

U.S. Fish and Wildlife Service
Wildlife Health Office
Avian Influenza Surveillance
Sample Collection and Shipment Protocol

Avian influenza live bird surveillance sample collections will include oropharyngeal swabs and cloacal swabs from apparently healthy birds (including hunter-killed). Whole carcasses will also be collected from wild bird mortality. Please note that shipping guidelines are general, and the submitter should always contact the receiving laboratory to determine their specific shipping requirements and schedules.

Mortality Collection and Shipment

1) PPE: Consult USFWS AI surveillance plan for guidance on appropriate PPE.

2) **Carcass Collection:**

- a) Collect the freshest carcasses you can find. Look for those with clear eyes, no scavenging, and that are representative of the age/gender/species affected by mortality event. For high priority species with few specimens, collect all that you can.
- b) Questions about shipping carcasses frozen versus chilled should be directed to the receiving lab to determine their capabilities and preferences. In general, for cause of death analysis, fresh chilled specimens are best. If the specimen must be kept more than 48 hours before shipping, freeze the carcass.
- c) The best AI samples come from carcasses shipped within 24 hours of collection, kept chilled.
- d) Some of the best specimens for diagnostic testing during mortality events are live animals exhibiting clinical signs of disease (e.g. drooping head, swimming in circles). Once you have caught the bird one acceptable method of euthanasia that maintains intact organs (including head) and does not contaminate the carcass with euthanasia drugs is cervical dislocation.
 - i.) **Cervical Dislocation:** Grip behind the back of the skull. With the other hand grip at the base of the neck (right in front of the body). Pull the body and head in opposite directions in a quick snapping motion and a slight twisting. This should dislocate the skull from the spinal column (Figure 1).

3) **Carcass Shipment:**

- a) Once you have collected the carcasses, attach a waterproof tyvek tag, labeled with an indelible waterproof pen such as a Sharpie. Information included on the tag should include date, location, GPS coordinates if available, and collector's information (name, address, and phone number) (Figure 2).
- b) Attach the tag to the leg of the bird using a zip tie.
- c) Bag the bird in a sealable plastic bag.
- d) Place the single bagged bird into a second bag. Several individually tagged birds can be placed into this second bag. Seal and label the second bag with collectors contact information and the number and species of birds included in the bag.
- e) Place a third bag inside an insulated shipping box with some absorbent material in the bottom of the bag to collect any fluids that might leak from the other bags. Place the double bagged birds into the third bag and seal.

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- f) Place enough blue ice packs on top of the birds to keep them cool during shipment. If shipping with dry ice, insulate the birds from direct contact with the dry ice to prevent burning. Caution: use dry ice only on the recommendation of the receiving lab.
- g) Replace the styrofoam lid.
- h) Place a copy of the shipping datasheet, sealed in a plastic bag for protection, on top of the styrofoam lid but under the cardboard flaps.
- i) Seal the cardboard shipping box. If shipping with dry ice, do not seal airtight. Leave a small area untaped to allow the evaporating CO₂ from the dry ice to seep out the box, preventing pressure from building up inside during shipment.
- j) Attach the air bill to the top of the box (Figure 3).
 - i) Note: record the tracking number at the top of the air bill to allow you to verify the shipment will arrive as scheduled via Fed Ex's website. When notifying the lab of the shipment, provide them with the tracking number as well.
 - ii) Under dangerous goods check "yes but designation not required", as the International Air Transportation Association (IATA) does not designate these type of samples as hazardous.
 - iii) The consignee's phone number is required for shipment. If there is any question about the delivery address or arrival time, Fed Ex can contact the lab to resolve these types of issues.
- k) On the side of the box attach the supplied "UN 3373 Biological Substance Category B" designation sticker (if not already on the box). Extras can be obtained from the FWS Wildlife Health Office.
- l) Also include both the shipper's and consignee's name and address on the side of the box in case something happens to the airbill.
- m) If shipping with dry ice include an UN 1845 designation sticker including the amount of dry ice included in the box. 5kg. should be more than sufficient to keep the contents at 0 C for 36-48 hours. Also check the Dry Ice designation box on the air bill, along with the estimated amount of dry ice. These can be obtained, upon request, from the FWS Wildlife Health Office
- n) Notify the lab with the expected delivery date and the number of boxes and samples to expect. Provide them with the airbill tracking number(s) so they can track the shipment from their end if needed. If shipping carcasses to the USGS National Wildlife Health Center, contact Krysten Schuler at (607) 270-2447 prior to carcasses being shipped. Fax a copy of the sampling datasheet to the USFWS Wildlife Health Office at (406) 994-4090, attention USFWS WHO.

Live Bird Sample Collection and Shipment:

- 1) PPE: can include coveralls, eye protection, and latex gloves.
- 2) Media:
 - a) Labs ship the media frozen, but if some arrives thawed, leave it refrigerated and use it first. If brain-heart infusion broth (BHI) is used the media is viable for up to a year after defrosting if kept refrigerated. The Viral Transport Medium (VTM) shipped directly from NWHC can be kept and used in a **refrigerated condition for one week**. The media may be **refrozen up to twice** after defrosting and can be kept indefinitely while frozen.

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- b) The day before sampling, pull out enough media for the next day's collections and put it in a refrigerator.
- c) During sampling and shipment keep the media refrigerated, not frozen.
- d) One media tube will be needed per bird, although 2 swabs will be collected, an oropharyngeal and a cloacal. Both swabs will be placed into the same media tube.

3) Collection team: 3 person team including a handler, sample collector/bander, and data collector is optimum.

