Section 5.2.10. Avian Sampling Methods

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Objectives: To safely collect biological samples from live and dead wild birds.

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The authors assert that animal capture and sampling should always occur in compliance with all applicable laws and regulations and should only be undertaken after securing all necessary permits and approvals, including ethical approvals.

For more information about the contents of this guide, please contact predict@ucdavis.edu.

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Section 5.2.10a. Confirmation of Knowledge

When you are familiar with the information in this guide, take the PREDICT quiz ***Section 8.4.9. Avian Sampling***.

Section 5.2.10b. Brief Overview of PPE

Minimum PPE Required for Handling Live, Dead, or Samples of Birds

The minimum PPE for bird sampling includes:

1. Designated clothing
2. Closed-toed shoes
3. Nitrile gloves
4. Protective glasses
5. N95 facemask for self-protection and to avoid contaminating samples.

(See the ***Biosafety and PPE Guide (Section 4.)*** for detailed instructions regarding PPE Use)

Section 5.2.10c. Avian Data Collection

Please refer to the following three templates for required data collection:

1. P2 Animal Data Collection Form
2. P2 Site Characterization Data Collection Form
3. P2 Specimen Data Collection Form

For more information on downloading templates from EIDITH see ***Section 5.2.3. General Data Collection Templates and Applications***.

Biometric Measurements

The P2 data templates mentioned above are required to be filled in. Additional biometric measurements may be collected at the discretion of the sampling party.

For many bird species, the sex or age of a captured individual may not be immediately evident with a simple visual inspection. Subtle but significant differences in morphology are often useful for differentiating between sexes and age classes.

Thus collecting biometric measurements can have important applications in disease sampling studies for determining differential infection or exposure rates based on sex or age.

Biometric measurements to be collected (optimal):

- Weight
- Culmen (bill) length and depth
- Tarsus length
- Wing length
- Tail length

Additional important data to establish breeding or physiological status of the bird:

- Presence of brood patches
- Moult stage

Age class:

- Usually the exact age will not be known and individuals should be assigned to a juvenile or adult age class

For collection methods for biometric measurements refer to FAO manual "Wild Birds and Avian Influenza: an introduction to applied field research and disease sampling techniques" (http://www.fao.org/docrep/010/a1521e/a1521e00.htm)

Photographs for Bird Identification

(Reference: European Commission DG Sanco 2006)

Digital photographs should be taken of each individual. The bird should fully fill the photographic frame, and wherever possible the image should include a ruler or other scale measure.

Photographs should be taken of:

- The whole bird, dorsal side, with one wing stretched out and tail spread and visible;
- The head in profile clearly showing the beak;
- Close-up photos of the tips of wing feathers can often determine whether the bird is an adult or a juvenile (bird in its first year);
- Ventral photographs should show the legs and feet (since leg color is often an important species diagnostic). If any rings (metal or plastic) are present on the legs, these should be photographed in situ as well as recording ring details.
- Any conspicuous markings/patterns should be photographed.

In late summer many water birds, especially ducks and geese, undergo moult and can be especially difficult to identify by non-specialists. At this time of year there is particular need for clear photographs to aid identification. The patch of color on the open wing's secondary feathers (called the "speculum") is often especially useful.

Section 5.2.10d. Avian Sample Collection

Samples are to be collected in duplicate from each animal. One sample must be collected into Trizol and one into viral transport media (VTM). Tubes must be labeled with a unique identifier number. Printed labels should be used (please see ***Section 5.2.3. General Data Collection Templates and Applications***).

The following basic set of samples should be collected from each animal where possible (If only one sample can be collected, then place into VTM):

1. **Two oral swabs** - one in 500 µL VTM and one in 500 µL Trizol
2. **Two cloacal swabs** - one in 500 µL VTM and one in 500 µL Trizol
   and/or
   Two fecal samples - one with max of 500ul/0.5cc feces in 500 µL VTM and one with max of 500ul/0.5cc feces in 1 mL Trizol
3. **Two whole blood samples** - one with max of 500 µL of whole blood in 500 µL VTM and one with max of 500 µL of whole blood in 500 µL Trizol
4. **Two serum samples** - 2 x 0.5ml aliquots frozen without media

Freeze all samples (except tissue in formalin) in liquid nitrogen immediately in the field and transfer to -80°C freezer once back in the lab.

