

Recommendations for Collecting Specimens from Poultry for Viral Diagnostic Testing

The purpose of this document is to provide recommendations to veterinary field and laboratory staff collecting and testing poultry specimens, specifically for influenza A viruses of birds (IAV) and avian paramyxoviruses (APMV) such as Newcastle disease (ND). These recommendations pertain to poultry virus or nucleic acid detection, and may not apply to other areas. Some viruses may be easily inactivated, so to avoid compromising the quality of the sample for diagnostic testing, the following procedures 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 (IAV and APMV have been shown to be stable in BHI when stored at 4°C for up to 96 hours) and is available from the NVSL at no cost; the order form can be accessed at: <http://www.aphis.usda.gov/library/forms/pdf/NVSLKitRequestForm.pdf>
 - The NVSL maintains stock of 3 ml BHI tubes for 1-5 swabs
 - A larger volume tube (5.5 ml BHI) is available upon request for the 11-swab tracheal/oropharyngeal pools (refer to 3.1.2.) and can be ordered in quantities of 500
- 1.1.2. Other acceptable VTM include any salt-balanced, buffered media with a protein component such as tris-buffered tryptose broth (TBTB)¹, nutrient broth (NB), and peptone broth (PB); including BD™ Universal Viral Transport 3 mL Collection Kit (<http://www.bd.com/ds/productCenter/CT-ViralTransport.asp>)
- 1.1.3. In the absence of appropriate VTM, phosphate buffered saline (PBS) or saline solution (contact lens solution) may be used to keep the swab moist during transport – dry swabs are not acceptable (refer to 1.1.4.)
Note: results may be affected, so PBS and saline should only be used when none of the preferable media are available.
- 1.1.4. Dry swab specimens are **not** recommended – heat and desiccation can inactivate IAV and ND in ≤24 hours; therefore, **negative results from dry swabs or swabs not collected in a VTM** are considered invalid and reported as a “No Test” with notation of the sample condition.

2.1. Swab collection (Figure 1)

- Use synthetic or semi-synthetic swabs (e.g. polyester, rayon, nylon) with 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

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

- 2.1.1. Tracheal/oropharyngeal (TR/OP) swabs are preferred for gallinaceous poultry (**Figure 1b-1d**)
 - The opening of the trachea and mouth can be swabbed (avoiding the esophagus), bringing the swab up through the choanal cleft (collectively referred to as TR/OP swab) - the sinuses drain into the choanal cleft, therefore swabbing will capture material from the upper respiratory tract (refer to **Figure 1a**)
 - Tracheal swabs, if needed, are best obtained from fresh carcasses
- 2.1.2. Cloacal (CL) swabs are preferred for domestic waterfowl and wild birds (**Figures 1e-1f**)
 - NOTE: National wild bird surveillance efforts recommend to put 1 OP and 1CL swab from an individual bird into a single tube; however, avoid pooling swabs from different wild birds into a single tube
- 2.1.3. Following sample collection, vigorously swirl the swab in the VTM, squeezing the excess liquid from the swab inside the specimen tube and then discarding the swab in an appropriate container – the **entire swab suspension** is submitted for diagnostic testing
 - *Note: swabs left inside the sample tube may result in media being drawn into the swab, leaving limited material for diagnostic testing*
- 2.1.4. If swabs remain in the specimen tube, all swab tips must be fully immersed in the VTM. For example, BHI tubes provided by the NVSL (or those with similar tube volumes) are not sufficiently wide to immerse more than 5 swabs in the VTM; therefore no more than 5 swabs are permitted to remain in the tube; **negative results from swab samples which were not fully immersed** are considered invalid and reported as a “No Test” with notation of the sample condition

3.1. Pooling procedures

- 3.1.1. Pooling of swabs may be performed for TR/OP swabs (maximum 11-swab pool for gallinaceous poultry, 5-swab pools for other species) or for CL swabs (maximum 5-swab pool) as follows:
 - the same sampling route – do NOT pool TR/OP and CL together when pooling poultry
 - the same premises, and
 - the same species
- 3.1.2. **TR/OP swabs:** maximum 11-swab pool in 5.5 mls of VTM for IAV and ND testing of gallinaceous poultry [*Note: not evaluated for other diseases such as infectious bronchitis, laryngotracheitis, or mycoplasma*], 5-swab pools² in a minimum of 3 mls of VTM **for FADs** and other species/disease testing
 - OPTION only for TR/OP swab samples for AI surveillance from gallinaceous poultry of the same species and same house and for those labs that conduct additional testing on

² Pools of 6 TR/OP swabs have not been specifically evaluated, but is considered an acceptable practice where initiated by field staff seeking to conduct the recommended 11-swab surveillance of gallinaceous poultry of the same species and same house only using 2 pooled samples instead of 3 (refer to 3.1.2). This does not apply to CL swabs (refer to 2.1.3 and 3.1.3) nor swabs from other species.

surveillance samples (refer to 3.1.2. above): a single LAB POOL may be tested at the laboratory by combining a representative 100ul aliquot from a 5-swab tube and a 6-swab² tube– if the LAB POOL is positive, BOTH sample tubes should be forwarded to NVSL for confirmation

- 3.1.3. **CL swabs** (maximum 5-swab pool in 3 mls of VTM) are the specimen of choice for domestic ducks/waterfowl
- 3.1.4. **Tissues:** pool appropriate tissues together from a single bird (e.g. respiratory vs enteric vs reproductive) - **do not pool tissues from more than one bird**

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

Sampling source	Preferred Specimen	Sample Collection	Detection of Virus
Gallinaceous poultry (chickens, turkeys, pheasants, quail)	TR/OP preferred, Cloacal swab (CL) may be used	Maximum of 11* swabs/pool from a single flock and species in 5.5 mls of VTM for surveillance * FOR FADs – max of 5 swabs/pool in 3 mls VTM	Detection of virus shed via respiratory route (predominant route for these species)
		Maximum of 5 swabs/pool from a single flock and species in a minimum of 3 mls of VTM	Detection of virus shed via enteric route
Domestic waterfowl	CL preferred, TR/OP swab may be used	Domesticated waterfowl may be pooled (5 per tube) from single flock and species	Detection of virus shed via enteric and/or respiratory routes
Wild bird species	1 CL and 1 OP swab from single bird in one tube	Do not pool different wild birds into a single tube ³	Detection of virus shed via enteric and/or respiratory routes
Any avian species	Tissue samples	Only pool tissues from a single ³ bird; pool by system (e.g. respiratory, enteric, urogenital/repro-ductive)	vND viruses may replicate to higher titres in tissues; brain is the specimen of choice for neurological forms of vNDV

³ Antibody from one bird may neutralize virus from another (e.g. mixed backyard poultry); potential to mix multiple viruses in a single sample

Figure 1. Swab collection: a) schematic of oral cavity; b-d) tracheal/oropharyngeal (TR/OP) swabs preferred for gallinaceous poultry; e-f) cloacal (CL) swabs preferred for domestic waterfowl and wild birds

