

NON-HUMAN PRIMATE SAMPLING METHODS

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This SOP is designed to orient trained veterinarians with experience working with non-human primates to sampling and data collection procedures for virus surveillance and discovery activities. It is not intended as introductory material for training in biological specimen collection from non-human primates and should not be used by individuals without extensive and formal non-human primate training.

This document was prepared as part of the One Health Workforce – Next Generation Project to support activities conducted under One Health Workforce Academies and is intended for an audience of qualified professionals trained in standard best practices. This guide is not intended for use by untrained individuals.*

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The authors assert that animal surveillance should always occur in compliance with all applicable laws and regulations and should only be undertaken by trained wildlife professionals after securing all necessary permits and approvals, including approval by an ethics review committee.

For more information about the contents of this guide, please contact onehealthnextgen@ucdavis.edu.

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Table of Contents

1.	Overview of PPE and Biosafety.....	3
1a.	Minimum PPE.....	3
1b.	First Aid Guidance for Bites, Scratches, Needlesticks, or Facial Splashes	4
1c.	Special Considerations for Handling NHPs.....	4
1d.	Potential for Pathogen Exposure	6
2.	Non-Human Primate Sampling	9
2a.	Sample Data Collection	10
2b.	Sample Collection Procedures from Live NHP During Anesthesia	13
2c.	Non-Invasive Primate Sample Collection	16
2d.	Sample Collection from Dead NHP	18
3.	References	20
A1.	Appendix 1: Field Inventory Checklist for NHP Sampling	21
A2.	Appendix 2: Accidental Needle Stick Exposure Emergency Protocol	23
A3.	Appendix 3: B Virus Exposure Emergency Protocol.....	24
A4.	Appendix 4: Ebola Virus Exposure Emergency Protocol	27
A5.	Appendix 5: Small Monkey Capture and Anesthesia Guide.....	28



1. Overview of PPE and Biosafety

This SOP is designed to orient trained veterinarians with experience working with non-human primates to sampling and data collection procedures for virus surveillance and discovery activities. It is not intended as introductory material for training in biological specimen collection from non-human primates and should not be used by individuals without extensive and formal non-human primate training.

Due to the COVID-19 pandemic, One Health workers who are actively working with non-human primates and other wildlife must recognize the potential for exposure to both known and novel zoonotic pathogens during animal capture, handling, and sampling activities. There is a high risk of “spill-back” of SARS-CoV-2 to non-human primates from infected humans, with potential dramatic impacts on the health of these important wildlife species. These risks can be mitigated through strict adherence to biosafety and biosecurity practices detailed below and in additional reference materials listed in this protocol.

All staff handling non-human primates, non-human primate products or their bodily fluids must be vaccinated for COVID-19. Ensuring that all staff have adequate protective antibody titers verified by laboratory testing is recommended. In addition to vaccination guidelines during times of COVID-19 transmission, field sampling teams should follow all relevant national recommendations, to maintain best practices to reduce virus transmission among local communities including physical distancing, face masks, and handwashing (or sanitizer) when engaging with community members and stakeholders at locations of animal capture and sampling.

1a. Minimum PPE

The minimum PPE required for handling Live, Dead, or Collecting Samples Invasively from NHPs includes:

- 1) Eye protection (goggles or face shields)
- 2) N95 (or better) respirator without exhalation valve
- 3) Tyvek/Tychem coveralls (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 disinfected¹, heavy-duty leather (or similar) gloves to protect against bites, worn over their nitrile gloves.
- 6) To protect both human handlers and sampled NHP, all personnel handling NHP should

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 covering 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 teams have had good success with Hexarmor Hercules 400R6E gloves.

be tuberculosis (TB) tested beforehand and vaccinated for COVID-19. In addition to COVID vaccination, daily temperature and symptom checks should be performed when handling NHP.

The minimum PPE for Non-Invasive NHP sampling includes:

- 1) Nitrile gloves
- 2) Cloth or surgical mask if sampling with other team members (due to COVID-19)
- 3) Dedicated clothing, long-sleeves and pants, with closed toed washable boots

Additional PPE may be required when particular pathogens are suspected or prevalent. See section 1d for more details.

