# BAT SAMPLING METHODS

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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.

The contents of this document are the responsibility of the authors and do not necessarily reflect the views of USAID or the United States Government. USAID, PREDICT, EEIDI, and the authors of this guide bear no responsibility for the actions of individuals implementing the material herein.

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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## **Table of Contents**

1.	Overview of PPE and First Aid	3
2.	Bat Capture, Handling, and Sampling	5
	2a. Capture Techniques	5
	2b. Handling Procedures	5
	2c. Sample Collection from Live Bats	6
	2d. Biological Data Collection	10
	2e. Sample Collection from Dead Bats	13
	2f. Non-invasive Colony Sampling	15
3.	Marking and Individual Identification of Bats	16
	3a. Temporary Marking Methods	16
	3b. Permanent Marking Methods	16
4.	References	18
A2.	Appendix 2: Key Features to Identify Microchiroptera Species	22



## 1. Overview of PPE and First Aid

Due to the COVID-19 pandemic, One Health workers who are actively working with bats and other wildlife must recognize the potential for exposure to both known and novel zoonotic pathogens during animal capture, handling, and sampling activities. A similar risk of "spillback" of SARS-CoV-2 to bats from infected humans also has the potential to dramatically impact the health of these important wildlife species. 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.

All staff handling bats, bat products or their bodily fluids must be vaccinated for rabies and should be vaccinated for COVID-19 if vaccine doses are available. Ensuring that all staff have adequate protective antibody titers verified by laboratory testing to both zoonotic viruses is recommended. As a general principle, all researchers and teams should be prepared to institute appropriate rabies post-exposure prophylaxis measures in the event a bat bite or scratch occurs. Before project sampling begins, teams should contact local referral health clinics and identify a facility with available post exposure anti-rabies IgG that would be sought in an exposure event. It is recommended that personnel with frequent bat contact follow CDC and WHO guidelines of checking anti-rabies antibody titers every two years: www.who.int/rabies/WHO Guide Rabies Pre Post Exposure Prophylaxis Humans 2013.pd f?ua=1).

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.

#### Minimum PPE required for handling live bats

The <u>minimum</u> PPE for handling bats during capture and sampling as well as non-invasive sampling includes:

- 1) Eye protection
- 2) N95 or P100 respirator without exhalation valve



- 3) Nitrile gloves for person collecting / handling specimens<sup>1</sup>
- 4) Puncture resistant gloves to be worn outside nitrile gloves for person handling bat<sup>2</sup>
- 5) Washable rubber shoes / boots
- 6) Tyvek/Tychem coveralls

#### Refer to the Biosafety and PPE SOP 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) Rabies post-exposure prophylaxis 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. Regardless, exposed personnel should immediately report to a medical clinic for administration of the booster doses. See WHO guidance for post-exposure prophylaxis at: <a href="http://www.who.int/rabies/human/prevvaccperson/en/">http://www.who.int/rabies/human/prevvaccperson/en/</a>



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<sup>1</sup> Nitrile gloves are recommended for handling bats.

<sup>2</sup> Examples of bite-resistant gloves include TurtleSkin NYCDoC leather gloves, Coverage series gloves (Warwick Mills Inc.), or Bitebuster gloves (BiteBuster LLC).

## 2. Bat Capture, Handling, and Sampling

## 2a. Capture Techniques

Capture techniques will vary based on the species being targeted and the characteristics of the location where the samples are being collected. Details of the main techniques including mist nets, harp traps, and hand capture are available in other documents such as the <u>FAO guide</u> <u>Investigating The Role Of Bats In Emerging Zoonoses</u>. Note that each project should establish its own, IACUC approved sample collection techniques, and therefore field staff should follow project-specific guidance for specimen collection. Always review your IACUC protocol for allowed activities.

Harp traps are recommended by the American Society of Mammalogists for use where larger concentrations of bats are expected, to minimize the time bats might remain entangled in a mist net (Sikes and Bryan 2016). Mist nets may also be used around roosts as bats emerge at dusk or return at dawn, but only when use of harp traps is not practical as the only means of capture. *Nets must be frequently monitored by study personnel, with bats removed within 15 minutes of capture,* as *recommended by the American Society of Mammologists.* For hand capture of perched bats, briefly shining an intense light may temporarily daze the animal to aid in its capture and restraint.

