

LaMMNA/IBP/UCLA CLOACAL SWAB AND FEATHER SAMPLING PROTOCOL

The Landbird Migration Monitoring Network of the Americas (LaMMNA) and the Institute for Bird Populations (IBP) are collaborating with the Center for Tropical Research at UCLA to collect samples of possible avian influenza to identify transmission paths in North American migratory landbirds, and to further the goal of developing custom vaccines against Influenza A. Our objective is to facilitate the collection of viral samples, through cloacal swabbing, from birds captured at MAPS (Monitoring Avian Productivity and Survival) stations during the summers, at MoSI (Monitoreo de Sobrevivencia Invernal) and MAWS (Monitoring Avian winter Survival) stations during the winters, and at LaMMNA stations primarily during the spring and fall migration seasons of 2006-2009.

During the 2007-2008 season, we will be sampling from North American migratory and non-migratory species, those that are banded with U.S. Fish and Wildlife Service bands. We are hoping that LaMMNA station operators are able to sample as many of these birds as possible once sampling equipment arrives beginning in late March. In order to gain information on connectivity between breeding and wintering grounds (and thus potential pathways for the spread of avian influenza) we ask that feather samples be collected for DNA and stable isotope analyses from all individuals from which cloacal swab samples are collected. This document provides information on LaMMNA/IBP's sampling strategy and details on cloacal-swabbing and feather-pulling techniques to be employed during the 2007-08 LaMMNA season.

Sampling Kits

All participants in this effort will be provided with cloacal swab sampling kits, which will be used to take cloacal swab samples from captured birds. Cloacal swab kits will be mailed to each operator by UCLA. Each kit will contain:

- Safety equipment (i.e., antiseptic skin cleanser, gloves, safety glasses, and respirator masks)
- Individually packaged 1-mm Dacron swabs
- Plastic vials containing 1ml of guanidine hydrochloride (SAFETY NOTE: GUANIDINE IS A SKIN AND EYE IRRITANT)
- Barcoded labels to place on sample vials
- A pair of diagonal cutting pliers
- A fine-point, felt-tipped pen
- A 35-mm film canister (for holding the lid of the plastic vial while the swab is being placed into the vial)
- Labeled envelopes for feather samples
- Cloacal swab sampling data sheets for recording cloacal swab data
- Extra plastic bags for packing vials for return shipment
- A return prepaid shipping form for sample vials and feather envelopes and datasheets

Sample forms and safety and protocol documentation will also be available on the IBP website.

Cloacal swab sampling kits will be mailed by UCLA to all participating station operators during late March 2007. Each sampling kit will contain 100 vials and 110 1-mm swabs (ten extra swabs that can be discarded in case of contamination; see below).

Your Safety and Protection

To date, no human has been diagnosed as having become infected with avian flu from contact with a live wild bird, and the highly pathogenic (HPAI) H5N1 variant has not been detected in the Western Hemisphere. It is important to realize, however, that a great many of the birds you handle and band (and have handled and banded in past years) have carried other forms of avian influenza. Thus, normal hygiene precautions, that include sanitizing hands before hand-to-mouth contact and before touching food, should always be taken when banding birds.

In order to protect LaMMNA operators, we require that all persons handling birds apply antiseptic skin cleanser to their hands immediately after all birds captured on each net run are released. To further minimize the probability of bird-to-bird transfer of any avian disease, we suggest that operators apply antiseptic skin cleanser to their hands after each individual bird is handled, both when removing birds from the nets and when processing birds at the central banding location. Operators should rub a small amount of the skin cleanser thoroughly over their hands and then let their hands dry (which only takes a few seconds). Please remember to keep the spout of the hand cleanser closed when not in use to avoid evaporation. Operators should not rub their eyes, eat, drink, or smoke during the banding session and all food items should be kept well away from the area where birds are banded. Water should be consumed only after hands have been cleaned using the antiseptic skin cleanser or washed with soap and water. Safety gloves and safety glasses are provided in each kit for operators who would feel more comfortable using them. The respirator mask will only need to be used in areas where HPAI-H5N1 has been detected (as of this writing, nowhere in the Western Hemisphere).

