

# Xpert<sup>®</sup> Xpress CoV-2/Flu/RSV *plus*

**REF** XP3COV2/FLU/RSV-10

Instructions for Use

**For Use Under an Emergency Use Authorization (EUA) Only**

For Use with GeneXpert<sup>®</sup> Dx or GeneXpert<sup>®</sup> Infinity Systems

**IVD**



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See Section 25, Revision History for a description of changes.

# Xpert<sup>®</sup> Xpress CoV-2/Flu/RSV *plus*

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## 1 Proprietary Name

Xpert<sup>®</sup> Xpress CoV-2/Flu/RSV *plus*

## 2 Common or Usual Name

Xpert Xpress CoV-2/Flu/RSV *plus*

## 3 Intended Use

The Xpert Xpress CoV-2/Flu/RSV *plus* test is a rapid, multiplexed real-time RT-PCR test intended for the simultaneous qualitative detection and differentiation of RNA from SARS-CoV-2, influenza A, influenza B, and/or respiratory syncytial virus (RSV) in either nasopharyngeal swab, anterior nasal swab or nasal wash/ aspirate specimens collected from individuals suspected of respiratory viral infection, consistent with COVID-19, by their healthcare provider. Clinical signs and symptoms of respiratory viral infection due to SARS-CoV-2, influenza, and RSV can be similar.

Testing of nasopharyngeal swab, anterior nasal swab, or nasal wash/aspirate specimens, run on the GeneXpert Dx and GeneXpert Infinity systems, is limited to laboratories certified under the Clinical Laboratory Improvement Amendments of 1988 (CLIA), 42 U.S.C. § 263a, that meet requirements to perform high or moderate complexity tests.

Testing of nasopharyngeal or anterior nasal swab specimens, run on the GeneXpert Xpress System (Tablet and Hub Configurations), is authorized for use at the Point of Care (POC), i.e., in patient care settings operating under a CLIA Certificate of Waiver, Certificate of Compliance, or Certificate of Accreditation.

Results are for the simultaneous detection and differentiation of SARS-CoV-2, influenza A virus, influenza B virus and RSV nucleic acids in clinical specimens and is not intended to detect influenza C virus. The SARS-CoV-2, influenza A, influenza B and RSV RNA is generally detectable in upper respiratory specimens during the acute phase of infection. Positive results are indicative of active infection, but do not rule out bacterial infection or co-infection with other pathogens not detected by the test.

Clinical correlation with patient history and other diagnostic information is necessary to determine patient infection status. The agent detected may not be the definite cause of disease. Laboratories within the United States and its territories are required to report all SARS-CoV-2 results to the appropriate public health authorities.

Negative results do not preclude SARS-CoV-2, influenza A virus, influenza B virus and/or RSV infection and should not be used as the sole basis for treatment or other patient management decisions. Negative results must be combined with clinical observations, patient history, and/or epidemiological information.

Testing with the Xpert Xpress CoV-2/Flu/RSV *plus* test is intended for use by trained operators who are proficient in performing tests using either GeneXpert Dx, GeneXpert Infinity and/or GeneXpert Xpress systems. The Xpert Xpress CoV-2/Flu/RSV *plus* test is only for use under the Food and Drug Administration's Emergency Use Authorization.

## 4 Summary and Explanation

An outbreak of respiratory illness of unknown etiology in Wuhan City, Hubei Province, China was initially reported to the World Health Organization (WHO) on December 31, 2019.<sup>1</sup> Chinese authorities identified a novel coronavirus (2019-nCoV), which has since spread globally, resulting in a pandemic of coronavirus disease 2019 (COVID-19). COVID-19 is associated with a variety of clinical outcomes, including asymptomatic infection, mild upper respiratory infection, severe lower respiratory disease including pneumonia and respiratory failure, and in some cases, death. The International Committee on Taxonomy of Viruses (ICTV) renamed the virus SARS-CoV-2.<sup>2</sup>

Influenza, or the flu, is a contagious viral infection of the respiratory tract. Transmission of influenza is primarily via aerosolized droplets (i.e., coughing or sneezing) and the peak of transmission usually occurs in the winter months. Symptoms commonly include fever, chills, headache, malaise, cough and sinus congestion. Gastrointestinal symptoms (i.e., nausea, vomiting or diarrhea) may also occur, primarily in children, but are less common. Symptoms generally appear within two days of exposure to an infected person. Pneumonia may develop as a complication due to influenza infection, causing increased morbidity and mortality in pediatric, elderly, and immunocompromised populations.<sup>3,4</sup>

Influenza viruses are classified into types A, B, and C, the former two of which cause the most human infections. Influenza A (Flu A) is the most common type of influenza virus in humans and is generally responsible for seasonal flu epidemics and potentially pandemics. Flu A viruses can also infect animals such as birds, pigs, and horses. Infections with influenza B (Flu B) virus are generally restricted to humans and less frequently cause epidemics.<sup>5</sup> Flu A viruses are further divided into subtypes on the basis of two surface proteins: hemagglutinin (H) and neuraminidase (N). Seasonal flu is normally caused by influenza A subtypes H1, H2, H3, N1 and N2.

Respiratory Syncytial Virus (RSV), a member of the *Pneumoviridae* family (formerly *Paramyxoviridae*), consisting of two strains (subgroups A and B) is also the cause of a contagious disease that affects primarily infants, the elderly, and those who are immunocompromised (e.g., patients with chronic lung disease or undergoing treatment for conditions that reduce the strength of their immune system).<sup>6</sup> The virus can cause both upper respiratory infections, such as colds, and lower respiratory infections manifesting as bronchiolitis and pneumonia.<sup>6</sup> By the age of two years, most children have already been infected by RSV and because only weak immunity develops, both children and adults can be re-infected.<sup>6</sup> RSV remains the leading cause for hospitalizations in infants worldwide.<sup>7</sup> Symptoms appear four to six days after infection and are usually self-limiting, lasting approximately one to two weeks in infants. In adults, infection lasts about 5 days and presents as symptoms consistent with a cold, such as rhinorrhea, fatigue, headache, and fever. The RSV season usually mirrors influenza as infections begin to rise during the fall and last through early spring.<sup>5,6</sup>

SARS-CoV-2, influenza, and RSV viruses can cause infections that present with very similar symptoms, making clinical differentiation between them very difficult.<sup>8</sup> Active surveillance programs in conjunction with infection prevention precautions are important components for preventing transmission of SARS-CoV-2, influenza and RSV. The use of assays providing rapid results to identify patients infected with these viruses can be an important factor for effective control, proper choice of treatment, and prevention of widespread outbreaks.

## 5 Principle of the Procedure

The Xpert Xpress CoV-2/Flu/RSV *plus* test is an automated *in vitro* diagnostic test for the simultaneous qualitative detection and differentiation of RNA from SARS-CoV-2, Flu A, Flu B, and RSV. The Xpert Xpress CoV-2/Flu/RSV *plus* test is performed on GeneXpert Instrument Systems (Dx and Infinity Systems). The primers and probes in the Xpert Xpress CoV-2/Flu/RSV *plus* test are designed to amplify and detect unique sequences in the following: nucleocapsid (N) and envelope (E) and RNA-dependent RNA polymerase (RdRP) genes of the SARS-CoV-2 virus genome, influenza A matrix (M), influenza A basic polymerase (PB2), influenza A acidic protein (PA), influenza B matrix (M), influenza B non-structural protein (NS), and the RSV A and RSV B nucleocapsid.

The GeneXpert Instrument Systems automate and integrate sample preparation, 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 systems consist of an instrument, computer, and preloaded software for running tests and viewing the results. The systems require the use of single-use disposable cartridges that hold the RT-PCR reagents and host the RT-PCR process. Because the cartridges are self-contained, cross-contamination between samples is minimized. For a full description of the systems, see the *GeneXpert Dx System Operator Manual* or the *GeneXpert Infinity System Operator Manual*.

The Xpert Xpress CoV-2/Flu/RSV *plus* test includes reagents for the detection of SARS-CoV-2, Flu A, Flu B and RSV viral RNA in either nasopharyngeal swab, anterior nasal swab, or nasal wash/aspirate specimens. A Sample Processing Control (SPC) and a Probe Check Control (PCC) are also included in the cartridge utilized by the GeneXpert instrument. The SPC is present to control for adequate processing of the sample and to monitor for the presence of potential inhibitor(s) in the RT-PCR reaction. The SPC also ensures that the RT-PCR reaction conditions (temperature and time) are appropriate for

the amplification reaction and that the RT-PCR reagents are functional. The PCC verifies reagent rehydration, PCR tube filling, and confirms that all reaction components are present in the cartridge including monitoring for probe integrity and dye stability.

The specimen is collected and placed into a transport tube containing 3 mL of viral transport medium, 3mL of saline or 2 mL of eNAT™. The specimen is briefly mixed by rapidly inverting the collection tube 5 times. Using the supplied transfer pipette, the sample is transferred to the sample chamber of the Xpert Xpress CoV-2/Flu/RSV *plus* cartridge. The GeneXpert cartridge is loaded onto the GeneXpert Instrument System platform, which performs hands-off, automated sample processing, and real-time RT-PCR for detection of viral RNA.

## 6 Reagents and Instruments

### 6.1 Materials Provided

The Xpert Xpress CoV-2/Flu/RSV *plus* kit contains sufficient reagents to process 10 specimens or quality control samples. The kit contains the following:

#### **Xpert Xpress CoV-2/Flu/RSV *plus* Cartridges with 10 Integrated Reaction Tubes**

- |   |                         |
|---|-------------------------|
| • Bead 1, Bead 2, and Bead 3 (freeze-dried) | 1 of each per cartridge |
| • Lysis Reagent                             | 1.0 mL per cartridge    |
| • Binding Reagent                           | 1.0 mL per cartridge    |
| • Elution Reagent                           | 3.0 mL per cartridge    |
| • Wash Reagent                              | 0.4 mL per cartridge    |

**Disposable Transfer Pipettes** **10-12 per kit**

**Flyer** **1 per kit**

- Instructions to locate (and import) the ADF and EUA documentation such as the Product Insert on [www.cepheid.com](http://www.cepheid.com).

**Quick Reference Instructions** **2 per kit**

(For use with the GeneXpert Xpress System only)

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**Note** Safety Data Sheets (SDS) are available at [www.cepheid.com](http://www.cepheid.com) or [www.cepheidinternational.com](http://www.cepheidinternational.com) under the **SUPPORT** tab.

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**Note** The bovine serum albumin (BSA) in the beads within this product was produced and manufactured exclusively from bovine plasma sourced in the United States. No ruminant protein or other animal protein was fed to the animals; the animals passed ante- and post-mortem testing. During processing, there was no mixing of the material with other animal materials.

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## 7 Storage and Handling

- Store the Xpert Xpress CoV-2/Flu/RSV *plus* cartridges at 2–28 °C.
- Do not open a cartridge lid until you are ready to perform testing.
- Do not use a cartridge that is wet or has leaked.

## 8 Materials Required but Not Provided

- GeneXpert Dx or GeneXpert Infinity systems (catalog number varies by configuration): GeneXpert instrument, computer, barcode scanner, operator manual.

For GeneXpert Dx System: GeneXpert Dx software version 4.7b or higher

For GeneXpert Infinity-80 and Infinity-48s systems: Xpertise software version 6.4b or higher

## 9 Materials Available but Not Provided

External controls in the form of inactivated virus(es) are available from ZeptoMetrix (Buffalo, NY).

