

Avian Sample Collection for Influenza A and Newcastle Disease

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This document contains sample collection recommendations from the National Veterinary Services Laboratories (NVSL) in Ames, IA, specifically for the detection of avian influenza A viruses (IAV) and avian paramyxoviruses (APMV-1), such as Newcastle disease (ND). This document supersedes all previous versions (WI-AV-0020) of *Avian Sample Collection for Influenza A and Newcastle Disease* (now NVSL-WI-0023).

- For guidance on the appropriate use of antigen capture immunoassay for influenza A, refer to https://www.aphis.usda.gov/animal_health/emergency_management/downloads/hpai/acia_testpolicy.pdf
- For guidance on the collection of environmental samples, please refer to the [Post C&D Environmental Sampling Guidance](#).
- For foreign animal disease (FAD)¹ investigations, the fastest route for confirmation is by the collection of duplicate samples for submission to a National Animal Health Laboratory Network (NAHLN) lab and NVSL in parallel – refer to the *Foreign Animal Disease (FAD) Investigation Manual (FAD PReP Manual 4-0)* for further guidance.
- For FAD investigations a **minimum of two pools** of 11-bird swab samples for gallinaceous poultry **or four pools** of 5-bird swab samples for any avian species are recommended.

1.1. Viral Transport Media (VTM)

- 1.1.1. Brain heart infusion broth (BHI; e.g., BD Bacto #237400) is the recommended VTM for these specimens as it contains a protein component which protects the virus from degradation during storage and shipping and is available from the NVSL for national avian influenza testing; the order form can be accessed [here](#) – refer to Table 1 – in summary:
 - 3 ml BHI Broth (40 tubes/box; no antibiotics): blue cap plastic tube for **up to 5-bird** swab pools from avian species.
 - 5.5 ml BHI with antibiotics (40 tubes/box): black cap glass tube for up to 5-bird swab pools for any avian species, and **up to 11-bird** swab pools from gallinaceous poultry only (refer to **3.1.2.**); may also be useful for environmental samples.²
 - NOTE: BHI when stored appropriately and visually inspected is fit-for-purpose for several years.
- 1.1.2. Other acceptable VTM include any salt-balanced, buffered media with a protein component such as tris-buffered tryptose broth (TBTB)³, nutrient broth (NB), and peptone broth (PB); or commercially available media, e.g., [BD™ Universal Viral Transport 3 mL Collection Kit](#), and Primestore MTM (currently for Wildlife Services wild bird surveillance; and where approved by APHIS – contact NVSL for appropriate sample volume).

¹ www.aphis.usda.gov/fadprep

² Refer to *HPAI Response Post C&D Environmental Sampling Guidance - Poultry* May 5, 2022 https://www.aphis.usda.gov/animal_health/emergency_management/downloads/hpai/env_sampling_proced.pdf

³ TBTB Formulation (NVSL media #10088): 1.21 g Trizma Base 77-86-1, 26 g Tryptose Broth, 1000 ml QH2O

- 1.1.3. If not an FADI, In the absence of appropriate VTM, phosphate buffered saline (PBS) or saline solution (contact lens solution – *not* disinfectant) may be used as a last resort to keep the swab moist during transport – dry swabs are **not** acceptable (refer to 1.1.4.); **NOTE:** PBS or saline should only be used when none of the preferable media are available as **negative results are not acceptable** for confirmation of disease status; appropriate samples should be obtained.
- 1.1.4. Dry swab specimens should be avoided – heat and desiccation can inactivate IAV and ND in ≤ 24 hours; therefore, **negative results are not acceptable** for confirmation of disease status; appropriate samples should be obtained.