Which Birds to Sample

As mentioned above, our goal is to sample as many individuals of migratory species as possible, those that are treated in Identification Guide to North American Birds and for which you use USFWS bands. We are also interested in non-migratory species, especially European starlings, brown-headed cowbirds, house sparrows and house finches. We request that you **not** sample hummingbirds (they are too small) or any unbanded bird. Because there is a limited time period during which active cloacal shedding of viruses occurs, recaptured birds, including within-year between-pulse recaptures, can be resampled to increase the probability of detecting any viruses or permit detection of new flu variants in the same individual. There is no need to sample from the same individual within the same pulse. During those times when large numbers of birds must be processed from a single net run, we recommend that viral and feather sampling be temporarily suspended in order to reduce handling and holding times for those birds.

Recommended Sampling Protocol

To avoid cross-contamination of samples, it is imperative to keep holding bags as clean as possible. Ideally, each holding bag should be used only once (to hold a single bird for cloacal swab sampling) during an entire day of LaMMNA banding, and should be washed (using one cup of Chlorox per wash load) and dried between days of banding. We realize that some stations can capture large numbers of birds in a single day or pulse and may not have enough holding bags to meet the above ideal situation. If a holding bag must be reused before being washed and dried, it must be thoroughly shaken out, turned inside-out, and shaken again before being used

for another bird. If you must resort to shaking out a holding bag, please wear safety glasses and a respirator mask while shaking out the bag, and do it well away from the banding area and from other persons.

We recommend that all of the normal banding, processing, and data collection, including weighing the bird and collecting tail feathers, be completed before taking the cloacal swab sample. This will allow the bird to be released as soon as the swab sample has been collected.

The entire banding, processing, data collection, and cloacal swabbing procedure can be performed by a single person, although it can be helpful to have a second person present to record data. If two bird handlers are present, it may be useful for one person to complete the normal banding, processing, and data collection and hand the bird to the second handler to obtain the cloacal swab sample. Alternatively, if many birds are captured on a given net run, it may be advantageous for each bird handler to completely process each bird from banding through cloacal swab sampling.

Feather Collection Protocol

When tail feathers are pulled, a small amount of skin cells remain attached to the quill of the feather. These skin cells are a valuable source of DNA that can be used to determine the population of origin of an individual bird. Moreover, a portion of the feather itself can be used for stable isotope analyses, which can provide important information on the location (at least latitude) where the feather was grown. Researches at UCLA and elsewhere use the results of DNA and stable isotope analyses to investigate patterns of migratory connectivity in birds, that is, to determine wintering locations for populations of breeding birds and vice-versa.

We recommend that two tail feathers be collected during the banding process from each bird that is to be swabbed, prior to collecting the cloacal swab sample. There is no need to collect feathers more than once during the same season, even if the individual is sampled for AI more than once during within-season recaptures (see above). Also, although we hope to get both AI and feather samples from all individuals, the collection of one of these samples without the other will still provide valuable data.

Pluck the outer tail feather from one side of the bird's tail, and the central tail feather from the other side of the tail. To pluck the feathers, just hold them firmly, relatively close to the base, and pull gently. Do not touch the quill, as the DNA is extracted from the skin cells attached to it. Place the feathers from each bird into one of the pre-printed envelopes provided by UCLA. Fill out the requested information on the envelope: species name (4-letter code is fine), band number, date (please use letters for the month instead of numbers), location code, station code, and, if possible, age, sex, and breeding condition (as determined by brood patch or cloacal protuberance). Please make a note if you notice that a central rectrix is of a different generation than one of the outer rectrices (e.g., alternate vs. basic feather).

