

Disease Testing and Sampling Requirements for Imported Live Poultry and Birds (and Hatching Eggs of Poultry, Birds, and Ratites) during Entry Quarantines

1. Purpose and Background

This guidance outlines sampling requirements for imported live poultry, birds, and hatching eggs of poultry, birds, and ratites during import entry quarantine at facilities approved by the Animal and Plant Health Inspection Service (APHIS) Veterinary Services (VS) division (hereinafter “VS”). The entry quarantine period for applicable poultry or hatching eggs is at least 30 days, and may be extended in case of infection or illness found during the quarantine. During the quarantine period, facilities will submit samples to the National Veterinary Services Laboratories (NVSL) for testing by virus isolation for avian influenza and Newcastle disease.¹ The NVSL must find the samples free of these viruses before the birds or eggs can be released from quarantine.

The National Animal Import Center Director or the Director of an Animal Import Center may approve the collection of additional specimens to be tested for other avian pathogens and may also ask the NVSL to use other testing protocols in addition to virus isolation. Requests for additional tests should only be made in the event of a disease outbreak during which quarantined birds manifest clinical signs other than those of avian influenza or Newcastle disease. Importers should consult the NVSL on specimen collection procedures for tests not covered by this guidance. Additional test requests will incur associated testing fees, for which the importer is responsible.

This guidance document represents the Agency’s position on this topic. It 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. The information it contains may be made available to the public. While this document provides guidance for users outside VS, VS employees may not deviate from the directions provided herein without appropriate justification and supervisory concurrence.

2. Document Status

- A. Review date: March 31, 2020.
- B. This document updates and replaces Veterinary Services Memorandum No 591.22, which is rescinded. The content of this document reflects program changes and enhances implementation.

3. Reason for Reissuance

Not applicable.

¹ 1) The NVSL charges a user fee directly to the importer separate from quarantine fees. Current fee rates can be found on the NVSL diagnostic testing Web site.

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4. Authority and References

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

- [7 CFR 371.4](#)
- [9 CFR 93 subparts A and B](#)

B. References

- [VS Guidance 13401.2, "APHIS Approval and Oversight of Privately Owned Avian Quarantine Facilities in the United States"](#)
- [VSG 5510.1, "Procedures for Processing Cooperative Service Agreements for Privately Owned Quarantine Facilities for Commercial Birds, Poultry and any Avian Eggs for Hatching," \(11/04\).](#)
- VS Notice 10-07, Diagnostic Submissions to the National Veterinary Services Laboratory is listed on the VS Share point for Notices.
- [VS Form 10-4, "Specimen Submission," \(8/09\).](#)
- [NVSL diagnostic testing user fees.](#)

5. Audience

VS employees, other Federal and State agencies, and members of the public.

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Note: VS personnel includes personnel VS contracts to perform quarantine oversight functions; they must physically collect all samples for disease testing. This function may not be delegated to the facility's representatives.

A. Instructions for Specimen Collection from Eggs Imported for Hatching

- 1) VS generally does not require sampling eggs before hatching. If VS determines that hatchlings show signs of illness, the NVSL may be consulted for guidance on specimen collection/submission procedures of any unhatched eggs.
- 2) VS personnel must maintain a daily log (VS Form 17-12 or equivalent, as approved by a VS representative). Any mortalities, hatchings, etc., must be accounted for using this form or an approved equivalent. See VS Guidance 13401.2 for recordkeeping requirements for poultry or hatching eggs during a quarantine period.
- 3) Eggs that have not hatched at the end of the hatching cycle, and which VS determines do not need to be sent to the NVSL, must be destroyed via incineration or other sterilization method as allowed by local ordinances.

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B. Instructions for Specimen Collection from Poultry Imported Live or Hatched from Imported Eggs

(Note: see Section C for swab sampling and pooling techniques.)

