2007 NWHC Protocol for collection, storage, and shipment of combined oral-pharyngeal (OP) and cloacal (CL) swab samples (v.5/29/07). Please coordinate shipments with Richard Zane (608-270-2481) or Diana Goldberg (608-270-2455) at the National Wildlife Health Center, Madison, Wisconsin.

Background information

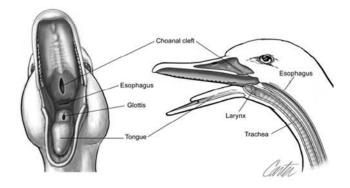
Both oral-pharyngeal (OP) and cloacal (CL) samples will be collected from each bird and combined in one vial. The 4 cc vials are pre-labeled with NWHC case and accession number and "OP & CL SWABS". Cryovials are shipped on dry ice and can be stored at standard freezer temp (-20°C) for the duration of the field season. Once thawed, virus transport media is good for 7 days refrigerated at 4°C. Unused media vials may be refrozen twice. As samples are collected, keep vials out of sunlight in a cooler on ice or blue ice packs during the day's work. After returning from the field, transfer vials to ultra-low freezers (-80°C), store on dry ice (double-bagged in sealed plastic bags, and separated from the dry ice by paper towels or newspapers), or in liquid nitrogen vapor shippers, but do not freeze at standard freezer temperature (-20°C). Alternatively, samples can be kept refrigerated or on frozen ice packs and shipped to NWHC if they will arrive at NWHC within a **maximum** of 72 hours of collection.

Oral-pharyngeal and cloacal swabs

- 1. Thaw appropriate number of vials of media at refrigerator temperature (4°C) or on ice and keep chilled with wet/blue ice packs in a cooler during the day of collection.
- 2. Unwrap a swab from the stem-end of the packaging (store swabs so they do not get wet). Open oral cavity (technique will depend on species and whether alive or dead; for live birds, seek advice if necessary to avoid injury to the bird), insert swab, and with gentle front to back motion swab along both sides of the base of the tongue.



Oral swabbing a dead ring-billed gull.



SCWDS, J. Brown

Continue toward the back of the oral cavity and swab the area behind the tongue, avoiding the glottis (opening to the trachea). Remove the swab from the oral cavity by bringing it forward in contact with the roof of the mouth; swab over the choanal slit along the top (dorsal side) of the oral cavity. Immerse swab in media in the 4cc vial and swirl.

- 3. Lift the swab about half way from the bottom of the vial and bend the stem over the edge of the vial to break off the stem (plastic stems) or cut the stem with scissors or wire cutters (metal stems) 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.
 - Scissors or wire cutters should be wiped with alcohol after each use.
- 4. Unwrap another swab from the stem-end of the packaging, remove swab and insert the entire head of the swab into the cloaca. Use gentle pressure and in a circular motion, swab the inside circumference of the cloaca two or three times. Shake off large pieces of feces and insert the swab into the **same** labeled vial as above, following step #3 above.
- 5. Write 4-letter species code on the vial with fine-tip Sharpie. Record data on Excel worksheet (see below). Keep vials on ice out of sunlight and store as described in Background, above.



Cloacal swabbing technique.

Shipping to NWHC

Ship samples on dry ice (preferred, 8-10 lbs.) or blue ice packs (equal volume of ice packs and sample vials, only if expected to arrive within 72 hours of sampling) via overnight courier (FedEx preferred). Vials should be placed in sequential order in chipboard cryovial boxes, with some description of the contents on the outside of the box. Enclose the boxes in double layers of leak proof plastic bags to protect the media in the vials from carbon dioxide, which will change the pH, and include absorbent material in case of spills. Separate the bagged boxes of vials from dry ice or blue ice packs with newspapers or paper towels to prevent damage to the plastic bags. Use freezer shipping containers (styrofoam cooler within cardboard box) as outer packaging. Put packing list (see below) on top of styrofoam cooler, so it is visible when cardboard box is opened. Label outside of container "Exempt Animal Specimens". If dry ice is used, apply dry ice IATA label and declare dry ice on air bill. Do not use hard plastic coolers or other "air-tight" containers with dry ice, to avoid build-up of pressure from carbon dioxide gases released. Vapor shippers can be also used for sample transport ("Not restricted – dry shipper" and "IATA A152" on air bill). Useful websites:

Shipment of Diagnostic Specimens:

http://www.ibc.umn.edu/shippingSpecimen.html

Information on transport of dry shippers:

http://www.zoo.ufl.edu/julian/dry_shipper/TipsDryShipper.pdf

Ship package by overnight express (FEDEX preferred; Mon-Wed, unless other prearrangement) to:

Richard Zane National Wildlife Health Center 6006 Schroeder Road Madison, WI 53711 Phone 608-270-2481

Note: Please notify NWHC of shipments through the web-based system found at http://wildlifedisease.nbii.gov/ai. Only authorized personnel have access: see your data administrator.

National Wildlife Health Center AI Sample Packing List

Sender's information	
Name, affiliation:	Date sent:
Phone:	email:
NWHC case #:	Species:
Number of vials:	Location:
Shipped on: dry ice blue ice	
NWHC use	
Received by:	Date:
Logged in by:	Date:

Field data to send to NWHC:

Use the National HPAI Early Detection System through the Wildlife Disease Information Node Visit http://wildlifedisease.nbii.gov/ai and download appropriate Excel worksheet Cross reference band or field number with NWHC case and accession number