Collection of Tissues
for Mercury Analysis
2024

Bat Field Sampling Methods

BAT FIELD SAMPLING METHODS

Collection of Tissues for Mercury Analysis

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Biodiversity Research Institute (BRI) is a 501(c)3 nonprofit organization located in Portland, Maine, USA. Founded in 1998, BRI is dedicated toward supporting global health through collaborative ecological research, assessment of ecosystem health, improving environmental awareness, and informing science-based decision making.

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Cover Photographs © Merlin Tuttle Foundation: Top: Egyptian slit-faced bat (*Nycteris thebaica*); Top row L to R: Damara woolly bat (*Kerivoula argentata*); Lappet-eared free-tailed bat (*Chaerephon major*); Dusky pipistelle (*Pipistrellus hesperidus*); Bottom row L to R: African yellow bat (*Scotophilus dinganii*); Egyptian fruit bat (*Rousettus aegyptiacus*); Rufous myotis (*Myotis bocagii*).

Table of Contents

1.0. Whole Blood Collection 1
1.1. Supplies for Venipuncture 1
1.2. Bat Blood Collection 2
1.3. Blood Sampling Labeling Format 2
2.0 Fur Sample Collection 4
2.1. Supplies for Fur Collection 4
2.2. Fur Collection Procedure 4
2.3. Fur Sample Labeling Format 5
3.0. Procedural Tables 6
4.0. Metadata Requirements 6
5.0. Storage of Samples 7
6.0. Shipment of Samples 7
7.0. Required Permits 8
8.0. References 9
Appendix
Bat Metadata Form 10

1.0 Whole Blood Collection

The nonlethal collection and analysis of whole blood samples in bats is used to identify the recent dietary uptake of contaminants, such as mercury (Evers 2018), and various stable isotopes (i.e., δ13C, δ15N, δ34S) that allow for diet reconstruction and identifying migratory origins. Blood samples can be obtained through venipuncture of veins of the wing or tail.

Micro-hematocrit capillary tubes are preferred for laboratory analyses but require specific storage and shipping arrangements. If capillary tubes are unavailable, vacutainers are an adequate replacement and can store a larger sample volume. IMPORTANT: Contaminant analyses require blood to be stored in Lithium heparinized receptacles, while stable isotope analyses require blood to be stored in sterile receptacles. For easy sample storage and shipping, blood dried on Whatman cards can be used for contaminant and stable isotope analyses (Perkins and Basu 2018; Barst et al. 2020).

Rule of thumb for the maximum volume of blood: Blood extraction should never exceed 1% of an individual's total body weight (Eshar & Weinberg 2010). Approximately 10% of a bats weight is blood (Eshar & Weinberg 2010); therefore, up to 10% of the volume of blood in a bat can be drawn. For example, a 1000g adult fruit bat has approximately 100 g of blood. From this, a maximum of 10 g of blood can be taken (1 g = 1 mL). A bat weighing 20 g has 2 g of blood; therefore, a maximum of 0.2 mL or 200 µL of blood can be collected.

IMPORTANT: BRI does not recommend collecting the maximum amount in any species.

1.1 Supplies for Venipuncture*

General Procedure	Piercing Procedure
☐ Data sheet	☐ 26–27 gauge needles (for small birds)
☐ Map of country/sampling site	☐ Micro-hematocrit capillary tubes (use heparinized for contaminants, sterile for stable iso-topes)
☐ Portable (~5L) cooler	☐ Leica Microsystems Critoseal™
☐ Ice pack	☐ 6 mL archive vacutainer
☐ Isopropyl alcohol pads	
☐ Dry cotton balls	
☐ Ultra-fine Sharpie™ permanent marker	
☐ Sandwich-size Ziploc™ plastic bags	
☐ Portable Sharps container	
☐ ***Optional:*** Whatman blood cards	
☐ ***Optional:*** Silica Gel Desiccant Packets (for Whatman card storage only)	

**For infomration on where to purchase supplies, email BRI (see page 8)*

1.2. Bat Blood Collection

Standard protocols for the collection of bat blood are outlined in this section. For each bat, a series of standard measurements must be recorded on the sample data sheet.

