Section 5.2.6. Non-Human Primate Sampling Methods

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Objective: To safely collect biological samples from non-human primates.

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The authors assert that animal capture and sampling should always occur in compliance with all applicable laws and regulations and should only be undertaken after securing all necessary permits and approvals, including ethical approvals.

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Section 5.2.6a. Confirmation of Knowledge
When you are familiar with the information in this Guide, take the PREDICT quiz Section 8.4.5. Non-Human Primate Sampling.

Section 5.2.6b. Brief Overview of PPE

Minimum PPE Required for Handling Live, Dead, or Samples of NHP
The minimum PPE for NHP sampling includes:

1. Eye protection (goggles or face shields)
2. N95 (or better) respirator
3. Long clothing/tyvek suits (Duct tape can be wrapped around the overlapping tyvek suit and gloves at the wrist to avoid skin exposure)
4. Nitrile gloves (double gloving is preferred, especially if sampling dead NHP)
5. In the rare cases where it is acceptable (see below), anyone hand-restraining NHP for sampling should wear disinfected\(^1\), heavy-duty leather (or similar) gloves to protect against bites
6. In order to protect both human handlers and sampled NHP, all personnel handling NHP should be tuberculosis (TB) tested beforehand as described below

Macaque Handling
Due to the risk of infection with Cercopithecine herpes 1 (‘B virus’), which can be fatal in humans, handling macaques (or other potential B virus carriers such as other NHP in close contact with macaques) requires special preparation. When handling macaques, it is imperative that before animals are handled all precautions are taken to minimize the risk of exposure to B virus and to minimize the risk of infection in the event of an accidental exposure. Please note that human to macaque transmission of herpesviruses may also occur. Protective measures include:

- Wearing a full-face shield (not just goggles) along with an N95 (or better) respirator.
- Having sufficient and immediately available eyewash (1 liter of saline if working in remote location) for a 15-minute continuous flush of any exposed mucous membranes.
- Having water and detergent soap (chlorhexidine or povidone-iodine) immediately available and in sufficient quantity to allow a 15 minute scrub of any exposed skin.
- Preferably also having freshly prepared 0.25% hypochlorite/Dakin’s solution (1:20 dilution of household bleach) for initial wash of skin - but NOT mucous membranes.

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\(^1\) Because they are porous, leather gloves cannot easily be disinfected. Spraying, wiping, or soaking in the best available disinfectants (e.g., 10% bleach) and allowing to sit or dry for >10 minutes can help destroy many potential pathogens. Likewise, wearing and changing over-sized disposable gloves over protective leather gloves can help to minimize cross contamination between handled animals. More easily cleaned protective gloves made from synthetic materials (heavy duty nitrile, Kevlar) can also be used. Some PREDICT teams have had good success with Hexarmor Hercules 400R6E gloves.
• Keeping extra swabs, viral culture media, and sero sampling materials available for post-exposure sampling of handler and macaque.
• Carrying medical alert cards.
• Consider having a cage ready for short term (2-3 week) captivity of suspect macaques for post-exposure sampling in the event of accidental exposure.

**First Aid Guidance for a Bite, Scratch, Needlestick, or Facial Splash**
The injured person must notify other research staff and work must stop immediately (with the possible exception of other workers ensuring the safety and containment of any live animals).

**All NHP -** Any bite, scratch, or needlestick site should be immediately washed well with soap and water for a full 5 minutes and then with betadine (povidone-iodine) or benzalkonium chloride (if available and especially if rabies virus exposure is suspected).

**Macaques (or other possible B virus carriers) -** Any possible exposure to B virus is potentially life threatening and must immediately trigger activation of the B Virus Emergency Exposure Protocol detailed in [Section 5.2.6g. Appendix II. B Virus Exposure Emergency Protocol](#).

**Suspect Ebola cases (e.g., ape carcasses) -** Any possible exposure to Ebola virus is potentially life-threatening and should immediately trigger activation of the Ebola Virus Emergency Exposure Protocol detailed in [Section 5.2.6h. Appendix III. Ebola Virus Exposure Emergency Protocol](#).

**Section 5.2.6c. Special Considerations for Handling NHP**

*Note:* This training guide supplements the Safe Animal Capture and Sampling (Section 5.2.5), which contains general information on working with wildlife species. This training guide also complements information in the Bushmeat Sampling Methods (Section 5.2.12.).

Handling NHP involves a number of special considerations.

1. Regardless of their specific status (e.g., endangered, threatened, protected or not), NHP are often high-profile species that engender special attention. Anyone handling NHP should strictly adhere to all regulations and follow all protocols and guidelines.
2. All primate species, regardless of size, are capable of inflicting serious injuries to their handlers; particularly bite wounds. Unlike most other taxa, many NHP have grasping hands and feet and are likely to grab (and then bite) rather than scratch or push their handlers during procedures. Heavy-duty leather gloves should be worn by anyone handling conscious (unanaesthetized or unsedated) NHP. Hand restraint is discouraged as a primary means of NHP immobilization. Chemical, rather than physical, restraint should be employed with a few exceptions. Hand restraint may be considered in rare instances when it can be done safely and without significant added stress or risk to the animal,
such as when handling infants, severely debilitated individuals, or during the process of chemically immobilizing very small NHP with hand injections.

3. NHP are typically very social animals and are likely to protect and defend other individuals in their group. Care must be taken, particularly during capture and immobilization, to protect against attacks, injuries, or disruptions from non-target individuals and especially from defensive adult males. Using visual blinds to hide activities and/or employing personnel fully dedicated to watching for aggressive or approaching animals can help minimize these risks.

4. Due to their size, considerable strength, and in some cases habituation to human visitors, great apes (and some larger monkeys) should be considered very dangerous. Even without aggressive intentions, field staff should be aware that great apes often grab, kick, strike, and drag humans for play and/or display behavior purposes.

5. If NHP need to be tracked for capture, or are opportunistically sampled as individuals or in low numbers, it may not be feasible or practical to set-up proper sampling stations as described below. In such situations, sampling station guidelines should be followed as closely as possible for both field collection sites and any later sample processing sites.

6. PREDICT personnel should already understand that due to their close genetic relationship to humans, NHP are considered to be more likely to share infectious agents (zoonoses) with humans. This means that they more likely to transmit infections to their human handlers, and they are also more susceptible to acquiring infections from their handlers.
   a. Proper use of PPE and related biosafety measures as described will help protect both handlers and the sampled NHP.
   b. To protect both staff and any handled NHP, all people working closely with NHP should be tuberculosis (TB) tested every 6 months with negative results documented and available before handling NHP. Any staff suspected of being infected with TB must not work with NHP. TB testing is typically done by intradermal tuberculin skin test (TST). Workers who have been vaccinated with BCG (Bacillus Calmette-Guerin, standard vaccine for many Europeans) should still be tested and the possibility of false positive results from vaccination needs to be discussed with their health care provider (see relevant information at: http://www.cdc.gov/tb/publications/factsheets/testing/diagnosis.htm). Personnel vaccinated with BCG and positive skin test should work with their health care provider to have additional confirmatory tests performed.
   c. To protect NHP from human infections, no persons with any current or recent (within a few days\(^2\)) clinical signs of illness (coughing, sneezing, fever, diarrhea, rash, cold sores, etc.) should handle or have close contact (<5 m) with any NHP. It must be remembered, however, that many agents are infectious to other animals before the infected individual becomes clinically ill (or after recovery). Ideally, personnel working

\(^2\) There are no distinct time rules because pathogen shedding depends on many host and pathogen-specific factors. Though infectivity can in some cases range up to many months after resolution of clinically apparent disease, in healthy adults most pathogens of concern here (e.g., respiratory viruses) are unlikely to be transmissible for more than a few days after recovering from illness.
regularly with NHP should participate in some level of an employee health program, and be up to date on all available vaccinations (especially measles, polio, hepatitis A, influenza(s), meningococcal meningitis, rabies, and tetanus). This helps to ensure their health and to protect their co-workers and any animals they may handle.

7. NHP are not typical sources of rabies virus transmission to humans, but like any mammal must be considered a risk, especially in areas where they might be regularly exposed to common, high-risk rabies reservoirs (e.g., domestic dogs in many countries). If there is any suspicion of rabies exposure (e.g., handler is bitten by or exposed to nervous tissues from a primate exhibiting neurologic signs), their physician should be contacted and post-exposure rabies vaccination should be obtained as soon as possible. Rabies symptoms in primates are variable (irritability, self-mutilation, paralysis, malaise).

