**PROCEDURE: Xpert® Xpress SARS-CoV-2/Flu/RSV**

1. **PRINCIPLE**

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

The assay is performed on GeneXpert Dx System, which automates and integrates sample purification, nucleic acid amplification, and detection of the target sequence 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 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.

1. **AVAILABILITY**

24/7, at all affiliate hospitals

1. **TEST CODE**

COVFR

1. **SPECIMEN**
	1. Nasopharyngeal (flocked) swab or nasal swab specimen collected in Viral/Universal Transport Medium.
	2. Minimum of 300 ul for testing.
	3. Stored at 2-8°C once received in lab. Stable for up to 7 days when stored at 2-8°C.
	4. Specimen integrity good for 24 hours, when stored at 9-30°C.
2. **MATERIALS AND EQUIPMENT**
3. Materials:
	1. Xpress SARS-CoV-2/Flu/RSV
		1. Xpert**®** Xpress SARS-CoV-2/Flu/RSV assay cartridges – 10 specimens
		2. Disposable 300 µL Transfer Pipettes – 1 bag of 12 per kit
		3. CD – 1 per kit
			* Assay definition files (ADF)
			* Instructions to import ADF into GeneXpert software
			* Package Insert
4. Materials available but not provided:
	1. Specimen collection kit: UTM vial / flocked swab.
	2. External Controls
		1. Positive (Flu/RSV/SARS-CoV-2) – Zeptometrix NATFRC-6C
		2. Negative (Coxsackievirus CVA9) – Zeptometrix NATCV9-6C
	3. Materials to properly clean the hood at the beginning of each shift, between testing or any time it is needed.
		1. 10% bleach
		2. DI H2O
		3. 70% Ethanol
5. Equipment:
	1. GeneXpert Dx System (software version 4.7b or higher) or GeneXpert Infinity Systems (-80 or -40s systems) (software version 6.4b or higher)
	2. Barcode scanner
	3. Printer
6. **STORAGE AND HANDLING**
7. Store the Xpert® Xpress SARS-CoV-2/Flu/RSV cartridges at 2-28º C.
8. Do not use UTM collection kits or cartridges passed the expiration date.
9. Do not open a cartridge lid except when adding sample.
10. Do not use a cartridge that has been shaken, dropped or damaged.
11. Do not use cartridges that appear wet or if the lid’s seal appears broken.
12. Do not reuse spent cartridges.
13. Start the test within 30 minutes of adding the sample to the cartridge.
14. **QUALITY CONTROL**
15. Maintenance
	* 1. Cleaning and maintenance of the instrument will be performed in accordance with the vendors Operator’s Manual. For further information, refer to the Infinity System’s Operator’s Manual.
16. Each test includes two internal controls to validate the assay:
	* 1. Sample processing control (SPC) and probe check.
17. 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 programmedto 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.
18. External quality control specimens are run on new shipments 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 in order for the lot and instrument to be used for patient samples.
19. Environmental “wipe” testing is performed monthly on the molecular hood in the Microbiology Lab. Any positive result will be brought up to a tech specialist. 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.
20. Refer to IQCP for complete Quality Control procedure.
21. Positivity Rate is monitored monthly.
22. **TEST PROCEDURE**

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

1. 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.
2. Preparing the Cartridge– *Refer to Figure Below*
	1. Remove the cartridge from the package.
	2. Mix the specimen by INVERTING the UTM tube five times.

**NOTE**: DO NOT VORTEX

* 1. Open the cartridge lid.
	2. 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.
	3. Close the cartridge lid.