2.1. Swab collection (Figure 1)

- Target sample collection from birds with the following priority⁴:
 - 1) Recent mortalities
 - 2) Sick birds
 - 3) When birds specified in items #1 or #2 above are not available and sampling of apparently healthy birds is needed, target birds next to building inlets or in cages adjacent to sick/dead birds
 - Use synthetic or semi-synthetic swabs (e.g., polyester, rayon, nylon) with a plastic handle (flocked or spun head).
 - **Avoid** cotton or calcium alginate swabs or swabs with wooden handles which have been shown to inactivate virus and inhibit PCR invalidating the laboratory test results.
- 2.1.1. Oropharyngeal (OP) swabs are preferred for gallinaceous poultry (**Figure 1b-1d**)
 - Swab the oral cavity and opening of the trachea, avoiding the esophagus, and bring the swab up through the choanal cleft where the sinuses drain to capture material from the upper respiratory tract (refer to **Figure 1a**).
 - Tracheal swabs (TR), if needed, are best obtained from fresh carcasses.
 - 2.1.2. Cloacal (CL) swabs are preferred for domestic waterfowl and other wild birds unless H5 goose/Guangdong lineage viruses are suspected in which case both OP and CL swabs are recommended (**Figure 1e**).
 - 2.1.3. The NVSL prefers submission of the **entire swab suspension** for diagnostic testing. After collecting the sample, swirl the swab vigorously in the VTM, squeeze the excess liquid from the swab inside the specimen tube and collect the swab in an appropriate container for proper disposal at the laboratory. Avoid leaving swabs or other collection devices in the tube; swabs left in the media may reduce the volume available for testing.
 - 2.1.4. Clearly label containers with appropriate ID using a waterproof marker or other label (barcode ID labels are available from NVSL on the same request form used for BHI).

3.1. Pooling procedures⁵

- 3.1.1. Swab samples may be pooled in accordance with **Table 1** and by:
 - the same species,
 - the same premises, and

⁴ Refer to *H5N1 Red Book*,

https://www.aphis.usda.gov/animal_health/emergency_management/downloads/hpai_response_plan.pdf.

⁵ Pooling procedures are based upon work conducted by Spackman et al. in coordination with the NVSL (BMC Veterinary Research 2013, 9:35 <http://www.biomedcentral.com/1746-6148/9/35>).

- the same sampling route – do NOT pool TR/OP and CL together,
 - additionally, pool mortalities, sick birds, and apparently healthy birds separately
- 3.1.2. The 5-swab pool in at least 3 mls of VTM was validated for both TR/OP and CL swabs from gallinaceous poultry and domestic ducks tested for IAV and ND; collection of up to 6 swabs in 3 mls VTM following the guidance in 3.1.1. allows for collection of the recommended 11 samples per Secure Supply plans and NPIP surveillance using two 3ml VTM tubes rather than three. ⁶
- A single 11-bird swab LAB POOL may be generated from one 5-bird swab pool and one 6-bird swab pool at the testing laboratory (testing labs should refer to NVSL SOP-AV-0068 for further details).
- 3.1.3. **The 11-bird swab pool is only valid for IAV/ND testing of TR/OP swabs from gallinaceous poultry and must be in 5 mls BHI**; NOTE: This has not been evaluated for other diseases such as infectious bronchitis, infectious laryngotracheitis, or mycoplasma. NOTE: The NVSL supplies 5.5ml BHI which contains antibiotics and is not appropriate for testing of bacterial diseases. For FADIs, a minimum of 2 11-bird swab pools per barn is required; or 4 5-bird swab pools for non-gallinaceous species.
- 3.1.4. **Tissues:** pool by system (respiratory, enteric, reproductive) typically from a single bird; it is not recommended to pool tissues from more than one bird especially for free-living waterfowl.

4.1 Specimen transfer and storage

- Maintain cold chain for all samples.
- Specimens should be held on ice pack immediately following collection until transferred to the testing laboratory or other refrigerated storage.
- Tubes should be stored and transferred in an upright position to reduce chances of leakage.
- IAV and APMV-1 have been shown to be stable in BHI when stored at refrigeration (4°C) for up to **96 hours**, with consideration given to the length of time needed at the laboratory for sample processing.
- If samples have been frozen (-70°C), they should remain frozen until delivered to the testing laboratory.
- Specimens should **never** be stored in the freezer portion (-20°C) of a standard refrigerator/freezer unit with an automatic defrost cycle (specimens will go through freeze/thaw, which is detrimental to the survival of virus and viral nucleic acid).

5.1 Forwarding samples to the NVSL in Ames, IA

- 5.1.1. Email ahead of shipment to: nvsl.ai.nd@usda.gov - Please include: 1) number of samples, 2) 10-4 form⁷, 3) tracking number, 4) pertinent case and contact information on the day the package is shipped. **Please use 8 AM delivery option if shipping by FedEx.**
- a. For FAD¹ investigations or samples from poultry, include in the email subject line:

⁶ While pools of 6 swabs have not specifically been evaluated, it is considered an acceptable practice where seeking to conduct recommended 11-bird swab surveillance using 2 pooled samples instead of 3 (refer to 3.1.3); NOTE: Testing of a single 11-bird swab pool only applies to TR/OP swabs from gallinaceous poultry.

