STANDARD OPERATION PROCEDURES FOR BIOLOGICAL SPECIMEN COLLECTION FOR SKYWALKER GIBBONS

THE MYANMAR SKYWALKER GIBBON CONSERVATION PROJECT

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UCDAVIS VETERINARY MEDICINE
Karen C. Drayer Wildlife Health Center

NCS MYANMAR

IUCN PRIMATES SECTION ON SMALL APES

FRIENDS OF WILDLIFE

FAUNA & FLORA INTERNATIONAL Myanmar

SUN YAT-SEN UNIVERSITY

DPZ

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The program: These standard operating procedures are an output of the Myanmar Skywalker Gibbon Conservation Project, a joint initiative of the Nature Conservation Society Myanmar (NCSM), University of California Davis School of Veterinary Medicine (UCD), IUCN Section on Small Apes (SSA), Friends of Wildlife (FOW), Fauna & Flora International (FFI), Wildlife Conservation Society (WCS), Sun-Yat-Sen University, the German Primate Center and the California National Primate Research Center.

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IUCN Section on Small Apes

TARGET SPECIES

- This project will be surveying some forest areas that have never been surveyed before. Therefore, while the Skywalker gibbon (*Hoolock tianxing*) is the priority species for acoustic population surveys, biological specimens should be collected from all primate species which are encountered.
- Take a photo for identification purposes if possible.
- Molecular identification will be used to confirm species identity.

Skywalker Hoolock Gibbon (Hoolock tianxing). Other gibbons which may be encountered: Hoolock leuconedys. Photo: Peng-Fei Fan.

Mount Popa Langur (Trachypithecus popa). Other langurs which may be encountered: Trachypithecus phyrei. Photo: Thaung Win.

Bengal Slow Loris (Nycticebus bengalencsis). Photo: Endangered Primate Rescue Center.

Rhesus macaque (Macaca mulatta). Other macaques which may be encountered: Macaca fascicularis, Macaca nemestrina. Photo: Tierra Smiley Evans.

MATERIALS NEEDED

- ❑ Disposable gloves
- ❑ 15 ml tubes pre-filled with 9 ml DNA / RNA shield
- ❑ 15 ml tubes pre-filled with 9 ml 95% ethanol
- ❑ 2 ml cryovials pre-filled with 1 ml DNA / RNA shield
- ❑ 5 ml cryovials pre-filled with 2 ml DNA / RNA shield
- ❑ Disposable scalpel blades
- ❑ Parafilm
- ❑ GPS
- ❑ Cryo-safe marker pen
- ❑ Cool box if available
- ❑ First aid kit
- ❑ Dacron tip plastic swabs
- ❑ Blood micro-sampling tips
- ❑ Desiccant packets
- ❑ Plastic Ziplock bags
- ❑ Sample Data Log (Appendix I)

PROPER HANDELING OF SAMPLES AND REAGENTS

- Avoid direct contact of your fingers / hands with the specimen. Alternatively use:
    - Gloves
    - Disposable sterile sticks
    - NEVER your bare hands
- DNA / RNA shield is meant to stabilize DNA and RNA in the samples. DNA and RNA remain stable under the following conditions:
    - 7 days at 35 – 40 °C
    - 30 days at 4 – 25 °C
    - 3 to 6 months at -20 °C
    - Indefinitely at -80 °C
- DNA / RNA shield is a toxic chemical and can cause chemical burns or dizziness if inhaled. This reagent is poisonous if ingested. Only authorized study personnel should handle this reagent and it should never be left behind in a village or forest area where humans or animals could contact it.
- In the event of an accidental spill on your body, flush the area with copious amounts of water.
- In the event of accidental inhalation and if dizziness occurs, move to a well-ventilated area.
- Try to avoid aerosolizing the liquid by always pipetting the liquid slowly and letting it fall down the side of the tube instead of producing bubbles, splashes or aerosolizing the liquid.