1. Starting the Test- Select **Xpert Xpress SARS-CoV-2\_Flu\_RSV** & Refer to *APPENDIX AP81 - GeneXpert Instrument Navigation*
2. 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 Manual* or the *GeneXpert* *Infinity System Operator Manual*.
3. **Reasons to Repeat the Assay**
	1. **ERROR (ONLY loading error) OR INVALID.**
	2. 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.
	3. 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.
	4. 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.
	5. If an External Control fails to perform as expected, repeat external control test and/or contact Cepheid for assistance.
4. **INTERPRETATION**
5. 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 test provides test results based on the detection of respective gene targets according to the algorithms. The format of the test results presented will vary depending on the user's choice to run either an Xpert Xpress\_SARS-CoV-2\_Flu\_RSV, Xpert Xpress\_SARS-CoV-2\_Flu or Xpert Xpress\_SARS-CoV-2 test.
	1. Table 1 shows the possible result outcomes when the Xpert Xpress SARS-CoV-2\_Flu\_RSV test mode is selected.

**Table 1. Xpert Xpress SARS-CoV-2\_Flu\_RSV Possible Results and Interpretation**

|  |  |
| --- | --- |
| **Result** | **Interpretation** |
| **SARS-CoV-2 POSITIVE** | The SARS-CoV-2 target nucleic acids are detected.• The SARS-CoV-2 signal has a Ct within the valid range andendpoint above the minimum setting.• SPC: NA (not applicable); SPC is ignored because SARS-CoV-2target amplification occurred.• Probe Check: PASS; all probe check results pass. |
| **Flu A POSITIVE** | The Flu A signal for either the Flu A1 nucleic acid target or the Flu A2 nucleic acid target or signals for both nucleic acid targets has a Ct within the valid range and endpoint above the threshold setting.• SPC - NA; SPC is ignored because the Flu A target amplificationoccurred.• Probe Check - PASS; all probe check results pass. |
| **Flu B POSITIVE** | 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 amplificationoccurred.• Probe Check: PASS; all probe check results pass |
| **RSV POSITIVE** | • The RSV signal has a Ct within the valid range and endpoint above the minimum setting.• SPC: NA; SPC is ignored because RSV target amplificationoccurred.• 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 notdetected.• SPC - PASS; SPC has a Ct within the valid range and endpointabove the minimum setting.• Probe Check - PASS; all probe check results pass. |
| **INVALID** | SPC does not meet acceptance criteria and all targets are not detected. Repeat test according to the Procedure.• SPC: FAIL; SPC and SARS-CoV-2, Flu A, Flu B and RSV signalsdo not have a Ct within valid range and endpoint is belowminimum setting.• Probe Check - PASS; all probe check results pass |
| **ERROR** | Presence or absence of SARS-CoV-2, Flu A, Flu B and RSV nucleic acids cannot be determined. Repeat test according to the Procedure.• SARS-CoV-2: NO RESULT• Flu A: NO RESULT• Flu B: NO RESULT• RSV: NO RESULT• SPC: NO RESULT• Probe Check: FAIL1; all or one of the probe check results fail1 If the probe check passes, the error is caused by the maximumpressure limit exceeding the acceptable range, no sample added, orby 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 |

1. 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 would change clinical management.
	1. Table 2 shows the possible result outcomes when the Xpert Xpress\_SARS-CoV-2\_Flu test mode is selected.

