

AVIAN INFLUENZA TESTING PROCEDURES

The following information is provided to introduce some of the techniques that are used in avian influenza diagnostics. Discuss with your veterinary professional which techniques, tests and procedures are appropriate for your collection.

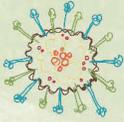
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TYPE OF SAMPLES FOR AI DIAGNOSTICS

Swabs of the trachea or oropharynx and cloaca are used to test for influenza virus. Tracheal swabs are often preferred over oropharyngeal swabs but in most cases, virus recovery from oropharyngeal swabs is close to as good or just as good as virus recovery from tracheal swabs. In the field, oropharyngeal swabs are usually preferred due to ease of collection. Gallinaceous birds typically shed AI viruses in respiratory secretions, so a tracheal or oropharyngeal swab is the primary source of virus detection from chickens and turkeys. Waterfowl typically shed AI viruses in their fecal secretions, so a cloacal swab is the primary source of virus detection from ducks and geese. For most bird species, it is prudent to collect both tracheal/oropharyngeal and cloacal swabs for testing.

Blood samples are also important to submit; however, with some very small species it may be difficult to collect enough blood without endangering the health of the animal. Blood samples are used for antibody testing. Seropositivity (positive for AIV antibodies) is indicative of one or more of the following: 1) current infection, 2) past exposure and/or 3) vaccination.



Influenza of Non-Domestic Species

Supplemental Training Information

SAMPLING EQUIPMENT

Polyester swabs with plastic shafts should be used to collect samples for diagnostic testing. Wooden shafted swabs should not be used as wood can act as a PCR inhibitor. Swabs come in a variety of sizes, so choose the swab size based on the size of the bird to be sampled.

After sample collection, place each swab into a sterile tube filled with 2 – 3 mls of brain heart infusion broth (BHI) or other viral transport media supplied by your NAHLN laboratory. Swabs should be swirled around vigorously in the BHI and then removed. Depending on directions from your veterinarian or surveillance program, swab samples can be pooled with up to 5 samples in a pool. Do not mix sample types in a pooled sample (pool tracheal/oropharyngeal samples with tracheal/oropharyngeal samples and cloacal samples with cloacal samples) and try to pool similar species together (duck samples with other duck samples)

SWABBING TECHNIQUES

To collect a tracheal swab sample, hold the bird's mouth open and watch the opening and closing of the larynx (opening to the trachea). Carefully introduce the swab down the trachea and gently rub the swab up and down along the trachea. The swab can also be drug along the tissues of the choanal slit.

To collect an oropharyngeal swab sample, hold the bird's mouth open and introduce the swab into the bird's mouth. Gently rub the swab around the tracheal opening, then up along the choana of the bird, making sure to drag the swab through the choanal slit as you pull the swab out of the bird's mouth.



[Video – Oropharyngeal Swabbing](#)
Example from Iowa State University

To collect a cloacal swab sample, hold the bird securely. Gently introduce the swab into the cloaca through the vent. A gentle twirling motion often helps introduce the swab into the cloaca. Insert the swab to the appropriate depth, to ensure contact with the mucous membranes, based on the size of the bird. Gently remove the swab and place the swab into the transport media tube. Keepers should be warned that the first few droppings after this procedure may have spots of frank blood.

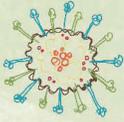


[Video – Cloacal Swabbing](#)
Example from Iowa State University

BLOOD COLLECTION

Blood is preferably collected from a bird from either the jugular vein or the metatarsal vein, by trained personnel. The amount of blood collected should be based on the size of the bird. Watch the bird for clinical signs of stress during blood collection.

Blood should be immediately transferred from the syringe to a red-top sterile tube. Bird serum can be difficult to separate from the blood clot, even after centrifugation. To maximize serum separation, immediately after filling the red top tube with blood, place the tube on its side. This will allow for maximum serum separation. Allow the blood to clot for a minimum of 15 minutes before centrifugation. Serum is needed for most antibody tests – plasma may not be an acceptable sample type. Check with your NAHLN laboratory.



Influenza of Non-Domestic Species

Supplemental Training Information

TISSUE COLLECTION

Tissues may be collected during necropsy for diagnostic testing. Tissues should be collected as sterilely as possible by using alcohol-flamed, autoclaved or disinfected instruments. Tissues can be placed in whirl-pak bags for storage and transport. Tissues should not dry out during shipment. If the tissues are small, place the tissue in a small amount of BHI or transport media. The following tissues are commonly collected for AIV testing: lung, liver, kidney, spleen and intestine.