- External Positive Control: Catalog #NATFRC-6C (NATtrol Flu/RSV/SARS-CoV-2)
- External Negative Control: Catalog #NATCV9-6C (Coxsackievirus A9)

eNAT Molecular Collection and Preservation Medium from Copan Italy S.p.A. (Brescia, IT):

- eNAT Molecular Collection and Preservation Medium, Copan Catalog #6U073S01
- eNAT Molecular Collection and Preservation Medium, Copan Catalog #6U074S01

## 10 Warnings and Precautions

### 10.1 General

- For *in vitro* diagnostic use.
- For emergency use only.
- For prescription use only.
- Positive results are indicative of presence of Flu A, Flu B, RSV, and/or SARS-CoV-2 RNA.
- Laboratories within the United States and its territories are required to report all SARS-CoV-2 results to the appropriate public health authorities.
- Treat all biological specimens, including used cartridges, as if capable of transmitting infectious agents. Because it is often impossible to know which might be infectious, all biological specimens should be handled using standard precautions. Guidelines for specimen handling are available from the U.S. Centers for Disease Control and Prevention<sup>9</sup> and the Clinical and Laboratory Standards Institute.<sup>10</sup>
- Follow safety procedures set by your institution for working with chemicals and handling biological specimens.
- Biological specimens, transfer devices, and used cartridges should be considered capable of transmitting infectious agents requiring standard precautions. Follow your institution's environmental waste procedures for proper disposal of used cartridges and unused reagents. These materials may exhibit characteristics of chemical hazardous waste requiring specific disposal. If country or regional regulations do not provide clear direction on proper disposal, biological specimens and used cartridges should be disposed per WHO [World Health Organization] medical waste handling and disposal guidelines.
- This product has not been FDA cleared or approved, but has been authorized for emergency use by FDA under an EUA for use by authorized laboratories.
- This product has been authorized only for the 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.
- The emergency use of this product is only authorized for the duration of the declaration that circumstances exist justifying the authorization of emergency use of *in vitro* diagnostics 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 declaration is terminated or authorization is revoked sooner.
- Testing of nasopharyngeal swab, anterior nasal swab, or nasal wash/aspirate specimens using the Xpert Xpress CoV-2/Flu/RSV *plus* test, run on the GeneXpert Dx and GeneXpert Infinity systems, is limited to laboratories certified under the Clinical Laboratory Improvement Amendments of 1988 (CLIA), 42 U.S.C. § 263a, that meet requirements to perform high or moderate complexity tests.
- Testing of nasopharyngeal or anterior nasal swab specimens using the Xpert Xpress CoV-2/Flu/RSV *plus* test, run on the GeneXpert Xpress System (Tablet and Hub Configurations), is authorized for use at the Point of Care (POC), i.e., in patient care settings operating under a CLIA Certificate of Waiver, Certificate of Compliance, or Certificate of Accreditation.

## 10.2 Specimens

- Maintain proper storage conditions during specimen transport to ensure the integrity of the specimen (see Section 12, Specimen Collection, Transport, and Storage). Specimen stability under shipping conditions other than those recommended has not been evaluated.

## 10.3 Assay/Reagent

- Do not open the Xpert Xpress CoV-2/Flu/RSV *plus* cartridge lid except when adding specimen.
- Do not use a cartridge that has been dropped after removing it from the packaging.
- Do not shake the cartridge. Shaking or dropping the cartridge after opening the cartridge lid may yield non-determinate results.
- Do not place the sample ID label on the cartridge lid or on the barcode label on the cartridge.
- Do not use a cartridge with a damaged barcode label.
- Do not use a cartridge that has a damaged reaction tube.
- Do not use reagents beyond their expiry date.
- Each single-use Xpert Xpress CoV-2/Flu/RSV *plus* cartridge is used to process one test. Do not reuse processed cartridges.
- Each single-use disposable pipette is used to transfer one specimen. Do not reuse disposable pipettes.
- Do not use a cartridge if it appears wet or if the lid seal appears to have been broken.
- Wear clean lab coats and gloves. Change gloves between the handling of each specimen.
- In the event of a spill of specimens or controls, wear gloves and absorb the spill with paper towels. Then, thoroughly clean the contaminated area with a 10% freshly prepared household chlorine bleach. Allow a minimum of two minutes of contact time. Ensure the work area is dry before using 70% denatured ethanol to remove bleach residue. Allow surface to dry completely before proceeding. Or, follow your institution's standard procedures for a contamination or spill event. For equipment, follow the manufacturer's recommendations for decontamination of equipment.

## 11 Chemical Hazards<sup>11, 12</sup>

- **Signal Word: Warning**
- **UN GHS Hazard Statements**
  - Harmful if swallowed
  - May be harmful in contact with skin
  - Causes eye irritation
- **UN GHS Precautionary Statements**
  - **Prevention**
    - Wash hands thoroughly after handling.
  - **Response**
    - Call a POISON CENTER or doctor/physician if you feel unwell.
    - If skin irritation occurs: Get medical advice/attention.
    - IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing.
    - If eye irritation persists: Get medical advice/attention.

## 12 Specimen Collection, Transport, and Storage

Proper specimen collection, storage, and transport are critical to the performance of this test. Inadequate specimen collection, improper specimen handling and/or transport may yield a false result. See Section 12.1 for nasopharyngeal swab collection procedure, Section 12.2 for nasal swab collection procedure, and Section 12.3 for nasal wash/aspirate procedure. Nasopharyngeal and nasal swab specimens can be stored at room temperature (15–30 °C) for up to 48 hours in viral transport medium, saline, or eNAT until testing is performed on the GeneXpert Instrument Systems. Alternatively, nasopharyngeal and nasal swab specimens can be stored refrigerated (2–8 °C) up to seven days in viral transport medium or saline, and up to six days in eNAT until testing is performed on the GeneXpert Instrument Systems.

Nasal wash/aspirate specimens can be stored at room temperature (15–30 °C) for up to 48 hours in viral transport medium or saline and refrigerated (2–8 °C) for up to seven days in viral transport medium or saline.

Samples collected into saline and eNAT should not be frozen.

Refer to the CDC Interim Guidelines for Collecting, Handling, and Testing Clinical Specimens from Persons Under Investigation (PUIs) for Coronavirus Disease 2019 (COVID-19)

<https://www.cdc.gov/coronavirus/2019-nCoV/lab/guidelines-clinical-specimens.html>

### 12.1 Nasopharyngeal Swab Collection Procedure

Insert the swab into either nostril, passing it into the posterior nasopharynx (see Figure 1). Rotate swab by firmly brushing against the nasopharynx several times. Remove and place the swab into the tube containing 3 mL of viral transport medium, 3 mL of saline, or 2 mL of eNAT. Break swab at the indicated break line and cap the specimen collection tube tightly.

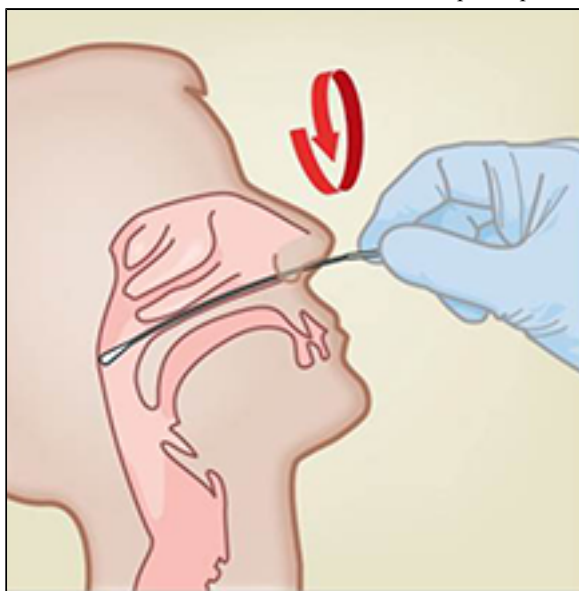
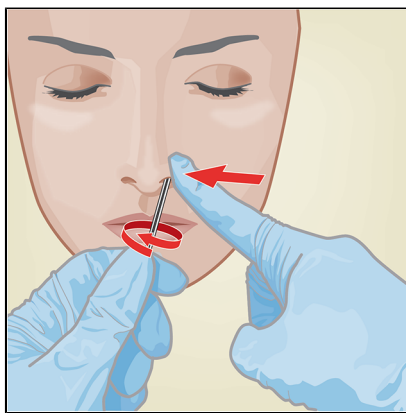


Figure 1. Nasopharyngeal Swab Collection

### 12.2 Nasal Swab Collection Procedure

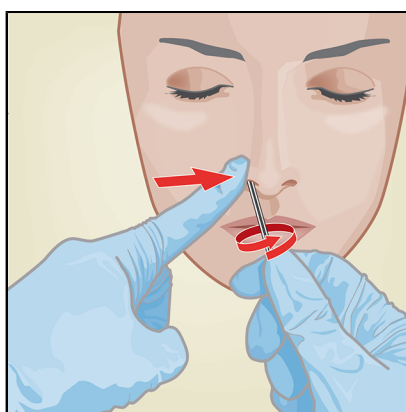
1. Insert a nasal swab 1 to 1.5 cm into a nostril. Rotate the swab against the inside of the nostril for 3 seconds while applying pressure with a finger to the outside of the nostril (see Figure 2).





**Figure 2. Nasal Swab Collection for First Nostril**

2. Repeat on the other nostril with the same swab, using external pressure on the outside of the other nostril (see Figure 3). To avoid specimen contamination, do not touch the swab tip to anything other than the inside of the nostril.



**Figure 3. Nasal Swab Collection for Second Nostril**

3. Remove and place the swab into the tube containing 3 mL of viral transport medium, 3 mL of saline, or 2 mL of eNAT. Break swab at the indicated break line and cap the specimen collection tube tightly.

## 12.3 Nasal Wash/Aspirate Procedure

Using a clean transfer pipette, transfer 600 µL of the sample into the tube containing 3 mL of viral transport medium or saline and then cap the tube.

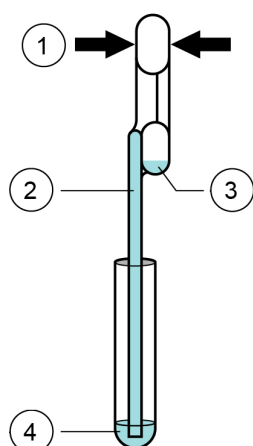
# 13 Procedure

## 13.1 Preparing the Cartridge

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

1. Remove a cartridge from the package.
2. Check the specimen transport tube is closed.
3. Mix specimen by rapidly inverting the specimen transport tube 5 times. Open the cap on the specimen transport tube.
4. Open the cartridge lid.
5. Remove the transfer pipette from the wrapper.

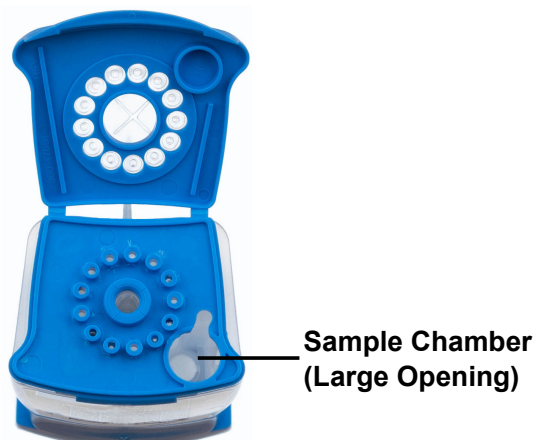
6. Squeeze the top bulb of the transfer pipette **completely until the top bulb is fully flat**. While continuing to hold the bulb fully flat, place the pipette tip in the specimen transport tube (see Figure 4).



