PROCEDURE: Xpert® Xpress SARS-CoV-2/Flu/RSV *plus* (FDA-cleared)

I. PRINCIPLE

The Cepheid Xpert® Xpress SARS-CoV-2/Flu/RSV *plus* (FDA-cleared) assay is a rapid, automated multiplexed real-time reverse transcriptase polymerase chain reaction (RT-PCR) test intended for the simultaneous *in vitro* diagnostic test qualitative detection and differentiation of SARS-CoV-2, Influenza A, Influenza B, and Respiratory Syncytial Virus (RSV) viral RNA in nasopharyngeal swabs, from individuals suspected of respiratory viral infection.

The assay is performed on the GeneXpert Dx and GeneXpert Infinity Systems, which automate and integrate sample purification, nucleic acid extraction and amplification, and detection of the target sequences in simple or complex samples using real-time PCR and RT-PCR assays. The system consists of an instrument, personal computer, and preloaded software for running tests and viewing the results. The system requires single-use disposable cartridges which hold the PCR reagents and hosts the PCR process. Because the cartridges are self-contained, cross-contamination between samples is minimized.

The Xpert® Xpress SARS-CoV-2/Flu/RSV *plus* (FDA-cleared) assay cartridges include reagents for the detection and differentiation of SARS-CoV-2, Influenza A, Influenza B and RSV. Sample Processing Control (SPC) and a Probe Check Control (PCC) are also included in the cartridge. The SPC is present to control for adequate processing of the target and to monitor the presence of inhibitors in the PCR reaction. The Probe Check Control (PCC) verifies reagent rehydration, PCR tube filling in the cartridge, probe integrity, and dye stability.

II. AVAILABILITY

24/7, at all affiliate hospitals

III. SPECIMEN

A. Nasopharyngeal (NP) flocked swab or nasal swab specimen collected in Viral/Universal Transport Medium.
B. Minimum of 300 ul for testing.
C. NP and/or nasal swabs can be stored at room temperature (15-30°C) for up to 48 hours, or at 2-8°C for up to 7 days.

IV. MATERIALS AND EQUIPMENT

A. Materials:
    1. Xpress SARS-CoV-2/Flu/RSV *plus*
        a. Xpert® Xpress SARS-CoV-2/Flu/RSV *plus* assay cartridges (10)
        b. Disposable 300 µL Transfer Pipettes – 1 bag (12)
B. Materials available but not provided:
    1. Specimen collection kits: Viral/Universal transport medium, Nylon flocked swabs.
    2. External Controls
        a. Positive (Flu/RSV/SARS-CoV-2) – Zeptometrix NATFRC-6C-IVD
        b. Negative (Coxsackievirus CVA9) – Zeptometrix NATCV9-6C-IVD
    3. BSC disinfecting supplies:
        a. 10% bleach
        b. DI H_2O
        c. 70% Ethanol
C. Equipment:
    1. GeneXpert Dx System (software version 4.7b or higher) or GeneXpert Infinity Systems (-80s or -48s systems) (software version 6.4b or higher)
    2. Barcode scanner

V. STORAGE AND HANDLING

A. Store the Xpert® Xpress SARS-CoV-2/Flu/RSV *plus* (FDA-cleared) cartridges at 2-28º C.
B. Do not use UTM collection kits or cartridges passed the expiration date.
C. Do not open a cartridge lid except when adding sample.
D. Do not use a cartridge that has been shaken, dropped or damaged.
E. Do not use cartridges that appear wet or if the lid's seal appears broken.
F. Do not reuse spent cartridges.
G. Start the test within 30 minutes of adding the sample to the cartridge.

