

**TESTING OF COMMERCIAL POULTRY AND
END OF PREMISES QUARANTINE
(For All Subtypes of AI)**

The RI DEM Division of Agriculture (DAG) may order depopulation with indemnity if the subtype is unknown, or if the subtype is known to be H5, H7, or other subtype that poses a threat to the poultry industry and/or human health. Release from quarantine without depopulation is apt to be an option only when the risk to the poultry industry and human health is demonstrably low (e.g., the premises was proven free of HPAI of all subtypes or to LPAI of H5 or H7 subtypes). The following protocol, then, is most likely to be used when a premises is only quarantined for trace back or suspicion of LPAI other than H5 or H7 subtypes.

Note: Poultry houses should not be repopulated until they are released from quarantine. By law, birds brought into a poultry house under quarantine are not eligible for indemnity.

The option of leaving dead birds at the end of the farm lane for swabbing by authorized personnel may be offered as an alternative to in-house collection and submission of swabs.

A. POSITIVE SEROLOGY: When the flock is initially quarantined based on a positive serology test (AGID), one of the protocols presented below must be followed:

If the subtype is unknown, or if the subtype is known to be H5, H7, or other subtype that poses a threat to the poultry industry and/or human health, choose one of the following options:

OPTION # 1:

- Collect tracheal/oropharyngeal swabs for virus detection testing from thirty (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**.
- Refer to the guidelines for collection and submission of samples included in this document.
- If any of these samples tests positive, submit 30 swabs a minimum of 14 days after the positive sample was collected. Continue this process until all samples submitted are virus detection test-negative.
- After all samples submitted are virus detection test-negative, place 60 laboratory-verified negative (blood and swabs) sentinel birds of the appropriate species in the house(s).
- Submit blood and swab samples collected from all of the sentinel birds for testing a minimum of 21 days after the birds were placed in the house(s).
- The flock is released from quarantine when all of the criteria listed below have been met:
 - There is no indication of clinical disease suggestive of avian influenza, and
 - Sentinel birds test negative when tested following twenty-one (21) days in the poultry house(s).

OR

OPTION # 2:

- Collect tracheal/oropharyngeal swabs for virus detection testing from one hundred and fifty (150) birds representative of the flock*. Take swab samples from any dead or sick birds first, and then swab other birds to collect 150 swabs**.
- Refer to the guidelines for collection and submission of samples included in this document.
- If any of these samples tests positive, repeat collection and submission of 150 swabs a minimum of 14 days after the positive sample was collected. Continue this process until all samples submitted are virus detection test-negative.
- The flock is released from quarantine when all of the criteria listed below have been met:
 - There is no indication of clinical disease suggestive of avian influenza, and
 - All samples submitted for testing are virus detection test-negative.

If the subtype is known to be a subtype other than H5, H7, or is not another subtype that poses a threat to the poultry industry and/or human health, choose one of the following options:

OPTION # 1:

- Collect tracheal/oropharyngeal swabs for virus detection testing from thirty (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**.
- Refer to the guidelines for collection and submission of samples included in this document.
- *If all samples are virus detection test-negative, and there is no indication of clinical disease suggestive of avian influenza, the flock is released from quarantine.*
- If any of these samples tests positive, submit 30 swabs a minimum of 14 days after the positive sample was collected. Continue this process until all samples submitted are virus detection test-negative.
- After all samples submitted are virus detection test-negative, place 60 laboratory-verified negative (blood and swabs) sentinel birds of the appropriate species in the house(s).
- Submit blood and swab samples collected from all of the sentinel birds for testing a minimum of 21 days after the birds were placed in the house(s).
- The flock is released from quarantine when all of the criteria listed below have been met:
 - There is no indication of clinical disease suggestive of avian influenza, and
 - Sentinel birds test negative when tested following twenty-one (21) days in the poultry house(s).

OR

OPTION # 2:

- Collect tracheal/oropharyngeal swabs for virus detection testing from thirty (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**.
- Refer to the guidelines for collection and submission of samples included in this document.
- *If all samples are virus detection test-negative, and there is no indication of clinical disease suggestive of avian influenza, the flock is released from quarantine.*
- If any of these samples tests positive, collect 150 swabs from birds representative of the flock minimum of 14 days after the positive sample was collected. Take swab samples from any dead or sick birds first, and then swab other birds to collect 150 swabs.
- Continue this process until all samples submitted are virus detection test-negative.
- The flock is released from quarantine when all of the criteria listed below have been met:
 - There is no indication of clinical disease suggestive of avian influenza, and
 - All samples submitted for testing are virus detection test-negative.

B. POSITIVE VIRUS DETECTION: When the flock is initially quarantined based on a positive virus detection test (PCR or Virus Isolation), one of the protocols presented below must be followed (for all subtypes of AI):

OPTION # 1:

- A minimum of 14 days after the date of collection of the positive sample, collect tracheal/oropharyngeal swabs for virus detection testing from thirty (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.**
- Refer to the guidelines for collection and submission of samples included in this document.
- If any of these samples tests positive, submit 30 swabs a minimum of 14 days after the positive sample was collected. Continue this process until all the samples submitted are virus detection test-negative.
- After all samples submitted are virus detection test-negative, place 60 laboratory-verified negative (blood and swabs) sentinel birds of the appropriate species in the house(s).
- Submit blood and swab samples collected from all of the sentinel birds for testing a minimum of 21 days after the birds were placed in the house(s).
- The flock is released from quarantine when all of the criteria listed below have been met:
 - There is no indication of clinical disease suggestive of avian influenza, and
 - Sentinel birds test negative when tested following twenty-one (21) days in the poultry house(s).

OR

OPTION # 2:

- A minimum of 14 days after the date of collection of the positive sample, collect tracheal/oropharyngeal swabs for virus detection testing from one hundred and fifty (150) birds representative of the flock*. Take swab samples from any dead or sick birds first, and then swab other birds to collect 150 swabs**.
- Refer to the guidelines for collection and submission of samples included in this document.
- If any of these samples tests positive, repeat collection and submission of 150 swabs a minimum of 14 days after the positive sample was collected. Continue this process until all the samples submitted are virus detection test-negative.
- The flock is released from quarantine when all of the criteria listed below have been met:
 - There is no indication of clinical disease suggestive of avian influenza, and
 - All samples submitted for testing are virus detection test-negative.

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 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 Premises ID.

SWABS

Tracheal/oropharyngeal swabs:

- Collect tracheal/oropharyngeal swabs for virus detection testing from birds representative of the flock. Take swab samples from any dead or sick birds first, and then swab other birds to collect the required number of swabs.
- 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 Premises ID on the side of the box of samples (not on the lid).
- Submit a completed submission form which includes Premises ID.

Cloacal swabs:**

- Collect cloacal swabs for virus detection testing from birds representative of the flock. Take swab samples from any dead or sick birds first, and then swab other birds to collect the required number of swabs.
- 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 Premises ID on the side of the box of samples (not on the lid).
- Submit a completed submission form which includes Premises ID.

* 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 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 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:
 - 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.

- 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.

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.