MAYO CLINIC LABORATORIES

Test Definition: FLUNP

Influenza Virus Type A and Type B, and Respiratory Syncytial Virus (RSV), Molecular Detection, PCR, Nasopharyngeal Swab

Overview

Useful For
Rapid and accurate detection of influenza A, influenza B, and respiratory syncytial virus in a single test for nasopharyngeal swab specimens

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

NY State Available
No

Specimen

Specimen Type
Swab

Specimen Required
Specimen Type: Nasopharyngeal swab
Container/Tube: Sterile container with viral 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 in viral transport media (for example, M4-RT, M4, or M4 media).

Specimen Minimum Volume
Nasopharyngeal swab submitted in minimum volume of 0.3 mL of viral transport media (eg, M4-RT)

Reject Due To

Swabs	E-swab, calcium alginate-tipped swab, wood swab, dry swab, or transport swab containing gel

Specimen Stability Information

Specimen Type	Temperature	Time	Special Container
Swab	Refrigerated (preferred)	7 days	
	Frozen	7 days	

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, the elderly, the immunocompromised, and those with impaired lung function, are at risk for serious complications. In the United States, influenza results in approximately 36,000 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. In 2009 to 2010, a novel influenza virus (called 2009 H1N1, previously "swine" flu) appeared in Mexico and quickly spread worldwide, causing the first influenza pandemic in more than 40 years. The resultant influenza season had an atypical distribution, with illness occurring during normally low-incidence months. Following a pandemic, disease incidence usually returns to the typical seasonal distribution within 1 to 2 years.(1)

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 respiratory system and can cause an influenza-like illness. Most otherwise healthy people recover from RSV infection in 1 to 2 weeks. However, 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, and is more frequently being recognized as an important cause of respiratory illness in older adults.(2)

RSV and influenza virus RNA can be detected by PCR in respiratory secretions, including upper and lower respiratory specimens. Nasopharyngeal swabs or aspirates are the preferred specimen types for detection of RNA from influenza A, influenza B, and RSV. Nasal swabs have also been shown to provide equivalent yield to nasopharyngeal specimens for molecular detection of influenza A and B RNA, but not RSV RNA.(3-4) Tracheal aspirates are generally not acceptable for testing due to the viscous nature of these specimens.

Reference Values

Negative

Interpretation

A 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 1 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.

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

Cautions

Given that influenza A and B and respiratory syncytial virus (RSV) are common and can cause an indistinguishable clinical disease, this test is offered only as a panel.

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 RSV RNA.

This test should not be performed unless the patient meets clinical and epidemiologic criteria for testing.

The test is specific for influenza A, influenza B, 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 virus RNA, but does not distinguish between the different subtypes of influenza A.

Negative results do not preclude infection with influenza A, influenza B, or RSV viruses 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 FDA approved for detection of Influenza A H7N9, though comparison of primer and probe sequences suggest that the assay will detect the H7N9 virus.

Supportive Data

Accuracy:

To assess accuracy, a combination of clinical or spiked nasopharyngeal swabs (n=60) were tested by the proposed Simplexa Flu A/B and respiratory syncytial virus (RSV) assay and the results compared to those of the current method (Gen-Probe/Hologic ProFlu assay). The results are summarized in the Table below:

Table 1. Comparison of the proposed Simplexa assay to the Gen-Probe/Hologic ProFlu assay using clinical and spiked nasopharyngeal swab samples (n=60).

Analyte	Positives	Negatives	Concordance
Influenza A	20/20	10/10	100%
Influenza B	10/10	10/10	100%

RSV	20/20	10/10	100%

Clinical Reference

1. Centers for Disease Control and Prevention. Influenza Accessed April 2020. Available at: www.cdc.gov/flu/index.htm
2. Lee N, Lui GC, Wong KT, et al: High morbidity and mortality of adults hospitalized for respiratory syncytial virus infections. Clin Infect Dis 2013;57(8):1069-1077
3. Meerhoff TJ, Houben ML, Coenjaerts FE, et al: Detection of multiple respiratory pathogens during primary respiratory infection: nasal swab versus nasopharyngeal aspirate using real-time polymerase chain reaction. Eur J Clin Microbiol Infect Dis 2010;29:365-371
4. Heikkinen T, Marttila J, Salmi AA, Ruuskanen O: Nasal swab versus nasopharyngeal aspirate for isolation of respiratory viruses. J Clin Microbiol 2002;40(11):4337-4339

Performance

Method Description

The Simplexa Flu A/B and respiratory syncytial virus (RSV) direct assay system is a real-time RT-PCR assay for the in vitro qualitative direct detection and differentiation of influenza A virus, influenza B virus, and RSV RNA directly from clinical samples. The system consists of the Simplexa Flu A/B and RSV direct assay, the 3M Integrated Cycler (with Integrated Cycler Studio Software), the Direct Amplification Disc and associated accessories.

The test is a real-time RT-PCR amplification and detection system that utilizes a bi-functional fluorescent probe-primer for the detection and differentiation of human influenza A virus RNA, human influenza B virus RNA and RSV RNA in nasopharyngeal swabs (NPS). The assay is composed of 2 principal steps: (1) extraction of RNA from patient specimens, (2) a bifunctional fluorescent probe-primer is used together with a reverse primer to amplify a specific target (for each analyte and the RNA internal control). The assay targets 3 viral gene segments, including conserved regions of influenza A virus (matrix gene), influenza B virus (matrix gene), and RSV (M gene). An RNA internal control is used to monitor the extraction process and to detect RT-PCR inhibition.(Package Insert: Simplexa Flu A/B and RSV Direct. Focus Diagnostics, Cypress, CA, Nov 2012)

PDF Report

No

Day(s) Performed

Monday through Sunday

Report Available

Same day/1 to 3 days

Specimen Retention Time

14 days

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Performing Laboratory Location

Mayo Clinic Jacksonville Clinical Lab

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 cleared, approved, or is exempt by the US Food and Drug Administration and is used per manufacturer's instructions. Performance characteristics were verified by Mayo Clinic in a manner consistent with CLIA requirements.

CPT Code Information

87631

LOINC® Information

Test ID	Test Order Name	Order LOINC® Value
FLUNP	Influenza A/B and RSV, PCR, NP Swab	78922-2

Result ID	Test Result Name	Result LOINC® Value
SS019	Specimen Source	31208-2
35975	Influenza A, PCR	76078-5
35976	Influenza B, PCR	76080-1
35977	Respiratory Syncytial Virus, PCR	76089-2