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PROTOCOL

Primate Sampling Methods

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Objective: To safely collect biological samples from live and dead wild non-human primates (hereafter referred to as NHP).

USAID Disclaimer

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CONTENTS

Section 1. Wildlife Sampling Ethics and Policy

Section 2. Permissions, Permits and Protocols

Section 3. Safety and Personal Protective Equipment

Section 4. Special Considerations for Handling NHP

Section 5. Field Sampling Station Set-Up

Section 6. Primate Processing

- Sample Data Collection
- Biological Sample Collection

Section 7. Sample Processing and Analysis

Section 8. Safe Disposal of Carcasses and Infectious Waste

Section 9. References

Appendix I. CDC-WHO Guide for Building a Waste Incinerator

Appendix II. B Virus Exposure Emergency Protocol

Appendix III. Ebola Virus Exposure Emergency Protocol

Appendix IV. Materials Glossary

SECTION 1. WILDLIFE SAMPLING ETHICS AND POLICY

All sampling of wildlife will be conducted in a humane and ethical manner, minimizing the impacts on wild populations. The "Three-Rs" of wildlife research will be observed:

- **Replacement** - Animals may be used only if the investigator's best efforts to find a replacement by which to obtain the required information have failed.
- **Reduction** - The fewest animals appropriate to provide valid information and statistical inference.
- **Refinement** - The most humane, least invasive techniques must be used with the goal of minimizing pain and distress.

Regarding Three-R's see: (http://www.ccac.ca/en/alternatives/wildlife_faune.html).

Even in situations when lethal sampling may be perceived as quicker, easier or less expensive, we will pursue humane and non-lethal methods for sampling.

In some instances, an unrecognized or previously undescribed species may be captured during sampling activities. Under these circumstances, the PREDICT partner may deem it necessary to collect a voucher specimen in order to identify the species of animal. Collecting a voucher specimen requires the euthanasia and preservation of an entire animal for the purpose of performing detailed genetic and morphological characterization. If the collection of a voucher specimen is necessary for identification, then that animal will be euthanized in accordance with the humane practices defined in the relevant IACUC protocol. Whenever a voucher specimen is collected, the PREDICT Executive Board will be notified.

Finally, no incentives, financial or otherwise, should be given to local hunters, vendors, or others that would lead to the capture or killing of additional animals or species that would not otherwise have been taken from the wild, either on the day of sampling or in the future. This includes not purchasing animals for sample collection purposes, even in market settings; as such purchases either through exchange of money or a financial equivalent, could exacerbate perceived demand. PREDICT partners will maintain vigilance regarding the potential for their presence to alter local market or trade dynamics, and PREDICT staff will modify their behavior and methodologies as needed if sellers or collectors seem to be tailoring their activities to match perceived PREDICT interests.

SECTION 2. PERMISSIONS, PERMITS, AND PROTOCOLS

Obtain all required permits and any other formal/informal approvals well in advance of beginning field sampling.

It is essential that all required permits and permissions for animal capture and handling, and sample acquisition and movement are received from the relevant governmental agencies/ministries and local community leaders (where appropriate) prior to commencement of field captures. As this process often takes time, it is recommended that the relevant agencies/ministries be contacted well in advance of the anticipated sampling start date.

Each country team should ensure that all activities are consistent with relevant PREDICT IACUC and IRB protocols and procedures.

All approvals and permits need to be properly documented and maintained and all relevant PREDICT guidelines and protocols need to be followed. Guidelines for sample packing and shipping, including relevant import and export permits, are covered in-depth in the ***PREDICT GUIDE: Packing and Shipping Biological Samples***. Remember that in all addition to all other permits, primate samples require special import permits from the Centers for Disease Control and Prevention (CDC).

SECTION 3. SAFETY AND PERSONAL PROTECTIVE EQUIPMENT

All personnel are responsible for knowing the safety guidelines and procedures that are relevant to all of their tasks. Personnel involved in capturing, handling, or sampling of NHP must understand and be able to apply all of the relevant safety guidelines provided in the PREDICT Guides and Protocols.

Before handling any NHP, all personnel must understand and follow this Primate Sampling Protocol and the most up-to-date versions of ALL of the following PREDICT guides:

- ***SAFETY GUIDE: Biosafety and PPE Use***
- ***SAFETY GUIDE: Laboratory Operations***
- ***Guide for Safe Animal Capture for Sampling***

Safety Responsibilities:

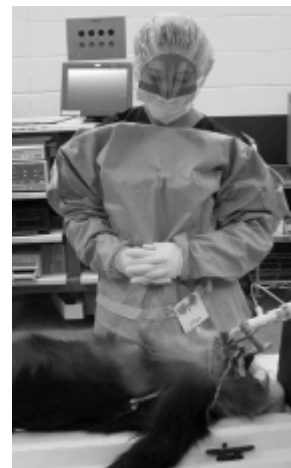
- The primary investigator and/or sampling team leader is responsible for safety and actions of field teams and for ensuring that the field team members have had all the appropriate safety training for the tasks to be performed.
- Each country team should ensure that all activities are in line with relevant PREDICT and IACUC protocols.
- Field personnel must immediately stop working and report to their supervisor and follow post-exposure guidelines for any injury or accident from handling NHP that may constitute an exposure to *Cercopithecine herpesvirus 1* (B virus), Ebola virus, or rabies virus (see details in Section 4). Even in cases where there is little concern for specific pathogen exposure, any breach of PPE (e.g., bite, needlestick, eye splash) should immediately be reported to the sampling supervisor.
- Each country team should ensure that appropriate PPE is used as described below.
- In order to protect both human handlers and sampled NHP, all personnel handling NHP should be tuberculosis (TB) tested beforehand as described in the following Section.

Brief Overview of Personal Protective Equipment (PPE)

The **PREDICT SAFETY GUIDE: Biosafety and PPE Use** should be used as a primary reference and any information and recommendations in that Guide supersede what is briefly outlined here.

The purpose of Personal Protective Equipment (PPE) is to:

1. Protect personnel from contamination or exposure to potential infectious agents.
2. Prevent the contamination of persons at a site thereby preventing the transmission of potential infectious agents to other sampling locations, animals, and people.
3. Though not strictly intended, it should be noted that proper PPE also helps to protect the health of animals from potential infectious agents in their human handlers. This is particularly true of NHP because of their close genetic relationship to humans.



Preparation for PPE Use in the Field

Use the most recent biosafety recommendations to decide what level of personal protection is needed to prevent contamination of the field personnel and sampling team, taking into account potential pathogens in the animal population (See '*Minimum PPE Required for Handling Live, Dead or Samples of NHP*' in Section 3 of the **PREDICT SAFETY GUIDE: Biosafety and PPE Use**). Planning and preparing field sampling kits should include making careful estimates of the number of sets of PPE that will be required by all personnel. Extra supplies should always be brought in case a sampler needs to change unexpectedly. Field sampling kits must also include supplies for disinfecting personnel on site and containing contaminated PPE for disposal (see Checklists in Section 5).

