GeneXpert®
Powered By CEPHEID INNOVATION

Xpert® Xpress CoV-2/Flu/RSV *plus*

REF XPRS4PLEX-10

Instructions for Use
CLIA Complexity: Waived
For Use with GeneXpert® Xpress System

℞ only **IVD**

Cepheid®

In Vitro Diagnostic Medical Device

302-8058, Rev. B
2025-05

Trademark, Patents and Copyright Statements

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

Xpert® Xpress CoV-2/Flu/RSV *plus*

℞only

For *In Vitro* Diagnostic Use

CLIA Complexity: Waived

A Certificate of Waiver is required to perform this test in a CLIA Waived setting. To obtain CLIA waiver information and a Certificate of Waiver, please contact your state health department. Additional CLIA waiver information is available at the Centers for Medicare and Medicaid website at www.cms.hhs.gov/CLIA.

Failure to follow the instructions or modification to the test system instructions will result in the test no longer meeting the requirements for waived classification.

1 Proprietary Name

Xpert® **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, performed on the GeneXpert® Xpress System, is an automated multiplexed real-time reverse transcriptase polymerase chain reaction (RT-PCR) test intended for use in the simultaneous *in vitro* qualitative detection and differentiation of severe acute respiratory syndrome coronavirus (SARS-CoV-2), influenza A, influenza B, and/or respiratory syncytial virus (RSV) viral RNA in nasopharyngeal swab and anterior nasal swab specimens collected from individuals with signs and symptoms of respiratory tract infection. Clinical signs and symptoms of respiratory tract infection due to SARS-CoV-2, influenza A, influenza B, and RSV can be similar.

The Xpert Xpress CoV-2/Flu/RSV *plus* test is intended for use in the differential detection of SARS-CoV-2, influenza A, influenza B and/or RSV RNA and aids in the diagnosis of COVID-19, influenza and/or RSV infections if used in conjunction with other clinical and epidemiological information, and laboratory findings. SARS-CoV-2, influenza A, influenza B, and RSV viral RNA are generally detectable in nasopharyngeal swab and anterior nasal swab specimens during the acute phase of infection.

Positive results are indicative of the presence of the identified virus, but do not rule out bacterial infection or co-infection with other pathogens not detected by the test. The agent(s) detected by the Xpert Xpress CoV-2/Flu/RSV *plus* test may not be the definite cause of the disease.

Negative results do not preclude SARS-CoV-2, influenza A, influenza B, and/or RSV infection. The results of this test should not be used as the sole basis for diagnosis, treatment or other patient management decisions.

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.[1] 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.[2]

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.[3]

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.[4] 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, 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).[5] The virus can cause both upper respiratory infections, such as colds, and lower respiratory infections manifesting as bronchiolitis and pneumonia.[5] 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.[5] RSV remains the leading cause for hospitalizations in infants worldwide.[6] 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.[4,5]

SARS-CoV-2, influenza, and RSV viruses can cause infections that present with very similar symptoms, making clinical differentiation between them very difficult.[7] 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 Xpress System. 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), envelope (E) and RNA-dependent RNA polymerase (RdRP) genes of the SARS-CoV-2 virus genome; matrix (M), basic polymerase (PB2), and acidic protein (PA) segments of the influenza A genome; matrix (M) and non-structural protein (NS) segments of the influenza B genome; and the nucleocapsid genes of RSV A and RSV B.

The GeneXpert Xpress System automates and integrates 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 system consists of an instrument, computer, and preloaded software for running tests and viewing the results. The system requires 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 system, see the *GeneXpert Xpress System User's Guide*.

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 or anterior nasal swab (NS) 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 (VTM)/Universal Transport Medium (UTM) 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, which performs hands-off, automated sample processing, and real-time RT-PCR for detection of viral RNA.

6 Reagents and Instruments

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 Integrated Reaction Tubes	**10**
• 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	**12 per kit**
Quick Reference Instructions (QRI) (For use with the GeneXpert Xpress System)	**1 per kit**
Flyer (with instructions to web location) for:	**1 per kit**
• Assay Definition File (ADF)	
• Instructions to import ADF into GeneXpert software	
• Instructions for Use	

Note Safety Data Sheets (SDS) are available at www.cepheid.com/edoc.

Note The protein stabilizer - bovine origin 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.

7 Kit 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

Sample Collection Swabs and Transport Media

Nylon flocked swabs, viral transport medium (VTM), Universal Transport Medium (UTM) and eNAT® Molecular Transport Medium are compatible for use with the Xpert Xpress CoV-2/Flu/RSV *plus* test.

The following materials are examples of those that are compatible with the Xpert Xpress CoV-2/Flu/RSV *plus* test:

Nasopharyngeal Sample Collection Kit for Viruses

- Copan UTM® 3C057N (Flexible Minitip Flocked Swab with UTM Medium)
- Copan eNAT Molecular Collection and Preservation Medium P/N 6U074S01 (Flexible Minitip Flocked Swab with eNAT Medium)
- BD Becton Dickinson Universal Viral Transport Kit P/N 220531 (Flexible Minitip Flocked Swab with UVT Medium)

Nasal Sample Collection Kit for Viruses

- Copan UTM 3C064N (Regular Flocked Swab with UTM Medium)
- Copan eNAT Molecular Collection and Preservation Medium P/N 6U073S01 (Regular Flocked Swab with eNAT Medium)

Alternatively, swabs and transport media can be obtained separately:

- Nylon flocked swab (Copan P/N 502CS01, 503CS01)
- Viral transport medium, 3 mL (Copan P/N 3C047N, Remel M4RT or Remel M5)

GeneXpert Xpress System

GeneXpert Xpress Instrument, GeneXpert Hub with integrated computer running proprietary GeneXpert Xpress Software Version 6.1 or higher, touchscreen monitor and barcode scanner, external CD drive, *Getting Started Guide*, and *GeneXpert Xpress System User's Guide*.

9 Materials Available but Not Provided

CD – available upon request

- ADF
- Import Instructions for ADF

External controls in the form of inactivated virus(es) are available from ZeptoMetrix (Buffalo, NY) for use with the Xpert Xpress CoV-2/Flu/RSV *plus* test.

- External Positive Control – NATtrol Flu/RSV/SARS-CoV-2; Cat # NATFRC-6C-IVD
- External Negative Control – Coxsackievirus A9; Cat # NATCV9-6C-IVD

10 Warnings and Precautions

10.1 General

- For *in vitro* diagnostic use.
- For prescription use only
- Positive results are indicative of presence of Flu A, Flu B, RSV, and/or SARS-CoV-2 RNA.
- Positive results for SARS-CoV-2 or suspected novel influenza should be reported to state, local, or federal health departments according to local reporting requirements.
- 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[8] and the Clinical and Laboratory Standards Institute.[9]
- Follow safety procedures set by your institution for working with chemicals and handling biological specimens.
- Refer to Copan eNAT Package Insert for safety and handling information.
- Avoid direct contact between guanidine thiocyanate and sodium hypochlorite (bleach) or other highly reactive reagents such as acids and bases. These mixtures could release noxious gas.
- Consult your institution’s environmental waste personnel on proper disposal of used cartridges, which may contain amplified material. This material may exhibit characteristics of federal EPA Resource Conservation and Recovery Act (RCRA) hazardous waste requiring specific disposal requirements. Check state and local regulations as they may differ from federal disposal regulations. Institutions should check the hazardous waste disposal requirements within their respective countries.
- 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.

- Used cartridges may contain potentially infectious materials, as well as PCR amplicons. Do not open or attempt to alter any part of the used cartridge for disposal.
- NPS and NS specimens should be collected with appropriate infection control precautions. Refer to the CDC Interim Guidelines for Collecting and Handling of Clinical Specimens for COVID-19 Testing for more information. https://www.cdc.gov/covid/hcp/clinical-care/clinical-specimen-guidelines.html. Viral culture should not be attempted in cases of positive results for SARS-CoV-2 and/or any similar microbial agents, unless a facility with an appropriate level of laboratory biosafety (*e.g.,* BSL 3 and BSL 3+, etc.) is available to receive and culture specimens.
- If infection with a novel Influenza A virus is suspected based on current clinical and epidemiological screening criteria recommended by public health authorities, specimens should be collected with appropriate infection control precautions for novel virulent influenza viruses and sent to state or local health departments for testing. Viral culture should not be attempted in these cases unless a BSL 3+ facility is available to receive and culture specimens.
- Quality control requirements must be performed in conformance with local, state, and/or federal regulations or accreditation requirements and your laboratory's standard quality control procedures.

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 collected in UTM/VTM 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.
- In the event of spill of specimens collected in Copan eNAT, refer to Copan eNAT Package Insert for proper handling of a spill.

11 Chemical Hazards[10, 11]

- **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.

Nasopharyngeal and anterior nasal swab specimens can be stored at room temperature (15–30 °C) for up to 48 hours in VTM/UTM or eNAT until testing is performed on the GeneXpert Xpress System. Alternatively, nasopharyngeal and anterior nasal swab specimens can be stored refrigerated (2–8 °C) up to seven days in viral transport medium and up to six days in eNAT until testing is performed on the GeneXpert Instrument Systems.

Nasopharyngeal and anterior nasal swab specimens collected in VTM/UTM or eNAT can be frozen at -20 °C or -80 °C and undergo 1 freeze/thaw cycle.

13 Starting the GeneXpert Xpress System

The recommended environmental operating conditions for Xpert Xpress CoV-2/Flu/RSV *plus* test are 15—30°C (59—86 °F), 20—80% relative humidity, noncondensing.

Note Before you start the test, make sure that the system is running GeneXpert Xpress software version 6.1 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 basic steps for running the test. For detailed instructions, see the *GeneXpert Xpress System User's Guide*.

1. Turn on the GeneXpert Xpress instrument.
2. Turn on the Hub computer.
   The **Windows Lock** screen appears.
3. Swipe up to continue.
   The **Windows Password** screen appears.
4. Touch **Password** to display the keyboard, then type your Windows password.
5. Touch the arrow button at the right of the password entry area.
   The GeneXpert Xpress software starts, and a **login** screen appears.
6. If enabled, you may log in by scanning a barcode on your institutional ID, using the barcode scanner (located behind the right side of the touchscreen). Then proceed to Step 10. Otherwise, follow the steps below to login manually.
7. Enter your User Name and Password (the virtual keyboard appears once you touch the entry fields).
8. Touch the **X**in the upper right of the virtual keyboard.
   The keyboard disappears and the **LOGIN** button appears at the bottom of the screen.
9. Touch the **LOGIN** button to continue.
10. The **Database Maintenance Reminder** screen and the **Archive Tests Reminder** dialog boxes may appear, depending on your system configuration. For more information, see the *GeneXpert Xpress System User's Guide*.

14 Procedure

14.1 Starting a Test

The following instructions showing how to prepare the sample and the cartridge are shown on screen in a video and are also described in the Quick Reference Instructions (QRI).

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

1. Put on a new pair of gloves if performing a new test.
2. Touch the **NEW TEST** button on the Home screen (see Figure 1).

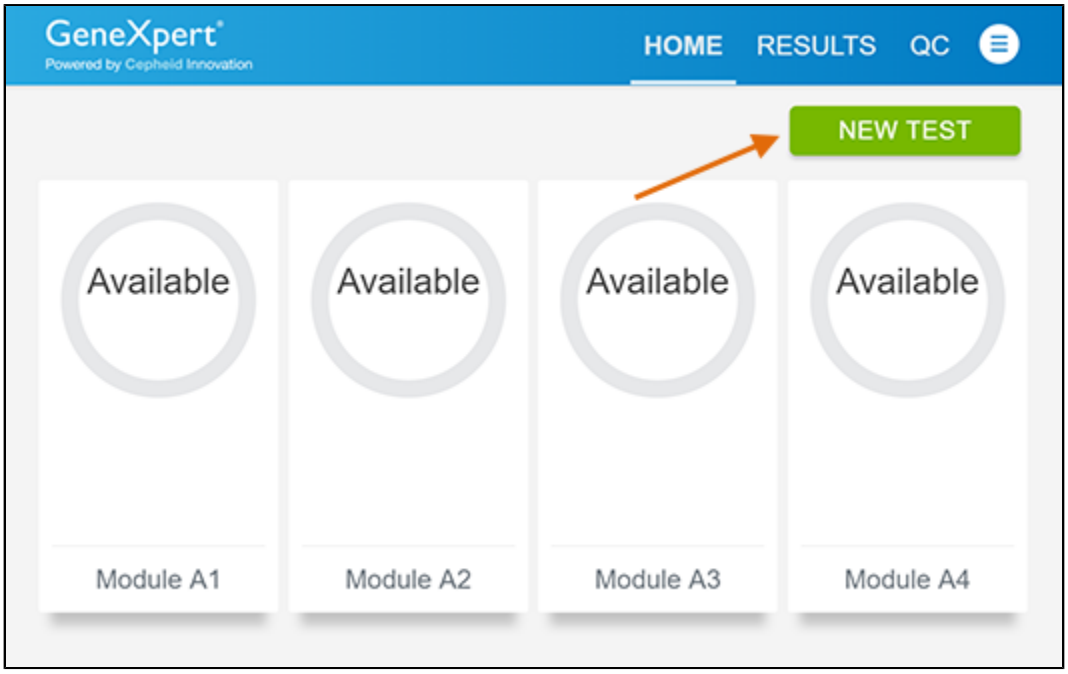


Figure 1. Home Screen

3. If Patient Information is configured by an administrator, then the **Patient Information** screen appears (see Figure 2). If Patient Information is not configured, the **Sample ID** screen appears. Skip to Section 14.2, Preparing the Specimen and Cartridge if the **Sample ID** screen appears.
4. Check that the specimen transport medium tube cap is closed.