Number	Description
1	Squeeze here
2	Pipette
3	Overflow Reservoir Bulb
4	Sample

**Figure 4. Transfer Pipette**

7. Keeping the pipette below the surface of the liquid, release the top bulb of the pipette slowly to fill the pipette with sample before removing from the tube. It is okay if liquid goes into the overflow reservoir (see Figure 4). Check that the pipette does not contain bubbles.
8. To transfer the sample to the cartridge, squeeze the top bulb of the pipette completely again until it is fully flat to empty the contents of the pipette (300 µL) into the large opening (Sample Chamber) of the cartridge shown in Figure 5. Some liquid may remain in the overflow reservoir. Dispose of the used pipette.



**Figure 5. Xpert Xpress CoV-2/Flu/RSV *plus* Cartridge (Top View)**

**Note** Take care to dispense the entire volume of liquid into the Sample Chamber. False negative results may occur if insufficient sample volume is added to the cartridge.

9. Close the cartridge lid.

## 13.2 External Controls

External controls described in Section 9 are available but not provided and may be used in accordance with local, state, and federal accrediting organizations, as applicable.

To run a control using the Xpert Xpress CoV-2/Flu/RSV *plus* test, perform the following steps:

1. Mix control by rapidly inverting the external control tube 5 times. Open cap on external control tube.
2. Open the cartridge lid.

3. Using a clean transfer pipette, transfer one draw of the external control sample (300 µL) into the large opening (Sample Chamber) in the cartridge shown in Figure 5.
4. Close cartridge lid.

### 13.3 Starting the Test

**Note** Before you start the test, make sure that the system contains modules with GeneXpert Dx software version 4.7b or higher or Infinity Xpertise software 6.4b or higher, and that the Xpert Xpress CoV-2/Flu/RSV *plus* Assay Definition File (ADF) is imported into the software.

This section lists the default steps to operate the GeneXpert Instrument System. For detailed instructions, see the *GeneXpert Dx System Operator Manual* or the *GeneXpert Infinity System Operator Manual*, depending on the model that is being used.

**Note** The steps you follow may be different if the system administrator has changed the default workflow of the system.

1. Turn on the GeneXpert Instrument System:

- **GeneXpert Dx:**

If using the GeneXpert Dx instrument, first turn on the instrument and then turn on the computer. Log into the Windows operating system. The GeneXpert software may launch automatically or may require double-clicking on the GeneXpert Dx shortcut icon on the Windows® desktop.

or

- **GeneXpert Infinity System:**

If using the GeneXpert Infinity instrument, power up the instrument by turning the power switch clockwise to the **ON** position. On the Windows desktop, double-click the Xpertise Software shortcut icon to launch the software.

2. Log on to the System software. The login screen appears. Type your user name and password.
3. In the GeneXpert System window, click **Create Test** (GeneXpert Dx) or **Orders** followed by **Order Test** (Infinity).
4. Scan or type in the Patient ID (optional). If typing the Patient ID, make sure the Patient ID is typed correctly. The Patient ID is shown on the left side of the View Results window and is associated with the test result.
5. Scan or type in the Sample ID. If typing the Sample ID, make sure the Sample ID is typed correctly. The Sample ID is shown on the left side of the View Results window and is associated with the test result.
6. Scan the barcode on the Xpert Xpress CoV-2/Flu/RSV *plus* cartridge. Using the barcode information, the software automatically fills the boxes for the following fields: Reagent Lot ID, Cartridge SN, Expiration Date, and Selected Assay.

**Note** If the barcode on the Xpert Xpress CoV-2/Flu/RSV *plus* cartridge does not scan, then repeat the test with a new cartridge.

7. Click **Start Test** (GeneXpert Dx) or **Submit** (Infinity) if Auto-Submit is not enabled. In the dialog box that appears, type your password, if required.

**For the GeneXpert Dx Instrument:**

- a. Locate the module with the blinking green light, open the instrument module door and load the cartridge.
- b. Close the door. The test starts and the green light stops blinking. When the test is finished, the light turns off and the door will unlock. Remove the cartridge.
- c. Dispose of used cartridges in the appropriate sample waste containers according to your institution's standard practices.

or

**For the GeneXpert Infinity System:**

- a. After clicking **Submit**, you will be asked to place the cartridge on the conveyor belt. After placing the cartridge, click OK to continue. The cartridge will be automatically loaded, the test will run, and the used cartridge will be placed onto the waste shelf for disposal.
- b. When all samples are loaded, click on the **End Order Test** icon.

**Note** Do not turn off or unplug the instruments while a test is in progress. Turning off or unplugging the GeneXpert instrument or computer will stop the test.

## 14 Viewing and Printing Results

For detailed instructions on how to view and print the results, see the *GeneXpert Dx System Operator Manual* or the *GeneXpert Infinity System Operator Manual*.

## 15 Quality Control

### 15.1 Internal Controls

Each cartridge includes a Sample Processing Control (SPC) and Probe Check Control (PCC).

**Sample Processing Control (SPC)** – Ensures that the sample was processed correctly. The SPC verifies that sample processing is adequate. Additionally, this control detects sample-associated inhibition of the real-time PCR assay, ensures that the PCR reaction conditions (temperature and time) are appropriate for the amplification reaction, and that the PCR reagents are functional. The SPC should be positive in a negative sample and can be negative or positive in a positive sample. The SPC passes if it meets the validated acceptance criteria.

**Probe Check Control (PCC)** – Before the start of the PCR reaction, the GeneXpert System measures the fluorescence signal from the probes to monitor bead rehydration, reaction tube filling, probe integrity, and dye stability. The PCC passes if it meets the validated acceptance criteria.

### 15.2 External Controls

External controls should be used in accordance with local, state, and federal accrediting organizations as applicable.

Cepheid recommends that all laboratories perform external QC with each new lot and shipment of reagents, at a minimum, while running the Xpert Xpress CoV-2/Flu/RSV *plus* test under Emergency Use Authorization (EUA).

If the expected results for the external control materials are not obtained, repeat the external controls, prior to releasing patient results. If the expected results for the external control material are not obtained upon repeat, contact Cepheid Technical Support.

## 16 Interpretation of Results

The results are interpreted automatically by the GeneXpert System and are clearly shown in the **View Results** window. The Xpert Xpress CoV-2/Flu/RSV *plus* 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 Xpress SARS-CoV-2\_Flu\_RSV *plus*, Xpress SARS-CoV-2\_Flu *plus* or Xpress SARS-CoV-2 *plus* test.

Table 1 shows the possible result outcomes when the Xpress SARS-CoV-2\_Flu\_RSV *plus* test mode is selected.

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

Result	Interpretation
<b>SARS-CoV-2 POSITIVE</b>	<p>The SARS-CoV-2 target RNA is detected.</p> <ul style="list-style-type: none"> <li>The SARS-CoV-2 signal has a Ct within the valid range and endpoint above the minimum setting</li> <li>SPC: NA (not applicable); SPC is ignored because SARS-CoV-2 target amplification occurred</li> <li>Probe Check: PASS; all probe check results pass</li> </ul>
<b>Flu A POSITIVE</b>	<p>The Flu A target RNA is detected.</p> <ul style="list-style-type: none"> <li>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</li> <li>SPC: NA; SPC is ignored because the Flu A target amplification occurred</li> <li>Probe Check: PASS; all probe check results pass</li> </ul>
<b>Flu B POSITIVE</b>	<p>The Flu B target RNA is detected.</p> <ul style="list-style-type: none"> <li>The Flu B signal has a Ct within the valid range and endpoint above the minimum setting</li> <li>SPC: NA; SPC is ignored because Flu B target amplification occurred</li> <li>Probe Check: PASS; all probe check results pass</li> </ul>
<b>RSV POSITIVE</b>	<p>The RSV target RNA is detected.</p> <ul style="list-style-type: none"> <li>The RSV signal has a Ct within the valid range and endpoint above the minimum setting</li> <li>SPC: NA; SPC is ignored because RSV target amplification occurred</li> <li>Probe Check: PASS; all probe check results pass</li> </ul>
<b>SARS-CoV-2 NEGATIVE; Flu A NEGATIVE; Flu B NEGATIVE; RSV NEGATIVE</b>	<p>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.</p> <ul style="list-style-type: none"> <li>SARS-CoV-2, Flu A, Flu B and RSV target RNAs are not detected</li> <li>SPC: PASS; SPC has a Ct within the valid range and endpoint above the minimum setting</li> <li>Probe Check: PASS; all probe check results pass</li> </ul>
<b>INVALID</b>	<p>SPC does not meet acceptance criteria and all targets are not detected. Repeat test according to the Retest Procedure in Section 17.2 of the IFU.</p> <ul style="list-style-type: none"> <li>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</li> <li>Probe Check: PASS; all probe check results pass</li> </ul>
<b>ERROR</b>	<p>Presence or absence of SARS-CoV-2, Flu A, Flu B and RSV RNA cannot be determined. Repeat test according to the Retest Procedure in Section 17.2 of the IFU.</p> <ul style="list-style-type: none"> <li>SARS-CoV-2: NO RESULT</li> <li>Flu A: NO RESULT</li> <li>Flu B: NO RESULT</li> <li>RSV: NO RESULT</li> <li>SPC: NO RESULT</li> <li>Probe Check: FAIL<sup>1</sup>; all or one of the probe check results fail</li> </ul> <p><sup>1</sup>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.</p>

Result	Interpretation
<b>NO RESULT</b>	<p>Presence or absence of SARS-CoV-2, Flu A, Flu B and RSV RNA cannot be determined. Repeat test according to the Retest Procedure in Section 17.2 of the IFU. A <b>NO RESULT</b> indicates that insufficient data were collected. For example, the operator stopped a test that was in progress.</p> <ul style="list-style-type: none"> <li>• SARS-CoV-2: NO RESULT</li> <li>• Flu A: NO RESULT</li> <li>• Flu B: NO RESULT</li> <li>• RSV: NO RESULT</li> <li>• SPC: NO RESULT</li> <li>• Probe Check: NA</li> </ul>
If the SPC is negative and the results for any of the targets are positive, the results for all targets are considered valid.	

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.

Table 2 shows the possible result outcomes when the Xpress SARS-CoV-2\_Flu plus test mode is selected.

