

Influenza A/B, SARS-CoV-2, and Respiratory Syncytial Viruses – Qualitative, Real-time PCR with Reflex to Influenza Virus A Subtyping – Qualitative, Real-time PCR

Use

Detection and differentiation of severe acute respiratory syndrome coronavirus (SARS-CoV-2), influenza A, influenza B, and/or respiratory syncytial virus (RSV) viral RNA in nasopharyngeal swab specimens collected from individuals with signs and symptoms of respiratory tract infection. Influenza A-positive specimens are reflexed for characterization of influenza A subtypes.

OSDH Infectious Disease Services pre-approval is required for submission of specimens from patients with suspected influenza A/H5 or Eurasian H7 to verify the patient meets clinical case criteria, including travel history to an area of novel influenza circulation. Call 24/7/365 for telephone consultation at 405-426-8710.

Clinical Significance

Influenza, SARS-CoV-2, and RSV often cause overlapping respiratory symptoms, making clinical distinction difficult without laboratory testing. Active surveillance and infection-prevention measures are essential for limiting transmission, and rapid diagnostic assays play a critical role in identifying infected individuals, guiding treatment, and preventing widespread outbreaks.

Influenza A and B viruses commonly cause acute respiratory illness characterized by fever, chills, headache, malaise, dry cough, body aches, and nausea; severe cases may lead to pneumonia, hemorrhagic bronchitis, or death, particularly in vulnerable populations. SARS-CoV-2, the virus responsible for COVID-19, presents with symptoms such as fever, cough, shortness of breath, fatigue, loss of taste or smell, sore throat, and headache, and can range from asymptomatic infection to severe or fatal disease. RSV primarily affects infants, young children, and older adults, causing congestion, runny nose, cough, fever, wheezing, and difficulty breathing, with severe illness occurring in high-risk individuals. Many other respiratory pathogens, including rhinoviruses, adenoviruses, and parainfluenza viruses, can cause illnesses similar to influenza-like illness, SARS-CoV-2, and RSV.

Further background information, fact sheets, statistics and educational resources may be found at the OSDH Infectious Disease Services [website](#).

Methodology

The Xpert Xpress CoV-2/Flu/RSV *plus* assay uses primers and probes to amplify and detect multiple unique sequences of the SARS-CoV-2 virus (N, E, RdRP genes), influenza A virus (M, PB2, PA genes), influenza B virus (M, NS genes) and RSV A and RSV B viruses (N genes). Samples positive for influenza A are reflexed to the Human Influenza Virus Real-time Reverse Transcriptase-PCR Diagnostic Panel, developed by the Centers for Disease Control and Prevention (CDC), which can differentiate influenza A subtypes H1pdm09, H3, H5, and H7. Influenza A H5, H7 and other subtypes may require confirmatory testing performed at the CDC.

Specimen Type

- Nasopharyngeal (NP) swab

Minimum Volume/Size

1 swab in tube containing 3 mL of a suitable viral transport medium; examples include Remel M4RT, M4, or M5, BD Universal Viral Transport (UVT®) Medium, and Copan Universal Transport Medium (UTM®).

M4RT is provided free of charge to OSDH County Health Departments only; use the [Laboratory Supply Request Form](#).

Collection Instructions

Respiratory virus detection depends on the collection of high-quality specimens, their rapid transport to the testing laboratory and appropriate storage before testing. Training in specimen collection is highly recommended due to the importance of specimen quality. Specimens should be collected using standard procedures of the submitting site. Follow storage and collection instructions applicable to the collection kit/medium used. Additional guidance is contained in the link below.

- See [Guidance for Collection of Nasopharyngeal Swab](#)

Common Causes for Rejection

- Incorrect collection device (cotton, wooden or calcium alginate swab)
- Transport media other than those indicated
- Received > 72 hours from time of collection and not frozen
- Swab without transport medium
- Specimens missing swabs
- Specimens that have leaked
- Specimen at ambient temperature

Shipping

- **Refrigerated** (2–8°C), use frozen cold packs: for fresh specimen(s) that will arrive at OSDH PHL within 72 hours of collection.
- **Frozen**, use dry ice: for specimen(s) that will **not** arrive at the OSDH PHL within 72 hours of collection.

Samples may be kept frozen (at least -20°C but -70°C or colder is preferred) at the collection site and submitted to the OSDH PHL if courier delivery cannot be arranged for same or next day delivery. If site is unable to provide dry ice for shipment, please call the OSDH PHL at 405-564-7750 at least 24 hours ahead of scheduled submission to request dry ice transport.

Turn-around Time

Within 5 working days of receipt

Specimens initially screened using the Xpert Xpress CoV-2/Flu/RSV *plus* assay that are influenza virus A-positive will be issued a preliminary report pending testing by the CDC Human Influenza Virus Real-time Reverse Transcriptase-PCR Diagnostic Panel at the OSDH PHL. Specimens in which variant or potential novel influenza viruses are detected by the CDC Human Influenza Virus RT-PCR Diagnostic Panel may be issued an additional preliminary report, pending further characterization by the CDC.