SAFE HANDELING OF PRIMATE SPECIMENS

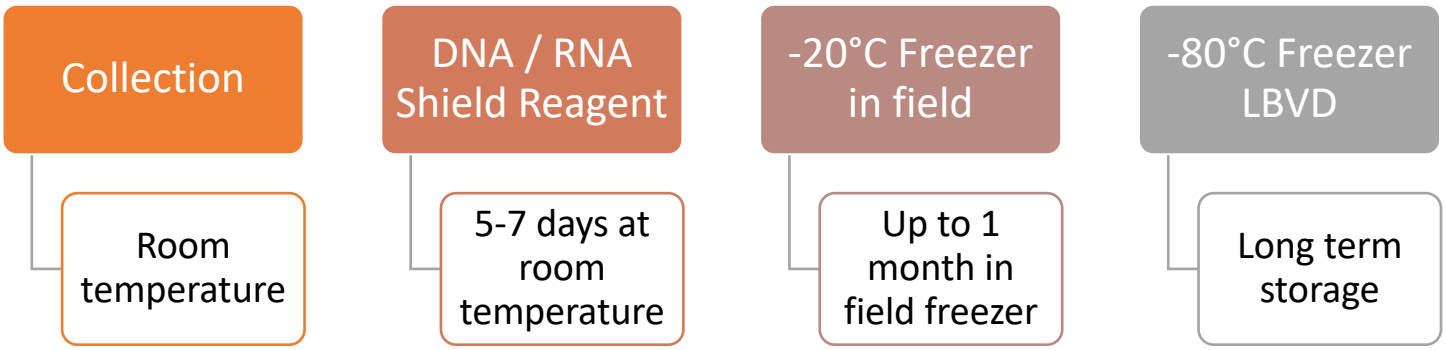
- Because primates are genetically similar to humans, we can share many of the same diseases. Specific precautions are needed whenever handling primate specimens.
- Diseases that we know primates carry and can pass to humans include:
    - Measles
    - Tuberculosis
    - Respiratory pathogens (bacterial, viral and parasitic)
    - Enteric pathogens (Shigella, Giardia, Campylobacter, Cryptosporidium)
    - Herpes B (carried by Macaque species) causes a rapidly fatal encephalitic disease in humans

- Personal protective equipment (PPE) should be worn at all times when collecting or handling primate specimens including:
    - Gloves
    - Goggles (when you anticipate a potential spray of bodily fluids)
    - Mask
    - Dedicated garments which can be discarded and or washed in a bleach solution
- You should not eat or drink when handling primate specimens.
- Hands should be washed before eating or drinking after working with specimens.
- If you are accidently injured while collecting a sample such as being stuck with a sharp object, scratched or bitten you should immediately immerse or pour 95% ethanol over your wound, which is available in the sample collection tubes.
- Next wash the wound. Use betadine, soap or an appropriate cleanser that is available. Continue to flush the wound with water that you have carried with you or an available stream for 10 minutes.
- Flush splashes to the nose, mouth or skin with copious amounts of water.
- Irrigate eyes with clean water, saline or eye wash.
- Seek immediate medical attention, particularly if bodily fluids from a macaque species come into contact with broken skin (i.e. a scratch or needle stick) or exposed mucous membranes (i.e. eyes, mouth, or nose) as herpes B infection in humans is a very serious disease.

COLD CHAIN CONSIDERATIONS

- This project's main objective for biological specimen collection is to molecularly identify species using genomic DNA. Genomic DNA is relatively stable in the environment and molecular identification of species has been successfully performed using samples stored under a variety of conditions including samples stored at room temperature, dried and frozen.
- A secondary consideration of this study is the opportunistic collection of specimens which may inform on the health of primate populations, including collection of RNA and DNA viruses, parasites and bacteria. These agents have a variety of cold chain requirements. RNA viruses for example, degrade very quickly in the environment and require careful handling and preservation as soon as possible.
- With this consideration, we have recommended a spectrum of sample collection and storage procedures, with priority given to DNA specimens for species identification.