Minimum PPE Required for Handling Live, Dead or Samples of NHP

For NHP, the *minimum* PPE includes eye protection, an N95 (or better) respirator, long clothing, and nitrile or latex gloves. 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. See additional notes about macaques below.

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 mins 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.

PPE Removal and Decontamination

All equipment used should be thoroughly cleaned and properly disinfected immediately after use and field personnel should be mindful that anything in contact with contaminated equipment or PPE must also be considered contaminated. Personnel should note that many breaches might occur during PPE removal. Thus, proper removal is one of the most critical procedures of PPE use.

Decontamination = cleaning + disinfection

1. Clean--remove organic material, dirt and grease
2. Disinfect--use an appropriate disinfectant

Thorough cleaning and disinfection of equipment are essential to protect personnel from pathogen exposure and to prevent the spread of pathogens to other wildlife or domestic animal populations. Used PPE gear must be removed in a manner to avoid contamination of the user or the environment, and disposed of appropriately and in a manner to avoid future contamination of other humans, animals, or the environment.

First Aid and Emergency Preparedness

It is recommended that at least two field staff have basic first aid and CPR training and be familiar with recommended first aid procedures for injuries likely to be encountered in the work tasks in the specific field setting. (Refer to the **PREDICT Guide for Field Emergency Preparedness**).

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. These 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.
- Keeping extra swabs, viral culture media, and sero-sampling materials 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 post-exposure sampling in the event of accidental exposure.

(See Section 4 and Appendix II for details and exposure emergency protocol.)

First Aid Protocol 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 (or benzalkonium chloride if available and 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 Appendix II.

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 III.

SECTION 4. SPECIAL CONSIDERATIONS FOR HANDLING NHP

This section supplements the ***PREDICT GUIDE: Safe Animal Capture for Sampling***, with which anyone handling NHP is expected to already be thoroughly familiar. Note also that sampling from dead NHP, destined for bushmeat or not, is also covered in the ***PREDICT PROTOCOL: Bushmeat Sampling Methods***. However, for completeness, much of that protocol is repeated here.

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 be careful to 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 (then bite) rather than scratch or push their handlers during procedures. Heavy-duty leather gloves should be worn by anyone handling conscious (unanesthetized or unsedated) NHP. Hand restraint is discouraged for any NHP not already chemically restrained. It 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, especially where they are locally hunted and may be very defensive, and during immobilization procedures. 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 not only are they more likely to transmit infections *to* their human handlers, but they are also more susceptible to acquiring infections *from* their handlers.
 - a. Proper use of PPE and related biosafety measures as described in the following section 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. TB testing is typically done by intradermal tuberculin skin test (TST). Workers who are already 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 facts sheets at: www.cdc.gov/tb/publications/factsheets/default.htm). Any staff suspected of being infected with TB must not work with NHP.
 - c. To protect NHP from human infections, no persons with any current or recent (within a few days¹) 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) so this precaution is only partly effective. Ideally, personnel working 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), and meningococcal meningitis). This helps to not only ensure their health, but secondarily to help protect their co-workers and any animals they may handle (see further discussion at: www.brookfieldzoo.org/pagegen/inc/ACNutter.pdf).
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

¹ 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.

dogs in developing 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), the post-exposure rabies vaccination should be obtained as soon as possible. Note that rabies symptoms in primates can be very variable (irritability, self-mutilation, paralysis) and/or very generalized (malaise, discomfort).

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 disposal of suspect carcasses are the most effective measures of preventing anthrax exposure and spread. See <http://www.bt.cdc.gov/agent/anthrax/> for additional information including post-exposure prophylactic protocols.
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 EITHER OF 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 exposure to 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 (e.g., bite, facial splash, needlestick) workers must stop **IMMEDIATELY** and trigger the **B Virus Emergency Exposure Protocol** detailed in **Appendix III**. TIMING IS CRITICAL and an immediate action can be the difference between life and death. Additional online information on B virus can be found at the following websites:



<http://www.cdc.gov/herpesvirus/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 or <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 ***Ebola Virus Emergency Exposure Protocol*** detailed in **Appendix III**. Potential post-exposure treatments are currently under investigation and may be available in the near future or through special arrangements by PREDICT partners (contact WCS in the event of a possible Ebola exposure emergency).

SECTION 5. FIELD SAMPLING STATION SET-UP

Proper selection, preparation, and arrangement of the sampling site will help to:

- Reduce handling time and resultant stress on animals;
- Avoid contamination of clean materials by used ones, and contamination and potential exposure to infectious agents of samples, animals and people;
- Allow efficient and discreet sampling without interference;
- Simplify post-processing clean-up and disinfection;
- Minimize negative environmental impacts.

A detailed sampling protocol for the taxonomic groups/species must be pre-established, and a data recording spreadsheet must be pre-prepared (see Section 6.1). It is beneficial to assign a duty to each member of the team (such as record keeping or sample tube labeling), to minimize confusion and avoid repetition or omission of any sampling steps. One team member (such as the PI) should be deemed officially in charge at the sampling station to ensure smooth processing of the animal(s) and to avoid disagreements on any decisions that may arise, which would delay processing of the animal(s).

Organization of the Sampling Area

Sampling materials should be located at a designated sampling station where investigators agree sampling will take place. A good field sampling station should be:

1. An area that is easy to disinfect.
2. Out of view of the general public and away from interference.
3. A location where, if disinfection efforts fail, it is not likely to put humans or other animals at risk (e.g., not at a picnic area).

Materials should be organized in a manner for easy access and swift processing of the animals (e.g., in chronological use order), to minimize stress on the animals and the handling time. Sampling materials must also be arranged for easy access by the individual doing the specific sampling technique (e.g., venipuncture, swabbing), and with sufficient space to avoid contamination of unused materials (by contaminated materials or other animals).

SUGGESTED LIVE* PRIMATE SAMPLING STATION CHECKLIST:

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
Monitoring equipment (pulse oximeter, stethoscopes, etc.)
Sharps container

PPE and emergency equipment

N95 (or better) respirators (enough for all team members plus extras)
Eye-protection, goggles (face shields if handling suspect B virus or Ebolavirus positive NHP)
Disposable/Nitrile gloves
Reusable and disinfect-able leather or heavy gloves
Protective suits, gowns, coveralls, or full length dedicated field garments
Basic first aid kit
Emergency exposure kits for B virus or Ebola (if applicable)
Working communications equipment (cell phone, satellite phone, etc.)
Emergency response plan (see **PREDICT Emergency Preparedness Guide, in development**)

Data collection materials

Field data collection sheets (sufficient # for animals to be sampled plus extras)
Writing instruments (pens, pencils and permanent markers)
Weather resistant holder for paperwork
Digital camera and extra charged batteries
GPS unit
Measuring tape, ruler, calipers as appropriate for size of animal

Sampling materials

Collection tubes
Collection media
Swabs
Slides
Needles
Syringes and/or vacutainer needle holders
Zip-loc bags
Cold storage container (cooler, ice packs, dry shipper, etc.)