1b. First Aid Guidance for Bites, Scratches, Needlesticks, or Facial Splashes

The injured or potentially exposed 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 NHPs: 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). For potential exposure of mucous membranes (for example via a facial splash), continuously flush the mucous membranes with sterile saline, eyewash, or clean water for 15 min. For facial splashes that occur while wearing full PPE, use disinfectant wipes to clean PPE and exposed skin, carefully remove PPE, and then wash exposed skin surfaces thoroughly for 5 minutes. More details on handling needlestick injuries in [Appendix 2](#).

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 [Appendix 3](#).

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 [Appendix 4](#).

1c. Special Considerations for Handling NHPs

Handling NHPs involves special considerations, including the following:

- 1) Regardless of their specific status (e.g., endangered, threatened, protected or not), NHPs are often high-profile species that engender special attention. Anyone handling NHPs 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 NHPs 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 (unanesthetized or un-sedated) NHPs. Hand restraint is discouraged as a primary means of NHP immobilization. Chemical, rather than physical, restraint should be employed with 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 (and never in the case of a macaque species), such as when handling infants, severely debilitated individuals, or during the process of chemically immobilizing very small NHP with hand injections.

- 3) NHPs 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 NHPs 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.

Special Consideration for Handling Macaques (for field activities in Asia)

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:

- **Only** directly handling a macaque **when it is properly anesthetized**
- Wearing a full-face shield (in addition to goggles) along with an N95 (or better) respirator with no exhalation valve.
- 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.

- 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.

1d. Potential for Pathogen Exposure

Due to their close genetic relationship to humans, NHPs are more likely to share infectious agents (zoonoses) with humans. This means that they are more likely to transmit infections **to** their human handlers, and they are also more susceptible to acquiring infections **from** their handlers.

- 1) Proper use of PPE and related biosafety measures as described will help protect both handlers and the sampled NHP.
- 2) 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 NHPs. **Any staff suspected of being infected with TB must not work with NHPs.** TB testing is typically done by intradermal tuberculin skin test (TST) or with a blood test, such as the QuantiFERON Gold TB test. 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 receiving a positive skin test should work with their health care provider to have additional confirmatory tests performed.
- 3) To protect both staff and any handled NHP, all people working closely with NHPs should be fully vaccinated for **COVID-19**. In addition, daily temperature and COVID symptoms checks are recommended when handling NHPs. Unvaccinated staff may participate in non-invasive NHP sample collection but should not participate in directly handling NHPs and should always wear masks and stay at least 7 meters away from animals.
- 4) To protect NHPs from human infections, no persons with any current or recent (within a few days²) clinical signs of illness (coughing, sneezing, fever, diarrhea, rash, cold sores, etc.) should handle or have close contact (<10 m) with any NHP. Also, any person having contact with person known to be infected with COVID-19 should not conduct field work for 10 days post exposure to that person. 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 regularly with NHPs should participate in some level of

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.

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.

Great apes and COVID-19.

Very strict measures should be implemented when working with great apes given their known susceptibility to SARS-CoV-2 infection³ and their delicate conservation status. All field personnel should be fully vaccinated and wear an N95 respirator without valve at all times. Strict respiratory, hands, clothes and waste hygiene must be observed (see Biosafety SOP). If possible, all personnel handling great apes should have a negative PCR test at least every 7 days (or an antigen test if PCR testing confirmation is available). No person exhibiting or reporting any sign of illness or who has been in contact with anybody diagnosed with COVID-19 in the preceding 14 days should be allowed to be near great apes. Any personnel arriving from outside the country should be vaccinated, quarantine for 10 days upon arrival, and have a negative PCR (last 24-48 hours) prior to heading out the field. More detailed information on recommendations and best practices can be found in [Great apes, COVID-19 and the SARS CoV-2. Section on Great Apes, IUCN SSC Primate Specialist Group](#)

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

Ebola virus and related *Filoviruses*: field personnel 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. For more detailed information, see:

- www.cdc.gov/ncidod/dvrd/spb/mnpages/dispages/ebola.htm
- <http://emedicine.medscape.com/article/216288-treatment>

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 [Appendix 4](#).