Procedure

1. Locate the acute ulnar in the wing (Figure 1) or a vein in the tail portion of the wing membrane (preferred).
2. Prick vein with a needle

   IMPORTANT —> do not go through both vein walls in patagium, only the top one; (only in uropatagium can you prick needle through both walls).
3. Allow blood to pool (usually happens very quickly).
4. Collect blood by placing the capillary tubes or microtainer below the pooled blood (a downward angle will allow the blood to be more easily pulled into the capillary tube or microtainer).
5. Collect 1-2 capillary tubes or at least 0.2 cc's in a microtainer.

   IMPORTANT —> only fill capillary tubes a minimum ¼ to ½ maximum.
6. Hold a fresh cotton swab on the collection area until bleeding has stopped (typically within 10 seconds).
   - If using Whatman cards, select one of the capillary tubes, prior to sealing, to be used to deposit blood onto the card.
   - Store card in a plastic bag with desiccant packets.
7. Use either Critocaps (preferred) or Critoseal to seal each end of the capillary tube.
8. Place capillary tubes in a vacuum vial and properly label all vials.
9. Store all blood samples in cooler with ice packs while in the field.
10. Transfer samples to a freezer for storage as soon as possible; they must remain frozen until analysis.

1.3. Blood Sampling Labeling Format

Include the following information:

1. Date of collection
2. Species
3. Age and sex
4. Identification number (ID #)
5. Sampling location (i.e., river or lake name)
6. State or Province

IMPORTANT:

All samples MUST have a unique sample I.D. # that is labeled correctly and legibly.

Figure 1. Direct draw blood collection from the cutaneous ulnar vein using a manual syringe