**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.

## **2b. 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 quiet, cool, dry, dark area until sampling time.
  - Bags should be marked with information on the individual bat and time of collection.
    - Ensure bags are placed and spaced sufficiently to avoid any accidental damage.
    - Bats held for more than 3 hours will be checked on hourly thereafter and fruit juice or sugar water should be offered at 1-hour intervals.
    - Any bats that display outward signs of stress will be assessed by the veterinarian and treated accordingly and/or immediately released. Signs of stress may include, but are not limited to, excessive vocalization or excessive movement within the bag, or reduced responsiveness.
- 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 reweighed and subtracted from previous total.
- 3) The bat should be removed from the bag and the samples listed 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 the 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 turned inside-out and washed/disinfected between uses.
- 4) Bats will not be held longer than 6 hours. Frugivorous and nectivorous bats will be given 100% fruit juice or sugar water prior to release. Animals will be released at the site of capture, taking care to avoid releases near deployed capture equipment.

## **2c. Sample Collection from Live Bats**

A suite of samples can be collected, and the specific samples and preservation methods will vary according to the goals of each specific study. Here we provide guidance on collection of samples aimed at virus surveillance and discovery.

The following basic set of samples should be collected from each animal when possible, in the quantities and media outlined below: **oral swab**, **feces/rectal swab**, **blood**, **urine/urogenital swab**. Additionally, a wing membrane swab should be taken to assist with molecular identification of species when the species identity is uncertain (Player et al. 2017). For any longitudinal sampling, the same individual may be sampled no more than once every 3 months.

The table below provides details on the suggested suite of samples to be collected from each animal handled and their preservation method. MagMAX lysis/binding buffer concentrate, also referred to as MagMAX lysis buffer, and viral transport medium (VTM) are the two primary sample preservation media, though serum and wing swabs are stored without media.

## Ideally, all samples will be frozen in liquid nitrogen immediately in the field and transferred to a -80°C lab freezer when field teams return.

#### NOTE: In all cases, if only one sample can be collected, then place it into VTM.

	Total samples	Pres			
Sample type		500 μL VTM	500 μL MagMAX lysis buffer	No Media	Comments
	One Health Institute		EcoHealth Ata Health St	rategies iccop Global Buyers	TREELEY ECHO UCI University of California Irvine

		#	Sample volume	#	Sample volume	#	Sample volume	
Oral swabs	2	1	swab	1	swab			
Feces/ Rectal swabs	2	1	0.5cc feces or swab	1	0.5cc feces or swab			Feces <b>OR</b> swab
Urine/ Urogenital swabs	2	1	100 μL urine or swab	1	100 μL urine or swab			Urine <b>OR</b> swab
Blood*	2-4	1-2	100 μL	1-2	100 μL			For larger species only, collect two 100 ul aliquots per media type (4 samples total)
Serum	2					2	250 μL	minimum 50 μL per aliquot recommended
Wing swab	1					1	swab	Interior of wing for species ID

\* 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.

## **Collection details**

- Two oral swabs: 1 in VTM and 1 in MagMAX lysis buffer: 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<sup>®</sup> Small Tapered Polyester-Tipped Swab from VWR, Catalog No.: 89133-756, select size based on species).
  - a) Place 1 swab in a 2mL screw-top cryovial filled with 500 μL VTM and use alcoholwiped (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 endswab without touching it. Scissors should be wiped with ethanol or isopropyl alcohol and flame sterilized after cutting each swab.
  - b) Place the other swab into 500  $\mu\text{L}$  MagMAX lysis buffer in a cryovial and cut the shaft as above.
  - c) Store in a liquid nitrogen dry shipper or dewar and transfer to -80°C freezer later.

#### 2) Rectal swabs / feces

- a) **Two rectal swabs: 1 in VTM and 1 in MagMAX lysis buffer.** Rectal swabs can be moistened with sterile saline prior to animal sampling.
  - i. Gently insert the sterile swab tips, one at a time, into the animal's rectum. Place 1 swab in a cryovial filled with 500  $\mu$ L 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).