**Table 2. Xpress SARS-CoV-2\_Flu plus Possible Results and Interpretation**

Result	Interpretation
<b>SARS-CoV-2 POSITIVE</b>	<p>The SARS-CoV-2 target RNA is detected.</p> <ul style="list-style-type: none"> <li>• The SARS-CoV-2 signal has a Ct within the valid range and endpoint above the minimum setting</li> <li>• SPC: NA (not applicable); SPC is ignored because SARS-CoV-2 target amplification occurred</li> <li>• Probe Check: PASS; all probe check results pass</li> </ul>
<b>Flu A POSITIVE</b>	<p>The Flu A target RNA is detected.</p> <ul style="list-style-type: none"> <li>• 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</li> <li>• SPC: NA; SPC is ignored because the Flu A target amplification occurred</li> <li>• Probe Check: PASS; all probe check results pass</li> </ul>
<b>Flu B POSITIVE</b>	<p>The Flu B target RNA is detected.</p> <ul style="list-style-type: none"> <li>• The Flu B signal has a Ct within the valid range and endpoint above the minimum setting</li> <li>• SPC: NA; SPC is ignored because Flu B target amplification occurred</li> <li>• Probe Check: PASS; all probe check results pass</li> </ul>
<b>SARS-CoV-2 NEGATIVE; Flu A NEGATIVE; Flu B NEGATIVE</b>	<p>SARS-CoV-2 target RNA is not detected; Flu A target RNA is not detected; Flu B target RNA is not detected.</p> <ul style="list-style-type: none"> <li>• SARS-CoV-2, Flu A, and Flu B target RNAs are not detected</li> <li>• SPC: PASS; SPC has a Ct within the valid range and endpoint above the minimum setting</li> <li>• Probe Check: PASS; all probe check results pass</li> </ul>
<b>INVALID</b>	<p>SPC does not meet acceptance criteria and all targets are not detected. Repeat test according to the Retest Procedure in Section 17.2 of the IFU.</p> <ul style="list-style-type: none"> <li>• SPC: FAIL; SPC and SARS-CoV-2, Flu A and Flu B signals do not have a Ct within valid range and endpoint is below minimum setting.</li> <li>• Probe Check: PASS; all probe check results pass</li> </ul>

Result	Interpretation
<b>ERROR</b>	<p>Presence or absence of SARS-CoV-2, Flu A and Flu B RNA cannot be determined. Repeat test according to the Retest Procedure in Section 17.2 of the IFU.</p> <ul style="list-style-type: none"> <li>• SARS-CoV-2: NO RESULT</li> <li>• Flu A: NO RESULT</li> <li>• Flu B: NO RESULT</li> <li>• SPC: NO RESULT</li> <li>• Probe Check: FAIL<sup>1</sup>; all or one of the probe check results fail</li> </ul> <p><sup>1</sup> 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.</p>
<b>NO RESULT</b>	<p>Presence or absence of SARS-CoV-2, Flu A and Flu B RNA cannot be determined. Repeat test according to the Retest Procedure in Section 17.2 of the IFU. A <b>NO RESULT</b> indicates that insufficient data were collected. For example, the operator stopped a test that was in progress.</p> <ul style="list-style-type: none"> <li>• SARS-CoV-2: NO RESULT</li> <li>• Flu A: NO RESULT</li> <li>• Flu B: NO RESULT</li> <li>• SPC: NO RESULT</li> <li>• Probe Check: NA</li> </ul>
If the SPC is negative and the results for any of the targets are positive, the results for all targets are considered valid.	

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.

Table 3 shows the possible result outcomes when the Xpress SARS-CoV-2 plus test mode is selected.

**Table 3. Xpress SARS-CoV-2 plus Possible Results and Interpretation**

Result	Interpretation
<b>SARS-CoV-2 POSITIVE</b>	<p>The SARS-CoV-2 target RNA is detected.</p> <ul style="list-style-type: none"> <li>• The SARS-CoV-2 signal has a Ct within the valid range and endpoint above the minimum setting</li> <li>• SPC: NA (not applicable); SPC is ignored because SARS-CoV-2 target amplification occurred</li> <li>• Probe Check: PASS; all probe check results pass</li> </ul>
<b>SARS-CoV-2 NEGATIVE</b>	<p>SARS-CoV-2 target RNA is not detected.</p> <ul style="list-style-type: none"> <li>• SARS-CoV-2 target RNA is not detected</li> <li>• SPC: PASS; SPC has a Ct within the valid range and endpoint above the minimum setting</li> <li>• Probe Check: PASS; all probe check results pass</li> </ul>
<b>INVALID</b>	<p>SPC does not meet acceptance criteria and SARS-CoV-2 is not detected. Repeat test according to the Retest Procedure in Section 17.2 of the IFU.</p> <ul style="list-style-type: none"> <li>• SPC: FAIL; SPC and SARS-CoV-2 signals do not have a Ct within valid range and endpoint is below minimum setting</li> <li>• Probe Check: PASS; all probe check results pass</li> </ul>

Result	Interpretation
<b>ERROR</b>	<p>Presence or absence of SARS-CoV-2 RNA cannot be determined. Repeat test according to the Retest Procedure in Section 17.2 of the IFU.</p> <ul style="list-style-type: none"> <li>• SPC: NO RESULT</li> <li>• Probe Check: FAIL<sup>1</sup>; all or one of the probe check results fail</li> </ul> <p><sup>1</sup> 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.</p>
<b>NO RESULT</b>	<p>Presence or absence of SARS-CoV-2 RNA cannot be determined. Repeat test according to the Retest Procedure in Section 17.2 of the IFU. A <b>NO RESULT</b> indicates that insufficient data were collected. For example, the operator stopped a test that was in progress.</p> <ul style="list-style-type: none"> <li>• SARS-CoV-2: NO RESULT</li> <li>• SPC: NO RESULT</li> <li>• Probe Check: NA</li> </ul>

The Xpert Xpress CoV-2/Flu/RSV *plus* test can be run to detect SARS-CoV-2, Flu and RSV by selecting Xpress SARS-CoV-2\_Flu\_RSV *plus* from the Select Test menu; SARS-CoV-2 and Flu only by selecting Xpress SARS-CoV-2\_Flu *plus*; or SARS-CoV-2 only by selecting Xpress SARS-CoV-2 *plus*. The Xpress SARS-CoV-2 *plus* test mode includes an Early Assay Termination (EAT) function that will provide earlier time to result in high titer specimens if the signal from the SARS-CoV-2 target reaches a predetermined threshold before the full 45 PCR cycles have been completed. When SARS-CoV-2 titers are high enough to initiate the EAT function, the SPC amplification curve may not be seen, and its results may not be reported.

## 17 Retests

### 17.1 Reasons to Repeat the Test

If any of the test results mentioned below occur, repeat the test once according to instructions in Section 17.2, Retest Procedure.

- 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.
- 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.
- 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.

If an External Control fails to perform as expected, repeat external control test and/or contact Cepheid Technical Support for assistance.

### 17.2 Retest Procedure

To retest a non-determinate result (**INVALID**, **NO RESULT**, or **ERROR**), use a new cartridge.

Use the leftover sample from the original specimen transport medium tube or new external control tube.

1. Put on a clean pair of gloves. Obtain a new Xpert Xpress CoV-2/Flu/RSV *plus* cartridge and a new transfer pipette.
2. Check the specimen transport tube or external control tube is closed.
3. Mix the sample by rapidly inverting the specimen transport medium tube or external control tube 5 times. Open the cap on the specimen transport tube or external control tube.
4. Open the cartridge lid.
5. Using a clean transfer pipette (supplied), transfer sample (one draw) to the sample chamber with the large opening in the cartridge.
6. Close the cartridge lid.



## 18 Limitations

- Performance of the Xpert Xpress CoV-2/Flu/RSV *plus* test has only been established in nasopharyngeal swab specimens. Use of the Xpert Xpress CoV-2/Flu/RSV *plus* test with other specimen types has not been assessed and performance characteristics are unknown.
- 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 CoV-2/Flu/RSV *plus* test but performance with these specimen types has not been established.
- The performance of this test was established based on the evaluation of a limited number of clinical specimens. Clinical performance has not been established with 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.
- The performance of this device has not been assessed in a population vaccinated against COVID-19.
- 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 virus or the virus being detected less predictably.
- This test cannot rule out diseases caused by other bacterial or viral pathogens.
- 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.
- 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.
- False negative results may occur if virus is present at levels below the analytical limit of detection.
- 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.
- 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.
- 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.
- This test has been evaluated for use with human specimen material only.
- This test is a qualitative test and does not provide the quantitative value of detected organism present.
- This test has not been evaluated for monitoring treatment of infection.
- This test has not been evaluated for patients without signs and symptoms of respiratory tract infection.
- This test has not been evaluated for screening of blood or blood products for the presence of SARS-CoV-2, influenza, or RSV.
- 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.
- 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.
- Cross-reactivity with respiratory tract organisms other than those described herein can lead to erroneous results.
- Recent patient exposure to FluMist® or other live attenuated influenza vaccines may cause inaccurate positive results.
- Zicam at 15% (w/v) may interfere with the detection of low levels of influenza B and RSV A.
- Samples collected into saline or eNAT should not be frozen.
- As the Xpert Xpress CoV-2/Flu/RSV *plus* test does not differentiate between the N2, RdRP and E gene targets, the presence of other coronaviruses in the B lineage, Betacoronavirus genus, including SARS-CoV may cause a false positive result. None of these other coronaviruses is known to currently circulate in the human population.
- 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.

## 19 Conditions of Authorization for Laboratory and Patient Care Settings

The Cepheid Xpert Xpress CoV-2/Flu/RSV *plus* Letter of Authorization, along with the authorized Fact Sheet for Healthcare Providers, the authorized Fact Sheet for Patients and authorized labeling are available on the FDA website:

<https://www.fda.gov/medical-devices/coronavirus-disease-2019-covid-19-emergency-use-authorizations-medical-devices/in-vitro-diagnostics-euas>.

However, to assist clinical laboratories and/or Patient Care Settings using the Xpert Xpress CoV-2/Flu/RSV *plus* (referred to in the Letter of Authorization as “Your Product”), the relevant Conditions of Authorization are listed below.

- Authorized laboratories<sup>a</sup> using your product must include with test result reports all authorized Fact Sheets. Under exigent circumstances, other appropriate methods for disseminating these Fact Sheets may be used, which may include mass media.
- Authorized laboratories using your product must use your product as outlined in the authorized labeling. Deviations from the authorized procedures, including the authorized instruments, authorized extraction methods, authorized clinical specimen types, authorized control materials, authorized other ancillary reagents and authorized materials required to use the Xpert Xpress CoV-2/Flu/RSV *plus* test are not permitted.
- Authorized laboratories that receive your product must notify the relevant public health authorities of their intent to run your product prior to initiating testing.
- Authorized laboratories using your product must have a process in place for reporting test results to healthcare providers and relevant public health authorities, as appropriate.
- Authorized laboratories must collect information on the performance of your product and report to DMD/OHT7-OIR/OPEQ/CDRH (via email: [CDRH-EUA-Reporting@fda.hhs.gov](mailto:CDRH-EUA-Reporting@fda.hhs.gov)) and Cepheid (+1 888 838 3222 or [techsupport@cepheid.com](mailto:techsupport@cepheid.com)) any suspected occurrence of false positive or false negative results and significant deviations from the established performance characteristics of the test of which they become aware.
- All operators using your product must be appropriately trained in RT-PCR techniques and use appropriate personal protective equipment when handling this kit, and use your product in accordance with the authorized labeling.
- Cepheid, authorized distributors, and authorized laboratories using your product must ensure that any records associated with this EUA are maintained until otherwise notified by FDA. Such records will be made available to FDA for inspection upon request.

