

## VS Guidance 13410.2

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### Disease Testing and Sampling Requirements for Live Avians and Imported Hatching Eggs of Avians During Import Quarantine

#### 1. Purpose and Background

This guidance outlines testing and sampling requirements for imported live avians and hatching eggs of avians required to undergo quarantine as a part of the import process. This guidance does not extend to pet avians; [VS Guidance 13417](#) describes testing requirements for pet birds.

Quarantine facilities are operated by the United States Department of Agriculture (USDA) Animal and Plant Health Inspection Service (APHIS) Veterinary Services (VS) or are privately owned and/or operated and approved by APHIS. The import quarantine period is at least thirty (30) days and may be extended for non-negative test results, illness, or days to hatch for hatching eggs. During quarantine, live avians and hatching eggs undergo testing for highly pathogenic avian influenza (HPAI) and for Newcastle disease. The National Veterinary Services Laboratories (NVSL) conduct all testing. NVSL results determine whether USDA will allow an import or refuse U.S. entry for the shipment.

APHIS may require additional testing when avians in the shipment present clinical signs of communicable diseases other than avian influenza or Newcastle disease, per title 9, *Code of Federal Regulations*, parts 104 and 205 ([9 CFR 93.104](#) and [9 CFR 93.205](#)). The importer must pay for any testing during quarantine. NVSL charges user fees directly to the importer for all tests performed by NVSL, separate from quarantine fees. Current testing user fee rates are online at [Diagnostic Testing at the NVSL](#).

**Notes:** [9 CFR 93.201\(c\)\(3\)\(e\)](#) allows for the import of *Columbiformes* species such as pigeons and doves from HPAI-affected countries and regions. Also, additional policy documents are available to VS staff upon request to assist with decision making when paramyxoviruses are detected during quarantine.

This guidance document represents the Agency's position on this topic and is intended solely as guidance. It does not have the force and effect of law, does not create, or confer any rights for or on any person, and does not bind the U.S. Department of Agriculture (USDA) or the public. Language suggesting that this guidance is mandatory (e.g., "shall," "must," "required," or "requirement") should not be construed as binding unless the terms quote from a statutory or regulatory requirement. The information this document contains may be made available to the public. While this document provides guidance for users outside APHIS, APHIS employees may not deviate from the directions provided herein without appropriate justification and supervisory concurrence.

#### 2. Document Status

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### 3. Reason for Reissuance

This document replaces VS Guidance 13410.1. VS is updating the document to reflect program changes and enhance implementation.

### 4. Authority and References

#### A. Authorities (*Code of Federal Regulations* (CFR)):

- [7 CFR 371.4](#)
- [9 CFR part 93 subpart A](#)
- [9 CFR part 93 subpart B](#)

#### B. References

- [VS Guidance 13401, APHIS Approval and Oversight of Privately Owned Avian Quarantine Facilities in the United States](#)
- [VS Guidance 13417, Procedures for Handling Pet and Performing Bird Importations \(Excluding Ratites and Hatching Eggs of Ratites\)](#)
- [NVSL Diagnostic Testing website \(for sample submission guidance\)](#)
- [NVSL Avian Sample Collection for Influenza A and Newcastle Disease](#)
- [Packaging and Labeling Submissions to the NVSL](#)
- [National Centers for Animal Health \(NCAH\) Portal](#)

#### C. Definitions

- 1) Avians: Animals of the class *Aves*, representing any avian species.
- 2) Pet birds: Birds, except ratites, which are imported for the personal pleasure of their individual owners and are not intended for resale.
- 3) Birds: For the purposes of this guidance, refers to all members of the class *Aves* (including eggs for hatching), other than poultry.
- 4) Poultry: For the purposes of this guidance, refers to chickens, ducks, geese, guinea fowl, partridges, pea fowl, pheasants, quail, swans, pigeons, doves, and turkeys (including eggs for hatching).
- 5) Columbiformes: Pigeons and doves.

### 5. Audience

Veterinary Services (VS) employees, other Federal and State agencies, and members of the public.

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### 6. Guidance

Note: VS personnel as mentioned below includes personnel VS contracts to perform quarantine oversight functions; such individuals must physically collect all specimens for disease testing. This function may not be delegated to the facility's representatives.

