OBJECTIVES: To collect biological samples from bushmeat (meat of wildlife, fresh hunter-killed or in markets) for genetic identification, pathogen detection, and characterization of the wildlife trade. See separate the detailed PREDICT protocol for personal safety and protective equipment, and the PREDICT guide for surveillance data collection.

USAID Disclaimer
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APPENDIX. CDC-WHO GUIDE TO BUILDING A WASTE INCINERATOR FROM A 220 LITER BARREL.
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.


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. Collection of a voucher specimen may require 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 of a live animal 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 or permissions for bushmeat sampling, and sample acquisition and movement are received from the relevant governmental agencies/ministries and local community leaders (where appropriate) prior to commencement of fieldwork. As this process often takes time, it is recommended the relevant agencies/ministries be contacted well in advance of the anticipated sampling start date.

If surveys or interviews are conducted with bushmeat sample collection, each country team should ensure that all activities are consistent with relevant PREDICT IRB protocols and procedures.
SECTION 3. SAFETY & PERSONAL PROTECTIVE EQUIPMENT

Safety Responsibilities:

- All personnel are responsible for knowing the safety guidelines and procedures that are relevant to their tasks. Personnel involved in handling, or sampling bushmeat must understand and be able to apply the safety guidelines below and in the PREDICT Safety Guide: Biosafety and PPE Use.

- The primary investigator and/or sampling team leader should verify that the field team members have had the appropriate safety training for the tasks to be performed.

- Each country team should ensure that all bushmeat sampling activities are conducted in accordance with this protocol.

- All people handling bats or their blood products must be vaccinated for rabies.

- Field personnel must report to his or her supervisor and follow post-exposure guidelines for any injury from handling bats that may constitute an exposure to rabies.

PPE Use for Handling and Sampling Bushmeat

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

- Protect personnel from contamination or exposure to potential infectious agents; and;
- Prevent the contamination of persons at a site thereby preventing the transmission of potential infectious agents to other sampling locations, animals, and people.

Each country team should ensure that appropriate PPE is used. Use current biosafety recommendations to decide what level of personal protection is needed to prevent pathogen exposure of the field personnel, taking into account potential pathogens in the bushmeats being sampled (See PREDICT Safety Guide: Biosafety and PPE Use for details regarding PPE Use).

The minimum PPE for bushmeat sampling includes double gloves, protective glasses, and an N95 facemask for self-protection and to avoid contaminating samples. (See the PREDICT Safety Guide: Biosafety and PPE Use for detailed instructions regarding PPE Use).

Preparation for PPE Use in the Field:
PPE is an essential component of surveillance sampling for zoonotic pathogens. 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. Field sampling kits must also include supplies for disinfecting personnel on site and containing contaminated PPE for disposal.

**PPE Removal and Decontamination:**

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. After removing PPE, persons should use disinfectant wipes to disinfect hands and forearms.

All reusable 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.

\[
\text{Decontamination} = \text{cleaning} + \text{disinfection}
\]

Decontaminate:

1. Clean--remove organic material, dirt and grease.
2. Disinfect--using a disinfectant known to kill the likely pathogens.

Thorough cleaning and disinfection of reusable equipment are essential to protect personnel from pathogen exposure and to prevent the spread of pathogens to other wildlife or domestic animal populations.

Gloves, boots and other reusable materials can be disinfected by submersion in the disinfectant solution for 10 minutes. Before leaving gloves or boots to soak, blood, tissue and other materials should be washed from the surface with the disinfectant. Items may then be removed and air-dried for the next usage.

**First Aid 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 field. (Refer to first aid field manuals).
SECTION 4. DATA COLLECTION

Important considerations for sample data collection:

- Follow the *PREDICT Guide: Surveillance Data Collection* and complete the PREDICT data forms as instructed.

- Sample labels must have unique IDs. If a barcode labeling system is used, these labels should be printed in advance of sampling work.