8. NHP are also not typical sources of anthrax exposure in humans, but are known to suffer and even die from anthrax, including in atypical forest environments. Proper PPE use and appropriate disposal of suspect carcasses are the most effective measures of preventing anthrax exposure. For additional information see http://www.bt.cdc.gov/agent/anthrax/.

9. Two particularly important and dangerous pathogens that workers may be exposed to by handling NHP are Ebola virus and Cercopithecine herpes-1 (B virus). EXPOSURE TO THESE PATHOGENS IS LIFE-THREATENING AND REQUIRES IMMEDIATE ACTION.

B virus- PREDICT staff are most likely to be exposed by handling live macaques, which should always be assumed to be infected with B virus, with or without any clinical signs. Macaques with oral lesions (right) should be handled with extreme caution and only by highly trained staff, if they are handled at all. Macaques shed the virus in their oral, gingival, and genital mucosa and transmission can occur via bites, scratches, percutaneous inoculation with infected materials (e.g., accidental needlestick), and mucosal splash exposure. There is risk of B virus exposure from macaque CNS (central nervous system) tissues and CSF (cerebrospinal fluid), but peripheral blood from macaques has not been known to cause infection in humans. To prevent exposure to B virus, workers must always follow all PPE procedures and the precautions outlined below. In the event of accidental exposure, workers must stop IMMEDIATELY and trigger the B Virus Emergency Exposure Protocol detailed in Section 5.2.6g, Appendix II. TIMING IS CRITICAL and an immediate action can be the difference between life and death. Additional information on B virus can be found here:
http://www.cdc.gov/herpesbvirus/index.html
http://www.cdc.gov/mmwr/preview/mmwrhtml/00015936.htm
http://www2.gsu.edu/~wwwvir/index.html
**Ebola virus** (and related Filoviruses) - PREDICT staff are most likely to be exposed by handling dead African ape carcasses, including bushmeat. Transmission can occur through contact with infected tissues, secretions, and body fluids and can be prevented through proper use of PPE and related barrier techniques (see [www.cdc.gov/ncidod/dvrd/spb/mnpages/dispages/ebola.htm](http://www.cdc.gov/ncidod/dvrd/spb/mnpages/dispages/ebola.htm) or [http://emedicine.medscape.com/article/216288-treatment](http://emedicine.medscape.com/article/216288-treatment) for more detailed information). Extreme caution must be taken by anyone sampling cases where Ebola infection is suspected. In the event of accidental exposure to Ebola virus (e.g., needlestick injury, any direct contact of eyes, skin or mucous membranes with infected fluids) workers must stop immediately and follow the details in Section 5.2.6h, Appendix III, Ebola Virus Exposure Emergency Protocol. Symptoms of Ebola and complications are treated as they appear. The following basic interventions, when used early, can significantly improve the chances of survival:

- Providing intravenous fluids (IV) and balancing electrolytes (body salts).
- Maintaining oxygen status and blood pressure.
- Treating other infections if they occur.


**Section 5.2.6d. Primate Sampling**

*Note: Capturing, trapping, darting, and immobilizing NHP should only be performed by experienced and skilled staff and are not entirely covered in this document (Hughes, T. 2010).*

PREDICT partners are expected to have detailed capture/immobilization protocols (and recording sheets, monitoring sheets, etc.) for any target primate species. This sampling protocol assumes a starting point of either a safely immobilized or an already dead primate. A vervet monkey capture and anesthesia guide is provided in Section 5.2.6i, Appendix IV, PREDICT Vervet Monkey Capture and Anesthesia Guide.

For the PREDICT project, post-capture processing will entail a number of sometimes concurrent activities. The main objectives during processing are:

1. Safeguard the health of all handlers and any live animals being processed.
2. Collect required sample data.
3. Collect required biological samples.
4. Collect supplemental data and samples.
5. Await animal recovery or dispose of carcass.
6. After recovery, release animals as close to their site of capture as possible and follow all other guidelines for release as stated in the PREDICT IACUC protocol.
In some cases, time constraints, anesthetic risk, inability to prolong immobilization, or other factors may necessitate prioritizing biological sample collection at the expense of collecting any physical measurements. At a minimum:

1. Obtain and record the animal’s weight (kg) as this can be important for proper drug dosing or emergency interventions and to estimate the age category.
2. Conduct a cursory physical exam before sampling in order to note any lesions or major abnormalities.
3. If capture wounds are observed, treat as needed.

**Sample Data Collection**

Please refer to the required data collection templates for data to collect:

1. P2 Animal Data Collection Form
2. P2 Site Characterization Data Collection Form
3. P2 Specimen Data Collection Form

*Note: See Section 5.2.3. General Data Collection Templates and Applications for help downloading templates from EIDITH.*

**Additional (Optimum) Data to Collect from NHP**

*Note: The P2 data templates mentioned above are required to be filled in. Additional data and biometric measurements may be collected at the discretion of the sampling party.*

Ideally, the following additional data should be collected from any NHP that are processed:

- body mass (kg)
- age class (see below)
- sex (and possibly reproductive status if adult female)
- whole body photograph(s)
- identifying characteristic photographs
- morphometric measurements

**Body mass:** Body mass may be one of the first measurements taken in order to ensure proper drug dosages, etc. Being careful to monitor breathing, and depending on size, NHP should be weighed (kg) in bags, slings, or a suitable container using a calibrated hanging spring scale or, if they are small enough, a tabletop scale with or without a tray or other container. If large NHP exceed the limit of spring scales two or more scales can be linked (one hanging from the other) to distribute the weight. The total weight is the measure of both scales added together. Scales should be zeroed (checked to make sure they measure ‘0.0’ units when empty) and any containers (bags, slings, trays, boxes) should be weighed beforehand and then both primate and container should be weighed together. Once the primate is removed from the container for sampling, the container should be re-weighed and subtracted from previous total. Alternatively, the weighing container can be tared so that the scale reads ‘0.0’ units with the container, and
then checked to verify it still measures exactly zero after the primate is removed. If scales are not available or accurate weights cannot be measured for any reason, a weight should still be estimated but the recording sheet **MUST note that it is an estimated and not a measured weight.**

**Age class:** If exact age is known (e.g., for habituated NHP) that should be recorded. Otherwise, for most primate species it will be possible to classify into one of the age classes in the table:

<table>
<thead>
<tr>
<th>Age Class</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>Neonate</td>
<td>Animal shows signs of being born within a few days.</td>
</tr>
<tr>
<td>Infant</td>
<td>Animal is unweaned and usually still clinging to mother and suckling.</td>
</tr>
<tr>
<td>Juvenile</td>
<td>Animal is mostly independent from mother, not yet adult-sized, and sexually immature.</td>
</tr>
<tr>
<td>Immature</td>
<td>Any individual not evidently sexually mature.</td>
</tr>
<tr>
<td>Subadult</td>
<td>Animal is fully independent, appears to be sexually mature, but not fully physically mature (e.g., less than full adult size).</td>
</tr>
<tr>
<td>Adult</td>
<td>Animal has secondary sexual characteristics, adult size, sexually mature.</td>
</tr>
<tr>
<td>Old Adult</td>
<td>Adult showing signs of age degeneration</td>
</tr>
</tbody>
</table>

**Sex determination (species identification/examination):** Based on morphology and unique characteristics, identify NHP to genus, and species (where possible) and sex. Sex determination for young individuals of many primate species is not always simple and photographs of genitalia should be taken, especially if there is any doubt. For female NHPs, note parity (e.g., presence of offspring, evidence of previous lactation), also determine pregnancy status by gently palpating the abdomen (at least for small NHP), and determine lactation status by gently attempting to express milk from the teats (for larger NHP, milk samples can be collected in an empty cryovial and stored frozen). If dependent offspring are captured along with their mothers, they should not be removed from their mothers unless absolutely necessary (e.g., to prevent injury or if they are nearly independent/weaned) and then only for the minimal time required for sampling.