Safety of field staff should be the #1 priority for projects involving wildlife sampling. Sampling of suspected Ebola viruses cases should only be carried out after careful consideration of risks and

³ APHIS, 2021. Confirmation of COVID-19 in Gorillas at a California Zoo. Animal and Plant Health Inspection Service. https://www.aphis.usda.gov/aphis/newsroom/stakeholder-info/sa_by_date/sa-2021/sa-01/ca-gorillas-sars-cov-2

with specifically trained and equipped personnel. In any scenario in which it is suspected that a filovirus may be involved (e.g., there is a known outbreak of ebolavirus in nearby humans, a dead African primate carcass is found with no other plausible cause of death, more than one animal carcass is found in a similar location), the situation should be treated as if Ebola is present. In this scenario the following precautions should be carried out:

- 1) Only highly experienced personnel (DVMs or equivalent) with training on biosafety protocols should be involved with sampling or handling of specimens.
- 2) Personnel should be limited to two individuals only.
- 3) In addition to minimum PPE described above and in the Biosafety and PPE SOP, the following pieces of protection should be added:
 - a) Double nitrile gloves should be worn
 - b) Tyvek suit with duct tape over connection between gloves and Tyvek
 - c) Tyvek surgical hood
 - d) Face shield
- 4) Donning of PPE should follow prescribed protocols outlined by the CDC. Training videos are available here: [https://www.cdc.gov/vhf/ebola/hcp/ppe-training/n95Respirator Coveralls/donning_01.html](https://www.cdc.gov/vhf/ebola/hcp/ppe-training/n95Respirator%20Coveralls/donning_01.html)
 - a) To view all videos (14 steps for donning), click through the steps in left sidebar.
- 5) Doffing of PPE should follow prescribed protocols outlined by the CDC. Training videos are available here: [https://www.cdc.gov/vhf/ebola/hcp/ppe-training/n95Respirator Coveralls/doffing_01.html](https://www.cdc.gov/vhf/ebola/hcp/ppe-training/n95Respirator%20Coveralls/doffing_01.html)
 - a) To view all videos (23 steps for doffing), click through the steps in left sidebar.
- 6) Only swab specimens should be collected.
- 7) No needles or other sharps should be used, and the carcass should not be opened.
- 8) The carcass should be buried as soon as possible after samples are collected by the team or instructions on safe disposal given to the appropriate wildlife or public health authority, if present and available to carry out the task immediately. Carcasses should be placed in a plastic body bag and buried at a minimum of 4 ft depth. Carcasses should be buried as close to where they were found as possible to avoid carrying them long distances and incurring additional exposure.
- 9) All used PPE should be burned on site.



See the Biosafety and PPE SOP for details on how to bury carcasses and incinerate infectious waste.

B virus: For project activities in Asia, 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 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 [Appendix 3](#). **TIMING IS CRITICAL** and an immediate action can be the difference between life and death.

*Macaques with oral lesions (right) should be handled with **extreme** caution and only by highly trained staff, if they are handled at all.*

Additional information on B virus can be found here:

- <http://www.cdc.gov/herpesvirus/index.html>
- <http://www.cdc.gov/mmwr/preview/mmwrhtml/00015936.htm>
- <http://www2.gsu.edu/~wwwvir/index.html>

Rabies: NHPs 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 saliva or nervous tissues from a primate exhibiting neurologic signs), the exposed individual should seek medical treatment and rabies post-exposure prophylaxis treatment should be started as soon as possible. Rabies symptoms in primates are variable (e.g., irritability, self-mutilation, paralysis, or malaise).

Anthrax: NHPs 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 <https://www.cdc.gov/anthrax/index.html>.

2. Non-Human Primate Sampling

Field personnel must have detailed capture/immobilization protocols (and recording sheets, monitoring sheets, etc.) for any target primate species. Protocols should be IACUC approved. This sampling guide assumes a starting point of either a safely immobilized or an already dead primate. A small monkey capture and anesthesia guide is provided in [Appendix 5](#).

For virus surveillance and discovery, 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 project's IACUC protocol.

2a. Sample Data Collection

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.

Minimum Data to Collect from NHP

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 to note any lesions or major abnormalities.
3. If capture wounds are observed, treat as needed.