- Data recording sheets should be designed to align with the larger PREDICT surveillance project. PREDICT required and optional data collection requirements are listed in the *PREDICT Guide: Surveillance Data Collection*.

- The entire sampling team should share the same understandings and definitions of terms used in the sheet (e.g. criteria for marking “poor” condition)

- The team should share understandings and definitions of how to describe contextual information about the sample collection, including the level of detail and common descriptive terms.

- Assign one team member the task of data recording.

Successful and consistent data recording is an essential component to disease surveillance. A data spreadsheet should be created well in advance with all required data fields included. The required data fields are listed in Table 1 below (Also see the *PREDICT Guide: Surveillance Data Collection*). The data recording sheet and sample labeling materials must be present during sampling, and updated after each sample taken (i.e., label each sample and record the corresponding data immediately after collecting each sample). As mentioned, it is ideal to assign the task of data recording to one team member to ensure accountability and that this key activity is completed accurately.
DATA TO COLLECT

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

Table 1. Data fields required for all specimens at all sites.

<table>
<thead>
<tr>
<th>Field Name</th>
<th>Explanation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Animal Classification</td>
<td>Class of the animal, e.g. Captive wild, wild, domestic, feral domestic</td>
</tr>
<tr>
<td>Animal ID</td>
<td>Unique ID of the Animal sampled</td>
</tr>
<tr>
<td>Condition At Sampling</td>
<td>The condition of the animal when sampling begins e.g. “apparently healthy”,</td>
</tr>
<tr>
<td></td>
<td>“injured” or “died (fresh carcass)”, etc.</td>
</tr>
<tr>
<td>Country</td>
<td>Country of collection site</td>
</tr>
<tr>
<td>Domestic Animals Present at Site</td>
<td>List of all domestic animals present at the site</td>
</tr>
<tr>
<td>Identification by</td>
<td>Who identified wildlife</td>
</tr>
<tr>
<td>Identification certainty</td>
<td>Level of certainty of wildlife identification</td>
</tr>
<tr>
<td>Laboratory storage method</td>
<td>The storage method used at the laboratory</td>
</tr>
<tr>
<td>Most Prominent Anthropogenic Change at Site</td>
<td>The most prominent anthropogenic change that has occurred in the last 6 months at the site e.g. “area with ecotourism” or “crop growing”, etc.</td>
</tr>
<tr>
<td>Primary Risk Interface</td>
<td>Describes the potential for human or domestic animal contact at the situation in which animals were handled or sampled.</td>
</tr>
<tr>
<td>Reason for collection</td>
<td>Description of reason for collection e.g. “opportunistic”, “active surveillance”, etc.</td>
</tr>
<tr>
<td>Recorder affiliation</td>
<td>Organization of person collecting data</td>
</tr>
<tr>
<td>Recorder ID (Name or email)</td>
<td>Name or email of person collecting data</td>
</tr>
<tr>
<td>Site Latitude</td>
<td>In Decimal degrees</td>
</tr>
<tr>
<td>Site Longitude</td>
<td>In Decimal degrees</td>
</tr>
<tr>
<td>Site name or ID</td>
<td>Unique name of site where data was collected</td>
</tr>
<tr>
<td>Species English name</td>
<td>Common English name of animal per Kingdon, Francis (field guides) or IUCN</td>
</tr>
<tr>
<td>Species scientific name</td>
<td>Specific genus species name based on ITIS; if the complete genus species is unknown use the next known level of taxonomic classification e.g. genus, subfamily, family</td>
</tr>
<tr>
<td>Specimen ID</td>
<td>Unique ID that references a specimen</td>
</tr>
<tr>
<td>Specimen Type</td>
<td>Type of specimen used. If “tissue”, describe further.</td>
</tr>
<tr>
<td>Start date</td>
<td>Date the sampling event started</td>
</tr>
<tr>
<td>State/Province</td>
<td>State or Province of collection site, e.g. “Sabah” or “Kampot”</td>
</tr>
<tr>
<td>Storage Location Facility Name</td>
<td>Name of the facility where the specimen are located</td>
</tr>
<tr>
<td>Storage Location within Facility</td>
<td>Description of where the specimens are located within the facility</td>
</tr>
<tr>
<td>Storage Medium</td>
<td>The storage medium used e.g. “10% formalin” or “Viral transport media”, etc.</td>
</tr>
<tr>
<td>Taxa Group</td>
<td>Taxa group that animal belongs</td>
</tr>
</tbody>
</table>
Additional (optimal) data:
- Date of animal death
- Age class
- Sex
- Condition of specimen
- Origin of specimen (for traded bushmeat originating from other countries)
- Characterization of sample pool (i.e., number of pieces/animals); see Surveillance Data Collection guide for details
SECTION 5. BUSHMEAT SAMPLE COLLECTION

