Supplement 1
HPAI Surveillance/Egg Movement Guidelines

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S1.1 INTRODUCTION

S1.1.1 Purpose

This supplement to the *Secure Egg Supply Plan* contains HPAI outbreak response surveillance measures intended to reduce the risk of HPAI spread through the movement of egg-industry products from within the Control Area. Measures were developed based on input from stakeholders participating in the Egg Sector Working Group, scientific publications and expert opinion. The surveillance protocol options recommended here were tailored to the risk of spread and the desired likelihood of detection - for the various commodity movements and their destinations. The impact of recommended surveillance options for each commodity on the risk of HPAI spread and other relevant criteria were evaluated in proactive commodity specific risk assessments and scientific publications.

S1.1.2 Overview

In general, the active surveillance sampling scheme recommends testing pools of 5 oropharyngeal swabs taken from the daily dead bird pool from each house on a commercial table-egg layer operation or breeder farm, either daily or at the time of product movement,

depending on the commodity. RRT-PCR test results are recommended for specific product movements such as washed and sanitized shell eggs moving to market. Testing pooled samples is also recommended in houses with a higher normal daily death loss to ensure a comparable probability of detecting HPAI.

S1.2 Active Surveillance Recommendations

According to the *SES Plan*, the flocks on monitored or at-risk premises in the Control Area that seek to move egg-industry products must be monitored for clinical signs of disease on a daily basis. In commercial table-egg layers, normal flock production parameters are exceeded when there is an increase in daily mortality greater than 3 times the past 7-day average and greater than 0.03 percent of the flock. *(4)* If the RRT-PCR test on the dead bird pool is not negative or if the daily mortality spikes (over 3 times the 7-day average daily mortality), additional diagnostic testing is conducted.*(12)*

A pooled sample consists of oropharyngeal swab samples taken from 5 dead birds from the pool of available mortality daily, from each house on the premises. The dead bird pool includes the daily mortality collected by the grower each morning. In situations where less than 5 dead birds are available, sick birds may be sampled to collect a total of 5 birds. Sick birds, are birds that have clinical signs consistent with HPAI infection.*(6)*[1] If fewer than 5 dead or sick birds are available, only the available dead or sick birds should be swabbed and pooled.[2] The absence of sick or dead birds is considered to be equivalent to a negative RRT-PCR test result based on testing of mortality pools. In caged table-egg layer houses, the predicted likelihood that there would no dead birds present for sampling on one day is very low, and the likelihood that there would be no dead birds present for sampling on two consecutive days is extremely low.[3] For breeder flocks (house), that absence of mortality is more common, and there is a low likelihood that there would be no dead birds on one day and very low on two consecutive days. When greater than 50 dead birds are present on a day in a house, then one pooled sample must be taken per 50 dead birds (e.g. 57 dead birds would require 2 pools, of 5 swabs). Swabs are pooled in media as required by the current NVSL protocol and each pool is independently tested by RRT-PCR at a NAHLN laboratory.

[1] Swollen combs and wattles, edema of the head which sometimes extends to the neck, combs are often cyanotic at the tips with dark areas of hemorrhage and necrotic foci, edema surrounding the eyes, conjunctivae are congested and swollen with occasional hemorrhage, severe congestion of the musculature, the legs between the hocks and feet may have areas of diffuse hemorrhage and edema, indications of watery diarrhea around the vent, nasal discharge, mucous accumulation with or without blood

[2] Euthanizing healthy birds from the flock to increase the number of swabs in the pool in order to meet a minimum number of 5 or 11 swabs for a pool provides negligible benefit, as there is a very small increase in the probability of detection in relation to the increased cost (labor and supplies) of swabbing healthy birds that must be considered.

[3] Estimate based on simulating weekly mortality data (™Agri Stats, Inc.) and adjusting randomly selected daily mortality counts according to the weekly mortality number. House size was assumed to be 100,000 for table-egg layers and 20,000 for breeder hens.