- ii. Place the other swab into a cryovial with 500 µL of MagMAX lysis buffer.
- iii. Store in a dewar or dry shipper with liquid nitrogen and transfer to -80°C freezer when possible.

## DO NOT USE VTM OR LYSIS BUFFER TO MOISTEN SWABS BEFORE SAMPLING. THEY ARE HIGHLY IRRITATING TO TISSUES.

## DO NOT FORCE TIP OF SWAB INTO RECTUM. IF IT DOES NOT ENTER EASILY, DO NOT COLLECT THIS SAMPLE.

- b) Alternatively, collect fresh feces: Add ~200 mg pea-sized piece of feces directly into two vials, one containing 500  $\mu$ L VTM (= maximum final ratio of 1:1) and one containing 500  $\mu$ L MagMAX lysis buffer (= maximum final ratio of 1:1) and mix each tube well. Freeze in dry shipper or dewar with liquid nitrogen and transfer to -80°C freezer when possible.
- 3) **Urogenital swabs/urine** When handling bats, collect two urogenital swabs and place one into 500  $\mu$ L VTM, and one into 500  $\mu$ L MagMAX lysis buffer. Swabs can be moistened with sterilesaline prior to animal sampling. If the bat urinates, collect two 100  $\mu$ L urine samples at an optimal ratio of 1 part urine: 5 parts VTM; and 1 part urine: 5 parts MagMAX lysis buffer (i.e., 100  $\mu$ L bat urine in 500  $\mu$ L MagMAX lysis buffer). 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, this should be avoided as contamination is more likely.

## 4) Whole blood in VTM and MagMAX lysis buffer, and serum divided into two aliquots

- a) 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 (e.g., 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 previous training in bat venipuncture to avoid injury to the animal.
  - 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). *Refer to your approved IACUC protocol drug table and its references as needed for dosage details.* 

- ii. The person restraining the bat is responsible for monitoring respiration and communicating respiratory status appropriately.
- b) 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 under 100g collect no more than 6 μL of blood per gram of body weight.
- c) Collect and process blood as described below based on bat's weight.
- d) 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.
- e) Bats must be fully recovered from anesthesia before release to prevent injury.

For bats under 100 g: Use a 100  $\mu$ L pipettor 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.,  $6\mu$ L per gram) using a pipette. Apply pressure to site of bleeding using a dry cotton ball until bleeding ceases (approximately 1 minute).

Expel ~100  $\mu$ L whole blood in a cryotube with 500  $\mu$ L of VTM and ~100 ul whole blood in a cryotube with 500  $\mu$ L of MagMAX lysis buffer. If there is not enough volume to fill two tubes, prioritize the VTM tube. If volume allows, pipette remainder of blood into a hematocrit tube. 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 with no media and freeze.

For bats over 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). Collect blood and then apply pressure to site of bleeding using a dry cotton ball until bleeding ceases (approximately 1 minute).

Place 100  $\mu$ L of whole blood directly in 500  $\mu$ L VTM, and 100  $\mu$ L of whole blood in 500  $\mu$ L MagMAX lysis buffer. When possible for larger species, save 4 x 100  $\mu$ L aliquots, two in 500  $\mu$ L VTM and two in 500  $\mu$ L MagMAX lysis buffer, if it will not interfere with obtaining adequate serum volume. Place the remainder of the blood into a serum separator tube. 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 50  $\mu$ L each).





Figure 1. Preferred venipuncture sites for large (>100g) bats. Note: Apply pressure with a dry 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)

5) Wing swab for species ID. This is a non-invasive alternative to wing membrane biopsies (Player et al. 2017). To collect DNA using a swab, open the bat's wing and rub a sterile rayon-polyester-tipped swab along the surface of the wing, using a motion that applies slight pressure to drag the swab while rolling it, resulting in most or all of the head of the swab making contact with the skin of the bat. Only enough pressure should be applied to make good contact of the skin with the head of the swab, without abrading the tissue. Rub the inside (ventral) surfaces of the wings only to avoid contamination. Place swab in a cryovial with no media and store frozen at -20° or -80°C.

## 2d. Biological Data Collection

There will be situations in which additional biological data can't be collected due to time constraints or condition of the animal. In that event, biological specimen sampling should be prioritized.

