

# BUSHMEAT SAMPLING METHODS

**Adapted by OHW-NG\* from the EEIDI\*\* and PREDICT\*\*\* Standard Operating Procedures (SOPs) developed by (in alphabetical order):** Mathew LeBreton, PREDICT Consortium, Tierra Smiley Evans, Kristine Smith, Benard Ssebide, Marcela Uhart, David Wolking

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

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

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## 1. Overview of PPE

Due to the COVID-19 pandemic, One Health workers who are actively working with wildlife must recognize the potential for exposure to both known and novel zoonotic pathogens during animal capture, handling, and sampling activities. These risks can be mitigated through strict adherence to biosafety and biosecurity practices detailed below and in additional reference materials listed in this protocol.

These documents are in no way a substitute for active hands-on training from experts in the field. We highly encourage persons seeking to do this type of research work with wildlife to seek out in-person training opportunities and guidance before initiating any hands-on work with wildlife.

In addition to vaccination guidelines during times of COVID-19 transmission, field sampling teams should follow all relevant national recommendations to maintain best practices to reduce virus transmission among local communities including physical distancing, face masks, and handwashing (or sanitizer) when engaging with community members and stakeholders at locations of animal capture and sampling.

The **minimum** PPE for sampling bushmeat includes:

- Double nitrile gloves
- Protective goggles
- N95 facemask without exhalation valve for self-protection and to avoid contaminating samples
- Dedicated clothing

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

*See the **Biosafety and PPE SOP** for detailed instructions regarding PPE Use.*

## 3.2. Bushmeat Sample Collection

### Overview of Samples to Collect

*Duplicate specimens are to be collected from each animal, if feasible. If only one sample can be collected, particularly in fresh kills, then place it into VTM.*

#### 1) **Blood**

a) **Fresh kill:** (listed in order of priority if small blood volumes are available)

i) **Whole blood micro-samples:** collect 2 microsamples using [HemaTIP™](#) collection



tips (Charles River, Inc) and / or [Nobuto strips](#) (Sterlitech, Inc) depending on availability. These can be collected from whole blood samples obtained as described below or from surface blood when cardiac / venipuncture is not possible. Store at room temperature in plastic clamshells provided or inside a ziploc bag with an appropriate desiccant (e.g., desiccant packet or rice).

- ii) **Whole blood on FTA Whatman cards:** collect 2 blood spots. Can be collected from whole blood samples obtained as described below or surface blood when cardiac / venipuncture is not possible. Air dry and store at room temperature in a ziploc bag with an appropriate desiccant (e.g., desiccant packet or rice).
  - iii) **Serum:** collect blood in one serum separator tube (tube size dependent on animal size). Allow blood to clot; if possible, centrifuge to maximize serum extraction. Store a minimum of 2 x 0.5mL serum aliquots (maximum of 6), frozen at -80°C without media.
  - iv) **Whole blood:** collect whole blood into one 2 ml lavender top tubes containing EDTA; cardiac puncture is recommended. Transfer a maximum of 100 µL of whole blood to a cryovial containing 500 µL VTM and another 100 µL of whole blood to a cryovial containing 500 µL MagMAX lysis buffer. Collect duplicates of each sample type as volume allows (maximum of 3 VTM and 3 MagMAX samples). Freeze in liquid nitrogen or -80°C freezer.
- 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:** collect 2 oral, 2 nasopharyngeal, and 2 rectal swabs, placing 1 of each sample type into 500 µL VTM and 500 µL MagMAX lysis buffer. If fresh kill, take duplicates of each swab type. Freeze in liquid nitrogen or -80°C freezer.

**Tissue:** Collect two, adjacent, approximately 200mg (pea-sized) samples from each of the following organs. Place one of each sample type in 500 µL MagMax Lysis buffer and 500 µL VTM.