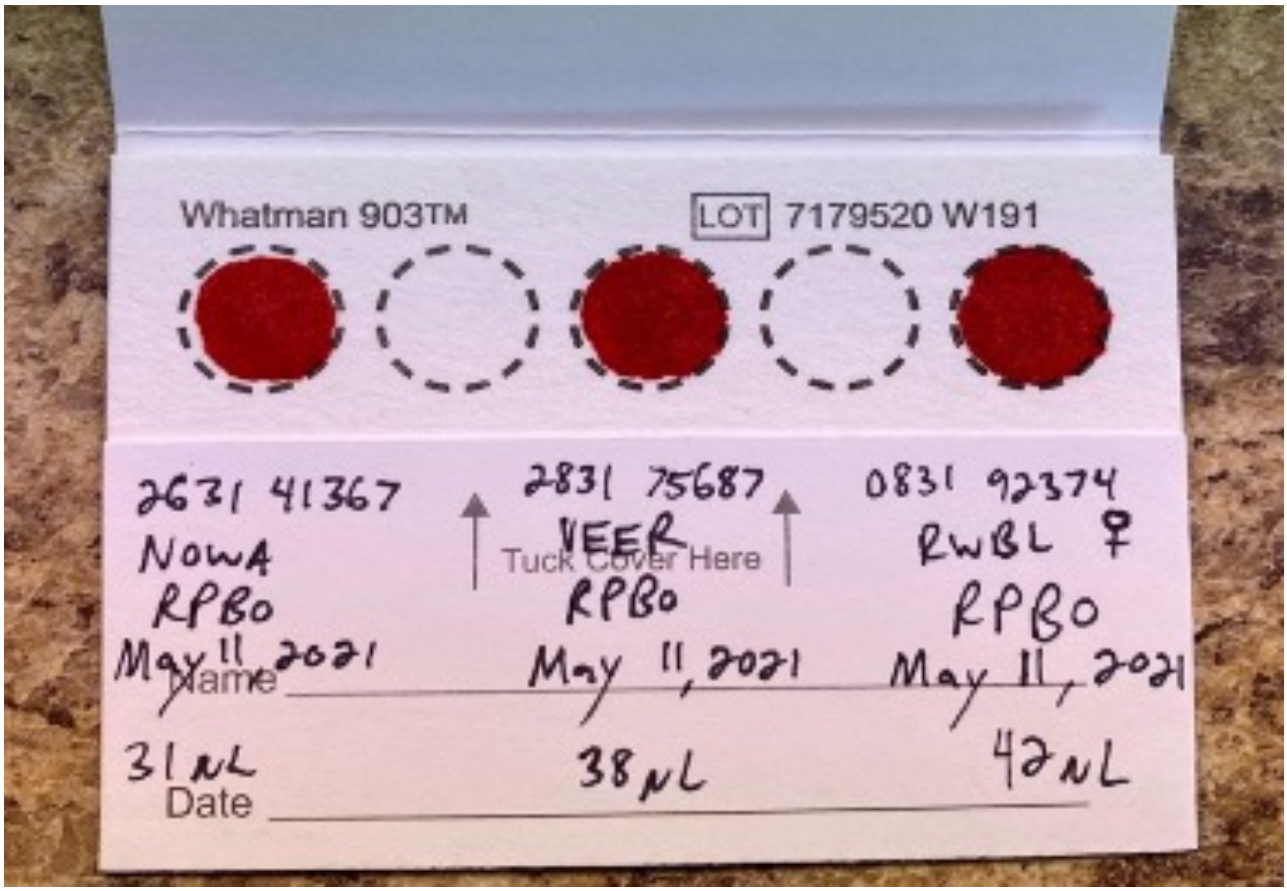

Figure 2. Completed Whatman card with appropriate metadata filled in.

2.0 Fur Sample Collection

The collection of fur samples is useful in identifying the body burden of heavy metals, such as mercury, because methylmercury is typically transferred to fur during fur growth (Evers 2018). Collected samples will provide important information in developing awareness about potential risks associated with mercury pollution. It is important to make sure all samples are collected in a safe and clean manner. Collaborating organizations are asked to record basic information for each sample. Sampling bat fur is indicative of chronic mercury exposure in bats.

2.1. Supplies for Fur Collection

- ☐ Data sheet
- ☐ Map of country/sampling site
- ☐ Stainless steel cuticle scissors
- ☐ Coin envelope, small plastic zip lock style bags, vials
- ☐ Permanent marker & ballpoint pen

2.2. Fur Collection Procedure

1. Record bat species and the common name on the datasheet.
2. Collect a fur sample using small stainless-steel cuticle scissors.
3. Clip fur from the belly and back and other locations as needed. Clip as close to the skin as possible without cutting the bat. The fur follicle does not need to be harvested. It will take 4-5 scissor swipes (~0.01 grams of fur).
4. Store fur in individually labeled sample containers (e.g., coin envelopes, plastic zip lock style bags, vials).
5. Ensure scissors are cleaned between each bat sampled by wiping the scissors with alcohol swabs and visually inspecting them to make sure there is no fur from a previous bat to avoid cross- contamination.
6. Samples do not need to be refrigerated or frozen

2.3. Fur Sample Labeling Format

Follow this format for labeling fur samples:

- ☐ Use an alpha-numeric code for species [conventional 2-letter code]
- ☐ Month, Day, Year
- ☐ Species (Latin name)
- ☐ Location (Site, Town, County, State or Province, Country)
- ☐ Length of forearm (mm), body mass (g),
- ☐ Age (AD = adult; J = juvenile),
- ☐ Sex (M=male; F = female),
- ☐ Reproductive condition for females (P = pregnant' L= lactation; PL = post-lactation; NR = non-reproductive),
- ☐ Reproductive condition for males (length and width of testes)