#### Minimum individual bat data to collect:

• Species (or identifying features to determine species through whole body photograph, in addition to interior of wing swab for molecular ID)



- Age class
- Sex
- Body weight

Optimal individual bat data to collect (in addition to the items above):

- Body condition
- Biometric measurements
- Additional morphometric measurements
- Reproductive status
- Health status
- Ectoparasites
- Sonic recording of Microchiroptera at release

**Species identification:** If species is known, note species utilizing the Integrated Taxonomic Information System (itis.gov)

When species is unknown or species identification is uncertain:

- 1) Refer to Appendix 2 for key features to focus on to help identify Microchiroptera.
- 2) Digital photographs\* should be taken of each bat:
  - 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)
- 3) Record biometric measurements\* as described below
- 4) Gather a specimen for molecular identification
  - a) Specimens for molecular identification:
    - i. whole blood sample, if collected
    - ii. wing membrane swab for epithelial cells, stored dry
    - iii. Oral and fecal swabs may contain epithelial cells
    - iv. For carcasses, other tissues may also be suitable

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

**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 sternum).

**Biometric measurements**: listed below should be taken in millimeters. However, collecting these measurements adds time to the sampling effort. Below are common measurements that are valuable for identification; nevertheless, the specific measurements needed 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, and consult with someone with experience in identifying Microchiroptera in the area.

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

- 1) Forearm/radius length ('elbow to wrist')
- 2) Ear length (most distal tip of ear to middle of the base)
- 3) Tragus length (top of tragus to base of ear)
- 4) Body length (measured with the bat in ventral recumbency from the tip of nose to the base of tail)
- 5) Hind foot length ('ankle to toe')
- 6) Tail length (from base to tip)
- 7) Tibia length ('knee to ankle')
- 8) Thumb

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

- 1) Forearm/radius length ('elbow to wrist')
- 2) Head length
- 3) Body length

**Reproductive status:** For female bats, determine pregnancy status by gently palpating the abdomen and lactation status by gently attempting to express milk from the teats.

**Health status:** Number of visual bite/scratch marks to be recorded as well as number of wing / ear notches and / or holes.

Ectoparasites: Ectoparasite species should be recorded if known and number of



ectoparasites on bat should be recorded (e.g. # ticks). One – three ectoparasite specimens of the same species should be carefully removed and placed <u>in a</u> tube with 95% ethanol.

**Sonic Recordings of Microchiroptera:** Passive acoustic recordings of bats may be taken at sampling sites. Acoustic recordings will be made using a sound-activated recorder, such as Wildlife Acoustics Song Meter SM4Bat or similar device, temporarily mounted at the sampling site to a tree or other suitable stable mount for a limited acoustic sampling timeframe (typically less than 1 week at a time). Passive acoustic recordings can be analyzed to assess species diversity at a sampling site, and are especially valuable for detecting species that are difficult for researchers to directly observe.

Additionally, calls of captured bats may be recorded at the time of release to help verify species identity and compare with passive acoustic recordings from the sampling site. These recordings will be made by releasing the bat near the point of capture, and allowing an ultrasonic acoustic recorder mounted nearby to detect and record the bat's echolocation calls as it flies away. For time-of-release echolocation recording, Wildlife Acoustics Echo Meter Touch 2, designed for use with a smartphone and recording app, may also be suitable.

## 2e. Sample Collection from Dead Bats

In case of accidental death before or during animal sampling, or where dead animals are available for opportunistic sampling, *collect blood, swab, and tissue samples (see details in table below).* As possible, also collect biological data outlined in section 2d.

**Accidental Deaths and Euthanasia:** Fully document any accidental deaths and cases that require euthanasia, including when and how death occurred, as these details must be reported to the IACUC. If euthanasia is required, see the <u>AVMA</u> and/or AAZV guidelines and follow all procedures outlined in your approved IACUC protocol.