- Liver
- Spleen
- Lung
- Lymph node (including retropharyngeal if possible)
- Muscle (if needed for species identification)

## Specimen Collection Techniques

Ensure all sample collection tubes or vials are labeled with appropriate information pertaining to sample ID (unique sample ID, or barcode and/or date).

**Wear appropriate PPE** according to species and pathogen-associated risk level (see above for minimum requirements).

As applicable, 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.,

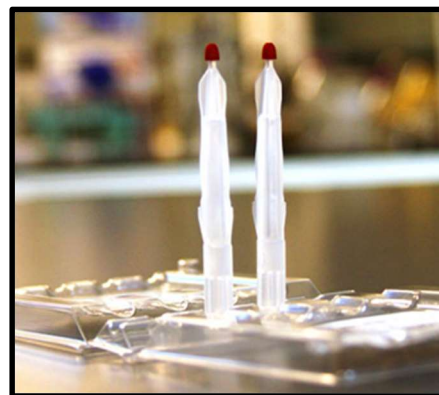


scissors and tweezers) before collecting each sample type.

## 1) Blood

### a) For whole blood and serum – syringe collection (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 from syringe to a serum separator or red top vacutainer and a lavender top EDTA tube if there is enough volume 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 red top vacutainer in rack in shade for up to 2 hours before following instructions below on “Blood clot”.
- iv) Transfer 100  $\mu$ L from the lavender EDTA tube to a cryotube with 500  $\mu$ L of VTM and an additional 100  $\mu$ L of blood to a cryotube with 500  $\mu$ L of MagMAX lysis buffer.



### b) For whole blood – micro-sample collection (fresh kill only):

- i) Identify source of blood from dead wildlife, prioritizing locations that do not have dirt, leaves or other materials on them. If the animal is being butchered, prioritize an internal blood source (i.e., heart) as opposed to cuts in the skin. If a cardiac stick is being performed to collect blood, this would be the ideal source of blood.
- ii) Take a micro-sampling tip (HemaTIP™, Charles River Laboratories or Nobuto strip, Sterlitech) and touch it to the blood source until the tip turns completely red (do not submerge in blood), following manufacturer’s protocols. This should take approximately 3-6 seconds and no blood should be dripping, once fully saturated. See protocols in Appendix 1.
- iii) Collect 2 micro-sampling tips or Nobuto strips per animal.
- iv) Place micro-sampling tips into the clamshell or designated non-sealed tube.
- v) Place clamshell (or tubes) in Ziploc bag with a desiccant packet. The goal is for these samples to be dry and have air circulating with the desiccant packet.
- vi) Air-dry and place Nobuto strips in a Ziploc bag or paper envelope with

dessicant.

- c) **FTA Whatman Cards:**
- i) pipette 125  $\mu$ L of whole blood directly onto the center of each circle on the card.
  - ii) Air dry cards then place inside a ziploc bag with an appropriate desiccant (e.g., desiccant packet or rice)
- d) **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  $\mu$ L VTM and frozen.
- 2) **For swabs (fresh kill only):** Using sterile polyester or Dacron-tipped (plastic shaft – not wooden) swabs, collect 2 oropharyngeal / respiratory and 2 rectal swabs. For the oropharyngeal / respiratory swab, place the swab as far down the respiratory tract as is feasible (ideally extending down the trachea). Place one oral and one rectal swab in separate cryovials filled with 500  $\mu$ L VTM. Place one oral and one rectal swab in separate 2 ml cryovials filled with 500  $\mu$ L MagMAX lysis buffer. 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  $-80^{\circ}\text{C}$  freezer.
- 3) **For muscle tissue:** Using a sterile scalpel blade, dissect beneath the exposed surface to take three  $\sim 200$  mg (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  $\mu$ L VTM and recap, and place another in a labeled cryovial with 500  $\mu$ L MagMAX lysis buffer and recap. Store immediately in liquid nitrogen (dry shipper or dewar) until transfer to  $-80^{\circ}\text{C}$  freezer. Place the third sample in 95% ethanol for genetics. Store at room temperature.
- 4) **For organ tissue:** Using sterile/clean scalpel blade, take two  $\sim 200$  mg (pea sized) 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  $\mu$ L VTM and another sample in a cryovial with 500  $\mu$ L MagMAX lysis buffer and freeze samples at  $-80^{\circ}\text{C}$  (or liquid nitrogen in the field).

