Section 5.2.7. Bat Sampling Methods

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Objective: To safely collect biological samples from bats.

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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 5.2.7a. Confirmation of Knowledge

When you are familiar with the information in this Guide, take the PREDICT quiz ***Section 8.4.6. Bat Sampling***.

Section 5.2.7b. Brief Overview of PPE

All staff handling bats or their blood products should be vaccinated for rabies. If possible, ensure that they have a protective antibody titer, and be prepared to institute appropriate post exposure prophylaxis measures in the event that a bat bite or scratch occurs. It is recommended that personnel with frequent bat contact follow CDC and WHO guidelines of checking titers every two years (www.who.int/rabies/WHO_Guide_Rabies_Pre_Post_Exposure_Prophylaxis_Humans_2013.pdf?ua=1).

Minimum PPE required for handling live bats

The minimum PPE for handling bats during capture and sampling includes:

1. Eye protection
2. N95 or P100 respirator
3. Designated long-sleeved clothing
4. Nitrile gloves[1]
5. Washable shoes

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

First aid protocol for a bite, scratch, or needlestick

1. The injured person must notify other research staff and work must stop.
2. The bite, scratch, or needlestick site should be washed well with water and betadine (povidone-iodine) or benzalkonium chloride (this is known to kill rabies virus) for a full 15 minutes. It is recommended that benzalkonium chloride be kept readily available in a first aid kit for such purposes.
3. If the injury (bite or scratch) is from a bat, the post-exposure rabies vaccination should be obtained as soon as possible. It is recommended that the field team develop a post-exposure vaccination plan with their physician prior to fieldwork if working in a remote location so that a booster dose can be administered soon after exposure. Otherwise, exposed personnel should immediately report to a medical clinic for administration of the booster doses. See WHO guidance for post-exposure prophylaxis at:
   http://www.who.int/rabies/human/postexp/en/
   http://www.who.int/rabies/human/prevvaccperson/en/

[1] Nitrile gloves are recommended for handling bats, in the absence of nitrile gloves and allergies to latex double latex gloves could be considered.

Section 5.2.7c. Data Collection

Please refer to the **required data collection templates** for data to collect. These include:

1. P2 Animal Data Collection Form
2. P2 Site and Event Characterization Data Collection Form
3. P2 Specimen Data Collection Form

For more information on downloading templates from EIDITH see ***Section 5.2.3. General Data Collection Templates and Applications*.**

Section 5.2.7d. Bat Capture, Handling, and Sampling

Capture techniques will vary based on the species being targeted and the location where the samples are being collected and details of the main techniques including mist nets, harp traps, and hand capture are available in other documents such as the FAO guide *Investigating The Role Of Bats In Emerging Zoonoses* (www.fao.org/docrep/014/i2407e/i2407e00.pdf). Note that not all the sample collection techniques in that guide are recommended for PREDICT2 and field staff should use the PREDICT2 guide for specimen collection guidance.

***Note**: The PPE requirements for handling animals during capture or during processing are the same. All animal capture, handling and sampling should be done in accordance with current IACUC protocols.*

Handling Procedures

1. Each bat should be placed into a porous cotton bag (with draw-string mouth), hung from a sturdy line over a polyethylene sheet (to catch urine), and kept in a cool dry place until sampling time.
2. Bats should be weighed (in grams) in bags using a Pesola hanging scale or a tabletop scale with or without a container (such as a cup). The container should be tared and both bat and bag should be weighed together. Once the bat is removed from the bag for sampling, the bag should be re-weighed and subtracted from previous total.
3. The bat should be removed from the bag and the samples below collected. The order of sampling may vary. For example, urine may be expelled on initial handling and urine would then be the first sample collected.
   Note: check bag for fresh feces before continuing. If fresh feces are available, these may be used as a sample and then a rectal swab is not necessary. The sampler must be certain that the feces belong to the bat being sampled. Bags should be either discarded after first use or washed/disinfected between uses.
4. Bats will not be held longer than 6 hours. Frugivorous and nectivorous bats will be give 100% fruit juice or sugar water prior to release.