#### A. Instructions for Eggs Imported for Hatching

- 1) VS generally does not require specimens from eggs before hatching. If VS determines that hatchlings show signs of illness, personnel will consult NVSL for guidance on collecting/submitting specimens of any unhatched eggs.
- 2) VS personnel must maintain a daily log ([VS Form 17-13](#) or equivalent, as approved by a VS employee) of any mortalities, hatchings, etc. See the current [VS Guidance 13401](#) for additional information on recordkeeping requirements for avians and hatching eggs during quarantine.
- 3) Eggs that have not hatched at the end of the hatching cycle, and which VS determines do not need to be sent to NVSL, must be destroyed via incineration or other sterilization method as allowed by local ordinances.

#### B. Instructions for Avians Imported Live or Hatched from Imported Eggs

- 1) Instructions before collecting specimens
  - a. Plan collections to ensure samples arrive at NVSL Monday - Thursday unless otherwise prearranged with NVSL, to allow for timely inoculation.
  - b. Refer to [Avian Sample Collection for Influenza A and Newcastle Disease](#) for sample collection guidance.
  - c. Segregate specimens collected by each quarantine lot/shipment and submit them separately to NVSL – **do not** mix specimens from different lots/shipments or originating countries on the same submission form.
  - d. Collect daily mortalities (dead avians) each day and refrigerate per the current [VS Guidance 13401](#).
  - e. Ensure that, by Day 16, specimen collection represents all species within the quarantine lot/shipment, and appropriately submit for testing by each quarantine lot/shipment.
- 2) Sampling strategy
  - a. Sampling frequency: Sampling should occur at least twice during quarantine for each lot/shipment, on Days 1-5 and Days 12-16, but at least 10 (ten) days

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apart. Samples collected on Days 1-5 will be tested using standard virus isolation. Samples collected on Days 12-16 will be tested by a single-pass virus isolation approach where possible. This will allow sufficient time to receive results from NVSL before the end of the quarantine period. Sample as described in Tables 1 and 2 below and be sure to note which sampling (see Section 3) f.3 – denote Last submission) to ensure the laboratory can apply the correct procedure.

1. If there are fewer than ten (10) birds in a lot/shipment, divide the samples as equally as possible between at least two (2) pools.
2. When pooling samples, include up to, but no more than, five (5) birds per pool.
3. If there are not enough daily dead or euthanized sick birds to fill the required minimum number of pools, evenly distribute the sick and dead between at least two (2) pools.
4. If the attending veterinarian identifies exceptional mortality, sample the lot/shipment in accordance with Table 2.

Table 1. Sampling during Days 1-2<sup>1</sup>

Total number of birds in a lot	Total number of birds to sample	Number of pooled samples
≤ 14	10 <sup>2</sup>	2
15–30	15	3
> 30	20	4

<sup>1</sup> Sampling expected to achieve 95 percent confidence in detecting at least one infected pool at 20 percent disease prevalence in the target population (lot) with 85 percent protocol sensitivity.

<sup>2</sup> If there are fewer than ten (10) birds in a lot/shipment, divide the samples as equally as possible between at least two (2) pools.

Table 2. Sampling during Days 14-16<sup>3</sup>

Total number of birds in a lot	Total number of birds to sample	Number of pooled samples (up to but no more than 5 birds per pool)
≤ 14	10 <sup>4</sup>	2
15–19	15	3
20–24	20	4
25–29	25	5
30–34	30	6
35–39	35	7
40–44	40	8

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45–94	45	9
95–250	50	10
> 250	55	11

<sup>3</sup> Sampling expected to achieve 99 percent confidence in detecting at least one infected pool at 10 percent disease prevalence in the target population (lot) with 85 percent protocol sensitivity.

<sup>4</sup> If there are fewer than ten (10) birds in a lot/shipment, divide the samples as equally as possible between at least two (2) pools.