In order to fulfill the permit requirements to move egg-industry products, the following diagnostic tests are required. The active surveillance testing described here is required for monitored or at-risk premises in the Control Area that are seeking to move egg-industry products, and have live poultry on the premises. The protocols are applicable for HPAI strains that cause clinical illnesses and rapidly increasing mortality in the infected flocks. Alternative surveillance protocols may be required when outbreaks are caused by avian influenza viruses that meet the molecular criteria for classification as highly pathogenic but do not cause elevated mortality that is considered to be representative of most HPAI strains[4]. *(3)*

- Pasteurized Liquid Egg *(7)*
  - No diagnostic testing is required.
- Non-pasteurized Liquid Egg to Pasteurization *(8)*
  - Negative RRT-PCR test results for HPAI on the first day of movement. Each result is from one, 5-bird pool sample per 50 dead birds from each house on the premises.
  - Subsequently, NPLE may be moved off the premises with consecutive daily negative RRT-PCR test results from one, 5-bird pool per 50 dead birds from every house on the premises, where the last test is within 24 hours of product movement.
- Washed and Sanitized Shell-eggs To Premises With or Without Poultry *(5, 9)*
  - One negative RRT-PCR test result is required to move washed and sanitized shell-eggs off the premises into storage or holding, for eggs collected on that day or prior. Each result is from one, 5-bird pool sample per 50 dead birds from each house on the premises.
  - Two negative RRT-PCR test results in conjunction with a 2-day hold, where at least 1 RRT-PCR test result is from a pooled sample taken on the second day of holding or later is required in order to move washed and sanitized shell-eggs to market. Each result is from one, 5-bird pool sample per 50 dead birds from each house on the premises.
- Nest Run Eggs to Processing *(5, 11)*
  - Two negative RRT-PCR test results in conjunction with a 2-day hold, where at least 1 RRT-PCR test result is from a pooled sample taken on the second day of

[4] H5 and H7 viruses which do not have an intravenous pathogenicity index of greater than 1.2 or cause less than 75% mortality in an intravenous lethality test, and that are sequenced to determine whether multiple basic amino acids are present at the cleavage site of the HA molecule, and determined to have an amino acid motif similar to that observed for other HPAI isolates.

holding or later is required to move nest run eggs to processing. Each result is from one, 5-bird pool sample per 50 dead birds from each house on the premises.

- Nest run eggs can move immediately to market after processing.

- Layer Hatching Eggs to the Hatchery *(10)*

  - Two negative RRT-PCR test results in conjunction with a 2-day hold, where at least 1 RRT-PCR test result is from a pooled sample taken on the second day of holding or later is required to move layer hatching eggs to a hatchery or to processing. Each result is from one, 5-bird pool sample per 50 dead birds from each house on the premises.

- Layer Day-old Chicks to a Pullet Farm *(13, 14)*

  - When the Control Area is initially established there may be eggs in the hatchery egg-room from flocks located in the Control Area. Two 5-bird pools from those flocks from each house on the premises should be immediately tested by RRT-PCR and found negative before permits are issued to reduce the risk of day-old chicks being moved off the premises from becoming infected via cross contamination from hatching eggs in the egg-room.

  - Subsequently movements of hatching eggs from within the Control Area will be permitted according to the Hatching Egg Product Summary.

  - Day-old chicks can move to pullet houses on quarantined premises as soon as permit requirements are met.

- Dry Eggshells to a Poultry Feed Mill *(15)*

  - One negative RRT-PCR test result within 24 hours of movement is required to move dry eggshells from a breaking plant to a feed mill. Each result is from one, 5-bird pool sample per 50 dead birds from each house on the premises.

- Wet Eggshells for Land Application or to a Landfill *(15)*

  - Two negative RRT-PCR test results are required before the first movement of wet eggshells to a land application site. Each result is from one, 5-bird pool sample per 50 dead birds from each house on the premises.

  - One negative RRT-PCR test result is required for daily movement thereafter. Each result is from one, 5-bird pool sample per 50 dead birds from each house on the premises.

- Wet Eggshells for Drying at a Standalone Breaking Facility Without Poultry Onsite *(15)*

- One negative RRT-PCR test result within 24 hours of movement is required to move wet eggshells to a drying facility without poultry onsite. Each result is from one, 5-bird pool sample per 50 dead birds from each house on the premises.

- One negative RRT-PCR test result is required for daily movement thereafter.

