

UCFWO SOP: Collection and Preservation of Blood from Small Birds for Laboratory Analysis  
Date Prepared: 6-4-02  
Date Revised: 6-10-03  
File Number: 1019.3765

## I. General

This standard operating procedure (SOP) outlines methods to collect blood from small birds (e.g., passerines) for analysis of blood chemistry and physiological parameters. Blood chemistry may include analysis of heavy metal content (e.g., lead), and analysis of physiological biomarkers such as packed cell volume (i.e., hematocrit) and delta-aminolevulinic acid dehydratase (ALAD) enzyme activity. This SOP outlines methods for blood collection and for measurement of packed cell volume by U.S. Fish and Wildlife Service, Upper Columbia Fish and Wildlife Office (UCFWO) personnel. Other blood chemistry and physiological parameter analysis will be conducted by other analytical facilities; methods for those analyses are outlined by those laboratories. This SOP is in addition to established procedures for bird collection and initial measurements on those birds. The SOP for bird collection is identified in the sampling and analysis plan or protocol that identified this procedure.

This SOP is intended to provide non-lethal methods of blood collection. However, blood collection from small birds is a difficult procedure that may result in some level of mortality. Birds that do not survive the procedure will be used for other tissue collection and analysis procedures to maximize the information gathered from those birds. The SOPs for tissue collections are identified in the sampling and analysis plan or protocol that identified this procedure.

## II. Equipment

Copies of approved State and Federal permits for bird collection  
25 to 27g hypodermic needles  
1 or 3 ml syringes  
Sodium heparin  
Powder free gloves  
Cryogenic tubes  
Hematocrit tubes  
Critoseal clay  
Centrifuge capable of spinning hematocrit tubes  
Power inverter for centrifuge and lights  
Work table and chairs  
Packed cell volume hematocrit tube reader

Ethanol  
Alconox  
Reagent-grade nitric acid  
Squirt bottles  
Deionized water  
Ethanol and deionized water squirt bottles  
Dissecting tools (scalpel, dissecting forceps (stainless), dissecting scissors (stainless))  
Dissecting tray  
Work lights (head or table mounted)  
Cooler  
blue or wet ice  
Liquid Nitrogen and dewer with sample holders  
Permanent black ink pins  
Data forms  
Paper towels  
Kimwipes

### III Jugular vein procedure

The jugular vein procedure will be the first attempted on each bird. This procedure is typically non-lethal to the bird.

Prepare the equipment for the procedure. Lay out dissecting equipment so that the tools are ready for use. Prepare a syringe for use by attaching the correct gauge needle, rinse syringe with sodium heparin, and slightly bend the needle so the opening of the needle can be inserted toward the vein. Place a new, clean paper towel (Kimwipe) on the dissecting tray. Ensure the tools and area have been cleaned with the decontamination procedure (see below) prior to handling the bird. Wear new powder-free gloves for each bird.

Remove the bird from the holding bag. Record the general information required from other SOP's outlined in the sampling and analysis plan or study protocol. Verify that the bird had not been sampled previously (by tail feather clip, see below).

The bird is held in one hand with the bird's right side up. The bird is secured in-hand to minimize movement, and the head is held between the fingers to gently extend the bird's neck. Gently blow on the neck to expose the unfeathered area on the lateral side of the neck. Once this area is found, apply a small amount of ethanol to the area to wet the feathers, sterilize the area, and expose the jugular vein. Pierce the skin directly over or beside the vein with the needle. Provide gentle suction on the needle by pulling the syringe plunger to 0.03 to 0.04 ml. Gently but decisively pierce the jugular vein; when the jugular vein is pierced, blood will enter the neck of the syringe. Extreme care must be taken to avoid pushing the needle through the vein and avoid excess lateral movement which could rupture the vein. To assist with this requirement, brace the hands against each other. Once blood is observed in the syringe, gently and slowly pull the syringe plunger to pull blood into the syringe. Do not pull faster than blood can enter the syringe; at no time should more than 0.1 ml of air space be between the syringe plunger and the blood

level. Extract a minimum of 0.45 ml from all songbird species sampled. Gently remove the needle and apply gentle pressure to the puncture site for 30 seconds to 2 minutes. After this recovery time, look for any excess bleeding either through the skin or under the skin; reapply pressure to the puncture site for another one to two minutes if continued bleeding is observed. If continued bleeding is observed under the skin, the vein has ruptured which is a fatal condition. Under these conditions, euthanize the bird by applying pressure on the sternum until the bird stops moving. Collect necessary tissues as indicated by the study protocol, and dispose of the remainder of the bird.

If bleeding stops, clip feather on the tail and offer sugar water solution to the bird by placing a drop of the solution on the side of the bird's beak. Continue to provide the solution until the bird rejects. Observe the bird behavior, release when the bird is alert and in apparent good condition. If the bird appears severely stressed (closed eyes, shivering, limp, otherwise not alert), place the bird back into the holding bag for 10 to 15 minutes to determine if the bird will survive. Remove surviving birds from the holding bag, clip the lateral tail feather on the right side, and release.

If the jugular vein procedure for blood collection is not successful (either by jugular vein rupture or technical problems), euthanize the bird and quickly prepare the bird for the cardiac puncture blood collection method outlined below.

#### IV Cardiac puncture method

The cardiac puncture method will be used if insufficient blood is collected from the jugular vein or if the jugular vein procedure ruptures the vein (see above). Alternatively, this method may be used to collect a replicate sample on birds that do not survive the jugular vein method but adequate blood is collected in that procedure.