**Photographs:** At a minimum, the following digital photographs should be taken:

a. Anterior/ventral view of full body with arms at sides, preferably with identification card or sheet displaying unique identifying number.
b. Full anterior facial view.
c. Full lateral facial/head view.
d. Views of full upper and lower dentition (to help determine/verify age and sex).
e. Frontal/ventral view of fully exposed genitalia.
f. Views of any lesions (e.g., cuts, scratches), physical abnormalities (e.g., missing toes), or individually identifying marks or characteristics (e.g., healed scars, abnormal coloration, facial spots or wrinkles, etc.)

**Body measurements:** Time permitting, the biometrics (in cm or mm) should be recorded with the *minimum standard mammal measurements (all linear)*:

a. Head and body length (measured dorsally and linearly from tip of nose to base of tail when head is stretched and aligned with back). Note: For many NHP (e.g., apes) this measure is adjusted to what is called “crown-rump” length that starts at the top of the head in order to produce the longest linear measurement (without wrapping over the head).

b. Tail length (from base to tip).

c. Hind foot length (heel to tip of longest toe - exclude nail and note which toe).

d. Tibia length ('knee to ankle').

e. Ear length: base of the notch below the ear opening (lower rim of external auditory canal) to the most distant point of the margin of the pinna.

**Biological Sample Collection**

*In addition to the standard PREDICT sampling and analyses, PREDICT partners are encouraged to collect additional samples and pursue routine diagnostics (e.g., blood counts and chemistries, urinalysis, etc.) where resources allow. Sample collecting for archival is also strongly recommended. Opportunities to collect biological samples and related health data from wild NHP are relatively uncommon and maximizing these opportunities can further advance wildlife health.*

The following basic set of samples should be collected from each animal where possible (If only one sample can be collected, then place into VTM):

1. **Two oral swabs** - one in 500 μL VTM and one in 500 μL Trizol
2. **Two rectal swabs/ fecal samples** - one swab in 500 μL VTM and one in 500 μL Trizol OR 0.5cc (pea size) feces in 500 μL VTM and 0.5cc (pea size) feces in 1 mL Trizol
3. **Two whole blood samples** - one with max of 500 μL of whole blood in 500 μL VTM and one with max of 500 μL of whole blood in 500 μL Trizol
4. **Two serum samples** - 2 x 500 μL aliquots, frozen without media
5. **Urogenital swab/urine samples** – one swab each in 500 μL VTM and 500 μL Trizol OR one 500 μL urine sample each in 500 μL VTM and in 500 μL Trizol

**Freeze all samples (except tissue in formalin) in liquid nitrogen immediately in the field and transfer to -80°C freezer once back in the lab.**
If there is no short-term access (i.e., within 24 hours) to cold chain such as in an emergency situation then samples can be collected in 500 μL of RNAlater instead of Trizol and VTM. Storage times and temperatures for samples in RNAlater are as follows:

- 1 day at 37°C (i.e., ambient temp)
- 1 week in the refrigerator
- Within one week freeze at -80°C for storage until analysis

**Sample Collection from Live NHP**

Live NHP should be chemically restrained during any invasive sample collection (e.g., blood collection). Two, preferably three, people are required for these manipulations: one person to safely restrain or position the primate, one to take samples, and a third to manage the tubes (e.g., unscrew the lids, hold them up to the sample taker, make sure the lids are replaced tightly and kept in order) and record sample data.

**Blood Collection**

*Note:* At least one person present should have previous experience in primate venipuncture to avoid injury to the animal. No more than 1 ml of blood per 100 g (= 10 ml/kg or 1%) of primate body weight should be collected at any one time.

**Collection procedure**

1. Select appropriate venipuncture site:
   - **Forearm veins** - In larger species (e.g., apes), the cephalic, radial, median, and ulnar veins might be large enough for safe blood collection.
   - **Femoral vein** - Best for small NHP and for large sample volumes. If the femoral artery (just lateral/anterior to the vein) is inadvertently pierced sampling can continue but extra effort must be made to apply post-collection pressure for at least 1 full minute to minimize hematoma formation.
   - **Jugular vein** - This may be the only option in very small NHP and must be accessed carefully.
   - **Caudal saphenous vein (Figure right with laboratory macaque)** - With compression of the upper thigh or knee, this vein can be prominent and superficial, but often collapses during collection.

2. Select appropriate size needle and syringe (or vacutainer) for the size of the primate.
3. Disinfect the site with iodine solution or alcohol.
5. **Do not recap needle.**
6. Apply pressure to site of bleeding using a cotton ball or gauze pad until bleeding ceases (approximately 1 minute).
7. Process blood (see below).
8. Properly dispose of sharps and other biohazard materials immediately upon transfer of sample to collection vials and slides.

**Blood processing**

Place whole blood or blood clots in VTM and Trizol: If animals are large enough, collect whole blood into 1 lavender top tube containing EDTA and in 1 serum separator/serum-clotting factor tube (red top or tiger top) tube. From the EDTA tube, store 500 μl whole blood in a cryovial with 500 μl VTM and a second sample of 500 μl whole blood in a cryovial with 500 μl Trizol.

For serum, from the red top/tiger top tube, allow blood to clot and/or centrifuge. Use a plastic pipette to take 1 ml of serum and transfer into 2 cryovial tubes, 0.5 ml each. You can harvest additional serum for serum bank as appropriate. Transfer the remaining blood clots to separate cryovials. If the animal is not large enough to collect two blood tubes (for whole blood and serum), save the blood clot after serum separation. The blood clot should be placed in a cryovial with 500 μl VTM. Freeze all samples in liquid nitrogen in dry shipper or dewar and transfer to -80°C freezer when possible.

(Optional) **Whole blood in EDTA:** If facilities are available to perform complete blood counts (CBCs) within 5 days, remaining whole blood in lavender top tubes can be refrigerated for analysis. Blood smears can also be prepared in the field.

**Oral Swabs**

**Swabs in VTM and Trizol:** Using two sterile, polyester-tipped swabs with a plastic shaft, rub the swab tip gently but thoroughly against the back of the primate’s throat, saturating the swab with saliva. Place 1 swab in a cryovial filled with 500 μl VTM and use flame-sterilized scissors to cut the shaft of the swab above the tip. If using plastic shaft swabs when scissors aren’t available, insert the swab to the bottom of the vial and then lift the tip and snap the plastic shaft of the swab on the edge of the cryovial. If the plastic shaft is snapped when the swab tip is resting on the bottom, the swab will be too long and the cryovial won’t close. Place the other swab into 500 μl of Trizol in a cryovial, following the same procedures. After inserting the swab and closing the vial lid, shake each tube to mix the sample well. Store both cryovials in a liquid nitrogen dry shipper or dewar & transfer to -80°C freezer when possible.

**Feces**

**200 mg in VTM and Trizol:** Collect either excreted feces or if primate is large enough (> 1 kg) use a gloved, lubricated (saline or medical lubricant) finger to collect feces directly from rectum. Place a ~200 mg (pea sized) sample of fresh feces in a cryovial with 500 μl VTM and another ~200 mg sample in a cryovial with 1 mL Trizol. Homogenize by shaking. Freeze in dry shipper or dewar with liquid nitrogen and transfer to -80°C freezer when possible.
If feces are not available, collect 2 rectal swabs- 1 in VTM and 1 in Trizol. Rectal swabs can be moistened with sterile saline prior to animal sampling. Gently insert one sterile swab tip at a time into the animal’s rectum. [Note: DO NOT USE TRIZOL AS A LUBRICANT – IT IS HIGHLY IRRITATING TO TISSUE.] Place 1 swab in a cryovial filled with 500 μl of VTM using a flame-sterilized scissors to cut the shaft of the swab above the tip. Place the other swab into a cryovial with 500 μl Trizol. Store in a dewar or dry shipper with liquid nitrogen dry shipper and transfer to -80°C freezer when possible.

Urine
Most NHP will urinate as a fear reaction prior to handling, but urine can sometimes be collected free catch or by bladder expression by trained personnel. Place 500 μl of urine in a cryovial with 500 μl VTM, and another 500 μl of urine in a cryovial with 500 μl Trizol. Store in dry shipper or dewar with liquid nitrogen and transfer to -80°C freezer when possible.

(Optional) Ectoparasites (e.g., mites, lice, nits, fleas)
Collect any obvious ectoparasites (and hairs if necessary, e.g., for louse nits- pictured right) using forceps and place in labeled, appropriate sized container of 95% ethanol and store at room temperature.

