Highly Pathogenic Avian Influenza A(H5N1)

Frequently Asked Questions & Resources

Contacts:
CDC Influenza Technical Assistance: flusupport@cdc.gov
APHL Influenza Program: fluquestions@aphl.org

CDC Resources:

- Summer 2024 Guidance to PHLs for Influenza Diagnostic Specimen Submission
- Highly Pathogenic Avian Influenza A(H5N1) Virus in Animals: Interim Recommendations for Prevention, Monitoring, and Public Health Investigations | Bird Flu | CDC
- Technical Update: Summary Analysis of the Genetic Sequence of a Highly Pathogenic Avian Influenza A(H5N1) Virus Identified in a Human in Michigan | Bird Flu | CDC
- How CDC is monitoring influenza data among people to better understand the current avian influenza A (H5N1) situation
- CDC Health Alert Network (HAN) Highly Pathogenic Avian Influenza A(H5N1) Virus: Identification of Human Infection and Recommendations for Investigations and Response
- CDC Laboratory Outreach Communication System (LOCS) Lab Advisory: Enforcement Discretion Granted for the Use of Conjunctival Swabs with the CDC Human Influenza Virus Real-Time RT-PCR Diagnostic Panel, Influenza A/H5 Subtyping Kit

FDA Resources:

- FDA Updates on Highly Pathogenic Avian Influenza (HPAI)
- FDA Questions and Answers Regarding Milk Safety During Highly Pathogenic Avian Influenza (HPAI) Outbreaks
- Interim HPAI Virus Biosafety Recommendations and Resources for Industry Laboratories and State Dairy & Dairy Product Laboratories
- Standard Operating Procedure for Subtyping of HPAI H5 in Milk and Milk Products Using RT-qPCR
- Standard Operating Procedure for Extraction and Detection of Influenza A virus in Milk and Milk Products

USDA Resources:

- UDSA APHIS: Highly Pathogenic Avian Influenza (HPAI) Detections in Livestock
- USDA Actions to Protect Livestock Health from Highly Pathogenic H5N1 Avian Influenza
- Fact Sheet: USDA, HHS Announce New Actions to Reduce Impact and Spread of H5N1

APHL Resources:

- APHL Risk Assessment Guide - Testing Raw Milk Samples that May Contain Highly Pathogenic Avian Influenza
- APHL Biosafety Considerations for Milk and Dairy Testing

OTHER Resources:

- Highly Pathogenic Avian Influenza (HPAI) Testing at Michigan Department of Health and Human Services Bureau of Laboratories/CDC

Review of Human Testing Recommendations
The recommended testing algorithm begins with either the CDC Human Influenza Real-Time RT-PCR Diagnostic Panel: Influenza A/H5 Subtyping Kit or the CDC Influenza SARS-CoV-2 (Flu SC2) Multiplex Assay. Positive specimens then move on to the appropriate subtyping or lineage typing kit. CDC recommends that most laboratories continue to follow the full influenza testing algorithm. However, if you are screening many individuals that have had a known exposure you can shift to use the Influenza A/H5 subtyping kit first. If those results are negative for the H5 markers, then proceed with the typical algorithm for influenza.

Specimen Collection
Specimen collection recommendations are dependent upon symptoms, but ideally two or three specimens are requested, each placed into a separate tube of VTM or UTM:

- Individuals with **respiratory symptoms**:
    1. Nasopharyngeal swab (NP)
    2. Combined nasal swab (NS) oropharyngeal swab (OS)
- Individuals with **conjunctivitis** (without respiratory symptoms):
    1. Conjunctival swab
    2. NP swab
- Individuals with **conjunctivitis** (with **respiratory symptoms**):
    1. Conjunctival swab
    2. NP swab
    3. A combined nasal swab and oropharyngeal (throat) swab
- Individuals with **severe respiratory disease** (collect lower respiratory tract specimens):
    1. Endotracheal aspirate
    2. Bronchoalveolar lavage fluid

FDA has granted enforcement discretion on the use of conjunctival specimens to be tested with the CDC Human Influenza Virus Real-Time RT-PCR Diagnostic Panel, Influenza A/H5 Subtyping Kit through November 1, 2024, provided laboratories implement the following risk mitigation measures. The enforcement discretion may be further extended if appropriate.