- b. Categories to sample: Prioritize categories below in order. Use appropriate personal protective equipment and change between these categories:
    - 1. Mortalities: Prioritize recently dead avians. **Do not** sample if more than seventy-two (72) hours after death (e.g., no need to sample mortalities during Days 3-11 unless considered exceptional).
    - 2. Morbidities: Sick avians.
    - 3. Apparently healthy avians: Sample only when needed to meet minimum sampling numbers in Tables 1 and 2 as appropriate.
  - c. Sample collection
    - 1. Swab collection: Collect the appropriate cloacal or oropharyngeal swab samples and follow the pooling guidance (also in Table 1 of [Avian Sample Collection for Influenza A and Newcastle Disease](#)).
    - 2. Pooling: Specimens from avians held together in closed, common housing may be pooled up to five (5) swabs per pool. Adhere to the sampling method as described by [Avian Sample Collection for Influenza A and Newcastle Disease](#), including route (e.g., location of sampling – oropharyngeal or cloacal route) and species group (e.g., passerines).
    - 3. Storage: Forward specimens to NVSL by the next business day after collection. Store collected specimens in the refrigerator (4°C) until shipment; **do not** freeze.
- 3) Packing, shipping, and identification of specimens
- a. Refer to [Packaging and Labeling Submissions to the NVSL](#).
  - b. Pack samples in a standard TC-34 specimen mailer or other packaging approved by NVSL. Tighten specimen tube caps securely to avoid specimen leakage in transit. Use frozen gel ice packs to keep contents cool.
  - c. To prevent contamination of outer shipping specimen containers, store them outside the facility quarantine area.

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- d. For this guidance, submit samples from each quarantine lot/shipment separately to NVSL – **do not** mix samples from different lots or originating countries on the same submission form.
- e. Clearly identify samples with consecutive numbers and make submissions distinguishable. Ensure the sample identification number on the sample label matches the numbers listed on the National Center for Animal Health (NCAH) packing list.
- f. Use the [NCAH NVSL Portal](#) for all submissions – NCAH guidance for submitters is posted at [NCAH Portal Guidance for NVSL Submitters](#):
  1. Under Submitter Information – the Referral Number is assigned by the submitter and serves as the submitter’s own reference.
  2. Under Specimen enter:
    - i. Category: Viral Diseases
    - ii. Disease or Causative Agent: Avian Influenza/Newcastle Disease Rule Out
    - iii. Laboratory test: At the Discretion of the Lab
  3. Additional Submission Information: Import permit number, expected release date, or any other optional information to provide NVSL about the specimens. The last submission from the quarantined lot should include the statement, "LAST SUBMISSION FROM THIS QUARANTINE."
- g. Place tubes by submission in a sealed plastic bag and disinfect the bag with a 2 percent solution of One Stroke Environ, Virkon S, or other APHIS-approved disinfectant before removing it from the quarantine facility. Place in refrigerator (do not freeze) until sending to NVSL.
- h. Place the disinfected bag into a second plastic bag along with the printed packing list(s) from the NCAH portal (or current [VS Form 10-4](#) in the rare case when the portal is not available) and seal. Place the doubled bagged package in the shipping container that has been held outside the bird holding area. Pack submissions from one quarantine station only in the shipping container with different submissions bagged separately. Shipment will be made by the most expedient courier service available and consistent with current NVSL protocols.

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### 4) Reporting laboratory results for imported avians

NVSL will generate a laboratory report for each submission received. VS personnel responsible for quarantine oversight will:

1. Be solely responsible for furnishing NVSL laboratory results to the importer **and** to the quarantine facility. Release only the final NVSL laboratory report to the importer.
2. Notify the Port Services Director by phone and email of any positive laboratory results for Newcastle disease or avian influenza.
3. Inform the importer if a virus other than Newcastle disease or avian influenza virus is isolated and/or should the quarantine be delayed due to the need for additional testing which would incur additional user fee charges.
4. Inform the importer when additional laboratory work will result in extending the quarantine beyond the minimum thirty (30)-day period.
5. The Port Services Director will notify the VS Strategy and Policy Director of Live Animal Imports (LAI) by phone and email of any positive laboratory results for Newcastle disease or avian influenza.
6. The VS staff responsible for quarantine oversights will consult with the Port Services Director on options to dispose of the shipment.
7. The staff responsible for quarantine oversights will notify the importer in writing of the final disposition decision and any associated user fees.

### 7. Inquiries

Direct questions about this guidance to the LAI avian import staff officer at 301-851-3300 or by email at [LAIE@usda.gov](mailto:LAIE@usda.gov).