	Total samples		Pre					
Sample type		50	00 μL VTM	500 μL MagMAX lysis buffer		Other		Comments
		#	Sample volume	#	Sample volume	#	Sample volume	
Oral swabs	2	1	swab	1	swab			
Feces/ Rectal swabs	2	1	0.5cc feces or swab	1	0.5cc feces or swab			Feces <b>OR</b> swab
Urine/ Urogenital swabs	2	1	100 μL urine or swab	1	100 μL urine or swab			Urine <b>OR</b> swab









Blood*	2-4	1-2	100 μL	1-2	100 μL			Collect two 100 ul aliquots per media type (4 samples total)
Serum	2					2	250 μL No media	minimum 50 μL per aliquot recommended
Tissues	10	1	200 mg (pea size)	1	200 mg (pea size)			Priority order for tissues 1. Liver 2. Spleen 3. Lung 4. Lymph node 5. Large intestine
Muscle	1					1	200 mg (pea size) 95% ethanol	

## **Collection details**

#### 1) Whole Blood by Cardiac Puncture in dead animals:

- a) Assemble the vacutainer holder and sheathed needle (18 22 G) with a Luer-lock adapter prior to the commencement of the procedure.
- b) Loosen the sheath from the needle using forceps (not fingers!) so that the needle can be easily removed from the sheath by lifting the holder/needle assembly and allowing gravity to pull the sheath off.
- c) Wet the sternum of the animal with 70% ethanol and locate the xiphoid process on the sternum visually or by palpating.
- d) Using the vacutainer holder/needle assembly, insert the needle (at approximately a 15° angle to the body plane) just posterior to the xiphoid process and into the thoracic cavity toward the heart.
- e) Keep the vacutainer holder steady and push the vacutainer tube (typically 3-6 mL) onto the internal needle. If the needle insertion was done correctly, blood will flow into the vacutainer. If blood does not flow, rotating or slightly moving the needle in or out may locate the cardiac chamber to start the flow.
- f) Collect as much blood as possible into both a serum separator tube (first) and lavender top EDTA tube (if additional volume allows).
- g) Follow guidelines for processing and storing whole blood as described above for live bats.

\*The procedures described above can also be accomplished with a standard needle and syringe if a vacutainer system is not available.

2) Collect and store swabs, feces, urine as described above for live bats.



3) Tissues: Collect two, adjacent, approximately 200 mg (pea-sized) pieces (maximum) of each tissue type: one in 500 μL VTM and one frozen in 500 μL MagMAX lysis buffer, both stored frozen at -80°C. For small bats, whole organs may need to be divided into 2 pieces for collection.

Tissues listed in order of priority:

- i. Liver
- ii. Spleen
- iii. Lung
- iv. Lymph node
- v. Large intestine

Collect a 200 mg (pea-sized) section **of muscle** and store in 95% ethanol for species ID. Can store at room temperature and later transfer to freezer.

As always, should you note any outstanding **gross lesions**, collect samples from the lesions as stated above, and store a third sample 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). Store at room temperature, NEVER FROZEN.

## 2f. Non-invasive Colony Sampling

## Saliva collection using dental rope boxes for fructivorous and nectivorous bats

When roosting sites have been identified for targeted fructivorous and / or nectivorous bats, monthly non-invasive saliva collection should be performed at the colony level. "Presentation boxes" should be constructed of hard plastic containers which can be used to insert dental ropes (Salivabio Children's Swab, Salimetrics.com) through holes drilled in the sides of the container.

Dental ropes should be coated with a sticky sweet substance regionally suitable (e.g., mango juice, papaya juice, sugar water). Plastic collection boxes can also be painted to resemble flowers for nectivorous bats. Plastic containers are to be hung in the roosting site and bats allowed to feed freely on the juice. Hang collection devices for no more than 1 hour to ensure saliva samples do not degrade.



Salimetrics children's oral swab and oral swab collection tube.

- Remove dental ropes from the collection container and place into salimetrics oral swabs tubes.
- Centrifuge samples in the field @ 3,000 rpm for 5 minutes to elute saliva into the bottom of the tube.



- Place 300 ul saliva in a cryovial tube containing 500 ul VTM.
- Place 300 ul saliva in a cryovial tube containing 500 ul MagMAX lysis buffer.
- Place 300 ul saliva in a cryovial tube with no storage media.
  - \*If inadequate volume is available, prioritize the sample in VTM.

#### **Guano Collection**

Tarps or plastic sheets may be placed underneath roosting sites to opportunistically collect fresh feces and urine from roosting bat colonies and/or guano farms. Wherever possible, data accompanying guano collection should include as many details as are known, including species, approximate number of animals roosting above tarp at the time of collection, and approximate colony size.

