MAYO CLINIC LABORATORIES

Test Definition: HPFLU

Influenza Virus Type A and Type B, and Respiratory Syncytial Virus (RSV) RNA, Molecular Detection, PCR, Varies

Overview

Useful For
Simultaneous detection of influenza A virus, influenza B virus, and respiratory syncytial virus in upper or lower respiratory tract specimens from individuals with flu-like illnesses

Testing Algorithm
For information see Coronavirus Disease 2019 (COVID-19), Influenza, and Respiratory Syncytial Virus Testing Algorithm.

Method Name
Multiplex Real-Time Polymerase Chain Reaction (RT-PCR)

NY State Available
Yes

Specimen

Specimen Type
Varies

Specimen Required
Preferred:
Specimen Type: Nasopharyngeal swab
Container/Tube: Sterile container with transport media
Specimen Volume: Entire specimen with a minimum of 1.5 mL (maximum 3 mL) of transport media.
Collection Instructions:
1. Collect specimen by swabbing back and forth over mucosa surface to maximize recovery of cells.
2. Swab must be placed into viral transport media (eg, M4-RT, M4 or M5), saline, or phosphate buffered saline (PBS).
Media should not contain guanidine thiocyanate (GTC).

Acceptable:
Specimen Type: Oropharyngeal (throat) swab, nasal mid-turbinate, or nares/nasal swab
Supplies:
-Culturette (BBL Culture Swab) (T092)
-Mid-turbinate (MT) Swab (Copan FLOQSwab) (T864)
-Swab, Sterile Polyester (T507)
Container/Tube: Sterile container with transport media
Specimen Volume: Entire specimen with a minimum of 1.5 mL (maximum 3 mL) of transport media.
Preferred: BBL Culture Swab, COPAN Mid-turbinate Swab

Acceptable: Dacron-tipped swab with plastic handle
Collection Instructions: Swab must be placed into viral transport media (eg, M4-RT, M4, or M5), saline, or PBS. **Media should not contain guanidine thiocyanate (GTC).**

Specimen Type: Lower respiratory tract
Sources: Bronchoalveolar lavage (BAL), bronchial washings, endotracheal aspirate, sputum
Container/Tube: Sterile container
Specimen Volume: 0.6 mL
Additional Information: Do not aliquot into viral transport media.

Specimen Minimum Volume

Swab in1.5 mL of media
Lower respiratory specimens: 0.3 mL

Reject Due To

Calcium alginate-tipped swab Wood swab Dry swab Transport swab containing gel	Reject

Specimen Stability Information

Specimen Type	Temperature	Time	Special Container
Varies	Frozen (preferred)	14 days	
	Refrigerated	72 hours	

Clinical & Interpretive

Clinical Information

Influenza, otherwise known as the "flu," is an acute, contagious respiratory illness caused by influenza A, B, and C viruses. Of these, only influenza A and B are thought to cause significant disease, with infections due to influenza B usually being milder than infections with influenza A. Influenza A viruses are further categorized into subtypes based on the 2 major surface protein antigens: hemagglutinin (H) and neuraminidase (N).

Common symptoms of influenza infection include fever, chills, sore throat, muscle pains, severe headache, weakness, fatigue, and a nonproductive cough. Certain patients, including infants, older individuals, patients who are immunocompromised, and those with impaired lung function, are at risk for serious complications. In the United States,

influenza results in 10,000 to 30,000 deaths and more than 200,000 hospitalizations each year.(1)

In the northern hemisphere, annual epidemics of influenza typically occur during the fall or winter months. However, the peak of influenza activity can occur as late as April or May, and the timing and duration of flu seasons vary.

Influenza infection may be treated with supportive therapy as well as antiviral drugs, such as the neuraminidase inhibitors, oseltamivir (Tamiflu) and zanamivir (Relenza). These drugs are most effective when given within the first 48 hours of infection, so prompt diagnosis and treatment are essential for proper management.

Respiratory syncytial virus (RSV) is a respiratory virus that also infects the human respiratory tract, causing an influenza-like illness. Most otherwise healthy people recover from RSV infection in 1 to 2 weeks, but infection can be severe in infants, young children, and older adults. RSV is the most common cause of bronchiolitis (inflammation of the small airways in the lung) and pneumonia in children under 1 year of age in the United States. It is increasingly recognized as a frequent cause of respiratory illness in older adults.(2)

RSV and influenza viruses can be detected in respiratory secretions, including upper and lower respiratory tract specimens, by molecular test methods. Nasopharyngeal swabs or aspirates are the preferred specimen types for detection of influenza A virus, influenza B virus, and RSV. Nasal swabs have also been shown to provide equivalent yield to nasopharyngeal specimens for molecular detection of influenza A and B viral RNA but not RSV RNA.(3-4)

Reference Values

Undetected

Interpretation

A "Detected" (positive) test result indicates that the patient is presumptively infected with the indicated virus. The test does not indicate the stage of infection. Rarely, more than one virus may be detected from the same patient specimen. Laboratory test results should always be considered in the context of clinical observations and epidemiologic data in making a final diagnosis.

An “Undetected” (negative) test result suggests that the patient is not infected with influenza A virus, influenza B virus, or respiratory syncytial virus (RSV).

An "Inconclusive" result indicates that the presence or absence of influenza A virus, influenza B virus, and RSV in the specimen could not be determined with certainty after repeat testing in the laboratory, possibly due to real-time, reverse transcription polymerase chain inhibition. Submission of a new specimen for testing is recommended.

