global protection and conservation of pangolins against IWT activities that threaten species survival.

Developing approaches and guidelines in the strategic sampling of pangolin seizures has become critical. Implementation of such approaches will ensure tactical sampling of confiscations can be integrated into forensic investigations. This will assist with obtaining intelligence data (e.g. species identification and population origin) for use by law enforcement authorities in prosecutions.

2. A GUIDELINE TO SAMPLING APPROACHES

The guidelines outlined in this manual describe general sampling techniques, from subjective and arbitrary methodology to statistical considerations for confiscations of pangolins. The current high levels of illegal wildlife trade in pangolins have resulted in large numbers of seizures of pangolins and their products (primarily scales). As illegal seizures of wildlife products constitute a wide array of species, and diverse pangolin by-products, in variable volumes

and sizes with an array of packaging, there is a need for establishing guidelines to ensure that sampling by law enforcement is adequate and reasonable. This is critical for forensic analyses in order to ensure that the most appropriate strategy is followed that addresses and covers the relevant questions required for successful crime prosecution and intelligence of each unique situation.

Thus, this guideline suggests a number of sampling strategies for a variety of scenarios from sampling small numbers involving single whole animal confiscations to large numbers or volumes of individuals or scales. Although, it is not possible to define a sampling strategy for each specific situation, these guidelines aim to support enforcement and forensic analysis laboratories in the selection of their sampling plan/strategy(ies) and best working practices for a wider application.

2.1. GUIDING PRINCIPLES

The best practice or approach for sampling for forensics is that the composition of the samples collected reflects, in principle, the composition of the entire seized item(s). Although only a fraction of a seizure can be investigated when dealing with large quantities of pangolin scales due to time constraints, cost effectiveness and efficiency, it is important that the sample still reflects the contents of the seizure. It is essential that the following principles are maintained when selecting a sampling strategy:

- 2.1.1.** The properties of the sample should be a true reflection of the seizure package(s) in terms of species or groups.
- 2.1.2.** The sampling process should adhere to **chain of custody** (COC) procedures to ensure that all collected evidence is documented, packaged and protected under custody. An example of the COC process developed by the Barcode of Wildlife Project (BWP) in South Africa is attached in APPENDIX I.
- 2.1.3.** Samples should be defensible in court by meeting the specific situational needs for relevant parties (police, prosecutors, courts, laboratories) based on:
 - 2.1.3.1.** Aims of the investigation and purpose of the results.
 - 2.1.3.2.** A statistical basis that is easy to explain.
 - 2.1.3.3.** Realistic cost implications and workloads of the laboratories (i.e. This is to enable acceptable turn-round times).

2.1.4. Sampling strategies should be relatively easy, practical and reasonable for use by enforcement and custom officials.

2.1.5. National, regional and international laws and legal practices may dictate the best strategy.

3. SAMPLING TECHNIQUES AND APPROACHES

The careful sampling of a seizure is critical as it is the first encounter between law enforcement and criminals in an investigation. The value chain of crime investigation and prosecutions is greatly dependent on how information is collected and shared.

Therefore, protocols and COC guidelines should be standardised and developed in such a way that they meet the needs of the end goal, which is crime prosecution and taking preventative measures in target areas. Figure 1 and 2 outline the decision making pipelines and options for sampling from pangolin seizures in each unique situation for whole animals (live or dead) and pangolin by-products.

There are several sampling approaches that can be applied for pangolins and these are highlighted below.

3.1. Representative sampling techniques

Representative sampling strategies can be performed on seized items with sufficient similar external characteristics such as whole individual animals, scales of different sizes and colour and seizures that are packaged in similar containers.

Therefore, evidence must be collected in such a way that the integrity of individuals or by-products contained in the confiscations is maintained in the representative sample used for forensics. This is especially vital for pangolin seizures as most of these are typically of scales and in large volumes with weights of up to 3 tonnes in a single confiscation while live pangolins or dead individuals recovered can weigh in the hundreds of kilograms.

A representative sampling procedure also needs to ensure that the number of samples selected from seizures is minimised (cost effective) while maintaining representativeness of species or locations of the seizure. It is important to also ensure that this sampling has statistical significance to inform on all species or populations contained within the confiscations and their origin.