- Testing of conjunctival specimens will be paired with testing of respiratory specimens collected using NPS, which will mitigate the risks associated with the misdiagnosis of patient cases presenting with conjunctivitis and no correlating respiratory symptoms;
- The conjunctival specimens will be collected and transported using the same swabs and transport media as are NPS used with the currently cleared CDC kit; and
- CDC will notify FDA of any complaints received regarding the performance of the assay when conjunctival swabs are used within 7 days of receipt of the complaint

Specimen Shipping and Select Agent Information
Positive results generated at PHLs are considered presumptive positive results and may be shipped Category B as a diagnostic specimen. On June 6, 2024 USDA announced a three year exemption on H5 viruses from select agent regulations.

HPAI Infection in Dairy Cows

- The June 6, 2024 recording of the Institute for Food Safety (IFS) at Cornell University special session of Dairy

Foods Virtual Office Hours titled June Update on HPAI in Cattle – Current Status and Testing Details is now available. Dr. Elisha Frye and Dr. Sam Alcaine from Cornell University provide an update on testing of dairy cow herds for HPAI, as well as on the virus stability and transmission.

- The April 3, 2024 recording of the Institute for Food Safety (IFS) at Cornell University special session of Dairy Foods Virtual Office Hours titled What Dairy Industry/Consumers should know about the Highly Pathogenic Avian Influenza Virus (HPAIV) is now available. Dr. Diego Diel provides an overview of HPAIV infections identified in several dairy cow herds and one person, as well as basic biology of this virus to help understand some of these recent events. This session also features Robert Lynch, DVM with an overview of biosecurity strategies that need to be implemented at the dairy farm level and explanation of what impact do these recent events have on the food safety of milk and dairy products.

Questions and Answers (Q&A)

Human Testing / CLIA

Q: Is CDC working to validate conjunctival swabs as a specimen type and share it with public health laboratories (PHLs)?
A: CDC has been granted FDA enforcement discretion on conjunctival specimens to be tested with the H5 subtyping kit. PHLs should work with CLIA to develop their verification plans to add this specimen type. This has been extended to November 1, 2024.

Q: Does APHL have resources on proper collection of conjunctival swabs?
A: CDC has developed guidelines for Conjunctival Swab Specimen Collection for Detection of Avian Influenza A(H5) Viruses. Specimens should be obtained from the everted eyelid by using a Dacron (DuPont)-tipped swab or the swab specified by the manufacturer's test kit (not cotton). Specimens must contain conjunctival cells, not exudate alone.

Q: Which types of specimen transport media is CDC accepting?
A: CDC will accept specimens that are collected in viral transport media (VTM) or universal transport media (UTM). The Instructions for Use (IFU) Package Inserts associated with the CDC Human Influenza Virus Real-Time RT-PCR Diagnostic Panels have been updated to include UTM. Relevant IFUs include:

- Influenza A/B Typing Kit
- Influenza A Subtyping Kit
- Influenza A/H5 Subtyping Kit

Q: Does CDC have Influenza A(H5N1) inactivation data on various extraction buffers?
A: CDC has validated for inactivation of Influenza H5N1 the following buffers:

- TRIzol LS
- 10% buffered formalin
- Beta-Propiolactone (BPL)
- Guanidinium-based Roche buffers
    - MagNA Pure 96 kit / MagNA Pure 96 External lysis buffer
    - MagNA Pure Compact / RNA isolation kit lysis buffer
    - MagNA Pure 96 Cellular RNA Large Volume Kit / MPLC RNA Isolation Tissue Lysis Buffer
- Guanidinium-based Qiagen buffers