## Sample Preservation and Storage

- **Micro-samples and FTA Whatman cards** can be stored at room temperature in a ziploc bag with an appropriate desiccant (e.g., desiccant packet or rice) in a cool dark place. For long-term storage, transfer to a  $-20^{\circ}\text{C}$  freezer if possible.

- **All other specimens** should be frozen in liquid nitrogen immediately in the field, stored in a dry shipper, dewar, or on dry ice until they can be transferred to -80°C freezer once back in the lab. Alternately, they may be stored long-term in a liquid nitrogen storage tank.

## Additional Sampling Considerations

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.).

*For ethical reasons it is recommended that there be no payment or incentive for wildlife samples.*

In many bushmeat market or hunter-killed sampling situations, it may not be acceptable to traders for you to take organ 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, and spleen, the easiest and cleanest organs to sample. From these hunter samples, collect a small part of each organ tissue (~200 mg) 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**. During sampling, be very careful not to contaminate carcasses with hazardous chemicals (e.g., lysis buffer) or to touch bushmeat with potentially contaminated gloved hands or non-sterile utensils.

### **4.3. 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.



## A. Appendix 1: Micro-sampling Procedures

### Charles River HemaTIP™ Microsampler

[www.criver.com/sites/default/files/resources/HemaTIP™Instructions.pdf](http://www.criver.com/sites/default/files/resources/HemaTIP™Instructions.pdf)

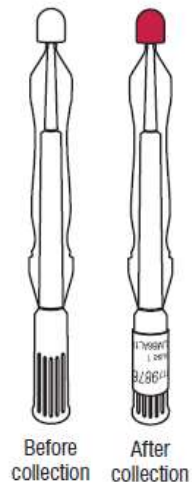
Instructional video at: <https://www.criver.com/products-services/research-models-services/animal-health-surveillance/serology-services/hematip-microsampler?region=3701>

Per manufacturer's website:

"With HemaTIP™, the collection media is the tip of an easy-to-hold stylus, simplifying the blood collection process. Once the sample is ready to be collected, the tip need only touch the blood and its super absorptive matrix media wicks the sample in approximately 3-6 seconds."

- 1) Label each HemaTIP™ around the base, overlapping the label to secure it in place.
- 2) Touch HemaTIP to blood until tip is completely saturated (red). Do not submerge in blood.
- 3) Place HemaTIP upright in clamshell case to dry for 1 hour at ambient temperature.
- 4) Following sample collection, HemaTIP can be stored refrigerated at 4 °C for up to 6 months.

charles river



### Sterlitech Nobuto Filter Strips

[www.sterlitech.com/blog/post/nobuto-filter-strips-for-studying-wild-animal-populations](http://www.sterlitech.com/blog/post/nobuto-filter-strips-for-studying-wild-animal-populations)

Per manufacturer's website:

"Made from high-purity cellulose fibers, Nobuto filter strips are structured for uniform sampling. Each strip holds approximately 0.1 mL of blood or 0.04 mL of serum. Once blood is applied and the strip has dried, researchers can analyze, store, or mail the samples without refrigeration."

Downstream testing for antibodies (i.e., ELISA) is employed to diagnose disease. They have proven a valuable tool in surveillance of avian influenza viruses, Newcastle disease virus (NDV), and West Nile virus (WNV) in wild birds. The strips can also be used in environmental studies, enabling researchers to monitor wild animals for heavy metal and toxin levels. Studies have shown that these filter strips are easy to use and enable delayed analysis of blood samples, enabling improved tracking of diseases within wild animal populations.