SAMPLES TO COLLECT (in order of importance)

1. Blood (if fresh carcass): plasma (frozen) and dried blood spots (Whatman SS903).

2. Swabs (if carcass fresh): 2 oral and 2 rectal swabs in VTM.

3. Tissue: 3 samples (0.5 cm x 0.5 cm) from each of the following organs: liver, lung, spleen, brain, heart, kidney, and intestine.
   a. One tissue sample frozen in lysis buffer (preferred for molecular analysis) or fresh frozen. (Laboratories recommend that organ tissue, if fresh, be fresh frozen at -80°C, particularly if it is to be used for pathogen discovery (high throughput sequencing), so that they can perform filtration on the sample and isolate viral nucleic acid or viral particles. If cold chain is a concern, lysis buffer will protect the sample if it should thaw (which is likely to happen) and the samples will be fine for any molecular technique other than high throughput sequencing).
   b. One tissue sample frozen in VTM (for viral isolation).
   c. One tissue in 10% neutral buffered formalin.

4. Tissue: 3 muscle samples (0.5 cm x 0.5 cm) each
   a. One fresh frozen or frozen in lysis buffer (preferred) – for molecular analysis
   b. One frozen in VTM (for viral isolation)
   c. One tissue sample in 95% ethanol (preferred for genetics, if species unknown) or fresh frozen.

Note: Depending on circumstance or freshness of a carcass, some of the above sample types will not be possible or reasonable (e.g., swabs from a dried carcass).
SECTION 6. BUSHMEAT SAMPLE COLLECTION METHODS
Sample Collection Technique for Researchers

(See Section 7 for sampling by hunters)

1. Affix pre-printed bar-scan labels with appropriate information pertaining to sample (unique sample ID, or bar code and/or date) on all vials.

2. **Wear appropriate PPE** according to species and pathogen-associated risk level (minimum PPE recommended for bushmeat sampling includes double gloves, protective glasses, and N95 face mask) for self-protection and to avoid contaminating samples.

3. Sample method:
   a. Use sterile, disposable sample collection utensils (tweezers/scalpels/needle and syringe) or wipe and flame with ethanol or isopropyl alcohol any metal instruments (e.g. scissors and tweezers) before collecting each sample type.
   b. **For plasma sample** (fresh carcass only):
      i. Label vacutainer and prop tube upright in tube holder.
      ii. Perform cardiac puncture (laterally between ribs or longitudinally under sternum) using 3 ml or 5 ml syringe and adequate (largest possible for size of species) size needle to reach heart and draw blood (e.g., 19g for larger animal).
      iii. While saving 1ml blood in syringe for blood spots, transfer blood from syringe to K3-EDTA vacutainer using blood transfer attachments or by disposing of the needle to the sharps box, and uncapping the vacutainer. If necessary to remove needle, recap the needle first using one handed recap technique (do not recap needle with cap in your hand) or use specially made needle disposal boxes. Do not contaminate outside of blood tube with blood. (If this occurs lightly clean outside of tube with ethanol-moistened gauze prior to moving on.) Place labeled vacutainer in rack in shade for up to 2 hours. Centrifuge blood samples and store plasma and buffy coat/red cells in separate cryovials and transfer to cryoboxes for storage in liquid nitrogen shipper/storage in the field (or transfer samples directly to liquid nitrogen dry shipper in field. In this case, once back in the lab or base camp, remove samples from dry shipper and place in cryoboxes and store immediately in -80 °C freezer or liquid nitrogen storage.) See instructions below on “Blood processing” for more details.
   c. **For dried blood spots (DBS)** (see also alternate instructions for samples taken by hunter):
i. Label paper envelope containing filter paper for DBS and sample data sheet with unique identifier/animal ID.