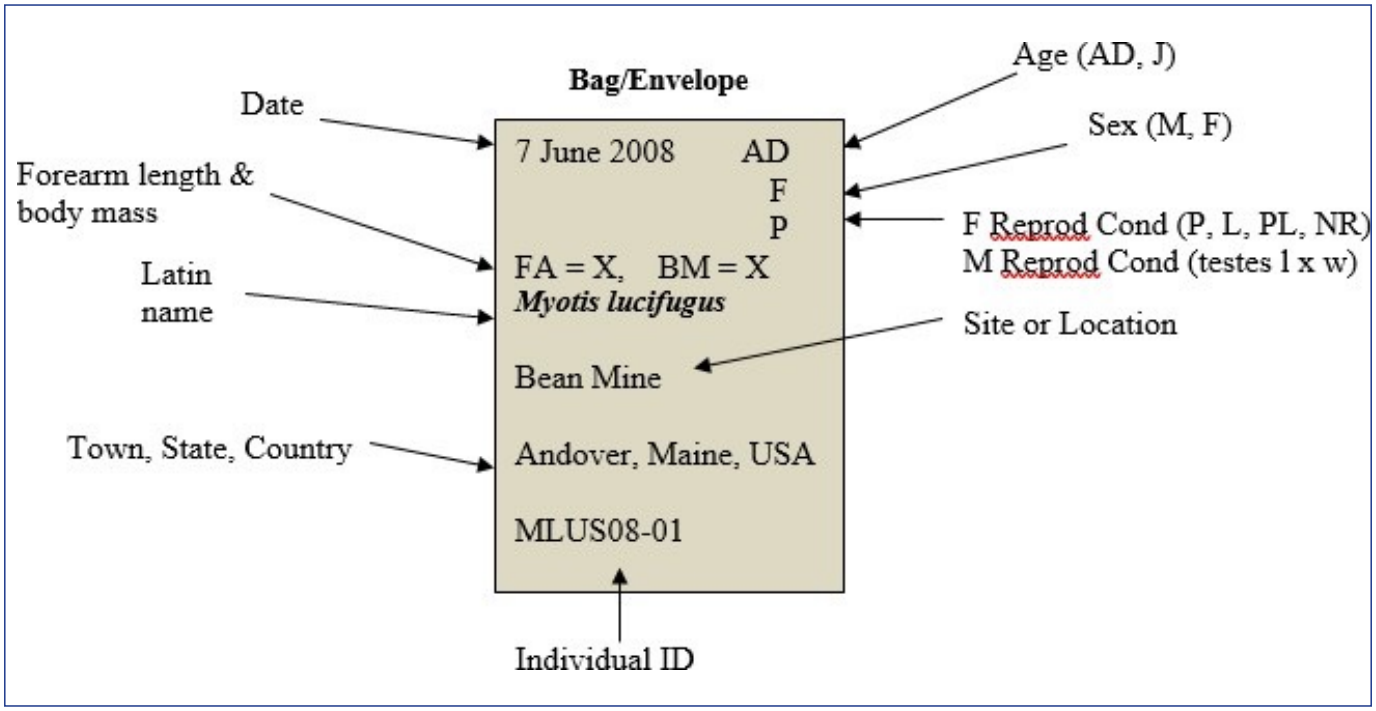

Figure 3: Correct labeling convention for individual samples to be shipped.

3.0 Procedural Tables

Table 1. Tissue sampling procedural flowchart for mercury analysis. Tissue selection is dependent on the focal taxonomic group(s) and research objective(s).

Desired tissue	Collection method	Suitable focal taxa	Receptacle	Minimum Amount	Storage
Blood	Piercing	All taxa	Lithium heparinized capillary tube	0.4 µL	Freezer
			Whatman card	1 card	Refrigerator or room tempera-ture
Fur	Clipping	All taxa	Paper coin envelope	2 symmetrical feathers	Refrigerator or room temperature

4.0 Metadata Requirements

Please clearly print at least the following information on each archive vial, paper coin envelope, or sample tag using a permanent marker:

- ☐ Band number, if applicable
- ☐ Species common name (please also include species Latin name if collected outside of the U.S.)
- ☐ Date (please use letters for the month instead of numbers, i.e., Mar 11, 2021)
- ☐ Sampling location name, state or province, and country
- ☐ Age and sex of individual (i.e., "After Second Year, Male"), if applicable

Please organize all metadata using the preferred templates and submit to BRI via the forms.

IMPORTANT: If contributing samples to BRI or another research group/agency, initiate collaboration at the earliest convenience.

Send any accompanying banding data for all sampled bats as an Excel spreadsheet or .csv file. A copy of these data sheets can be obtained from josh.guilbert@gmail.com.

The identification of individual bats can be achived via photo ID of one or both wings. Collecting this data is advisable if banding is not possible or if the work is conducted in an area where photo ID has been done in the past. Take a clear ventral side photo of the outreach wing with the collagen fibers visible. Note the photo numbers on the datasheet.

IMPORTANT:
If a bat is unable to be banded in the field, the individual and the corresponding sample MUST be given a unique identification number (i.e., "Organization-Country-Unbanded0001"), which should be available on both the sample receptacle and data sheet(s).

5.0 Storage of Samples

Please follow these requirements:

- Whole blood samples in capillary tubes or vacutainers should be immediately stored in a cooler with ice or ice packs in the field. IMPORTANT: blood samples should then be transferred to a freezer AS SOON AS POSSIBLE, or within 24 hours of collection, and should remain frozen until analysis. While heavy metals, such as mercury, in blood are stable, freezing samples prevents blood degradation.
- Dried blood spots on Whatman cards should be stored with a desiccant packet in separate sealed plastic bags (to reduce the influence of moisture). Ideally, these should be stored in a 4°C refrigerator (to prevent molding), but can also be stored at room temperature away from direct sunlight prior to shipping and analysis.
- Fur sample envelopes should ideally be stored in a sealed plastic bag in a 4°C refrigerator (to prevent molding), but can also be stored in a sealed plastic bag at room temperature away from direct sunlight prior to shipping and analysis.