*A separate list for field necropsy procedures is anticipated in other protocols in development.

SECTION 6. PRIMATE PROCESSING

Capturing, trapping, darting, and immobilizing NHP should only be performed by experienced and skilled staff and are not covered in this document.

PREDICT partners are expected 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.

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. Recover animal 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. Measure and record the animal's weight/mass initially as this can be important for proper drug dosing or emergency interventions.
2. Conduct a cursory physical exam before sampling in order to note any wounds or major abnormalities and to protect the health of both handler and animal.

As discussed in the ***PREDICT Guide for Safe Animal Capture and Sampling***, if during capture, handling or processing a primate sustains a life-threatening injury or one that will render the animal incapable of surviving in the wild, humane euthanasia should be considered and should only be performed by experienced individuals using lethal injection.

Sample Data Collection

Successful and consistent data recording is an essential component to disease surveillance. A data spreadsheet (electronic and/or printed) should be created well in advance with all required data fields included. The required data fields are listed in the most up-to-date version of the ***PREDICT Guide: Surveillance Data Collection***. The field data recording sheet and sample labeling materials must be present during sampling, and updated after each sample is taken (i.e., label each sample and record the corresponding data immediately after collecting each sample). It is ideal to assign the task of data recording to one team member to ensure that this key activity is completed accurately, consistently, and with accountability.

Important considerations for sample data collection:

- *****Follow the MOST RECENTLY UPDATED PREDICT Guide: Surveillance Data Collection and complete the PREDICT data forms as instructed.*****
- Sample labels must have unique animal and sample IDs as per the above Guide. If a barcode labeling system is used, these labels should be printed in advance of sampling work.
- If field data cannot be collected directly into a computer, printed field data recording sheets should be designed to align with the larger PREDICT surveillance project. Required and optional PREDICT data are listed in the data collection guide. Taxa specific sample field data collection sheets are currently under consideration and if/when completed will be available to PREDICT partners.
- The entire sampling team should share the same understandings and definitions of terms used in the sheet (e.g., criteria for marking “poor” condition) and how to describe contextual information about the sample collection, including the level of detail and common descriptive terms. Specific training should be given and a ‘data dictionary’ to clearly define specific terms should be compiled and approved beforehand. Regardless of previous training, the sampling team leader is responsible for ensuring that the team collects consistent and accurate data.
- Assign one team member to record data.

Minimum Data to Collect with Samples

The minimum set of data (fields) that should be recorded for all specimens is listed in Table 1. **See the *PREDICT Guide: Surveillance Data Collection* for details about data collection and submission.**

Table 1. Data fields currently required at all sites (CHECK GAINS FOR MOST UP TO DATE LIST**).**

Field Name	Explanation
Date	Date observation made, required for event, optional for others
Recorder name	Name of person collecting data
Recorder affiliation	Affiliation of person collecting data
Animal ID	See Appendix, Individual tab
Taxonomic descriptor	If exact species is not known, taxonomy to most precise level possible (e.g., genus, subfamily, family, etc.)
Species scientific name	Scientific name of animal (genus and species)
Identification certainty	Level of certainty of wildlife identification
Identified by?	Who identified wildlife
Site name	Unique name of site where data was collected
Country	Country of collection site
State/Province	State or Province of collection site, e.g., "California" or "Kampot"
Site Longitude	Decimal degrees
Site Latitude	Decimal degrees
Source type	Type of individual/facility in possession of wildlife, or means by which the wildlife came to be in possession
Reason for collection	Description of reason for collection (e.g., opportunistic, active surveillance etc.

Additional (Optimum) Data to Collect from NHP

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

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

Body weight: As mentioned previously, body weight may be one of the first measurements taken (prior to required data) 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. (Note: 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 other 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 below:

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.
Immature	Any individual not evidently sexually mature.
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 NHP to genus, and species

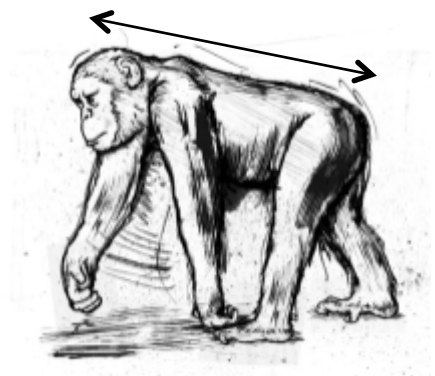
(where possible) and sex. Sex determination for young individuals of many primate species is not always simple and photographs of genitalia should be taken, especially if there is any doubt. For female NHPs, note parity (e.g., presence offspring, evidence of previous lactation) also determine pregnancy 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 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 to safely complete sampling.

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

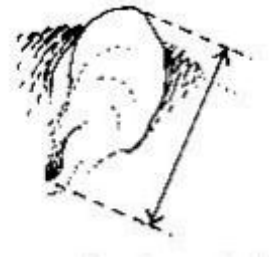
- a. Anterior/ventral view of full body with arms at sides, preferably with identification card or sheet displaying unique identifying number.
- b. Full anterior facial view.
- c. Full lateral facial/head view.
- d. Views of full upper and lower dentition (which can help determine/verify age and sex).
- 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/mm) should be recorded with the *minimum standard mammal measurements (all linear) as follows:*

- 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 [See figure]: base of the notch below the ear opening (lower rim of auditory canal = meatus) to the most distant point of the margin of the pinna.



Additional optional measurements

- a. Head length, trunk height, hip breadth, hand length and breadth, foot breadth, limb segments (thigh, lower leg, upper arm, forearm).
- b. Chest circumference (at nipples), abdominal circumference (at umbilicus), and cranial circumference (at or above brow).

Biological Sample Collection

OVERVIEW

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. Opportunities to collect biological samples and related health data from wild NHP are relatively uncommon and maximizing these opportunities can further advance wildlife health monitoring.

Live NHP

Live NHP should be chemically restrained during any invasive sample (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 samples.

As described in detail below, the basic set of PREDICT samples collected from each live animal will include **blood**, **saliva**, and **feces**. Optional collection of urine, skin/hair, ectoparasite, and milk (when available) is advised whenever possible.

Dead NHP

If carcasses are not whole or are fairly decayed then the ***PREDICT Protocol for Bushmeat Sampling*** may be more applicable. If bodies are relatively whole and fairly fresh then **blood**, organ **tissues**, and (optionally) external parasites and urine should be collected as detailed below.