1) Sampling within 24 hours of arrival or hatching

- a. Swab collection: Collect cloacal or oropharyngeal swab samples from all animals (up to a maximum of 150) that die within 24 hours of arrival at the quarantine facility (or within 24 hours of hatching for eggs). The oropharyngeal (OP) site should be used for sample collection from chickens, turkeys, and game birds (gallinaceous species). For waterfowl use only the cloacal site.
- b. Submit samples to the NVSL as described in section D.

2) Sampling during days 2-16 (from dead poultry only)

a. Tissue samples

1. Collect tissue samples from dead poultry on either days 2, 7, and 12 or days 3, 8, and 13 (every 5th day).
2. Sample all dead birds up to a maximum of 30 dead poultry collected per individual collection day (and up to a maximum of 90 animals sampled between days 2 and 16).
3. Up to a total of 5 tissue specimens (from no more than 5 different animals of the same species) can be pooled into one tube. Do not pool tissue specimens from multiple species (for example, do not mix chicken with duck tissue specimens).
4. VS should instruct the importer, the quarantine operator, or both to hold all mortalities in a cooler in between days designated for VS sampling.

b. Swab samples

1. In addition to tissue samples, collect swab samples from dead poultry, one swab per animal, on either days 2, 7, and 12 or days 3, 8, and 13 (every 5th day). Swab no more than 40 dead poultry per individual collection day.
2. Swabs from five animals (from the same species) should be pooled into one broth tube. Submit up to 40 samples (8 broth tubes) per collection day, with a maximum submission for the 3 collection days of 24 broth tubes which represents a **maximum** of 120 animals. If there are fewer dead animals, sampling numbers will be fewer. These numbers reflect maximums. If there is much additional mortality, consult the NVSL to determine if sampling should be increased.

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- 3) Sampling during days 7-14 (live poultry)
 - a. 75 live poultry should be sampled by swabbing only (one swab per animal) as a one-time event during days 7 through 14. (If the quarantined lot contains fewer than 75 animals, sample the whole shipment.)
 - b. Swab the oropharynx of non-waterfowl species (for waterfowl, swab the cloaca).
 - c. Any clinically ill poultry should be sampled first, with the remainder from apparently healthy animals.
 - d. Sample poultry located in different areas throughout the facility.
 - e. All species within the facility should be represented in the samples selected, but do not pool more than one species together in a tube.
 - f. VS encourages collection between days 12 and 14 whenever possible.
- 4) If exceptional mortality occurs during days 2 through 16 (or later during the quarantine), additional sampling may be advisable. Personnel collecting samples should consult with the NVSL regarding unusual mortality events.

C. Tissue and Swab Sampling Protocols

- 1) General tissue collection from dead poultry

Remove spleen, trachea, lungs, and a portion of the terminal gut. Place the organs (or parts thereof, depending on the size of the birds) in 5.5 ml of brain-heart infusion (BHI) broth and freeze promptly. Ensure that BHI broth covers all tissue within the tube. (Note: Dead poultry should be collected daily (by the importer) and refrigerated per VS Guidance 13401.2.)

- 2) General swab collection from dead poultry:

Sampling must be vigorous to obtain tissue of the cloaca or oropharynx. Use one swab per animal. Do not use cotton swabs. The NVSL recommends pooling five swabs per tube of broth; however, do not pool swabs collected from different species, and do not pool tracheal and cloacal swabs. If VS personnel need further instructions on how to collect swabs, they should consult the NVSL "[Disease-Specific Guide to Sample Collection.](#)" Note: The oropharyngeal (OP) site should be used for sample collection from chickens, turkeys, and game birds (gallinaceous species). Only the cloacal site should be used for waterfowl.

- 3) VS offices must plan collections to ensure samples arrive at the NVSL during the business week (unless otherwise prearranged with the NVSL).

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Table 1 Summary of Sampling

Time	Submission Type	Sample types Tissue or Swabs	Max. # poultry sampled	Maximum # pooled* samples sent to NVSL	Notes
Arrival-24 hours	Dead poultry	Swabs from cloacal or oropharyngeal sites	150	30	
Days 2-16 (sample every 5th day)	Dead poultry	Tissue: Sample no more than 30 dead animals per 5- day period	90	18	If less than 30 dead per sampling period sample all
		Swabs: Sample no more than 40 dead animals per 5 day period	120	24	
Days 7-14 (one time sampling)	Live poultry	Swabs: cloacal or oropharyngeal	75	15	Sample the whole shipment, if less than 75 animals
Total			435	87 pooled samples	

* Pooled is defined as collected tissues or swabs from no more than 5 different animals of the same species.