- QIAamp Viral RNA mini kit / AVL buffer
- QIAamp DSP Viral RNA mini kit / AVL buffer
- Qiagen RNeasy RNA extraction kit / RLT buffer

Testing was done following the manufacturer's standard protocols then inactivated materials were inoculated into embryonated chicken eggs and examined for growth in eggs. For buffers that result in embryo lethality, CDC first determined the buffer's toxicity concentration in eggs using a non-lethal buffer dilution for inactivation. Guanidinium concentrations did not kill the embryo, but still completely inactivated the virus.

Peer Reviewed Journal Articles on Inactivation of Influenza A(H5N1):

- Avelin V, Sissonen S, Julkunen I, Österlund P. Inactivation efficacy of H5N1 avian influenza virus by commonly used sample preparation reagents for safe laboratory practices. *Journal of Virological Methods*. 2022;304:114527. Doi:10.1016/j.jviromet.2022.114527
- Elveborg S, Monteil VM, Mirazimi A. Methods of Inactivation of Highly Pathogenic Viruses for Molecular, Serology or Vaccine Development Purposes. *Pathogens*. 2022;11(2):271. Doi:10.3390/pathogens11020271

Q: What steps should my laboratory take if we detect Influenza A(H5N1) in a clinical specimen?
A: The laboratory should notify flusupport@cdc.gov immediately of the presumptive positive result. The specimen will need to be sent to CDC (Cat B diagnostic specimen) for confirmation.

Q: What is the turnaround time (TAT) on specimens sent to CDC?
A: The current turnaround time on specimens received for HPAI testing is within one day of receipt. Depending on specimen testing requests, TAT could be up to 7 days. Testing for the most recent human case was confirmed by CDC the same day the sample arrived at CDC.

Q: Does FDA enforcement discretion apply only to testing performed at CDC or does it apply to PHLs using the CDC assay as well?
A: This would apply to all laboratories using the CDC assays. The enforcement discretion allows PHLs to utilize the assay as FDA approved so you can go through your verification process for CLIA rather than doing a full validation as an LDT.

Q: Are there positive controls available as conjunctival swabs or will PHLs need to spike for verification?
A: CDC does not have conjunctival specimens to share for positive controls and PHLs will likely need to spike samples for their verification processes.

Q: How should conjunctival swabs be verified as a specimen type?
A: Labs can use conjunctival matrix that is negative for influenza to serve as either negative controls or spiked positive controls. CDC is working with a company to develop a synthetic positive control as well and hope to have more information in the near future. There is purified RNA available commercially that may be used as a positive control.

Q: Will CDC provide verification panels for conjunctival swabs?
A: APHL and CDC are working together to develop a plan to provide verification material. Because conjunctival swabs are not a common specimen type, the matrix is difficult to obtain in sufficient quantities to provide panels.

Several public health laboratories have developed specimen collection guidance that requests collection of two

conjunctival swabs so one can be retained at the public health laboratory to assist with verification.

Q: Does CDC have reagents available for Influenza A/H5 testing?
A: CDC has cleared diagnostic influenza in-vitro diagnostic (IVD) kits available for PHLs through the International Reagent Resource (IRR) at no cost. There are certain limits to kits, but if your PHLs needs additional kits, describe the situation in your IRR submission form comments.

- Influenza A/B typing kit: 1st line influenza virus diagnosis
- Flu SC2 Multiplex: 1st line influenza virus or SARS-CoV-2 diagnosis
- Influenza A-Subtyping kit: Determines if typical human epidemic/endemic ("seasonal") influenza virus
- Influenza B-lineage typing kit: Determines if typical human epidemic/endemic ("seasonal") influenza virus
- Influenza A/H5 Subtyping kit: Determines if it is H5 avian influenza virus
    - Should not be performed unless the patient meets clinical and epidemiological criteria for testing suspect specimens (see Highly Pathogenic Avian Influenza A(H5N1) Virus in Animals: Interim Recommendations for Prevention, Monitoring, and Public Health Investigations | Bird Flu | CDC)
    - Test results with both H5a and H5b markers* positive are considered presumptive positive and the sample should be sent to CDC as a diagnostic specimen for confirmation (i.e. Category B shipping).
    - Test results with either H5a or H5b markers positive should also be sent to CDC for additional testing.
      *Lot **220307**. H5b primers should not be used.