VI. QUALITY CONTROL

A. Maintenance
    1. Cleaning and maintenance of the instrument will be performed in accordance with the manufacturer's Operator's Manual. For further information, refer to the Infinity System's Operator's Manual.
B. Each test includes two internal controls to validate the assay and test samples are controlled according to the following procedures:
    1. **Sample processing control (SPC) -** The SPC is present to control for adequate processing of the target and to monitor the presence of specimen associated inhibitors in the PCR reaction. The SPC verifies that the release of RNA from the influenza and RSV viruses has occurred if the organism is present. It also verifies that the specimen processing was adequate. The SPC is considered to pass if it meets the validated acceptance criteria. If the SPC does not meet acceptance criteria, the sample was not properly processed or PCR is inhibited, the SPC will fail. The SPC should be positive in a negative sample and can be negative or positive in a positive sample.
    2. **Probe check –** Before the start of the PCR reaction, the GeneXpert is programmed to perform a probe check to monitor reagent bead rehydration, reaction tube filling, probe integrity, and dye stability. Probe check is considered to pass if it meets the validated acceptance criteria. If a problem is detected or the assay aborts, the probe check will fail.
C. External quality control specimens are run on new shipments, new lots, and/or every 30 days, whichever is more frequent. External controls are run after major system maintenance including software upgrade, annual PM, and if 3 or more modules are replaced at the same time. External controls are repeated if the controls are out of range or invalid. QC must be acceptable for the lot and instrument to be used for patient samples.
D. Environmental "wipe" testing is performed monthly on all equipment involved in testing. Any positive result will be brought up to a Sr. technologist. A thorough cleaning protocol using 10% bleach, deionized water, and 70% ethanol will be performed. Environmental wipe tests will be repeated. Environmental testing is acceptable when results for all targets are negative.
E. Refer to IQCP for complete Quality Control procedure.
F. Positivity Rate is monitored monthly.

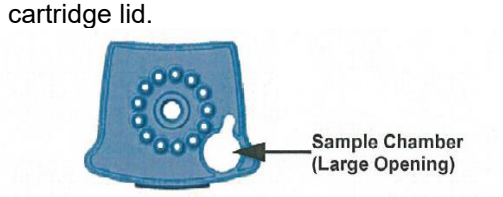
VII. TEST PROCEDURE

NOTE: Start the test within 30 minutes of adding the sample to cartridge.

A. Pre-analytical
    1. Clean designated hood with 10% bleach; rinse with deionized water; clean with 70% ethanol. This cleaning procedure must be performed before and after each specimen.

B. Preparing the Cartridge– *Refer to Figure Below*

1. Remove the cartridge from the package.
2. Mix the specimen by rapidly inverting the specimen tube 5 times.
   NOTE: DO NOT VORTEX
3. Open the cartridge lid.
4. Using supplied clean transfer pipette, squeeze the top bulb of the transfer pipette **completely until the top of the bulb is fully flat**. Transfer 300uL (one draw) of the specimen into the sample chamber with the large opening in the cartridge.
5. Close the cartridge lid.



C. Load the cartridge onto the GeneXpert system.
   1. For detailed instructions on how to program and load cartridge onto the Genexpert system, see *APPENDIX AP81 - GeneXpert Instrument Navigation*. You may also refer to the *GeneXpert Dx System Operator's Manual* or the *GeneXpert Infinity System Operator's Manual*.

D. Printing and Viewing Results
   1. For detailed instructions on how to view and print the results, see *APPENDIX AP81 - GeneXpert Instrument Navigation*. You may also refer to the *GeneXpert Dx System Operator's Manual* or the *GeneXpert Infinity System Operator's Manual*.

E. **Reasons to Repeat the Assay**
   1. **ERROR, INVALID, or NO RESULT**
      a. An **INVALID** result indicates that the control SPC failed. The sample was not properly processed, PCR is inhibited, or the sample was not properly collected.
      b. An **ERROR** result could be due to, but not limited to, Probe Check Control failure, system component failure, no sample added, or the maximum pressure limits were exceeded.
      c. A **NO RESULT** indicates that insufficient data were collected. For example, cartridge failed integrity test, the operator stopped a test that was in progress, or a power failure occurred.
      d. If an External Control fails to perform as expected, repeat external control test and/or contact Cepheid for assistance.