Sampling Procedures

The following basic set of samples should be collected from each animal where possible (If only one sample can be collected, then place into VTM):

1. **Two oral swabs** - one in 500 µL VTM and one in 500 µL Trizol
2. **Two fecal samples** - one with max of 500 µL/0.5cc feces in 500 µL VTM and one with max of 500 µL/0.5cc feces in 1 mL Trizol

   Or

   Two rectal swabs - one in 500 µL VTM and one in 500 µL Trizol
3. **Two blood samples** - 2 x 500 µL aliquots, one in 500 µL VTM and one in 500 µL Trizol
4. **Two serum samples** - 2 x 500 µL aliquots (only if more than 2ml of blood available), frozen without media. A minimum of 100 µL serum (single aliquot) should be collected to be useful for PREDICT diagnostic testing

   ***Note**: If animals are too small to collect two blood tubes (for whole blood and serum), collect serum and save remaining clot in VTM after serum separation*
5. **Two urogenital swabs/urine samples** - one with max of 500 µL of urine in 500 µL VTM and one with max of 500 µL of urine in 500 µL Trizol

Freeze all samples in liquid nitrogen immediately in the field and transfer to -80°C lab freezer.

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 200 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

Collection of samples from bats:

a. **Two oral swabs: 1 in Trizol, 1 in VTM**: Using sterile, polyester-tipped swabs with either an aluminum or plastic shaft, rub the swab tip gently but thoroughly against the back of the animal's throat, saturating the swab with saliva (recommend Puritan® Small Tapered Polyester-Tipped Swab from VWR, Catalog No.: 89133-756).
   i) Place 1 swab in a 1mL screw-top cryovial filled with 500 µL VTM and use alcohol-wiped (or ethanol-wiped), flame-sterilized scissors to cut the shaft of the swab about 1cm above the tip. Swabs should be cut on the shaft as close as possible to the end-swab without touching it. Scissors should be wiped with ethanol or isopropyl alcohol and flame sterilized after cutting each swab.
   ii) Place the other swab into 500 µL Trizol in a cryovial and cut the shaft as above.
   iii) Store in a liquid nitrogen dry shipper or dewar and transfer to -80˚C freezer later.

b. **Two rectal swabs: 1 swab in VTM, 1 swab in a tube with Trizol.** Rectal swabs can be moistened with sterile saline prior to animal sampling.

DO NOT USE VTM TO MOISTEN SWABS BEFORE SAMPLING
DO NOT USE TRIZOL AS A LUBRICANT *– IT IS HIGHLY IRRITATING TO TISSUE.*
DO NOT FORCE TIP OF SWAB INTO RECTUM*, IF IT WON'T ENTER EASILY, DO NOT COLLECT THIS SAMPLE.*

Gently insert the sterile swab tips, one at a time, into the animal's rectum. Place 1 swab in a cryovial filled with 500 µL of VTM and using isopropyl alcohol-wiped (or ethanol-wiped), flame-sterilized scissors cut the shaft of the swab above the tip (or snap as mentioned above). Place the other swab into a cryovial with 500 µL of Trizol. Store in a dewar or dry shipper with liquid nitrogen and transfer to -80˚C freezer when possible.

c. **Alternatively, collect fresh feces:** Add 500 µL or pea-sized pieces of feces directly into two vials, one containing 500 µL VTM (= maximum final ratio of 1:1) and one containing 1 mL Trizol (= maximum final ratio of 1:2) and mix each tube well. Freeze in dry shipper or dewar with liquid nitrogen and transfer to -80˚C freezer when possible.

d. **Whole blood in VTM and Trizol, and serum divided into two aliquots;**
   i) Manually restrain bats during blood collection. For larger bats, two or preferably three people are required for these manipulations: one person to safely restrain the bat, one to take samples, and a third to manage the tubes (i.e. unscrewing the lids, holding them up to the sample taker, making sure the lids are replaced tightly and kept in order) and record samples. Smaller insectivorous bats may be restrained and sampled by a single person. Anyone sampling bats should have had previous training in bat venipuncture to avoid injury to the animal. In addition:
      - It is recommended that large fruit bats (*Pteropus, Aceradon,* and other large species) be anesthetized using either injectable medetomidine (50 µg/kg) + ketamine (5 mg/kg) or gas anesthesia (isoflurane 4-5% induction, 2% maintenance).
      - The person restraining the bat is responsible for monitoring respiration and communicating respiratory status appropriately.
   ii) Bats must be bled with caution to maintain a ratio no greater than 10 µL of collected blood to 1 g of bat body weight (equivalent to 1% bodyweight).
   NOTE: for bats <100g we use the maximum amount of 6 µL per gram of body weight.
   iii) **For bats > 100 g**: Use a non-heparinized syringe to collect blood (not to exceed 1% of the total body weight). Recommended venipuncture sites include the propetagial (cephalic) vein, the uropetagial (saphenous) vein, or the brachial vein (Figure 1.) If volume allows, place some blood in an EDTA (lavender top) tube and some in a serum vacutainer (red-top) tube containing serum-clotting factor. From the lavender tubes collect 500 µL of whole blood and place in 500 µL VTM, and 500 µL of whole blood in 500 µL Trizol. After allowing the blood to clot in the red top tubes, either

