



National Animal Health Laboratory Network (NAHLN) Frequently Asked Questions Highly Pathogenic Avian Influenza (H5N1) in Dairy Herds:

Updated August 29, 2024

Since late March 2024, the U.S. Department of Agriculture, Food and Drug Administration, Centers for Disease Control and Prevention, and state veterinary and public health officials have been investigating the spread of highly pathogenic avian influenza into dairy cows, with multiple human infection. This is a rapidly evolving situation and USDA, as well as state and federal partners, are committed to sharing updates as information becomes available. Here, we are answering some of the most frequently asked questions by members of the National Animal Health Laboratory Network (NAHLN).

What is the appropriate nomenclature for this virus, now that it has appeared in dairy cows?

From USDA's perspective, highly pathogenic avian influenza (HPAI) or H5N1 are the most scientifically accurate terms to describe this virus.

Is H5N1 a Select Agent?

No. All influenza A H5 viruses have been temporarily removed (for 3 years) from the select agent list.

- There is no longer a requirement to complete Form 4.
- Laboratories (diagnostic and research) may store and work with H5 viruses if the correct permit is in place. NAHLN labs and researchers may contact apie@usda.gov with questions concerning permits.
- HPAI H5N1 virus is a biosafety level 3 agent.
- All non-negative samples must be forwarded to NVSL.
- H7 positive samples are still considered Select Agent.

Has any other country detected HPAI in livestock, especially goats and dairy cattle, other than USA?

We are not aware of any reports in other countries. To remain aware visit the World Organization for Animal Health (WOAH)'s World Animal Health Information System (WAHIS) site here [WAHIS Portal](#).

What does "confirmed state" mean for the H5N1 livestock incident?

Results confirmed at NVSL for H5N1 (H5.2.3.4.4.b) based on the [case definition](#).

When did the Federal Order go into effect?

April 29, 2024

BILLING/INVOICING AND TASK ORDERS

What Task Orders (TO) do we need in place to test which species for HPAI?

- Foreign Animal Disease TO can be used for testing of any FAD investigations (FADI).
- Avian HPAI TO can be used for testing of domestic avian samples associated with the current HPAI outbreak, including FADIs.
- Livestock HPAI TO can be used for IAV-A testing in any species, including peri-domestic mammals and birds, associated with a livestock premises. This includes cats. Any bovine IAV-A testing including FADIs can be charged to this TO.

How do I get a Livestock HPAI TO?

A Livestock TO has been requested for all NAHLN laboratories that are approved for IAV-A testing as

of 04/25/2024. If your TO is close to being expended, please contact Tari Moody tari.a.moody@usda.gov or the NAHLN. NAHLN.Coordinators@usda.gov

What testing is paid for by NAHLN?

- All dairy cattle displaying clinical signs.
- Pre-movement (domestic) testing.
- Producers interested in the disease status their asymptomatic animals.
- Samples taken from other animals including cats with potential exposure (from dairies, exposed to raw milk, etc.) or reported clinical signs associated with this disease event.
- We do not pay for Research covered by cooperative agreements or Commercial Product testing.

Will NAHLN Cover shipping costs?

NAHLN labs needing to ship non-negative samples to NVSL for confirmatory testing may request pre-paid shipping labels [for shipping Monday through Saturday](#) from NCAH.Shipping@usda.gov Labels will be delivered electronically in sets of 10. Please include the name and phone number of a contact person, address of your NAHLN laboratory, and the email address where labels should be sent.

Will the task order cover subtyping for H5 and H5 2.3.4.4 PCRs?

Yes. NAHLN will cover cost of matrix and H5/H5.2.3.4.4.b for any matrix non-negative samples. If matrix testing is non-negative the sample must be sent to NVSL before sequencing at a NAHLN laboratory.

Why is USDA paying for movement testing in livestock but not poultry?

For HPAI detections in poultry, USDA is paying for depopulation and indemnity which is not occurring for detections in livestock.

SAMPLING AND TESTING PROTOCOLS

Who will sample the cows and submit the samples?

Not specified in Federal Order. Defer to state specific requirements for each surveillance stream.

What are sample types for HPAI mammalian samples?

For lactating dairy cows, the best sample is milk. For non-lactating cattle, nasal swabs can be collected but this sample is not as reliable in identifying the presence of virus. Other mammalian species on dairy farms thus far have displayed neurologic signs and the best sample has been brain tissue.

What are the expectations of NAHLN labs for pre-movement testing?

- Expected Turnaround time: 3 calendar days (including weekends)
 - If samples submitted on a Friday, results are expected no later than Monday.
- Communicate as soon as possible if your laboratory anticipates reaching capacity to meet this TAT; NAHLN.Coordinators@usda.gov.
- Premises ID number (PIN) is required for pre-movement testing.
 - Results may be held until the PIN is provided.
- All laboratories testing for influenza must report results. See Federal Order announcement linked above.

Is the pre-movement testing for all states or just those with confirmed cases in livestock?

All states must adhere to the Federal Order.

Are there any estimates of the average number of lactating dairy cattle moving each day by state?