Reference Range

Influenza A RNA Not Detected; Influenza B RNA Not Detected; SARS-CoV-2 Virus RNA Not Detected; Respiratory Syncytial Virus RNA Not Detected

Reportable Results

- **Xpert Xpress CoV-2/Flu/RSV *plus* Assay:**
 - Influenza A Not Detected
 - Influenza B Not Detected
 - SARS-CoV-2 Virus Not Detected
 - Respiratory Syncytial Virus Not Detected
 - Influenza A Detected (see note)
 - Influenza B Detected

- SARS-CoV-2 Virus RNA Detected
- Respiratory Syncytial Virus RNA Detected
- Influenza Virus B Detected, possible co-infection or recent live attenuated influenza virus vaccination
- Inconclusive: Presence or absence of Influenza A and B, SARS-CoV-2 and RSV RNA cannot be determined. Potential PCR inhibitor or poor quality of sample; recommend recollection and submission of fresh sample, if clinically appropriate

Note: *Influenza A-positive specimens are issued a preliminary report while awaiting testing by the CDC Human Influenza Virus RT-PCR Diagnostic Panel at the OSDH PHL.*

- **CDC Human Influenza Virus RT-PCR Diagnostic Panel:**

- Influenza Not Detected
- Influenza Virus A Detected, Subtype: H1 2009 pandemic strain
- Influenza Virus A Detected, Subtype: H3 strain
- Influenza Virus A Detected, Subtype: H1 2009 pandemic strain; possible coinfection or recent live attenuated influenza virus vaccination
- Influenza Virus A Detected, Subtype: H3 strain; possible co-infection or recent live attenuated influenza virus vaccination
- Influenza Virus A Detected, Subtype: Undetermined (see note)
- Influenza Virus A Detected, Subtype: Undetermined, referred to CDC for subtyping (see note)
- Presumptive Positive for Influenza A/H3N2 variant; referred to CDC for confirmation
- Indeterminate: Potential PCR inhibitor or poor quality of sample; recommend recollection and submission of fresh sample, if clinically appropriate

Note: *The OSDH PHL does not have the ability to subtype all strains of influenza A virus. Also, some strain results require confirmation by the CDC before they can be reported. Specimens must meet specific quality criteria to be acceptable for testing by the CDC; if the specimen is acceptable, the OSDH PHL report will indicate "Subtype: Undetermined, referred to CDC for subtyping" and a CDC report will follow, whereas if unacceptable by CDC, the OSDH PHL report will indicate "Subtype: Undetermined" as a final report.*

Interpretation

- Failure to detect RNA for any of the targeted viruses suggests an absence of viruses in the sample; however, negative results do not preclude infection with targeted or other viruses and should not be used as the sole basis for treatment or other patient management decisions.
- Detection of RNA for any of the targeted viruses suggests the presence of targeted virus in the sample; however, positive results do not imply infectivity or rule out other viral or bacterial co-infections and should not be used as the sole basis for treatment or other patient management decisions.
- An indeterminate or inconclusive test result is likely due to a PCR inhibitor or poor-quality specimen.

Limitations/Interferences

- As with any molecular test, mutations within targeted sequences could affect primer and/or probe binding resulting in failure to detect the presence of target viruses or newly emerging variants.
- The performance characteristics of the Xpert Xpress CoV-2/Flu/RSV *plus* assay may vary depending on influenza A and SARS-CoV-2 variants circulating and their prevalence, which are expected to change over time.
- The performance characteristics of the CDC Human Influenza Virus RT-PCR Diagnostic Panel may vary depending on influenza A variants circulating and their prevalence, which are expected to change over time.
- Viral nucleic acid may persist *in vivo*, independent of virus infectivity. Detection of analyte target(s) does not imply that the corresponding virus(es) are infectious or are the causative agents for clinical symptoms.
- Erroneous test results might occur from improper specimen collection; failure to follow the recommended

sample collection, handling, and storage procedures; technical error; or sample mix-up.

- Individuals immunized with live attenuated influenza nasal spray vaccine may be positive for one or more influenza virus targets for several days post-vaccination; vaccination history should be considered when interpreting positive test results, especially early in the respiratory virus season.
- FluMist was shown to interfere with detection of low levels of SARS-CoV-2 and RSV A and B using the Xpert Xpress CoV-2/Flu/RSV *plus* assay.
- Snuff was shown to interfere with detection of low levels of influenza A and B using the Xpert Xpress CoV-2/Flu/RSV *plus* assay.
- Nasal decongestants were shown to interfere with detection of low levels of influenza A and B and RSV A using the Xpert Xpress CoV-2/Flu/RSV *plus* assay.

CPT Code

Variable depending on test results.

Notes

These tests are approved for *in vitro* diagnostic use by the U.S. Food and Drug Administration.