



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

*Updated April 27, 2024*

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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 one 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 or H5N1 are the most scientifically accurate terms to describe this virus.

## **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 of new incursions of high-consequence animal diseases, 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 livestock incident?**

NVSL confirmed results for H5N1 (H5.2.3.4.4.b)

## **When did the Federal Order go into effect? [USDA Announcement](#)**

April 29, 2024

## **What Task Orders do we need in place to test which samples for HPAI?**

Foreign Animal Disease task orders can be used for testing of FAD investigations from either avian or bovine species.

Avian HPAI task orders can be used for testing of domestic avian samples associated with the current HPAI outbreak, including FADIs.

Livestock HPAI task orders can be used for IAV-A testing any species, including peri-domestic and avian, associated with a livestock premises. This type of TO should be used for all pre-movement and "worried well" testing.

A Livestock TO has been requested for all NAHLN laboratories that are approved for IAV-A testing as of 04/25/2024.

## **What testing is paid for by NAHLN?**

All dairy cattle displaying clinical signs.

Pre-movement (domestic) testing

Producers interested in the disease status of their asymptomatic animals.

Samples taken from other animals on dairies associated with this disease event.

NAHLN cannot pay for shipping of samples. Please check with your state on possible umbrella agreement funding to assist with shipping costs.

**Will testing of milk-based feed ingredients be covered by NAHLN funding?**

No- reimbursement is for animal testing only.

**How much testing has been conducted on cattle across the NAHLN at this point?**

As of 04/26/3034 NAHLN Laboratories have messaged 3,771 test results for HPAI in cattle.

**Will the task order cover subtyping? If matrix non-negative, do we follow up with H5 and H5 2433 PCRs?**

NAHLN will cover cost of matrix and H5/H5.2.3.4.4.b for any matrix non-negative samples. No H7 testing on these samples.

If matrix testing is non-negative the sample must be sent to NVSL before sequencing at a NAHLN laboratory.

- If sequencing is completed the sample will be classified as a select agent and the NAHLN laboratory will be responsible for shipping costs to NVSL.

**Does NAHLN pay for retroactive export testing of cattle (completed prior to the federal order)?**

No

**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.

**Who will sample the cows and who will submit the samples? Are there any requirements for the shippers to ensure that sampling is “adequate” / legit?**

Not specified in Federal Order. Defer to state specific requirements at this time.

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

Expected Turnaround time: 3 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 in order to meet this TAT; [NAHLN.Coordinators@usda.gov](mailto:NAHLN.Coordinators@usda.gov).

PIN is required for pre-movement and worried well testing.

All laboratories testing for influenza must report results. See Federal Order announcement linked above.

Additional guidance pending on movement for slaughter and exhibition.

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

All states

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

75,000 CVIs issued annually for US, 1 per pod, 15 cows per pod

- Approximately 1400/week

**Should we contact our SAHO/AVID 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.

**Should all milk samples that come to the diagnostic lab be screened before other testing is done for a mastitis workups?**

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)

**Is necropsy recommended or advisable on a dead cow that tested HPAI-positive even it's done in a BSL3 necropsy area?**

Please adhere to your laboratory’s policies and procedures.

**Just to clarify, are you suggesting we test \*all\* dead (lactating) dairy cattle that come in for necropsy? Or**

**only if they come in from a herd that is "suspect" for HPAI infection?**

It is advised testing occurs for any suspect herds/animals or if clinical signs are suggestive of HPAI.

**Has Life Tech been notified? Are they still going to be able to keep up with supply chain demands?**

The NAHLN Program Office is working with vendors to assess possible supply chain issues.

**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.

**Is Milk an approved sample type for NAHLN testing?**

Milk is approved with an approved deviation request in place. Please contact the NAHLN Program Office for more information [NAHLN.Coordinators@usda.gov](mailto:NAHLN.Coordinators@usda.gov).

**Can nasal swabs be pooled?**

No, do not pool nasal swabs.

**How are milk samples to be pooled?**

Please follow the same guidelines that have been set for ASF/CSF: Samples should be pooled by the smallest epidemiological unit (pen>room>barn>premise). Samples can only be pooled from one premise. Milk may be pooled up to 5 samples per pool.

**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.

**Can milk be diluted in anything other than MTM (ex. 1X PBS)?**

Dilution of raw milk samples should be conducted 1 part milk to 3 parts MTM or PBS prior to extraction. Extraction requires 200µl input for these sample types.

**To verify the milk pooling procedure, would you pool equal volumes of milk and then dilute 1/3 (PrimeStore or PBS) or would you dilute the milk and then pool?**

Pool milk first then dilute 1 part milk:3 parts PrimeStore/PBS.  
Recommend all milk is diluted.

**If we receive 5 milk samples that are to be pooled - are we putting 200ul of milk from each of the 5 samples into one singular MTM tube?**

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 PrimeStore/PBS.

**Is pooling and diluting of milk still appropriate for samples submitted for pre-movement testing?**

Yes: same guidance applies.

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

Yes: samples should be a composite of milk from all quarters.