Approximately 75,000 Certificates of Veterinary Inspection issued for cattle movement annually in the US with each assumed to average 15 cows. This represents approximately 1400 cattle/week.

Should the SAHO/AVIC be contacted for FAD determination on samples/animals submitted for testing that have a history of clinical signs?

Yes. Clinical animals on farm should be part of an FAD investigation. Communication with your SAHO/AVIC on all submissions coming from other sources is highly recommended.

Should milk be screened for influenza before other testing is done (mastitis workup, etc.)?

Please assess the history and clinical signs if known. This blanket testing would most likely result in large numbers of additional samples for testing and is not required.

Should we test necropsy samples?

If clinical signs/history indicate possible HPAI, yes. Possible tissues: fetal pools (lung, liver, spleen, kidney) and placenta; adult lactating animals: mammary tissue; young or non-lactating animals: tissue pool (lung, liver, spleen, kidney).

Please adhere to your laboratory's policies and procedures for biosafety/biosecurity.

Have vendors been notified of potential increased demand on supply chain for reagents and supplies?

The NAHLN Program Office is working with vendors to assess possible supply chain issues. Contact NAHLN Coordinators with any concerns.

Is Milk an approved sample type for NAHLN testing?

Milk can be tested with an approved deviation request in place. Please contact the NAHLN Program Office for more information NAHLN.Coordinators@usda.gov.

Should all four quarters be sampled (submitted as one sample) from each cow?

Yes. Samples should be a composite of milk from all quarters.

What is the recommended volume of milk that should be collected for a milk sample?

We recommend collecting a minimum of 3ml fluid

milk to ensure sufficient volume for confirmatory testing as limited volume has been an issue with some samples submitted for confirmatory testing.

How are milk samples to be pooled?

Milk may be pooled from up to 5 animals by the smallest epidemiological unit (pen>room>barn>premises). Samples can only be pooled from one premises.

Please follow the guidance provided in the Blanket Deviation for NVSL-SOP-0068.05 Testing of milk and mammary tissue from dairy cows.

Equal parts of all milk samples pooled, then diluted 1:3 with MTM/PBS.

If a pool is non-negative do individual samples need to be tested?

Please use laboratory discretion: if you have the capacity for this additional testing it will be reimbursed by NAHLN.

- All non-negative samples (individuals or individuals and pools must be forwarded to NVSL for confirmatory testing.

What samples other than milk and mammary tissue are acceptable? Can nasal swabs be pooled?

No, do not pool nasal swabs.

Can we test the samples for Matrix and H5 simultaneously?

If samples are from clinical animals and submitted as an FADI, they can be run simultaneously.

If samples are from healthy animals submitted for pre-movement or worried well testing, matrix should be run first before proceeding with H5/H5 2.3.4.4.b on non-negative matrix samples.

Do the H5/H5 2.3.4.4b results need to be reported the same day as the matrix non-negative result?

H5/H5 2.3.4.4b could be reported next day.

What extraction methods are recommended for milk samples?

Please follow the guidance provided in the Blanket

Deviation for NVSL-SOP-0068.05 Testing of milk and mammary tissue from dairy cows and NVSL-WI-0042.

Regarding the CORE kit, should we add the third 80% ethanol wash that was mentioned in an earlier call?

The additional 80% ethanol wash is optional. DVL uses the CORE extraction with the additional 80% ethanol wash on most samples due to the amount of downstream sequencing performed and their need for harmonized extraction procedures.

Can we use the MagMAX 96 Viral Isolation kit?

No. must use protocols with 200µl for extraction of milk.

If the MagMax-96 Viral RNA Isolation kit is typically used for AI testing, must our analysts take a PT for use of the MagMax Pathogen RNA/DNA kit for testing of milk/mammary tissue? (We currently use and are PT'd on the MagMax Pathogen RNA/DNA kit for CSF, ASF, and FMD.)

No. You will not need to PT to use the pathogen RNA/DNA for milk/mammary tissue testing if you are PT'd for other NVSL assays with that kit.

Would it be acceptable to use 300 µl input from the Complex Core extraction?

It will be acceptable to use 300µl as long as the xeno values remain in range.

What Ct range is being observed in positive milk samples from individual cows?

10-39

Is HPAI sequencing conducted from milk or VI?

Sequencing is occurring from both, dependent on CT and other factors.

When sending samples to NVSL (serum, whole blood) that have not been tested but are from non-negative cows how should they be included on the spreadsheet sent with the notification emails?

Can be listed as serial samples, previously confirmed herd (premises), etc. in notification emails.

Can NAHLN labs forward fluA non-negative samples from other livestock and companion species (e.g. horses) for characterization by sequencing?

Yes. NVSL appreciates receiving these samples for further characterization.

Will the NVSL continue to provide MTM PrimeStore at no charge for this movement testing?

As long as they are able to they will. May request up to 30 boxes per order.

What should NAHLN labs do if they experience internal control failures in dairy samples?

If a Ct value is obtained, please forward the sample to NVSL and note the shifted internal control Ct values.