In this procedure, live birds are euthanized by gently pressing on the sternum until movement stops. This euthanasia procedure quickly suffocates the bird without compromising physiological measurements that may be made.

Place the dead bird on its back on the dissecting tray. Gently blow on the breast of the bird to expose the unfeathered area along the sternum. Squirt ethanol on this area to dampen the feathers and sterilize the area. Cut the skin from the posterior region of the sternum to the abdomen with a scalpel. Cut the musculature with dissecting scissors at the edge of the breast muscles to expose the peritoneal cavity. Gently lift the breast from the cardiac region and cut the connective tissue with the scalpel or scissors. Insert the hypodermic needle into the heart and extract the required blood. This procedure must be completed rapidly after the bird dies. Deceased birds will be disposed of properly as indicated on state and federal bird collection permits.

#### V. Blood handling after collection

Once blood is collected, the needle will be removed (and placed into a sharps container), and the hematocrit sample will be collected from the syringe. Insert the hematocrit tube into the syringe

barrel and fill the tube one third to one half full. Place your finger on the clean end of the tube to prevent blood loss from the tube. Gently invert the tube and release your finger to allow the blood to flow approximately 5 mm from the fill end of the tube. Recap the tube with your finger, then insert the fill end into critoseal clay to seal that end of the tube. Record the location number of the tube in the clay tray.

Blood remaining in the syringe will be transferred to cryogenic tubes. To transfer blood to cryogenic tubes, first label and preweigh each cryogenic tube to at least 0.001 g accuracy. Wear clean powder-free gloves for labeling and handling of vials because oils from hands can change vial weights. Make certain the balance is level and a stable “zero” is obtained. Weigh the cryogenic vial with cap, also ensuring stable readings. Invert the syringe and gently tap the syringe so all blood collects against the syringe plunger. Note the total blood volume in the syringe. A minimum of 200  $\mu\text{l}$  is needed for blood lead and 150  $\mu\text{l}$  is needed for ALAD analysis. The following table is a guide to target volumes needed for each sample type.

<b>Target volumes for each sample type</b>		
<b>Total volume (<math>\mu\text{l}</math>) collected after hematocrit removal</b>	<b>Blood lead sample target volume (<math>\mu\text{l}</math>)</b>	<b>ALAD sample target volume</b>
< 350 $\mu\text{l}$	entire sample	not collected
350 to 400 $\mu\text{l}$	200 $\mu\text{l}$	150 to 200 $\mu\text{l}$
400 to 600 $\mu\text{l}$	200 to 400 $\mu\text{l}$	200 $\mu\text{l}$
600+ $\mu\text{l}$	300+ $\mu\text{l}$	300 $\mu\text{l}$

To transfer blood to the cryogenic tube, introduce a small amount of air into the syringe and gently tap the syringe to move most of the blood away from the plunger. This procedure will allow most of the blood to be expelled from the syringe. Note volume of blood in the syringe and only transfer the target amounts as indicated above. Check zero on balance, then weigh the cryogenic tube, cap, and blood sample to the nearest 0.001 g. Ensure both the zero and the weighed sample are recorded at stable readings. Record data on data sheets as indicated. After weight is collected, immediately place blood sample into liquid nitrogen for preservation.

#### VI. Hematocrit analysis

Several hematocrit samples can be collected prior to centrifugation. Place the centrifuge tube into the centrifuge. Plug the centrifuge into the power inverter connected to the vehicle or other 110V power supply. Centrifuge the samples for one minute. Remove and read the packed cell volume with the percent hematocrit reader card.

#### VII. Dissection tool cleaning procedure

All dissecting tools and trays contacting bird tissue must be cleaned prior to use with another bird. The solutions used for cleaning include a weak alkanox soap solution, 5% nitric acid solution, and deionized water, all in squirt bottles. The weak alkanox soap solution is sprayed on the tool and the edges of the tool are gently scrubbed with a kimwipe. The tool is sprayed with deionized water to wash soap off. The tool is rinsed with nitric acid solution, then rinsed with deionized water and placed back onto a clean kimwipe at the dissecting area.

The alkanox solution is made in one liter increments by placing approximately one gram of alkanox in the pre-labeled bottle, and diluting with tap water. The nitric acid solution is also made in one liter increments by filling the bottle 50% full with deionized water, adding 50 ml of reagent-grade concentrated nitric acid, then filling the bottle to the neck with deionized water. DO NOT add acid first, a violent reaction will result when the deionized water is added.

#### VIII. Sample handling and documentation

Hematocrit samples can be “stockpiled” for approximately 2 hours prior to centrifugation. After centrifugation and recording the packed cell volume, the used hematocrit tube will be placed in a safe “sharps” container to avoid injury.

The blood sample in the cryogenic tube is placed into liquid nitrogen immediately after collection. The sample ID on the cryogenic tube and data sheet are verified for accuracy prior to placing into liquid nitrogen. The sample ID will be written on chain of custody forms and verified against the data sheet prior to starting procedures on the next bird.

Data sheets will be placed into a dedicated three-ringed binder immediately after being completed. Data sheets will be photocopied weekly and copies placed in an archive three-ringed binder that is kept at the UCFWO.

#### IX. Safety

Powder-free gloves will be worn during the procedure to avoid contaminating samples and as a safety procedure for field technicians.

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