4) Cloacal swab collection:

- a) Unwrap the swab by pressing the shaft end through the paper wrapper and grip the swab by the exposed end. Extract the swab and ensure it doesn't contact external surfaces.
- b) "Rolling technique": roll the swab between your index finger and thumb as you press the swab into the cloaca. Be careful not to touch feathers or any other surface with the swab as this could cause contamination of the sample.
- c) The swab only needs to penetrate as deep as the Dacron fiber end.
- d) Keep rolling the swab between your thumb and index finger while circling the inside of the cloaca, being careful not to damage the penis in males.
- e) Withdraw the swab and shake off the excess feces downwind of the sampling crew. The goal is to collect cells lining the cloaca, not fecal material.

5) Oropharyngeal swab collection:

- a) Open the bird's mouth by gently pressing on the outside of the head near the base of the bill.
- b) Swab the pharynx surfaces behind the tongue using the same technique as for the cloaca. Swab the area around (but not into) the glottis (the opening of the windpipe, or trachea), and finish by withdrawing the swab head along the choanal slit (a slit located on the roof of the mouth that connects to the nostrils). Again, be careful not to touch anything else with the swab to avoid contamination.

6) Inserting swab into media:

- a) Insert the swabs into the open media tube being careful to not let the swabs touch the outside of the tube.
- b) Swirl the swabs in the media.
- c) With the tip of the swab ¼ inch from the bottom of the tube, bend the swab shaft over the edge of the tube breaking off the excess. Remove only enough swab shaft to secure the vial cap.
- d) Discard the excess shaft, and tightly replace the media tube cap.

7) Data Collection:

- a) The shipping datasheet will be a COPY of the sampling datasheet.
 - i) Record the sample ID as the NWHC case number – vial number (case number 44330 vial number 005 is sample ID 44330-005). If the bird has a band, write this number in the comments section.

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8) Live Bird Swab Sample Shipment:

- a) Samples are best shipped within 24 hours of collection. If samples can be shipped within 48-72 hours after collection, refrigerate or store on blue ice packs.
- b) Tubes are placed back into media tube box. If submitting through NWHC the vials must be packed in sequential order by NWHC vial number.
- c) Place box into sealable plastic bag.
- d) Double bag with absorbent material in between bags.
- e) Place into insulated shipping box with blue ice packs on top.
- f) Replace foam lid.
- g) Place shipping datasheet into a sealable plastic bag and place on top of the Styrofoam lid.
- h) Seal box and apply airbill
 - i) The airbill is made out the same as above for carcass shipment (Figure 3) EXCEPT marking "No" under the dangerous goods declaration.
- j) Write the shipper's and consignee's name and address on the side of the box in case something happens to the airbill.
- k) On the side of the box write "Exempt Animal Specimen". If there is an UN3373 designation on the box, cross it out.
- l) Contact the lab and notify them how many samples you are sending and when to expect delivery. Provide the lab with the airbill tracking number(s). If shipping swabs to the USGS National Wildlife Health Center, contact **Richard Zane at (607) 270-2481** prior to shipping. Fax a copy of the sampling datasheet to the **USFWS Wildlife Health Office at (406) 994-4090**, attention USFWS WHO.

9) Hunter Harvested Swab Sample Collections:

- a) Swabs are collected and shipped in the same manner as live bird collections.
- b) The check station is recorded as the collection site.

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Figure 1: Cervical Dislocation. (Friend, et al.)

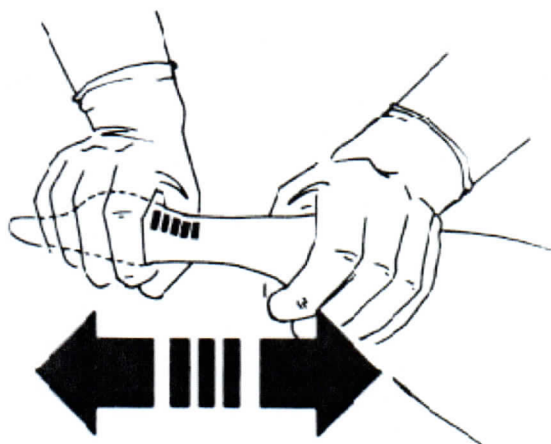


Figure 2: Tyvek leg tag for carcass submissions.

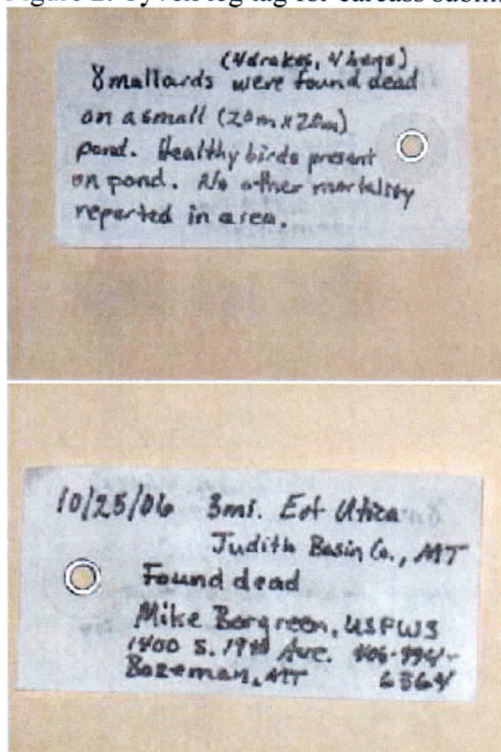


Figure 3: Fed Ex airbill

References:

Friend, Milton; Franson, J. Christian. Field Manual of Wildlife Diseases General Field Procedures and Diseases of Birds. USGS.