- Reasons for maintaining cold chain: cells that are present in tissues, feces, blood, saliva and other bodily fluids begin to lyse (break down) immediately upon exit from the body. During this process, enzymes called RNAses begin to rapidly degrade RNA viruses. RNAses are highly prevalent in specimens such as saliva and whole blood. Cooling the enzymes diminishes their activity and allows the sample to remain in good condition so that diagnostic tests can be performed at a later time.
- Sunlight can also rapidly degrade samples, particularly for pathogens such as RNA viruses.
    - Select sample collection and processing sites in a shady area
    - Do not let samples sit out in the sun once collected
- All samples for this project should be stored under the following conditions (*unless an exception is noted as in for dried specimens).



TUBE LABELING AND DATA RECORDING

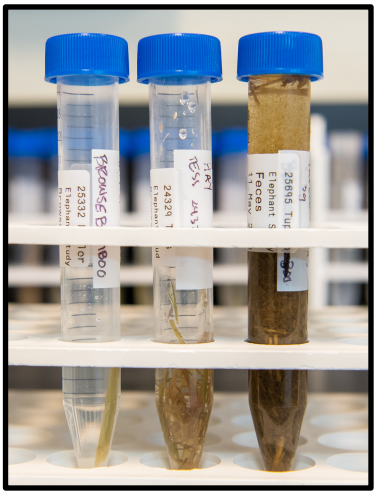
- The following information should be recorded directly onto sample tubes using a cryo-safe pen:
    - Species (If known; if in doubt, label at genus level i.e. gibbon, langur, macaque)
    - Date
    - Location name (All survey sites should be given a designated site name in the site data collection form (Appendix II))
    - Sample type
    - Storage media (i.e. DNA/RNA Shield, ethanol, none)
- The following information should be recorded on the specimen data collection form (Appendix I)
    - GPS coordinates
    - IUCN Habitat Classification of site location

ALIQUOT NUMBERS

Sample Type Encountered	DNA / RNA Shield Sample Aliquot	95% Ethanol Sample Aliquot	Dry / No media Sample Aliquot
Feces	1	2	
Chewed plants	2		
Whole recently killed / dead body	1 muscle tissue, 1 of each organ tissue (as feasible), 1 oral swab, 1 nasal swab, 1 rectal swab		2 micro-blood samples
Dried parts (bones, skull, etc.)			1

FECAL SAMPLE COLLECTION

- Samples must be fresh and ideally collected immediately after defecating
- Samples can be a maximum of 12 hours old
- Try to only include portions of fecal sample that were not in contact with ground
- Transfer 1 gram (equivalent of 5 peas) to a 15 ml tube prefilled with 9 ml DNA / RNA shield
- Mix thoroughly by crushing the fecal sample with the end of a disposable plastic applicator tip
- Close the tube and mix thoroughly by shaking
- Seal tube with parafilm

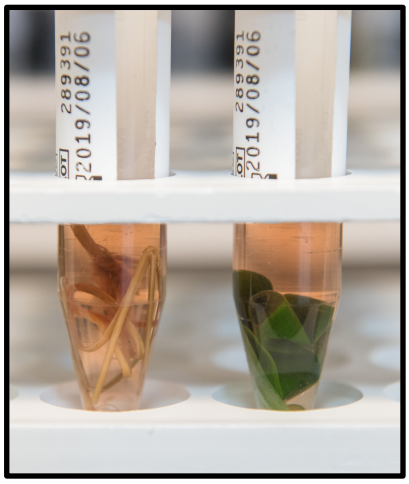


A total of 3 aliquots will be collected per primate (2 in ethanol, 1 in DNA / RNA shield.)