(Optional) Milk
If lactating females are handled, milk may be collected into cryovial(s) and stored frozen at -80°C. For basic analysis 0.5-2.0 ml is adequate and even small NHP (less than 500 g) can be milked to full evacuation one time and provide ~1 ml of milk without risking the health of their infants. Dependent offspring are typically best left with the nursing mothers and separation of nursing young prior to sampling should never be done strictly for the purpose of collecting milk.

Non-Invasive Primate Saliva Sampling (Rope method)

Field Collection Supplies:
- Six inch nylon oral swab ropes (Salimetrics, Inc)
- Nylon swab retrieval strings (if necessary)
- 5 ml compartmental swab storage tubes (Salimetrics, Inc)
- Cryovials
- Backpack/bag for concealing collection supplies
- Attractant (jam, bananas, juice, honey, etc.)
- Viral transport media
- Pipettor and tips (or disposable pipettors)
- Cooler bags and ice packs
- Field centrifuge
- Trash bags
- Spray bottle of disinfectant
- PPE (N95 masks, goggles, gloves)
Primate Groups for Sampling
Saliva sampling using distributed ropes is intended for use with semi-habituated primate species that will allow researchers to approach within a reasonable distance for sample collection. Precautions should be taken to avoid baiting primates into closer contact with local people or further habituating primate groups by limiting the number of times a single group is sampled and avoiding primates associating humans with distributing food.

Collecting Non-invasive Saliva Samples
1. While wearing gloves, dip 6-inch nylon swab ropes (Salimetrics, Inc) into an appropriate attractant (juice, jam, crushed banana, etc.). For some species (i.e. baboons), disguising the ropes completely inside a banana is more effective.
2. Walk around and look for isolated individual primates that are out of sight of the rest of the primate group. Try to identify individuals that are in the lower canopy or on the ground. When deciding on an area to sample, make sure there are no more than three primates in your perspective sampling area. Distributing ropes where large numbers of primates are present can initiate aggression between individuals.
3. Observe the social structure of the primates present carefully. Throw the rope to the most dominant primate (either adult male if present, or the largest adult female present). Juveniles may get the treat after the adult has discarded it. If you want to sample a juvenile primate make sure they are out of sight of other individuals.
4. Throw the rope when no primates are watching you and continue walking so the primate does not associate you with the treat.
5. Watch the primate as they chew on the rope. Keep a reasonable distance to avoid disturbing them. Follow the primate until it discards the rope. Do not approach a dropped rope until the primate has left the area and is no longer watching you.
6. When collecting the sample, have a designated person wearing PPE (N95 mask, eye goggles, gloves) approach the sample. Compress the chewed nylon swab rope with a gloved hand into a swab collection tube (Salimetrics, Inc.). Pipette 1ml of viral transport media (VTM) over the compressed rope in the swab collection tube. Store tubes on ice packs.
7. Move to a different location within the site to collect the next sample so no primates begin to associate you with distributing food. If it is difficult to retrieve dropped ropes because they are lost in tree branches, a thin nylon string can be sewn onto distributed 6-inch ropes to aid with retrieval.
8. In the laboratory or field processing station, centrifuge ropes for 15 minutes at 3,000rpm to elute saliva/VTM into the bottom collection compartment. Pour or pipette the saliva/VTM into labeled cryovial tubes and store in freezer at -80 degrees C.
Sample Collection from Dead NHP
If animals are found dead or must be euthanized by trained personnel per acceptable guidelines (see Section 8.5.2. AVMA’s Euthanasia Guidelines and Section 8.5.3. AAZV’s Euthanasia Guidelines) due to health or welfare reasons, necropsy samples may be taken. When full necropsies are performed, following the American Association of Zoo Veterinarians (AAZV) great ape necropsy protocol is recommended and can be adapted for all primate species (see Section 5.2.6j. Appendix V. Occupational Primate Disease Safety Guidelines for Zoological Institutions).

Note: properly following this extensive necropsy procedure and collecting and measuring all samples can require 4-6 hours per animal.

If carcasses are not whole or are fairly decayed, see Section 5.2.12. Bushmeat Sampling Methods. If bodies are relatively whole and fairly fresh then blood, organ tissues, urine and (optionally) external parasites may be collected as described below.

Post-Mortem Blood Collection
From recently dead animals, it may be possible to collect whole blood (often clotted) from the right side of the heart where the largest volume of blood is available. Collect all available blood into an appropriate size container (typically one or more blood tubes). Allow the tubes to sit undisturbed for at least 30 minutes, and then centrifuge at high speed (2000 x G for 20 minutes). Use a plastic pipette to take 1 ml of serum and transfer into 2 cryovial tubes, 0.5 ml each. Transfer the remaining blood clots to separate cryovials. Freeze all samples in liquid nitrogen immediately in the field and transfer to -80°C freezer once back in the lab. If a centrifuge is not available, allow the clots and cells to settle as much as possible and collect serum as above.

Tissue Collection
Collect three, adjacent, approximately 200 mg (pea-sized) samples of the following tissues:

- Adrenal
- Colon
- Heart
- Liver
- Lymph node
- Ovary
- Testes
- Cecum
- Duodenum
- Kidney
- Lung
- Spleen
- Pancreas
- Other, if required

One specimen should be frozen in 500 μL VTM in a cryovial, one should be frozen in 1 mL Trizol in a cryovial, and one should be stored at room temperature in a small vial or jar in 10% buffered formalin at a volume of fixative 10 times the volume of the tissue (once fixed, the tissue may be transferred to a smaller volume for shipment).
Urogenital Swab/Urine
A urine sample should be collected if the carcass contains an intact bladder holding uncontaminated urine. Ideally, the sample should be collected with a 3 ml syringe attached to a 25 to 22 gauge needle. Insert the needle through the bladder wall and use the syringe to withdraw a maximum of 1 ml of urine. Do NOT stabilize the bladder by placing your hand beneath it, as this will put you at risk for needle injury. If the bladder is contracted (appears grossly empty of urine), use a sterile blade to make a small incision in the bladder wall. Small amounts of urine might be present and possible to suction up with a needleless 1 ml syringe inserted through the open incision. Place 500 μl of urine in a cryovial with 500 μl VTM, and another 500 μl of urine in a cryovial with 500 μl Trizol. Alternatively, if urine is not available, two urogenital swabs can be taken, with one placed in 500 μl VTM and one in 500 μl Trizol. Swabs can be moistened with sterile saline prior to animal sampling. Store samples in a dry shipper or dewar with liquid nitrogen and transfer later to -80°C freezer.
Section 5.2.6e References


Section 5.2.6f. Appendix I. Field Inventory Checklist

General Field Supplies:
- Headlamps
- AAA batteries and AA batteries
- Leatherman/Pocket knife
- GPS unit to mark site coordinates (in decimal degrees)
- Binoculars
- Brightly colored flagging tape

Workstation materials:
- Drapes, sheets, blankets, tarps, towels, plastic sheeting, etc.
- Scale and sacks, harnesses, ropes for weighing
- Disinfectants and clean-up supplies
- Biohazard bags (or plain bags and biohazard stickers) and sealing tape
- Hard, coverable container for transporting biohazard bags (if necessary)
- Anesthetic or immobilization drugs, medications, vaccinations
- Anesthesia monitoring equipment (pulse oximeter, stethoscopes, thermometer, etc.)