If no Ct value is obtained, options are:

- 1) to forward noting shifted internal control, or
- 2) proceed with dilution 1:3 (if not already done). If initial dilution was done, try diluting the diluted sample again 1:3 in MTM or PBS;
- 3) Particularly viscous samples may benefit from centrifugation per tissue homogenate clarification process in section 5.1.1 of NVSL SOP 0068

What protocol will be used for official serology testing?

NVSL-SOP-1255 Use of IDEXX Influenza A ELISA for Antibody Detection in Bovidae-origin Milk and Serum

- Each analyst must be PT'ed individually.
- Each NAHLN laboratory must have a CLIN in place IDIQ contract.
- Electronic messaging guidance is available on the NAHLN website.

How will serology in NAHLN labs be used for official testing?

NAHLN labs that are PT'd for serology will be able to support both PCR and ELISA for the National Herd Status surveillance program.

Is there routine sequencing ongoing for these samples?

DVL is sequencing samples and releasing into public database on a weekly basis, but with no collection date or state of origin.

Where are the sequences deposited? Who can have access to the sequences?

PRJNA1102327: bioproject where all are available. Published on Fridays.
Also, the nicely curated FASTA files that represent the entire outbreak are available on GSAID.
- Approximately 1400 sequences since 2022.

Have the sequences among the affected bovine (dairy) herds been the same across affected states?

The sequences seem to indicate movement from the affected dairies to one another and to the near by poultry facilities.
It remains important to sample/sequence enough animals in each herd.

Reporting - MESSAGING GUIDANCE

Messaging guidance can be found here: [Reporting Guidance](#)

Should NAHLN labs message IAV-A test results from cattle and other mammals?

Yes. messaging these results is expected and guidance can be found on the NAHLN website.

Do we report and message non-negative results after testing is completed, or do we need to hold off until NVSL confirms a non-negative?

Report and message as testing is completed like all other HPAI testing is currently being messaged/ reported. This includes matrix, H5, and H5 2.3.4.4.b.

If samples are submitted as an FADI and they are NOT DETECTED, should NAHLN labs still message results?

Yes. All HPAI testing should be messaged, including those that are part of an FAD investigation.

How should Testing Purpose be chosen for messaging?

Please refer to the current version of the guidance posted here on the NAHLN website: [Reporting Guidance](#)

How are samples identified in the message?

SNOMED codes are used and can be found for approved sample types in the Messaging Guide. [Reporting Guidance](#)

Should NAHLN Labs use the same LOINC and Program OID that is used for avian testing?

Yes. Same LOINC and OID for PCR testing are used for influenza testing in cattle.

How are non-NAHLN Laboratories expected to report influenza testing?

Laboratories that are not part of the NAHLN, are required to report all nonnegative results to APHIS using a specific excel spreadsheet. The completed spreadsheet should be submitted weekly to the email address: HPAI.Results@usda.gov.

Are positive test results for influenza A in all species reportable?

All influenza A, with the exception of H1/H3 in swine should be reported to USDA.

What are testing requirements for movement to slaughter?

Refer to [APHIS Requirements & Recommendations](#)

HPAI TESTING IN CATS

What samples should be tested from rabies negative cats?

Brain tissue or brain swabs

Is a PIN or FAD # required for testing of cats?

Neither is required. If they from a dairy premises use that PIN and/or FAD #. If not associated and required by your LIMS, use NA.

Who might be submitting these samples?

These may be coming from public health laboratories, private veterinarians, etc.

How to message IAV-A Testing of Cats?

- Submission Purpose: EE
- Feline SNOMED: 448169003
- Specimens: Swab of brain, 364781000009104 or Brain tissue, 128157004

If cat samples from public health lab do not have location info, can we put NA for messaging purposes?

Yes. Please also ensure the state of animal origin is sent in xad.4. If your LIMS requires a PremID, it is acceptable to also enter NA into this field as well.

Any guidance on testing healthy cats from worried owners?

If cats have flu symptoms, clinicians should rule out feline flu strains and test as normal. However, NVSL will sequence and characterize any positive detection on any diagnostic case regardless of species (provided the sample meets the quality criteria) and will support characterization once a flu detection is identified.

This testing can be charged on your livestock task order.

What about testing stray cats rescued by animal control those might have contact with wildlife?

Cats that are neurologic should be tested for rabies first and should really go through the process outlined in the states.

Is there a specific section of cat brain that is preferred for H5 testing?

No. Swabbing any portion of the brain seems to work well.

Is freezing cat samples ok if they want to batch them once a week?

Yes. Ideally freeze at -80C.

Can cat swabs be pooled like poultry?

No. This is individual animal testing only.

Are NAHLN laboratories allowed to test samples for the rabies-negative cat study?

Yes. Testing these samples is covered under the mammalian blanket deviation.

NVSL Support

What can NAHLN labs do to support NVSL?

- Clear communication with the reference laboratory when forwarding samples:
 - Provide 3-5ml of sample whenever possible.
- Provide sample tubes in boxes, not in bags.
- Confirm tubes are closed securely and labeled appropriately.
- Complete the submission form completely.
- Provide excel spreadsheets with information for multiple animals.
- Provide Ct values for PCR results.