- Transfer 1 gram (equivalent of 5 peas) to a 15 ml tube prefilled with 9 ml 95% ethanol
- Mix thoroughly by crushing the fecal sample with the end of a disposable plastic applicator tip
- Close the tube and mix thoroughly by shaking
- Seal tube with parafilm
- Repeat for a second ethanol sample

CHEWED PLANT COLLECTION

- Chewed plants have been used successfully to identify host genomic DNA and viruses in a variety of species (Smiley Evans et al 2012, Smiley Evans et al. 2020).
- Identify trees where primates have been feeding and dropped plants
- Samples should be as fresh as possible; ideally you will have observed primates eating and dropping items
- Identify ends and/or most chewed portions of plants
- Cut most chewed portion of plants and place in 10 ml tube containing 2 ml DNA / RNA shield
- Fill the tube with chewed plant material until the liquid no longer can cover added material (repeat for 2 samples)
- Invert tube to "wash" the chewed plants with liquid
- Take a photo of the plants (chewed portion and whole intact plant for ID)



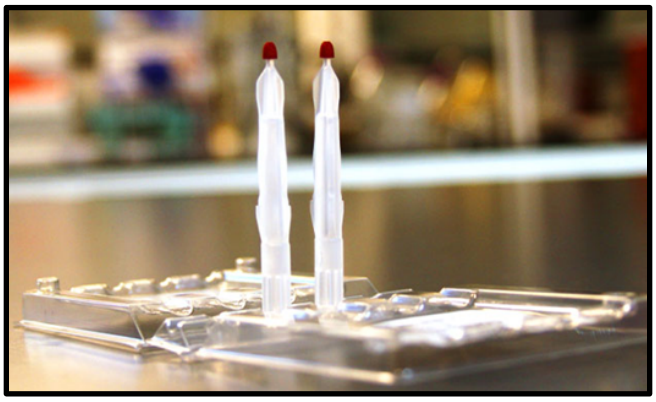
A total of 2 aliquots will be collected per primate (both in DNA / RNA shield).

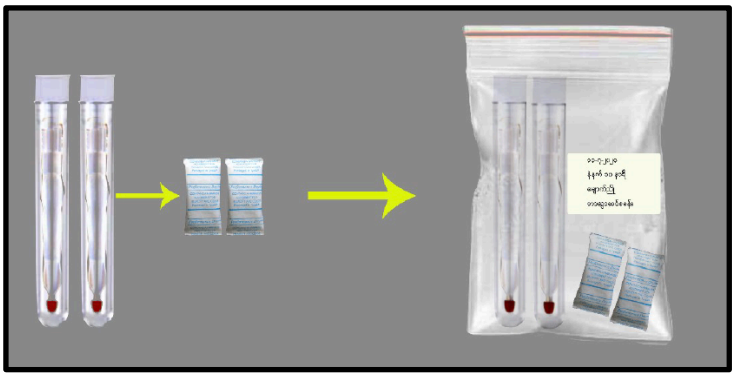
FRESH TISSUE COLLECTION

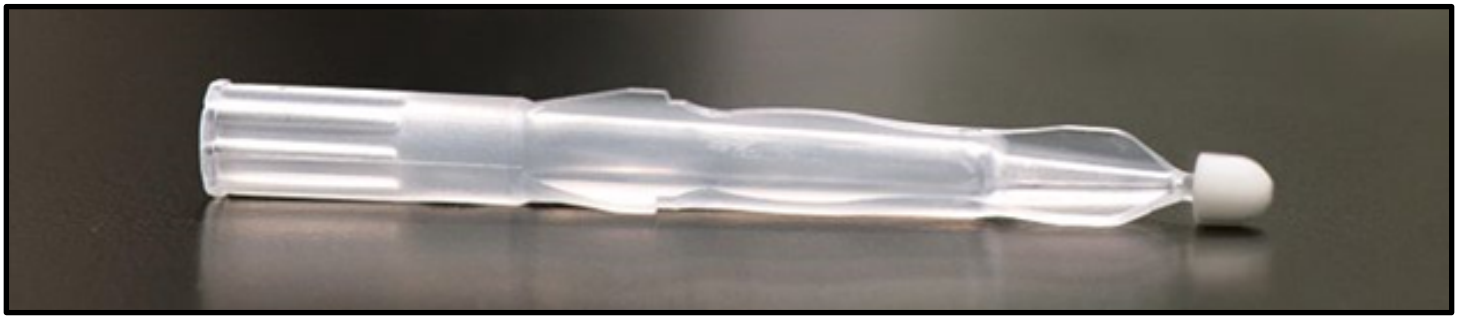
- Fresh tissue should be collected from dead bodies when encountered in the forest or when hunted animals are found in markets or villages.
- Cut 1 gram (size of 5 peas) fresh tissue using a disposable scalpel blade
- Transfer tissue to a 15 ml tube prefilled with 9 ml DNA / RNA shield
- Close the tube and mix thoroughly by shaking
- Seal tube with parafilm
- Note type of tissue (i.e. organ type, muscle type, etc. on sample log)