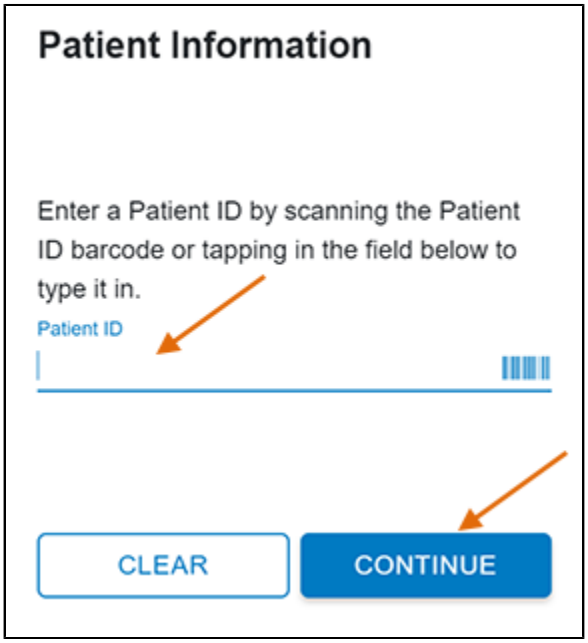


Figure 2. Patient Information Screen

5. Scan patient ID barcode or manually enter the Patient ID.
6. Touch **CONTINUE**. The **Confirm Patient Information** screen appears.

7. Verify the Patient ID and touch **CONFIRM**. The **Sample ID** screen appears.

14.2 Preparing the Specimen and Cartridge

1. Remove a cartridge and a transfer pipette from the cartridge kit box.
2. Check that the transport medium tube cap is closed. Scan Sample ID barcode or manually enter the Sample ID for patient specimen.
3. Touch **CONTINUE**. The **Confirm Sample ID** screen appears.
4. Verify the Sample ID and touch **CONFIRM**. The **Scan Cartridge Barcode** screen appears (see Figure 3).

   In the following steps, keep the cartridges upright when handling or scanning. Do not rotate or tip the cartridge, because damage to the contents or injury to personnel may occur.

Note If the barcode on the Xpert Xpress CoV-2/Flu/RSV *plus* cartridge does not scan or scanning the barcode results in an error message stating that the cartridge is expired, then repeat the test with a new cartridge. If you have scanned the cartridge barcode in the Xpress software and the assay definition file is not available, a screen will appear indicating the assay definition file is not loaded on the system. If this screen appears, refer to the kit flyer for instructions to web location to access the ADF or contact Cepheid Technical Support.

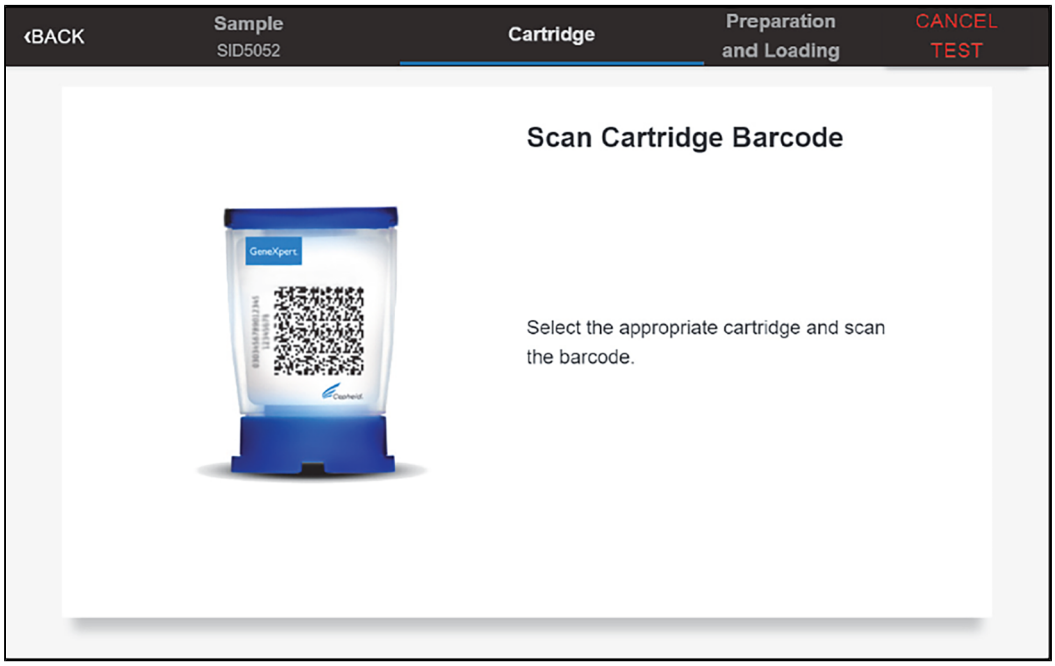


Figure 3. Scan Cartridge Barcode Screen

5. Select the appropriate cartridge with the sample and scan the cartridge barcode. After scanning, the **Select Test** screen appears.
6. Select the test to run (see Figure 4)

   - Flu A and Flu B: Select **Xpress Flu plus**
   - Flu A, Flu B and RSV: Select **Xpress Flu RSV plus**
   - SARS-CoV-2: Select **Xpress SARS-CoV-2 plus**
   - SARS-CoV-2, Flu A and Flu B: Select **Xpress SARS-CoV-2 Flu plus**
   - SARS-CoV-2, Flu A, Flu B and RSV: Select **Xpress SARS-CoV-2 Flu RSV plus**

   Only the test result for the assay selected at this step will be collected once the test is started. SARS-CoV-2, Flu A, Flu B, and RSV results will only be collected if the **Xpress_SARS-CoV-2_Flu_RSV** plus assay is selected.

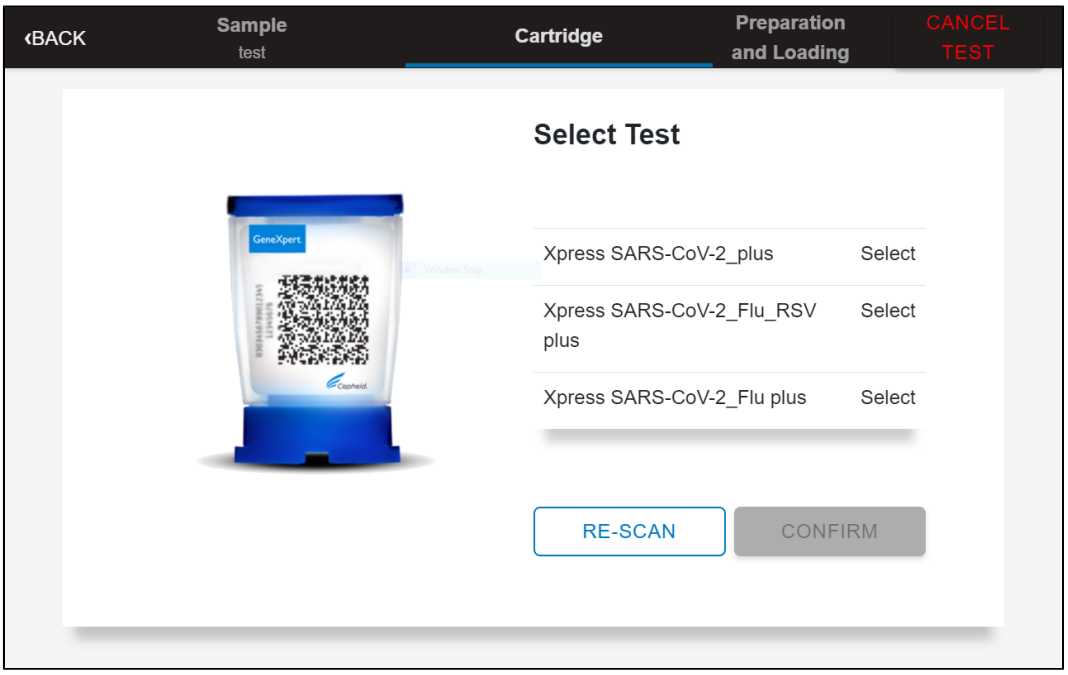


Figure 4. Select Test Screen

7. Verify that the correct cartridge has been scanned and that the assay name matches the name of the assay on the cartridge (see Figure 5).

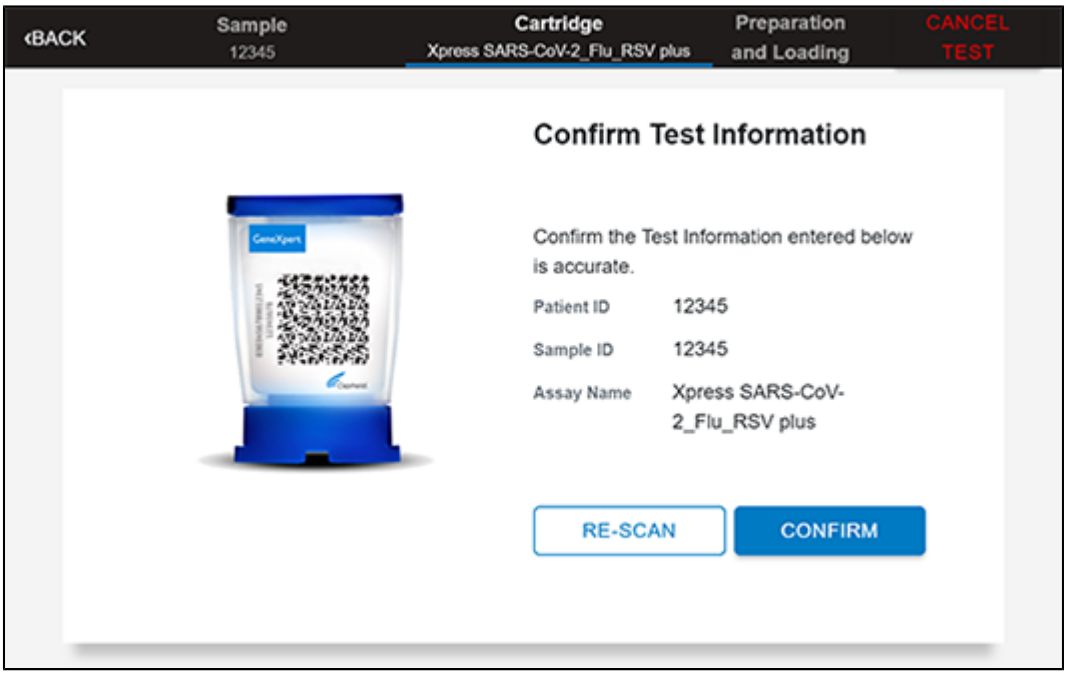


Figure 5. Confirm Test Information Screen

8. Touch **CONFIRM** if the displayed information is correct.
9. Depending on your configuration, the **Enter Credentials to Continue** screen may appear (see Figure 6). If enabled, you may log in by scanning your institutional ID. Otherwise, manually enter your User Name and Password and touch **LOGIN** to continue.

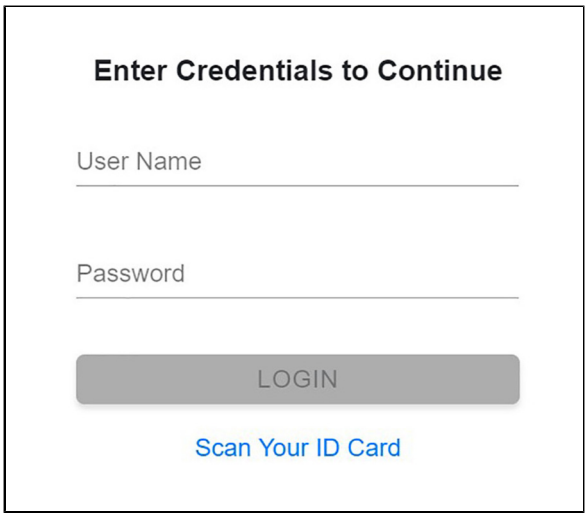


Figure 6. Enter Credentials to Continue Screen

10. The Cartridge Preparation screen appears (see Figure 7).

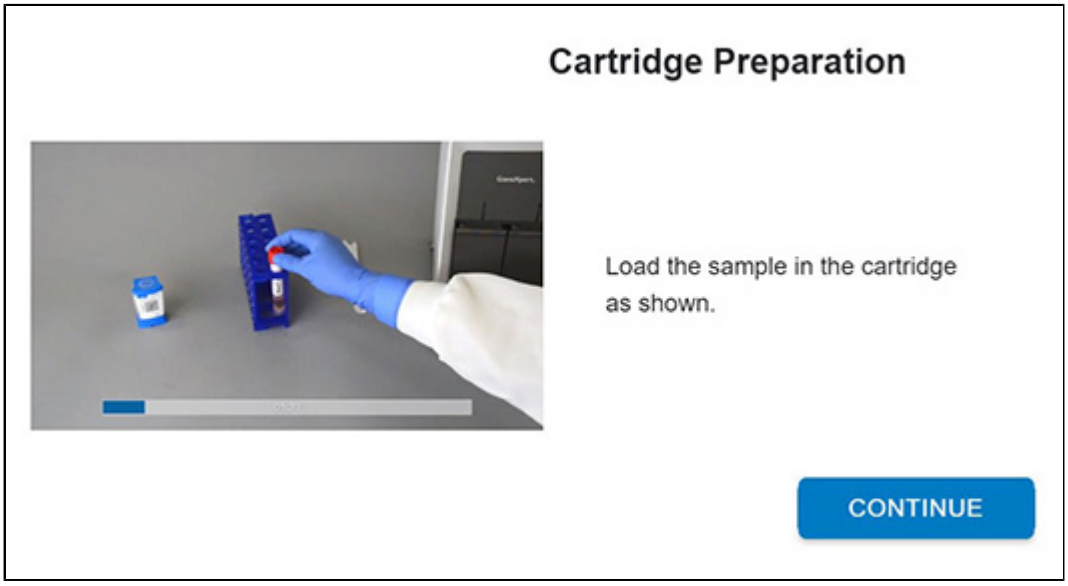


Figure 7. Cartridge Preparation Screen

11. Watch the video before continuing. The video will repeat. Touch the **SKIP VIDEO AND CONTINUE** button to exit video.