ii. Remove filter paper handling only the corner of the DBS paper.

iii. Slowly drip blood from syringe onto SS903 DBS paper avoiding clots. Tilt the filter paper if necessary to allow the blood spot to spread and soak evenly. Allow drips to fall at same rate as absorbed by paper (i.e., do not rapidly place large amounts of blood onto the circles). Complete one spot before starting the next. Create multiple (up to 5) spots on the paper in the preprinted circles ensuring the blood soaks right through the filter paper. Once complete, note the number of complete spots on the data sheet. Do not touch sample areas.

iv. Bend the filter paper to put a curve in the paper. Place the filter paper on the preprinted envelope labeled with the animal species, locality and date and keep in a safe place to allow the spots to completely dry, avoiding direct sunlight, fire, and moisture.

When blood spots are completely dry, place in pre-labeled paper envelope.

**DO NOT SEAL THE ENVELOPE STICKY STRIP.** (The samples will have to be checked and sampled for testing, so access to the envelope is important.)

v. Place envelope in a zip-lock plastic bag (e.g. http://www.royalbag.com/8_x_10_2_mil_clear_reclosable_bags.asp) with silica gel packs to protect from moisture/humidity.

vi. Dry samples can be grouped together (up to ~50) in zip-lock bags and bags can be placed in plastic sealable containers to reduce air penetration and kept at room temperature/air-conditioned space.

vii. When possible, transfer to long-term storage in bitran bags (leakproof poly zipper specimen bags) and store at -20C.

(An alternative method for very humid environments where blood spots take hours to dry is to place each individual incompletely dried 903 blood spot card into small plastic bag rather than directly into paper envelopes. The plastic bags are left open and placed in an airtight plastic box with loose desiccant crystals to finish drying the blood spot cards. After drying completely, the DBS cards are then place into labeled paper envelopes and handled as described above. Care must be taken to prevent desiccant crystals from moving from one plastic bag to another and cross contaminating samples.)

Blood spot cards must also be protected from insects such as ants and flies while they are drying. If insects are a problem, a
small food net dome or cover made from mosquito netting may be used to protect drying samples.

d. **For swabs (fresh carcass only):** Using sterile Dacron-tipped (aluminum or plastic shaft – not wooden) swabs, 2 oral and 2 rectal swabs are taken. Place one oral and one rectal swab in separate 2 ml cryovials filled with 1ml lysis buffer. Place one oral and one rectal swab in separate 2 ml cryovials with filled with 1 ml VTM. When being placed into the tubes, swab tips are cut (with ethanol-flamed scissors) on the shaft as close as possible to the swab tip without touching/contaminating it. Scissors should be wiped and flamed with ethanol or isopropyl alcohol between each sample. Sealed, labeled vials with samples are immediately stored in liquid nitrogen (dry shipper or dewar) until transferred to -80°C freezer.

e. **For muscle tissue:** Using a sterile scalpel blade, dissect beneath the exposed surface to take ~0.5 cm³ (small pea-sized) sample of muscle tissue ensuring no contamination from external environment/surface bacteria or fungus. Take muscle sample from most fresh area available (raw tissue preferable). Place sample in labeled 2 ml cryovial with 1 ml lysis buffer and recap. Repeat step above and place second sample in labeled 2 ml cryovial with 1 ml VTM. Repeat again and place third sample in labeled 2 ml cryovial with no media, or with 1 ml ethanol for genetic analysis (if species unknown). Sealed, labeled vials with samples in VTM and lysis buffer are immediately stored in liquid nitrogen (dry shipper or dewar) until transferred to -80°C freezer.