VIII. INTERPRETATION

A. The results are interpreted automatically by the GeneXpert System and are shown in the **View Results** window. The Xpert® Xpress SARS-CoV-2/Flu/RSV *plus* (FDA-cleared) test provides test results based on the detection of respective gene targets according to the algorithms.

1. Table 1 shows the possible result outcomes when the Xpert Xpress SARS-CoV-2_Flu_RSV plus test mode is selected.

Table 1. Xpert Xpress SARS-CoV-2_Flu_RSV *plus* Possible Results and Interpretation

Result	Interpretation
SARS-CoV-2 POSITIVE	The SARS-CoV-2 target RNA is detected. • The SARS-CoV-2 signal has a Ct within the valid range and endpoint above the minimum setting. • SPC: NA (not applicable); SPC is ignored because SARS-CoV-2 target amplification occurred. • Probe Check: PASS; all probe check results pass.
Flu A POSITIVE	The Flu A target RNA is detected. • The Flu A signal for either the Flu A1 RNA target or the Flu A2 RNA target or signals for both RNA targets has a Ct within the valid range and endpoint above the threshold setting. • SPC - NA; SPC is ignored because the Flu A target amplification occurred. • Probe Check - PASS; all probe check results pass.
Flu B POSITIVE	The Flu B target RNA is detected. • The Flu B signal has a Ct within the valid range and endpoint above the minimum setting • SPC: NA; SPC is ignored because Flu B target amplification occurred. • Probe Check: PASS; all probe check results pass
RSV POSITIVE	The RSV target RNA is detected. • The RSV signal has a Ct within the valid range and endpoint above the minimum setting. • SPC: NA; SPC is ignored because RSV target amplification occurred. • Probe Check: PASS; all probe check results pass.
SARS-CoV-2 NEGATIVE; **Flu A NEGATIVE;** **Flu B NEGATIVE;** **RSV NEGATIVE**	SARS-CoV-2 target RNA is not detected; Flu A target RNA is not detected; Flu B target RNA is not detected; RSV target RNA is not detected. • SARS-CoV-2, Flu A, Flu B and RSV target RNAs are not detected. • SPC - PASS; SPC has a Ct within the valid range and endpoint above the minimum setting. • Probe Check - PASS; all probe check results pass.
INVALID	SPC does not meet acceptance criteria and all targets are not detected. Test should be repeated. • SPC: FAIL; SPC and SARS-CoV-2, Flu A, Flu B and RSV signals do not have a Ct within valid range and endpoint is below minimum setting. • SARS-CoV-2 amplification fails specification • Probe Check - PASS; all probe check results pass
ERROR	Presence or absence of SARS-CoV-2, Flu A, Flu B and RSV RNA cannot be determined. Test should be repeated. • SARS-CoV-2: NO RESULT • Flu A: NO RESULT • Flu B: NO RESULT • RSV: NO RESULT • SPC: NO RESULT • Probe Check: FAIL[1]; all or one of the probe checks results fail [1] If the probe check passes, the error is caused by the maximum pressure limit exceeding the acceptable range, no sample added, or by a system component failure.
NO RESULT	Presence or absence of SARS-CoV-2, Flu A, Flu B and RSV nucleic acids cannot be determined. Repeat test according to the Procedure. A **NO RESULT** indicates that insufficient data were collected. For example, the operator stopped a test that was in progress. • SARS-CoV-2: NO RESULT • Flu A: NO RESULT • Flu B: NO RESULT • RSV: NO RESULT • SPC: NO RESULT • Probe Check: NA

B. If only one viral target is positive but coinfection with multiple targets is suspected, the sample should be re-tested with another FDA-cleared, approved, or authorized test, if coinfection changes clinical management.

