TESTING TRACE-BACK/TRACE-FORWARD FLOCKS FOR AI STATUS  
(For All Subtypes of AI)

Following the detection of Avian Influenza, flocks that may have been exposed may be discovered during the epidemiological investigation. These flocks are labeled as “trace-back” or “trace-forward” flocks and are required to undergo testing to determine their AI status.

*For birds 3 weeks of age and older*

TESTING REQUIREMENTS:

**Blood samples from live birds***:

- Collect blood samples from thirty (30) birds representative of the flock (from all areas of the house or cage banks). If there are less than 30 birds in the flock, collect samples from all birds.
- Refer to the guidelines for collection and submission of samples included in this document.

**Cloacal swab samples from live birds (waterfowl)***:

- Collect cloacal swabs for virus detection testing from 30 birds representative of the flock. Take swab samples from any dead or sick birds first, and then swab other birds to collect 30 swabs. If there are fewer than 30 birds in the flock, collect samples from all birds.

*All samples must be taken by government authorized personnel (DAG, USDA, or their designated laboratory personnel). If a foreign animal disease (a reportable FAD, such as HPAI) is suspected, sampling can only be conducted by a certified FADD (Foreign Animal Disease Diagnostician) or an authorized representative. Prior notification of USDA or DAG is required.

**Waterfowl: Cloacal swabs and virus isolation required.

GUIDELINES FOR COLLECTION AND SUBMISSION OF SAMPLES

*Please Note*: In collecting and submitting samples, identify the Premises precisely. All submission forms should include a Premises ID Number. If you do not know the number, request one from the RI DEM Division of Agriculture (DAG at 401-222-2781). If a Premises ID number is unavailable, on all submission forms record the street address where the samples were taken. (Note: That address may differ from the grower’s mailing address.)

COLLECTION OF SAMPLES

**BLOOD**

- Collect blood samples from thirty (30) birds representative of the flock (from all areas of the house or cage banks). If there are fewer than 30 birds in the flock, collect samples from all birds.
• Collect at least 0.50 ml of blood in tubes available from the DAG or its designated laboratory (i.e., microfuge tubes must be at least one-third full).
• Allow blood to stand for 4 to 12 hours at room temperature.
• Place blood in refrigerator until it can be transported to the laboratory.
• Complete the submission form and send it with the samples.
• Write the farm name and Premises ID on the side of the box of samples (not on the lid).
• Submit a completed submission form which includes a Premises ID.

CLOACAL SWABS
• Collect cloacal swabs for virus detection testing from 30 birds representative of the flock. Take swab samples from any dead or sick birds first, and then swab other birds to collect 30 swabs. If there are fewer than 30 birds in the flock, collect samples from all birds.
• Use dry swabs for dead bird sample collection; use dry swabs or swabs moistened with VTM for live bird sample collection.
• Insert the swab and rub the mucosa vigorously.
• Use 1 swab for each bird.
• Place swabs into tubes containing enough VTM to moisten and cover the swabs.
• Place 11 swabs (from 11 different birds) into one tube.
• Submit tubes to a DAG-designated laboratory.
• Write the farm name and a Premises ID on the bottom of the box of samples (not on the lid).
• Submit a completed submission form which includes a Premises ID.

* All samples must be taken by government authorized personnel (DAG, USDA, or their designated laboratory personnel)

**Waterfowl: Cloacal swabs required

SUBMISSION OF SAMPLES TO A LABORATORY
• Samples can be tested only at a USDA- or DAG-designated laboratory, normally the Connecticut Veterinary Medical Diagnostic Laboratory (CVMDL) at the University of Connecticut. In accordance with USDA / APHIS NVS protocols, confirmatory testing/subtyping will normally be done at the National Veterinary Services Laboratory (NVSL) in Ames, Iowa.
• Call the laboratory in advance to These let them know when the samples will arrive:
  Connecticut Veterinary Medical Diagnostic Laboratory
  University of Connecticut, 61 N. Eagleville Rd., Storrs, CT 06269
  Phone 860-486-0837; Fax 860-486-3738
• When leaving dead birds at the end of the farm lane for swabbing, place birds in a sturdy, leak-proof container. Authorized personnel will normally collect swab samples and leave the dead birds at that site for disposal.
• To ensure the integrity of swab samples:
  o VTM should be an orange color when fresh. Check that the VTM has not changed to a violet color and has not passed the date of expiration.
  o VTM should be refrigerated at all times.
Keep the swabs in VTM on fresh ice packs until transporting to a DAG-designated laboratory (you may need to change ice packs at least every 24 hours).

If samples are stored in a freezer for more than 72 hours, place a plastic bag around each box and seal to prevent drying of the VTM.

Place a completed copy of an AI Sample Submission Form in each box with the corresponding samples and a Premises ID number.

**Reporting**

Report any signs suggestive of AI in the flock immediately to the RI DEM Division of Agriculture at: (401) 222-2781.

Signs suggestive of AI include the following:

- Increased mortality;
- Decreased egg production;
- Swollen eyelids/sinuses/combs or wattles;
- Purple or bluish discoloration of wattles and combs;
- Respiratory snicking; and
- Generally depressed birds.

These are general guidelines only.

Commonly, the producer will notice mortality increases and, in the case of layers, decreased egg production, which usually trails the mortality by several days.

The RI DEM Division of Agriculture reserves the right to amend the above mentioned requirements for Avian Influenza with the goal of any changes still being to prevent, contain and eliminate the disease. Changes to the general guidelines of the protocol may result from information including, but not limited to, virus strain, pathogenicity, morbidity and mortality, movement of birds and products, and additional epidemiological information obtained as a result of avian influenza investigations.