Q: If laboratories receive specimens from a suspect case would there be a need to run the Influenza A/H7 subtyping kit as well?
A: No, not at this time.

Q: Are other high throughput platforms (such as Thermo FIsher King Fisher Flex) being considered for the H5 assay?
A: CDC is currently working with FDA on the mechanism to add additional platforms. The Thermo Fisher King Fisher Flex would not be one considered, however, because it is not IVD labeled.

Q: Is the use of CDC Human Influenza Real-Time RT-PCR Diagnostic Panel: Influenza A/H5 Subtyping Kit being made available to local public health labs that are not part of the of the regional laboratory network?
A: The H5 assay is available to all laboratories approved to run the CDC influenza diagnostic panels and actively ordering from IRR. There are not plans to add additional local laboratories at this time.

Q: What kind of shipping (i.e. standard, rapid) should be used for sending specimens to CDC?
A: Overnight Category B shipping should be sufficient, with delivery as early in the day as possible.

Q: Will influenza assays other than the CDC Human Influenza Real-Time RT-PCR Diagnostic Panel: Influenza A/H5 Subtyping Kit detect Influenza A(H5N1)?
A: The CDC assay appears to be the only assay that is currently in production and is able to give an H5N1 result. FDA expects that other influenza assays do detect the virus and give a diagnosis of influenza A positive. Specimens from patients with known exposure to infected animals should be sent to PHLs for further testing and subtyping using the CDC kits.

Q: What is the test result interpretation if one swab tests presumptive positive and the other swab is not detected?

A: The swabs are interpreted separately. If either one is positive, it is a presumptive positive, but the conjunctival swab result may not be reported to patients until you have verified the assay.

Biosafety/ Biosecurity

Q: If laboratories identify a sample as positive with the CDC Influenza A H5 subtyping kit, will this be considered a positive confirmation of a Select Agent?

A: Positive results generated at PHLs are considered presumptive positive results and may be shipped Category B as a diagnostic specimen. If confirmed at CDC, the agency will file the appropriate select agent paperwork. PHLs are encouraged to send all original material of presumptive positives to CDC. Extracted material of confirmed positives is not considered a select agent if the specimen is confirmed positive at CDC.

Q: Should suspect Influenza A (H5N1) specimens be processed in BSL-3 facilities until extracted or inactivated?

A: Technically diagnostics can be run at BSL-2, but with potential select agent considerations PHLs may choose to use BSL-3 until inactivated. This is at the discretion of your biosafety officer. PHLs are encouraged to conduct their own risk assessments. APHL Risk Assessment Guide - Testing Raw Milk Samples that May Contain Highly Pathogenic Avian Influenza

Q: What level of biosafety PPE is used in the rabies labs that are also testing for H5?

A: BSL-2 with additional enhancements; working in a biosafety cabinet and using full respiratory protection (PAPRs or medically fitted N-95 masks) until inactivation.

H5N1 Virus

Q: Is the current H5N1 virus expected to be responsive to Tamiflu?

A: Yes.

Q: In severe human cases associated with clade 2,3,4,4b are there hallmark mutations that should be monitored for or are there good references to review those details?

A: Yes, there are typical mutations, primarily in the polymerase genes, that are often associated with mammalian adaptations, likely leading to increased severity and replication in the lower respiratory tract. Refer to published articles describing mutations to be on the lookout for and the CDC H5N1 Genetic Changes Inventory for more information.