If there is no **short-term** access (i.e., within 24 hours) to cold chain such as in an emergency situation, then samples can be collected in 500 µL of RNAlater instead of Trizol and VTM. Storage times and temperatures for samples in RNAlater are as follows:

- 1 day at 37 °C (i.e., ambient temp)
- 1 week in the refrigerator
- Within one week freeze at -80°C for storage until analysis

Details on Sample Collection and Storage Media

1. **Two oral swabs**: 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 (see Figure 1).

   Place 1 swab in a cryovial filled with 500 µL Trizol and use alcohol-wiped (or ethanol-wiped), flame-sterilized scissors to cut the shaft of the swab above the tip. [Note: If the plastic shaft can be snapped, then scissors are not necessary and the risk of cross-contamination is reduced. To snap the swab, lift the swab a little above the bottom of the vial then snap it. This will ensure the swab will not block the cap]. Place the other swab into 500 µL of VTM (= maximum final ratio of 1:1) in a cryovial.

Figure Avian 1: Oral swab sample collection from a bird *(Photo credit: Taronga Zoo/Karrie Rose from FAO Wildlife bird highly pathogenic avian influenza surveillance manual)*

Store in a liquid nitrogen dry shipper or dewar and transfer to -80˚C freezer when possible.

2. Two cloacal swabs/fecal samples: Gently and slowly insert the head of the swab into bird's cloacal cavity (cloacal cavities of small birds can be very shallow; thus the swab head should not be inserted very far into the cloaca). Swabs can be moistened with sterile saline prior to animal sampling. **Do not use VTM or Trizol to moisten swabs.** Gently twirl or rotate the swab back and forth 2-5 times to exfoliate (collect) cells from the cloacal wall (see Figure 2). Remove the swab from the cloacal cavity and place in a cryovial filled with 500 µL Trizol and use alcohol-wiped (or ethanol-wiped), flame-sterilized scissors to cut the shaft of the swab above the tip. [Note: If the plastic shaft can be snapped, then scissors are not necessary and the risk of cross-contamination is reduced. To snap the swab, lift the swab a little above the bottom of the vial then snap it. This will ensure the swab will not block the cap]. Repeat above process with second swab, and place into 500 µL of VTM (= maximum final ratio of 1:1) in a cryovial.

For fecal samples, add 500 µL or pea-sized piece of feces directly into 2 cryovials, one containing 1 mL Trizol (= maximum final ratio of 1:2) and one containing 500 µL VTM (= maximum final ratio of 1:1) and mix each tube well.

Store in a liquid nitrogen dry shipper or dewar and transfer to -80˚C freezer when possible.

Figure Avian 2: Cloacal swab sample collection from a bird *(Photo credit: Taronga Zoo/Karrie Rose from FAO Wildlife bird highly pathogenic avian influenza surveillance manual)*

3. Whole blood and serum samples: Blood can be collected from the jugular vein (right side of the bird's neck; see Figure 3), brachial/ulnar vein (wing vein) or medial metatarsal vein (leg vein; see Figure 4) using a 22G, 23G, 25G, or 27G hypodermic needle hypodermic needs or butterfly needle and a 12 mL, 10 mL, 6 mL, 3 mL or 1 mL syringe, depending on the size of the bird and the amount of blood to be collected.

In general, it is safe to collect 0.3-0.6 cc of blood per 100 g of body mass from live birds, however, it is always best to collect as little blood as is necessary to conduct the testing required. If you plan to do hematology tests in addition to disease surveillance, it is recommended that you use a 22G through 25G needle as a 27G needle or smaller damages cells as they pass through this narrow diameter needle. After blood is collected, cover the venipuncture site with gauze and apply digital pressure until bleeding stops (30-60 seconds).

Add up to 500 µL of whole blood directly into 2 cryovials, one containing 500 µL Trizol and one containing 500 µL VTM (= maximum final ratio of 1:1) and mix each vial well.

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.*

For serum samples, collect blood using a non-heparinized syringe and place blood into a serum vacutainer (red-top) tube containing serum-clotting factor. After allowing the blood to clot, either spin tube in a centrifuge or allow tube to stand vertically on ice as much as possible. Use a sterile pipette tip and pipette gun to draw off serum and collect 2 x 0.5ml aliquots (no Trizol or VTM).

Store in a liquid nitrogen dry shipper or dewar and transfer to -80˚C freezer when possible.