Cautions

This test has been designed to minimize the likelihood of false-positive test results. However, should false-positive results occur, risks to patients could include a recommendation for quarantine of household or other close contacts, a recommendation for patient isolation that might limit contact with family or friends, the ability to work, or the ability to receive certain medical care, prescription of an antiviral drug or other therapy, or other unintended adverse effects.

The sensitivity of the assay is very dependent upon the quality of the specimen submitted. A nasopharyngeal swab is the

preferred specimen type for optimal detection of respiratory syncytial virus (RSV) RNA.

The test is specific for influenza A virus, influenza B virus, and RSV; therefore, the results do not exclude the possibility of infection with other respiratory viruses. Influenza C virus is not detected by this assay.

This assay detects influenza A viral RNA but does not distinguish among the different viral subtypes.

"Undetected' (negative) results do not preclude infection with influenza A virus, influenza B virus, or RSV and should not be used as the sole basis for treatment or other patient management decisions.

This assay detects both viable and nonviable virus. Test performance depends on viral load in the specimen and may not correlate with cell culture performed on the same specimen.

The assay has not been US Food and Drug Administration approved for detection of influenza A H7N9, though comparison of primer and probe sequences indicates that the assay will detect the H7N9 viral subtype.

Clinical Reference

1. Centers for Disease Control and Prevention. Information for clinicians on influenza virus testing. Updated August 29. 2022. Accessed August 4, 2023. Available at www.cdc.gov/flu/professionals/diagnosis/index.htm
2. Centers for Disease Control and Prevention. Respiratory syncytial virus infection (RSV). Updated July 21, 2023. Accessed August 4, 2023. Available at www.cdc.gov/rsv/clinical/index.html
3. Anderson NW, Binnicker MJ, Harris DM, et al. Morbidity and mortality among patients with respiratory syncytial virus infection: a 2-year retrospective review. Diagn Microbiol Infect Dis. 2016; 85(3):367-371
4. Boerger AC, Binnicker MJ. Comparison of the panther fusion respiratory panels to routine methods for detection of viruses in upper and lower respiratory tract specimens. Diagn Microbiol Infect Dis. 2020;97(2):115014

Performance

Method Description

The Panther Fusion Flu A/B/RSV (influenza virus type A and type B, and respiratory syncytial virus) assay is a multiplex, real-time, reverse-transcription polymerase chain reaction diagnostic test for the rapid and qualitative detection and differentiation of influenza A virus, influenza B virus, and RSV. This assay is US Food and Drug Administration approved for use with nasopharyngeal (NP) swab specimens only, and the performing laboratory has validated other upper respiratory tract specimens (eg, NP aspirate, oropharyngeal swab, nasal mid-turbinate swab, or nares/nasal swab) and lower respiratory tract specimens (eg, bronchoalveolar lavage, bronchial washings, endotracheal aspirate, sputum).

Specimens are transferred first to a specimen lysis tube containing that lyses cells, releases target nucleic acid, and protects them from degradation during storage. The internal control-S (IC-S) is added to each test specimen and controls via the working Panther Fusion Capture Reagent-S. The IC-S in the reagents monitors specimen processing, amplification, and detection. Capture oligonucleotides hybridize to nucleic acid in the test specimen. Hybridized nucleic acid is then separated from the specimen in a magnetic field. Wash steps remove extraneous components from the reaction tube. The elution step elutes purified nucleic acid. During the nucleic acid capture and elution step, total nucleic

acid is isolated from specimens. Eluted nucleic acid is transferred to a reaction tube already containing oil and reconstituted master mix. Target-specific forward and reverse primers and probes simultaneously amplify, detect, and discriminate the following target sequences: matrix genes of influenza A virus, influenza B virus, and RSV A/B, along with IC-S. Influenza A virus is detected on the FAM channel, influenza B virus is detected on ROX, and RSV A/B is detected on HEX. The assay software then compares the fluorescence signals generate to predetermined cutoff values to produce a qualitative result for the presence or absence of each virus.(Instruction manual: Flu A/B/RSV Assay [Panther Fusion System]. Hologic, Inc; AW-16832 Rev. 002, 04/2019)

PDF Report
No

Day(s) Performed
Monday through Sunday

Report Available
Same day/1 to 2 days

Specimen Retention Time
4 days

Performing Laboratory Location
Rochester

Fees & Codes

Fees
- Authorized users can sign in to Test Prices for detailed fee information.
- Clients without access to Test Prices can contact Customer Service 24 hours a day, seven days a week.
- Prospective clients should contact their account representative. For assistance, contact Customer Service.

Test Classification
This test has been modified from the manufacturer's instructions. Its performance characteristics were determined by Mayo Clinic in a manner consistent with CLIA requirements. This test has not been cleared or approved by the US Food and Drug Administration.

CPT Code Information
87631

LOINC® Information

Test ID	Test Order Name	Order LOINC® Value
HPFLU	Influenza A/B and RSV, PCR, Varies	92143-7

Test Definition: HPFLU

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Result ID	Test Result Name	Result LOINC® Value
HPFLS	Influenza A/B and RSV, Source	31208-2
610412	Influenza A virus PCR	In Process
610413	Influenza B virus PCR	In Process
610414	Respiratory Syncytial Virus, PCR	In Process