f. **For organ tissue:** Using sterile/clean scalpel blade, take 0.5cm cube of each organ tissue (see recommended list of organs above) ensuring no contamination from external environment. Organ samples should each be placed in individual, labeled cryovials. Duplicate samples for molecular analysis and viral isolation is ideal, but if not possible due to space, one set of samples stored frozen in VTM is preferred (unless sample is one of few designated for pathogen discovery techniques). Sealed, labeled vials with samples are immediately stored in liquid nitrogen (dry shipper or dewar) until transferred to -80°C freezer.

**Additional sampling considerations:**

In many bushmeat market or hunter-killed sampling situations, it may not be acceptable to traders for you to take organ samples. Remember that under PREDICT ethical guidelines, you CANNOT pay or trade anything for the samples. If allowed, intestinal/lymph node samples can often easily be obtained by inserting long hemostats into rectum and pulling out sample of colon tissue. If the animal is to be butchered, you may also ask the owner/hunter to cut small samples of liver, lung, small intestine, large intestine, spleen, and kidneys. From these hunter samples, collect a small part of each organ tissue (~0.5 cm cube) while maintaining sterility to the extent possible (i.e. avoiding surface of original hunter-taken tissue and asking the trader to clean her/his knife between cuttings of samples of various organs). Remember that (legal or not) bushmeat is intended for human consumption so, if
during sampling be very careful not to contaminate carcasses with hazardous chemicals (e.g. lysis buffer, formalin) or to touch bushmeat with potentially contaminated, gloved hands or non-sterile utensils.

The researcher must consider quality of specimens and the pathogens of interest when deciding whether or not to sample a carcass for pathogens. Tissue from animals that have been smoked, dried, or dead longer than 24 hours are much less likely to harbor live pathogens or detectable RNA viruses, and are more likely to contain contaminating agents and bacterial overgrowths. Other pathogens, such as DNA viruses, may be detectable in tissues for an extended period of time. Most types of tissue (including skin or hair) can be used for genetic analysis (species identification), even from specimens that are of lesser quality (dried, processed, etc.).

Nuclisens Lysis buffer is recommended for all sample collections. This buffer is optimal for both DNA and RNA viruses and is compatible with the equipment at the Center for Infection and Immunity at the School of Public Health at Columbia University. However, RNA later or alternative lysis buffers may be appropriate for diagnostics at local laboratories. It is a good idea to discuss preferred media type with the laboratory that will analyze your samples prior to collection.

Only cryovials with twist-on cap and o-ring should be used (i.e., vs. snap-on cap). Specialized cryolabels and cryomarkers are recommended over the alternatives.

Prevent fungal contamination of dried blood spots caused by excess moisture of sample paper. Fungal contamination occurs when specimens are not completely dried. It can be avoided by:

- Always keeping plastic storage bags properly sealed, ensuring no punctures or damage.
- Completely drying the blood spots prior to storage.
- Always using fresh silica gel. Reusing silica gel can be a source of cross-contamination of samples.
- Transferring to -20°C for long-term storage.
SECTION 7. BLOOD PROCESSING INSTRUCTIONS

1. Ensure proper PPE is worn by everyone working with blood sample (at minimum: gloves, eye protection, facemask).
2. Place labeled vacutainers in centrifuge ensuring balance in opposing tubes.
3. Centrifuge at 3000 rpm for 15 minutes.
4. Carefully remove vacutainers avoiding mixing plasma and red blood cells.
5. Label cryovials for plasma and buffycoat.
6. Open packet of sterile transfer pipettes at bulb end.
7. Avoid contamination of the pipette (do not touch tip and do not put pipette down).
8. Collect (aspirate) plasma, avoiding disturbance to the buffycoat and red blood cells.
9. Maintain pressure on pipette bulb to avoid air entering the pipette and formation of foam.
10. Transfer plasma to cryovial(s). Note volume of each aliquot or of single aliquot. (Because only small volumes are typical needed for diagnostic assays, plasma or serum should generally not be stored in aliquots larger than 1.0 ml in order to avoid repeated freezing and thawing.)
11. Collect buffy coat from surface of red blood cells and a small quantity of red blood cells.
12. Transfer to cryovial.
13. Collect the remaining red blood cells and transfer to a labeled cryovial.
14. Use a fresh, sterile pipette for each blood sample.
15. Note the samples collected in your data spreadsheet.

