Section 5.2.12. Bushmeat Sampling Methods

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Objective: Guidance on how to collect biological samples from hunted wildlife and wildlife byproducts in the context of the PREDICT project.

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

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Section 5.2.12a. Confirmation of Knowledge

When you are familiar with the information in this guide, take the PREDICT quiz ***Section 8.4.11 Bushmeat Sampling***.

Section 5.2.12b. Brief Overview of PPE

Minimum PPE Required for Bushmeat Sampling

The minimum PPE for sampling small carnivores includes:

- Double gloves
- Protective glasses
- N95 facemask for self-protection and to avoid contaminating samples

***Note: Wear appropriate PPE** according to species and pathogen-associated risk level.*

(See the ***Biosafety and PPE Guide (Section 4.)*** for detailed instructions regarding PPE Use)

Section 5.2.12c. Bushmeat Sample Collection

Samples to Collect

Duplicate specimens are to be collected from each animal (if feasible). If only one sample can be collected, then place into VTM.

1) Blood
   a) **Fresh kill**: collect whole blood and serum.
      i) **Whole blood: Collect as much blood as possible. Cardiac puncture is recommended.** Collect whole blood into 1 lavender top tube containing EDTA. Transfer a max of 500 µL of whole blood to cryovial containing 500 µL VTM and another 500 µL of whole blood to cryovial containing 500 µL Trizol. Freeze in liquid nitrogen or -80°C freezer.
      ii) **Serum**: collect blood in at least 1 serum separator tube. Allow blood to clot and store a minimum of 2 x 0.5mL serum aliquots, frozen without media.

Note: If blood volume recovered is too small to collect two blood tubes (for whole blood and serum), collect serum and save remaining clot in 500 µL VTM after serum separation.

   b) **Carcass**: collect blood clot. Place in at least one cryovial containing 500 µL VTM, and freeze in liquid nitrogen or -80°C freezer.
2) **Swabs** (if fresh kill – x2 of each swab type): Collect 2 oral and 2 rectal swabs placing 1 of each sample into 500 µL VTM and Trizol, respectively. Rectal swabs can be moistened with sterile saline prior to animal sampling.

3) **Tissue:** Collect three, adjacent, approximately 200mg (pea-sized) samples from each of the following organs:

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

One specimen should be frozen in 500 µL VTM in a cryovial, one should be frozen in 1 mL Trizol in a cryovial, and one should be stored at room temperature in a small vial or jar in 10% buffered formalin at a volume of fixative 10 times the volume of the tissue (once fixed, the tissue may be transferred to a smaller volume for shipment).

Freeze all samples (except tissue in formalin) in liquid nitrogen immediately in the field and transfer to -80°C freezer once back in the lab.

If there is no **short-term** access (i.e., within 24 hours) to cold chain such as in an emergency situation then samples can be collected in 500 µl of RNAlater instead of Trizol and VTM. Storage times and temperatures for samples in RNAlater are as follows:

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

Section 5.2.12d. Bushmeat Sample Collection Methods

Sample Collection Technique

1. Ensure all sample collection tubes or vials are pre-labeled with appropriate information pertaining to sample ID (unique sample ID, or barcode and/or date).
2. **Wear appropriate PPE** according to species and pathogen-associated risk level (see above for minimum requirements).
3. Sample methods:
    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 whole blood and serum** (fresh kill only):
        i. Label vacutainer and prop tube upright in tube holder.
        ii. If possible, 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) without opening the carcass. Alternatively, open thoracic cavity to reach the heart.

iii. Transfer blood (retaining ~1 ml in the syringe if volume permits) from syringe to a serum separator or red top vacutainer by disposing of the needle to the sharps box, and uncapping the vacutainer. 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 before following instructions below on "Blood clot".

iv. Transfer up to 500 µL of the blood remaining in the syringe to a cryotube with 500 µL of VTM and an additional 500 µL of remaining blood to a cryotube with 500 µL of Trizol (maximum final ratio of 1:1 in both cases).

c. **For blood clot** (carcass where collection of whole blood is not feasible):
   i. Using a sterile scalpel blade or forceps, collect blood clot ensuring no contamination from the external environment. Blood clot should be placed directly into 500 µL VTM and frozen.

d. **For swabs (fresh kill only):** Using sterile polyester or Dacron-tipped (aluminum or plastic shaft – not wooden) swabs, collect 2 oral and 2 rectal swabs. Rectal swabs can be moistened with sterile saline prior to animal sampling. Place one oral and one rectal swab in separate cryovials filled with 500 µL VTM. Place one oral and one rectal swab in separate 2 ml cryovials with filled with 500 µL Trizol. After placement into the tubes, cut swab tips (with ethanol-flamed scissors) on the shaft as close to the swab tip without touching/contaminating it. Scissors should be wiped with ethanol or isopropyl alcohol and flamed between each sample. Alternatively, snap swab shafts above the tip. After closing tubes, mix each tube well. Sealed, labeled vials with samples are to be immediately stored in liquid nitrogen (dry shipper or dewar) until transfer to -80˚C freezer.

e. **For muscle tissue:** Using a sterile scalpel blade, dissect beneath the exposed surface to take three ~0.5 cm^3 (small pea-sized) samples of muscle tissue ensuring no contamination. Take muscle samples from most fresh area available (raw tissue preferable). Place one sample in a labeled cryovial with 500 µL VTM and recap, and place another in a labeled cryovial with 1 mL Trizol and recap. Store immediately in liquid nitrogen (dry shipper or dewar) until transfer to -80˚C freezer. Place third sample in labeled jar or vial with 10% buffered formalin at a volume of fixative 10 times the volume of the tissue, and store at room temperature.

f. **For organ tissue:** Using sterile/clean scalpel blade, take three 0.5 cm^3 samples 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. Place one sample in a cryovial with 500 µL VTM and another sample in a cryovial with 1 mL Trizol, and freeze samples at -80°C (or liquid nitrogen in the field). Place third tissue sample in a labeled jar or vial with 10%

neutral buffered formalin at a volume of fixative 10 times the volume of the tissue, and store at room temperature.

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 a 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^3) 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, during sampling be very careful not to contaminate carcasses with hazardous chemicals (e.g., Trizol or 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.).

Required sample storage conditions:

- Store all collected specimens immediately in liquid nitrogen or -80 °C freezer.
- Keep all samples frozen in liquid nitrogen in a dry shipper or dewar until transfer to -80˚C freezer for long-term storage.
- Do not allow samples to thaw once frozen.
- Tissues in buffered formalin must be kept at room temperature.

Section 5.2.12e. References

CDC and WHO (Centers for Disease Control and Prevention and World Health Organization). 1998. Infection Control for Viral Haemorrhagic Fevers in the African Health Care Setting. Atlanta, Centers for Disease Control and Prevention, pp. 198.

WCS Field Veterinary Program 2006. Bushmeat-Handling Protocol for Ecoguards, Congo-Gabon.