Laboratory Testing of Highly Pathogenic Avian Influenza A(H5N1)

FAQ For Clinical Laboratories: Developed Jointly by ASM and APHL

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1. What is Highly Pathogenic Avian Influenza A(H5N1) (HPAI H5N1)?

HPAI H5N1 does not normally infect people, but rare, sporadic cases of human infection have occurred. Illnesses in humans from HPAI H5N1 infections have ranged in severity from no symptoms or mild illness (e.g., eye infection, upper respiratory symptoms) to severe disease (e.g., pneumonia) that resulted in death. Human infections with HPAI H5N1 have most often occurred after close or lengthy contact without PPE (Personal Protective Equipment i.e., gloves, respiratory protection, or eye protection) with infected birds or environments contaminated with virus shed from infected birds. Very rarely, human infections with HPAI H5N1 have happened through an intermediary animal, including dairy cows.[1,2]

HPAI H5N1 has been found in wild birds worldwide and is causing outbreaks in poultry and U.S. dairy cows, with several recent human cases in U.S. dairy and poultry workers. While the current public health risk is low for the general population, the CDC is watching the situation carefully and working with state health departments to monitor symptomatic people with animal exposure.

2. When should a person be tested for HPAI H5N1?

If a patient has influenza-like symptoms, they should first be tested for seasonal influenza. If a symptomatic patient has appropriate risk factors (see below) or an influenza test shows an unsubtypable influenza A result, the sample should be sent to a public health laboratory for HPAI H5 testing. The CDC recommends HPAI H5 specific testing in individuals who exhibit influenza-like illness and/or conjunctivitis with the appropriate epidemiological risk factors for exposure (e.g., exposure to an animal known or suspected to be infected with HPAI H5 virus).[3] When possible, clinical labs should support the public health initiative for enhanced surveillance.[4]

Local jurisdictions may have different algorithms (e.g., low risk and high risk), submission criteria, and which laboratory to contact (e.g., local vs state). Laboratories should contact their local health department for the most up to date information. More information regarding risk factors and testing can be found on the CDC website.[4]

3. What testing is available and where?

HPAI H5-specific PCR testing is available at state and local public health laboratories and at the CDC using the CDC Human Influenza Virus Real-Time RT-PCR Diagnostic Panel, Influenza A/H5 Subtyping Kit, which has 510k clearance from the FDA.
While the algorithm may vary based on local jurisdiction, HPAI H5 testing in public health laboratories currently generally follows a multi-test algorithm:

- Specimen is tested for influenza A and B first
- If the specimen is influenza A positive, then seasonal subtyping (H1N1 and H3N2) is performed
    - Note: H5 subtyping may be performed at the same time as seasonal influenza subtyping PCR and in cases where the patient has risk factors for HPAI H5N1.
- If the specimen is influenza A positive and seasonal subtyping negative, then HPAI H5 PCR is performed
- Positive results obtained at a public health laboratory will be reported to the health department and the specimen submitter as "presumptive positive" and be sent to the CDC for confirmatory testing per a requirement in the test's Instructions for Use (IFU).

Clinical laboratories should reach out to their local public health contacts to confirm the hours of operation and potential testing cut-off times to coordinate delivery and testing.

4. What specimens should be collected and sent to public health to confirm HPAI H5N1?

For the most up to date information regarding appropriate specimen collection, current CDC guidelines should be reviewed.[4,5] For patients presenting with symptoms of conjunctivitis only, conjunctival and nasopharyngeal swabs must both be collected separately as two individual swabs and submitted for parallel testing. Both swabs are required for testing to be performed. Both swabs should be placed in separate tubes containing Viral Transport Medium (VTM) or Universal Transport Medium (UTM).

- NOTE: Aluminum shafted calcium alginate swabs commonly used in ophthalmology are not an acceptable collection device for molecular testing

For patients presenting with classic respiratory symptoms, a nasopharyngeal and a nasal swab combined with an oropharyngeal swab (e.g., two swabs combined into one transport media vial) should be submitted.