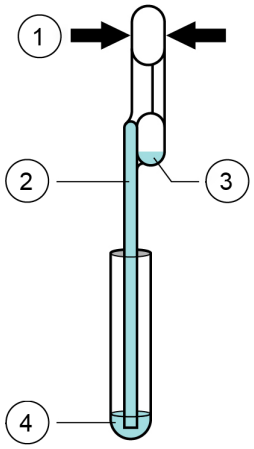
12. Mix specimen by rapidly inverting the specimen transport tube 5 times. Open the lid on the specimen transport tube.

13. Open the cartridge lid by lifting the front of the cartridge lid.

14. Remove the transfer pipette from the wrapper.

Note Do not place unwrapped pipette on the workbench.

15. 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 8).



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

Figure 8. Transfer Pipette

16. 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 8). Check that the pipette does not contain bubbles.
17. 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 9. Some liquid may remain in the overflow reservoir. Dispose of the used pipette.

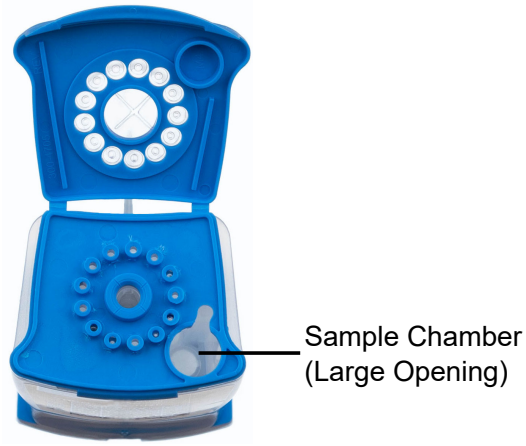


Figure 9. Xpert Xpress CoV-2/Flu/RSV *plus* Cartridge (Top View)

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

18. Close the cartridge lid.
19. Go to Section 14.4, Loading the Cartridge.

14.3 Running External Controls

It is recommended that external controls be tested at the frequency noted below.

- Each time a new lot of Xpert Xpress CoV-2/Flu/RSV *plus* kits is received.
- Each time a new shipment of Xpert Xpress CoV-2/Flu/RSV *plus* kits is received even if it is the same lot previously received.

- Each time a new operator is performing the test (i.e., operator who has not performed the test recently).
- When problems (storage, operator, instrument, or other) are suspected or identified.
- If otherwise required by your institution's standard Quality Control (QC) procedures.

1. Put on a new pair of gloves if performing a new test. Touch the **QC** button on the Home screen (see Figure 10).

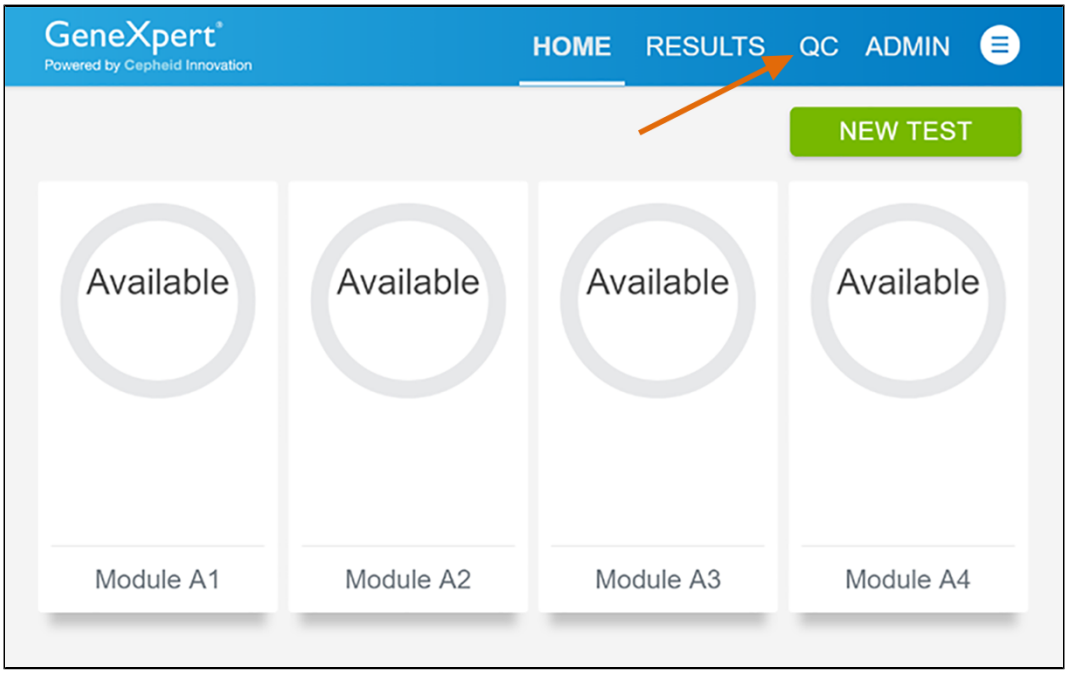


Figure 10. Home Screen

2. The Quality Control screen appears. Touch **RUN QC POSITIVE Test**, **RUN QC NEGATIVE TEST**, or **RUN PROFICIENCY TEST** option (Figure 11).

Figure 11. Quality Control Screen

3. The Sample ID appears.
4. Enter the Sample ID, by typing **Positive Control** or **Negative Control** or scan the Sample ID barcode.

5. Touch **CONTINUE**. The Confirm Sample ID screen appears.
6. Verify the Sample ID and touch **CONFIRM**. The Scan Cartridge Barcode screen appears (see Figure 12).

In the following steps, keep the cartridges upright when handling or scanning. Do not rotate or tip the cartridge, because damage to the contents or injury to personnel may occur.

Note If the barcode on the Xpert Xpress CoV-2/Flu/RSV *plus* cartridge does not scan or scanning the barcode results in an error message stating that the cartridge is expired, then repeat the test with a new cartridge. If you have scanned the cartridge barcode in the Xpress software and the assay definition file is not available, a screen will appear indicating the assay definition file is not loaded on the system. If this screen appears, refer to the kit flyer for instructions to web location to access the ADF or contact Cepheid Technical Support.

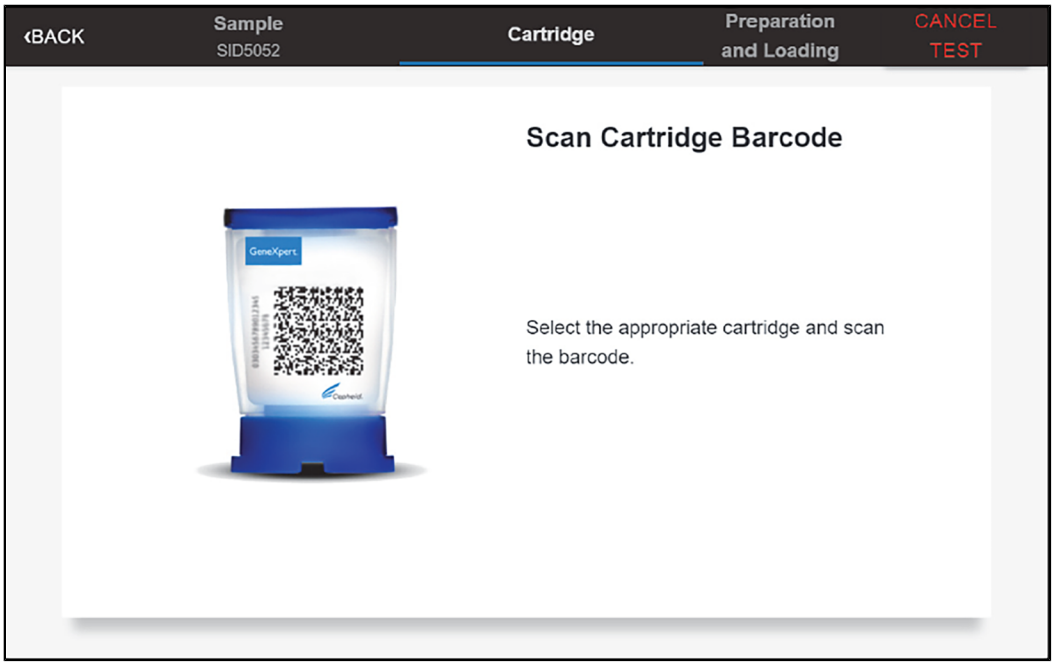


Figure 12. Scan Cartridge Barcode Screen

7. Select the appropriate cartridge with the sample and scan the cartridge barcode. After scanning, the **Select Test** screen appears.
8. Select **Xpress SARS-CoV-2 Flu RSV plus** from the Select Assay menu.
9. Confirm the test information is correct then touch **CONFIRM** (see Figure 13).

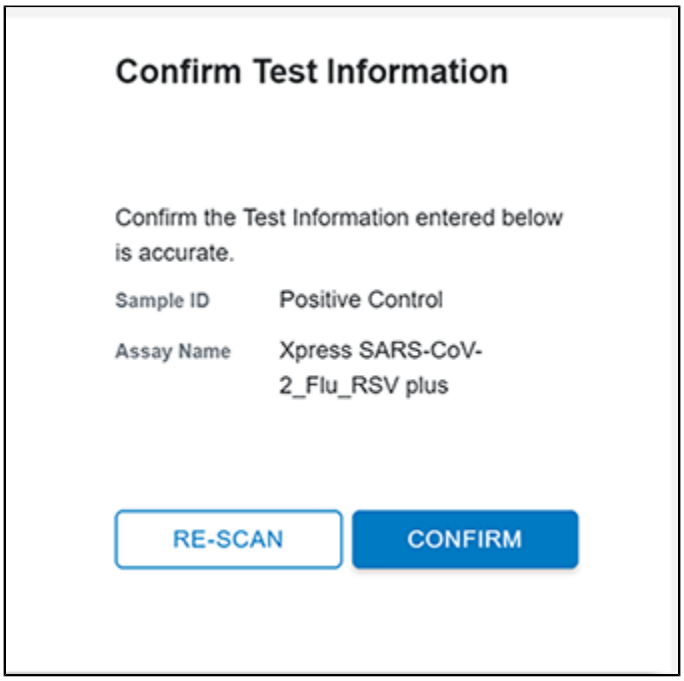
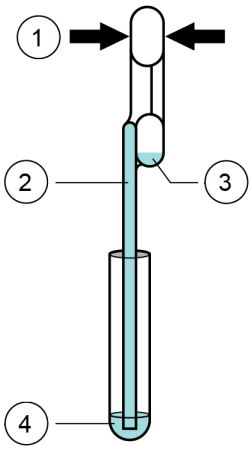


Figure 13. Confirm Test Information

10. Watch the video before continuing. The video will repeat. Touch the **CONTINUE** button to exit video.
11. Mix control by rapidly inverting the external control tube 5 times. Open the lid on the external control tube.
12. Open the cartridge lid by lifting the front of the cartridge lid.
13. Remove the transfer pipette from the wrapper.

Note Do not place unwrapped pipette on the workbench.

14. Squeeze the top bulb of the transfer pipette completely until the bulb is fully flat. While continuing to hold the bulb fully flat, place the pipette tip in the specimen transport tube (see Figure 14).



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

Figure 14. Transfer Pipette

15. Keeping the pipette below the surface of the liquid, release the top bulb of the pipette slowly to fill the pipette before removing from the tube. It is okay if liquid goes into the overflow reservoir (see Figure 14). Check that the pipette does not contain bubbles.
16. To transfer the external control to the cartridge, squeeze the top bulb of the pipette completely again until it is fully flat to empty the contents of the pipette into the large opening (Sample Chamber) of the cartridge shown in Figure 15. Dispose of the used pipette.

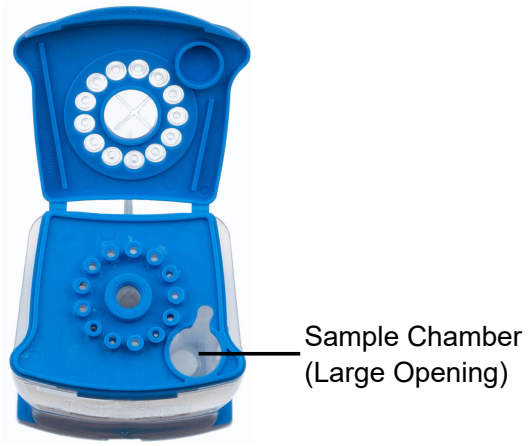


Figure 15. Xpert Xpress CoV-2/Flu/RSV *plus* Cartridge (Top View)

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

17. Close the cartridge lid.
18. Go to Section 14.4, Loading the Cartridge.

14.4 Loading the Cartridge

1. Touch the **CONTINUE** button on the Cartridge Preparation screen. The Load Cartridge into Module screen appears (see Figure 16).
2. Open the module door with the flashing green light.