Sample Labeling

Tubes must be labeled with a unique identifier number. PREDICT is evaluating using a bar code labeling system. In the meantime printed labels should be used. One option is the printers and labels made by Electronic Imaging Materials (<http://www.eiminc.com/>)

SAMPLE COLLECTION FROM DEAD OR EUTHANIZED NHP

As discussed throughout this protocol, NHP should be considered potentially infectious for a wide variety of dangerous pathogens and dead NHP in particular should be sampled only following all safety measures including proper PPE use, proper work station decontamination, and proper carcass disposal as outlined here and in other PREDICT documents.

Though not required for PREDICT sampling, thorough necropsy procedures can be very beneficial and might pertain to some animals (e.g., valuable or known

individuals, suspicious deaths, etc.) and are addressed in separate documents. Time and skill permitting, when full necropsies are performed, following the Association of Zoos and Aquariums/AZA (or similar) great ape necropsy protocol is recommended and can be adjusted for application to all primate species. *(Note that properly following this extensive necropsy procedure and collecting and measuring all samples can require 4-6 hours for a single animal.)*

Post-mortem blood collection

In 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) and allow to sit undisturbed for at least 30 minutes. Then centrifuge at high speed (2000 x G for 20 minutes), then remove the serum (clear, yellow or red-tinged fluid at the top), then transfer clots to cryovials, and then refrigerate or freeze. If a centrifuge is not available, allow clots and cells to settle as much as possible and then collect serum and then clots. If the animal died recently and the blood has not yet clotted and no centrifuge is available, invert the blood tubes after collection and allow it to clot. Then turn right side up and carefully remove the stopper, which should also remove the clot and leave clean serum sample.

At a minimum, as many of the following blood samples as possible should be collected:

- **2 thin whole blood smears**, dried, fixed, and stored at room temperature
- **2** or more aliquots (60 ul – 1.0 ml) of **separated serum**, frozen
- 1/2 of **blood clot**: 2 volumes **VTM** (aliquotted), then frozen
- 1/2 of **blood clot**: 3 volumes **lysis buffer** (aliquotted), then frozen

Tissue collection

Three separate samples of each of the following tissues should be collected at a minimum:

- large intestine
- small intestine
- liver
- lung
- kidney
- spleen
- brain (if possible)
- any abnormal appearing tissue*

The three pieces should be collected as follows:

- ~200 mg (pea-sized) piece placed in an **empty cryovial** then **frozen**
- ~200 mg piece placed in vial of **VTM** then **frozen**.
- ~1 gm (with no part thicker than 1 cm) sample of each organ tissue should be placed together in a jar of **10% buffered formalin** at 10 parts formalin for each part tissue and kept at **room temperature** for histopathology.

*It will usually require experience to identify abnormally appearing tissues but potentially recognizable gross lesions include masses, discolored areas, ulcerations, etc. Samples for pathology (i.e., in formalin) should be collected at the abnormal margins to include both normal and abnormal sections in the same piece of tissue. Collection of any obvious internal parasites in ethanol is also recommended.

External parasites (*Optional*)

Care should be taken NOT to collect post-mortem arrivals (flies, maggots, beetles, ants, etc.) and to only collect obvious external parasites (ticks, lice, fleas, etc.). These should be collected into tubes or vials of 90% (or stronger) **ethanol**.

Urine (*Optional*)

If carcass/urinary bladder is intact and contains uncontaminated urine, a sample should be collected. Place ½ of the collected urine sample in a cryovial with **lysis buffer** at an optimal ratio of 1 part urine: 3-part lysis buffer. Note that for very small volumes of urine, a minimum of 300 ul lysis buffer is required. Place the other half in a cryovial with 1 part urine: 2 parts **VTM**. Store in dry shipper or dewar with liquid nitrogen and transfer to -80°C freezer when possible.

SAMPLE COLLECTION FROM LIVE NHP:

Blood Collection

Precautions

- **At least one person present should have previous experience in primate venipuncture to avoid injury to the animal.**
- NHP should be immobilized using either injectable or gas anesthesia according to appropriate guidelines.
- The person restraining the primate is responsible for monitoring respiration and other vital signs and communicating the status of the animal appropriately.
- No more than 1 ml of blood per 100 gm (= 10 ml/kg or 1%) of primate body weight should be collected at any one time.

- Primate blood should always be considered highly infectious and hazardous.

Collection procedure

1. Select appropriate venipuncture site:

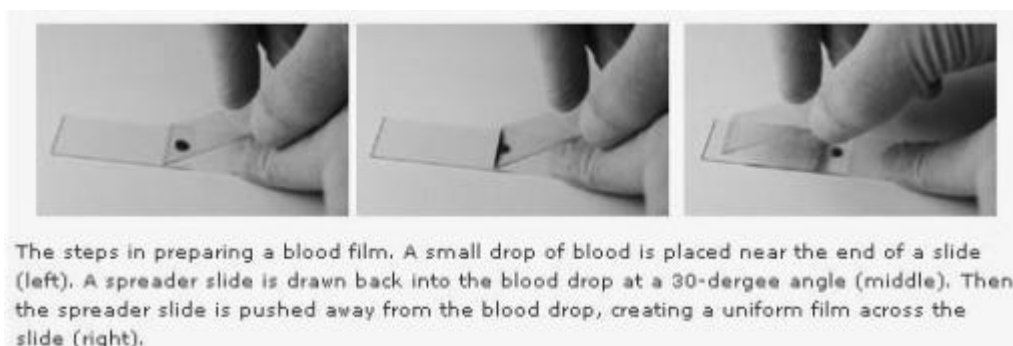
- **Femoral vein**- Best for small NHP and for large sample volumes. If the femoral artery (just lateral/anterior to the vein) is inadvertently pierced sampling can continue but extra effort must be made to apply post-collection pressure for at least 1 full minute to minimize hematoma formation.
- **Caudal 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.
- **Forearm veins**- In larger species (e.g., apes) the cephalic, radial, median, and ulnar veins might be large enough for safe blood collection.
- **Jugular vein**-This may be the only option in very small NHP and must be accessed carefully.



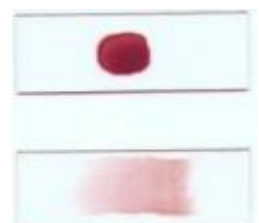
2. Select appropriate size needle and syringe (or vacutainer) for the size of the primate.
3. Disinfect the site with iodine solution or alcohol.
4. Collect sample.
5. **Do not recap needle.**
6. Apply pressure to site of bleeding using a cotton ball or gauze pad until bleeding ceases (approximately 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

1. **Two thin blood smears:** Use initial drops to make two (2) thin blood smears on glass slides. Allow slides to air-dry and fix with methanol or ethanol and store at room temperature.