6.0 Shipment of Samples

If you are submitting samples to BRI for collaboration, *thank you for contributing!* At least two weeks prior to shipping any samples, please complete the metadata submission form to allow sufficient time for BRI to file the proper permits. BRI will send all completed forms required for transport once the permits have been issued. After all other permits have been approved (see 7.1. Required Permits), please schedule a shipment with your carrier service of choice.

To package the tissue samples, please follow these instructions:

- Use a small cooler to secure and insulate all blood samples during shipment.
- Place ice pack(s) inside the cooler to insulate the frozen blood.
- If archive vials are glass, it is **IMPORTANT** to pad the samples with bubble wrap or newspaper inside the cooler to avoid breakage.
- Fur and dried blood spots on Whatman cards do not need special packing and do not need to be kept cold (if shipped within 3–4 months after collection).
- Place the cooler in a cardboard box and fill the empty space with additional packing materials, such as bubble wrap or newspaper, to secure the cooler during shipment.
- Include a set of all necessary permits and forms at the top of the packing material before sealing the package with packing tape (see 7.0. Required Permits). Attach a second set of permit copies in a plastic pouch on the exterior of the package.

Please include the following details on the shipping label and package exterior:

Biodiversity Research Institute
276 Canco Road
Portland, Maine 04103 USA

WILDLIFE :: USFWS :: MBTA

EXTRA COPIES OF DOCUMENTS INSIDE BOX

IMPORTANT:
To avoid postal or customs delays, ship samples on a Monday or Tuesday, and never just before a federal holiday.

When asked by the shipping representative if you are shipping anything perishable, answer **NO**.

7.0. Required Permits

For shipments within the United States, please include the following:

- A copy of the collector's federal USFW Threaten and Endangered permit.
- A copy of the collector's state scientific collecting permit.
- A copy of the importer's USDA import permit
  (not required if samples were collected within the U.S.)

For shipments outside of the United States, please include the following:

- A copy of the importer's USDA import permit*
- A copy of the importer's CDC permit.
- USFWS Form 3-177 (Declaration of importation or exportation of fish or wildlife)
- FedEx Declaration of Biological Shipments*
- FedEx Commercial Invoice
- CITES export permit, if applicable
- A copy of the origin country's export permit, if applicable

*Forms provided by BRI via email - see contact information below

IMPORTANT:
Shipments arriving in the United States may be denied entry, destroyed, or returned if they do not include the appropriate permits. For further questions or clarifications, or if you are interested in collaborating, feel free to contact BRI via phone, email, or visit us at our website:

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8.0 References

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BAT Metadata Form

SITE:_____ COUNTRY:_____ X:_____ Y:_____

DATE (YYYY-MM-DD) PAGE: OF STAFF:

		Recapture?	CODES (U)nknown	SEX: (M)ale, (F)emale AGE: (A)dult, (J)uvenile			MALE REPRODUCTIVE: (N)on-Reproductive, (TD)Testes Descended FEMALE REPRODUCTIVE: (N)on-Reproductive, (L)actating, (PR)egnant, (PO)st-Lactating							Net Info	
				Demographic Information											
	TIME		BAND #	SPECIES GGG SSS	AGE	SEX	REPRO	Weight (g)	Forearm (mm)	Ear (mm)	Tragus (mm)	Tail (mm)	Wing Score	#	Height M
#	23:03	Y	BRM42069	TAD BRA	A	F	PR	11	43	19	10	16	0	A	2
1															
2															
3															
4															
5															
6															
7															
8															
9															
10															
11															
12															
13															
14															
15															

Wing Score 0: <5% splotches, no discolored flaking forearm, no necrotic tissue, no holes, membrane intact.

Wing Score 1: 5-50% splotches, discolored flaking forearm, no necrotic, no holes, membrane intact

Wing Score 2: 50-90% splotches, discolored flaking forearm, few areas of necrotic issue, small holes <50 mm in diameter holes, Necrosis edges no loss of area

Wing Score 3: >90% splotches, discolored flaking forearm, Abundant necrosis, large holes >50 mm in diameter holes, Noticeable loss of membrane area

Discolored flaking forearm alone automatically scores Wing Score of 1