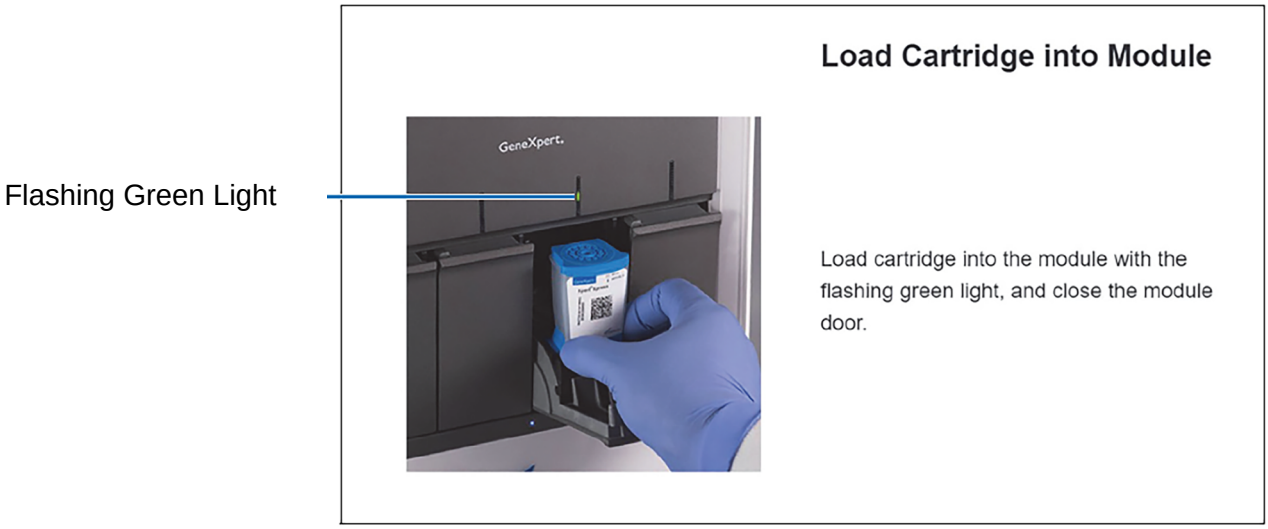


Figure 16. Load Cartridge into Module Screen

3. Load the cartridge with the barcode facing the operator on the cartridge bay platform. Do not try to insert the cartridge past the cartridge bay platform.
4. Close the door until it clicks. The green light will stop blinking and the test starts.
5. When the cartridge is loaded, the **Test Loading** screen appears, followed by the **Test Running** screen showing that the test is running. A circular graphic indicator at the right indicates the progress of the test and the time remaining until a test result is available.

Note While a test is running, you can start another test. See Section 14.5, Start a New Test While a Test is Running.

Note Do not turn off or unplug the instrument while a test is in progress. Turning off or unplugging the GeneXpert Xpress instrument stops the test. If necessary, touch the **STOP TEST** button to cancel a test while it is loading or running.

6. When the test is done, the green light goes out and the door automatically unlocks. The screen text changes to **Test Completed**. The **Test Completed** screen provides the results for the test just completed.

Note If an unexpected result occurs (e.g., Negative Quality Control result is positive or Positive Quality Control result is negative), test a new Quality Control sample using a new cartridge. If an unexpected result occurs upon retest, contact Cepheid Technical Support.

7. Open the module door, remove the used cartridge, and properly dispose of the cartridge according to your institution's policy.
8. Touch **HOME** to go back to the Home screen.
9. To log out, touch the **User Menu** icon, then select **Logout**.

14.5 Start a New Test While a Test is Running

You can start a new test while another test is in progress.

1. Touch the **HOME** button on the Test Running screen.
2. For a new user log in, touch the **User Menu** icon to log in.
3. Repeat the steps in Section 14.1, Starting a Test, Section 14.2, Preparing the Specimen, and Section 14.4, Loading the Cartridge.
4. After a second test has started, touch the **HOME** button. The status of both tests appears. The **Home** screen displays the module(s) in use with a circular graphic indicator around each test, and Patient Identification below the module graphic (see Figure 17).

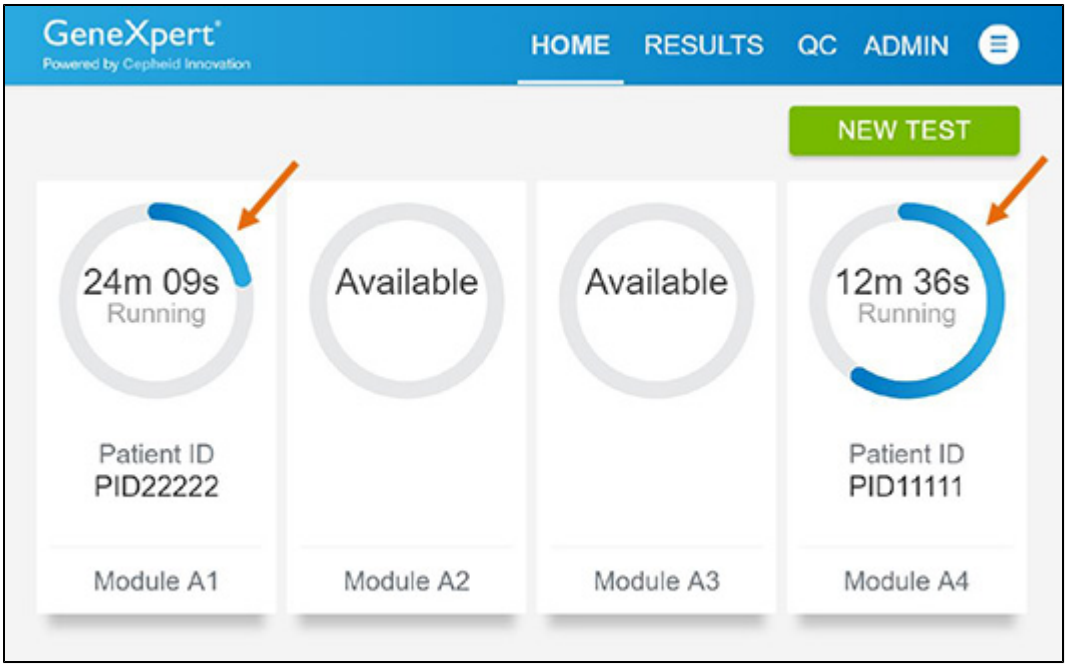


Figure 17. Home Screen Showing Two Tests Running

5. After a test has completed, the module icon text changes to Complete (see Figure 18). Touch **Complete View Result** to view test results.

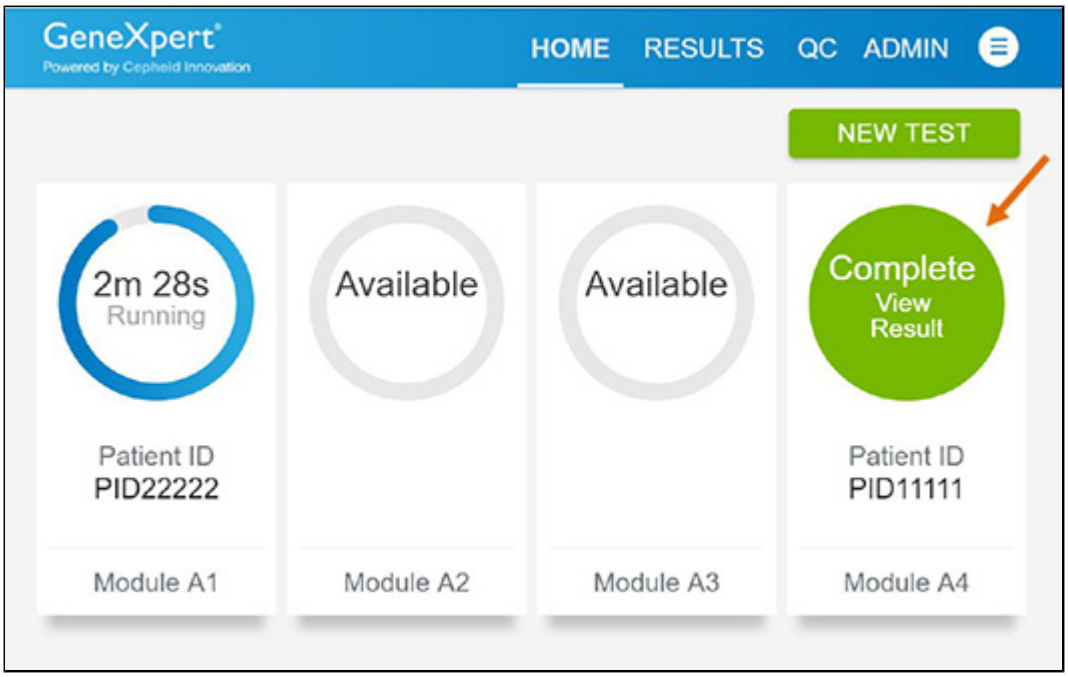


Figure 18. Home Screen with One of Two Tests Completed

14.6 Viewing Test Results

1. Touch the **RESULTS** button located on the panel at the top of the screen (see Figure 18). The **Results** screen appears (see Figure 19). Test results are, by default, in order of the date and time that the test was run. Navigate through the test result pages by touching the numbered buttons or arrows at the bottom of the screen.

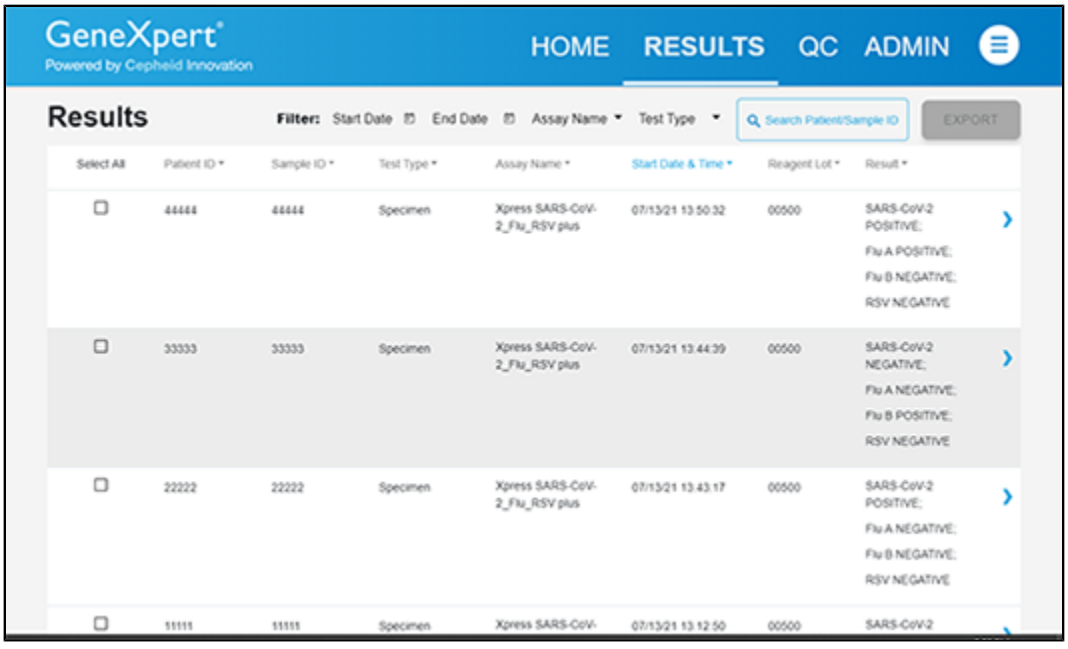


Figure 19. Results Screen

2. Touch the desired result to open the **Test Result** screen (see Figure 20).
3. To view test report, touch the **REPORT** button then swipe across the screen from left to right to minimize screen and view report.

Figure 20. Test Result Screen (Example)

Note If an unexpected result occurs (e.g., Negative Quality Control result is positive or Positive Quality Control result is negative), test a new Quality Control sample using a new cartridge. If an unexpected result occurs upon retest, contact Cepheid Technical Support.

15 Quality Control

15.1 Internal Controls

Each Xpert Xpress CoV-2/Flu/RSV *plus* cartridge includes two internal controls: 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 described in Section 9 are available but not provided and must be used in accordance with local, state, and/or federal regulations or accreditation requirements, as applicable.

16 Interpretation of Results

The results are interpreted automatically by the GeneXpert Xpress 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 one of the following tests:

- **Xpress SARS-CoV-2 Flu RSV plus**
- **Xpress SARS-CoV-2 Flu plus**
- **Xpress SARS-CoV-2 plus**
- **Xpress Flu plus**
- **Xpress Flu RSV plus**

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
SARS-CoV-2 POSITIVE	The SARS-CoV-2 target RNA is detected. ● The SARS-CoV-2 signal has a Ct within the valid range and endpoint above the minimum setting ● SPC: NA (not applicable); SPC is ignored because SARS-CoV-2 target amplification occurred ● Probe Check: PASS; all probe check results pass
Flu A POSITIVE	The Flu A target RNA is detected. ● The Flu A signal for either the Flu A1 RNA target or the Flu A2 RNA target or signals for both RNA targets have a Ct within the valid range and endpoint above the threshold setting. (Note: Flu A1 and Flu A2 are in different channels and a Flu A POSITIVE result requires signals from either one, or both, of the channels). ● SPC – NA; SPC is ignored because Flu A target amplification occurred. ● Probe Check – PASS; all probe check results pass.
Flu B POSITIVE	The Flu B target RNA is detected. ● The Flu B signal has a Ct within the valid range and endpoint above the minimum setting. ● SPC: NA; SPC is ignored because Flu B target amplification occurred. ● Probe Check: PASS; all probe check results pass.
RSV POSITIVE	The RSV target RNA is detected. ● The RSV signal has a Ct within the valid range and endpoint above the minimum setting. ● SPC: NA; SPC is ignored because RSV target amplification occurred. ● Probe Check: PASS; all probe check results pass.
SARS-CoV-2 NEGATIVE; **Flu A NEGATIVE;** **Flu B NEGATIVE;** **RSV NEGATIVE**	SARS-CoV-2 target RNA is not detected; Flu A target RNA is not detected; Flu B target RNA is not detected; RSV target RNA is not detected. ● SARS-CoV-2, Flu A, Flu B and RSV target RNAs are not detected. ● SPC – PASS; SPC has a Ct within the valid range and endpoint above the minimum setting. ● Probe Check – PASS; all probe check results pass.
NO RESULT-REPEAT TEST	If result is **NO RESULT - REPEAT TEST**, retest with a new cartridge according to the Section 17.2, Retest Procedure of the IFU. If retest is **NO RESULT - REPEAT TEST**, obtain a new specimen for testing.