♦ Inedible Egg Product (INEP) to Pasteurization or Landfill *(15)*

- Two negative RRT-PCR tests are required before the first movement of INEP to pasteurization at an inline facility. Each result is from one, 5-bird pool sample per 50 dead birds from each house on the premises.

- One negative RRT-PCR test result is required within 24 hours prior to movement on subsequent days.

S1.2.1 Surveillance Design Rationale

Targeting daily dead birds: Targeting the daily dead bird pool to detect HPAI is more efficient than randomly sampling live birds in the house because the prevalence of HPAI in the daily dead bird pool increases at a greater rate relative to the HPAI prevalence among live clinically normal birds in the total population (house). HPAI in a house will be detected earlier and with fewer samples, by targeting the daily dead bird pool, than by testing a random selection of live birds from the total population.

For example, the probability of detecting at least one HPAI-infected bird is greater than 95 percent in a house containing 100,000 birds, with normal daily deaths of 32 birds plus 18 or more birds infected with HPAI in the dead bird pool (50 total dead birds) from 2 pools (on consecutive days or on the same day) if each pool is independently tested by RRT-PCR because the test sensitivity on a 5-bird pool is approximately 86.5 percent. Table S1-1 gives the daily probability of detection of HPAI by targeted sampling for pools containing at least one infected swab where 5 birds were sampled.

The probabilities of detection can be determined using the hypergeometric probability of selecting an HPAI infected bird and the sensitivity of the RRT-PCR test. The sensitivity of the RRT-PCR test is assumed to be 86.5 percent if a swab from at least one infected bird is included in the pooled sample.*(1)* Assuming 86.5 percent sensitivity is a conservative assumption as it is unknown as to whether the sensitivity of the test improves if swabs from two or more HPAI infected birds are included in the sample pool.

Assuming random sampling, in order to achieve a 95 percent probability of detection from a population of 100,000 birds in which 18 live birds are infected with HPAI virus, over 19,200 birds must be selected and tested (AusVet FreeCalc, 86.5% sensitivity, 100% specificity). Even if the number of infectious birds in the population is three times the number of birds with clinical signs in this example (i.e. 51 infectious birds), over 7,400 live birds must be selected and tested to achieve a 95 percent level of detection, assuming random sampling. Therefore, by targeting the daily sick and dead bird population, fewer birds need to be sampled.

Table S1-1. Daily probability of detecting HPAI in table-egg layer houses by targeted sampling of the daily dead bird pool with 5 swabs per tube*

Target population 50 dead birds, RRT-PCR Test Sensitivity 86.5%		
Consecutive Days Tested	Scheme # 1◊ One 5-Bird Pool** Per Day	Scheme #2◊◊ Two 5-Bird Pools** First Day
1	78.3%	95.5%
2	95.3%	99%
3	99%	99.8%
4	99.8%	99.9%

* The example gives the probability of detecting at least one HPAI-infected bird where the HPAI prevalence is at least 36 percent in the target population of the daily dead birds each day. The detection probabilities were calculated using the same number of dead birds for each day. No assumptions were made on the prevalence of HPAI-infected birds in the house or an increased number of dead birds to calculate the consecutive day's probability of detection due to HPAI spread in the house.

** Bird- pool samples taken from five dead birds and placed in one pool and tested as a single sample.

◊ Scheme # 1: One bird pool tested each day for the duration of outbreak.

◊◊ Scheme # 2: Two bird pools tested first day, then one 5-bird pool tested each day for duration of outbreak.

Number of test results: The movement of various egg-industry products is associated with different risks for HPAI disease spread. Some of the product movements (e.g. movement of washed and sanitized shell-eggs to market) may also require a higher probability of detection based on the end use. The recommended surveillance options were developed considering the risk of spread associated with each product movement and the desired probability of detection for the various products. For products such as movement of washed and sanitized shell eggs to market where a higher probability of detection is desired so that that eggs are not contaminated, obtaining two negative RRT-PCR test results was recommended. Obtaining two negative RRT-PCR test results also provides a 95% probability of detecting at least one diseased bird in the target population of dead birds at a certain minimum prevalence (36% when testing pooled samples of 5 birds per 50 dead birds) (Table S1.1).