<sup>a</sup> The letter of authorization refers to “authorized laboratories as follows: (1) testing of nasopharyngeal swab, nasal swab, or nasal wash/aspirate specimens using the Xpert Xpress CoV-2/Flu/RSV *plus* test run on the GeneXpert Dx and GeneXpert Infinity systems is limited to laboratories certified under the Clinical Laboratory Improvement Amendments of 1988 (CLIA), 42 U.S.C. § 263a, that meet requirements to perform high or moderate complexity tests and (2) testing of nasopharyngeal or nasal swab specimens using the Xpert Xpress CoV-2/Flu/RSV *plus* test run on the GeneXpert Xpress System (Tablet and Hub Configurations) is authorized for use at the Point of Care (POC), i.e., in patient care settings operating under a CLIA Certificate of Waiver, Certificate of Compliance, or Certificate of Accreditation.

## 20 Performance Characteristics

### 20.1 Clinical Evaluation

The performance of the Xpert Xpress CoV-2/Flu/RSV *plus* test was evaluated using archived clinical nasopharyngeal (NP) swab specimens in viral transport medium or universal transport medium. Archived specimens were selected consecutively by date and previously known analyte result. A total of 279 NP swab specimens were tested with Xpert Xpress CoV-2/Flu/RSV *plus* side by side with FDA authorized SARS-CoV-2 RT-PCR test and the FDA-cleared influenza/RSV molecular test in a randomized and blinded fashion.

Positive Percent Agreement (PPA), Negative Percent Agreement (NPA), and non-determinate rate were determined by comparing the results of the Xpert Xpress CoV-2/Flu/RSV *plus* test relative to the results of FDA authorized SARS-CoV-2 RT-PCR test for the SARS-CoV-2 target, and FDA-cleared influenza/RSV molecular test for the Flu A, Flu B, and RSV targets, respectively.

Xpert Xpress CoV-2/Flu/RSV *plus* demonstrated a PPA and NPA of 100.0% and 100.0% for SARS-CoV-2, respectively; 100.0% and 100.0% for Flu A, respectively; 100.0% and 100.0% for Flu B, respectively; 100.0% and 100.0% for RSV, respectively (Table 4). The non-determinate rate for the Xpert Xpress CoV-2/Flu/RSV *plus* test was 1.4% (4/279).

**Table 4. Xpert Xpress CoV-2/Flu/RSV *plus* Performance Results**

Target	Number of Specimens	TP	FP	TN	FN	PPA (95% CI)	NPA (95% CI)
SARS-CoV-2	279	66	0	213	0	100.0% (94.5% - 100.0%)	100.0% (98.2% - 100.0%)
Flu A	264	51	0	213	0	100.0% (93.0% - 100.0%)	100.0% (98.2% - 100.0%)
Flu B	264	46	0	218	0	100.0% (92.3% - 100.0%)	100.0% (98.3% - 100.0%)
RSV	264	47	0	217	0	100.0% (92.4% - 100.0%)	100.0% (98.3% - 100.0%)
TP: True Positive; FP: False Positive; TN: True Negative; FN: False Negative; CI: Confidence Interval							

## 20.2 Analytical Sensitivity (Limit of Detection)

The analytical sensitivity of the Xpert Xpress CoV-2/Flu/RSV *plus* test was first estimated using two reagent lots by testing limiting dilutions of seven respiratory viruses (NATrol SARS-CoV-2, Flu A H1, Flu A H3, Flu B Victoria lineage, Flu B Yamagata lineage, RSV A and RSV B) into pooled negative clinical NP swab matrix, following the guidance in Clinical and Laboratory Standards Institute (CLSI) document EP17-A2. The estimated LoD values as determined by Probit regression analysis were verified using two lots of Xpert Xpress CoV-2/Flu/RSV *plus* reagents. The verified LoD values for the viruses tested are summarized in Table 5.

**Table 5. Xpert Xpress CoV-2/Flu/RSV *plus* Limit of Detection**

Virus/Strain	LoD Concentration
SARS-CoV-2 (USA-WA1/2020)	138 copies/mL
Influenza A/Idaho/07/2018	0.007 TCID <sub>50</sub> /mL
Influenza A/Hong Kong/45/2019	0.44 FFU/mL
Influenza B/Washington/2/2019	12.9 CEID <sub>50</sub> /mL
Influenza B/Wisconsin/10/2016	2.4 TCID <sub>50</sub> /mL
RSV A/2/Australia/61	0.33 TCID <sub>50</sub> /mL
RSV B/9320/MA/77	0.37 TCID <sub>50</sub> /mL

## 20.3 Analytical Reactivity (Inclusivity)

The inclusivity of Xpert Xpress CoV-2/Flu/RSV *plus* was evaluated on June 30<sup>th</sup>, 2021 using *in silico* analysis of the assay amplicons in relation to 1,566,123 SARS-CoV-2 sequences available in the GISAID gene database for three targets, E, N2 and RdRP.

For analysis of the E target, 1,626 sequences were excluded due to ambiguous nucleotides, which reduced the total to 1,564,497 sequences. Of the 1,564,497 GISAID sequences, 1,555,036 (99.4%) were an exact match to the SARS-CoV-2 E target amplicon generated in the Xpert Xpress CoV-2/Flu/RSV *plus* test. Single nucleotide mismatches were observed for 9,406 sequences and two mismatches were observed for 55 sequences. Of the 55 sequences with two or more mismatches, 16 sequences contained 2 mismatches in the forward primer region, and three sequences contained 2 mismatches in the probe region. These double mismatches could have an impact on probe or reverse primer binding.

For analysis of the N2 target, 2,046 sequences were excluded due to ambiguous nucleotides, which reduced the total used in the evaluation to 1,564,077 sequences. Of the 1,564,077 GISAID sequences, 1,511,700 (96.65%) were an exact match to the SARS-CoV-2 N2 target amplicon generated in the Xpert Xpress CoV-2/Flu/RSV *plus* test. Single nucleotide mismatches were observed for 50,466 sequences and two or three mismatches were observed for 1,911 sequences. For the 31 sequences with three variant positions, five sequences have two of the mismatched nucleotides in the probe region and two of the sequences have two of the mismatched nucleotides in the reverse primer region. These double mismatches could have an impact on probe or reverse primer binding. None of the other mismatches are predicted to have a negative impact on the performance of the assay.

The RdRP is amplified using a semi-nested primer/probe set; only the inner amplicon is used for the *in silico* analysis. For analysis of the RdRP target, 3,616 sequences were excluded due to ambiguous nucleotides, which reduced the total to 1,565,149 sequences. Of the 1,565,149 GISAID sequences, 1,550,310 (99.05%) were an exact match to the SARS-CoV-2 RdRP target amplicon generated in the Xpert Xpress CoV-2/Flu/RSV *plus* test. Single nucleotide mismatches were observed for 14,791 sequences and two or more mismatches were observed for 48 sequences. Two sequences have 5 mismatches, three located in the probe region and two in the reverse primer region, and 19 sequences have two nucleotide mismatches in the forward primer or probe region. These mismatches could have an impact on probe or reverse primer binding. None of the other mismatches are predicted to have a negative impact on the performance of the assay.

In addition to the *in silico* analysis of the SARS-CoV-2 primers and probes for inclusivity, the inclusivity of the Xpert Xpress CoV-2/Flu/RSV *plus* test was evaluated by bench testing against multiple strains of SARS-CoV-2, influenza A H1N1 (seasonal pre-2009), influenza A H1N1 (pandemic 2009), influenza A H3N2 (seasonal), avian influenza A (H5N1, H5N2, H6N2, H7N2, H7N3, H2N2, H7N9, and H9N2), influenza B (representing strains from both Victoria and Yamagata lineages), and respiratory syncytial virus subgroups A and B (RSV A and RSV B) at levels near the analytical LoD. A total of 84 strains comprised of 5 SARS-CoV-2 virus strains, 4 SARS-CoV-2 in vitro RNA transcripts representing variant

strains, 69 influenza viruses (48 influenza A and 21 influenza B) and 6 RSV strains (4 RSVA and 2 RSV B) were tested in this study with the Xpert Xpress CoV-2/Flu/RSV *plus* test. Three replicates were tested for each strain. All SARS-CoV-2, Flu and RSV strains tested positive in all three replicates. Results are shown in Table 6.

**Table 6. Analytical Reactivity (Inclusivity) of the Xpert Xpress CoV-2/Flu/RSV *plus* Test**

Virus	Strain	Tested Titer	SARS-CoV-2	Flu A	Flu B	RSV
SARS-CoV-2	NATrol SARS-CoV-2 USA-WA1/2020	412 copies/mL	POS	NEG	NEG	NEG
	SARS-CoV-2/Hong Kong/VM20001061/2020	0.5 TCID <sub>50</sub> /mL	POS	NEG	NEG	NEG
	SARS-CoV-2/Italy-INMI1	4 TCID <sub>50</sub> /mL	POS	NEG	NEG	NEG
	SARS-CoV-2/South_Africa/KRISP-K005325/2020	0.2 TCID <sub>50</sub> /mL	POS	NEG	NEG	NEG
	SARS-CoV-2/England/204820464/2020	0.5 TCID <sub>50</sub> /mL	POS	NEG	NEG	NEG
	SARS-CoV-2 RNA USA/WA2/2020(C09) <sup>a</sup>	100 copies/mL	POS	NEG	NEG	NEG
	SARS-CoV-2RNA/England/205041766/2020(C14) <sup>a</sup>	100 copies/mL	POS	NEG	NEG	NEG
	SARS-CoV-2 RNA /England/MILK-9E05B3/2020 (C15) <sup>a</sup>	200 copies/mL	POS	NEG	NEG	NEG
	SARS-CoV-2 RNA /Japan (Brazil)/IC-0564/2021 (C17) <sup>a</sup>	100 copies/mL	POS	NEG	NEG	NEG
Influenza A H1N1 (pre-2009)	A/swine/Iowa/15/30	30 TCID <sub>50</sub> /mL	NEG	POS	NEG	NEG
	A/WS/33	5.0 CEID <sub>50</sub> /mL	NEG	POS	NEG	NEG
	A/PR/8/34	20 CEID <sub>50</sub> /mL	NEG	POS	NEG	NEG
	A/Mal/302/54	0.156 CEID <sub>50</sub> /mL	NEG	POS	NEG	NEG
	A/Denver/1/57	10 CEID <sub>50</sub> /mL	NEG	POS	NEG	NEG
	A/New Jersey/8/76	5.0 CEID <sub>50</sub> /mL	NEG	POS	NEG	NEG
	A/New Caledonia/20/1999	0.10 TCID <sub>50</sub> /mL	NEG	POS	NEG	NEG
	A/New York/55/2004	30 TCID <sub>50</sub> /mL	NEG	POS	NEG	NEG
	A/Solomon Island/3/2006	0.0159 TCID <sub>50</sub> /mL	NEG	POS	NEG	NEG
	A/Taiwan/42/06	0.0159 TCID <sub>50</sub> /mL	NEG	POS	NEG	NEG
	A/Brisbane/59/2007	0.060 TCID <sub>50</sub> /mL	NEG	POS	NEG	NEG
	A/Swine/NY/02/2009	20 TCID <sub>50</sub> /mL	NEG	POS	NEG	NEG
Influenza A H1N1 (pdm2009)	A/Colorado/14/2012	0.13 TCID <sub>50</sub> /mL	NEG	POS	NEG	NEG
	A/Michigan/45/2015	100 CEID <sub>50</sub> /mL	NEG	POS	NEG	NEG
	A/Iowa/53/2015	100 CEID <sub>50</sub> /mL	NEG	POS	NEG	NEG
	A/Michigan/272/2017	1.0 TCID <sub>50</sub> /mL	NEG	POS	NEG	NEG