Result	Interpretation
INSTRUMENT ERROR	If result is **INSTRUMENT ERROR**, touch **CLEAR ERROR** and follow the on-screen instructions. When the **Home** screen appears, repeat the test using a new cartridge according to the Section 17.2, Retest Procedure of the IFU.

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
SARS-CoV-2 POSITIVE	The SARS-CoV-2 target RNA is detected. ● The SARS-CoV-2 signal has a Ct within the valid range and endpoint above the minimum setting. ● SPC: NA (not applicable); SPC is ignored because SARS-CoV-2 target amplification occurred. ● Probe Check: PASS; all probe check results pass.
Flu A POSITIVE	The Flu A target RNA is detected. ● The Flu A signal for either the Flu A1 RNA target or the Flu A2 RNA target or signals for both RNA targets have a Ct within the valid range and endpoint above the threshold setting. (Note: Flu A1 and Flu A2 are in different channels and a Flu A POSITIVE result requires signals from either one, or both, of the channels). ● SPC – NA; SPC is ignored because the Flu A target amplification occurred. ● Probe Check – PASS; all probe check results pass.
Flu B POSITIVE	The Flu B target RNA is detected. ● The Flu B signal has a Ct within the valid range and endpoint above the minimum setting. ● SPC: NA; SPC is ignored because Flu B target amplification occurred ● Probe Check: PASS; all probe check results pass.
SARS-CoV-2 NEGATIVE; **Flu A NEGATIVE;** **Flu B NEGATIVE**	SARS-CoV-2 target RNA is not detected; Flu A target RNA is not detected; 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 above the minimum setting. ● Probe Check – PASS; all probe check results pass.
NO RESULT-REPEAT TEST	If result is **NO RESULT - REPEAT TEST**, retest with a new cartridge according to Section 17.2, Retest Procedure of the IFU. If retest is **NO RESULT - REPEAT TEST**, obtain a new specimen for testing.
INSTRUMENT ERROR	If result is **INSTRUMENT ERROR**, touch **CLEAR ERROR** and follow the on-screen instructions. When the **Home** screen appears, repeat the test using a new cartridge according to Section 17.2, Retest Procedure of the IFU.

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
SARS-CoV-2 POSITIVE	The SARS-CoV-2 target RNA is detected. ● The SARS-CoV-2 signal has a Ct within the valid range and endpoint above the minimum setting. ● SPC: NA (not applicable); SPC is ignored because SARS-CoV-2 target amplification occurred. ● Probe Check: PASS; all probe check results pass.
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 above the minimum setting. ● Probe Check – PASS; all probe check results pass.
NO RESULT-REPEAT TEST	If result is **NO RESULT - REPEAT TEST**, retest with a new cartridge according to the Section 17.2, Retest Procedure of the IFU. If retest is **NO RESULT - REPEAT TEST**, obtain a new specimen for testing.
INSTRUMENT ERROR	If result is **INSTRUMENT ERROR**, touch **CLEAR ERROR** and follow the on-screen instructions. When the **Home** screen appears, repeat the test using a new cartridge according to the Section 17.2, Retest Procedure of the IFU.

Table 4 shows the possible result outcomes when the **Xpress Flu *plus*** test mode is selected.

Table 4. Xpress Flu *plus* Possible Results and Interpretation

Result	Interpretation
Flu A POSITIVE	The Flu A target RNA is detected. ● The Flu A signal for either the Flu A1 RNA target or the Flu A2 RNA target or signals for both RNA targets have a Ct within the valid range and endpoint above the threshold setting. (Note: Flu A1 and Flu A2 are in different channels and a Flu A POSITIVE result requires signals from either one, or both, of the channels). ● SPC: NA; SPC is ignored because Flu A target amplification occurred. ● Probe Check: PASS; all probe check results pass.
Flu B POSITIVE	The Flu B target RNA is detected. ● The Flu B signal has a Ct within the valid range and endpoint above the minimum setting. ● SPC: NA; SPC is ignored because Flu B target amplification occurred. ● Probe Check: PASS; all probe check results pass.
Flu A NEGATIVE; Flu B NEGATIVE	Flu A target RNA is not detected; Flu B target RNA is not detected. ● Flu A and Flu B target RNAs are not detected. ● SPC: PASS; SPC has a Ct within the valid range and endpoint above the minimum setting. ● Probe Check: PASS; all probe check results pass.
NO RESULT – REPEAT TEST	If result is **NO RESULT – REPEAT TEST**, retest with a new cartridge according to the Section 17.2, Retest Procedure of the IFU. If retest is **NO RESULT – REPEAT TEST**, obtain a new specimen for testing.
INSTRUMENT ERROR	If result is **INSTRUMENT ERROR**, touch **CLEAR ERROR** and follow the on-screen instructions. When the **Home** screen appears, repeat the test using a new cartridge according to the Section 17.2, Retest Procedure of the IFU.

Table 5 shows the possible result outcomes when the Xpress Flu RSV test mode is selected.

Table 5. Xpress Flu RSV *plus* Possible Results and Interpretation

Result	Interpretation
Flu A POSITIVE	The Flu A target RNA is detected. ● The Flu A signal for either the Flu A1 RNA target or the Flu A2 RNA target or signals for both RNA targets have a Ct within the valid range and endpoint above the threshold setting. (Note: Flu A1 and Flu A2 are in different channels and a Flu A POSITIVE result requires signals from either one, or both, of the channels). ● SPC: NA; SPC is ignored because Flu A target amplification occurred. ● Probe Check: PASS; all probe check results pass.
Flu B POSITIVE	The Flu B target RNA is detected. ● The Flu B signal has a Ct within the valid range and endpoint above the minimum setting. ● SPC: NA; SPC is ignored because Flu B target amplification occurred. ● Probe Check: PASS; all probe check results pass.
RSV POSITIVE	The RSV target RNA is detected. ● The RSV signal has a Ct within the valid range and endpoint above the minimum setting. ● SPC: NA; SPC is ignored because RSV target amplification occurred. ● Probe Check: PASS; all probe check results pass.
Flu A NEGATIVE; Flu B NEGATIVE; RSV NEGATIVE	Flu A target RNA is not detected; Flu B target RNA is not detected; RSV target RNA is not detected. ● Flu A, Flu B and RSV target RNAs are not detected. ● SPC: PASS; SPC has a Ct within the valid range and endpoint above the minimum setting. ● Probe Check: PASS; all probe check results pass.
NO RESULT – REPEAT TEST	If result is **NO RESULT – REPEAT TEST**, retest with a new cartridge according to the Section 17.2, Retest Procedure of the IFU. If retest is **NO RESULT – REPEAT TEST**, obtain a new specimen for testing.
INSTRUMENT ERROR	If result is **INSTRUMENT ERROR**, touch **CLEAR ERROR** and follow the on-screen instructions. When the **Home** screen appears, repeat the test using a new cartridge according to the Section 17.2, Retest Procedure of the IFU.

The **Xpress SARS-CoV-2 *plus*** and **Xpress Flu *plus*** test modes include 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 or Flu A/B target reaches a predetermined threshold before all PCR cycles have been completed. When SARS-CoV-2 or Flu A/B titers are high enough to initiate the EAT function, the SPC and/or other target amplification curves may not be seen, and their results may not be reported.

17 Retests

17.1 Reasons to Repeat the Test

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

- An **INSTRUMENT ERROR** result could be due to, but not limited to, a system component failure, or the maximum pressure limits were exceeded.
- A **NO RESULT-REPEAT TEST** indicates that insufficient data were collected. For example, cartridge failed integrity test, Probe Check Control failure, no sample added, the operator stopped a test that was in progress, or a power failure occurred. Alternatively, other assay analysis settings intended to produce a valid test result were not met.

2. If an External Control fails to perform as expected, repeat external control test and/or contact Cepheid Technical Support for assistance.
3. Because the incidence of co-infection with three or more viruses (Influenza A, Influenza B, RSV and SARS-CoV-2) is low, it is recommended that specimens undergo repeat testing if nucleic acids from three or more viruses are detected in a single specimen.

17.2 Retest Procedure

To retest a non-determinate result (**NO RESULT-REPEAT TEST**, **INSTRUMENT ERROR**), use a new cartridge.

Use the leftover sample from the original specimen transport 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 by lifting the front of 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 and anterior nasal 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.
- The performance of the Xpert Xpress CoV-2/Flu/RSV *plus* test has not been specifically evaluated for nasopharyngeal swab and anterior nasal swab specimens from immunocompromised individuals.
- The clinical performance has not been established for all circulating variants of SARS-CoV-2 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.
- Performance characteristics for influenza A were established when influenza A/H3 and influenza A/H1-2009 were the predominate influenza strains. When other influenza A viruses are emerging, performance characteristics may differ.
- As with any molecular test, mutations within the target regions of the Xpert Xpress CoV-2/Flu/RSV *plus* test could affect primer and/or probe binding resulting in failure to detect the presence of target viruses or newly emerging variants.
- Positive and negative predictive values are highly dependent on prevalence. The likelihood of a negative result being false is higher during peak activity when prevalence of disease is high. The likelihood of a positive result being false is higher during periods when prevalence is moderate to low.
- This test cannot rule out diseases caused by non-target 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.
- When using the Xpert Xpress CoV-2/Flu/RSV *plus* test in the Flu Only mode, in the event of a mixed Flu A and Flu B infection where one target crosses the cycle threshold >5 cycles prior to the other target, the target with the higher titer of the two infections will be reported as POSITIVE and the lower titer target will be reported as NEGATIVE.
- False negative results may occur if on-panel viruses are present at levels below the analytical limit of detection.
- Negative results do not preclude SARS-CoV-2, influenza A, influenza B, or RSV infections and should not be used as the sole basis for diagnosis, 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.
- The Xpert Xpress CoV-2/Flu/RSV *plus* test is a qualitative test that reports Ct values for individuals that test positive for SARS-CoV-2, influenza A, influenza B, and/or RSV. These Ct values should not be interpreted as a measure of viral levels.
- The performance of this test has not been established for monitoring treatment of infection with any of the on-panel organisms. The performance of this test has not been established with postmortem specimens.
- The Xpert Xpress CoV-2/Flu/RSV *plus* test has not been validated for the testing of pooled specimens or the screening of specimens from asymptomatic individuals that do not have signs and symptoms of respiratory infection.
- The performance of this test has not been established screening of blood or blood products.
- Anterior nasal swab and nasopharyngeal swab specimens collected in 2 mL Copan eNAT, Remel M4RT, and Remel M5 are compatible for use with the Xpert Xpress CoV-2/Flu/RSV *plus* test. Performance of the Xpert Xpress CoV-2/Flu/RSV *plus* test with specimens collected in Copan eNAT, Remel M4RT and Remel M5 has been established in analytical studies, however, clinical performance of the assay in these media types was not established.
- The effect of interfering substances has only been evaluated for those listed within the labeling. Interference by substances other than those described may lead to erroneous results.
- FluMist was shown to interfere with detection of low levels of SARS-CoV-2 and RSV B at concentrations $>6.7x10^{-6}$ % (v/v) and was shown to interfere with detection of low levels of RSV A at concentrations $>6.7x10^{-7}$ % (v/v).
- Recent patient exposure to FluMist® or other live attenuated influenza vaccines may cause inaccurate positive influenza results.
- Human peripheral blood mononuclear cells (PBMC) at concentrations > 2.5×10^5 cells/mL were shown to interfere with detection of low levels of influenza B.
- Snuff at > 0.25% (w/v) was shown to interfere with the detection of low levels of influenza A and at > 0.1% (w/v) with the detection of low levels of influenza B.
- Zicam at 15% (w/v) was shown to interfere with the detection of low levels of influenza A, influenza B and RSV A.
- 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 may lead to erroneous results.
- As the Xpert Xpress CoV-2/Flu/RSV *plus* test does not differentiate between the N, RdRP and E gene targets, the presence of other coronaviruses in the B lineage, *Betacoronavirus* genus, including SARS-CoV and bat coronaviruses may cause a false positive result. None of these other coronaviruses are known to currently circulate in the human population.
- The RSV A primers and probes have a high degree of identity to Pangolin RSV A sequences and therefore may cross-react with Pangolin RSV A if the organism is circulating in a human population and present in a sample tested with the Xpert Xpress CoV-2/Flu/RSV *plus* test. None of these Pangolin RSV A strains are known to currently circulate in the human population.
- This test is not intended to differentiate RSV subgroups (i.e., A or B), influenza A subtypes (i.e., H1N1, H3N2) or influenza B lineages (i.e., Yamagata, Victoria). If differentiation of specific RSV or influenza subtypes and strains is needed, additional testing, in consultation with state or local public health departments, is required.
- In some samples with very high SARS-CoV-2 viral concentrations, analysis settings intended to reduce the risk of false positive results caused by non-specific or irregular fluorescence detection may trigger a **NO RESULT-REPEAT TEST**.