Data Collection Supplies:
- Field Data Collection sheets (Site/Event, Animal, and Specimen sheets)
- Clipboard or other weather resistant writing surface
- Pens and permanent markers
- Digital camera and charger
- Blank index cards or paper to label animals in photos
- Printed labels
- Waterproof paper (for use in formalin specimen jars)

Personal Protective Equipment (PPE)
- N95 (or better) respirators and a small stapler* (enough for all team members plus extras)
- Safety goggles or face shields (for every person handling monkeys). Use face shields if handling suspect B virus or Ebolavirus positive NHP.
- Long clothing/tyvek suits
- Disposable nitrile gloves
- First Aid Kit (with soap and betadine for cleaning wounds)
- Thicker gloves for primate handlers (i.e., leather to be worn over the nitrile gloves) that can be disinfected and re-used (e.g., Hexarmor Hercules 400R6E)
- Emergency exposure kits for B virus or Ebola (if applicable)
- Working communications equipment (cell phone, satellite phone, etc.)
- Emergency response plan (see Section 3. Emergency Preparedness Guide)
* A small stapler can be used to staple the elastic straps back onto the N95 mask if they snap off

Biological data and sample collection supplies:
- Ruler, tape measure, or calipers appropriate for the size of the animal
- Serum separator blood tubes
- EDTA blood tubes
- Appropriate gauge needles or butterfly needles for smaller primates
- Syringes: 1mL, 3, 6 or 12mL
- Gauze or cotton to apply iodine to blood collection site
- Iodine for preparing blood collection site
- Sterile polyester swabs with plastic shaft for oropharyngeal, nasal, and fecal swabs
Sterile saline

Cryovials for storing serum, blood clots, swabs, feces, etc.
  "Cryovials" refers to plastic, internally threaded screw-top vials with a silicon O-ring to prevent leakage. NUNC or Corning brand are recommended

Viral Transport Media (VTM) and Trizol for storing specimens

95% Ethanol for storing ectoparasites

1.5mL microcentrifuge tubes for storing ectoparasites

Necropsy kit for post-mortem exam in case of accidental death
  21 gauge needles (for cardiocentesis)
  22 to 25 gauge needles (for urine collection)
  1 and 6 mL syringes (for cardiocentesis and urine collection)
  Scalpel and surgical blades
  Forceps
  Sharp and blunt tip scissors
  75%-80% Ethanol in small screw-capped vials for storing forensic entomological specimens

Small jars containing 10% buffered formalin for histopathology specimens

Sample processing and storage supplies:
  Tube racks or cryovial boxes for storing tubes
  Field centrifuge to spin blood to separate serum
  Sterile plastic pipettes (for aliquoting serum)
  Surgical blades for dividing blood clot
  Cold Storage Container - Cooler and ice packs for specimens during collection
  Charged dry shipper for specimen field storage
  Cryoboxes, canes, or nylon stockings for organizing specimens in dry shipper

Waste Disposal Supplies:
  Sharps container (for needles and pipette tips)
  Trash bags or containers that can be disinfected
  Virkon or similar disinfectant that kills viruses
  Spray bottle for disinfectant
  Portable waste incinerator or other biohazard disposal plan
Section 5.2.6g. Appendix II. B Virus Exposure Emergency Protocol

(Adapted from Cohen et al., 2002. Recommendations for Prevention of and Therapy for Exposure to B Virus (Cercopithecine Herpesvirus 1). Clinical Infectious Diseases, 35: 1191-203.)

FIRST AID ***MOST IMPORTANT STEP***

*Mucous membrane exposure:* flush eye or mucous membranes with sterile saline solution or water for 15 min (or 1 liter).

*Skin exposure:* Wash skin thoroughly with a solution containing detergent soap (e.g., chlorhexidine or povidone iodine) for 15 min. Consider washing skin with freshly prepared 0.25% hypochlorite solution, followed by detergent solution, for 10–15 min.

INITIAL EVALUATION

*Exposed worker*

- Assess the adequacy of cleansing; the health care provider should repeat cleansing.
- Determine and document the date, time, location, and description of the injury, and the type of fluid or tissue contacted.
- Evaluate general health (including medications) and determine when the last tetanus booster was received.
- Determine the need for post-exposure prophylaxis with antibiotics or rabies vaccine and immunoglobulin.

*NHP (partly intended for laboratory NHP)*

- Identify the monkey associated with the exposure, the species of that monkey, and the responsible veterinarian.
- Assess general health (including medications and involvement in past and present research studies).
- Evaluate prior serologic history (including infection with B virus or simian immunodeficiency virus).
- Consider confining monkey for further evaluation and testing.

EXAMINATION AND LABORATORY TESTING

*Exposed worker*

- Physical examination, especially evaluation of the site of the exposure and neurologic examination.
- Consider obtaining serum samples at baseline for serologic analysis (pair at 2-3 weeks).
- Consider culturing specimens from the wound site or exposed mucosa after cleaning.

*NHP*

- Examine the animal for mucosal lesions (e.g., vesicles, ulcers), conjunctivitis, etc.
- Consider culturing specimens from the lesions, conjunctiva, and buccal mucosa.
- Consider serologic testing for B virus (if the animal is not known to be seropositive) and follow-up paired sample at 2-3 weeks.

EDUCATION AND TREATMENT

- Counsel the patient regarding the significance of the injury.
- Provide the patient with information on the signs and symptoms of B virus infection.
- Ensure that the patient has a card (to carry in his or her wallet) that includes information on B virus and a phone number to call for advice in an emergency.
- Ensure that the patient’s occupational health care provider and supervisor are notified.
• Review with the patient and his or her work supervisor the safety precautions in place at the time of injury.
• Schedule a follow-up appointment.

**CONSIDER POST-EXPOSURE PROPHYLAXIS**

**Pros and cons of post-exposure prophylaxis for persons exposed to B virus:**

**Pros**
- Initiation of acyclovir therapy within 24 h after exposure to B virus prevents death among animals.
- Initiation of acyclovir therapy within hours of exposure may prevent or modify symptomatic B virus disease.

**Cons**
- Infection with B virus is very rare relative to the number of possible exposures.
- There are no controlled studies that document the ability of immediate empirical therapy to prevent infection or symptomatic B virus infection in humans.
- Acyclovir therapy can suppress virus shedding and seroconversion, which may make diagnosis more difficult.

**Recommendations for post-exposure prophylaxis for persons exposed to B virus.**

**Prophylaxis recommended:**
- Skin exposure (with loss of skin integrity) or mucosal exposure (with or without injury) to a high-risk source (e.g., a macaque that is ill, immunocompromised, or known to be shedding virus or that has lesions compatible with B virus disease).
- Inadequately cleaned skin exposure (with loss of skin integrity) or mucosal exposure (with or without injury).
- Laceration of the head, neck, or torso.
- Deep puncture bite.
- Needlestick associated with tissue or fluid from the nervous system, lesions suspicious for B virus, eyelids, or mucosa.
- Puncture or laceration after exposure to objects (a) contaminated either with fluid from monkey oral or genital lesions or with nervous system tissues, or (b) known to contain B virus.
- A post-cleansing culture is positive for B virus.

**Prophylaxis considered:**
- Mucosal splash that has been adequately cleaned.
- Laceration (with loss of skin integrity) that has been adequately cleaned.
- Needlestick involving blood from an ill or immunocompromised macaque.
- Puncture or laceration occurring after exposure to objects (a) objects contaminated with body fluid (other than that from a lesion), or (b) potentially infected cell culture.

**Prophylaxis not recommended:**
- Skin exposure in which the skin remains intact.
- Exposure associated with non-macaque species of NHP.
Section 5.2.6h. Appendix III. Ebola Virus Exposure Emergency Protocol
(adapted from http://www.cdc.gov/ncidod/dvrd/spb/mnpages/vhfmanual/section5.htm)

Accidental needlestick injury: Assume any needlestick injury is a suspected contact for viral hemorrhagic fever (VHF) whether or not a break in the skin can be seen. If an accidental needlestick injury occurs, contact the health care provider and treat the exposure site.

1. Immerse the exposed site in 70% alcohol for 20 to 30 seconds, and wash with soap and clean water.
2. Flush the site in running water for 20 to 30 seconds.
3. If needed, cover with a dressing.
4. Report the incident to a supervisor or the physician-in-charge.

The purpose of notifying the physician-in-charge is:
- To identify what caused the problem.
- To take corrective action to solve the problem and prevent accidental transmission.
- To provide appropriate care for the possible case of VHF.

Remind the exposed worker that accidents do happen even when every precaution to prevent them has been taken. Reassure worker that reporting the accidental exposure will have no negative consequences. Explain that reporting the accidental exposure is essential for protecting themselves, their families, other health workers and patients.

Accidental contact with infectious body fluids: An accidental contact can occur if there is unprotected contact between infectious body fluids and broken skin or the mouth, nose or eye. For example, vomit may run under a glove, a primate might cough blood which runs into the health care worker's eye, or splashed blood may run underneath a health care worker's mask and get into the mouth. Treat any accidental contact as a suspected contact with VHF. As soon as the contact occurs:

1. Flush the area in the most appropriate manner with soap and clean water. If a splash occurs in the eye, flush it with clean water.
2. Leave the isolation area and remove the protective clothing as recommended.
3. Take a shower and put on street clothes.
4. Report the exposure to a supervisor or the physician-in-charge. Complete the necessary forms.