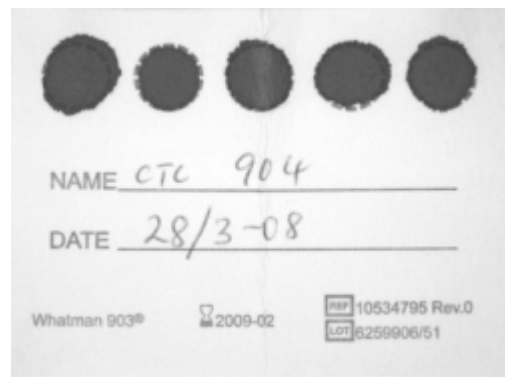


Too thick



Good

2. **Aliquot serum into cryovials:** Place collected blood into serum tubes (red top or tiger top, if >1 ml blood is collected, or into 1.5 ml conical Eppendorf tubes). Place labeled blood tubes in a rack on ice (optimally) for up to 2 hours prior to centrifuging. Centrifuge the blood samples. If a centrifuge is not available, red top tubes with blood can be left standing on ice overnight to allow serum to separate. Use a pipette to draw off serum, aliquot into 60 ul to 1.0 ml volumes per cryovial, and store cryovials in a dry shipper or dewar. As soon as possible, remove samples and place in cryoboxes and store in an -80 °C *freezer*.
3. **Blood clots in VTM and LB:** The blood clot should be divided with half placed in a cryovial with at least 3X volumes or 0.5 ml of VTM, and the other half into at least 2X volumes or 0.5 ml of lysis buffer. Place tubes into liquid nitrogen in dry shipper or dewar and transfer to -80°C *freezer* when possible.
4. **Blood spots on filter paper (optional):** Using any remaining blood in syringe, fill all the circles or make small (<1 cm) spots on the Whatman 903 (or similar) filter paper/cards. Once well dried (~2 hrs), either place inside envelope and store at *room temperature* (or, for long term storage, place in sealable plastic bag with desiccant and refrigerate).
5. (Optional) **Whole blood in EDTA:** If facilities are available to perform complete blood counts (CBCs) within 5 days, whole blood can be collected in EDTA and refrigerated (not frozen) for analysis.



Throat Swabs

Swabs in VTM and LB: Using sterile, polyester-tipped swabs with either an aluminum or 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 0.5-1 ml lysis buffer and use a flame-sterilized scissors to cut the shaft of the swab above the tip. Place the other swab into 1 ml of VTM in a cryovial and cut shaft as above. Store both cryovials in a liquid nitrogen dry shipper or dewar & transfer to -80°C *freezer* when possible.

Feces

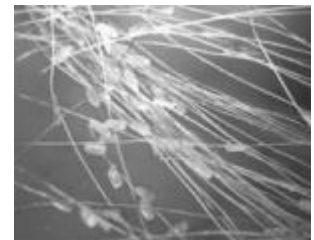
200 mg in VTM and empty cryovial: Collect either excreted feces or *if primate is large enough (> 1 kg)* use a gloved, lubricated (saline or medical lubricant)

finger to collect directly from rectum. Place a ~200 mg (pea size) sample of fresh feces in a 1.0 ml empty cryovial and another ~200 mg sample in 1.0 ml of VTM in a cryovial and 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 swab in lysis buffer** : [Note: VTM can be used as a lubricant to decrease the risk of trauma to the rectum. **DO NOT USE LYSIS BUFFER AS A LUBRICANT – IT IS HIGHLY IRRITATING TO TISSUE**]. Dip sterile swab tips briefly into VTM before inserting gently into the animal's rectum. Place 1 swab in a cryovial filled with 1.0 ml of VTM using a flame-sterilized scissors to cut the shaft of the swab above the tip. Place the other swab into a tube with lysis buffer. Store in a dewar or dry shipper with liquid nitrogen dry shipper and transfer to -80°C *freezer* when possible.

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*.

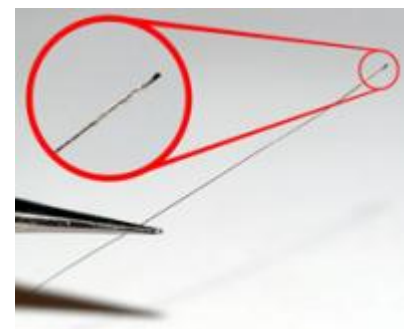


Urine (Optional)

Most NHP will urinate as a fear reaction prior to handling but urine can sometimes be collected free catch or by bladder expression from trained personnel. Place ½ of collected urine sample in a cryovial with **lysis buffer** at an optimal ratio of 1 part urine: 3-part lysis buffer. Note that for very small volumes of urine, a minimum of 300 ul lysis buffer is required. Place the other half in a cryovial with and 1 part urine: 2 parts **VTM**. Store in dry shipper or dewar with liquid nitrogen and transfer to -80°C *freezer* when possible.

Hair samples or skin biopsies (Optional)

Plucked hair samples (with root tissue) are easy to collect and are usually sufficient for DNA analysis if needed for species identification of individual NHP. For DNA analysis hairs should be collected (by plucking with forceps) early in the procedure to minimize potential contamination including with human cells. Plucked hairs can be stored dry in clean envelopes or empty vials.



Primate skin biopsies should not be needed for PREDICT sampling but if collected (e.g., for other protocols) they should be collected only from properly anesthetized NHP. A sterile punch biopsy should be used on a surgically prepared section of skin over the shoulder blade avoiding obvious blood vessels. In some small or thin-skinned NHP it may be necessary to surgically glue (best) or suture biopsy sites after collecting punches (recommend single simple interrupted or cruciate pattern and only with absorbable suture assuming animal will be released). If collected for DNA, place each biopsy in a cryovial with 0.5 ml **95% ethanol** and store at *room temperature*.

Milk (Optional)

If lactating females are handled, collect milk into cryovial(s) and store frozen. For basic analysis 0.5-2.0 ml is adequate and even small NHP (less than 500 gm) 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 (see above) and separation of nursing young prior to sampling should never be done strictly for the purpose of collecting milk.

Summary of samples to collect from live NHP:

Required PREDICT samples

- Blood:** 2 thin whole blood smears, dried, fixed, and stored at room temperature
2 or more aliquots (60 ul – 1.0 ml) of separated serum, frozen
1/2 of blood clot: 2 volumes VTM (aliquotted), then frozen
1/2 of blood clot: 3 volumes lysis buffer (aliquotted), then frozen
- Saliva:** 1 oropharyngeal/throat swab in VTM, then frozen
1 oropharyngeal/throat swab in lysis buffer, then frozen
- Feces:** ~200mg fresh fecal sample in empty cryovial, frozen
~200mg fresh fecal sample in 1.0 ml VTM, frozen
Or
1 rectal swab in VTM, then frozen
1 rectal swab in lysis buffer, then frozen

Optional samples

Urine: 1 volume free catch urine: 2 volumes VTM, then frozen
1 volume free catch urine: 3 volumes lysis buffer, then frozen

Hair/skin: ~5-10 plucked hairs with roots, dry in a clean envelope or vial
or 2 mm skin punch biopsies in 95% ethanol (for DNA)

Ectoparasites: whole parasites in 95% ethanol at room temperature.