19 Expected Values

Expected values as determined by Xpert Xpress CoV-2/Flu/RSV *plus* are presented for Category I and II specimens, stratified by nasopharyngeal swab (NPS) and anterior nasal swab (NS) specimen types (Table 6) and by age group (Table 7).

Table 6. Positivity Rate Stratified by Specimen Type for Category I and II Specimens

Analyte	Prospectively Collected Fresh and Frozen Specimens[a] (Category I and Category II)			Prospectively Collected Frozen Specimens[b] from 2016-2017 Influenza Season (Category II)		
	Overall	NPS	NS	Overall	NPS	NS
SARS-CoV-2	19.9% (625/3147)	20.1% (314/1565)	19.7% (311/1582)	NA[c]	NA[c]	NA[c]
Flu A	6.2% (197/3186)	5.5% (87/1583)	6.9% (110/1603)	23.9% (269/1124)	25.5% (151/592)	22.2% (118/532)
Flu B	0.0% (0/3186)	0.0% (0/1583)	0.0% (0/1603)	8.5% (96/1124)	10.0% (59/592)	7.0% (37/532)
RSV	0.4% (12/3186)	0.4% (6/1583)	0.4% (6/1603)	15.8% (178/1124)	14.4% (85/592)	17.5% (93/532)

[a] One prospectively collected specimen was frozen and retrospectively tested.

[b] Prospectively collected and stored thereafter at -70°C.

[c] Not applicable – Specimens collected prior to the COVID-19 pandemic were expected to be negative for SARS-CoV-2 and were tested only for the Flu A, Flu B and RSV analytes.

Table 7. Positivity Rate Stratified by Age Group for Category I and II Specimens

Analyte	Prospectively Collected Fresh and Frozen Specimens[a] from 2022 (Category I and Category II)					Prospectively Collected Frozen Specimens[b] from 2016-2017 Influenza Season (Category II)				
	Overall	Age Group (years)				Overall	Age Group (years)			
		≤5	6-21	22-59	≥60		≤5	6-21	22-59	≥60
SARS CoV-2	19.9% (625/3147)	12.5% (23/184)	14.1% (122/867)	22.9% (381/1661)	22.8% (99/435)	N/A[c]	N/A[c]	N/A[c]	N/A[c]	N/A[c]
Flu A	6.2% (197/3186)	7.3% (13/179)	10.3% (91/880)	4.7% (80/1690)	3.0% (13/437)	23.9% (269/1124)	18.7% (82/438)	42.0% (87/207)	18.7% (67/358)	27.3% (33/121)
Flu B	0.0% (0/3186)	0.0% (0/179)	0.0% (0/880)	0.0% (0/1690)	0.0% (0/437)	8.5% (96/1124)	7.3% (32/438)	14.5% (30/207)	8.4% (30/358)	3.3% (4/121)
RSV	0.4% (12/3186)	0.6% (1/179)	0.7% (6/880)	0.3% (5/1690)	0.0% (0/437)	15.8% (178/1124)	33.1% (145/438)	0.5% (1/207)	3.4% (12/358)	16.5% (20/121)

[a] One prospectively collected specimen was frozen and retrospectively tested.

[b] Prospectively collected and stored thereafter at -70°C.

[c] Not applicable - Specimens collected prior to the COVID-19 pandemic were expected to be negative for SARS-CoV-2 and were tested only for the Flu A, Flu B and RSV analytes.

20 Performance Characteristics

20.1 Clinical Evaluation

The clinical performance of the Xpert Xpress CoV-2/Flu/RSV *plus* test was evaluated in a multi-site, observational and method comparison study that included 23 geographically diverse sites in the United States (US) using specimens collected from individuals showing signs and symptoms of respiratory infection. Of the 23 sites, 22 performed Xpert testing and specimen collection, and 1 site performed comparator and discrepant testing.

Specimens tested included prospective clinical NPS and NS specimens collected in UTM/VTM. Prospectively collected fresh clinical specimens (Category I) tested in the study were from a larger US specimen collection protocol. Fresh (3333/3334) and frozen (1/3334) specimens meeting the eligibility criteria were prospectively collected and tested in 2022. Due to low prevalence of Flu/RSV in 2022, archived prospectively collected frozen clinical specimens (Category II) collected during the 2016-2017 influenza season were used to supplement the sample size. These specimens represent contemporary Flu/RSV strains. Since these specimens were collected prior to the COVID-19 pandemic, they were expected to be negative for SARS-CoV-2 and therefore tested only for the Flu A, Flu B, and RSV targets. Available demographic data from the individuals from whom Category I and II specimens were collected are presented in Table 8.

Table 8. Demographic Summary for Category I and II Specimens

Prospectively Collected Clinical Specimens	NPS (N=2300)	NS (N=2261)	Overall (N=4561)
Gender			
Female	1296 (56.3%)	1374 (60.8%)	2670 (58.5%)
Male	1004 (43.7%)	887 (39.2%)	1891 (41.5%)
Age Group (Years)			
≤5	273 (11.9%)	402 (17.8%)	675 (14.8%)
6-21	600 (26.1%)	550 (24.3%)	1150 (25.2%)
22-59	1129 (49.1%)	1015 (44.9%)	2144 (47.0%)
≥60	298 (13.0%)	294 (13.0%)	592 (13.0%)
Specimen Testing			
Fresh	1654 (71.9%)	1679 (74.3%)	3333 (73.1%)
Frozen	646 (28.1%)	582 (25.7%)	1228 (26.9%)
Race			
Prospectively Collected in 2022			
American Indian or Alaska Native	1 (0.0%)	3 (0.1%)	4 (0.1%)
Asian	53 (2.3%)	58 (2.6%)	111 (2.4%)
Asian, White	6 (0.3%)	2 (0.1%)	8 (0.2%)
Black or African American	403 (17.5%)	389 (17.2%)	792 (17.4%)
Black or African American, White	6 (0.3%)	7 (0.3%)	13 (0.3%)
Native Hawaiian or Other Pacific Islander	1 (0.0%)	1 (0.0%)	2 (0.0%)
White	1042 (45.3%)	1035 (45.8%)	2077 (45.5%)
Other Mixed	2 (0.1%)	4 (0.2%)	6 (0.1%)
Missing, Declined to Answer or unknown	141 (6.1%)	180 (8.0%)	321 (7.0%)
Prospectively Collected Pre- Pandemic			
Not Available	645 (28.0%)	582 (25.7%)	1227 (26.9%)

Prospectively Collected Clinical Specimens	NPS (N=2300)	NS (N=2261)	Overall (N=4561)
Ethnicity			
Prospectively Collected in 2022			
Hispanic	136 (5.9%)	146 (6.5%)	282 (6.2%)
Non-Hispanic	1411 (61.3%)	1413 (62.5%)	2824 (61.9%)
Missing, Declined to Answer or Unknown	108 (4.7%)	120 (5.3%)	228 (5.0%)
Prospectively Collected Pre-Pandemic			
Not Available	645 (28.0%)	582 (25.7%)	1227 (26.9%)
COVID-19 Vaccination Status			
Prospectively Collected in 2022			
Vaccinated	1293 (56.2%)	1184 (52.4%)	2477 (54.3%)
Not Vaccinated	328 (14.3%)	468 (20.7%)	796 (17.5%)
Unknown	34 (1.5%)	27 (1.2%)	61 (1.3%)
Prospectively Collected Pre-Pandemic			
Not Available (Not Vaccinated)	645 (28.0%)	582 (25.7%)	1227 (26.9%)

Specimens were tested using Xpert Xpress CoV-2/Flu/RSV *plus* side-by-side with a U.S. FDA-cleared molecular respiratory panel that includes SARS-CoV-2 and a U.S. FDA-cleared molecular Flu A/B//RSV test, in a randomized and blinded fashion.

Positive Percent Agreement (PPA) and 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 a U.S. FDA-cleared molecular respiratory panel for the SARS-CoV-2 target, and a U.S.FDA-cleared molecular Flu A/B/RSV assay for the Flu A, Flu B, and RSV targets, respectively.

Discrepant results between Xpert Xpress CoV-2/Flu/RSV *plus* and the comparator for the SARS-CoV-2 target were investigated using a U.S. FDA EUA SARS-CoV-2 molecular test. Discrepant results between the Xpert Xpress CoV-2/Flu/RSV *plus* and the comparator for the Flu A/B/RSV targets were investigated using a U.S. FDA-cleared molecular respiratory panel.

A total of 3147 specimens, including 1565 NPS and 1582 NS specimens that yielded valid results by both the Xpert Xpress CoV-2/Flu/RSV *plus* and the U.S. FDA-cleared molecular respiratory panel, were included in the performance evaluation for SARS-CoV-2. A total of 4310 prospective (Category I and II) specimens, including 2175 NPS and 2135 NS specimens that yielded valid results by both the Xpert Xpress CoV-2/Flu/RSV *plus* and the U.S. FDA-cleared molecular Flu A/B/RSV assay were included in the performance evaluation for Flu A, Flu B, and RSV targets.

For the NPS specimens (both fresh and frozen specimens, combined), Xpert Xpress CoV-2/Flu/RSV *plus* demonstrated a PPA and NPA of 98.7% and 98.5% for SARS-CoV-2, respectively; 99.1% and 98.5% for Flu A, respectively; 96.6% and 99.9% for Flu B, respectively; 97.8% and 100.0% for RSV, respectively (**Table 9**). The initial non-determinate rate for the Xpert Xpress CoV-2/Flu/RSV *plus* test using NPS specimens was 2.5% (58/2300). On repeat testing, 51 specimens yielded valid results. The final non-determinate rate for the Xpert Xpress CoV-2/Flu/RSV *plus* test was 0.3% (7/2300).

Table 9. Xpert Xpress CoV-2/Flu/RSV *plus* Performance Results for NPS Specimens

Analyte	Specimen Collection	Numbers of Specimens	True Positive	False Positive	True Negative	False Negative	PPA (%)	95% CI	NPA (%)	95% CI
SARS-CoV-2	Fresh	1564	294	4[a]	1247	19[b]	98.7	96.6 - 99.5	98.5	97.7 - 99.0
	Frozen	1	1	0	0	0	100	20.7 - 100.0	NA	NA
	Overall	1565	295	4	1247	19	98.7	96.6 - 99.5	98.5	97.7 - 99.0
Flu A	Fresh	1582	79	0	1495	8[c]	100	95.4 - 100.0	99.5	99.0 - 99.7
	Frozen	593	130	2[d]	440	21[e]	98.5	94.6 - 99.6	95.4	93.1 - 97.0
	Overall	2175	209	2	1935	29	99.1	96.6 - 99.7	98.5	97.9 - 99.0
Flu B	Fresh	1582	0	0	1582	0	NA	NA	100	99.8 - 100.0
	Frozen	593	57	2[f]	532	2[g]	96.6	88.5 - 99.1	99.6	98.6 - 99.9
	Overall	2175	57	2	2114	2	96.6	88.5 - 99.1	99.9	99.7 - 100.0
RSV	Fresh	1582	6	0	1576	0	100	61.0 - 100.0	100	99.8 - 100.0
	Frozen	593	84	2[h]	506	1[i]	97.7	91.9 - 99.4	99.8	98.9 - 100.0
	Overall	2175	90	2	2082	1	97.8	92.4 - 99.4	99.9	99.7 -100.0
CI: 95% two-sided Confidence Interval; NA: Not Applicable										

[a] Discrepant test results based on a U.S. FDA EUA SARS-CoV-2 molecular test: 1/4 SARS-CoV-2 positive; 3/4 SARS-CoV-2 negative
[b] Discrepant test results based on a U.S. FDA EUA SARS-CoV-2 molecular test: 6/19 SARS-CoV-2 positive; 13/19 SARS-CoV-2 negative
[c] Discrepant test results based on a U.S. FDA-cleared molecular respiratory panel: 6/8 Flu A positive; 2/8 Flu A negative
[d] Discrepant test results based on a U.S. FDA-cleared molecular respiratory panel: 2/2 tests not performed due to the specimens being stored for a longer duration than allowed per the package insert
[e] Discrepant test results based on a U.S. FDA-cleared molecular respiratory panel: 21/21 tests not performed due to the specimens being stored for a longer duration than allowed per the package insert
[f] Discrepant test results based on a U.S. FDA-cleared molecular respiratory panel: 2/2 test not performed due to specimens being stored for a longer duration than recommended per the package insert
[g] Discrepant test results based on a U.S. FDA-cleared molecular respiratory panel: 2/2 test not performed due to specimens being stored for a longer duration than recommended per the package insert
[h] Discrepant test results based on a U.S. FDA-cleared molecular respiratory panel: 2/2 test not performed due to specimens being stored for a longer duration than recommended per the package insert
[i] Discrepant test results based on a U.S. FDA-cleared molecular respiratory panel: 1/1 test not performed due to specimens being stored for a longer duration than recommended per the package insert

For the NS specimens (both fresh and frozen specimens, combined), Xpert Xpress CoV-2/Flu/RSV *plus* demonstrated a PPA and NPA of 98.4% and 99.3% for SARS CoV-2, respectively; 97.6% and 98.9% for Flu A, respectively; 100.0% and 99.9% for Flu B, respectively; 97.0% and 99.9% for RSV, respectively (**Table 10**). The initial non-determinate rate for the Xpert Xpress CoV-2/Flu/RSV *plus* test using NS specimens was 2.9% (65/2261). On repeat testing, 46 specimens gave valid results upon retest. The final non-determinate rate for the Xpert Xpress CoV-2/Flu/RSV *plus* test was 0.8% (19/2261).