**Table 2. Xpert Xpress\_SARS-CoV-2\_Flu Possible Results and Interpretation**

|  |  |
| --- | --- |
| **Result**  | **Interpretation** |
| **SARS-CoV-2 POSITIVE** | The SARS-CoV-2 target nucleic acids are detected.• The SARS-CoV-2 signal has a Ct within the valid range and endpointabove the minimum setting.• SPC: NA (not applicable); SPC is ignored because SARS-CoV-2 targetamplification occurred.• Probe Check: PASS; all probe check results pass. |
| **Flu A POSITIVE** | The Flu A signal for either the Flu A1 nucleic acid target or the Flu A2nucleic acid target or signals for both nucleic acid targets has a Ctwithin the valid range and endpoint above the threshold setting.• SPC – NA; SPC is ignored because the Flu A target amplificationoccurred.• Probe Check – PASS; all probe check results pass. |
| **Flu B POSITIVE** | The Flu B signal has a Ct within the valid range and endpoint abovethe minimum setting.• SPC: NA; SPC is ignored because Flu B target amplification occurred.• Probe Check: PASS; all probe check results pass. |
| **SARS-CoV-2 NEGATIVE;** **Flu A NEGATIVE;** **Flu B NEGATIVE** | SARS-CoV-2 target RNA is not detected; Flu A target RNA is notdetected; Flu B target RNA is not detected.• SARS-CoV-2, Flu A, and Flu B target RNAs are not detected.• SPC – PASS; SPC has a Ct within the valid range and endpoint abovethe minimum setting.• Probe Check – PASS; all probe check results pass. |
| **INVALID** | SPC does not meet acceptance criteria and all targets are not detected.Repeat test according to the Procedure.• SPC: FAIL; SPC and SARS-CoV-2, Flu A, and Flu B signals do nothave a Ct within valid range and endpoint is below minimum setting.• Probe Check - PASS; all probe check results pass |
| **ERROR** | Presence or absence of SARS-CoV-2, Flu A, and Flu B nucleic acidscannot be determined. Repeat test according to the Procedure• SARS-CoV-2: NO RESULT• Flu A: NO RESULT• Flu B: NO RESULT• SPC: NO RESULT• Probe Check: FAIL1; all or one of the probe check results fail1 If the probe check passes, the error is caused by the maximum pressurelimit exceeding the acceptable range, no sample added, or by a systemcomponent failure. |
| **NO RESULT** | Presence or absence of SARS-CoV-2, Flu A, and Flu B nucleic acidscannot 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• SPC: NO RESULT• Probe Check: NA (not applicable) |

**Table 3. Xpert Xpress\_SARS-CoV-2 Possible Results and Interpretation**

|  |  |
| --- | --- |
| **SARS-CoV-2 POSITIVE** | The SARS-CoV-2 target nucleic acids are detected.• The SARS-CoV-2 signal has a Ct within the valid range and endpointabove the minimum setting.• SPC: NA (not applicable); SPC is ignored because SARS-CoV-2 targetamplification occurred.• Probe Check: PASS; all probe check results pass. |
| **SARS-CoV-2 NEGATIVE** | SARS-CoV-2 target RNA is not detected.• SARS-CoV-2 target RNA is not detected.• SPC – PASS; SPC has a Ct within the valid range and endpoint abovethe minimum setting.• Probe Check – PASS; all probe check results pass |
| **INVALID** | SPC does not meet acceptance criteria and SARS-CoV-2 is not detected.Repeat test according to the Retest Procedure in Section 17.2.• SPC: FAIL; SPC and SARS-CoV-2 signals do not have a Ct within validrange and endpoint is below minimum setting.• Probe Check - PASS; all probe check results pass |
| **ERROR** | Presence or absence of SARS-CoV-2 nucleic acids cannot bedetermined. Repeat test according to the Retest Procedure inSection 17.2.• SPC: NO RESULT• Probe Check: FAIL1; all or one of the probe check results fail1 If the probe check passes, the error is caused by the maximum pressurelimit exceeding the acceptable range, no sample added, or by a systemcomponent failure |

.

1. **REPORTING RESULTS**
	1. Refer to *Critical Call Policy Procedure*. If result is called use the canned comment @CALM.
		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

* + 1. **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

* + 1. **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

* + 1. **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

* + 1. **INVALID, NO RESULT or ERROR (EXCEPT for loading errors)** 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. Specimens that incur a loading error will be repeated.

* + 1. **MIXED POSITIVE RESULTS**:

**NOTE:** Repeat tests with a mixed infection result on a different platform. Floor/ER should be notified of delay.

**NOTE:** The possibility of mixed infections is rare. If the repeat confirms the original result it must be brought to the attention of the laboratory Director or Associate laboratory Director. The Pathology Resident on call should be notified of mixed infections on 2nd or 3rd shift.