Figure Avian 3: Blood sample collection from the jugular vein. *(Photo credit: FAO, 2007)*

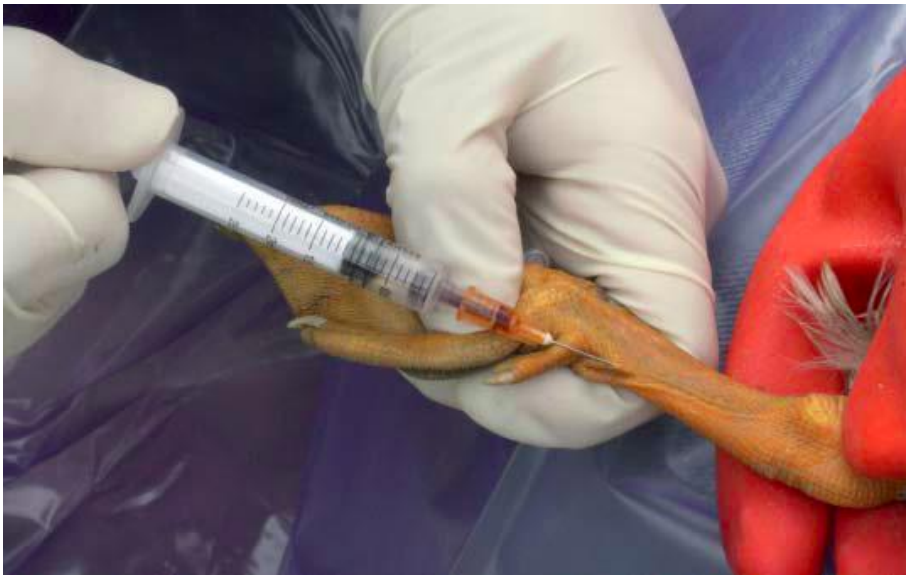

Figure 4: Blood sampling from the medial metatarsal vein. *(Photo credit: FAO, 2007)*

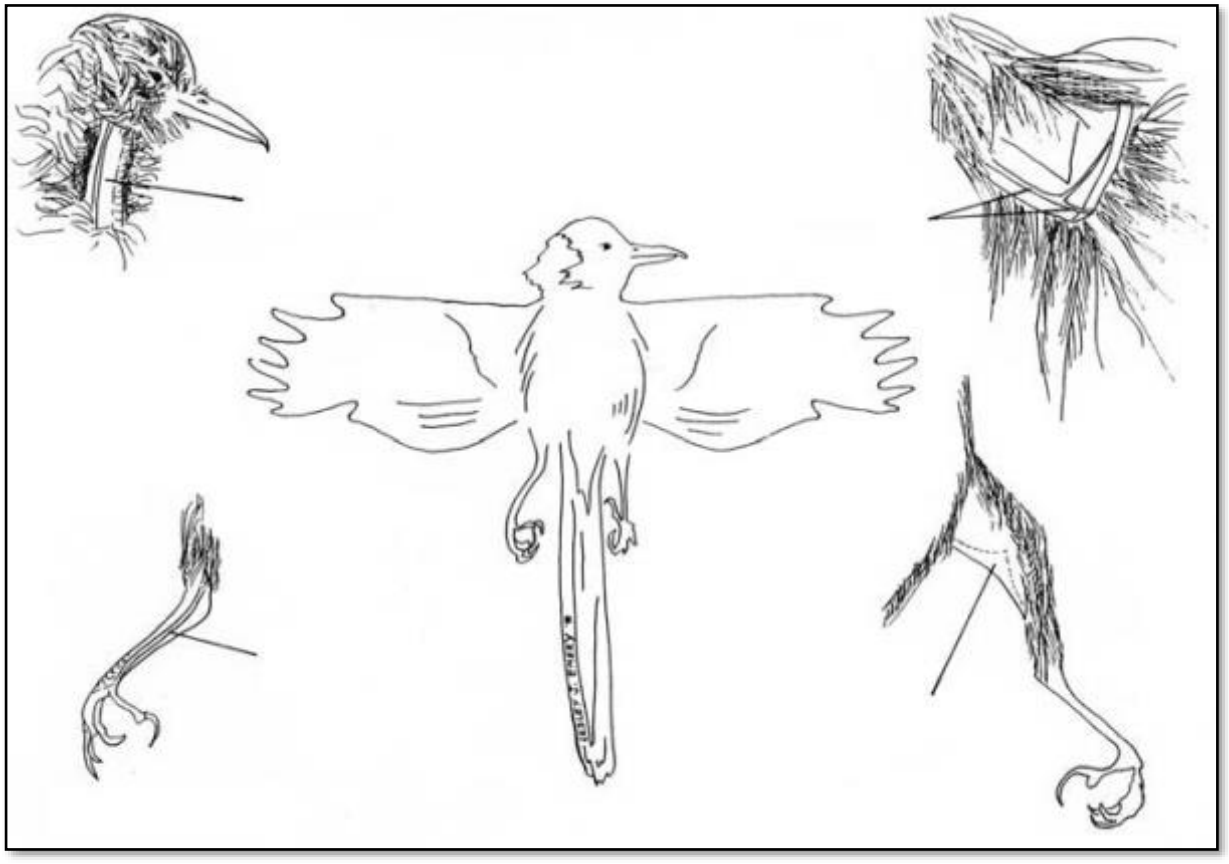

Figure Avian 5: Common sites of venipuncture and administration of subcutaneous fluids in birds *(Photo credit: FAO, 2006)*