⁷ http://www.aphis.usda.gov/library/forms/pdf/VS_Form10_4.pdf

State FAD reference number (or 2-digit state if no FAD number)/**Priority X/Fed Ex tracking #.**

- b. If wildlife, email subject line should contain:
2-digit State/ wild bird/Fed Ex tracking #.
- 5.1.2. If the investigation is Priority 1 or A, **email first to nvsl.ai.nd@usda.gov**. If there is no reply, it is critical to directly speak to someone in Diagnostic Virology at the NVSL, **call 515-337-7551** (after normal business hours the number will roll to security personnel who can get in contact with relevant personnel). Other contacts for DVL can be found at the following site: [USDA APHIS | NVSL Diagnostic Virology Laboratory](#).
- 5.1.3. Please contact the Diagnostic Virology Laboratory at the number above for further instructions when sending driver/courier after hours/holidays/weekends.

Table 1. Preferred specimens for Influenza A and Newcastle disease diagnostics.

Sampling source	Preferred Specimen	Sample Collection	Comment
Gallinaceous poultry (e.g., chickens, turkeys, pheasants, quail)	Tracheal or oropharyngeal (TR/OP) preferred Cloacal swab (CL) may be used	<ul style="list-style-type: none"> FOR FADs – typically 5 swabs/pool in at least 3 mls of VTM Up to 11 swabs/pool in at least 5mls of VTM pooled is valid only for TR/OP swabs from gallinaceous species^a Up to 5 swabs/pool at least 3 mls of VTM pooled by sample route and species	Virus usually shed via respiratory route; may be strain dependent
Domestic waterfowl (production)	CL preferred , TR/OP swab may be used	Up to 5 swabs/pool from a single flock and species in at least 3 mls of VTM	Virus usually shed via the enteric route; may be strain dependent – e.g., for H5 goose/Guangdong lineage, both OP and CL are recommended
Wild/captive waterfowl species ^c	TR/OP and CL swabs may be used	<ul style="list-style-type: none"> Collect USDA Wildlife Services Surveillance samples by pooling 1 CL and 1 OP swab from a single bird in one 3ml VTM tube^b; this approach may also be used for captive waterfowl that are openly housed Captive flocks in closed, common housing may be pooled 5 swabs/pool in at least 3mls VTM by sample route and species 	Wild migratory waterfowl are the natural reservoir for influenza A viruses (typically enteric shed)
Other wild/free living/captive /pet species	Typically, CL swabs ; fresh fecal samples may be used – call the NVSL for guidance	<ul style="list-style-type: none"> Captive flocks in closed, common housing may be pooled 5 swabs/pool in at least 3mls VTM by sample route and species group (e.g., passerines) 	Shedding of influenza from non-host species can be variable and dependent on other factors such as immune status and virus strain
Any avian species	Tissue samples	Pool by system from a single bird (e.g., respiratory, enteric, reproductive) ^b - mince tissue and place in 3mls VTM	vND viruses may replicate to higher titres in tissues; brain tissue is preferred if neurological signs are noted