Additional Data to Collect from NHP (Optimal)

Note: 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:

- 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, the NHP should be weighed (kg) in bags, slings, or a suitable container (as appropriate based on size) using a calibrated hanging spring scale or, if they are small enough, a tabletop scale with or without a tray or other container. If a large NHP exceeds 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 a 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:

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

Sex determination (species identification/examination): Based on morphology and unique characteristics, identify NHPs 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 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.

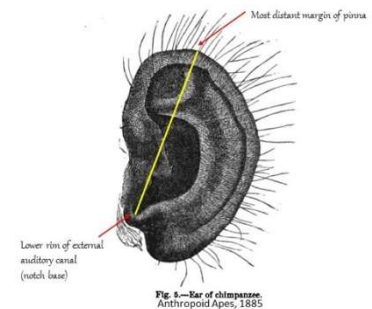
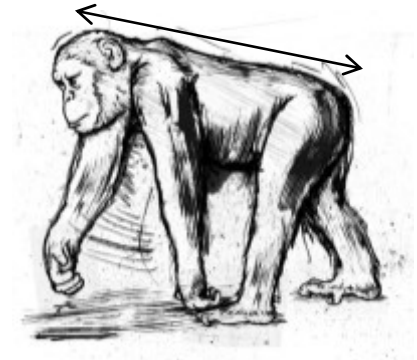
Photographs: At a minimum, the following digital photographs should be taken:

- Anterior/ventral view of full body with arms at sides, preferably with identification card or sheet displaying unique identifying number.
- Full anterior facial view.
- Full lateral facial/head view.
- Views of full upper and lower dentition (to help determine/verify age and sex).
- Frontal/ventral view of fully exposed genitalia.
- 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)*:

- 1) 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 NHPs (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).
- 2) Tail length (from base to tip).
- 3) Hind foot length (heel to tip of longest toe- exclude nail and note which toe).
- 4) Tibia length (‘knee to ankle’).
- 5) 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.
- 6) For very small primates (e.g. marmosets) include a complete view of the animal next to a ruler.



List of Biological Samples to collect:

The table below provides details on the suggested suite of samples to be collected from each animal handled and their preservation method. MagMAX lysis/binding buffer concentrate, also referred to as MagMAX lysis buffer, and viral transport medium (VTM) are the two primary sample preservation media for viral discovery and surveillance. Serum is stored without media.

Ideally, all samples will be frozen in liquid nitrogen immediately in the field and transferred to a -80°C lab freezer when field teams return.

NOTE: In all cases, if only one sample can be collected, then place it into VTM.

Sample type	Total samples	Preservation method						Comments
		500 µL VTM		500 µL MagMAX lysis buffer		No Media		
		#	Sample volume	#	Sample volume	#	Sample volume	
Oral swabs	2	1	swab	1	swab			

Nasopharyngeal swabs	2	1	swab	1	swab			Deep into each nostril and rotate for 10 seconds
Feces/ Rectal swabs	2	1	0.5cc feces or swab	1	0.5cc feces or swab			Feces OR swab
Urine/ Urogenital swabs	2	1	100 µL urine or swab	1	100 µL urine or swab			Urine OR swab
Blood	4	2	100 µL	2	100 µL			
Serum	4					4	500 µL	

2b. Sample Collection Procedures from Live NHP During Anesthesia

Live NHPs 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:
 - a. **Forearm veins** - In larger species (e.g., apes), the cephalic, radial, median, and ulnar veins might be large enough for safe blood collection.
 - b. **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.
 - c. **Jugular vein** - This may be the only option in very small NHP and must be

accessed carefully.

- d. **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.
- 4) Collect sample.
- 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

Whole blood in VTM and MagMAX lysis buffer: 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 2 aliquots of 100 μ l whole blood in cryovials with 500 μ l VTM and 2 aliquots of 100 μ l whole blood in cryovials with 500 μ l MagMAX lysis buffer concentrate.

For serum, from the red top/tiger top tube, allow blood to clot and/or centrifuge. Use a plastic pipette to take 2 ml of serum and transfer into 4 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 μ l 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 2-3 days, remaining whole blood in lavender top tubes can be refrigerated for analysis. Blood smears can also be prepared in the field.