Follow up accidental exposures:
1. Monitor the condition of the exposed worker. Take a measured temperature two times per day.

If a fever occurs -- temperature is 38.5°C (101°F) or higher -- the worker should not do any work activities and should seek immediate medical attention. Treat as a suspected case of VHF if the worker’s signs and symptoms meet the case definition.
Section 5.2.6i. Appendix IV. PREDICT Vervet Monkey Capture and Anesthesia Guide

Monkey Capture
Information in this guide is based on field training with Mountain Gorilla Veterinary Project/Rwanda PREDICT staff and the PREDICT Primate Capture Training Protocol by Chris Whittier.

1. Assess monkey sleeping, foraging, and movement patterns in a given location. Working with local scouts to monitor monkey troop movements can make trapping efforts much more efficient.
2. After assessing movement patterns, identify a trapping location. During initial capture efforts, morning and late afternoon (as monkeys were leaving and returning to sleeping areas) were effective trapping times.
3. Prepare the Tomahawk non-collapsible metal traps by securely baiting them with a fresh banana before placing them near the target monkey troop. A long piece of duct tape folded in half lengthwise makes an effective strip for tying the banana to the bottom of the cage. The banana should be positioned just behind the treadle, so that it doesn’t interfere with the ability of the treadle to trigger the trap to close (Figure 1). Tying the banana to the cage with at least 2 loops of duct tape ensures that the monkey can’t steal the banana without triggering the trap (Figure 2).

Figure 1: Tying a banana to the trap. Photo credit: PREDICT Tanzania Team

Figure 2: Vervet Monkey triggering the trap while trying to remove a banana. Photo credit: PREDICT Tanzania Team
4. Weigh the baited Tomahawk traps (in kg) using the appropriate spring scale. Set Tomahawk non-collapsible metal traps near the monkey troop. Place the traps on level ground, in a shaded location when possible. Capture staff should monitor the set traps from a distance to avoid disturbing the monkeys.

5. Once a monkey has been captured, weigh the trap with the monkey (in kg), and subtract the trap weight from the total weight to calculate the weight of the monkey. This weight is necessary for calculating doses of anesthetic drugs. Male vervet monkeys typically weigh 3-6.5 kg. Females are usually 1.5-5 kg.

6. Following capture and weighing, traps containing monkeys should be removed to a sampling site and placed in a quiet, shaded location away from humans or other disturbances. Continue monitoring open traps to capture additional individuals. Monkeys in troops that have been captured previously may be extremely hesitant to enter traps.

**Monkey Processing**

**Note:** Members of the country/field team should determine the specimens to be collected based on the interface sampled, the species of primates captured, feasibility, safety, and diagnostic requirements.

1. Fill in site/event information on the data sheet and prepare animal and specimen data sheets for recording individual animal data. Use the GPS unit to collect site latitude/longitude in decimal degrees.

2. Set up a monkey processing station. Place leaves or other vegetation on the ground to insulate the anesthetized monkey from a cold or hot surface. Place a disposable plastic sheet or apron over the vegetation to create a sampling area that can be easily disinfected (Figure 3). Organize supplies in advance for easy access during sampling.

![Figure 3: An anesthetized vervet monkey at the processing station with a PREDICT field team member identifying the femoral vein. Photo credit: PREDICT Tanzania Team](image-url)
3. All individuals handling monkeys and samples should wear appropriate PPE: N95 or better respirators, nitrile gloves, long clothing/tyvek suits, and safety glasses or face shields. Duct tape can be wrapped around the overlapping tyvek suit and gloves at the wrist to avoid skin exposure.

4. Using the weight of the captured monkey, calculate the appropriate anesthetic drug volumes. Vervet monkeys can be safely anesthetized using a combination of ketamine and medetomidine (Dormitor®) with the following doses:

   → 3.5 mg/kg of ketamine
   → 0.035 mg/kg of medetomidine‡

‡Dexmedetomidine may also be used at a dose of 0.0175 mg/kg

Volume of drug needed (mL) = animal weight (kg) * safe and effective dose (mg/kg) ÷ drug concentration (mg/mL).

Ketamine is commonly available in a concentration of 100mg/mL or 200mg/mL, and medetomidine is commonly 1mg/mL. Using these concentrations (100mg/mL for ketamine), a 5 kg monkey would require:

5 kg * 3.5 mg/kg Ketamine ÷ 100mg/mL = 0.175 mL
5 kg * 0.035 mg/kg Medetomidine ÷ 1 mg/mL = 0.175 mL

The total volume of anesthetics (0.175 + 0.175 = 0.35 mL) can be drawn up in a single syringe for hand-injection of the monkey. A 22 or 25 gauge needle can be used to deliver the drugs.

5. Prepare the Atipamezole reversal (dose = 0.175 mg/kg) in a syringe and set aside in a cool place until sampling is complete. Note: when using 5mg/mL concentration of Atipamezole, the reversal volume is the same as the Medetomidine volume with 1mg/mL concentration of Medetomidine.

6. Once the drugs have been drawn up and combined in a single syringe, the monkey can be hand-injected through the bars of the trap. To reduce stress and to ensure that the full anesthetic dose is administered, the monkey must be confined to a small area of the trap where its movements are restricted. As a pilot method, the PREDICT Tanzania and Rwanda teams used 2 tools in combination to restrain the monkey in the cage. First, the comb (Figure 4, left) is inserted under the entry door of the trap to block the monkey from leaving. The entry door is then opened, and the plunger (Figure 4, right) is inserted. The comb is removed and then the plunger is pushed gently downward to confine the monkey to the bottom portion of the trap.

Figure 4: PREDICT tram inserting the comb just under the trap door so that the door can be opened without the monkey escaping (left). Anesthetic drugs are hand-injected through the bars at the bottom of the trap where the monkey is confined by the plunger tool (right). Photos courtesy of Mike Cranfield, Mountain Gorilla Veterinary Project.
7. Inject the anesthetic drugs intra-muscularly (target muscles of the thigh, shoulder or upper arm) and observe the monkey for initial signs of anesthesia: eyelids drooping, decreased movement, leaning against walls of trap, lying on floor of trap, unresponsive to stimuli. Signs of anesthesia should be visible within 5-10 minutes post-injection.

8. Once the monkey is anesthetized, carefully remove it from the trap. Open the sliding rear door of the trap a few inches to avoid escape by a partially anesthetized monkey. Quickly pull the monkey’s arms behind its back (Figure 5). This safe strategy for holding small primates prevents a partially anesthetized or even fully aware monkey from biting the handler.

9. Place the anesthetized monkey on its back on the plastic sheet of the sampling station (see Figure 3). Ensure that the monkey has a clear airway, with unimpeded chest movements and unrestricted airflow in and out of the lungs. Assess and continually reassess the monkey’s plane of anesthesia. During anesthesia, team members should monitor the monkey’s breathing, heart rate, mucous membrane color, and capillary refill time. Rectal temperature and blood oxygen saturation (using a Pulse-oximeter) can also be assessed. Grip reflexes are among the first to return when a monkey is coming out of anesthesia.

10. Collect a blood sample. The femoral vein is the most reliable blood collection site in small primates. The femoral vein is found lateral and parallel to the femoral artery, which can be easily palpated in the inguinal region. Prepare the blood collection site by swabbing the area with iodine soaked gauze. Use a 25 gauge or smaller butterfly needle inserted at a roughly 45 degree angle to the skin to locate the femoral vein (Figure 6). Once blood is visible in the tubing of the butterfly needle, attach a syringe and apply gentle section. Avoid pulling the syringe too quickly, as too much vacuum pressure can collapse the vein.

Figure 5: Dr. Mike Cranfield, from the Mountain Gorilla Veterinary Project, demonstrates the proper technique to restrain a vervet monkey. Photo credit: PREDICT Tanzania Team
11. Once the appropriate amount of blood has been collected, remove the needle and use gauze to apply pressure to the collection site for 1 minute to avoid hematoma formation. Store the collected blood in a serum separator tube or EDTA collection tube if whole blood is needed. Place the blood sample tubes in the cooler immediately after collection. **Do not recap the needle!**

**Dispose of needle in the sharps container and the syringe in a biohazard or designated bag.**

12. Collect additional biological samples as described in the PREDICT biological sample collection protocol.

13. Before recovery, perform a physical exam of the captured monkey, noting any evidence of disease or injury. Capture related injuries can occur, and severity should be assessed prior to release. Small wounds and lacerations can be cleaned with iodine or betadine. More serious wounds may require veterinary intervention.