**Just a clarification, is the 200µl of ORIGINAL sample that goes into MTM, or is the 200µl what needs to go into the extraction plate?**

Extraction plate

**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.

**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? Are both the H5 and 2344 PCR's required for non-negatives?**

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.

**Will the testing guidance mandate use of the VetMax Gold AI PCR Kit and the MagMax Core Kit for extraction? What are the types of extraction kits that may be utilized?**

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

**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.

**We normally use the MagMax-96 Viral RNA Isolation kit for AI and are PT'd for that kit. Since that kit is not permitted for the deviation, 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 since you are PT'd for other NVSL assays with that kit.

**Can we use the MagMAX 96 Viral Isolation kit?**

No- must be 200µl for extraction of milk.

**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.

**Will the KingFisher program be shared or the specific name of the program.....heated or non-heated script?**

Pending from DVL

**What are the recommended individual sample types? Nasal swabs? Milk? Can nasal swabs be pooled?**

Current federal order is for lactating dairy cattle only.

- Sample must be milk.

No pooling of nasal swabs.

**Can you please share the Ct range that is being observed in positive milk samples?**

10-30

**Is HPAI sequencing being conducted directly from milk or VI?**

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

**If we are sending samples to NVSL (serum, whole blood) that have not been tested at our lab but are from non-negative cows so you want them included on the spreadsheet we send with the notification emails? Or is there a different place you want them listed?**

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

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

As long as they are able to they will.

**Can we request more MTM tubes from NVSL?**

Yes; may order up to 30 boxes per order.

**Can we expect a uniform sample container to be used for submissions?**

Unsure how that is possible without NAHLN laboratories providing those to the field for collection.

**Any guidance on testing bulk tank samples for surveillance? Not movement of cattle**

Development of possible bulk milk tank testing is in process. Refer to federal testing guidance document.

**Any insight or discussion on use of antibody testing (NP ELISA - on Serum or Milk) to understand exposure or status of animals / herds?**

NVSL is working to get a plan in place for implementation of serology testing. An exact timeline is not known at this time. Specificity testing has been completed at Wisconsin NAHLN Laboratory.

Few serum and milk samples from affected cows are available to complete this work.

The NPO is working on using retired CLIN for this testing/reimbursement.

**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.

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

Yes- messaging of these results is expected and guidance can be found on the NAHLN website. (insert link when posted)

**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.

**Should we use Interstate movement or Emerging Event as Testing Purpose for messaging?**

Reporting guidance will be posted on the NAHLN website and will include the most up-to-date information on all aspects of messaging.

Possible submission reasons: F: FAD, M: interstate movement, E: Export, H: Health Monitoring, EE: Emerging Event

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

**Is there a SNOMED for pooled milk samples?**

No- use the SNOMED for individual milk, 119321005.

**Can we continue to use same test code that we in Avian testing (LOINC: 44263-2, OID: 2.16.840.1.113883.3.5.8.4) or there is a different LOINC assigned for IAV-A test in cattle?**

Same LOINC and OID for PCR testing

**From USDA Guidance: 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).**

This only applies to non-NAHLN laboratories. All NAHLN laboratories are required to message all testing results via HL7 messaging.

**Would this be an opportunity to make the HPAIV results messaged to the USDA NAHLN to the SAHO's?**

Laboratories will need to continue to provide laboratory reports to appropriate recipients.

**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.

**Are we paying for export testing to Canada since they have imposed the same pre-movement testing requirement?**

Yes

**What are testing requirements for movement to slaughter?**

Refer to [APHIS Requirements & Recommendations](#)

**Select Agent Guidance**

**Is there any extension available for the 21 day exemption for dealing with SA?**

At this time there is no extension available to the 21 day

requirement to transfer or destroy any remaining SA.

**Can you confirm that nucleic acid is not considered SA.**

Only nucleic acids that can produce infections forms of a select agent virus are considered SA. The expectation is that there is scientific justification/or other information that provides assurance that there is no viable virus remaining in the nucleic acid extracts.

**Can they inactivate serum to create a sample no longer SA?**

To qualify for the diagnostic exemption, any remaining SA must be destroyed on-site by a recognized sterilization or inactivation process. The expectation is that there is scientific justification and/or other information that provides assurance that no viable virus remains in the serum. FSAP does not consider a test result identifying the presence of antibodies in a sample as positive identification of the select agent therefore antibody positive diagnostic samples are not subject to the select agent regulations.

**Is there a protocol for inactivating serum available?**

FSAP does not provide inactivation protocols. It is the responsibility of the entity to ensure that any inactivation protocols effectively inactivate SA. If the diagnostic lab is performing the inactivation of select agent as part of their FSAP registration, they must in-house validate their inactivation protocols. There is no requirement for non-registered entities or registered entities that have non-registered diagnostic labs to in-house validate their inactivation protocols, but the expectation is that there is scientific justification/or other information that provides assurance that there is no viable virus remaining in the serum after inactivation.

**Are subsequent serial milk samples from a cow previously confirmed to have had HPAI in a milk sample automatically SA or do the subsequent samples need to also be confirmed with HPAI to be considered SA?**

Subsequent serial milk samples from cows previously confirmed to have HPAI are not SA. The samples need to be confirmed with HPAI to be consider SA.