

SOP - Collection of Blood and Feathers for Contaminant and Disease Sampling

April 2010

BLOOD COLLECTION

Whole blood

The collection and analysis of whole blood samples in birds is used to identify the recent dietary uptake of mercury (Hg) and other contaminants. Direct draw using a manual syringe is typically used on sea ducks. Blood can be drawn from any location and can be determined by the biologist/veterinarian on site. Typical sites are the cutaneous ulnar (wing)(Fig. 1), jugular vein (Fig. 2), or caudial tibial (leg). The **jugular is preferred**; wing vein is a potential, but probably will have to control bleeding afterwards).

Collect 2 cc of whole blood from each bird. A minimum of 1.0 cc is needed for archive purposes, useful in analyses such as metals, and possibly stable isotopes. A minimum of 0.2 mL is needed for Hg analysis.

Maximum recommended blood collection is 1% of body weight; a potential issue for small birds but generally not for sea ducks. Unless there are specific requests for larger volumes, we will be collecting only 2 cc from each bird.

Target sample sizes: Initially, 50-75 samples for each species per capture site.

Blood Drawing Procedure:

- Sterilize collection area with a cotton ball and isopropyl alcohol.
- Insert needle parallel with the vein.
- Draw **2 cc** of blood.
- Remove needle from vein and place needle in sharps container.
- Place a clean dry cotton ball on collection area to stop bleeding.
- Remove needle from syringe, then inject 1 cc of drawn blood into one 1.3 cc heparinized vial and the remainder into another 1.3 cc heparinized vial . Do not squirt the blood through the needle into the tube. **IMPORTANT: AVOID FILLING VIALS COMPLETELY AS THE TOP WILL POP OFF UNDER PRESSURE. HALF FULL VIALS IS PLENTY.**
- Invert vials slowly (do not shake) at least 6 times to mix blood with heparin to prevent clots.
- Label the 1.3 cc vials with a permanent Sharpie® marker.
- Store blood samples in a cooler with ice packs in the field and transfer to a freezer within 24 hours of collection.
- Store vials at standard temperature freezer (-20C)

Figure 1. Blood collection from the cutaneous ulnar.



Figure 2. Blood collection from the jugular

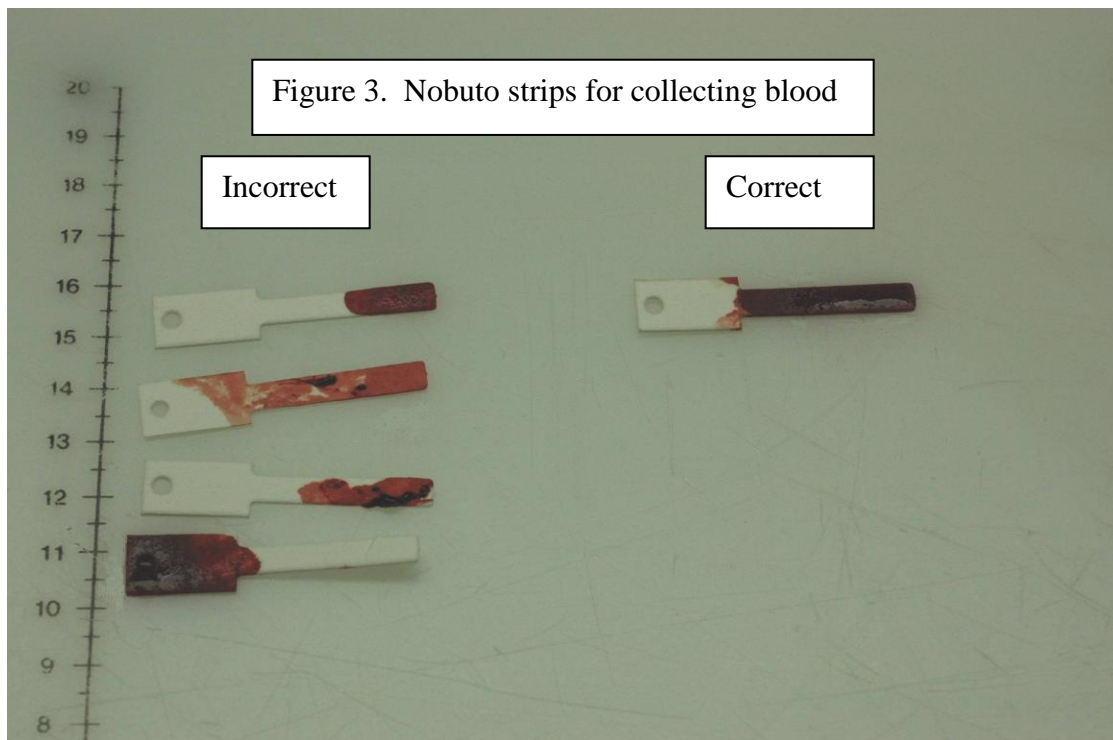


Collection of blood using Nobuto strips: Nobuto strips are filter paper strips (Fig. 3) for storing whole blood or extracting serum **for disease testing**.