Milk: up to 2.0 ml in empty cryovials then frozen

SECTION 7. SAMPLE PROCESSING AND ANALYSIS

Samples collected for PREDICT may be used for a number of activities including ongoing surveillance, pathogen discovery, specific research projects, long-term biobanking, and activities and analyses that are yet to be defined. The PREDICT sampling protocols are designed and intended to ensure that collected samples are suitable for the expected diagnostics and/or long term storage or archiving with the intent to complete diagnostics over the course of the project.

The exact processing and analyses of collected samples will likely vary by species, geography, available laboratories, time of collection and related epidemiologic factors. In addition, each PREDICT partner may have different aims and intentions for their collected samples. As the PREDICT Project advances some diagnostics may become standardized, at least within certain geographic regions and/or for certain species.

SECTION 8. SAFE DISPOSAL OF CARCASSES AND INFECTIOUS WASTE

Improper disposal of surveillance sampling waste, animal carcasses, and necropsy waste may harm human and animal health and the environment.

Prior to conducting field-sampling activities, plans should be made for safe handling and disposal of all infectious waste materials, necropsy waste and carcasses. Safe handling of infectious materials includes containment, disinfection, local burning and burial or transport of the materials to a health institution (that has a health care waste incinerator or burial site). The preferred procedure for most field sampling generated waste is to safely contain (i.e., with triple layered protection, see below) and transport the material to a health institution, such as a medical or veterinary facility, that has the capacity to autoclave and incinerate the waste, or has a safe disposal site. There are exceptional circumstances for which the best option may be to dispose of the infectious waste in the field (See recommended procedure for field disposal below.)

Carcass disposal is fraught with technical difficulties. Burning – if done properly (i.e., reducing the carcass to ash) is usually done using gasoil (diesel) or wood for fuel. It requires a long time to burn and uses a lot of fuel. In addition, anthrax spores can become airborne if the fire is not managed appropriately. Burial has the advantages of being generally less time-consuming and less expensive, but it does not guarantee destruction of all infectious organisms (e.g., anthrax spores may persist in the soil for decades). Burial also leaves open the possibility that someone could dig up and recover the meat to eat or sell. Ultimately the field supervisor must determine the best method for disposal based on the guidance provided below, his/her judgment and resources available.

Collecting and Containing Infectious Materials for Transport

Guidelines for collecting and transporting infectious waste:

- Use appropriate PPE to handle and move infectious waste – At a minimum, gloves, respirators/masks (N95, N100 or P100), goggles, and an apron should be worn when packaging, handling or moving infectious waste bags or containers. (See the *PREDICT Safety Guide: Biosafety and PPE Use* for more details regarding PPE).
- Collect infectious waste in strong plastic bags (preferably red or orange colored bags). Bags should not be filled more than $\frac{3}{4}$ full so they can easily be tied or taped closed without spillage or over stretching the bag. Once sealed, the exterior of the bag should be sprayed with a disinfectant.

- Use containers with tight lids and secure them on the vehicle – The preferred way to contain bags of infectious waste for transport is in plastic buckets, barrels or boxes with lids that may be secured tightly. These containers should be lined with plastic bags that are tied or otherwise sealed. Infectious waste containers should be strapped securely on a roof cargo rack or in the cargo compartment of a vehicle. Loose containers are more likely to be damaged or tossed from the vehicle.
- Disinfect waste bags and containers—Prior to moving infectious waste bags or containers the exterior should be sprayed with a disinfectant. After transporting infectious waste containers, all containers that will be reused must be disinfected.
- Disinfect all vehicles surfaces where infectious waste containers were stowed.
- Use a disinfectant known to kill the pathogens likely to be found in the waste.

Best Options for Disposal of Infectious Materials and Carcasses

Determining the best option for disposal of infectious material must be carefully evaluated, including consultation with local environmental and health authorities. Local permits may be required for disposal of necropsy or infectious waste. Options for infectious waste or carcass disposal and the rationale for each option are described below.

OPTION 1: Delivery of Waste to a Health Facility for Safe Disposal.

This is generally the best option when:

- a. The volume of infectious waste is limited to the number of plastic bags or containers that can be properly secured on the available transport vehicle. The volume of waste associated with most daily field sampling activities fits in this category. (Necropsy waste from medium to large-sized animals may not fit in this category).
- b. The waste can be delivered to the health facility for disposal within 48 hours. The longer waste is temporarily stored, the greater the risk that the containment bags or containers will break and expose humans and other animals to infectious materials.
- c. The local health facility has agreed to dispose of the waste and is expecting your delivery of infectious waste.
- d. A transport vehicle is available with either a roof rack or outside bed in which to transport the infectious waste containers. *Do not transport infectious waste bags or containers in the passenger compartment of a vehicle.* All materials transported must be securely attached to the vehicle so that containers will not

break or spill from the vehicle.

To deliver infectious waste to a health facility for disposal:

- a. Secure an agreement with the facility to accept waste. This agreement should include the costs, delivery times, and infectious waste containment requirements.
- b. Be sure have available the required PPE, disinfection and containment materials necessary to safely contain and transport waste: masks, gloves, coveralls, sharps containers, sturdy plastic bags and ties, disinfectant spray, buckets with tight fitting lids, and/or liquid waste containers as needed.
- c. Check vehicle and vehicle cargo space requirements for anticipated waste bags and containers. Sealed bagged or bottled waste should be transported in outside racks or cargo areas rather than inside vehicle passenger compartments. All such materials should be secured so they are unlikely to break open or fall off the vehicle.
- d. Notify the authorities of the facility to which the infectious material will be delivered in advance of the scheduled sampling activities, so they may anticipate your delivery of infectious materials.
- e. Contain and deliver the waste to the facility in accordance with the guidelines of the facility. It is recommended that high-risk waste be triple-bagged and sprayed with 10% sodium hypochlorite solution to disinfect the outside of the bags. Contaminated waste may include gloves, mask, face shield, Tyvek suits and other soiled and disposable materials. The triple-bagged materials are delivered to a facility for burning and burial.
- f. Disinfect the transport vehicle immediately after each delivery.

OPTION 2: Field Disposal—Burning and Burying of Infectious Materials

Under certain circumstances, field disposal may be the best (safest and most practical) option for disposing of carcasses, necropsy waste and other infectious materials as long as field disposal (burning and burying) can occur in the vicinity of the site where the waste was generated. For field disposal of infectious waste, PREDICT recommends the combination of both burning and burying. Burying waste contained in plastic bags, without burning, will likely allow pathogens to survive longer, posing greater risk of exposing people and animals. The best option for carcass disposal may be burning and burying, just burying, or just leaving the carcass where it is found.