Virus	Strain	Tested Titer	SARS-CoV-2	Flu A	Flu B	RSV
	A/Idaho/07/2018	0.0159 TCID <sub>50</sub> /mL	NEG	POS	NEG	NEG
	A/Wisconsin/505/2018	0.25 TCID <sub>50</sub> /mL	NEG	POS	NEG	NEG
	A/Hawaii/66/2019	100 CEID <sub>50</sub> /mL	NEG	POS	NEG	NEG
	A/Indiana/02/2020	NA <sup>b</sup>	NEG	POS	NEG	NEG
Influenza A H3N2 (Seasonal)	A/Aichi/2/68	2.0 CEID <sub>50</sub> /mL	NEG	POS	NEG	NEG
	A/Hong Kong/8/68	2.0 CEID <sub>50</sub> /mL	NEG	POS	NEG	NEG
	A/Port Chalmers/1/73	100 CEID <sub>50</sub> /mL	NEG	POS	NEG	NEG
	A/Hawaii/15/2001	100 CEID <sub>50</sub> /mL	NEG	POS	NEG	NEG
	A/Wisconsin/67/05 <sup>c</sup>	0.22 TCID <sub>50</sub> /mL	NEG	POS	NEG	NEG
	A/Brisbane/10/2007	0.025 TCID <sub>50</sub> /mL	NEG	POS	NEG	NEG
	A/Minnesota/11/2010	30 CEID <sub>50</sub> /mL	NEG	POS	NEG	NEG
	A/Indiana/08/2011	0.25 TCID <sub>50</sub> /mL	NEG	POS	NEG	NEG
	A/Texas/50/2012	0.050 TCID <sub>50</sub> /mL	NEG	POS	NEG	NEG
	A/Alaska/232/2015	20 CEID <sub>50</sub> /mL	NEG	POS	NEG	NEG
	A/Singapore/INFIMH-16-0019/2016	20 CEID <sub>50</sub> /mL	NEG	POS	NEG	NEG
	A/Texas/71/2017	1.0 FFU/mL	NEG	POS	NEG	NEG
	A/Kansas/14/2017	1.0 FFU/mL	NEG	POS	NEG	NEG
	A/Wisconsin/04/2018	1.0 FFU/mL	NEG	POS	NEG	NEG
	A/Arizona/45/2018	2.0 FFU/mL	NEG	POS	NEG	NEG
	A/Hong Kong/45/2019	2.0 FFU/mL	NEG	POS	NEG	NEG
Avian influenza A <sup>d</sup>	A/Mallard/NY/6750/78 (H2N2)	<1 pg/μL	NEG	POS	NEG	NEG
	A/duck/Hunan/795/2002 (H5N1)	<1 pg/μL	NEG	POS	NEG	NEG
	A/Vietnam/1194/2004 (H5N1)	<1 pg/μL	NEG	POS	NEG	NEG
	A/Anhui/01/2005 (H5N1)	<1 pg/μL	NEG	POS	NEG	NEG
	A/Japanese white eye/Hong Kong/1038/2006 (H5N1)	<1 pg/μL	NEG	POS	NEG	NEG
	A/mallard/WI/34/75 (H5N2)	<1 pg/μL	NEG	POS	NEG	NEG
	A/chicken/CA431/00 (H6N2)	<1 pg/μL	NEG	POS	NEG	NEG
	A/duck/LTC-10-82743 (H7N2)	<1 pg/μL	NEG	POS	NEG	NEG
	A/chicken/New Jersey/15086/3 (H7N3)	<1 pg/μL	NEG	POS	NEG	NEG
	A/Anhui/1/2013 (H7N9)	0.612 ng/μL	NEG	POS	NEG	NEG
	A/Shanghai/1/2013 (H7N9)	NA <sup>e</sup>	NEG	POS	NEG	NEG

Virus	Strain	Tested Titer	SARS-CoV-2	Flu A	Flu B	RSV
	A/chicken/Korea/38349-p96323/1996 (H9N2)	<1 pg/μL	NEG	POS	NEG	NEG
Influenza B	B/Lee/40	1.0 PFU/mL	NEG	NEG	POS	NEG
	B/Allen/45	0.25 CEID <sub>50</sub> /mL	NEG	NEG	POS	NEG
	B/GL/1739/54	0.50 CEID <sub>50</sub> /mL	NEG	NEG	POS	NEG
	B/Maryland/1/59	1.0 CEID <sub>50</sub> /mL	NEG	NEG	POS	NEG
	B/Taiwan/2/62	1.0 CEID <sub>50</sub> /mL	NEG	NEG	POS	NEG
	B/Hong Kong/5/72	1.0 CEID <sub>50</sub> /mL	NEG	NEG	POS	NEG
Influenza B Victoria Lineage	B/Panama/45/90	1.0 TCID <sub>50</sub> /mL	NEG	NEG	POS	NEG
	B/Malaysia/2506/04	0.025 TCID <sub>50</sub> /mL	NEG	NEG	POS	NEG
	B/Florida/02/06	0.025 TCID <sub>50</sub> /mL	NEG	NEG	POS	NEG
	B/Brisbane/60/2008	0.05 TCID <sub>50</sub> /mL	NEG	NEG	POS	NEG
	B/Maryland/15/2016	0.25 TCID <sub>50</sub> /mL	NEG	NEG	POS	NEG
	B/Colorado/6/2017	0.25 TCID <sub>50</sub> /mL	NEG	NEG	POS	NEG
	B/Hawaii/01/2018	8.0 TCID <sub>50</sub> /mL	NEG	NEG	POS	NEG
	B/Missouri/12/2018(NA D197E)	10 TCID <sub>50</sub> /mL	NEG	NEG	POS	NEG
	B/Washington/02/2019	60 TCID <sub>50</sub> /mL	NEG	NEG	POS	NEG
Influenza B Yamagata Lineage	B/Florida/07/2004	0.50 TCID <sub>50</sub> /mL	NEG	NEG	POS	NEG
	B/Florida/04/06	0.25 TCID <sub>50</sub> /mL	NEG	NEG	POS	NEG
	B/Wisconsin/01/2010	0.50 CEID <sub>50</sub> /mL	NEG	NEG	POS	NEG
	B/Wisconsin/10/2016	20 TCID <sub>50</sub> /mL	NEG	NEG	POS	NEG
	B/Indiana/17/2017	10 TCID <sub>50</sub> /mL	NEG	NEG	POS	NEG
	B/Oklahoma/10/2018	10 TCID <sub>50</sub> /mL	NEG	NEG	POS	NEG
RSV A	RSV-A/NY	0.386 TCID <sub>50</sub> /mL	NEG	NEG	NEG	POS
	RSV-A/WI-629.8.2/2007	0.50 TCID <sub>50</sub> /mL	NEG	NEG	NEG	POS
	RSV-A/WI/629-11-1_2008	0.50 TCID <sub>50</sub> /mL	NEG	NEG	NEG	POS
	RSV-A, Strain: 4/2015 Isolate #1	0.25 TCID <sub>50</sub> /mL	NEG	NEG	NEG	POS
RSV B	RSV-B/WV14617/85	0.10 TCID <sub>50</sub> /mL	NEG	NEG	NEG	POS
	RSV-B-CH93(18)-18-01	0.10 TCID <sub>50</sub> /mL	NEG	NEG	NEG	POS

a *in vitro* RNA transcripts

b Titer A/Indiana/02/2020 virus was without titer and was diluted 100,000-fold in simulated background matrix for testing.

c One of three replicates reported ERROR. The run was successfully repeated to obtain three valid replicates.

d Purified viral RNA in simulated background matrix was used for avian influenza A viruses due to biosafety regulations.

e Inactivated avian influenza A (H7N9) viruses without viral titer was diluted 100,000-fold in simulated background matrix and tested due to biosafety regulations.

## 20.4 Analytical Specificity (Exclusivity)

An *in silico* analysis for possible cross-reactions with all the organisms listed in Table 7 was conducted by mapping the SARS-CoV-2 primers and probes in the Xpert Xpress CoV-2/Flu/RSV *plus* test individually to the sequences downloaded from the GISAID database. E primers and probes are not specific for SARS-CoV-2 and will detect Human and Bat SARS-coronavirus. No potential unintended cross reactivity with other organisms listed in Table 7 is expected based on the *in silico* analysis.

**Table 7. Microorganisms Analyzed in the *in silico* Analysis for the SARS-CoV-2 Target**

Microorganisms from the Same Genetic Family	High Priority Organisms
Human coronavirus 229E	Adenovirus (e.g. C1 Ad. 71)
Human coronavirus OC43	Human metapneumovirus (hMPV)
Human coronavirus HKU1	Parainfluenza viruses 1-4
Human coronavirus NL63	Influenza A
SARS-coronavirus	Influenza B
MERS-coronavirus	Influenza C
Bat coronavirus	Enterovirus (e.g. EV68)
	Respiratory syncytial virus
	Rhinovirus
	<i>Chlamydia pneumoniae</i>
	<i>Haemophilus influenzae</i>
	<i>Legionella pneumophila</i>
	<i>Mycobacterium tuberculosis</i>
	<i>Streptococcus pneumoniae</i>
	<i>Streptococcus pyogenes</i>
	<i>Bordetella pertussis</i>
	<i>Mycoplasma pneumoniae</i>
	<i>Pneumocystis jirovecii</i> (PJP)
	<i>Parechovirus</i>
	<i>Candida albicans</i>
	<i>Corynebacterium diphtheriae</i>
	<i>Legionella non-pneumophila</i>
	<i>Bacillus anthracis</i> (Anthrax)
	<i>Moraxella catarrhalis</i>
	<i>Neisseria elongata</i> and <i>N. meningitidis</i>
	<i>Pseudomonas aeruginosa</i>
	<i>Staphylococcus epidermidis</i>
	<i>Streptococcus salivarius</i>
	<i>Leptospira</i>
	<i>Chlamydia psittaci</i>
	<i>Coxiella burnetii</i> (Q-Fever)



Microorganisms from the Same Genetic Family	High Priority Organisms
	<i>Staphylococcus aureus</i>

In addition to the *in silico* analysis of the SARS-CoV-2 primers and probes for cross-reactivity, the analytical specificity of the Xpert Xpress CoV-2/Flu/RSV plus test was evaluated by bench-testing a panel of 48 microorganisms comprising 4 human coronaviruses, 1 MERS coronavirus and 43 common respiratory pathogens or those potentially encountered in the nasopharynx. The panel was tested in different pools of microorganisms; if a pool produced a positive result, then each member of the pool would have been tested individually. Three replicates of each pool were tested. A sample was considered negative if all three replicates were negative. The bacterial and yeast strains were tested at concentrations of  $\geq 1 \times 10^6$  CFU/mL with the exception of *Chlamydia pneumoniae* which was tested at  $1.2 \times 10^6$  IFU/mL and *Lactobacillus reuteri* which was tested at  $5 \times 10^7$  copies/mL of genomic DNA. Viruses were tested at concentrations of  $\geq 1 \times 10^5$  TCID<sub>50</sub>/mL. The analytical specificity was 100%. Results are shown in Table 8.