3.1.1. GENERAL: A step by step guide for collecting a representative sample

- 3.1.1.1.** A dedicated work area must be identified prior to sampling, where possible.
- Worksheets must be made available for detailed record keeping throughout sampling (See APPENDIX II –NZG COC sampling datasheet for whole animals).
- 3.1.1.2.** All packages should be counted, assigned field identification (numbered), origin of the shipment recorded (and any other history) and sorted into groups based on sizes and similarities in packaging containers before sampling.
- 3.1.1.2.1.** Carefully evaluate the number of units present in the confiscation.
- 3.1.1.2.2.** Visually inspect each unit as well as the contents carefully and record the number of items and any other information (date, contents, specific characteristics, weight etc.).
- Total weight of seizure must be recorded, and each package weighed separately.
- 3.1.1.2.3.** Record the number of unique items such as individuals, pieces of a carcass, scales and other pangolin by-products in packages.
- Packages must be accurately and consistently numbered to ensure accurate representation of data/results.
- 3.1.1.3.** Packages can be sorted into groups after opening the units, if there are differences in the appearance of their contents, such as differences among individuals based on species or differences in scales types.
- 3.1.1.3.1.** In this case, the sampling procedure should follow the specific criteria for whole individuals (3.1.2.) or sampling scales (3.1.3.) mentioned below.
- 3.1.1.4.** Sampling a representative sample will depend on type of confiscations at hand.
- 3.1.1.4.1.** Representative sampling shall not apply in:
- Single unit confiscations: in this case sample the unit.
 - Homogenous package contents: sufficient sampling has to be done for species identifications, i.e. 2-5 sub-samples.

3.1.2. WHOLE INDIVIDUALS: a step by step guide for representative sampling

3.1.2.1. Pangolins must be separated into as many groups as possible based on distinct species characteristics or external features and dissimilarities before sampling.

3.1.2.2. In the case of live individuals, where possible a veterinarian must assess the health status of each pangolin. Blood samples must be taken (1 ml) and expedited to forensic labs. A microchip tag can also be inserted at this time for tracking/ monitoring.

3.1.2.3. In the case of dead individuals, blood clots, tissue and/or scale samples (2-5) must be taken.

3.1.2.3.1. A veterinarian may perform a post-mortem where possible for forensic pathology. Samples to be stored include the brains, heart tissue, lungs, spleen, liver, intestines, ribs (bone marrow) or gonads e.t.c.

3.1.2.4. Where confiscations contain whole live or dead animals only, all individuals must be sorted by size and other external species specific characteristics prior to sampling. Live animals should be prioritised for forensic analysis to determine origin of individuals where possible.

PLEASE NOTE: This will assist with successful and rapid rehabilitation and reintroduction of animals as pangolins have low stress tolerance and cannot be kept in captivity for prolonged periods².

3.1.2.5. Each unique group will be considered as a whole population or species and should be sampled as a distinct group for analysis.

3.1.2.6. The best quality samples must be selected for forensic analysis e.g. sufficient blood (1 ml) or tissue (1 cm³).

3.1.2.7. PLEASE NOTE that the sampling approach will depend on the information required for forensic analysis.

3.1.2.7.1. **Species ID:** a minimum of 2-5 individuals must be selected per distinct species group or cluster.

3.1.2.7.2. **Population origin:** A minimum of 2-5 individuals must be selected per distinct group/cluster of each species. In the case that the whole pangolins are;

- Less than 20: it is advised that all individuals are sampled.
- More than 20: sample from each distinct group/ cluster based on practicability and cost effectiveness.
- More than a 100: Between 2-5 individuals can be selected from each distinct species group. Each individual must represent observed differences (e.g. scale types within species). Can use statistical approach (see 3.3).

3.1.3. SCALES AND PROCESSED PRODUCTS: A step by step guide for representative sampling

The main constituents found in pangolin seizures are scales and other body parts. Estimating the number of individuals from confiscated scale packages is complex as scale seizures are usually in large volumes (e.g. 3 tonnes in a single confiscation). Because it is impractical to sample the whole seizures, different methods and approaches can be employed depending on the size/volume and the question that should be answered for enforcement.

In this manual, we describe options for representative sampling technique of packages with different scale morph types (shapes and colour) and body parts (e.g. claws). It is important that the sample collected for forensic analysis must be of the best quality. All sampling must follow COC procedures.