Criteria for Choosing a Field Disposal (Burn and Bury) Method

Field disposal may be the best option when:

- The volume of infectious waste exceeds what can be safely contained and transported to a health facility for disposal. This may include large amounts of necropsy waste or liquid waste, or animal carcasses. Moving the infectious materials poses great risk of spreading the infection to other areas.
- It is not possible to transport the waste to a facility for disposal within 48 hours.
- There is no vehicle with adequate cargo space for the waste bags or containers.
- There are places nearby where waste can be safely buried.

Considerations for Determining the Best Site to Burn and Bury Infectious Materials

- Nature and amount of material for disposal (size and quantities of waste.)
- Availability of sites nearby suitable for digging a waste pit and burning waste, away from houses and other structures that constitute human communities.
- Accessibility of site by the vehicle used to move the waste.
- Features of the soil (e.g., easy to dig), low to no slope.
- Depth of groundwater: water table level should be at least 1.5 meters below the bottom of the pit.
- At least 50 meters from water catchments, bore holes and wells.
- Away from livestock, poultry or dogs.
- Away from wildlife that may dig up the material.
- Risk that humans may dig up the material.
- Subsequent plans for use of the area.
- Whether or not fencing will be required to exclude animals.

Procedure for Burning and Burying Infectious Waste and Necropsy Waste

1. The pit placement should not be dug in wet (swampy) soil and should be at least 50 m from any water source or human habitation.
2. Wear PPE (gloves, masks, goggles and apron) when handling or moving a carcass for burial, and while burning and burying the waste.
3. Contain infectious necropsy waste and other infectious materials in sealed plastic bags. Spray the exterior of the bags with disinfectant prior to handling or moving to the burn-burial pit.
4. Dig a hole, generally at least 1.5 m to 2 m deep-- enough to allow the waste to be covered with at least 1 meter of soil. Place wood fuel in the pit prior to placing the waste bags in the pit. (See the Illustration below for dimensions of a burn-burial pit).
5. Pour a cup of diesel fuel (gasoil) over the waste material and wood fuel and ignite carefully with a torch on a stick, while staying clear of the fire pit. (If burning

waste repeatedly at a base compound consider building a 220-liter (55-gallon) steel drum waste incinerator as specified by WHO-CDC. See Appendix I.)

6. The fire should be tended with a long stick to move burning contents to ensure all is burned. Fuel may need to be added to completely burn all waste.
7. Disinfect shovels and any other reusable equipment or containers used to move and bury the waste. Disinfect with 70% ethanol (metal items like the shovel) or 10% bleach (plastic or rubber items like boots).

Procedure for Burning and Burying Carcasses

- Place the animal material in a safe place, at least 100 meters from human settlements, and at least 50 m from any water source (stream, well, etc.).
- If diesel fuel (gasoil) is being used, place the carcass/parts in a shallow (10 cm) deep hole to help contain the burn.
- If using brush or wood as fuel, make sure that it is dry enough to burn easily.
- Typically a pyre is constructed, with the carcass/parts placed on top of a large pile of fuel.
- In either case, the carcass/parts should be burned until reduced to ashes.
- Shovel dirt over the remaining ashes to completely cover them.
- Disinfect shovels and any other reusable equipment or containers used to move and bury the waste. Disinfect, with 70% ethanol, metal items like the shovel or with 10% bleach for plastic or rubber items like boots.

Procedures for Burying Carcasses

- Wear PPE (gloves, masks, goggles and apron) when handling or moving a carcass for burial.
- The pit should not be dug in wet (swampy) soil and should be at least 50 m from any water source.
- Dig a pit, generally at least 1 m (and ideally 2 m) deep-- enough to allow the carcass to be covered with at least 60 cm of soil.
- Disinfect shovels and any other reusable equipment or containers used to move and bury the waste. Disinfect with 70% ethanol metal items like the shovel or with 10% bleach for plastic or rubber items like boots.

Option 3: Leave an Infectious Carcass Where it is Found

Leaving a carcass where it is found may be the best option when:

- a. The carcass is not near people or domestic animals.

b. The animal carcasses are large or numerous such that it is not safe or practical to move and bury them. For example, there may be numerous large animals during a disease outbreak. In this case, local authorities will decide how to deal with the carcasses.

c. Burial of the carcass presents an unacceptably high risk of infection to the handler. For example, burial of suspected Ebolavirus or anthrax-infected carcasses in a forest environment requires extensive manipulation of carcass and tools, posing significant risk of breaching PPE.

Considerations for leaving infectious carcasses where they are found

The safest and most practical option for handling an infectious animal carcass or numerous carcasses may be to leave them where they are found. If the decision is made to not move a carcass that may be infected with Ebola, anthrax or some other dangerous pathogen, local public health and animal health officials should be notified of the location and suspected infectious risk of the carcasses.

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APPENDIX I. CDC-WHO Guide for Building a Waste Incinerator from a 220-Liter Barrel.

Steps for Building an Incinerator



- 1 Find a 220-litre (55-gallon) drum.



- 2 Cut open the drum. Remove and save the top cutaway piece.

- 3 Hammer the edges of the drum so they are not sharp.



- 4 Cut 3 half-moon openings just above the top end of the drum.

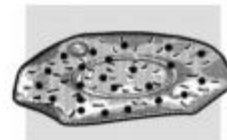


- 5 Turn the drum upside down. The bottom of the drum now is the top.

- 6 Cut 4 holes on the sides of the drum. Thread 2 metal rods through these holes so that they cross inside the drum.



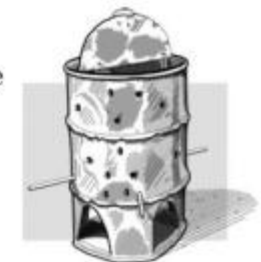
- 7 Punch holes in the top cutaway piece to make a platform.



- 8 Pierce a series of holes on the side of the drum and above the crossed rods to improve the draw of the fire.



- 9 Cut away half of the top. Attach the wire loops to the cutaway half to make a trap door. Attach another loop for a handle to open the trap door.



- 10 Place the platform inside the drum on top of the rods.



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 (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 NHP.

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, 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:

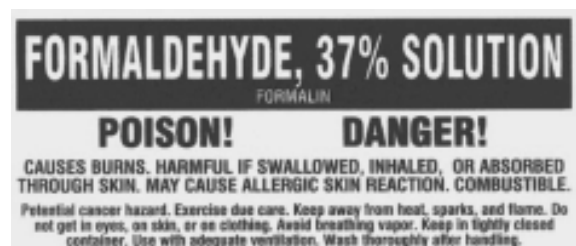
1. Monitor the condition of the exposed worker. Take a measured temperature two times per day.
2. If a fever occurs -- temperature is 38.5°C (101°F) or higher -- the worker should not do any work activities and should seek immediate medical attention. Treat as a suspected case of VHF if the worker's signs and symptoms meet the case definition.

APPENDIX IV. MATERIALS GLOSSARY

PREDICT sampling protocols use a number of different types of collection tubes or vials and transport/storage media. All personnel need to be familiar with these supplies and materials in order to properly and safely collect samples.