Oral Swabs: 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.

- a) Place 1 swab in a cryovial filled with 500 μ l VTM and use alcohol- wiped (or ethanol-wiped), 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.

- b) Place the other swab into 500 µl of MagMAX lysis/binding buffer concentrate in a cryovial, following the same procedures.
- c) 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.

Nasopharyngeal swabs: Same as oral swab but instead the swabs must be inserted 'all the way in' (gently) into each nostril and rotated for 10 seconds. The pharynx and nostrils are swabbed equally and via each nostril for 10 seconds each. The [CDC Nasopharyngeal Specimen Collection Steps](#) offers a good guide and visual reference for this procedure (in humans).

Feces or fecal swabs

- a) **Feces:**
 - I. 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.
 - II. 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 500 µl MagMAX lysis buffer.
 - III. Homogenize by shaking. Freeze in dry shipper or dewar with liquid nitrogen and transfer to -80°C freezer when possible.
- b) If feces are not available, collect 2 **rectal swabs**. 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 LYSIS BUFFER AS A LUBRICANT – IT IS HIGHLY IRRITATING TO TISSUE.]
 - I. 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 MagMAX lysis buffer.
 - II. Store in a dewar or dry shipper with liquid nitrogen dry shipper and transfer to -80°C freezer when possible.

Urine

Most NHPs 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 100 µl of urine in a cryovial with 500 µl VTM, and another 100 µl of urine in a cryovial with 500 µl MagMAX lysis buffer. If the animal does not urinate you can try collecting urogenital swabs. 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 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.

2c. Non-Invasive Primate Sample Collection

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 minimum PPE (as appropriate for the species) approach the sample. Compress the chewed nylon swab rope with a gloved hand into a swab collection tube (Salimetrics, Inc.). 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) Pipet 200 μ l of Phosphate Buffered Saline (PBS) directly onto the rope once inside the storage/centrifuge tube.
- 9) Centrifuge tubes in the field @ 3,000 rpm for 5 minutes to elute saliva into the bottom of the tube.
- 10) Place 300 μ l saliva in a cryovial tube containing 300 μ l VTM (*note, if tubes are pre-aliquoted with 500 μ l VTM. Remove and discard the appropriate volume of VTM so that there is a 1:1 volume of saliva to VTM).
- 11) Place 300 μ l saliva in a cryovial tube with no storage media*.
- 12) Place 300 μ l saliva in a cryovial tube containing 300 μ l MagMAX lysis buffer (*note, if tubes are pre-aliquoted with 500 μ l MagMax lysis buffer. Remove and discard the appropriate volume of MagMax lysis buffer so that there is a 1:1 volume of saliva to MagMax lysis buffer)*.

*If inadequate volume of saliva is available, prioritize the sample in VTM, followed by tubes with no storage media. Minimum saliva solution (saliva plus PBS) volume per tube is 200 μ l.

Collecting Non-invasive Fecal Samples

- 1) Primates should be observed defecating to assure that samples collected are fresh (ideally collected within 15 minutes of defecation).
- 2) Using gloved hands and a disposable spatula or stick (the plastic shaft of a swab works well), select a ~200 mg (pea sized) sample of fresh feces from the inside of the fecal bolus. The goal is for the sample collected to not have come into contact with the ground.
- 3) Place one ~200 mg fecal sample in a cryovial with 500 μ l VTM. Homogenize by shaking.
- 4) Place another ~200 mg fecal sample in a cryovial with 500 μ l MagMax lysis buffer. Homogenize by shaking.
- 5) Freeze samples in dry shipper or dewar with liquid nitrogen and transfer to -80°C freezer when possible.