- Swabs should be made of a synthetic material (e.g., Dacron) with a plastic shaft. Swabs should be put into tubes containing viral or universal transport media.

5. For labs who perform influenza assays that provide a subtype, how should labs handle unsubtypable specimens?

If an assay provides Influenza A seasonal subtyping (H1N1 or H3N2), specimens that are unsubtypable or result as inconclusive for these targets should be sent to a public health laboratory for HPAI H5 testing and/or additional characterization to determine if the unsubtypable specimen contains a novel influenza A virus.

6. For labs utilizing a courier system, are there concerns with transporting the specimen from the collection site to the laboratory?

Courier transport should follow standard Department of Transportation (DOT) Category B guidance.

7. Do current commercial molecular or antigen-based influenza tests detect HPAI H5N1?

Laboratories should reach out to manufacturers directly to determine if their assays will detect HPAI H5N1. Some companies have issued official letters stating whether their assay detects HPAI H5N1 or

does not. Laboratories should review the IFU, which may also be referred to as the package insert, to check for inclusivity panels with influenza A subtypes surveyed. The FDA maintains a database of in vitro diagnostics (IVD) influenza assays and established lineages/subtyping.[6]

8. Can specimens that are suspected to potentially contain HPAI H5N1 be tested for other routine respiratory viruses in the clinical microbiology laboratory (e.g., SARS-CoV-2, RSV)?

Clinical laboratories can continue testing the specimens, but labs should prioritize testing for influenza A and sending the specimen to public health if HPAI H5N1 is suspected.

9. What precautions should be taken when handling the specimens?

Laboratories should practice universal respiratory precautions. Additional precautions and considerations can be found on the CDC website.[7]

10. What lysis buffers inactivate HPAI H5N1?

APHL has provided guidance on lysis buffers validated to inactivate HPAI H5N1.[8]

11. Can suspected cases of HPAI H5N1 be tested in a point of care testing (POCT) setting?

If a patient is at high risk for HPAI H5N1 due to exposure and appropriate symptoms, samples should be sent to a clinical laboratory to be tested instead of in a POCT setting. If the patient is not considered high risk for HPAI H5N1, samples can be tested for influenza in the POCT setting. However, institutions may have their own guidance and institutional infection prevention teams should be consulted. CDC has published additional guidance on infection control.[9]

12. What should a clinical laboratory do if they have a specimen in their possession that contains confirmed HPAI?

HPAI H5N1 avian influenza viruses are exempted from the requirements of the Select Agent regulations listed in 9 C.F.R. Part 121 for a period of three years starting June 6, 2024.[10] With the select agent exemption in place (as of July 19, 2024), specimens containing confirmed HPAI H5N1 would not need to be destroyed as noted in APHIS/CDC Form 4. Waste specimens could follow a similar disposal path to specimens with confirmed seasonal influenza, SARS-CoV-2, or TB. Note that this exemption is time limited, so it is not recommended that the laboratories retain their positive specimens long-term. Once the exemption runs out, the laboratories will need to register with the Federal Select Agent Program to possess confirmed specimens or transfer or destroy the specimens within a specific time period.

13. If a patient is at high risk due to exposure but a commercial PCR flu test is negative, what should be done?

If a patient has risk factors and appropriate symptoms but tests negative on a commercial influenza PCR assay, the sample should be sent to public health regardless of commercial assay result.

14. What is the turnaround time (TAT) for testing to confirm HPAI H5N1?

The typical TAT for HPAI H5 testing at a public health laboratory is 1-2 days after the specimen is received. This TAT may vary depending on what time of day the specimen arrives, the volume of samples received at the laboratory, and other factors. Check with your specific public health laboratory about their TAT. Testing for other clinical diagnostic assays can be performed in parallel and does not need to be paused while waiting for the result from public health.

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Resources

1. https://www.cdc.gov/media/releases/2024/p0401-avian-flu.html
2. https://www.cdc.gov/bird-flu/php/avian-flu-summary/reported-human-infections.html
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