



This document contains sample collection recommendations from the Avian Viruses Section at 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).

Updated 03/2016, this document supersedes previous versions of **WI-AV-0020**.

- For guidance on the collection of environmental samples, please refer to the Post C&D Environmental Sampling Guidance.
- For foreign animal disease (FAD)<sup>1</sup> 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.

### 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; the order form can be accessed [here](#):
  - 3 ml BHI Broth (40 tubes/box; no antibiotics): blue cap plastic tube for up to 5 swab pools from avian species.
  - 5.5 ml BHI with antibiotics (40 tubes/box): black cap glass tube for up to 11 swab pools from gallinaceous poultry (refer to **3.1.2.**); also for environmental samples.<sup>2</sup>
- 1.1.2. Other acceptable VTM include any salt-balanced, buffered media with a protein component such as tris-buffered tryptose broth (TBTB)<sup>3</sup>, nutrient broth (NB), and peptone broth (PB); or commercially available media, e.g., [BD™ Universal Viral Transport 3 mL Collection Kit](#).
- 1.1.3. 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 clinical signs (daily morbidity and mortality)
- Use synthetic or semi-synthetic swabs (e.g., polyester, rayon, nylon) with a plastic handle (flocked or spun head).

<sup>1</sup> [www.aphis.usda.gov/fadprep](http://www.aphis.usda.gov/fadprep)

<sup>2</sup> Refer to *HPAI Outbreak 2014-2015 Post C&D Environmental Sampling Guidance* August 19, 2015  
[https://www.aphis.usda.gov/animal\\_health/emergency\\_management/downloads/hpai/env\\_sampling\\_proced.pdf](https://www.aphis.usda.gov/animal_health/emergency_management/downloads/hpai/env_sampling_proced.pdf)

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

- **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 (**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. The tips of any swabs remaining in the specimen tube must be fully immersed in VTM; swabs left in the media may reduce the volume available for testing.
    - No more than 5 swabs are permitted to remain in the VTM tubes provided by the NVSL; **negative results** from swab samples not fully immersed in VTM **are not acceptable** for confirmation of disease status; appropriate samples should be obtained.
  - 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 procedures<sup>4</sup>

- 3.1.1. Swab samples may be pooled in accordance with **Table 1** and by:
  - the same species,
  - the same premises, and
  - if poultry, the same sampling route – do NOT pool TR/OP and CL together.
- 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.<sup>5</sup>
  - A single 11-swab LAB POOL may be generated from one 5-swab pool and one 6-swab pool at the testing laboratory (testing labs should refer to *NVSL SOP-AV-0068* for further details).
- 3.1.3. **An 11-swab pool in 5.5 mls of VTM can be collected only for IAV/ND testing of TR/OP swabs from gallinaceous poultry**; 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.

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<sup>4</sup> 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>).

<sup>5</sup> While pools of 6 swabs have not specifically been evaluated, it is considered an acceptable practice where seeking to conduct recommended 11-swab surveillance using 2 pooled samples instead of 3 (refer to 3.1.3); NOTE: Testing of a single 11-swab pool only applies to TR/OP swabs from gallinaceous poultry.

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

- 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.dvl.avian@aphis.usda.gov](mailto:nvsl.dvl.avian@aphis.usda.gov) - Please include: 1) number of samples, 2) 10-4<sup>6</sup> form, 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 FAD<sup>1</sup> investigations or samples from poultry, include in the email subject line:  
**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 1-2 or A it is critical to directly speak to someone in the Avian Viruses section 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).
- 5.1.3. Please contact the Avian Viruses section at the number above for further instructions when sending driver/courier after hours/holidays/weekends.

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<sup>6</sup> [http://www.aphis.usda.gov/library/forms/pdf/VS\\_Form10\\_4.pdf](http://www.aphis.usda.gov/library/forms/pdf/VS_Form10_4.pdf)

**Table 1.** Preferred specimens for influenza A and Newcastle disease diagnostics (rev. 03/2016).

Sampling source	Preferred Specimen	Sample Collection	Comment
Gallinaceous poultry (e.g., chickens, turkeys, pheasants, quail)	<b>Tracheal or oropharyngeal (TR/OP) preferred</b>	<ul style="list-style-type: none"> <li>FOR FADs – typically 5 swabs/pool in at least 3 mls of VTM</li> <li>Up to 11 swabs/pool in at least 5.5 mls of VTM pooled by sample route and species for TR/OP swabs from gallinaceous species only <sup>a</sup></li> </ul>	Virus usually shed via respiratory route; may be strain dependent
	Cloacal swab (CL) may be used	Up to 5 swabs/pool at least 3 mls of VTM pooled by sample route and species	
Domestic waterfowl (production)	<b>CL preferred</b> , 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
Wild/captive waterfowl species	<b>TR/OP and CL</b> swabs may be used	<ul style="list-style-type: none"> <li>Collect USDA Wildlife Services Surveillance samples by pooling 1 CL and 1 OP swab from a <b>single bird</b> in one 3ml VTM tube<sup>b</sup>; this approach may also be used for captive waterfowl that are openly housed</li> <li>Captive flocks in closed, common housing may be pooled 5 swabs/pool in at least 3mls VTM by sample route and <b>species</b></li> </ul>	Wild migratory waterfowl are the natural reservoir for influenza A viruses (typically enteric shed)
Other wild/free living/captive /pet species	Typically <b>CL swabs</b> ; fresh fecal samples may be used – call the NVSL for guidance	<ul style="list-style-type: none"> <li>Captive flocks in closed, common housing may be pooled 5 swabs/pool in at least 3mls VTM by sample route and <b>species group</b> (e.g., passerines)</li> </ul>	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 <b>single</b> bird (e.g., respiratory, enteric, reproductive) <sup>b</sup> - 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

<sup>a</sup> The 5-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.

<sup>b</sup> 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.

**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.