**Table 8. Respiratory Microorganisms and Human Coronavirus Tested, Concentrations and Xpert Xpress CoV-2/Flu/RSV plus Test Results**

Strain	Tested Concentration	SARS-CoV-2	Flu A	Flu B	RSV
Negative Control	NA	NEG	NEG	NEG	NEG
Positive Control	NA	POS	POS	POS	POS
Human coronavirus NL63	1.17e5 TCID <sub>50</sub> /mL	NEG	NEG	NEG	NEG
MERS-coronavirus	1.17e5 TCID <sub>50</sub> /mL	NEG	NEG	NEG	NEG
Human coronavirus 229E	1.21e5 TCID <sub>50</sub> /mL	NEG	NEG	NEG	NEG
Human coronavirus OC43	1.02e5 TCID <sub>50</sub> /mL	NEG	NEG	NEG	NEG
Human coronavirus HKU1	1.23e6 copies/mL	NEG	NEG	NEG	NEG
Adenovirus Type 1	4.07e5 TCID <sub>50</sub> /mL	NEG	NEG	NEG	NEG
Adenovirus Type 7	1.14e5 TCID <sub>50</sub> /mL	NEG	NEG	NEG	NEG
Cytomegalovirus	1.0e5 TCID <sub>50</sub> /mL	NEG	NEG	NEG	NEG
Echovirus	1.14e5 TCID <sub>50</sub> /mL	NEG	NEG	NEG	NEG
Enterovirus	2.80e5 TCID <sub>50</sub> /mL	NEG	NEG	NEG	NEG
Epstein Barr Virus	5.60e6 TCID <sub>50</sub> /mL	NEG	NEG	NEG	NEG
HSV	1.97e5 TCID <sub>50</sub> /mL	NEG	NEG	NEG	NEG
Human metapneumovirus	4.07e5 TCID <sub>50</sub> /mL	NEG	NEG	NEG	NEG
Human parainfluenza Type 1	1.0e5 TCID <sub>50</sub> /mL	NEG	NEG	NEG	NEG
Human parainfluenza Type 2	1.2e5 TCID <sub>50</sub> /mL	NEG	NEG	NEG	NEG
Human parainfluenza Type 3	1.2e5 TCID <sub>50</sub> /mL	NEG	NEG	NEG	NEG
Human parainfluenza Type 4	1.19e6 TCID <sub>50</sub> /mL	NEG	NEG	NEG	NEG
Measles	1.2e5 TCID <sub>50</sub> /mL	NEG	NEG	NEG	NEG
Mumps virus	1.2e5 TCID <sub>50</sub> /mL	NEG	NEG	NEG	NEG
Rhinovirus Type 1A	1.0e5 TCID <sub>50</sub> /mL	NEG	NEG	NEG	NEG
<i>Acinetobacter baumannii</i>	1.30e7 CFU/mL	NEG	NEG	NEG	NEG
<i>Bordetella pertussis</i>	6.40e7 CFU/mL	NEG	NEG	NEG	NEG

Strain	Tested Concentration	SARS-CoV-2	Flu A	Flu B	RSV
<i>Burkholderia cepacia</i>	1.90e8 CFU/mL	NEG	NEG	NEG	NEG
<i>Candida albicans</i>	6.30e6 CFU/mL	NEG	NEG	NEG	NEG
<i>Candida parapsilosis</i>	1.45e6 CFU/mL	NEG	NEG	NEG	NEG
<i>Citrobacter freundii</i>	1.73e8 CFU/mL	NEG	NEG	NEG	NEG
<i>Corynebacterium sp.</i>	1.27e7 CFU/mL	NEG	NEG	NEG	NEG
<i>Enterococcus faecalis</i>	5.87e7 CFU/mL	NEG	NEG	NEG	NEG
<i>Escherichia coli</i>	1.55e8 CFU/mL	NEG	NEG	NEG	NEG
<i>Hemophilus influenzae</i>	6.62e6 CFU/mL	NEG	NEG	NEG	NEG
<i>Lactobacillus reuteri</i>	5.0e7 copies/mL	NEG	NEG	NEG	NEG
<i>Legionella spp.</i>	1.42e8 CFU/mL	NEG	NEG	NEG	NEG
<i>Moraxella catarrhalis</i>	2.46e6 CFU/mL	NEG	NEG	NEG	NEG
<i>Mycoplasma pneumoniae</i>	2.7e6 CFU/mL	NEG	NEG	NEG	NEG
<i>Neisseria meningitidis</i>	4.2e6 CFU/mL	NEG	NEG	NEG	NEG
<i>Neisseria mucosa</i>	1.0e8 CFU/mL	NEG	NEG	NEG	NEG
<i>Propionibacterium acnes</i>	8.25e7 CFU/mL	NEG	NEG	NEG	NEG
<i>Pseudomonas aeruginosa</i>	1.05e7 CFU/mL	NEG	NEG	NEG	NEG
<i>Staphylococcus haemolyticus</i>	2.66e6 CFU/mL	NEG	NEG	NEG	NEG
<i>Staphylococcus aureus</i>	5.87e7 CFU/mL	NEG	NEG	NEG	NEG
<i>Staphylococcus epidermidis</i>	2.47e7 CFU/mL	NEG	NEG	NEG	NEG
<i>Streptococcus agalactiae</i>	1.75e7 CFU/mL	NEG	NEG	NEG	NEG
<i>Streptococcus pneumoniae</i>	2.26e7 CFU/mL	NEG	NEG	NEG	NEG
<i>Streptococcus pyogenes</i>	9.0e6 CFU/mL	NEG	NEG	NEG	NEG
<i>Streptococcus salivarius</i>	4.19e6 CFU/mL	NEG	NEG	NEG	NEG
<i>Streptococcus sanguinis</i>	8.67e6 CFU/mL	NEG	NEG	NEG	NEG
<i>Chlamydia pneumoniae</i>	1.20e6 CFU/mL	NEG	NEG	NEG	NEG
<i>Mycobacterium tuberculosis (avirulent)</i>	1.20e6 CFU/mL	NEG	NEG	NEG	NEG

## 20.5 Microbial Interference

Microbial interference of the Xpert Xpress CoV-2/Flu/RSV plus test caused by the presence of bacterial or viral strains that might be encountered in human upper respiratory tract specimens, was evaluated by testing a panel of 10 commensal microorganisms, consisting of 7 viral strains and 3 bacterial strains. Contrived samples consisted of SARS-CoV-2, Flu A, Flu B, RSV A, or RSV B viruses seeded at 3x the Limit of Detection (LoD) into simulated nasopharyngeal swab (NPS)/ nasal swab (NS) matrix in the presence of Adenovirus Type 1C, Human Coronavirus OC43, Rhinovirus Type 1A, Human metapneumovirus, Human parainfluenza Types 1, 2, and 3 (each seeded at 1x10<sup>5</sup> units/mL), *Hemophilus influenzae* (seeded at 1x10<sup>6</sup> CFU/mL), *Staphylococcus aureus* or *Staphylococcus epidermidis* (each seeded at 1x10<sup>7</sup> CFU/mL).

Replicates of 8 positive samples were tested for each target virus (SARS-CoV-2, Flu A, Flu B, RSV A, or RSV B) and each potential microbial interference strain combination. For each target, all 8 of 8 replicate samples were correctly identified using the Xpert Xpress CoV-2/Flu/RSV plus test. No interference by the commensal viral or bacterial strains was reported.

## 20.6 Competitive Interference

Competitive interference of the Xpert Xpress CoV-2/Flu/RSV *plus* caused by co-infections were evaluated by testing contrived samples of individual SARS-CoV-2, Flu A, Flu B or RSV strains at 3X LoD in the presence of different target strains at a higher concentration in a simulated background matrix. The concentration at 3X LoD was 414 copies/mL for SARS-CoV-2 (inactivated USA-WA1/2020); 0.021 TCID<sub>50</sub>/mL for Flu A/Idaho/072018, 38.7 CEID<sub>50</sub>/mL for Flu B/Washington/2/2019; 0.99 TCID<sub>50</sub>/mL for RSV A/2/Australia/61), and 1.11 TCID<sub>50</sub>/mL for RSV B/9320/MA/77. The competitive strains were evaluated at 10<sup>5</sup> or higher titer units (copies/mL, TCID<sub>50</sub>/mL, CEID<sub>50</sub>/mL or PFU/mL). The corresponding concentration of RNA (copies/mL) for the Flu and RSV strains was determined by droplet digital PCR (ddPCR). Replicates of 3 were tested for each target strain and each competitive strain combination. The virus at high concentration shows no competitive inhibitory effects if 3 of 3 replicates for the target strain report positive results. If the results reported less than 3 of 3 positive replicates, the concentration of the competing virus was reduced by 10-fold increments until no interference was observed. Below is a summary of the results:

**Table 9. Summary of Competitive Interference Study with Flu A at High Concentration**

Test Viruses at 3X LoD	Interferent Virus	Correct Calls (n/3)			
		at 1.7e8 RNA copies/mL	at 1.7e7 RNA copies/mL	at 1.7e6 RNA copies/mL	at 1.7e5 RNA copies/mL
Flu B	Flu A	0/3	0/3	2/3	3/3
RSV A		0/3	0/3	3/3	Not tested
RSV B		3/3	Not tested	Not tested	Not tested
SARS-CoV-2		3/3	Not tested	Not tested	Not tested

**Table 10. Summary of Competitive Interference Study with Flu B at High Concentration**

Test Viruses at 3X LoD	Interferent Virus	Correct Calls (n/3) at 1.4e5 RNA copies/mL
Flu A	Flu B	3/3
RSV A		3/3
RSV B		3/3
SARS-CoV-2		3/3

**Table 11. Summary of Competitive Interference Study with RSV A at High Concentration**

Test Viruses at 3X LoD	Interferent Virus	Correct Calls (n/3) at 4.6e6 RNA copies/mL
Flu A	RSV A	3/3
Flu B		3/3
SARS-CoV-2		3/3

**Table 12. Summary of Competitive Interference Study with RSV B at High Concentration**

Test Viruses at 3X LoD	Interferent Virus	Correct Calls (n/3) at 1.9e5 RNA copies/mL
Flu A	RSV B	3/3
Flu B		3/3
SARS-CoV-2		3/3

**Table 13. Summary of Competitive Interference Study with SARS-CoV-2 at High Concentration**

Test Viruses at 3X LoD	Interferent Virus	Correct Calls (n/3)	
		at 1e6 RNA copies/mL	at 1e5 RNA copies/mL
Flu A	SARS-CoV-2	3/3	Not tested
Flu B		1/3	3/3
RSV A		3/3	Not tested
RSV B		3/3	Not tested

The study showed that Flu A/Idaho/07/2018 at concentrations above 1.7e5 RNA copies/mL inhibited detection of Flu B at 3X LoD, and at concentrations above 1.7e6 RNA copies/mL inhibited detection of RSV A at 3X LoD (Table 9). In addition, SARS-CoV-2 at concentrations above 1e5 RNA copies/mL inhibited detection of Flu B at 3X LoD (Table 13). No other competitive interference was observed for the potential co-infections tested in the study at the concentrations tested.

## 20.7 Potentially Interfering Substances

Substances that could be present in the nasopharynx (or introduced during specimen collection and handling) and potentially interfere with accurate detection of SARS-CoV-2, Flu A, Flu B and RSV were evaluated with direct testing on the Xpert Xpress CoV-2/Flu/RSV *plus*.

