HANDLING LITTER/MANURE, CLEANING AND DISINFECTION
FOR REVOCATION OF SPECIAL QUARANTINE

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.

IF DEAD BIRDS ARE REMOVED FROM THE HOUSE
Requirements for handling litter/manure if the dead birds are removed from the poultry house:

OPTION #1 – LITTER/MANURE IS KEPT IN THE POULTRY HOUSE:
• Litter/manure shall be kept in the house for thirty (30) days after the birds have been removed.
• A minimum of twenty (20) days after the birds have been removed from the poultry house, personnel designated by USDA or the RI DEM Division of Agriculture (DAG) may obtain environmental samples to test for avian influenza.
  o For commercial flocks, refer to Sampling Commercial Poultry Houses for AI (6-9-AI-P17 in the DEM ERP).
  o For flocks with fewer than 3,000 birds, refer to Sampling Small Flock Poultry Houses for AI (6-9-AI-P15 in the DEM ERP).
• Litter/manure must remain in the poultry house for at least 30 days, even if samples collected at day 20 are negative for AI.
• If litter/manure samples test positive for AI, additional samples must be collected and tested at least 20 days after the positive samples were collected.
• If the second set of samples tests negative for AI, litter/manure must remain in the poultry house for at least 10 days after the negative samples were collected.
• Therefore, if positive samples are collected on day 20, additional samples may be collected and tested on day 40. If the second set of samples tests negative, litter/manure may not be removed before at least day 50.

IF STANDARD CLEANING AND DISINFECTION ARE DONE IN THE HOUSE
• Litter/manure shall be removed from the house following negative environmental test results and with adherence to the protocols listed above, before cleaning and disinfection of the house occurs.
• The house must be cleaned by wet wash until all areas are considered none or slight on evaluation for organic material by DAG or USDA personnel.
• The house must be disinfected with products approved by the DAG.

IF ALTERNATIVE CLEANING AND DISINFECTION ARE DONE IN THE HOUSE
• 60 test negative (blood and swabs) sentinel birds may be placed in the house after litter/manure is removed and house is cleaned to remove other excess organic material.
  □Sentinels may be placed on day 30 if environmental tests are negative.
• □ Sentinels are eligible for testing a minimum of 21 days after placement.
• Blood samples and oropharyngeal swabs (cloacal swabs, if waterfowl) collected from the all of the sentinels must be submitted to a DAG-designated laboratory for testing.
• The house may be released from quarantine when all of the criteria listed below have been met:
  o There is no indication of clinical disease suggestive of avian influenza in the sentinels, and
  o Sentinel birds test negative when tested after twenty-one (21) days in the poultry house.
  o □ If the sentinels test positive, environmental testing must be repeated 20 days after the samples were collected from the sentinels. If the environmental tests are negative, fresh sentinels must be placed in the house after 30 days and the cycle begins again.

OR (If both dead birds and litter/manure will removed, then)

OPTION #2 – LITTER/MANURE IS REMOVED FROM THE POULTRY HOUSE:
• Litter/manure shall be removed immediately and stored in a dedicated structure, or stored outside of the building and covered with vinyl tarps or plastic (as approved by DAG or USDA), for a minimum of thirty (30) days prior to spreading on or moving off of the premises.
• Litter/manure samples must be tested for AI at least 30 days after removal from the poultry house and must have a negative test result before being moved off of the premises.
• If litter/manure is stored under a tarp, the tarp shall not be lifted for at least 30 days after removal of litter/manure from the poultry house.

IF STANDARD CLEANING AND DISINFECTION IS DONE IN THE HOUSE
• The house must be cleaned by wet wash until all areas are considered none or slight on evaluation for organic material by DAG or USDA personnel.
• The house must be disinfected with products approved by the DAG.
• When the house is dry or nearly dry, personnel designated by USDA or the DAG will collect environmental samples from each house for avian influenza testing. The Order of Special Quarantine will be revoked when the steps listed above are successfully completed, and final virus culture isolation results of environmental samples are negative for Avian Influenza virus.