spin tube in a centrifuge or allow tube to stand vertically on ice overnight. Use a sterile pipette tip and pipette gun to draw off serum and place even aliquots into 2 cryovials (minimum 60 µL).

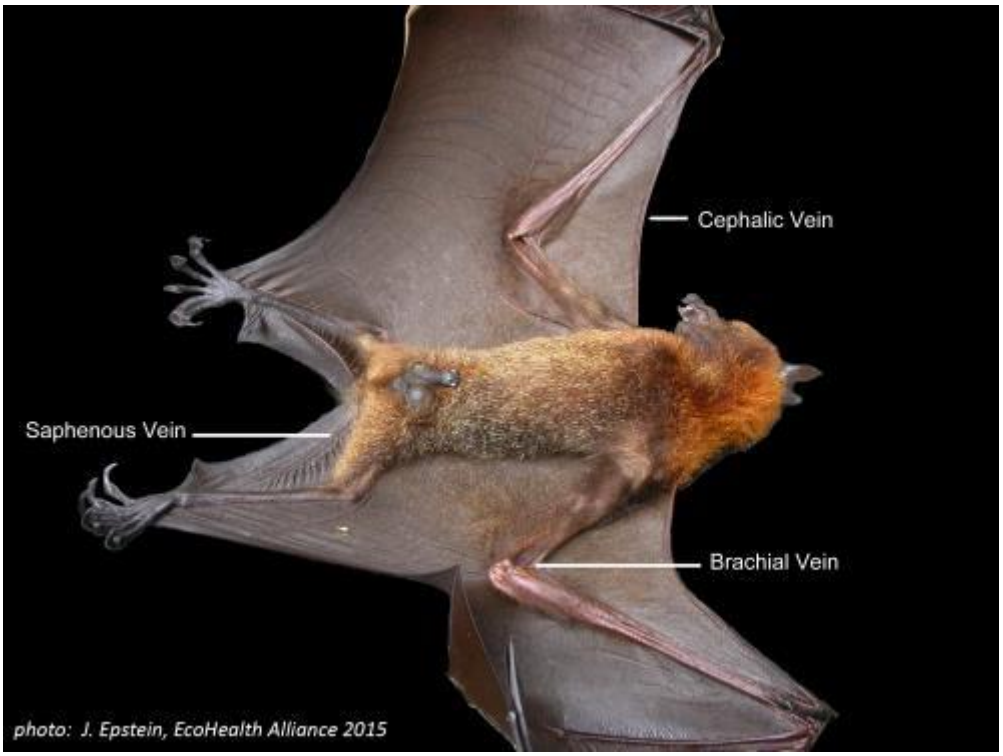

Figure 1. Preferred venipuncture sites for large (>100g) bats. Note: Apply pressure with a cotton ball to ensure that hemostasis is achieved after blood draw, especially with the brachial vein or artery, which are closely associated and higher pressure vessels compared to the cephalic or saphenous vein. (From Newman, Field, de Jong and Epstein, FAO 2011)

v) **For bats <100 g**: Use a 75 µL heparinized glass hematocrit tube to collect blood. Bat is restrained in one hand and the wing is gently extended by the wrist. The radial artery or vein is punctured using the tip of a sterile 25 G (gauge) needle and a droplet of blood is allowed to form. Collect up to 0.6% body mass of blood (e.g., 6µL per gram) using hematocrit tubes. Use a bulb to expel the whole blood in a cryotube with 500 µL of VTM. **Apply pressure to site of bleeding using a cotton ball until bleeding ceases (approximately 1 minute).** Hematocrit tubes can be centrifuged using a portable hematocrit centrifuge to separate serum. Score glass tube (using a razor blade or X-acto knife) where the serum meets the red cell fraction and carefully snap the tube. Use a bulb to expel serum into a micro-cryovial and freeze. If two or more capillary tubes are filled, collect two aliquots of serum. Preserve the remaining red cell clots in a separate cryovial and freeze.