Potentially interfering substances in the nasal passage and nasopharynx may include, but are not limited to: blood, nasal secretions or mucus, and nasal and throat medications used to relieve congestion, nasal dryness, irritation, or asthma and allergy symptoms, as well as antibiotics and antivirals. Positive and negative samples were prepared in simulated nasopharyngeal swab (NPS)/ nasal swab (NS) matrix. Negative samples (N = 8) were tested in the presence of each substance to determine the effect on the performance of the sample processing control (SPC). Positive samples (N = 8) were tested per substance with viruses spiked at 3x the LoD determined for each strain. Positive samples tested with the Xpert Xpress CoV-2/Flu/RSV *plus* included one SARS-CoV-2, one influenza A H1N1, one influenza A H3N2, one influenza B and two RSV (RSV A and RSV B) strains. The substances, with active ingredients, that were evaluated are listed in Table 14.

**Table 14. Potentially Interfering Substances Tested**

Substance ID	Substance/Class	Substance/Active Ingredient
No substance	Control	Copan Universal Transport Medium (UTM)
Albuterol Sulfate	Beta-adrenergic bronchodilator	Albuterol Sulfate (5mg/mL)
Anefrin	Nasal Spray	Oxymetazoline, 0.05%
BD Universal Transport Medium	Transport Media	N/A
Copan 3U045N.PH (Cepheid Swab/M)	Transport Media	N/A
Blood	Blood	Blood (Human)
Fluticasone Propionate Nasal Spray	Nasal corticosteroid	Fluticasone Propionate

Substance ID	Substance/Class	Substance/Active Ingredient
Menthol	Throat lozenges, oral anesthetic and analgesic	Benzocaine, Menthol
Mucin	Mucin	Purified Mucin protein (Bovine or porcine submaxillary gland)
Mupirocin	Antibiotic, nasal ointment	Mupirocin (20 mg/g=2%)
PHNY	Nasal Drops	Phenylephrine, 1%
Saline	Saline Nasal Spray	Sodium Chloride (0.65%)
Remel M4RT	Transport Media	N/A
Remel M5	Transport Media	N/A
Tamiflu	Anti-viral drugs	Zanamivir
Tobramycin	Antibacterial, systemic	Tobramycin
Zicam	Nasal Gel	Luffa operculata, Galphimia glauca, Histaminum hydrochloricum Sulfur (0.05%)
Zinc	Zinc supplement	Zinc Gluconate

The results from the study (Table 15) show that for most cases, 8 out of 8 replicates reported positive results for each combination of virus and substance tested and no interference was observed. When Zicam was initially tested at 15% w/v, interference was observed in the detection of Flu B and RSV A. However, when Zicam was tested at 7.5% w/v, no interference was observed.

**Table 15. Mean Ct values for Xpert Xpress CoV-2/Flu/RSV *plus*  
Targets Tested in the Presence of Potentially Interfering Substances**

Substance	Concentration Tested	Number of Correct Results/Number Tested					
		SARS-CoV-2/ USA-WA-1	Influenza A/Idaho/07/2018	H3N2 Flu A/ Hong Kong/ 45/2019	Flu B/ Washington /02/2019	RSV A/2/ Australia/61	RSV B/9320/ MA/77
Control Simulated NPS/NS Matrix (No substance)	100% (v/v)	8/8	8/8	8/8	8/8	8/8	8/8
Anefrin	15% (v/v)	8/8	8/8	8/8	8/8	8/8	8/8
Albuterol Sulfate	0.83 mg/mL	8/8	8/8	8/8	8/8	8/8	8/8
BD Universal Transport Medium	N/A	8/8	8/8	8/8	8/8	8/8	8/8
Blood	2% (v/v)	8/8	8/8	8/8	8/8	8/8	8/8
Copan Swab M	N/A	8/8	8/8	8/8	8/8	8/8	8/8
Fluticasone Propionate Nasal Spray	5 µg/mL	8/8	8/8	8/8	8/8	8/8	8/8
Menthol	1.7 mg/mL	8/8	8/8	8/8	8/8	8/8	8/8
Mucin	0.1% (w/v)	8/8	8/8	8/8	8/8	8/8	8/8
Mupirocin	10 mg/mL	8/8	8/8	8/8	8/8	8/8	8/8

Substance	Concentration Tested	Number of Correct Results/Number Tested					
		SARS-CoV-2/ USA-WA-1	Influenza A/Idaho/07/2018	H3N2 Flu A/ Hong Kong/ 45/2019	Flu B/ Washington /02/2019	RSV A/2/ Australia/61	RSV B/9320/ MA/77
PHNY	15% (v/v)	8/8	8/8	8/8	8/8	8/8	8/8
Remel M4RT	N/A	8/8	8/8	8/8	8/8	8/8	8/8
Remel M5	N/A	8/8	8/8	8/8	8/8	8/8	8/8
Saline	15% (v/v)	8/8	8/8	8/8	8/8	8/8	8/8
Tamiflu	7.5 mg/mL	8/8	8/8	8/8	8/8	8/8	8/8
Tobramycin	4 µg/mL	8/8	8/8	8/8	8/8	8/8	8/8
Zicam	15% (w/v)	8/8	8/8	8/8	5/8 <sup>a</sup>	7/8 <sup>b</sup>	8/8
Zinc	0.1 µg/mL	8/8	8/8	8/8	8/8	8/8	8/8

<sup>a</sup> With 15% (w/v) Zicam, a statistically significant difference was observed between the control mean Ct and the test mean Ct. Testing was repeated with 7.5% (w/v) Zicam and no clinically significant difference was observed between the control mean Ct and the test mean Ct.

<sup>b</sup> With 15% (w/v) Zicam, a statistically significant difference was observed between the control mean Ct and the test mean Ct. Testing was repeated with 7.5% (w/v) Zicam and no statistically significant difference was observed between the control mean Ct and the test mean Ct.

## 20.8 Carry-over Contamination

A study was conducted to assess whether the single-use, self-contained Xpert Xpress CoV-2/Flu/RSV *plus* cartridge prevents specimen and amplicon carryover by testing a negative sample immediately after testing of a very high positive sample in the same GeneXpert module. The negative sample used in this study consisted of simulated NPS/NS matrix and the positive sample consisted of high Flu B and high SARS-CoV-2 virus concentrations (Flu B/Wisconsin/10/2016 at 1.0e6 TCID<sub>50</sub>/mL and inactivated SARS-CoV-2 USA-WA1/2020 at 1e4 copies/mL) seeded into negative NPS/NS matrix. The negative sample was tested in a GeneXpert module at the start of the study. Following the initial testing of the negative sample, the high positive sample was processed in the same GeneXpert module immediately followed by another negative sample. This was repeated 20 times in the same module, resulting in 20 positives and 21 negatives for the module. The study was repeated using a second GeneXpert module for a total of 40 positive and 42 negative samples. All 40 positive samples were correctly reported as **SARS-CoV-2 POSITIVE; Flu A NEGATIVE; Flu B POSITIVE; RSV NEGATIVE**. All 42 negative samples were correctly reported as **SARS-CoV-2 NEGATIVE; Flu A NEGATIVE; Flu B NEGATIVE; RSV NEGATIVE** with the Xpert Xpress CoV-2/Flu/RSV *plus* test. No specimen or amplicon carry-over contamination was observed in this study.

## 21 References

1. Centers for Disease Control and Prevention. <https://www.cdc.gov/coronavirus/2019-ncov/index.html>. Accessed February 9, 2020.
2. bioRxiv. (<https://www.biorxiv.org/content/10.1101/2020.02.07.937862v1>). Accessed March 3, 2020.
3. Petric M, Comanor L, Petti CA. Role of the laboratory in diagnosis of influenza during seasonal epidemics and potential pandemics. *J Infect Dis*. 2006;194:S98-110.
4. Schweiger B, Zadow I, Heckler R, et al. Application of a fluorogenic PCR assay for typing and subtyping of influenza viruses in respiratory samples. *J Clin Micro*. 2000;38:1552-1558.
5. <http://www.cdc.gov/flu/about/viruses/types.htm>. Accessed on May 19, 2016.
6. <http://www.cdc.gov/RSV/index.html>. Accessed on March 14, 2013.
7. Acero-Bedoya, S., Wozniak, P. S., Sánchez, P. J., Ramilo, O., & Mejias, A. (2019). Recent trends in RSV immunoprophylaxis: clinical implications for the infant. *American journal of perinatology*, 36(S 02), S63-S67.
8. Solomon, D. A., Sherman, A. C., & Kanjilal, S. (2020). Influenza in the COVID-19 Era. *Jama*, 324(13), 1342-1343.
9. Centers for Disease Control and Prevention. Biosafety in Microbiological and Biomedical laboratories (refer to latest edition). <http://www.cdc.gov/biosafety/publications/>
10. Clinical and Laboratory Standards Institute. Protection of Laboratory Workers from Occupationally Acquired Infections; Approved Guideline. Document M29 (refer to latest edition).
11. REGULATION (EC) No 1272/2008 OF THE EUROPEAN PARLIAMENT AND OF THE COUNCIL of 16 December 2008 on the classification labeling and packaging of substances and mixtures amending and repealing, List of Precautionary Statements, Directives 67/548/EEC and 1999/45/EC (amending Regulation (EC) No 1907/2007).
12. Occupational Safety and Health Standards, Hazard Communication, Toxic and Hazard Substances (March 26, 2012) (29 C.F.R., pt. 1910, subpt. Z).

## 22 Cepheid Headquarters Locations

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## 23 Technical Assistance

Before contacting Cepheid Technical Support, collect the following information:

- Product name
- Lot number
- Serial number of the instrument
- Error messages (if any)
- Software version and, if applicable, Computer Service Tag Number

### US

Telephone: + 1 888 838 3222  
Email: [techsupport@cepheid.com](mailto:techsupport@cepheid.com)















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Contact information for all Cepheid Technical Support offices is available on our website: [www.cepheid.com/en\\_US/support/contact-us](http://www.cepheid.com/en_US/support/contact-us).



## 24 Table of Symbols

Symbol	Meaning
	Catalog number
	<i>In vitro</i> diagnostic medical device
	Do not reuse
	Batch code
	Consult instructions for use
	Caution
	Manufacturer
	Country of manufacture
	Contains sufficient for <i>n</i> tests
	Control
	Expiration date
	Temperature limitation
	Biological risks
	For prescription use only



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**For use under Emergency Use Authorization (EUA) Only**

## 25 Revision History

**Description of Changes:** 302-6991, Rev. A to Rev. B

**Purpose:** To address Conditions of Authorization for the Xpert Xpress CoV-2/Flu/RSV *plus* test

Section	Description of Change
18	Updated limitations statements to include additional information about performance characteristics and competitive interference.
20.6	<p>Updated value at which competitive strains were evaluated from “10<sup>4</sup> or higher titer units” to “10<sup>5</sup> or higher titer units”.</p> <p>Added “If the results reported less than 3 of 3 positive replicates, the concentration of the competing virus was reduced by 10-fold increments until no interference was observed.”</p> <p>Updated Table 13 to include data regarding Correct Calls at 1e5 RNA copies/mL.</p> <p>Added “In addition, SARS-CoV-2 at concentrations above 1e5 RNA copies/mL inhibited detection of Flu B at 3X LoD (Table 13).”</p>
25	Updated Revision History section.