IX. REPORTING RESULTS

A. Refer to *Appendix AP64- Soft Instructions for Cepheid GeneXpert®*

1. **SARS-CoV-2 POSITIVE; Flu A NEGATIVE; Flu B NEGATIVE; RSV NEGATIVE** report as:
   SARS-CoV-2- DETECTED
   FLUA – Not Detected
   FLUB - Not Detected
   RSV – Not Detected

2. **SARS-CoV-2 NEGATIVE; Flu A POSITIVE; Flu B NEGATIVE; RSV NEGATIVE** report as:
   SARS-CoV-2- Not Detected
   FLUA – DETECTED
   FLUB – Not Detected
   RSV – Not Detected

3. **SARS-CoV-2 NEGATIVE; Flu A NEGATIVE; Flu B POSITIVE; RSV NEGATIVE** report as:
   SARS-CoV-2- Not Detected
   FLUA – Not Detected
   FLUB – DETECTED
   RSV – Not Detected
4. **SARS-CoV-2 NEGATIVE; Flu A NEGATIVE; Flu B NEGATIVE; RSV POSITIVE** report as
   SARS-CoV-2- Not Detected
   FLUA – Not Detected
   FLUB – Not Detected
   RSV – DETECTED

5. **INVALID (after initial repeat)** result as:
   @IR (Indeterminate) for all ordered tests: A patient's sample is considered "indeterminate" when the curve associated with that sample fails to cross the user-defined cycle threshold and the specimen's internal control fails to amplify.

6. **MIXED POSITIVE RESULTS**:
   NOTE: Repeat tests with ≥3 detected targets and notify care team of the delay.
   NOTE: The possibility of mixed infections is rare. If repeat testing matches the original result, they should be brought to the attention of the laboratory Director or Associate Director.

X. LIMITATIONS

A. Performance of the Xpert® Xpress CoV-2/Flu/RSV *plus* (FDA-cleared) test has only been established in nasopharyngeal and anterior nasal swab specimens. Use of the Xpert® Xpress CoV-2/Flu/RSV *plus* (FDA-cleared) test with other specimen types has not been assessed and performance characteristics are unknown.
B. The performance of the Xpert Xpress CoV-2/Flu/RSV plus test has not been specifically evaluated for nasopharyngeal swab and anterior nasal swab specimens from immunocompromised individuals.
C. The clinical performance has not been established in all circulating variants but is anticipated to be reflective of the prevalent variants in circulation at the time and location

of the clinical evaluation. Performance at the time of testing may vary depending on the variants circulating, including newly emerging strains of SARS-CoV-2 and their prevalence, which change over time.

D. Performance characteristics for influenza A were established when influenza A/H3 and influenza A/H1-2009 were the predominate influenza strains. When other influenza A viruses are emerging, performance characteristics may differ.
E. As with any molecular test, mutations within the target regions of the Xpert Xpress CoV-2/Flu/RSV plus test could affect primer and/or probe binding resulting in failure to detect the presence of target viruses or newly emerging variants.
F. Positive and negative predictive values are highly dependent on prevalence. The likelihood of a negative result being false is higher during peak activity when prevalence of disease is high. The likelihood of a positive result being false is higher during periods when prevalence is moderate to low.
G. This test cannot rule out diseases caused by other bacterial or viral pathogens.
H. The performance of this test was validated using the procedures provided in this package insert only. Modifications to these procedures may alter the performance of the test.
I. 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. Careful compliance with the instructions in this insert is necessary to avoid erroneous results.
J. False negative results may occur if on-panel viruses are present at levels below the analytical limit of detection.
K. Negative results do not preclude SARS-CoV-2, influenza or RSV infection and should not be used as the sole basis for treatment or other patient management decisions.
L. Use of this test is limited to personnel who are trained in the procedure. Failure to follow these instructions may result in erroneous results. Results from the Xpert Xpress CoV-2/Flu/RSV plus test should be correlated with the clinical history, epidemiological data, and other data available to the clinician evaluating the patient.
M. 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.
N. This test has been evaluated for use with human specimen material only.
O. The Xpert Xpress CoV-2/Flu/RSV plus test is a qualitative test that reports Ct values for individuals that test positive for SARS-CoV-2, influenza A, influenza B, and/or RSV. These Ct values should not be interpreted as a measure of viral levels.
P. The performance of this test has not been established for monitoring treatment of infection with any of the on-panel organisms. The performance of this test has not been established with postmortem specimens.
Q. The Xpert Xpress CoV-2/Flu/RSV plus test has not been validated for the testing of pooled specimens or the screening of specimens from asymptomatic individuals that do not have signs and symptoms of respiratory infection.
R. The performance of this test has not been established in screening of blood or blood products.
S. Anterior nasal swab and nasopharyngeal swab specimens collected in 2 mL Copan eNAT, Remel M4RT, and Remel M5 are compatible for use with the Xpert Xpress CoV-2/Flu/RSV plus test. Performance of the Xpert Xpress CoV-2/Flu/ RSV plus test with specimens collected in Copan eNAT, Remel M4RT and Remel M5 has been established in analytical studies, however, clinical performance of the assay in these media types was not established.
T. The effect of interfering substances has only been evaluated for those listed within the labeling. Interference by substances other than those described may lead to erroneous results.
U. FluMist was shown to interfere with detection of low levels of SARS-CoV-2 and RSV B at concentrations >6.7x10-6 % (v/v) and was shown to interfere with detection of low levels of RSV A at concentrations >6.7x10-7 % (v/v).