3.1.3.1. Records of sampling details and field identifications must be kept at all times throughout sampling on a datasheet.

3.1.3.2. Packages must be weighed individually and weights recorded. This is important as;

The number of individuals can be estimated by dividing the total weight of the bulk package by average weight of scales of one pangolin, which is species dependent ³. Below are some estimates for African species (Prof Ray Jansen Pers. Com);

- Temminck's ground pangolin – scales are estimated to be 25-30% of total body mass (Ave: 12 kg).
- Giant ground pangolin - scales are estimated to be 25-30% of total body mass (Ave: 30 kg).
- Black and white bellied tree pangolins – scales are estimated to be 20-25% of total body mass (Ave: 2 kg).

3.1.3.3. Sort, group, label and assign unique field identifications to all scale packages carefully by identifying characteristics and similarities.

- Mixing of scales from different packages must be avoided.

3.1.3.4. The sorting of packages by scale morph type can be by physical features or types of scales.

3.1.3.5. If scales of different shape and colours are present, sort all scales first by colour then by shape. There are four main shapes (folded scale, broad rhombic, elongated kite, small rhombic). See APPENDIX III for examples of different scale morph types.

- Colour (e.g. light, medium, dark, reddish-brown, olive) or shape (elongated kite, small rhombic, folded scale, broad rhombic).

- Physical features: longitudinal grooves, indentations on scales (sometimes absent).
- Claws and other body parts must also be sorted according to colour, shape or size where possible.
- Unique scale sub-groups can be sorted into individual bags in each package where possible.

3.1.3.6. If sorting is not possible, different types of scales (5 – 20) must be selected evenly from the entire contents of the package following protocols for subsampling package units in small to large seizures described below;.

3.1.3.6.1. **Species ID** – from each scale type sub-group representing a species, select a minimum sample of five scales. (See Appendix IV for example photos of African species).

3.1.3.6.2. **Population origin** – from each scale colour or shape morph type per sub-group, select as many scales as possible (5 – 20).

3.1.3.7. All samples must be stored in good a condition (dry or refrigerated) to prevent sample degradation. Ensure all items are sealed to prevent contamination.