Number of pooled samples per test result: The probability of including a diseased bird in a pooled sample taken randomly from the daily dead bird pool depends on the normal morality relative to the mortality caused by HPAI. For flocks with greater normal mortality, either due to a larger flock size or other operational factors, the probability of detection with testing a single pooled sample would be lower because the probability of selecting a HPAI infected bird to be placed in the 5-bird pool will be lower. In the example provided in table S1-1, the normal mortality is 32 dead birds while the HPAI disease mortality is 18 dead birds. Here, if the normal mortality were 42 birds and 18 birds dead from HPAI for a total of 60 dead birds, then the probability of detection with 2 pooled samples under scheme #1 would decrease to 92.7%. The recommendation to test a pooled sample of 5 birds per each 50 dead birds among the daily mortality in each house would ensure a comparable probability of detection for layer houses with higher normal mortality levels.

Number of swabs per pooled sample: The *SES Plan* recommends pooling swabs from 5 dead birds, per 50 dead birds from each house for RRT-PCR testing, as this protocol has been determined to adequately reduce the risk of HPAI spread through egg-industry products, if the active surveillance measures recommended for each commodity as described in section S1.2 are strictly followed. Recently, protocols for RRT-PCR testing with swabs from 11 dead birds per pool for detecting avian influenza virus (AIV) by RRT-PCR have been validated *(2)*. Using a pool size of 11 dead birds instead of 5 dead birds is acceptable as an option provided that the number of pooled samples tested remains the same as recommended in the *SES Plan*. (Table S1.2).

In some cases, collecting 11 swabs provides an equivalent 95 percent probability of detection for movement of egg-industry products at a cost savings or detects the presence of HPAI at a lower prevalence rate in the target population (Table S1.3). For layer houses with a greater daily mortality, sampling two pooled samples of 11 birds per 100 dead birds among the daily mortality is (95.3 percent) comparable to sampling four 5-bird pools per 100 dead birds (>96.7 percent) when the total number of HPAI infected birds is the same (i.e., 18 percent). Testing two 11-bird pooled samples achieves the 95 percent probability of detection when the prevalence in the target population is 18 percent (Table S1.3) whereas testing of two 5-bird pools achieves the 95 percent detection probability at a prevalence of 36% (Table S1.2). In other words, using the two 11-bird pool protocol detects HPAI in the target population at a lower HPAI prevalence.

Holding Period: A holding time of 2 or more days after egg production in conjunction with daily RRT-PCR testing can significantly reduce the number of contaminated eggs moved from a flock before infection is detected.*(5)* Holding time increases the probability that HPAI infection is detected via diagnostic testing or through observation of clinical signs before moving virus positive product. A 48 hour holding period was recommended by members of the Egg Sector Working Group for some product movements depending on the level of risk.

Table S1-2. Daily probability of detecting HPAI in table-egg layer houses by targeted sampling of the daily dead bird pool with 11 swabs per tube*

Target population 50 dead birds, RRT-PCR Test Sensitivity 86.5%		
Consecutive Days Tested	Scheme # 1◊ One 11-Bird Pool** Per Day	Scheme #2◊◊ Two 11-Bird Pools** First Day
1	86.2%	98.1%
2	98.1%	99.7%
3	99.7%	99.9%
4	99.9%	99.9%

* The example gives the probability of detecting at least one HPAI-infected bird where the HPAI prevalence is at least 36 percent in the target population of the daily dead each day. The detection probabilities were calculated using the same number of dead birds for each day. No assumptions were made on the prevalence of HPAI-infected birds in the house or an increased number of dead birds to calculate the consecutive day's probability of detection due to HPAI spread in the house.

** Bird- pool samples taken from eleven dead birds and placed in one pool and tested as a single sample.

◊ Scheme # 1: One bird pool tested each day for the duration of outbreak.

◊◊Scheme # 2: Two bird pools tested first day, then one 11-bird pool tested each day for duration of outbreak.