[Video – Tissue Collection](#)

Example from Iowa State University

SAMPLE STORAGE AND SHIPMENT

Sample collection tubes or whirl-pak bags should be properly labeled with identifying information. Samples should be stored in the refrigerator and transported to the laboratory as soon as possible. Samples should be shipped overnight on ice (cold, not frozen) for priority delivery in the laboratory. Submit complete paperwork with all samples.

Be conversant with the principles of biosafety with shipping samples to ensure the safety of those handling the packages. There are extensive US and international requirements for shipping samples by air transportation. Shipping guidelines are required by the International Air Transportation Association (IATA) and are compliant with US Code of Federal Regulations guidelines Class 6.2 Dangerous Goods. All packages containing diagnostic animal specimens that are transported by air should be labeled appropriately. All personnel shipping diagnostic specimens should be appropriately trained. Further information on shipping diagnostic specimens is detailed below.

DIAGNOSTIC TESTS

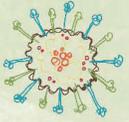
Antigen and virus detection methods are typically performed on swab or tissue samples. A positive test result would indicate that the animal is currently infected with AI virus. The following diagnostic tests can be used to detect antigen or virus in a sample:

- Virus isolation in embryonated eggs
- Real-time reverse-transcription polymerase chain reaction (qRT-PCR)
- Antigen detection kits (Flu Detect, Directigen, etc)

Antibodies are detected in serum samples. The following diagnostic tests are used to detect antibodies to avian influenza virus in a serum sample:

- Agar Gel Immunodiffusion
- Enzyme-Linked Immunosorbent Assay
- Hemagglutination-Inhibition

Serology techniques should be used to determine if the bird has antibodies to avian influenza. Detection of antibodies is an indicator of current infection, past exposure or previous avian influenza virus vaccination.



Regulations for Shipping Diagnostic Specimens

Labeling Shipping Boxes for Air Transport

Over the past few years, regulations for the transportation of Class 6.2 Dangerous Goods have changed. All packages containing diagnostic animal specimens that are transported by air should be labeled appropriately. While many diagnostic samples are considered part of this class, some of the samples sent from veterinary hospitals to the CSU VDL can be labeled as exempt. There are specific requirements for labeling, including size, wording and location of the marking.

All packages containing diagnostic animal specimens should be marked with one of the following designations:

Category A – Infectious Substances Affecting Animals Only (UN 2900) or Infectious Substances Affecting Humans (UN 2814)

These are substances that are **expected** to contain highly pathogenic infectious agents, such as agents from the USDA/CDC Select Agent lists. **Most diagnostic samples do not need to be shipped under this designation**, as they are not known to contain these agents (the presence of the agent is unconfirmed at this time – it is not known until the agent is detected by diagnostic testing at the CSU Veterinary Diagnostic Laboratory). If the sample is highly suspected to contain a Category A agent, it should be shipped under this designation. Further training should be completed by shippers to fully understand the regulations for shipping these substances.

Category B – Biological Substance, Category B (UN3373)

If a diagnostic specimen is EXPECTED to contain a pathogen, the package should be labeled with a UN3373 diamond-on-point mark with the wording “Biological Substance, Category B.” Minimum dimensions for this marking are 50mmx50mm.

Examples of specimens that should be sent with this mark include, but are not limited to:

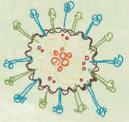
- Fresh tissues or swab samples sent for PCR or virus isolation
- Fresh tissues or swab samples sent for bacterial culture
- Fecal samples expected to contain a pathogen
- Culture plates of organisms sent for further testing

Exempt Animal Specimen

When there is **minimal likelihood** that the sample contains a pathogen, the package may be marked as “Exempt Animal Specimen”.

Examples of specimens that may be sent with this mark include, but are not limited to:

- Serum sent for antibody testing
- Tissues sent in 10% formalin (higher than 10% formalin requires further marking – UN 3334)



Influenza of Non-Domestic Species

Supplemental Training Information

- Samples to be tested for therapeutic drug monitoring or toxins
- Environmental samples not expected to contain a pathogen
- Dried blood spots placed on absorbent filter paper

All markings should be placed on the outside of the box in a prominent location. UN3373 – Biological Substance, Category B label stickers and other package labels can be purchased online from www.saftpak.com, www.casingcorp.com or www.uline.com.

Please Note: It is the shipper's responsibility to insure that the package complies with all current regulations. Regulations change frequently. Shippers may be fined for violations of the transport regulations. Average fines exceed \$ 1200 for basic infractions.

For training materials, including manuals, CDs and pamphlets, we recommend www.saftpak.com or www.iata.org. Any staff responsible for shipping Dangerous Goods should be trained by an approved training program and training documentation should be kept on file with the employer.