1. **LIMITATIONS**
	1. Performance of the Xpert Xpress SARS-CoV-2/Flu/RSV test has only been established in nasopharyngeal swab specimens.
	2. Use of the Xpert Xpress SARS-CoV-2/Flu/RSV test with other specimen types has not been assessed and performance characteristics are unknown.
	3. Nasal swabs (self-collected under supervision of, or collected by, a healthcare provider) and nasal wash/aspirate specimens are considered acceptable specimen types for use with the Xpert Xpress SARS-CoV-2/Flu/RSV test but performance with these specimen types has not been established.
	4. As with any molecular test, mutations within the target regions of the Xpert Xpress SARS-CoV-2/Flu/RSV test could affect primer and/or probe binding resulting in failure to detect the presence of virus or the virus being detected less predictably.
	5. This test cannot rule out diseases caused by other bacterial or viral pathogens.
	6. 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.
	7. 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.
	8. False negative results may occur if virus is present at levels below the analytical limit of detection.
	9. 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.
	10. Results from the Xpert Xpress SARS-CoV-2/Flu/RSV test should be correlated with the clinical history, epidemiological data, and other data available to the clinician evaluating the patient.
	11. Viral nucleic acid may persist *in vivo*, independent of virus viability. Detection of analyte target(s) does not imply that the corresponding virus(es) are infectious or are the causative agents for clinical symptoms.
	12. This test has been evaluated for use with human specimen material only.
	13. This test is a qualitative test and does not provide the quantitative value of detected organism present.
	14. This test has not been evaluated for monitoring treatment of infection.
	15. This test has not been evaluated for screening of blood or blood products for the presence of SARS-CoV-2, influenza or RSV.
	16. The effect of interfering substances has only been evaluated for those listed within the labeling. Interference by substances other than those described can lead to erroneous results.
	17. Results from analytical studies with contrived co-infected samples showed potential for competitive interference when SARS-CoV-2, Flu or RSV was present at 1X LoD levels.
	18. Cross-reactivity with respiratory tract organisms other than those described herein can lead to erroneous results.
	19. Recent patient exposure to FluMist® or other live attenuated influenza vaccines may cause inaccurate positive results.
	20. As the Xpert Xpress SARS-CoV-2/Flu/RSV test does not differentiate between the N2 and E gene targets, the presence of other coronaviruses in the B lineage, *Betacoronavirus* genus, including SARS-CoV-1 may cause a false positive result. None of these other coronaviruses is known to currently circulate in the human population.
	21. This test is not intended to differentiate RSV subgroups, influenza A subtypes or influenza B lineages. 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.
	22. This test has not been FDA cleared or approved.
	23. This test has been authorized by FDA under a EUA for use by authorized laboratories.
	24. This test has been authorized only for the simultaneous qualitative detection and differentiation of nucleic acids from SARS-CoV-2, influenza A, influenza B, and respiratory syncytial virus (RSV), and not for any other viruses or pathogens.
	25. This test is only authorized for the duration of the declaration that circumstances exist justifying the authorization of emergency use of *in vitro* diagnostic tests for detection and/or diagnosis of COVID-19 under Section 564(b)(1) of the Federal Food, Drug and Cosmetic Act, 21 U.S.C. § 360bbb-3(b)(1), unless the authorization is terminated or revoked sooner.
2. **TECHNICAL SUPPORT**
3. Contact Cepheid Technical Support At 888-838-3222 techsupport@cepheid.com
4. Before contacting, collect the following information:
	* + 1. Product name
			2. Lot number
			3. Serial number on instrument
			4. Error messages (if any)
			5. Software version
5. Restarting the System:
6. Make sure instrument is not processing a sample.
7. Remove all cartridges from the modules.
8. Quit GeneXpert Software (USER menu, click EXIT)
9. Turn off the computer
10. Turn off instrument
11. Wait a few minutes
12. Turn on the instrument
13. Turn on the computer
14. Start the GeneXpert software.
15. **REFERENCES**
	1. Cepheid Xpert Xpress SARS-CoV-2/Flu/RSV Package insert. Ref. VPCOV2/FLU/RSV-10. 302-4421, Rev. A September 2020.