Table 10. Xpert Xpress CoV-2/Flu/RSV *plus* Performance Results for NS Specimens

Analyte	Specimen Collection	Numbers of Specimens	True Positive	False Positive	True Negative	False Negative	PPA (%)	95% CI	NPA (%)	95% CI
SARS-CoV-2	Fresh	1582	302	5[a]	1266	9[b]	98.4	96.2 - 99.3	99.3	98.7 - 99.6
	Frozen	0	0	0	0	0	NA	NA	NA	NA
	Overall	1582	302	5	1266	9	98.4	96.2 - 99.3	99.3	98.7 - 99.6
Flu A	Fresh	1603	107	4[c]	1489	3[d]	96.4	91.1 - 98.6	99.8	99.4 - 99.9
	Frozen	532	99	1[e]	413	19[f]	99.0	94.6 - 99.8	95.6	93.2 - 97.2
	Overall	2135	206	5	1902	22	97.6	94.6 - 99.0	98.9	98.3 - 99.2
Flu B	Fresh	1603	0	0	1603	0	NA	NA	100	99.8 - 100.0
	Frozen	532	34	0	495	3[g]	100	89.9 - 100.0	99.4	98.2 - 99.8
	Overall	2135	34	0	2098	3	100	89.9 - 100.0	99.9	99.6 - 100.0
RSV	Fresh	1603	6	1[h]	1596	0	85.7	48.7 - 97.4	100	99.8 - 100.0
	Frozen	532	91	2[i]	437	2[j]	97.8	92.5 - 99.4	99.5	98.4 - 99.9
	Overall	2135	97	3	2033	2	97.0	91.6 - 99.0	99.9	99.6 - 100.0
CI: 95% two-sided Confidence Interval; NA: Not Applicable										

[a] Discrepant test results based on a U.S. FDA EUA SARS-CoV-2 molecular test: 1/5 SARS-CoV-2 positive; 4/5 SARS-CoV-2 negative.

[b] Discrepant test results based on a U.S. FDA EUA SARS-CoV-2 molecular test: 2/9 SARS-CoV-2 positive; 4/9 SARS-CoV-2 negative; 2/9 invalid results; 1/9 discrepant testing was inadvertently not performed.

[c] Discrepant test results based on a U.S. FDA-cleared molecular respiratory panel: 2/4 Flu A positive; 2/4 Flu A negative.

[d] Discrepant test results based on a U.S. FDA-cleared molecular respiratory panel: 2/3 Flu A positive; 1/3 Flu A negative.

[e] Discrepant test results based on a U.S. FDA-cleared molecular respiratory panel: 1/1 tests not performed due to the specimens being stored for a longer duration than allowed per the package insert.

[f] Discrepant test results based on a U.S. FDA-cleared molecular respiratory panel: 19/19 tests not performed due to the specimens being stored for a longer duration than allowed per the package insert.

[g] Discrepant test results based on a U.S. FDA-cleared molecular respiratory panel: 3/3 tests not performed due to the specimens being stored for a longer duration than allowed per the package insert.

[h] Discrepant test results based on a U.S. FDA-cleared molecular respiratory panel: 1/1 RSV positive

[i] Discrepant test results based on a U.S. FDA-cleared molecular respiratory panel: 2/2 test not performed due to the specimens being stored for a longer duration than allowed per the package insert.

[j] Discrepant test results based on a U.S. FDA-cleared molecular respiratory panel: 2/2 test not performed due to the specimens being stored for a longer duration than allowed per the package insert.

The number of specimens with positive results for more than one target as detected by Xpert Xpress CoV-2/Flu/RSV *plus* is presented in **Table 11** and **Table 12**, where **bolded** values indicate concordant results.

Table 11. Multi-Target Detection by Xpert Xpress CoV-2/Flu/RSV *plus* for Specimens Collected in 2022

Infection		Comparator Results						
		SARS-CoV-2 only	Flu A only	RSV Only	SARS-CoV-2 and Flu A	Negative	Total	Co-Infection Rate (%)
Xpert Xpress CoV-2/ Flu/RSV *plus*	SARS-CoV-2 only	**568**	0	0	1	25	594	0.1
	Flu A only	0	**179**	0	0	11	190	
	RSV only	0	0	**12**	0	0	12	
	SARS-CoV-2 and Flu A	0	0	0	**1**	0	1	
	Negative	9	3	1	0	**2218**	2231	
	Total	577	182	13	2	2254	3028	
	Co-Infection Rate (%)	0.3						

As presented in **Table 11**, a total of 3028 Category I and II specimens collected in 2022 yielded valid results for SARS-CoV-2, Flu A, and RSV targets for both the Xpert Xpress CoV-2/Flu/RSV *plus* test and the comparator test. The co-infection rate for Xpert Xpress CoV-2/Flu/RSV *plus* was 0.1% (1/797) and the rate of co-infection by the comparator was 0.3% (2/774).

Table 12. Flu A, Flu B, and RSV Multi-Target Detection by Xpert Xpress CoV-2/Flu/RSV *plus* for Specimens Collected in 2016–2017 and 2022

Infection		Hologic Panther Fusion Flu A/B/RSV								
		Flu A only	Flu B only	RSV only	Flu A and Flu B	Flu A and RSV	Flu B and RSV	Negative	Total	Co-infection Rate (%)
Xpert Xpress CoV-2/ Flu/RSV *plus* Test	Flu A only	**406**	0	0	1	1	0	44	452	2.0
	Flu B only	0	**85**	0	0	0	0	3	88	
	RSV only	0	0	**179**	0	0	0	3	182	
	Flu A and Flu B	1	4	0	**1**	0	0	1	7	
	Flu A and RSV	0	0	2	0	**5**	0	0	7	
	Flu B and RSV	0	0	0	0	0	**1**	0	1	
	Negative	7	1	4	0	0	0	**3561**	3573	
	Total	414	90	185	2	6	1	3612	4310	
	Co-infection Rate (%) for the Comparator	1.3								

As presented in **Table 12**, of the 4310 Category I and II specimens evaluated for Flu A, Flu B and RSV targets, the co-infection rate for Xpert Xpress CoV-2/Flu/RSV *plus* was 2.0% (15/737) and the rate of co-infection by the comparator was 1.3% (9/698).

20.2 Analytical Sensitivity (Limit of Detection)

Clinical Nasopharyngeal Swab (NPS) Matrix

The analytical sensitivity of the Xpert Xpress CoV-2/Flu/RSV *plus* test was first estimated by using 2 reagent lots and testing limiting dilutions of viruses (NATtrol SARS-CoV-2, 1st World Health Organization (WHO) International Standard for SARS-CoV-2, Flu A H1, Flu A H3 , Flu B Victoria linage, Flu B Yamagata lineage, RSV A and RSV B) in pooled negative clinical NPS-UTM/VTM matrix, following the guidance in Clinical and Laboratory Standards Institute (CLSI) document EP17-A2. The LoD is defined as the lowest concentration for each strain at which 95% (19/20) of replicates yield a positive result. The estimated LoD values as determined by Probit regression analysis were verified using 2 lots of Xpert Xpress CoV-2/Flu/RSV *plus* reagents, by testing 20 replicates per virus/lot combination. The highest (least sensitive) LoD value for the two lots was reported as the final, verified LoD. The verified LoD values for the viruses tested are summarized in Table 13.

Table 13. Xpert Xpress CoV-2/Flu/RSV *plus* Limit of Detection in Clinical NPS-UTM/VTM Matrix

Virus/Strain	LoD Concentration
USA-WA1/2020 (NATtrol)	138 copies/mL
1st WHO International Standard	94 IU/mL
Flu A/Idaho/07/2018	0.007 $TCID_{50}$/mL
Flu A/California/07/2009	0.0022 $TCID_{50}$/mL
Flu A/Hong Kong/45/2019	0.44 FFU/mL
Flu A/Victoria/361/2011	0.05 $TCID_{50}$/mL
Flu B/Washington/2/2019	12.9 $CEID_{50}$/mL
Flu B/Wisconsin/10/2016	2.4 $TCID_{50}$/mL
RSV A/2/Australia/61	0.33 $TCID_{50}$/mL
RSV A/Long/MD/56	0.17 $TCID_{50}$/mL
RSV B/9320/MA/77	0.37 $TCID_{50}$/mL
RSV B/Wash/18537/62	0.2 $TCID_{50}$/mL

Clinical Anterior Nasal Swab (NS) Matrix

The analytical sensitivity of the Xpert Xpress CoV-2/Flu/RSV *plus* test in clinical anterior nasal swab (NS) matrix was first estimated by using 2 lots and testing limiting dilutions of viruses (NATtrol SARS-CoV-2, 1st World Health Organization (WHO) International Standard for SARS-CoV-2, Flu A H1, Flu A H3, Flu B Victoria linage, Flu B Yamagata lineage, RSV A and RSV B) in pooled negative clinical NS UTM/VTM matrix, following the guidance in Clinical and Laboratory Standards Institute (CLSI) document EP17-A2. The LoD is defined as the lowest concentration for each strain at which 95% (19/20) of replicates yield a positive result. The estimated LoD values as determined by Probit regression analysis were verified using 2 lots of Xpert Xpress CoV-2/Flu/RSV *plus* reagents. The highest (least sensitive) LoD value for the two lots was reported as the final, verified LoD. The verified LoD values for the viruses tested are summarized in Table 14.

Table 14. Xpert Xpress CoV-2/Flu/RSV *plus* Limit of Detection in Clinical NS-UTM/VTM Matrix

Virus/Strain	LoD Concentration
USA-WA1/2020 (NATtrol)	64 copies/mL
1st WHO International Standard	143 IU/mL

Virus/Strain	LoD Concentration
Flu A/Idaho/07/2018	0.012 $TCID_{50}$/mL
Flu A/California/07/2009	0.0028 $TCID_{50}$/mL
Flu A/Hong Kong/45/2019	0.49 FFU/mL
Flu A/Victoria/361/2011	0.065 $TCID_{50}$/mL
Flu B/Washington/2/2019	26.3 $CEID_{50}$/mL
Flu B/Wisconsin/10/2016	2.41 $TCID_{50}$/mL
RSV A/2/Australia/61	0.28 $TCID_{50}$/mL
RSV A/Long/MD/56	0.22 $TCID_{50}$/mL
RSV B/9320/MA/77	0.27 $TCID_{50}$/mL
RSV B/Wash/18537/62	0.4 $TCID_{50}$/mL

20.3 Analytical Reactivity (Inclusivity)

SARS-CoV-2 in silico Analyses

The inclusivity of Xpert Xpress CoV-2/Flu/RSV *plus* was evaluated using *in silico* analysis of the assay amplicons in relation to SARS-CoV-2 sequences available in the GISAID gene database as of June 15, 2022. The sequences were separated into the lineages of interest based on the Pango Lineage assigned to each genome by GISAID, and those with ambiguous nucleotides were removed. Thus, the following inclusivity analyses focus on the combined, non-ambiguous sequences from the variants of interest and variants of concern as of June 15, 2022. These constituted 10,310,839 sequences for the E target, 10,428,014 sequences for the N2 target, and 10,178,602 sequences for the RdRP target. Table 15 summarizes the effective predicted inclusivity for E, N2 and RdRP amplicons for the variants of interests and concern.

Table 15. Predicted Inclusivity for E, N2 and RdRP Amplicons for SARS-CoV-2 Variants of Interests and Concern

Amplicon	Exact Match	1 Mismatch[a]	2 or More Mismatches	% Total <2 Mismatches
CEP-COV-E-PLUS	10,262,080 of 10,310,839 (99.5%)	47,959 (0.5%)	800 (0.01%)	100%
CEP-COV-N2	10,228,739 of 10,428,014 (98.1%)	194,319 (1.9%)	4,956 (0.05%)	99.9%
CEP-COV-RDRP	10,092,873 of 10,178,602 (99.2%)	84,595 (0.8%)	1,134 (0.01%)	100%

[a] Single-nucleotide mismatches are predicted to not impact the performance of the test.

Based on the built-in redundancy of the Xpert Xpress CoV-2/Flu/RSV *plus* test's SARS-CoV-2 amplification system (i.e., 3 independent targets, only 1 of 3 must be detected to assign a positive result), it is not anticipated that any of the evaluated SARS-CoV-2 sequences would be missed by the Xpert Xpress CoV-2/Flu/RSV *plus* test.

SARS-CoV-2, Flu A, Flu B, and RSV Inclusivity Wet-Testing

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 concentrations of ~3x LoD in simulated matrix. A total of 102 respiratory viral strains comprised of 18 SARS-CoV-2 strains, 69 influenza viruses (48 influenza A

and 21 influenza B) and 15 RSV strains were evaluated for analytical reactivity (inclusivity) 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 3 replicates. Results are shown in Table 16.