Detailed Cloacal Swab Protocol

Virus cells are located in the gastrointestinal tract in birds and can be present on the cloacal walls and in feces. Correct cloacal swabbing provides a sample of cells from the inner surface of the cloacal wall, and is accomplished by inserting the swab into the cloaca and slowly rotating the swab to collect these cells.

To obtain a cloacal swab sample, take the following steps:

- 1) Hold the bird in the bander's grip and remove the Dacron swab from its package, being very careful to let nothing touch the tip of the swab
- 2) Use your fingers to secure the bird's legs and tail.
- 3) Blow on the vent of the bird to part the feathers, exposing the cloaca. An optical binocular magnifier can make it easier to see what you are doing.
- 4) Gently and slowly insert the Dacron head of the swab into bird's cloacal cavity. Remember that cloacal cavities of small birds can be very shallow; thus the swab head should not be inserted very far into the cloaca. Once the swab is initially inserted change its angle slightly such that the swab is more aligned with the bird's body and the head of the swab is pointing more toward the head of the bird. This will help prevent damage to the softer membranes along the back wall of the cloaca.
- 5) Gently twirl or rotate the swab back and forth 2-5 times to exfoliate (collect) cells from the cloacal wall.
- 6) Remove the swab from the cloacal cavity and release the bird.

To place the cloacal swab sample in a plastic vial and store the used vials, take the following steps:

- 1) While still holding the cloacal swab in your hand, remove the cap from a plastic vial and place the cap in the clean, empty film canister provided for this purpose. Continue to assure that nothing touches the tip of the cloacal swab.
- 2) Place the swab head-first into the guanidine in the plastic vial and briefly but rapidly spin the swab 2-5 times in the vial to suspend any collected material in the guanidine. **Note that Guanidine hydrochloride is an eye and skin irritant. Please refer to the Material Safety Data Sheet for Handling/Exposure instructions. If you get Guanidine on your skin, immediately wash with soap and water, if you get it in your eyes, immediately flush with water for 15 minutes. Safety glasses and gloves are provided to protect against skin and eye exposure.**
- 3) Pull the swab partially out of the guanidine and use the diagonal cutting pliers to cut the aluminum shaft of the swab near (above) the top of the Dacron swab head. This will allow the swab head to remain in the vial while you screw the cap back onto the vial and seal the contents. Tighten the cap as much as possible to prevent evaporation of the guanidine during storage.
- 4) Use the fine-point felt-tipped pen to mark the cap of the vial to indicate that it has been used, and to record the four-letter species code and band number on the vial.
- 5) Fill out the cloacal swab sampling data sheet by entering the band number, sample vial number (from the bar code), four-letter station code, date, four-letter species code, age,

sex, feathers pulled (yes or no), and any relevant comments onto the data sheet. Fill in LaMMNA Organization and contact names at the top of the data sheet. If it is also a MAPS location, fill in the four-letter MAPS location code on the top of the data sheet.

Comment: I would rather they were able to fill out both the full name of the Location as well as a code.

- 6) Place the used vial into a plastic bag with the other used vials and store them in a shaded, cool location until the end of the day's banding session.
- 7) At the end of the day of banding, store the plastic bag containing all used vials in a cool and dry location until all fifty vials in a given kit have been used (they do NOT need to be refrigerated).

Returning Completed Samples to the Redwood Sciences Laboratory

Finally, as soon as the season is over, seal the sample boxes of vials with two ziplock bags and ship the boxes with LaMMNA samples back to the Redwood Sciences Laboratory (RSL) (Linda Long, USFS Redwood Sciences Laboratory, 1700 Bayview Drive, Arcata, CA 95521, 707-825-2947), along with the associated feather samples and the completed cloacal swab sampling data sheets associated with those vials. Use the prepaid shipping form provided by UCLA. RSL will check the sample sheets for completion, log in the data, and send the package on to UCLA.

Thank you very much for your participation in this important work!