14. Mark the monkey with a temporary marker in a visible location such as the upper thigh (Figure 6) to avoid resampling the same animal when a troop is targeted for multiple days of capture efforts. The mark should wear off within one week.

15. Once sampling and physical examination are complete and the monkey has been anesthetized for a **MINIMUM** of 45 minutes, administer the Atipamezole reversal by intra-muscular injection. Earlier administration (<45 minutes post-induction) may result in ketamine-induced seizures. Place the monkey in a large dog kennel (or locally constructed container of similar size) in a quiet, shaded location away from humans to allow it to recover from anesthesia. It is extremely important to inspect and choose a safe release area away from hazards such as roads, people, or cliffs that could endanger the recently anesthetized monkey. Monitor the monkey regularly, and when it is fully aware and moving in a coordinated manner, open the door to allow it to return to its troop.
Section 5.2.6j. Appendix V. Occupational Primate Disease Safety Guidelines for Zoological Institutions
(Appendix 3 – Standard Necropsy Report for Non-Human Primates Work Sheet; From http://www.aazv.org)

Appendix 3: Standard Necropsy Report for Non-Human Primates Work Sheet

Pathology #: ________________ Species: ________________ Date: ________________

Animal #/Name: ________________ Sex: ________________ Age (DOB): ________________

Date of death/euthanasia: ________________ Time: ________________ (am/pm)

Method of euthanasia: _______________________________________________________________________

Time and date of necropsy: ________________ Duration of necropsy: ________________

Post mortem state: ________________ Nutritional state: ________________

Pathologist or prosector and institution: _______________________________________________________________________

Gross diagnosis:

Abstract of clinical history:
Please check tissues submitted for histopathology.

External Examination (note evidence of trauma, exudates, diarrhea etc):

- Hair coat:
- Skin:
- Scent glands:
- Mammary glands and nipples:
- Umbilicus (see neonatal/fetal protocol):
- Subcutis (note: fat, edema, hemorrhage, parasites):
- Mucous membranes (note: color, exudates):

Ocular or nasal exudate?:

- Eyes and ears:
- External genitalia:
- Oral cavity, cheek pouches and pharynx: Dentition (see attached dental form):
- Tongue:

Musculoskeletal System (Note fractures, dislocations, malformations?):

- Bone growth plate (rib, distal femur, sternabra)
- Muscles:
- Bone marrow (femur):
- Joints (note any exudates or arthritis):
- Spinal column (examine ventral aspect when viscera removed)

Examination of the neck region:

- Larynx:
- Laryngeal air sac (see protocol for great apes):
- Mandibular and parotid salivary glands:
- Thyroids and parathyroids:
- Cervical/cranial lymph nodes:
- Esophagus:

Thoracic Cavity (Note any effusions, adhesions, or hemorrhage):

Note amount, color and any lesions in mediastinal and coronary fat:

- Thymus:
- Heart (see attached protocol):
- Great vessels:
- Trachea and bronchi:
- Lungs:
- Esophagus:
- Lymph nodes:
**Abdominal Cavity** (Note any effusions, adhesions, or hemorrhage?):
Note amount, color or lesions in omental, mesenteric and perirenal fat:

- Liver and gall bladder:
- Stomach:
- Pancreas:
- Duodenum:
- Jejunum:
- Ileum:
- Cecum and (in apes) appendix:
- Colon and rectum:
- Lymph nodes:
- Kidneys and ureters:
- Adrenals:
- Gonads:
- Uterus:
- Bladder and urethra:
- Male accessory sex glands (prostate and seminal vesicles):
- Umbilical vessels, round ligaments of bladder in neonates:
- Abdominal aorta and caudal vena cava:

**Nervous System:**
- Meninges:
- Brain:
- Pituitary:
- Trigeminal (gasserian) ganglia:
- Spinal cord (please note to which lumbar segment the cord extends):
- Brachial plexus and sciatic nerves:

**Is there an identifiable pineal gland?**

**WEIGHTS AND MEASUREMENTS** (in grams, kilograms, and cm, please)

Body weight: _____________________________________________________________

**Lymphoid tissue:**

- R. axillary LN: ______________ L. axillary LN: ____________________________
- R. inguinal LN: ______________ L. inguinal LN: ____________________________
- Jejunal LN: ____________________________
- Spleen: ____________________________ Thymus: ____________________________
Abdominal Organs:
Liver: _________________________
R. Kidney: _________________________ L. Kidney: _________________________
R. Adrenal: _________________________ L. Adrenal: _________________________
R. Ovary: _________________________ L. Ovary: _________________________
Uterus: ____________________________
Placenta (weight and measure disc(s)): ________________________________

Thoracic Organs:
Heart wt: _________________________ Thymus (above): _________________________
Height: ____________________________ Circumference at coronary groove: _________________________
Left Vent. Thickness: _________________________ Rt. Vent. Thickness: _________________________
Septum: ____________________________
Lt. AV valve circ. _________________________ Rt. AV valve circ. _________________________
Aortic valve circ. _________________________ Pulmonary v. circ. _________________________
Rt. Lung: ____________________________ L. Lung: ____________________________

Other:
Brain: ____________________________ Tumors? _________________________________
R. Testes (wt.): _________________________ L. Testes: ____________________________
Length x dia: ____________________________
Penis (length x diameter): ____________________________

STANDARDIZED BODY MEASUREMENTS FOR NONHUMAN PRIMATES INCLUDING APES:
Crown rump length (linear) ____________________________
Crown rump length (curvilinear) ____________________________
Cranial circumference (above brow ridge) ____________________________
Length of head (tip of jaw to top of crest) ____________________________
Width of brow ridge ____________________________
Chest circumference (at nipples) ____________________________
Abdominal circumference (at umbilicus) ____________________________

Left arm: Shoulder-elbow:____________________________ elbow-wrist:____
wrist-tip of middle finger:____________________________ pollex:____
Right arm: Shoulder elbow:____________________________ elbow- wrist:____
wrist-tip of middle finger:____________________________ pollex:__________________________

Left leg: hip-knee:____________________________ knee-ankle:____________________________
ankle-tip of big toe:____________________________ heel-tip of big toe:____
hallux: ____________________________
Right leg: hip-knee:____________________________ knee-ankle:____________________________
ankle-tip of big toe:____________________________ heel-tip of big toe:____
hallux: ____________________________
ANCILLARY DIAGNOSTICS (CHECK IF PERFORMED, GIVE RESULTS IF AVAILABLE, NOTE LOCATION IF STORED, OR TO WHOM SENT):

Cultures:
bacterial: fungal:
viral:

Heart blood:
serum:
filter paper blot:

Parasitology:
feces:
direct smears:
parasites:

Tissues fixed in 10% formalin (list tissues or specific lesions other than those checked above):
Tissue fixed for EM: ___________________________ Tissue frozen: ___________________________
Impression smears: ___________________________
Comments: ___________________________
NONHUMAN PRIMATE POST MORTEM EXAMINATION

Collection of tissues
Tissues to be fixed in 10% neutral buffered formalin should be less than 0.5 cm thick to ensure penetration of formalin for fixation.

Initial fixation should be in a volume of fixative 10 times the volume of the tissues. Agitation of the tissues during the first 24 hrs is helpful to prevent pieces from sticking together and inhibiting fixation. Once fixed tissues may be transferred to a smaller volume for shipment.

Labeling of specimens
If pieces are small or not readily recognizable (e.g., individual lymph nodes) they can be fixed in cassettes or embedding bags or wrapped in tissue paper labeled with pencil or indelible ink. Another alternative is to submit lymph nodes with attached identifiable tissue, e.g., axillary with brachial plexus, inguinal with skin, bronchial with bronchus, etc.

Sections from hollow viscera or skin can be stretched flat on paper (serosal side down) and allowed to adhere momentarily before being placed in formalin with the piece of paper. The paper can be labeled with the location from which the tissue came.

The formalin container should be labeled with the animals name or number, the age and sex, the date and location, and the name of the prosector.