vi) **Do not recap needle**. Place needle in sharps container and syringe in biohazard bag. Deliver medical waste to an incinerator or other secure medical waste disposal where possible.

vii) Bats must be fully recovered from anesthesia before release to prevent injury.

e. **Urogenital swabs/urine** - When handling bats, collect two urogenital swabs and place one into 500 µL VTM, one into 500 µL Trizol. Swabs can be moistened with sterile saline prior to animal sampling. If the bat urinates, collect two 500 µL urine samples at an optimal ratio of 1 part urine: 1 part VTM; and 1 part urine: 1 part Trizol (e.g., ~500 µL bat urine in 500 µL Trizol). Store samples in dry shipper or dewar with liquid nitrogen and transfer to -80˚C freezer when possible.
***Note**: Larger fruit bats tend to urinate as they are removed from the cotton bag. Urine may be collected at this point using a pipette or tube. Urine may also be collected using a pipette from a surface however contamination is more likely and this should be avoided.*

f. **Necropsy sampling** - In case of accidental death before or during animal sampling, or where dead animals are available for opportunistic sampling, **collect tissue samples** – three, adjacent, approximately 200mg (pea-sized) pieces of each tissue type: one frozen in 500 µL VTM at -80°C, one frozen in 1 mL Trizol at -80°C, and one 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). In these cases also collect as much blood as possible. Cardiac puncture is recommended.

Collect approximately 200 mg (pea-sized) samples of the following tissues:

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

If euthanasia is required see the AAZV and ***AVMA guidelines (Section 8.5.2)***.

Additional Data To Collect:

Additional identification and biometric measurements may be collected at the discretion of the sampling party, although they are not mandatory (unless they are needed for species identification).

- Whole body photograph
- Identifying characteristic photographs
- Age class*
- Sex
- Body weight

- Body condition **
- Biometric measurements (see Biometrics section below for details)
- Additional morphometric measurements
- Reproductive status

***Age classes** - For some bat species it will be possible to classify bats into one of three age classes:

- **Pre-weaned juvenile** -- pup is still clinging to dam and suckling.
- **Juvenile** -- pup is independent from dam, may be adult sized, but sexually immature. Absence of secondary sexual characteristics such as elongated teats, not gravid at the time of capture, and for male Pteropodid bats, tiny barbules present on the glans penis. Incomplete fusion of phalangeal symphysis (head of phalanx not yet fused with shaft of phalanx as viewed when wing is backlit – this is more apparent with larger bats).
- **Adult** -- secondary sexual characteristics present, pregnant or lactating, adult size. Complete fusion of phalangeal symphysis.

****Body Condition:** For larger fruit bats, it is also useful to evaluate body condition based on pectoral muscle mass--a quick and subjective measurement of nutritional status and robustness, which is a useful when assessing health in the context of infection. Record pectoral muscle mass as one of three categories: “Poor” (emaciated, prominent sternum), “Fair” (flat across pectoral muscles and sternum), “Good” (pectoral muscles are rounded and extend/bulge past the sternum).

Species identification:
1. The following digital photographs* should be taken of each bat where there is uncertainty about species identification:

a. Full body in anterior-posterior presentation and wings extended with identification card displaying unique identifying number
b. Full anterior facial (macro setting)
c. Full lateral facial/head (macro setting)
d. View of parted pelage on ventrum and dorsum (macro setting)

**Proper PPE should be worn at all times while holding animals, including while holding animals for photos or measurements.*

2. The biometric measurements (in millimeters) listed below should be taken. However, collecting these measurements adds time to the sampling effort. For micro-bats these are common measurements and they are valuable for identification; nevertheless the specific needs vary by species and by region. If you are in doubt of an identification look at reference texts for the genus or family and try to determine which the characteristics that

are relevant for that group. Someone with experience in identifying Microchiroptera in the area is usually required for this.