Required sample storage conditions:

- Keep plasma, swabs, tissues, other “frozen” samples for diagnostic analysis in cool, shaded area until frozen.
- Ideally, do not let samples sit more than 4 hours before freezing.
- Store frozen in liquid nitrogen in dry shipper or dewar until transfer to -80°C freezer.
- Do not allow samples to thaw once frozen.
- Blood spots should be stored in a dry place with low humidity at a cool or room temperature (or frozen at -20°C).
- Tissues in ethanol may be kept at room temperature.
Cleaning after handling samples:

1. Place all sharps in a sharps container.
2. Collect all waste and place in biohazard bag.
3. Clean all reusable metal equipment with 10% bleach and then 70 and 10% bleach for plastic items.

See Section 9 of this protocol for details regarding infectious waste disposal.
SECTION 8. HUNTER INSTRUCTIONS FOR COLLECTION OF BLOOD SPOTS

1. Find a clean source of slowly dripping blood (e.g., a meat cut that is dripping blood; try not to have a mix of blood and other body fluids).
2. Hold the filter paper flat, by the corner, between thumb and index finger.
3. Keeping hands clean and dry, start the left first circle of filter paper by allowing the blood to slowly drip from meat/carcass.
4. Tilt the filter paper if necessary to allow the blood spot to spread and soak evenly.
5. Create multiple spots of blood within the circles on the filter paper ensuring the blood soaks right through the filter paper. Complete one spot before starting the next.
6. Bend the filter paper to put a curve in the paper.
7. Place the filter paper on the preprinted paper envelope and keep in a safe place to allow the spots to completely dry, avoiding direct sunlight and fire.
8. When blood spots are completely dry, place in pre-labeled envelope. **DO NOT SEAL THE ENVELOPE. (The samples will have to be checked and sampled for testing, so access to the envelope is important.)**
9. Put the preprinted envelope inside provided plastic bag with silica gel packet inside. (The purpose of the silica gel pack is to help absorb moisture.)
10. Press all the air out of sac and seal the bag (if available place plastic bag in airtight plastic box). Keep the clip lock plastic sac in a dry, safe place, out of reach of children.

For very humid environments see alternative method of drying blood spot cards in open plastic bags, as described under Section 6.

During follow-up visit by research staff should:
1. Ask hunters about any problems they may have encountered with the sampling process or the sampling materials.
2. Take the opportunity to reinforce the training hunters received from your team. Address any issues or confusion immediately. Record, in writing for the Country Coordinators convenience, any problems or safety issues encountered and explain how these issues were addressed.
3. Collect samples from collectors.
4. Place samples in new undamaged plastic/clip-lock bags.
5. Compile list of collected samples and verify local names of sampled animals prior to departure from site.
6. Change silica gel and check that no fungus is growing on the blood spots (white spots indicate fungus). Any samples with fungal growth should be kept separately from uncontaminated collection.
7. Check quality of samples – On each piece of SS903 filter paper there are five printed circles. Record a score for each filter paper from 0-5 for the number of
printed circles with complete blood spots on the SS903 filter paper. Record on data sheets.

Dry samples can be grouped together (up to ~50) in bitran bags.