Tissues to be preserved
From the skin submit at least one piece without lesions, a nipple and mammary gland tissue, scent gland, any lesions and subcutaneous or ectoparasites.

Axillary and or inguinal lymph nodes may be submitted whole from small animals and should be sectioned transversely through the hilus in large primates.

Mandibular, and/or parotid salivary glands should be sectioned to include lymph node with the former and ear canal with the latter. Thyroids, if it is a small primate, may be left attached to the larynx and submitted with the base of tongue, pharynx, esophagus as a block. In larger primates, take sections transversely through the thyroids trying to incorporate the parathyroids in the section.

Trachea and esophagus and laryngeal air sac sections may be submitted as a block.

Cervical lymph nodes may be submitted whole if small or sectioned transversely.

A single sternebra should be preserved as a source of bone marrow. A marrow touch imprint may be made from the cut sternebra and air dried for marrow cytology.

Section of thymus or anterior pericardium should be taken perpendicular to the front of the heart.
Heart: weigh and measure heart after opening but before sectioning. Please fix longitudinal sections of left and right ventricles with attached valves and atria in large animals and the whole heart opened and cleaned of blood clots in smaller animals. In tiny animals the heart may be fixed whole after cutting the tip off the apex.

Lungs: if possible inflate at least one lobe by instilling clean buffered formalin into the bronchus under slight pressure. Fix at least one lobe from each side and preferably samples from all lobes. In little animals the entire "pluck" may be fixed after perfusion and sampling for etiologic agents.

Gastrointestinal Track: Take sections of all levels of the GI track including: gastric cardia, fundus and pylorus (or presaccus, saccus, tubular stomach and pylorus in colobines); duodenum at the level of the bile duct with pancreas attached; anterior, middle and distal jejunum; ileum; ileoceccocolic junction with attached nodes; cecum and (in apes) appendix; ascending, transverse and descending colon. Open loops of bowel to allow exposure of the mucosa and allow serosa to adhere momentarily to a piece of paper before placing both bowel section and paper in formalin; or gently inject formalin into closed loops.

Liver: Take sections from at least two lobes, one of which should include bile ducts and gall bladder.

Spleen: Make sure sections of spleen are very thin if the spleen is congested; formalin does not penetrate as far in very bloody tissues.

Mesenteric (jejunal) nodes: section transversely; colonic nodes may be left with colon sections.

Kidneys: Take sections from each kidney: Cut the left one longitudinally and the right one transversely so they will be identifiable (or label). Please make sure the sections extend from the capsule to the renal pelvis. Adrenals: small adrenals may be fixed whole but larger ones should be sectioned (left - longitudinal and right transversely) making sure to use a very sharp knife or new scalpel blade so as not to squash these very soft glands.

Bladder: sections should include fundus and trigone. Please make sure to include round ligaments (umbilical arteries) in neonates.

Male gonads and accessory sex glands: Section the prostate with the urethra and seminal vesicles transversely. Section testes transversely. If testes are being collected perimortem for sperm retrieval, try to arrange to take small sections before the gonads are manipulated.

Female reproductive organs: Fix the vulva, vagina, cervix, uterus and ovaries from small and medium sized primates as a block (after making a longitudinal slit to allow penetration of formalin). Rectum and bladder (opened) can also be included in this block. In somewhat larger animals make a longitudinal section through the entire track.

In great apes make transverse sections of each part of the track and the ovaries. (See reproductive track protocols from the contraception advisory group if animals are to be included in their database.)
CARDIAC EXAMINATION FOR GREAT APES (AND OTHER PRIMATES IN WHICH CARDIAC DISEASE IS PRESENT)
Examine heart in situ. Check for position, pericardial effusions or adhesions. Collect for culture or fluid analysis if present.
Remove heart and entire thoracic aorta with "pluck".

Examine heart again. Check the ligamentum (ductus) arteriosus for patency. Check position of great vessels. Open pulmonary arteries to check for thrombi.

Remove heart and thoracic aorta from the rest of the "pluck". Examine for presence of coronary fat. Examine external surfaces especially coronary vessels. Note relative filling of atria and state of contracture (diastole or systole at death) and general morphology. (The apex should be fairly pointed.)

Measure length from apex to top of atria. Measure circumference at base of atria (around coronary groove).

Open the heart:

Begin at the tip of the right auricle and open the atrium parallel to the coronary groove continuing into the vena cava. Remove blood clot and examine the AV valves. Cut into the right ventricle following the caudal aspect of the septum and continuing around the apex to the anterior side and out the pulmonary artery. Remove postmortem clots and examine inner surface. Open left atrium beginning at the auricle and continuing out the pulmonary vein. Remove any clots and examine valves. Open the left ventricle starting on the caudal aspect and following the septum as for the right ventricle. When you reach the anterior aspect, clear the lumen of blood and identify the aortic outflow. Continue the incision around the front of the heart and into the aorta, taking care to cut between the pulmonary artery and the auricle. Open the entire length of the thoracic aorta. Remove all postmortem clots. You may gently wash the heart in cool water or dilute formalin to better visualize the internal structures and valves. Sever the thoracic aorta from the heart just behind the brachiocephalic arteries. Examine intima and adventitia and section aorta for formalin. Sever the pulmonary vessel and vena cava close to the heart.
Weigh and measure the heart and record.

Measure thickness of right and left ventricular free walls and the septum. (On the left side, do not measure directly through a papillary muscle.)

Measure the circumference of the right and left AV valves and the aortic and pulmonary valves using a pliable measuring tape (or use a piece of string and measure the string on a straight ruler).

Take sections for histopathology:
Sections should include:
longitudinal sections of left and right ventricles AV valves and atria.

Sections of myocardium from left and right ventricles including coronary vessels. Sections of papillary muscles. Sections from the septum at the vase of the AV valves (area of conduction system).

In small animal like callitichids, you may fix the heart whole.
Fix the entire heart, if possible by immersion in 10% buffered formalin for more detailed examination by a cardiac pathologist.

Other vessels:
Make sure to examine the abdominal aorta, iliac arteries and popliteal arteries (frequent sites of aneurysms in humans).

Note the location and severity of fibrous plaques, fatty streaks and atherosclerotic plaques and presence of mineralization or thrombosis.

**POSTMORTEM EXAMINATION OF NONHUMAN PRIMATE FETUSES AND NEONATES**

External examination of the fetus:

Weigh the fetus and make body measurements.

Measure the placental disc(s) and weigh the placenta. Note umbilical length and vascular patterns on the placenta.

Note presence of hair, freshness of the carcass (if dam is dead, is the decomposition of the fetus consistent with that of the dam) and any evidence of meconium staining.

Internal examination of the fetus:

Follow the general nonhuman primate necropsy protocol.

Make sure to note whether ductus arteriosus and foramen ovale are patent. Note also whether the lungs are aerated and to what extent.

Note dentition / erupted teeth.

Identify umbilical vein and arteries and check for inflammation. Make sure to save umbilicus and round ligaments of the bladder (umbilical arteries) for histology.

Mae sure to save a growth plate (e.g. costochondral junction or distal femur) in formalin. Cultures:
Take as many of the following as possible: Stomach content or swab of the mucosa; lung; spleen or liver; placental disc and extra-placental membranes. Do both aerobic and anaerobic cultures if possible.

**POST MORTEM EXAMINATION OF THE AIR SACS OF ORANGUTANS AND OTHER NONHUMAN PRIMATES**

Examine the skin over the air sac for signs of fistulae or scars. Note thickness of the skin and presence of fat or muscle overlying the air sac.

Incise the air sac through the skin on the anterior aspect. Note color and texture of air sac lining. Note presence or absence of exudate.

Note presence or absence of compartmentalization by connective tissue and presence of diverticulae.
Note extent of air sacs (e.g., under clavicle, into axilla, etc.)

Identify and describe the opening(s) from the larynx into the air sac (e.g. single slit-like opening, paired oval openings etc.). Note any exudate.

Note the location, size and shape of the opening in the larynx (e.g. from lateral saccules or centrally at the base of the epiglottis). Note length of any connecting channel between larynx and air sac and direction a probe must take to go from inside the larynx to the air sac.

Cultures:

Please culture several different sites within the air sacs (we need data to determine normal flora and if infections are "homogeneous" or compartmentalized).

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