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*Please note: This situation is evolving rapidly; this is guidance is subject to change. Check back frequently for updated versions. The intended audience for this document is all laboratories testing for Influenza A in livestock.*

**Testing Guidance for Labs for Influenza A in Livestock**

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**Background**

In order to continue to monitor and understand the extent of this virus and reduce the risk of further spread of Highly Pathogenic Avian Influenza (HPAI) H5N1 virus, resulting in greater threats to poultry and livestock, APHIS issued a [Federal Order](#) on April 24, 2024 that requires pre-movement testing for lactating dairy cattle moving interstate and reporting of positive Influenza A nucleic acid detection diagnostics results from all laboratories and State Animal Health officials.

**Required Testing of Lactating Dairy Cattle for Interstate Movement at an Approved National Animal Health Laboratory Network (NAHLN) Laboratory**

Approved NAHLN Laboratories – Please check the [NAHLN website](#) for NAHLN laboratories approved to test for avian influenza.

Lactating dairy cows must be tested prior to movement (Samples must be submitted to a NAHLN Laboratory):

Collect milk/udder secretions from individual cows. Ensure each quarter is sampled, as there have been reports of only one quarter having virus. Combine the milk from each quarter into one sample for submission to the laboratory. Submit between 3-10 ml of milk per animal.

NAHLN Laboratories may pool milk samples from up to 5 cows together; this must be done in the laboratory.

NAHLN laboratories will conduct NAHLN-approved PCR testing: FluA matrix, H5 and optionally 2.3.4.4b.

## Voluntary Testing of Cattle

While interstate movement testing of lactating dairy cattle is required per the [Federal Order](#), producers and veterinarians may choose to conduct additional testing to manage HPAI suspect or affected herds to better protect their herds. If this testing is performed at NAHLN laboratories, APHIS will support the testing costs provided they obtain a FAD number (for sick cattle) or include a premises ID and follow the testing guidance below. APHIS will reimburse for Influenza A testing at NAHLN laboratories associated with this event for the following submission reasons:

- Suspect cattle
- Apparently healthy cattle that have been exposed to or epidemiologically linked to suspect or confirmed positive HPAI cattle
- Cattle from producers concerned their cattle may have HPAI
- Sick or dead domestic animals near affected premises
- Wildlife (reason for submission must indicate emerging event)
- Monitoring of healthy cattle via bulk tank samples (approved participation per protocol specified below)

### *Testing suspect cattle*

See the [case definition](#) for a complete description. Briefly, dairy cattle may experience a sudden drop in feed intake, a marked drop in herd level milk production with some more severely affected cows having thickened milk or may have essentially no milk, or respiratory signs including clear nasal discharge.

Suspect cattle may also include exposed cattle showing no clinical signs.

### Samples to collect:

- If cows are lactating, one sample must be milk (mammary tissue if animal has died).
  - Milk/udder secretions. It is very important for each quarter to be sampled, as there have been reports of only one quarter having virus. Pool the milk from each quarter into one sample for submission to the laboratory. Submit between 3-10 ml of milk per animal.
- If cows are not lactating, deep nasal swabs are the best sample type.
  - A single sterile swab with at least a 6 in handle may be used to sample both nostrils. Ensure the mucosa in the nasal passage is brushed firmly with the swab. After both nasal passages have been sampled, the swab is placed in PrimeStore MTM, BHI, or Saline (least preferable). Vigorously swirl the swab in the media, express fluid by pressing the swab against the side of the tube and remove the swab. Do not retain the swab in the media. PrimeStore MTM is the only media that can be submitted without ice packs; do not submit dry swabs. Nasal swabs cannot be pooled.

### *Testing higher risk cattle*

- Because the sensitivity of nasal swabs is not known, animals introduced into the herd within 6 months of lactation should be sampled upon freshening.
- Non-lactating cattle such as springing heifers can be voluntarily tested using nasal swabs during movement and/or after quarantine.

### *Testing pooled milk*

Testing pooled (bulk tank) milk for herd characterization or recovery is currently under validation. Participation requires pre-approval. Please contact [NVSL.Info@usda.gov](mailto:NVSL.Info@usda.gov) for details.

### **Testing Wildlife**

Sampling dead and dying birds as well as mammals particularly around affected premises is encouraged. Contact the local wildlife agency or call USDA Wildlife Services at 1-866-4USDA-WS (or 1-866-487-3297). They can assist with identification and sampling. Birds are sampled by collecting oropharyngeal and cloacal swabs.

- Recommended samples from mammals include brain (preferred if clinically affected) or oral, nasal, or rectal swabs. After sampling, swabs should be placed in MTM or BHI.
- Wild bird mortality events in and around affected premises should be reported to the State Wildlife Agency or APHIS Wildlife Services at 1-866-4USDA-WS.
- Wildlife samples should be designated as “other” with “wildlife” specified on laboratory submission forms.
- Apparently healthy wildlife should not be euthanized solely for sampling. Nuisance wildlife removed with the appropriate State and Federal permits can be sampled with appropriate coordination with your local WS office or State Wildlife Agency.

### **Testing Sick or Dead Cats or Other Companion Animals near Affected Premises**

Cats are particularly susceptible to H5N1 2.3.4.4b viruses; cats with neurologic disease and dead cats on affected dairies are widely reported.

- Brain swabs from affected cats (or whole carcasses) can be submitted to NAHLN laboratories.
- Other domestic animals associated with affected farms showing illness should be tested. Contact your NAHLN laboratory for consultation.

### **Reporting Requirements for Laboratories**

NAHLN laboratories already have reporting via electronic messaging in place with those electronic messaging requirements provided by the NAHLN Program Office.

All other laboratories testing samples from livestock for Influenza A virus must report all positive results regardless of the test method.

Laboratories are required to report testing results and accompanying sample information on a weekly basis to APHIS by emailing a copy of the spreadsheet to [HPAI.Results@usda.gov](mailto:HPAI.Results@usda.gov).