2d. Sample Collection from Dead NHP

If animals are found dead or must be euthanized by trained personnel per acceptable guidelines (following your IACUC approved procedures 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. In addition, specimens can be taken following the lead veterinarians institutionally approved necropsy protocols. *The necropsy sampling protocols below are suggestions to accompany any protocols that your organization may already have in place for primate diagnostics.*

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

If carcasses are fairly decayed, a minimal set of specimens should be collected, prioritizing one muscle tissue specimen for species identification. 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 up to 2 ml of serum and transfer into 4 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:

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

One specimen should be frozen in 500 μ L VTM in a cryovial, one should be frozen in 500 μ L MagMAX lysis buffer in a cryovial, and one should be stored at room temperature in a large jar with 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 100 μ L of urine in a cryovial with 500 μ L VTM, and another 100 μ L of urine in a cryovial with 500 μ L MagMAX lysis buffer. Alternatively, if urine is not available, two urogenital swabs can be taken, with one placed in 500 μ L VTM and one in 500 μ L MagMAX lysis buffer. Store samples in a dry shipper or dewar with liquid nitrogen and transfer later to -80°C freezer.

Rectal Swab/Feces

Collect either feces within the rectum or 2 rectal swabs. Place a ~200 mg (pea sized) sample of fresh feces in a cryovial with 500 μ L VTM and another ~200 mg sample in a cryovial with 500 μ L MagMAX lysis buffer and homogenize by shaking. For rectal swabs, insert one sterile swab tip at a time into the animal's rectum and store 1 swab in VTM and 1 swab in MagMAX lysis buffer. Freeze samples in dry shipper or dewar with liquid nitrogen and transfer to -80°C freezer when possible.

Nasopharyngeal swab

Insert one sterile swab tip at a time into each nostril, all the way back to the nasopharynx, and rotate for 10 seconds. Place 1 swab in VTM and 1 swab in MagMAX lysis buffer. Freeze samples in dry shipper or dewar with liquid nitrogen and transfer to -80°C freezer when possible.

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A1. Appendix 1: Field Inventory Checklist for NHP Sampling

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 directly 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.)

* 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 MagMAX lysis buffer 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
- ☐ Salimetrics oral swab ropes if doing non-invasive sampling
- ☐ Salimetrics oral swab centrifuge tubes if doing non-invasive sampling

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

A2. Appendix 2: Accidental Needle Stick Exposure Emergency Protocol

Accidental needlestick injury: Assume any needlestick injury is a suspected contact for a zoonotic pathogen 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 thoroughly 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 your supervisor and the physician-in-charge. *Reporting forms may be found in the Biosafety and PPE SOP.*

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 possible exposure to a zoonotic pathogen.

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.

A3. Appendix 3: 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 (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.

A4. Appendix 4: Ebola Virus Exposure Emergency Protocol

Adapted from <http://www.cdc.gov/ncidod/dvrd/spb/mnpages/vhfmanual/section5.htm>

Accidental needle stick: Assume any needlestick injury is a suspected contact for a 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 following protocols described in Appendix II for needle sticks.

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.

A5. Appendix 5: Small Monkey Capture and Anesthesia Guide

Adapted from Vervet Monkey Protocols in the PREDICT Non-Human Primate SOP, based on field training with Mountain Gorilla Veterinary Project/Rwanda PREDICT staff and the PREDICT Primate Capture Training Protocol by Chris Whittier.

Monkey Capture

- 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).
- 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.



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

- 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.
- 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 field team should determine the specimens to be collected based on 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.
- 3) All individuals handling monkeys and samples should wear appropriate PPE: N95 or better respirators with no exhalation valve, nitrile gloves, Tyvek suits, and safety goggles



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

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. Refer to your approved IACUC protocol drug table and its references as needed for dosage details. **These drugs should only be handled by a trained veterinarian.**

For example, 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

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

$$5 \text{ kg} * 3.5 \text{ mg/kg Ketamine} \div 100 \text{ mg/mL} = 0.175 \text{ mL}$$
$$5 \text{ kg} * 0.035 \text{ mg/kg Medetomidine} \div 1 \text{ mg/mL} = 0.175 \text{ mL}$$

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



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



Figure 6: Blood collection from the femoral vein of a vervet monkey. Photo credit: PREDICT Tanzania Team 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 suction. Avoid pulling the syringe too quickly, as too much vacuum pressure can collapse the vein.
- 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 biological sample collection protocol above.
- 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 7) 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.



Figure 7: A vervet monkey with a temporary mark recovers from anesthesia. Photo by PREDICT Tanzania team