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D. Packing, Shipping, and Identification of Specimens

- 1) Pack samples in dry ice (or with multiple frozen gel packs) in a standard TC-34 specimen mailer or other replacement form approved by the NVSL. Tighten specimen tube caps securely to avoid specimen leakage in transit.
- 2) To prevent contamination of outer shipping specimen containers, store them outside the quarantine area in the facility.
- 3) For this guidance, a submission consists of all of the specimens collected on a given date. Identify samples with consecutive numbers. Ensure the sample identification number on the sample label matches the numbers listed on VS Form 10-4.
- 4) All blocks on VS Form 10-4, "Specimen Submission," should be filled out as completely as possible. Record all information related to the given group of birds on VS Form 10-4 or current equivalent. The following items are of particular importance:
 - a. Block 9: Examinations Requested -- Newcastle disease and avian influenza by virus isolation.
 - b. Block 15: Referral Number assigned by the submitter; serves as the submitter's own reference.
 - c. Block 21: Identification – assigned by the submitter. Should be consecutive numbers, and must correspond with the number placed on the tube.
 - d. Block 22: Additional Data: Import permit number, expected release date or any other optional information to provide the NVSL about the specimens. The last submission from the quarantined lot should include the statement, "LAST SUBMISSION FROM THIS QUARANTINE."
- 5) Specimens should be frozen as soon after collection as possible. Place tubes with submission forms (VS Form 10-4 or current equivalent) in a sealed plastic bag. Disinfect the bag with a 2 percent solution of One Stroke Environ, Virkon S, or an APHIS-approved disinfectant, before removing it from the quarantine facility. Place the sealed and disinfected bag in the shipping container that has been held outside the bird holding area. Pack submissions from one quarantine station only in the shipping container. Shipment will be made by the most expedient courier service available and consistent with current NVSL protocols. Current shipping requirements and mailing addresses can be found on the [NVSL Web site](#).

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E. Reporting Laboratory Results for Imported Poultry

- 1) The NVSL will generate a laboratory report for each submission received.
- 2) VS personnel responsible for quarantine oversight will:
 - a. Be solely responsible for furnishing laboratory results to the importer and to the quarantine facility. Only final and conclusive laboratory results should be released to the importer.
 - b. Report positive laboratory results of Newcastle disease and/or avian influenza to the importer via a written report on receipt of the laboratory report. The written report will list the location of the quarantine, the importer's name and contact information, the species involved, the date of the collection, and the date of viral isolation by the NVSL.
 - c. Notify the supervising director by phone and email of any positive laboratory results for Newcastle disease or avian influenza.
 - d. Inform the importer when virus isolates, other than Newcastle disease or avian influenza virus, are made; and/or whether embryo (hatching egg) deaths that may occur during quarantine require additional laboratory work and additional user fee charges.
 - e. Inform the importer when additional laboratory work will result in holding the poultry beyond the minimum 30-day quarantine period.
 - f. Furnish copies of laboratory reports to the importer on request.
- 3) The supervising director will notify the VS National Import Export Services Director of Live Animals, Riverdale, MD, by phone and email of any positive laboratory results for Newcastle disease or avian influenza.

F. Disposition of Poultry in the Event of Positive Lab Results

- 1) The VS administrative unit in the field or the VS staff responsible for quarantine oversights will consult with the supervising director on options to dispose of the shipment.
- 2) The VS administrative unit in the field or VS staff responsible for quarantine oversights will notify the importer in writing of the final disposition decision and any associated user fees.

7. Inquiries

Questions should be directed to the avian import staff officer at 301-851-3300, opt.2, or by email at VS-Live.Animals_Import.Permits@aphis.usda.gov.