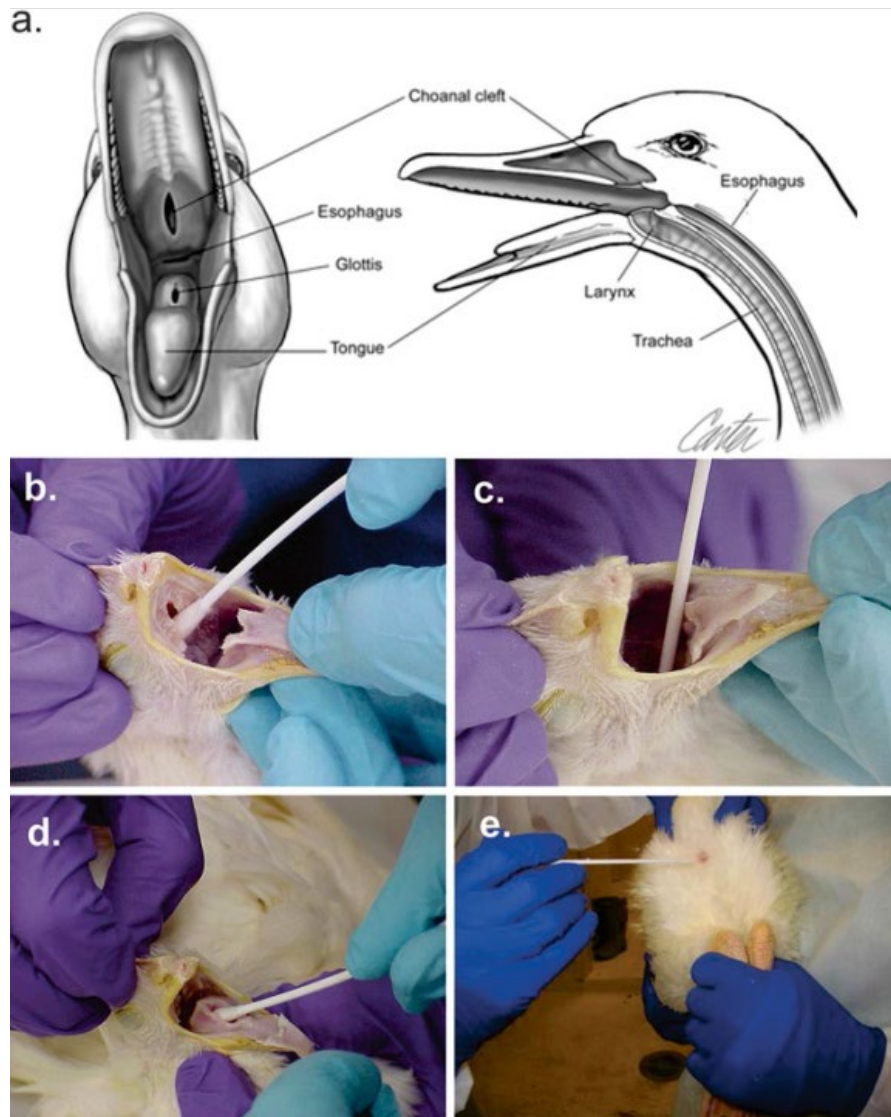
^a The 5-bird swab pool in 3 mls of VTM was validated for IAV and ND testing of both TR/OP and CL swabs from gallinaceous poultry and domestic ducks; pooling of up to 6 swabs from the same species, location, and sampling route in 3 mls allows for collection of 11 samples per Secure Supply plans and NPIP surveillance using two tubes rather than three. Either 3ml (any for domestic species) or 5.5 ml with abx (for TR/OP swabs from gallinaceous poultry only) may be used for zone surveillance.

^b Antibody from one bird may neutralize virus from another (e.g., mixed backyard poultry); avoid potential of mixing viruses from different birds in a single sample when sampling migratory waterfowl.

^c The H5 goose/Guangdong HPAI viruses often shed higher via the OP route in waterfowl; sampling of both routes is recommended.

Figure 1.

Swab collection: a) schematic of oral cavity; b-d) tracheal/oropharyngeal (TR/OP) swabs preferred for gallinaceous poultry; e) cloacal (CL) swabs preferred for domestic waterfowl and wild birds. From: *Methods in molecular biology*, vol. 436: Avian influenza virus. E. Spackman, ed. Humana Press, Totowa, NJ. pp. 95. 2008.



Appendix 1: BHI Stability

Stability studies were conducted on 3 separate lots of BHI media containing antibiotics (NVSL media # 50067) and 3 separate lots containing no antibiotics (media # 10009). Each lot of media was spiked with live virus then frozen and thawed two days later to mimic shipping conditions from field to lab. Virus isolation was conducted and harvested material was evaluated for bacterial contamination and confirmation of virus recovery. Testing was conducted three separate times for each of the 3 lots of media over the course of approximately one year.

BHI broth was determined to be stable a **minimum of 6 years from manufacture date**. Expiration dates are provided on the outer box containing BHI tubes prepared at the National Veterinary Services Laboratories for quality management. The expiration of unopened BHI tubes may be extended in 6 month increments up to 6 years past expiration using the following criteria.

- 1) The expiration on the box applies to the tubes provided in the original shipment. Tubes can be individually labeled with the expiration date after receipt.
- 2) Individual laboratory policies for expired reagents should be used if applicable. If the policy is to NOT use expired reagents, then a deviation including a plan must be put into place to proceed. A laboratory may have a policy to requalify media or obtain documentation supporting an extended expiration. If there is no policy regarding expired media, follow the recommendations below.
- 3) BHI expiration can be extended as long as the unopened media has been stored at 4°C or colder (-20°C recommended), the tubes have not been compromised, no obvious color change is observed, and the media does not appear to be cloudy.
- 4) The following should be documented: storage conditions of the expired media since the time of receipt, who confirmed the criteria in bullet 3 and when the media was evaluated.