Sampling of Dead or Euthanized Birds

If carcasses are not whole, the PREDICT guide for ***Bushmeat Sampling*** may be more applicable. If bodies are relatively whole and fairly fresh then blood, organ tissues and urine should be collected.

As discussed throughout this protocol, all wildlife should be considered potentially infectious for a wide variety of dangerous pathogens and dead animals in particular should be sampled only following all safety measures including proper use of PPE, proper work station decontamination and proper carcass disposal as outlined here and in other PREDICT documents (***Section 4. Biosafety and PPE Use***, ***Section 2.5. Safe Disposal of Carcasses and Infectious Waste Guide***, and ***Section 5.2.4. General Field Sampling Station Setup***).

Thorough necropsy procedures can be very beneficial and might pertain to some animals (e.g., valuable or known individuals, suspicious deaths, etc.); these procedures are addressed in separate documents. Time and skill permitting, when full necropsies are performed, following any Association of Zoos and Aquariums/AZA (or similar) necropsy protocol is recommended. Most of these protocols can be adjusted for application to other species. (Note: properly following extensive necropsy procedures and collecting and measuring all samples can require 4-6 hours for a single animal.)

Post-Mortem Blood Collection

In recently dead animals, it may be possible to collect whole blood (often clotted) from the right side of the heart, where the largest volume of blood is available. Blood may also be found in the chest cavity. If the animal died recently and the blood has not yet clotted, collect whole blood into 1 lavender top tube containing EDTA and add up to 500 µL of whole blood directly into 2 cryovials, one containing 500 µL Trizol and one containing 500 µL VTM. Also collect whole blood separately to obtain serum. Collect available blood into an appropriate sized container (typically one or more blood tubes) and allow it to sit undisturbed for at least 30 minutes. Then centrifuge at high speed (2000 x G for 20 minutes), remove the serum (clear, yellow or red-tinged fluid at the top), transfer clots into cryovials containing 500 µL Trizol and 500 µL VTM, and freeze the samples. If a centrifuge is not available, allow clots and cells to settle as much as possible and then collect serum into 2 x 0.5ml aliquots and blood clots into cryovials containing 500 µL Trizol and 500 µL VTM.

Tissue Collection from Dead Birds

Collect three, adjacent, approximately 200mg (pea-sized) samples of the following tissues:

- Adrenal
- Colon
- Heart
- Liver
- Lymph node
- Ovary
- Cecum
- Duodenum
- Kidney
- Lung
- Spleen
- Pancreas

- Testes
- Other, if required

One specimen should be frozen in 500 µL VTM in a cryovial, one should be frozen in 1 mL Trizol in a cryovial, and one should be stored at room temperature in a small vial or jar 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).

It will usually require experience to identify abnormal tissues, but potentially recognizable gross lesions include masses, discolored areas, ulcerations, etc. Samples for histopathology (i.e., in formalin) should be collected at the abnormal margins to include both normal and abnormal sections in the same piece of tissue. Collection of any obvious internal parasites in ethanol is also recommended.

Section 5.2.10e. Health and Welfare of Birds during Capture and Handling

The health and well-being of the birds is the primary concern during all phases of capture. There are multiple methods for trapping and handling varying bird types, and examples can be found in the FAO Animal Production and Health Manual No. 5 ("Wild Bird HPAI Surveillance: sample collection from healthy, sick and dead birds", available at: http://www.fao.org/avianflu/en/animalhealthdocs.html)

The following principles should be adhered to, to ensure birds are captured and handled correctly, safely and with minimum disturbance (FAO, 2007):