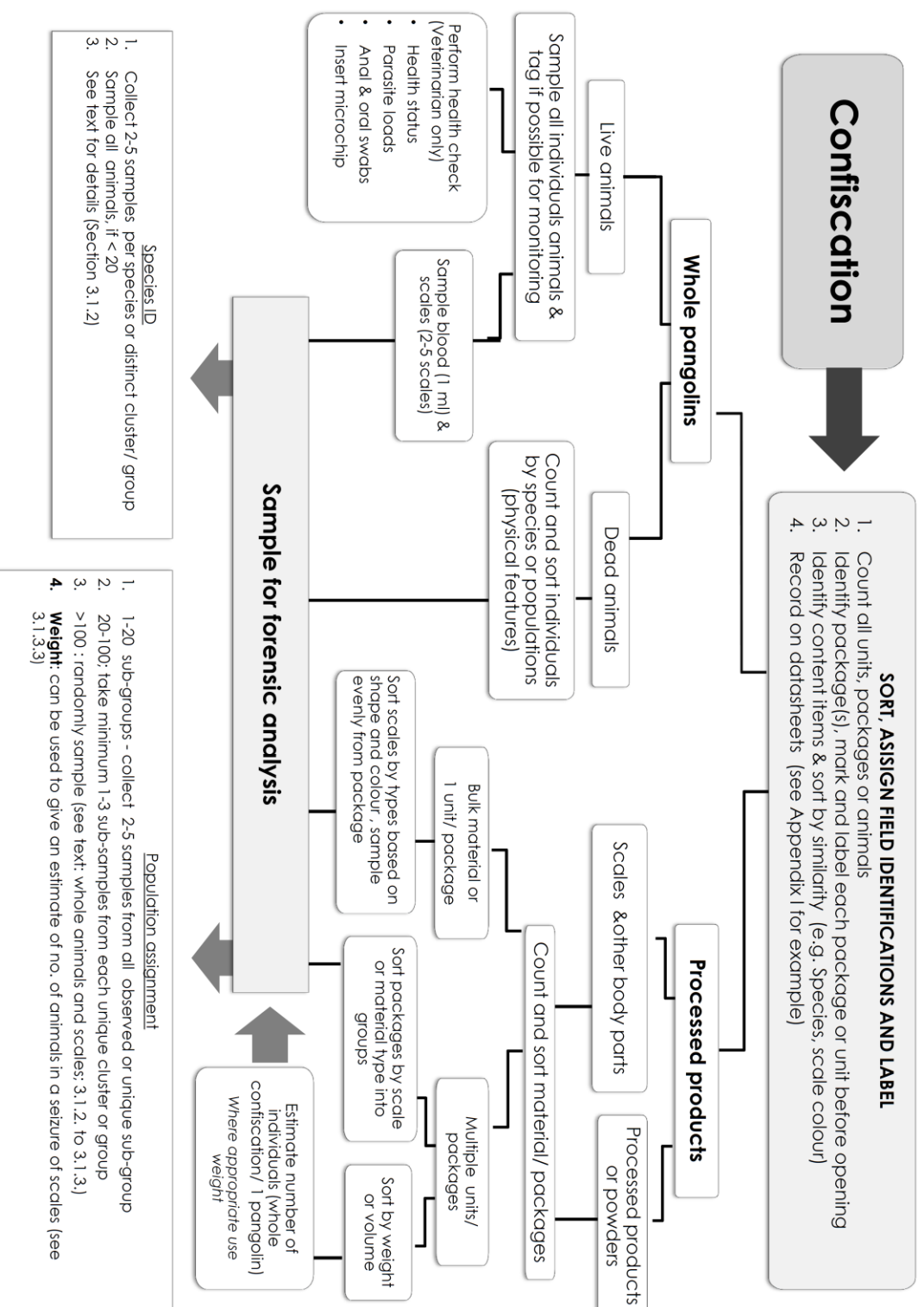


Figure 1. Sampling plan approach for processing pangolin seizures of different materials.

3.2. Random (non-statistical) sampling

There are a number of different random or arbitrary sampling methods. The objective of a sampling procedure is to reduce the number of samples or ensure representation for use in subsequent forensic analysis. Several random sampling protocols and formulas that have been used for sampling large confiscations (e.g. drugs) will be discussed here. The different protocols are outlined in Table 1.

Table 1: Random sampling protocols for selecting samples from a seizure, (modified from ref ⁴). N is defined as the seizure and n is the sample selected from the confiscation.

Options	Formula	Procedure	Limitation
Protocol 1	$n = N$	Sample everything	Less information Suitable for smaller and live animal confiscations
Protocol 2	$n = 0.05(N)$	Only sample 5% of each confiscation e.g. if $N = 40$ therefore, sample ~3 packages	Excessive sample size in case of large confiscations
Protocol 3	$n = 20 + 10\%(N - 20)$ $N > 20$	e.g. where $N = 100$ therefore, sample $n = 28$	Excessive sample size in case of large confiscations
Protocol 4	$N < x, n = N$ $x \leq N \leq y, n = z$ $N > y, n = \sqrt{N}$	Where $x = 10, y = 100, z = 10$ If above true, $n = N$ follows line protocol 1, sample all Where $n = z$ e.g. $n = 10, n < 20$ therefore sample all $n = \sqrt{N}$ e.g. $n = \sqrt{300}$, sample 22 packages	Excessive sample size in case of large confiscations

Suppose $N = x$ for each of the protocols mentioned above, n will be the number of units to be sampled from the confiscation that adequately represents the characteristics of the seizure. N = seizure size, n = sample size; where x, y and z are arbitrary numbers.

The protocols defined above offer a simple approach to random sampling from large seizures to gain information that characterises the seizure. Any of the listed protocols can be used based on specific limitations or capacity for efficiency and cost effectiveness. An example of the use of the protocols listed in Table 1 is shown in Table 2. It is important to note that these approaches do not bear any statistical basis⁴.

Table 2: Using Protocol 3 to select samples from a seizure of pangolin scale packages.