Note: the following procedure can be done in the field at time of blood collection, or if more convenient, strips taken indoors from blood in vials but before freezing the blood samples.

Collect two strips from each bird, if possible. Dip the narrow end of the swab in the blood of the bird from one of the filled 1.3 cc vials. Allow the blood to soak up until it reaches the wide end of the swab. Once the narrow end of the swab is soaked with blood, remove from blood. If possible, allow the swab to air dry by propping them on something (e.g. egg carton or within the dividers in a cryovial box) so that air circulates all around them. Once dry, place into a 9cc archival tube. If it's not possible to air dry at time of collection (e.g., field setting), then place both strips wet into a 9cc archival tube and label the tube (see below). Freeze at standard freezer temperature (-20C).

Target sample sizes: 50-100 samples for each species per capture site.

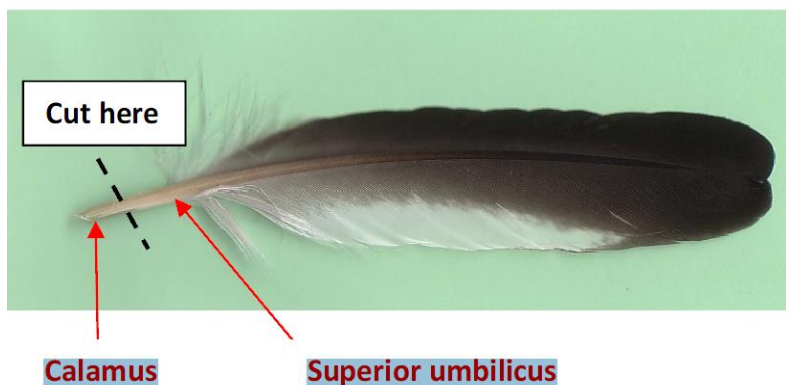


FEATHER COLLECTION

Feathers will be collected for stable isotope analysis (see separate protocol) and also for identifying the body burden of Hg in birds. Mercury is transferred to growing feathers during the molting period. Any feather can be analyzed for Hg (including shed feathers), but adult secondaries are a useful standard. Secondaries should be cut above the superior umbilicus (unless the bird is molting and it is easily plucked). For some species, secondaries may not be feasible and therefore symmetrical collection of tail feathers is recommended.

The second secondary feather should be clipped from each wing (i.e., two total feathers). The second secondary feather should be determined from where the primaries and secondaries meet in the middle of the wing (if difficult to determine, 12th feather from the wing tip). Each feather should be clipped along the calamus (shaft), well below the end of the feather vane (i.e. below the superior umbilicus on the calamus), located near the skin (Fig. 4). Feathers should be placed in coin envelopes and kept dry.

Figure 4. Standardized field clipping of secondary flight feathers.



STORING FEATHER SAMPLES

Feather samples should be placed in the labeled coin envelope and stored in a dry place. Put a packet of silica crystals (as a desiccant) in the Ziploc containing the feather envelopes.

LABELING BLOOD AND FEATHER SAMPLES

Each vial and envelope used should be labeled to include:

- * Band #
- * Tissue type
- * Date
- * Species
- * Age/sex
- * Sampling Location

SHIPPING SAMPLES

- All blood samples should be shipped in a plastic cooler with an enclosed master packing list. Use attached form. An emailed Excel file containing the collection data is also acceptable. Use packing or duct tape to seal the cooler.
- If samples are shipped Next Day Air, use frozen blue ice packs for blood samples. The receipt of these samples after two days is acceptable. Contact recipient to make sure their office is open to receive samples as scheduled.
- Feathers do not need special packing and do not need to be kept cold (if shipped within 3-4 months after collection).

Send one 1.3 cc tube (in 9cc archival tube) (blood for archival) to: **To be Determined**

Send one 1.3 cc tube (in 9cc archival tube) (blood for Hg) and feather samples (for Hg) to: Biodiversity Research Institute, 19 Flaggy Meadow Road, Gorham, Maine, USA. (207-839-7600/7655 tel/fax) (lucas.savoy@briloon.org)