Table S1-3. Daily probability of detecting HPAI in table-egg layer houses by targeted sampling of the daily dead bird pool with 11 swabs per tube*

Target population 100 dead birds, RRT-PCR Test Sensitivity 86.5%		
Consecutive Days Tested	Scheme # 1◊ One 11-Bird Pool** Per Day	Scheme #2◊◊ Two 11-Bird Pools** First Day
1	77.9%	95.3%
2	95.1%	99%
3	98.9%	99.8%
4	99.8%	99.9%

* The example gives the probability of detecting at least one HPAI-infected bird where the HPAI prevalence is at least 18 percent in the target population of the daily dead birds each day. The detection probabilities were calculated using the same number of dead birds for each day. No assumptions were made on the prevalence of HPAI-infected birds in the house or an increased number of dead birds to calculate the consecutive day's probability of detection due to HPAI spread in the house.

** Bird- pool samples taken from eleven dead birds and placed in one pool and tested as a single sample.

◊ Scheme # 1: One bird pool tested each day for the duration of outbreak.

◊◊Scheme # 2: Two bird pools tested first day, then one 11-bird pool tested each day for duration of outbreak.

S1.2.2 Assumptions

The following assumptions were made when estimating the probability of detection for the active surveillance protocols.

- HPAI surveillance occurs at the house level. Dead birds are randomly selected for testing from the daily pool of dead birds.
- Each morning, the producer, collects and places all dead birds into the target population from which the bird pool is drawn.
- A producer is equally as likely to miss a HPAI infected dead bird when collecting the daily mortality as any other dead bird in the house.
- Sampling to achieve 95 percent confidence in detecting at least one infected bird in the target population (dead bird pool) is an adequate level of detection.

S1.2.3 Background Information

Daily Mortality: Based on analysis of data provided by the egg-industry, the normal daily death rate for table-egg layers varies from 0.00005 (5/100K) birds to 0.0006 (60/100K) per house. An increase in mortality greater than three times the past 7-day average and greater than 0.03 percent of the flock is a trigger producers to take "diagnostic action" due to observing a unexpected increase in mortality. *(4)* Major factors influencing the mortality rate are: bird strain (death rate: 2.3 to 9.5 percent per year), bird age (0.0003 early in cycle, 0.0001 mid-cycle and 0.0003 at cycle end), and house construction design and age.

House Size: On commercial operations, the number of table-egg layer hens per house varies from 50,000 to 350,000 birds. Fifteen years ago, the average house size was 50,000 birds, but in the last 5 years, newer operations have built houses as large as 300,000 to 350,000 birds. However, this represents a small proportion of table-egg layer producers in operation in the U.S. Breeder house sizes are considerably smaller.

Production Size: Eighty to 85 percent of the total U.S. egg production occurs on complexes that contain 50,000 to 6 million birds. The average number of houses per complex is 10 and a complex may consist of 15 or more houses.

Egg Storage: Most production units have the capacity to store eggs for 2 days, but a minority of premises (especially small producers or producers with older facilities) has a storage capacity of 5–7 days.

Probability of Detection: The probability of detection depends on the number of daily HPAI infected dead birds among the number of normal daily dead birds (HPAI dead bird prevalence). Increased transmission rates are likely to result in more HPAI diseased (dead) birds per day, increasing the HPAI prevalence from which the pools are taken, which also increases the probability of detection by a given day post infection.

S1.3 CONCLUSION

In order for permits to be issued to move egg-industry products from within a HPAI Control Area during an outbreak, the active surveillance protocols described here are to be implemented by industry in conjunction with APHIS during an outbreak. If the RRT-PCR test on the dead bird pool is not negative or if the daily mortality spikes (mortality greater than three times the past 7-day average and greater than .03 percent of the flock), additional diagnostic testing is conducted.

S1.4 INFORMATION SOURCES

This document was prepared by USDA-APHIS-VS-CEAH based on input from members of the Egg Sector Working Group. Additional information was provided through personal communication between Dr. Alex Thompson, USDA-APHIS-VS-CEAH-National Surveillance Unit and Drs. Simon Shane, international poultry consultant; Gregg Cutler, private poultry veterinarian working in a three-person poultry practice in California; Ken Anderson, poultry scientist, North Carolina State University College of Agriculture and Life Sciences, Extension Poultry Science; and Dave Halvorson, extension poultry veterinarian and professor emeritus, University of Minnesota, College of Veterinary Medicine. Additional sources of information were "The North Carolina Layer Performance and Management Test" (2009), the United Egg Producers Web site, and the APHIS National Avian Influenza Response Plan, June 29, 2007.

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