V. Recent patient exposure to FluMist® or other live attenuated influenza vaccines may cause inaccurate positive influenza results.
W. Human peripheral blood mononuclear cells (PBMC) at concentrations > 2.5 x 105 cells/mL were shown to interfere with detection of low levels of influenza B.
X. Snuff at > 0.25% (w/v) was shown to interfere with the detection of low levels of influenza A and at > 0.1% (w/v) with the detection of low levels of influenza B.
Y. Zicam at 15% (w/v) was shown to interfere with the detection of low levels of influenza A, influenza B and RSV A.
Z. Results from analytical studies with contrived co-infected samples showed potential for competitive interference of influenza B or RSV A at low concentrations (~3x LoD) when influenza A concentration is >1.7e5 RNA copies/mL or 1.7e6 RNA copies/mL, respectively. In addition, there is potential for competitive interference of influenza B at low concentration (~3x LoD) when SARS-CoV-2 concentration is >1e5 RNA copies/mL.
AA. Cross-reactivity with respiratory tract organisms other than those described in manufacturer's PI may lead to erroneous results.
BB. As the Xpert Xpress CoV-2/Flu/RSV plus test does not differentiate between the N, RdRP and E gene targets, the presence of other coronaviruses in the B lineage, Betacoronavirus genus, including SARS-CoV and bat coronaviruses may cause a false positive result. None of these other coronaviruses are known to currently circulate in the human population.
CC. The RSV A primers and probes have a high degree of identity to Pangolin RSV A sequences and therefore may crossreact with Pangolin RSV A if the organism is circulating in a human population and present in a sample tested with the Xpert Xpress CoV-2/Flu/RSV plus test. None of these Pangolin RSV A strains are known to currently circulate in the human population.
DD. This test is not intended to differentiate RSV subgroups (i.e., A or B), influenza A subtypes (i.e., H1N1, H3N2) or influenza B lineages (i.e., Yamagata, Victoria). If differentiation of specific RSV or influenza subtypes and strains is needed, additional testing, in consultation with state or local public health departments, is required.
EE. In some samples with very high SARS-CoV-2 viral concentrations, analysis settings intended to reduce the risk of false positive results caused by non-specific or irregular fluorescence detection may trigger an INVALID test result.

XI. TECHNICAL SUPPORT

A. Contact Cepheid Technical Support At 888-838-3222 techsupport@cepheid.com
    1. Before contacting, collect the following information:
        a. Product name
        b. Lot number
        c. Serial number on instrument
        d. Error messages (if any)
        e. Software version

XII. REFERENCES

A. Cepheid Xpert Xpress SARS-CoV-2/Flu/RSV *plus* Package insert. Ref. Xpert® Xpress CoV-2/Flu/RSV plus 302-8057, Rev. C 2025-05