MICRO-BLOOD SAMPLING

- Identify source of blood from dead primate
- Prioritize locations that do not have dirt, leaves or other materials on them.
- If the animal is being butchered, prioritize an internal blood source (i.e. heart) as opposed to cuts in the skin.
- Take a micro-sampling tip and touch it to the blood source until it turns completely red (do not submerge in blood)
- This should be approximately 3-6 seconds and no blood should be dripping once fully saturated
- Collect 2 micro-sampling tips per animal
- Place micro-sampling tips into the clamshell or designated (non-sealed tube)
- Place clamshell (or tubes) in Ziplock bag with a desiccant packet
- The goal is for these samples to be dry and have air circulating with the desiccant packet







SWABS

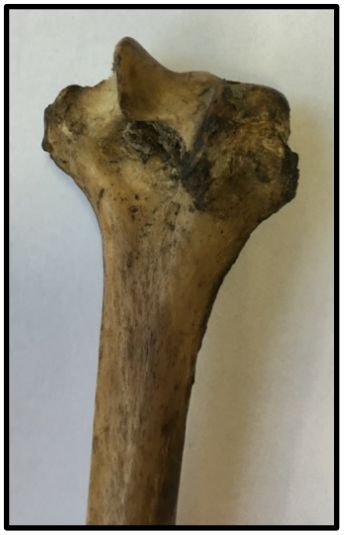
- Using sterile polyester or Dacron-tipped swabs, collect 1 nasal, 1 oral and rectal swab.
- Place each swab in separate 2 ml cryovials filled with 1 ml DNA/RNA shield.
- After placement into the tubes, break swab tips on the shaft as close to the swab tip as possible.
- After closing tube mix tubes well by inverting.

Collection of rectal swab from hunted langur.

Collection of nasal swab from hunted langur.

DRY TISSUES

- Dry tissue samples can be collected from dead bodies found in the forest or from hunter trophies (skins, soft tissue attached to bones/skulls)
- Cut 1 gram of skin / tissue (size of 5 peas) with a disposable scalpel and transfer to a 15 ml (non-sealed) tube
- When only soft tissue attached to bones/skulls is available, scratch whatever is possible, and transfer to a 15 ml tube
- Place tube inside a Ziplock back with a desiccant packet



LEACHES

- Leaches have been successfully used to detect blood meal hosts and may potentially be useful for identifying primate species in which they have fed on (Schnell et al 2012).
- Wait until leeches invade your body and collect them with tweezers. You want to collect the leach before it starts feeding on your blood, otherwise the blood meal host identified will be you!
- Only collect larger leeches as only those contain blood meals
- 5-10 leeches per site and transfer them to a 15 ml tube prefilled with 9 ml DNA / RNA shield
- Close the tube and mix thoroughly by shaking
- Seal tube with parafilm

REFERENCES

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APPENDIX I

SPECIMEN DATA COLLECTION LOG

Genus	Species	Specimen Collection Date	Storage Media	Location Description	Latitude	Longitude	Sample Type (Feces, tissue, blood, chewed plant, etc.) Please specify tissue or organ type.	Age Category (Infant, Juvenile, Adult, Unknown)	IUCN Habitat Classifcation (See Appendix II)	Plant Type (If Applicable)

APPENDIX II

IUCN HABITAT CLASSIFICATION SCHEME

We will be assessing the location of each sample collected using reported GPS locations but would also like each person collecting specimens to note the general habitat surrounding them. Please refer to the detailed guidelines provided by the IUCN on classifications of habitats available at:
https://www.iucnredlist.org/resources/habitat-classification-scheme