Influenza A in Swine Testing Algorithm

Effective as of: 06/01/17

Not funded by USDA

Lung tissue, Nasal Swabs

Matrix PCR
Complete within 2 business days of receipt

Stop

CT > 25

Oral Fluids

Matrix PCR
Complete within 2 business days of receipt

Stop

CT > 20

Only one (1) sample may be subtyped at USDA expense. Choose lung/nasal swab sample over an oral fluid sample for this reimbursement if both have the lowest CT value. Remaining samples may be subtyped at submitter’s expense.

Funded by USDA

Please refer to the instructions regarding unusual results and alternate protocols.

Sample Submission:

CT < 25

Continue to Subtyping PCR
Select 1 sample with lowest CT value (lung/nasal swab samples preferred) to Subtype
Complete within 1 business day of Matrix PCR results

CT < 20

Mixed subtype identified

Stop

Mixed subtype identified

Subtype identified

No subtype identified

If subtyping was conducted on original material, attempt VI then repeat subtyping on the isolate.
Contact NVSL if unable to obtain virus or sequencing not successful

Perform VI on sample. Complete VI within 3 weeks of sample receipt. Send aliquot to NVSL. Ship to the repository on 1st and 3rd Tuesdays of month.

Sequence virus isolates for H and N gene. Complete within 3 weeks of obtaining virus.

Deposit sequence in GeneBankwithin 1 week of completing sequencing.

Use standardized naming convention:
GenBank Accession number and virus name.
Virus name = A/Swine/State/NAHLN Barcode/Year of collection
Influenza A in Swine Testing Algorithm Instructions

IMPORTANT: Please note new cut-off CT values for Matrix PCR. Please follow timelines.

NVSL Notification and Shipping

1. **Samples associated with human illness**-- should NOT be tested at NAHLN labs. All samples associated with human illness should be sent to NVSL. Please notify DVL/Bovine, Porcine, and Aquaculture Viruses (BPA) staff if you receive samples associated with human illness.

2. **Unusual results**-- If no subtype or partial subtype was obtained on original material, attempt VI. If no isolate is obtained, subtyping or sequencing is unsuccessful on the isolate, or sequencing on the original material indicates an unusual virus, then contact DVL, BPA by phone, fax or email for next steps BEFORE SHIPPING.
   a. Phone (515) 337-7551
   b. Fax to NVSL DVL at fax number (515) 337-7348.

3. **Samples submitted for follow-up testing**-- use a VS 10-4 form; include the following information:
   - NAHLN Laboratory name
   - Date of collection
   - Barcode (In Section 21 under Animal Id, list the barcodes)
   - NAHLN lab accession number
   - Collection site state
   - Age class of swine associated with case/sample(s)
   - Specimen type
   - Reason for submission; clinical signs associated with the case
   - Laboratory results received to-date
   - Indicate testing requested in Examination Requested section
   - Owner information- For Traceable samples only

4. **IAV-S Isolate Repository**-- Please submit aliquots of isolates in cryogenic vials to DVL for REPOSITORY -- send the original virus isolate (first passage material - **DO NOT regrow virus prior to shipment**). Ship on 1st and 3rd Tuesdays of each month. Use a VS 10-4 form completed with the following information:
   - Submitter name and contact information—Laboratory’s information
   - NVSL Submitter ID—This is critical!
   - Name of Owner—USDA Swine Surveillance
   - Owner information and Location of animals—N/A
   - Examinations Requested—IAV-S Repository- BPA distribution
   - Purpose of Submission—Reagent Evaluation
   - Referral Number—Use if referring lab needs this
   - Animal ID—Include barcode(s)
   - Preservation
   - Specimens Submitted, including total number of Specimens Submitted
   - Species
   - Number of Animals Sampled
   - All other fields can be entered as N/A
   - Specify each virus isolate passage number via email or on the submission documents
5. **Shipping to NVSL**— Routine repository isolates should be frozen at -65°C or colder prior to shipment and shipped on dry ice.

**IAV-S Data Reporting**

1. **Reporting instructions**— Test results can be submitted monthly, but must be reported by the 5th of each month through either:
   - Electronically messaging into LMS (preferred reporting method).
   - Submitting cumulative spreadsheet to NAHLN program office at NAHLN@aphis.usda.gov.
   - Ad-hoc requests for data may be made in the event of cases of concern.
   - If an unusual subtype is detected, notify NVSL and the NAHLN office within 2 business days.

2. **GenBank**— Use the GenBank standardized naming convention: Virus name = **A/Swine/State/NAHLN Barcode/Year of collection**.
   - Can be submitted under USDA Swine Surveillance as Last Name for anonymous samples
   - State needs to be spelled out in full.
   - For date- use collection date; if collection date is not available, then use date received.

**Alternate protocols**

If an isolate is obtained from an alternative methodology other than that approved through the NAHLN Methods Technical Working Group processes, the isolate can be subtyped and sequenced (H, N); approved subtyping and sequencing of the H and N gene will be eligible for reimbursement from NAHLN; however the alternative methods will NOT be subject for reimbursement.