For long-term storage, transfer to bitran bags and store at -20C. Long-term storage is when the samples will only be removed from the freezer for testing. Ice after arrival in final storage laboratory. This helps avoid major temperature variations that might affect samples.
SECTION 9. DISPOSAL OF BUSHMEAT 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 sampling 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 and transport the material to a health institution such as a medical or veterinary facility that has the capacity to autoclave and/or incinerate the waste, or has a safe disposal site. There are exceptional circumstances where 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 or an animal could dig up and recover the meat to eat. Ultimately the field supervisor must determine the best method for disposal based on the guidance provided below, their judgment and resources available.

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.

Criteria for choosing to deliver infectious waste to a health facility for disposal

This is generally the best option when:

- The volume of infectious waste is limited so the number of plastic bags or containers 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).
- The waste can be delivered to the health facility for disposal within a couple of
days. The longer waste is temporarily stored the greater the risk that the containment bags or containers will be breached and exposure of humans and animals may occur.

- The local health facility has agreed to dispose of the waste and is expecting your delivery of infectious waste.
- 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.

**Delivering Infectious Waste to a Health Facility for Disposal**

The preferred option for disposal of infectious field sampling materials and necropsy waste of infected carcasses is to contain the waste and deliver it to a health facility that maintains a safe disposal system. This option requires the following tasks:

1. Secure an agreement with the facility to accept waste. Such an agreement includes the costs, delivery times, and infectious waste containment requirements.
2. Prepare sampling supplies, to include the required PPE, disinfection and containment materials necessary to safely contain and transport waste. This will include masks, gloves, coveralls, sharps containers, sturdy plastic bags and ties, disinfectant spray, buckets with tight fitting lids, and/or liquid waste containers as needed.
3. 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.
4. 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.
5. 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.
6. Disinfect the transport vehicle immediately after each delivery.

**Option 2: Field Disposal—Burning and burial of infectious waste near where the waste is generated.**

Under certain circumstances, field disposal may be the best (safest and most practical) option for disposing of carcasses, necropsy waste and other infectious materials. For example, moving infectious waste always adds the risk of spreading the disease to other areas. For field disposal of infectious waste, PREDICT
recommends the combination of both burning and burial. 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 just burning and burial, burial, 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 a couple days.
- 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.
- Accessibility of site by the vehicle used to move the waste.
- Features of the soil (easy to dig), little slope.
- Depth of groundwater—groundwater should be at least 1.5 meters below the bottom of the pit.
- At least 50 meters from water-catchments, bore holes and wells.
- Proximity to livestock, poultry or dogs.
- Presence of wildlife that may dig up the material.
- Likelihood that humans may dig up the material.
- Subsequent plans for use of the area.
- If fencing will be required to exclude animals.

Procedures for burning and burying infectious waste and necropsy waste:

1. Hole placement should not be in wet (swampy) soil and should be at least 50m 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 earth. 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 the Appendix.)

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).

Procedures for burning and burying carcasses:

1. 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.).
2. If diesel fuel (gasoil) is being used, place the carcass/parts in a shallow (10 cm) deep hole to help contain the burn.
3. If using brush or wood as fuel, make sure that it’s dry enough to burn easily and burn hot.
4. Typically a ‘pyre’ is constructed, with the carcass/parts placed on top of a large pile of fuel.
5. In either case, the carcass/parts should be reduced to ashes.
6. Shovel dirt over the remaining ashes to completely cover them.
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).

Procedures for burying carcasses:

1. Wear PPE (gloves, masks, goggles and apron) when handling or moving a carcass for burial.
2. Hole placement should not be in wet (swampy) soil and should be at least 50m from any water source.
3. Dig a hole, generally at least 1 m (and ideally 2 m) deep—enough to allow the carcass to be covered with at least 60 cm of earth.
4. Carcass/parts should be covered with 1/2 - 1 inch (1.5-2.5 cm) of lye before being covered with packed earth.
5. 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).

Option 3: Leave an infectious carcass where it is found

Leaving a carcass where it is found may be the best option when:
• The carcass is not near people or domestic animals.

• 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.

Considerations to leave 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 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.

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, 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 ¾ 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 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.
SECTION 10. REFERENCES


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.