

## PROTOCOL FOR ACQUIRING AVIAN BLOOD SAMPLES

The following procedures describe and refer to methods of collecting avian blood samples. Bleed within about 5 minutes of retrieval, if possible, to minimize stress to the bird via one of the three methods outlined below.

### Collection from Toe Clip:

1. Support leg of bird at knee joint and clip an accessible toe nail with nail clippers, scissors, or other appropriate instrument. Cut should extend to beginning of vascular area in the nail bed, but no deeper than necessary. The nail clippers, scissor blades, or other instrument used must be cleaned in disinfectant before each use.
2. Apply drop of blood forming at the site of the nail clip directly to a glass slide and make a blood smear or collected into a capillary tube.
3. Any residual bleeding can be stopped with a cotton swab which has been dipped in styptic powder, with silver nitrate sticks, or electro cautery.

### Collection from Wing Vein:

1. Place the bird in the right hand with back to palm and left wing extended with index and middle fingers.
2. Moisten the wing joint with a cotton swab that has been dipped in 70% alcohol. Use swab to move feathers away from the joint so that the brachial vein is exposed.
3. Lightly prick the brachial vein with a 27 gauge needle and immediately collect the blood by inserting one end of a Fisher Red-tipped, heparinized microhematocrit capillary tube into the bubble of blood or collect blood with a 26, 27, or 28 gauge needle on a 0.5 or 1 cc syringe. Hold the tube at a slight decline from the wing to maximize flow into the tube. Fill the tubes to within about 2 cm of the end (2/3 - 3/4 full). Seal each tube on the blood-drawing end with critoseal. Place the tube on wet ice until centrifugation (i.e., do not freeze). It is best to centrifuge the blood immediately, but it may be kept on ice or refrigerated for longer if necessary (usually no more than 24 hours later, however). If you create a hematoma (blood clotting under the skin), you can often get the blood flowing again by rubbing back and forth over the wing vein with your thumb.
4. Collect one microhematocrit tube per 10 grams of body weight so that no more than 1% of the body weight is removed.
5. Stop any residual bleeding with a cotton swab which has been dipped in styptic powder.
6. Centrifuge the tubes at about 13,400 G for 5 minutes in a centrifuge with a microhematocrit tube rotor head (with the sealed ends pointed outwards).

7. After centrifugation, measure hematocrit as the ratio of the length of the red blood cells in the tube of the total length of blood in the tube.
8. Saw the tube with the edge of a small file just at the line between the erythrocytes and the plasma portions. Break the tube at this line (after a very small nick is placed in the tube it breaks cleanly, and easily, at the site of the nick).
9. Lightly blow the plasma half into a “nunc” or other cryogenic tube (if the blood is to be stored in liquid nitrogen). Use a syringe or pipette to blow the erythrocyte half into a different nunc tube. Label the tubes and place in dry ice or liquid nitrogen, or into a freezer if one is available.

#### Collection from Jugular Vein:

NOTE: Only persons trained in the removal of blood from the jugular vein are authorized to employ this procedure. These persons must also be knowledgeable in the use of antishock therapy.

1. Place bird in hand with back to palm, index and middle fingers placed on either side of neck with tips pressed against mandible.
2. Gently stretch neck with index and middle fingers to expose feather tracts on either side of right jugular vein.
3. Wet feathers with a cotton swab moistened with 70% alcohol to reveal the featherless space between feather tracts.
4. Gently depress neck area with thumb at a spot immediately anterior of point of folded wing joint to restrict jugular flow and raise vein.
5. Insert 26, 27, or 28 gauge needle on a syringe into the jugular vein and withdraw no more than 0.1 ml of blood per 10 grams of body weight (no more than 1% of body weight).
6. Remove needle and apply light pressure with thumb for 30 seconds or as long as required to prevent formation of hematoma.

NOTE: *The above information was extracted from protocol adapted by the U.S. Fish and Wildlife Service, National Biological Survey, 1849 C Street, N.W., ARLSQ 725, Washington, D.C. 20240 (202/208-6394).*