Microchiroptera biometric measurements **(as per Menzel et al., 2002)**

a. Forearm/radius length ('elbow to wrist')
b. Ear length (most distal tip of ear to middle of the base)
c. Tragus length (top of tragus to base of ear)
d. Body length (measured with the bat in ventral recumbancy from the tip of nose to the base of tail).
e. Hind foot length ('ankle to toe')
f. Tail length (from base to tip)
g. Tibia length ('knee to ankle')

Megachiroptera biometric measurements **(as per Menzel et al., 2002)**

a. Forearm/radius length ('elbow to wrist')
b. Head length
c. Body length

For larger fruit bats, it is also useful to evaluate body condition based on pectoral muscle mass--a quick and subjective measurement of nutritional status and robustness, which is useful when assessing health in the context of infection. Record pectoral muscle mass as one of three categories: "Poor" (emaciated, prominent sternum), "Fair" (flat across pectoral muscles and sternum), "Good" (pectoral muscles are rounded and extend/bulge past the sternum).

3. Based on these morphometrics and other appropriate unique characteristics, identify bats to genus, species (where possible), age class, and sex. For female bats, determine pregnancy status by gently palpating the abdomen and lactation status by gently attempting to express milk from the teats.

4. Release bats as close to their site of capture as possible.

5. If a sonic recording device is available, for Microchiroptera record the bat's calls upon release. These recordings can assist with identification of the specimens and with compiling resources for identifying bats in the area.

Section 5.2.7e. References

Food and Agriculture Organization of the United Nations. 2011. Investigating the role of bats in emerging zoonoses: Balancing ecology, conservation and public health interests. Edited by S.H. Newman, H.E. Field, C.E. de Jong and J.H. Epstein. FAO Animal Production and Health Manual No. 12. Rome. http://www.fao.org/docrep/014/i2407e/i2407e00.pdf

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Menzel M.A., J. M. Menzel, S. B. Castleberry, J. Ozier, W.M. Ford and J.W. Edwards (2002) Illustrated Key to Skins and Skulls of Bats in the Southeastern and Mid-Atlantic States. USDA Forest Service, PA. 10p (Accessed 25.04.09 : http://www.fs.fed.us/ne/newtown_square/publications/research_notes/pdfs/2002/rnne376.pdf)

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Section 5.2.7f. Appendix I. Supply and Equipment List

Note: Supply details, availability, and vendor sources may vary.

PPE

- ☐ Designated clothing (e.g. overalls or other clothes which can be put on before sampling and removed following sampling)
- ☐ Flexible face shield or other eye protection
- ☐ N95 or P100 respirator
- ☐ Nitrile examination gloves
- ☐ Washable shoes

First Aid

- ☐ Betadine or (or benzalkonium chloride)
- ☐ First aid kit (with post-exposure prophylactic vaccine if working in remote areas where vaccine is not rapidly accessible)

Data Collection

- ☐ Datasheets (or EIDITH tablet for direct data entry)
- ☐ Pencils
- ☐ GPS

Capture and Handling

- ☐ Mist nets, poles and ropes
- ☐ Flagging tape
- ☐ Leather gloves
- ☐ Holding bags
- ☐ Spring/electronic balance
- ☐ Dial/digital caliper
- ☐ Stainless steel wing rulers
- ☐ Large ziplock bag
- ☐ Chemical restraint requirements
- ☐ Camera
- ☐ Identification guides

Sampling

- ☐ Processing trays
- ☐ Permanent lab markers for tube numbering
- ☐ Cryotubes

- ☐ Needles 25G, 27G
- ☐ Needles and syringes for blood draws
- ☐ Sterile swabs (dacron/polyester)
- ☐ Sterile saline
- ☐ Cryo resistant tube labels
- ☐ Cryovial rack
- ☐ Cryoboxes and dividers
- ☐ 75 µL glass hematocrit tubes (heparinized)
- ☐ Plastic vacutainers (EDTA and dry)
- ☐ Pipetters and disposable tips
- ☐ Portable centrifuge for vacutainers
- ☐ Portable centrifuge for hematocrit tubes
- ☐ Cryo gloves
- ☐ Fine point forceps
- ☐ Scissors
- ☐ Dissection kit
- ☐ Trizol reagent
- ☐ Viral Transport Medium (VTM)
- ☐ RNAlater reagent
- ☐ Buffered formalin
- ☐ 95% ethanol
- ☐ Lighter
- ☐ Liquid nitrogen shipper/liquid nitrogen

Waste Disposal and Decontamination

- ☐ Paper towel
- ☐ Sharps containers
- ☐ Bleach
- ☐ 95% ethanol
- ☐ Biohazard bags
- ☐ Sprayers