Collect with swabs or add three  $\sim$ 200 mg pea-sized piece of feces directly into vials, as follows:

- one cryovial with 500 µL VTM (maximum ratio of 1:1)
- one cryovial with 500 μL MagMAX lysis buffer (maximum ratio of 1:1)
- one cryovial with no media

Mix each tube well and freeze in dry shipper or dewar with liquid nitrogen and transfer to a -80°C freezer when possible.

## 3. Marking and Individual Identification of Bats

Both temporary and permanent methods can be used to avoid repeated sampling within a short time period and to help identify individual recaptures in successive sampling efforts as needed to meet research objectives. Longitudinal surveillance may occur only at some research sites.

## **3a. Temporary Marking Methods**

Short-term marking methods help avoid repeated sampling within a short time period. Methods may include fur clipping, bleaching a small patch of fur using hydrogen peroxide or hair dye, marking wing membranes with a permanent marker, or painting claws of one foot with nail polish (quick drying, formaldehyde-free). Field team should be consistent about where they apply markings (e.g., left wing membrane between specific digits, nails of left foot, etc.), so that it is easy to find and identify these marks if recaptured in the future.

## **3b. Permanent Marking Methods**

Where repeated sampling of the same individuals is desired for longitudinal surveillance at a site, animals may need to be permanently marked, such as with cold-branding through applying liquid nitrogen to the wing membranes (Sherwin et al. 2002). If numerous individuals need to be uniquely identified, bats may be marked by inserting a sterile microchip containing a unique ID



number subcutaneously between the scapulae. Commonly used microchips include passive integrated transponder (PIT) tags from Avid, Co. https://www.avidid.com, or Trovan https://www.trovan.com/.

**PIT tag placement**: PIT tags will be inserted subcutaneously to provide a reliable unique alpha-numerical code serving as an animal ID. Skin will be disinfected with 70% ethanol prior to tag placement using a plastic syringe with an appropriate gauge single-use sterile needle (usually provided with the PIT tag kit). The tag should be implanted on the back, between the scapulae. After implantation, the puncture hole may be sealed with cyanoacrylate adhesive. For trained personnel, the procedure should take less than 1 min. The tags are activated by a handheld reader through radio frequency identification (RFID), through the generation of a close-range electromagnetic field. The size of PIT tags is species and size dependent. PIT tags will be used only in animals with sufficient body size where tag insertion has negligible impact (generally animals weighing more than 30 grams for 11-12mm standard length tags which weigh about 0.1 grams, or 15 grams for 7-8mm mini tags). Subcutaneous PIT tagging is a minimally invasive procedure that does not require anesthesia; however, if animals are anesthetized for other procedures, PIT tag deployment should be done prior to reversal or recovery.



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#### A1. Appendix 1: Supply and Equipment List

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

## <u> PPE</u>

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 without exhalation valve

□ Nitrile examination gloves

UWashable shoes

Tyvek/Tychem suits

## First Aid

Betadine 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 tablet for direct data entry)

Pencils

GPS

#### **Capture and Handling**

☐ Mist nets, poles and ropes

Flagging tape

Leather gloves

breathable cloth holding bags

Spring/electronic scale

Dial/digital caliper

Stainless steel wing rulers

Large ziploc bag

Chemical restraint drugs and equipment

🗌 Camera

☐ Identification guides

#### **Sampling**

Processing trays

Permanent lab markers for tube numbering

Cryotubes

Needles 25G, 27G

Needles and syringes for blood draws

USAID SEA UCDAVIS One Health Institute AFROHUN SEA HUN

Sterile swabs (dacron/polyester)

EcoHealth Alliance

20



Ara Health Strategies icop Gold Beckley CFD UCI Chiversity of Curana

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 MagMAX lysis buffer □ Viral Transport Medium (VTM) ☐ 10% Buffered formalin 95% ethanol Lighter Liquid nitrogen shipper/liquid nitrogen

#### Waste Disposal and Decontamination

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

EcoHealth Alliance At a Health Strategies icop Fields Berkeley ECHO UCI Chiversity of Cationia Invine

## A2. Appendix 2: Key Features to Identify Microchiroptera Species



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