Q: Are there other resources that cover molecular markers of illness severity in humans?

A: There are a number of review articles and publications that have looked at H5 specifically. The GISAID FluSurver can be used to search for molecular markers that are associated with phenotypic changes in H5N1 viruses.

Funding

Q: Can you clarify if HPAI response falls under an appropriate expense for ELC COVID-19 supplemental funds given that it is respiratory focused?
A: CDC has stated that it will be difficult to redirect COVID-19 dollars at this time. ELC recipients may request to redirect funds to support this response with appropriate justifications. Activities that are part of a broader activity that furthers COVID-19 surveillance as well may be easier to justify.

Q: Are there plans to expand testing capacity for influenza A/H5 beyond public health laboratories and CDC?
A: CDC has licensing agreements in place with ten commercial laboratories or diagnostic manufacturers. These agreements allow these companies to develop their own Influenza A(H5N1) assays based on the CDC test. CDC continues to meet with these companies to discuss the H5 assay licensing agreements and interest in development of commercial H5 tests. CDC's Technology Transfer Office and the Influenza Division are actively pursuing establishing licensing agreements with multiple companies and several have been completed.

Milk Testing

Q: Can laboratories use the CDC Influenza assay for milk testing?
A: CDC Influenza A/H5 subtyping kits should not be used for milk testing and only used on human testing. FDA's Bacteriological Analytical Manual has a standard operating procedure for Extraction and Detection of Influenza A virus in Milk and Milk Products that may be used.

Q: Raw milk is collected from the farms for FDA required testing at public health labs. What information is available relevant to risk assessments for those handling and processing raw milk?
A: Laboratories receiving raw or pre-processed milk samples for testing should re-examine safety considerations of working with these and other cattle-derived samples. In August 2024, APHL developed a risk assessment guide that provides a framework for laboratories to assess their risk of exposure to HPAI when testing raw milk samples. In June 2024, FDA shared with APHL their Interim HPAI Virus Biosafety Recommendations and Resources for Industry Laboratories and State Dairy & Dairy Product Laboratories.

Q: Many states have dairy testing programs in place, but what influenza surveillance activities should be done through milk testing?
A: Federal agencies, working with dairy farmers, state veterinarians, NAHLN laboratories, and the dairy product industry, continue to monitor for newly detected infections, analyze herd epidemiology data, gather information on transmission route(s), and confirm the safety of the US milk supply. FDA is beginning to report their results from testing milk samples throughout the dairy production process and has confirmed detection of viral particles in some of the samples. States that are interested in testing milk and/or milk products for influenza A may use FDA's standard operating procedure.

Q: Is it required that samples from cattle or milk must be analyzed in a lab separate from human specimens?
A: Under the Pasteurized Milk Ordinance, the 2400 form for Cultural Procedures states, within a work area, only compatible laboratory functions should be performed (item 1.e). The PMO also references Good Laboratory Practices. In routine practice, separating human and food testing is prudent if a potential regulatory action could be taken on the results, but some laboratories manage contamination risks using separated spaces, biosafety hoods, and dedicated personnel.

Q: Can state laboratories leverage some of our wastewater testing capacity to screen dairy samples, using the H5

primer sets if that's allowed?
A: CDC Influenza H5 subtyping kits should not be used for non-human testing. APHL encourages public health laboratories to communicate with your state NAHLN laboratory counterparts and state GRADE 'A' Milk Sanitation personnel or federal liaisons to these programs for questions around H5N1 testing within your state.

Q: What are the CT values for milk vs. NP swab?
A: APHIS has reported that milk from infected cows contains high levels of H5N1 virus. Ct values in milk have been reported to be low. To date, APHIS has not found significant concentration of virus in respiratory related samples from cattle, which could indicate that respiratory transmission is not a primary means of transmission in cattle.

Q: Has HPAI been detected only in dairy cows or also in beef cattle?
A: To date, APHIS has received no reports of signs in beef herds.