Size of seizure (N)	Number of samples to select (n)
1 - 20	Follow protocol 1
30	21
40	22
50	23
60	24
70	25
80	26
90	27
100	28

3.3. Statistical approach

Statistical approaches that have confidence levels for representative, unbiased sampling are available. These approaches can be applied by forensic labs with the relevant expertise and capacity during sampling to make a statistical inference about the whole confiscation. The two basic assumptions are that ^{4,5};

1. The proportion in the sub-sample is an accurate estimate the composition of the seizure (Frequentist approach) or
2. The sub-sample proportion is known, fixed and an accurate estimate the composition of the seizure as it takes prior information into account (Bayesian approach).

Statistical approaches are important if conclusions about the whole confiscation are to be drawn based on the selected samples. The requirements of the question being asked will determine the appropriate statistical approach. However because of complexity, these have not been included in these standard guidelines. List of published methods that can be consulted include two frequentist approaches: hypergeometric distribution^{4,6}, binomial distribution⁴ and the Bayesian approach^{4,7} for determining sample size.

4. REFERENCES

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APPENDIX I: Barcode of Wildlife project (BWP) South Africa step-by-step sampling protocol.

STEP-BY-STEP PROCEDURE AND PHOTOGRAPHIC SEQUENCE FOR CHAIN OF CUSTODY (COC) REFERENCE SAMPLE COLLECTION

Make sure that each photo has:

- CAMERA SETTINGS WITH CORRECT Date and time
- A SCALE reference (e.g. standard ruler)
- WHITEBOARD indicating: *SPECIES, FIELD ID, LOCALITY* and *DATE*
- Each photo must be in LANDSCAPE view (horizontal)

ACTION	STEPS IN COC COLLECTION PROCESS	TICK
Process	1. Check that the date, time & number sequence SETTINGS ON CAMERA is correct.	
Process	2. Put on Latex GLOVES .	
Process	3. SET UP a suitable surface for doing the sampling: plastic sheet/Cloth provided/clean, stable and even surface.	
Process	4. Set up the WHITEBOARD : WRITE the details for (1) Species, (2) Field ID, (3) Locality, (4) Date and (5) Time. Write the word START on the whiteboard.	
Photo	5. Photograph the LABELLED whiteboard.	
Process	6. ERASE the words START and the TIME from whiteboard.	
Photo	7. Photograph the packaged sampling kit with the whiteboard.	
Photo	8. Photograph laid out UNLABELLED sampling kit items & the BLANK field data sheet <i>Ensure that the evidence sealing bag seal numbers are readable / visible.</i>	
Process	9. LABEL all sampling kit items (falcon tubes, evidence bags etc) with field ID Enter collection event data on relevant field datasheet.	
Photo	10. Photograph the laid out sampling items & the field datasheet. <i>Ensure that the new labels on the bags, datasheet and the sampling tubes are readable / visible.</i>	
Photo	11. Photograph the WHOLE ANIMAL (This will be the e-voucher) showing the labelled whiteboard.	
Photo	12. Photograph all relevant DIAGNOSTIC characters of the species (as many photos). <i>Knowledge of the diagnostic features of a particular taxon will facilitate the capture of additional, more detailed photographs that are critical for identification purposes.</i>	
Photo / Process	13. Photograph the sample COLLECTION PROCESS, showing the position on the specimen from which the sample was removed (e.g. take a photo of the syringe drawing a blood sample from animal).	
Photo	14. Photograph the TRANSFER of collected sample into the labelled sampling kit (e.g. Photograph the process of transferring the collected blood into a labelled EDTA tube).	

Tips for ensuring good quality photographs:

- Try to take photos at a 90° angle rather than at a slant
- Use natural light rather than a flash to avoid reflection or glare obscuring the numbers on plastic surfaces. If a flash is used direct this upwards – away from the item to be photographed.

STEP-BY-STEP PROCEDURE AND PHOTOGRAPHIC SEQUENCE FOR CHAIN OF CUSTODY (COC) REFERENCE SAMPLE COLLECTION


	15. REPEAT steps 13 and 14 for EACH SAMPLE that is collected (e.g. tissue, blood, FTA paper, hair, scales, feathers etc).	
Photo	16. Photograph the tubes/kits containing the collected samples & the field data sheet, NEXT TO THE OPEN empty tamper evident evidence bag into which they will be placed & the field data sheet. <i>Ensure that all labels are readable / visible and that tubes are tightly sealed.</i>	
Photo	17. Photograph the tubes/kits containing the samples & the field data sheet INSIDE THE OPEN empty tamper evident evidence bag. <i>Ensure that all labels are readable / visible.</i>	
Photo	18. SEAL the tamper evident evidence bag and photograph the sealed bag with the collected samples. <i>Ensure that all labels are readable / visible.</i>	
Process / Photo	19. Write the word END on the white board and the time, with the sample code, date, and photograph the board.	
FINISH	20. STORE evidence bags in cool dry space avoiding sunlight.	