IF ALTERNATIVE CLEANING AND DISINFECTION IS DONE IN THE HOUSE
• After the house is cleaned to remove other excess organic material, 60 test negative (blood and swabs) sentinel birds may be placed in the house.
• □ Sentinels may be placed on day 30 if environmental tests are negative.
• □ Sentinels are eligible for testing a minimum of 21 days after placement.
• Blood samples and oropharyngeal swabs (cloacal swabs, if waterfowl) collected from the all of the sentinels must be submitted to a DAG-designated laboratory for testing.
The house may be released from quarantine when all of the criteria listed below have been met:
  o There is no indication of clinical disease suggestive of avian influenza in the sentinels, and
  o Sentinel birds test negative when tested after twenty-one (21) days in the poultry house(s).

If the sentinels test positive, environmental testing must be repeated 20 days after the samples were collected from the sentinels. If the environmental tests are negative, fresh sentinels must be placed in the house after 30 days and the cycle begins again.

IF DEAD BIRDS AND LITTER/MANURE ARE COMPOSTED IN THE HOUSE
Requirements for handling of litter/manure if the birds (and litter/manure) are composted in the Poultry House:

• Composted material shall be kept in the house for thirty (30) days and will be composted according to established guidelines.
• A minimum of twenty (20) days after composting has begun, personnel designated by DAG or USDA may obtain environmental samples to test for avian influenza.
• Composted material must remain in the poultry house for at least 30 days, even if samples collected at day 20 are negative for AI.
• If composted material/manure samples test positive for AI, additional samples must be tested at least 20 days after the positive samples were collected.
• If the second set of samples tests negative for AI, composted material must remain in the poultry house for at least 10 days after the negative samples were collected.
• Therefore, if positive samples are collected on day 20, additional samples may be collected and tested at day 40. If the second set of samples test negative, composted material may not be removed before at least day 50.

IF STANDARD CLEANING AND DISINFECTION IS DONE IN THE HOUSE
• Composted material shall be removed from the house following negative environmental test results, with adherence to the protocols listed above, and when DAG or USDA personnel have determined that adequate composting has occurred, before cleaning and disinfection of the house occurs.
• DAG personnel are responsible for ensuring that composting is in compliance with applicable State regulations.
• The house must be cleaned by wet wash until all areas are considered none or slight on evaluation for organic material by DAG or USDA personnel.

IF ALTERNATIVE CLEANING AND DISINFECTION IS DONE IN THE HOUSE
• Composted material shall be removed from the house following negative environmental test results, with adherence to the protocols listed above, and when DAG or USDA personnel have determined that adequate composting has occurred.
• AG personnel are responsible for ensuring that composting is in compliance with applicable State regulations.
• After the house is cleaned to remove other excess organic material, 60 test negative (blood and swabs) sentinel birds may be placed in the house.  
• Sentinels may be placed on day 30, if environmental tests are negative.  
• Sentinels are eligible for testing a minimum of 21 days after placement.  
• Blood samples and oropharyngeal swabs (cloacal swabs, if waterfowl) collected from all of the sentinels must be submitted to a DAG-designated laboratory for testing.  
• The house may be released from quarantine when all of the criteria listed below have been met:  
  o There is no indication of clinical disease suggestive of avian influenza in the sentinels, and  
  o Sentinel birds test negative when tested after twenty-one (21) days in the poultry house(s).  
• If the sentinels test positive, environmental testing must be repeated at least 20 days after the samples were collected from the sentinels. If the environmental tests are negative, fresh sentinels must be placed in the house after 30 days and the cycle begins again.

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 2.5 milliliters (cc) of blood in tubes available from the DAG or its designated laboratory.  
• 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 bottom 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 bottom 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)

**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:
  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.
- Write the farm name and Premises ID on the bottom of the box of samples (not on the lid).
- Submit a completed submission form which includes Premises ID.

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.