- Wild bird capture is strictly controlled in most countries; those engaged in capture activities should be aware of and comply with local and national laws and obtain all required local, state, provincial & federal permits well in advance.
- Capture techniques and equipment that expose birds to foreseeable risk of injury should be avoided at all costs.
- Approved restraint techniques and handling guidelines should be used e.g., those described by FAO (2007); consult with experienced wildlife veterinarians and biologists if modifications to restraining and handling techniques are required.
- Those conducting capture efforts should take all precautions to avoid disturbing nesting birds at breeding sites or enhancing vulnerability to nest site predation following human intrusion.
- Monitor weather forecasts prior to conducting capture efforts to ensure birds are not captured during extreme climatic conditions that would expose them to an increased risk of hypothermia or hyperthermia.
- Always have a sufficient number of experienced personnel (at least four) available before undertaking any capture operation.
- Check operative traps and nets at appropriate time intervals; birds should not remain in traps or nets any longer than is necessary. This is capture technique and weather dependent, and could be as short as every 15 minutes to twice a day.
- Close or dismantle traps and nets that are inoperative and not checked regularly.

- Maintain a calm and quiet environment at the bird-handling site.
- Conditions at the bird-processing site should be appropriate for the environmental conditions: in cold, wet conditions, birds should be kept warm and dry; in hot, sunny conditions, birds should be processed in a sheltered, shaded and cool site.
- Processing stations should be located as near as possible to the capture site to avoid holding birds for transportation any longer than is absolutely necessary.

Bird Welfare (FAO, 2007)

There is always the risk of distress or injury when handling wild birds. Preferably, an appropriately trained veterinarian will be available to examine and treat any injured or distressed bird, but, at the very minimum, a basic first aid kit should be included in the equipment list of every field study. In no instance should a seriously injured bird be released into the wild without first being examined and treated by a veterinarian. If euthanasia is required see the AAZV and ***AVMA guidelines (Section 8.5.2)***.

Common Maladies and Treatments

Scratches, cuts, and abrasions

These may be unavoidable during capture and confinement and simple treatment by rinsing the injury with clean water or sterile saline before releasing the bird should suffice for most minor injuries. More serious injuries should be brought to the attention of a veterinarian.

Shock/Inertia

Birds are susceptible to the stress of capture and handling and may suffer a physiological (shock) or neurological (inertia) reaction where birds become unresponsive to external stimuli to the point that they appear “frozen”. Shock may be accompanied by rapid breathing (not evident in inertia).

Birds should be allowed to recover in a quiet, sheltered and well-ventilated area, well away from any human activity. Limiting time in captivity, maintaining a calm and quiet captive environment, and working at a site appropriate for the environmental conditions will help prevent shock and inertia.

Hypothermia and Hyperthermia

Capturing, transporting and handling birds during extreme temperatures, rain or foul weather makes them vulnerable to hypothermia or heat stress (hyperthermia) and should be avoided where possible.

Hypothermia can occur in cold conditions when feathers become wet and lose their insulating properties. Signs of hypothermia include shivering, lethargy and skin that is cold to the touch. Birds suffering from hypothermia should be dried and placed near a heat source such as a heating lamp (compact fluorescents bulbs should be at least 4-6” (10-15.25cm) from the

animal's head and UV bulbs 12-20" (30.5-50.8cm)) or a hot water bottle (non-insulated). Holding wet birds in dry airy crates, at sufficiently low density and away from human disturbance usually allows them to preen themselves dry.

Handlers should avoid use of petroleum-based lotions (e.g., common in hand-creams and moisturizers) that may cause plumage to lose its insulating properties.

Hyperthermia can occur in hot conditions when birds are held in direct sunlight, at high ambient temperatures, or in overcrowded crates without adequate ventilation or water. Hyperthermia may also occur if birds are subject to a prolonged chase during capture. Signs of hyperthermia include panting, wings held away from the body, lethargy, seizures or prostration.

Birds suffering from hyperthermia should not be handled, but should be placed in a well-ventilated box/crate, moved to a cool, shaded area and provided with abundant drinking and swimming water. It may be beneficial to mist the bird with water or apply alcohol or water to the bird's feet to accelerate heat dissipation.

Section 5.2.10f. References

European Commission, DG SANCO (2007).
EU Guidelines for AI surveillance in wild birds and poultry in 2007.

FAO 2006. Wild Bird Highly Pathogenic Avian Influenza Surveillance: Sample collection from healthy, sick and dead birds. Edited by K. Rose, S.H. Newman, M. Uhart and J. Lubroth. FAO Animal Production and Health Manual, No. 4. Rome

FAO. 2007. Wild Birds and Avian Influenza: an introduction to applied field research and disease sampling techniques. Edited by D. Whitworth, S.H. Newman, T. Mundkur and P. Harris. FAO Animal Production and Health Manual, No. 5. Rome. (also available at www.fao.org/avianflu)