Note: Steps 11-12 can be done before steps 8 depending on the environment and sampling conditions.

Tips for ensuring good quality photographs:

- Try to take photos at a 90° angle rather than at a slant
- Use natural light rather than a flash to avoid reflection or glare obscuring the numbers on plastic surfaces. If a flash is used direct this upwards – away from the item to be photographed.

APPENDIX II: Example of a COC sampling datasheet (NZG)



NRF NZG
National Research Foundation National Zoological Gardens of South Africa

Chain of Custody: Pangolin Collection Datasheet

For Biobank use only	
Acc. No.	
Cat. No.	

Note: Duplicate samples to be collected and placed into 2 forensic bags

General Information:

Sampling date: _____ Sampler: _____

Field ID: _____ Sex: Male Female Age: _____

Forensic bag seal ID: Bag 1: _____ Bag 2: _____

Microchip number: _____ New/Previously inserted: _____

Veterinarian sign: _____ Date: _____

GPS co-ordinates: South: East:

Origin of the animal: _____

Animal submitted by:

Name: _____

Institution: _____

Contact number: _____ Email: _____

Veterinarian details:

Name	Designation	E-mail	Telephone	Signature

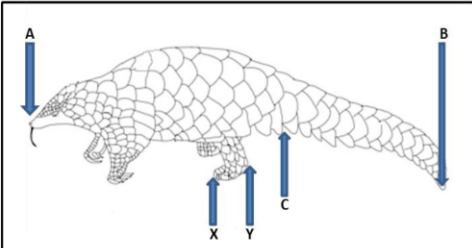
Sampled by:

Name	Designation	E-mail	Telephone	Signature

Conservation official details:

Name	Designation	E-mail	Telephone	Signature

Measurements:



Body length:	mm
Tail length:	mm
Foot length:	mm
Mass:	g

Measurements guide (along contours):	
A - B	Body length: from tip of nose to tip of tail
C - B	Tail length: from tip of tail to mid-anus (measured underneath the pangolin)
X - Y	Foot length: length of hind foot measured from heel to end of the middle toe

Health check:

	1	2	3	4
Body condition score	Bony, skeletal. Emaciated and bone structure visible	Thin neck and shoulder. Flattened tailhead, hip and pelvic bones	Moderate fat around tailhead, flattened pelvic and hip bones	Overweight. Thick neck with visible folds and rounded shoulders

General appearance / Initial observations: _____









Body temperature:	°C	External parasites:	yes	no
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Eye examination: _____

Dehydration: _____

Visible injuries: _____

APPENDIX III: Pangolin scale types, features and topography (Examples are for Temminck’s ground pangolin and the two African tree pangolins).

<p>1. Broad rhombic shape</p> 	<p>Topography Broad, presence of longitudinal grooves. Indentations sometimes present from wear and tear i.e. friction between scales or interaction with environment</p> <p>Location on pangolin body Back and tail</p> 
<p>2. Elongated kite shape</p> 	<p>Topography Presence of longitudinal, median keel that extends to the tip</p> <p>Location on pangolin body Shoulder flanks and limbs</p> 
<p>3. Folded shape</p> 	<p>Topography Folded scales found from the first row beginning of tail and extend only on the lateral side of the tail.</p> <p>Location on pangolin body Tail</p> 
<p>4. Claws</p> 	<p>Topography Claws are different sizes due to their function in digging. Pangolins limbs extend into five digits each with a claw.</p> <p>Location on pangolin body Fore limbs and Hind limbs</p> 

APPENDIX IV: African pangolin species (Photos: © Copyright African Pangolin Working Group 2017)

a) Giant ground pangolin -*Smutsia gigantea*



b) Temminck's ground pangolin -*Smutsia temminckii*



c) **Black bellied tree pangolin** - *Phataginus tetradactyla*



d) **White bellied tree pangolin** - *Phataginus tricuspis*

