

## ATTACHMENT 9

### Protocol for the Collection of Tracheal/Cloacal Swab Samples

1. Contact Laboratory to determine specific protocol to use. Laboratories may request samples be placed in tubes containing Viral Transport Medium (VTM) or brain-heart infusion broth (BHI).
2. Thaw appropriate number of pre-labeled tubes of Viral Transport Medium (VTM) or brain-heart infusion broth (BHI) at refrigerator temperature (4 °C) overnight and keep chilled with wet/blue ice packs in a cooler during the day of collection.
3. Unwrap a Dacron swab from the stem-end of the packaging.
4. Remove swab and insert the entire head of the swab into the trachea or cloaca. Use gentle pressure and in a circular motion, swab the inside circumference of the trachea/cloaca two or three times.
5. For Cloacal swabs, shake off large pieces of feces.
6. Inserting the swab into the tube containing VTM or BHI broth. With the swab in the media, swirl the stem end of the swab between fingers vigorously. Lift the swab approximately ¼” from the bottom of the vial and bend the stem over the edge of the vial to break off the stem so that the swab remains in the vial and the cap can be screwed tight.  
The entire swab end and a portion of the stem will be left in the tube.  
If the stems are unable to be broken (some small swabs will have metal stems) then they can be cut with scissors. Scissors should be wiped with 70% alcohol each time they are used to cut a stem.
7. Record sample tube number on banding sheet or the Sample History Sheet along with date, species, age, sex, and location data (GIS coordinates if possible)..
8. Replace tube into cooler for transport back to the base camp. Samples should be kept cold (<4 °C, frozen if possible) and out of direct sunlight.
9. At camp, transfer tubes into liquid nitrogen shippers or into a freezer as soon as possible. Note any exceptions to the collection or storage conditions in field sheets and note such information on the "Sample History and Packing List Form".
10. Place tubes into a hard plastic shipping container with enough frozen gel packs to keep samples cold for at least two days.
11. Notify laboratory that samples are being shipped, the method of shipment (FEDEX is preferred), and the expect date of arrival. Packages should only be shipped on Monday, Tuesday, or Wednesday.



**Sample History and Packing List  
H5N1 Avian Influenza Project**

Submitter \_\_\_\_\_  
 Address \_\_\_\_\_  
 \_\_\_\_\_  
 Phone \_\_\_\_\_  
 E mail \_\_\_\_\_

Sample ID	Bird Species	Age	Sex	Band Number	Location	Comments or Sampling problems

## ATTACHMENT 10

### Fecal Sampling and Shipping Protocol

#### ***Fecal Sampling***

##### Purpose

The purpose of this standard operating procedure is to describe the essential elements of proper handling and collection of field fecal samples for surveillance of avian influenza.

##### Procedures

- Before collecting samples, personnel should don the appropriate personal protective equipment (PPE). These include latex or nitrile gloves and face shields, and if it is determined to be necessary, PAPRs.
- Label plastic whirl pack bags with necessary information including date, location (GPS coordinate if possible), species (if possible), investigator and sample identification. Record all required information on data sheets.
- Feces must be less than 24 hours old. Feces should appear moist.
- For collection, turn a sterile Whirl-Pak inside out and pick up feces using the Whirl-Pak as a glove, then turn the bag right side in with the feces inside the closed bag. Release as much air from the inside of the bag.
- Label the Whirl-Pak using an indelible ink marker. The sample should be labeled with the sample number, date, time, collector's name, location, and Quality Assurance number (Protocol Number). This latter information describes in detail the context, purpose, and other procedural and review information of the samples collected.
- Place the Whirl-Paks with fecal samples into a large zip-lock bag, tape (e.g., duct tape or packaging tape) the opening and label the outer bulk bag with name, date, location, and Protocol number.
- Place the bulk collection bag in a cooler with wet or blue ice to keep the specimen cool. This precaution is for maximizing the chances for subsequent viral isolation. Pack samples with enough ice or frozen gel packs to keep samples cold for at least two days.
- Maintain the temperature of samples as constant as possible.

- Change gloves if soiled or contaminated. When finished collecting, wash hands with suitable antibacterial agent.
- Notify laboratory that samples are being shipped, the method of shipment (FEDEX is preferred), and the expect date of arrival. Packages should only be shipped on Monday, Tuesday, or Wednesday; this allows the laboratories time to process samples during a normal work-week, or allows for tracking if the shipment is delayed.

## ***Shipping***

### Purpose

The purpose of this standard operating procedure is to ensure diagnostic specimens are shipped safely and in compliance with governing regulations and requirements. Shippers of diagnostic specimens where a relatively low probability exists that infectious substances are present (diagnostic specimens being transported to undergo routine screening tests or for the purpose of initial diagnosis may be considered to fall under this category) must comply with the International Air Transportation Association (IATA) Dangerous Goods Regulations. The shipper must also ensure that shipments are prepared in such a manner that they arrive at their destination in good condition and that they present no hazard to persons or animals during shipment.

### Procedures:

- Federal Express is the preferred carrier for the USDA/NWRC. Use next day service.
- The inner packaging (appropriately labeled) must be comprised of a watertight primary receptacle, and must no exceed 500 ml total volume. Primary receptacles include those of glass, metal, or plastic (i.e., test tube, plastic jar, or taped zip-loc bag). Positive means of ensuring a leak-proof seal must be provided. Screw caps on primary receptacles must be reinforced with adhesive tape.
- A secondary packaging (also water tight) must be used, but must not exceed 4 L total volume.
- An absorbent material sufficient to absorb the entire contents of all primary receptacles must be placed between the primary receptacle and the secondary packaging.
- The outer packaging (i.e., cardboard box or cooler) must be of adequate strength for its capacity, weight, and intended use (capable to withstand being dropped at least 1.2 meters, without leakage of the primary receptacle or significant damage to the outer packaging).

- An itemized list of contents must be enclosed between the secondary packaging and the outer packaging. To protect against possible leakage, the list should be enclosed in a sealed plastic bag.
- A Shippers Declaration for Dangerous Goods is not required. However, both the air waybill and the outer box must show the text "***DIAGNOSTIC SPECIMEN PACKED IN COMPLIANCE WITH IATA PACKING INSTRUCTION 650***".