- **Viral transport media (VTM)**- is a material designed to preserve viruses for later viable recovery. The media is usually a gel sold in a collection tube (sometimes with a swab) and contains a number of ingredients for sustaining viruses, preventing bacterial contamination, and ensuring viability through freezing and thawing. VTM has a *limited shelf life* and must be checked for expiration dates and needs to be stored according to specific directions, *typically refrigerated before and after use* and can be frozen for long term storage at -70° C or colder. Collected samples in VTM should always be considered infectious and as biohazards.
- **Lysis buffer**- is a generic term for a solution that is used to preserve nucleic acids (DNA and RNA). The name 'lysis' is based on the ingredients that lyse bacterial, viral, or other cell walls in order to expose the nucleic acids (NAs). There is a wide range of lysis buffer products and each has different properties, handling hazards, and storage requirements that all personnel should know before handling specific products. Some of these products can be very expensive.
- **Ethanol**- Ethyl alcohol or ethanol has many uses, including storage of biological specimens. Strong ethanol (>65% volume/volume solution in water) is a cheap and effective disinfectant because it denatures proteins and dissolves lipids thereby killing most bacteria, viruses and fungi. At higher concentrations (>90% v/v) ethanol can also be a good preservative for nucleic acids, though the long-term storage of unpurified nucleic acids in ethanol is not advised.
- **Formalin**- Formaldehyde is a gas that is called formalin when dissolved in solution. Like ethanol, formalin has many uses and is an effective disinfectant, but it also can be toxic, allergenic, and carcinogenic and its use is restricted in many countries. It is commonly used as a buffered 10% solution for tissue storage because of its properties in 'fixing' or preserving tissues for various analyses.

A fully saturated formalin solution ("full strength") is about 37 gms formaldehyde / 100 mls of water, typically expressed as 37% w/v (or 40% v/v). For tissue preparation we typically use what is referred to as "10% formalin" (i.e., a 1:9



dilution of full strength), which is actually only a 4% solution of formaldehyde and is also buffered with sodium phosphate. If you have to mix/dilute it yourself, remember that what is often labeled as 40% “formalin” is actually 40% *formaldehyde* and is therefore 100% formalin that needs to be diluted 1:9 not 1:3.

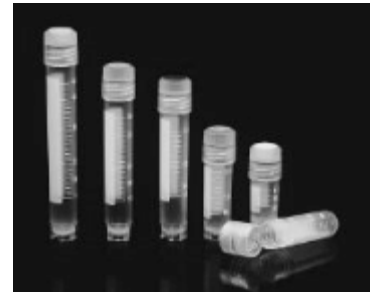
Key point: 1 part full strength formalin (40% formaldehyde)/9 parts water = 10% formalin for tissues (and should be buffered or switched to 10% buffered formalin ASAP).

A recipe for preparing 10% buffered formalin (there are others)

Formaldehyde, 37-40%	100 ml
Distilled water	900 ml
*Sodium phosphate, monobasic	4.0 gm
*Sodium phosphate, dibasic	6.5 gm
Total	1.0 liter

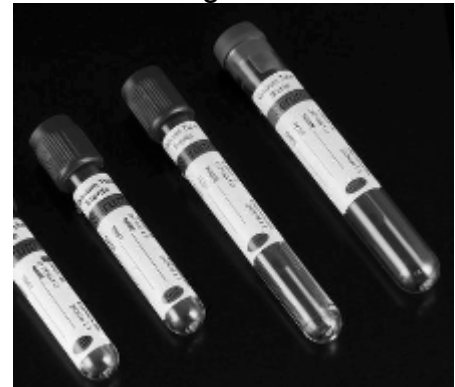
**if above not available can substitute 4.0 gm sodium chloride (table salt)*

- **Cryovials-** This name refers to plastic, internally threaded screw-top vials with a silicon O-ring to prevent leakage. They are usually small (1-10 ml) and made with certain plastics that can be safely stored in ultra cold (-80° F) conditions. NUNC™ or Corning™ brands are recommended.

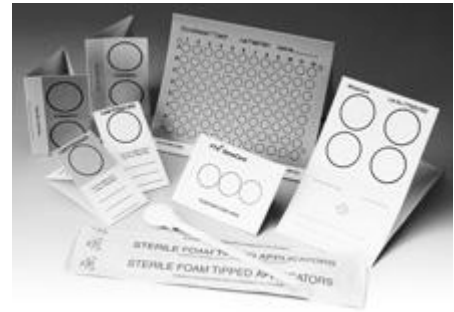


- **Blood or Vacutainer® tube-** These are sealed, internally sterile and usually negatively pressured (i.e., vacuum) tubes designed for collecting blood (but also used for other samples). They can be made from certain plastics but are usually glass and have rubber or plastic, colored caps that are coded to indicate what preservatives or other media they contain, if any. For PREDICT sampling, staff should be familiar with the following tubes:

- *Red top:* either empty or containing separator gel and clot activator. Though screw capped vials are better, empty red top tubes can be used for urine, parasites, biopsies, etc, but tubes containing gel should not be used for anything other than whole blood that needs serum separated.
- *“Tiger” top* (marbled red and black) or *Gold top:* contain gel and clot activator; used for separating serum.
- *Purple/lavender top:* contain EDTA to *prevent* clotting; used for blood counts and can be used for blood smears; not currently necessary for PREDICT sampling. Note: when spun, the clear supernatant contains platelets and is therefore plasma not serum, which should be noted.



- **Dried Blood Spots-** A variety of products/filter papers are available for collecting dried blood spots in addition to the Whatman 903 cards specifically mentioned. Many of these products are sufficient for PREDICT sampling, which aims to preserve dried blood spots mainly for recovery of pathogen DNA and RNA. Because some products are designed for toxicology, pharmacology, protein analysis, and preservation of molecules other than nucleic acids, PREDICT partners are advised to ensure that the products they use are appropriate for DNA and RNA preservation.



- **Respirators-** Disposable *surgical masks* and disposable *mechanical/particulate respirators* are traditionally not the same devices. Surgical masks are typically made of paper and are designed mostly to prevent the mask wearer from contaminating their patients. Mechanical respirators (MRs) are usually made of filtering materials more complex than paper and are designed mostly to prevent the wearer from inhaling particulate matter (chemicals, gases, toxins, infectious agents, etc.). In the US the National Institute for Occupational Safety and Health (NIOSH) certifies MRs that are oil aerosol proof (P), resistant (R), or non-resistant (N) and able to filter out at least 95, 99, or 99.97 (referred to as “100”) percent of airborne particles greater than 0.3 microns. ‘N95’ is therefore the minimum standard for NIOSH certification (i.e., R95 or P95 are at least as effective as N95 and also usually more expensive) but is certified unlike a standard surgical mask. There are now many devices marketed as surgical masks that are in fact N95 certified respirators

Standard Surgical Masks



N95 Respirators