Table 16. Analytical Reactivity (Inclusivity) of the Xpert Xpress CoV-2/Flu/RSV *plus* Test

Virus	Strain	Target Conc.	Result			
			SARS-CoV-2	Flu A	Flu B	RSV
SARS-CoV-2	NATtrol SARS-CoV-2 USA-WA1/2020	412 copies/mL	POS	NEG	NEG	NEG
	SARS-CoV-2/HongKong/VM20001061/2020	0.03 $TCID_{50}$/mL	POS[a]	NEG	NEG	NEG
	SARS-CoV-2/Italy-INMI1	1 $TCID_{50}$/mL	POS	NEG	NEG	NEG
	SARS-CoV-2/Africa/KRISPK005325/ 2020 (Beta)	0.025 $TCID_{50}$/mL	POS	NEG	NEG	NEG
	SARS-CoV-2/England/204820464/2020	0.05 $TCID_{50}$/mL	POS	NEG	NEG	NEG
	SARS-CoV-2 (NY-Wadsworth-21033899-01/2021) P1_2021 (Gamma)	0.01 $TCID_{50}$/mL	POS	NEG	NEG	NEG
	SARS-CoV-2 (NY-Wadsworth-21006055-01/2021) P2_2021 (Zeta)	0.03 $TCID_{50}$/mL	POS	NEG	NEG	NEG
	SARS-CoV-2 (NY-Wadsworth-21025952-01/2021) B.1.526_2021 (Iota)	0.1 $TCID_{50}$/mL	POS	NEG	NEG	NEG
	SARS-CoV-2 (NY-Wadsworth-103677-01/2020) B.1_2020	0.003 $TCID_{50}$/mL	POS	NEG	NEG	NEG
	SARS-CoV-2 (NY-Wadsworth-33126-01/2020) B.1.595_2020	0.0015 $TCID_{50}$/mL	POS	NEG	NEG	NEG
	SARS-CoV-2 (USA/CA-Stanford-15_S02/2021) B.1.617.1 (Kappa)	1.7 $TCID_{50}$/mL	POS	NEG	NEG	NEG
	SARS-CoV-2 (USA/PHC658/2021) B.1.617.2 (Delta)	0.01 $TCID_{50}$/mL	POS	NEG	NEG	NEG
	SARS-CoV-2 (USA/MDHP01542/ 2021) B.1.351 (Beta)	100 (genome equivalents/mL)	POS	NEG	NEG	NEG
	SARS-CoV-2 (USA/GA-EHC-2811C/20221) B.1.1.529 (Omicron)	100 (genome equivalents/mL)	POS	NEG	NEG	NEG
	SARS-CoV-2 RNA, USA/WA2/2020 (C09)[b]	100 copies/mL	POS	NEG	NEG	NEG

Virus	Strain	Target Conc.	Result			
			SARS-CoV-2	Flu A	Flu B	RSV
	SARS-CoV-2 RNA,England/205041766/2020 (C14) (alpha)[b]	100 copies/mL	POS	NEG	NEG	NEG
	SARS-CoV-2 RNA, England/ MILK-9E05B3/2020 (C15) (alpha)[b]	200 copies/mL	POS	NEG	NEG	NEG
	SARS-CoV-2 RNA /Japan (Brazil)/ IC-0564/2021 (C17) (gamma)[b]	100 copies/mL	POS	NEG	NEG	NEG
Flu A H1N1 (pre-2009)	A/swine/Iowa/15/30	10 $TCID_{50}$/mL	NEG	POS	NEG	NEG
	A/WS/33	0.6 $CEID_{50}$/mL	NEG	POS	NEG	NEG
	A/PR/8/34	1.25 $CEID_{50}$/mL	NEG	POS	NEG	NEG
	A/Mal/302/54	0.156 $CEID_{50}$/mL	NEG	POS	NEG	NEG
	A/Denver/1/57	1.5 $CEID_{50}$/mL	NEG	POS	NEG	NEG
	A/New Jersey/8/76	5 $CEID_{50}$/mL	NEG	POS	NEG	NEG
	A/New Caledonia/20/1999	0.10 $TCID_{50}$/mL	NEG	POS	NEG	NEG
	A/New York/55/2004	9 $TCID_{50}$/mL	NEG	POS	NEG	NEG
	A/Solomon Island/3/2006	0.0159 $TCID_{50}$/mL	NEG	POS	NEG	NEG
	A/Taiwan/42/06	0.002 $TCID_{50}$/mL	NEG	POS	NEG	NEG
	A/Brisbane/59/2007	0.008 $TCID_{50}$/mL	NEG	POS	NEG	NEG
	A/Swine/NY/02/2009	3.2 $TCID_{50}$/mL	NEG	POS	NEG	NEG
Flu A H1N1 (pdm 2009)	A/Colorado/14/2012	0.04 $TCID_{50}$/mL	NEG	POS	NEG	NEG
	A/Michigan/45/2015	15 $CEID_{50}$/mL	NEG	POS	NEG	NEG
	A/Iowa/53/2015	6 $CEID_{50}$/mL	NEG	POS	NEG	NEG
	A/Michigan/272/2017	0.07 $TCID_{50}$/mL	NEG	POS	NEG	NEG
	A/Idaho/07/2018	0.0159$TCID_{50}$/mL	NEG	POS	NEG	NEG
	A/Wisconsin/505/2018	0.08 $TCID_{50}$/mL	NEG	POS	NEG	NEG
	A/Hawaii/66/2019	100 $CEID_{50}$/mL	NEG	POS	NEG	NEG
	A/Indiana/02/2020	NA[c]	NEG	POS	NEG	NEG
Flu A H3N2	A/Aichi/2/68	2 $CEID_{50}$/mL	NEG	POS	NEG	NEG
	A/Hong Kong/8/68	0.25 $CEID_{50}$/mL	NEG	POS	NEG	NEG
	A/Port Chalmers/1/73	8 $CEID_{50}$/mL	NEG	POS	NEG	NEG
	A/Hawaii/15/2001	33 $CEID_{50}$/mL	NEG	POS	NEG	NEG
	A/Wisconsin/67/05c	0.22 $TCID_{50}$/mL	NEG	POS	NEG	NEG
	A/Brisbane/10/2007	0.003 $TCID_{50}$/mL	NEG	POS	NEG	NEG

Virus	Strain	Target Conc.	Result			
			SARS-CoV-2	Flu A	Flu B	RSV
	A/Minnesota/11/2010	2.4 $CEID_{50}$/mL	NEG	POS	NEG	NEG
	A/Indiana/08/2011	0.02 $TCID_{50}$/mL	NEG	POS	NEG	NEG
	A/Texas/50/2012	0.008 $TCID_{50}$/mL	NEG	POS	NEG	NEG
	A/Alaska/232/2015	2 $CEID_{50}$/mL	NEG	POS	NEG	NEG
	A/Singapore/INFIMH-16-0019/2016	2.5 $CEID_{50}$/mL	NEG	POS	NEG	NEG
	A/Texas/71/2017	1 FFU/mL	NEG	POS	NEG	NEG
	A/Kansas/14/2017	0.15 FFU/mL	NEG	POS	NEG	NEG
	A/Wisconsin/04/2018[d]	0.15 FFU/mL	NEG	POS	NEG	NEG
	A/Arizona/45/2018	2 FFU/mL	NEG	POS	NEG	NEG
	A/Hong Kong/45/2019	0.8 FFU/mL	NEG	POS	NEG	NEG
Avian Flu A[e]	A/Mallard/NY/6750/78 (H2N2)	< 1 pg/uL	NEG	POS	NEG	NEG
	A/duck/Hunan/795/2002 (H5N1)	< 1 pg/uL	NEG	POS	NEG	NEG
	A/Vietnam/1194/2004 (H5N1)	< 1 pg/uL	NEG	POS	NEG	NEG
	A/Anhui/01/2005 (H5N1)	< 1 pg/uL	NEG	POS	NEG	NEG
	A/Japanese white eye/Hong Kong/1038/2006 (H5N1)	< 1 pg/uL	NEG	POS	NEG	NEG
	A/mallard/WI/34/75 (H5N2)	< 1 pg/uL	NEG	POS	NEG	NEG
	A/turkey/Massachusetts/3740/1965 (H6N2)	0.1 fg/uL	NEG	POS	NEG	NEG
	A/duck/LTC-10-82743 (H7N2)	5 fg/uL	NEG	POS	NEG	NEG
	A/chicken/New Jersey/15086/3 (H7N3)	4 fg/uL	NEG	POS	NEG	NEG
	A/Anhui/1/2013 (H7N9)	0.612 ng/uL	NEG	POS	NEG	NEG
	A/Shanghai/1/2013 (H7N9)	NA[f]	NEG	POS	NEG	NEG
	A/chicken/New Jersey/12220/1997 (H9N2)	0.05 pg/uL	NEG	POS	NEG	NEG
Flu B	B/Lee/40	0.08 PFU/mL	NEG	NEG	POS	NEG
	B/Allen/45	0.25 $CEID_{50}$/mL	NEG	NEG	POS	NEG
	B/GL/1739/54	0.50 $CEID_{50}$/mL	NEG	NEG	POS	NEG
	B/Maryland/1/59	0.2 $CEID_{50}$/mL	NEG	NEG	POS	NEG
	B/Taiwan/2/62	0.7 $CEID_{50}$/mL	NEG	NEG	POS	NEG
	B/Hong Kong/5/72	1 $CEID_{50}$/mL	NEG	NEG	POS	NEG

Virus	Strain	Target Conc.	Result			
			SARS-CoV-2	Flu A	Flu B	RSV
Flu B (Victoria Lineage)	B/Panama/45/90	0.125 $TCID_{50}$/mL	NEG	NEG	POS	NEG
	B/Malaysia/2506/04	0.001 $TCID_{50}$/mL	NEG	NEG	POS	NEG
	B/Florida/02/06	0.004 $TCID_{50}$/mL	NEG	NEG	POS	NEG
	B/Brisbane/60/2008	0.005 $TCID_{50}$/mL	NEG	NEG	POS	NEG
	B/Maryland/15/2016	0.06 $TCID_{50}$/mL	NEG	NEG	POS	NEG
	B/Colorado/6/2017	0.01 $TCID_{50}$/mL	NEG	NEG	POS	NEG
	B/Hawaii/01/2018	1 $TCID_{50}$/mL	NEG	NEG	POS	NEG
	B/Missouri/12/2018 (NA D197E)	1.2 $TCID_{50}$/mL	NEG	NEG	POS	NEG
	B/Washington/02/2019	60 $TCID_{50}$/mL	NEG	NEG	POS	NEG
Flu B (Yamagata Lineage)	B/Florida/07/2004	0.03 $TCID_{50}$/mL	NEG	NEG	POS	NEG
	B/Florida/04/06	0.03 $TCID_{50}$/mL	NEG	NEG	POS	NEG
	B/Wisconsin/01/2010	0.025 $CEID_{50}$/mL	NEG	NEG	POS	NEG
	B/Wisconsin/10/2016	2 $TCID_{50}$/mL	NEG	NEG	POS	NEG
	B/Indiana/17/2017	0.5 $TCID_{50}$/mL	NEG	NEG	POS	NEG
	B/Oklahoma/10/2018	1 $TCID_{50}$/mL	NEG	NEG	POS	NEG
RSV A	RSV-A/NY	0.386 $TCID_{50}$/mL	NEG	NEG	NEG	POS
	RSV-A/WI-629.8.2/2007	0.50 $TCID_{50}$/mL	NEG	NEG	NEG	POS
	RSV-A/WI/629-11-1_2008	0.50 $TCID_{50}$/mL	NEG	NEG	NEG	POS
	RSV-A, Strain: 4/2015 Isolate #1	0.03 $TCID_{50}$/mL	NEG	NEG	NEG	POS
	RSV-A (2014, Isolate 342)	0.38 IU/mL	NEG	NEG	NEG	POS
	RSV-A (A2 cpts-248 mutant)	1600 copies/mL	NEG	NEG	NEG	POS
	RSV-A (2000/3-4)	0.0015 $TCID_{50}$/mL	NEG	NEG	NEG	POS
	RSV-A (2001/3-12)	0.28 $TCID_{50}$/mL	NEG	NEG	NEG	POS
	RSV-A (1997/12-35)	0.5 $TCID_{50}$/mL	NEG	NEG	NEG	POS
	RSV-A (*Homo sapiens*/ARG/177/2006)	0.089 $TCID_{50}$/mL	NEG	NEG	NEG	POS
	RSV-A (1998/3-2)	0.0089 $TCID_{50}$/mL	NEG	NEG	NEG	POS
RSV B	RSV-B/WV14617/85	0.04 $TCID_{50}$/mL	NEG	NEG	NEG	POS
	RSV-B-CH93(18)-18-01	0.004 $TCID_{50}$/mL	NEG	NEG	NEG	POS
	RSV-B (12/2014, Isolate #1)	0.008 $TCID_{50}$/mL	NEG	NEG	NEG	POS
	RSV-B (cp23 Clone 1A2)	4200 copies/mL	NEG	NEG	NEG	POS

[a] One of three replicates was Invalid. The run was successfully repeated to obtain three valid replicates.

[b] *In vitro* transcripts from Twist Biosciences.

[c] Influenza A/Indiana/02/2020 virus was without titer and the stock was diluted 48,000-fold in simulated matrix for testing.

[d] One of three replicates yielded an **ERROR** result. The run was successfully repeated to obtain three valid replicates.

[e] Purified viral RNA in TE and diluted in simulated matrix was tested due to biosafety regulations.

[f] Inactivated avian influenza A (H7N9) viral RNA without viral titer was diluted 100,000-fold in simulated matrix for testing due to biosafety regulations.

20.4 Analytical Specificity (Exclusivity)

In silico Analyses

An *in silico* analysis for possible cross-reactions with all the organisms listed in **Table 17** was conducted by mapping the SARS-CoV-2 oligonucleotides and amplicons in the Xpert Xpress CoV-2/Flu/RSV *plus* test individually to the sequences downloaded from the GISAID database. E gene primers and probes are not specific for SARS-CoV-2 and will detect Human and Bat SARS-coronavirus.

In silico exclusivity analysis using Flu A, Flu B and RSV primer and probe oligonucleotides against the GenBank database (which encompasses essentially all species) produced a large number of matches with at least 80% similarity to each oligonucleotide. The vast majority of these matches are to species (e.g., plant species) that would not be expected to occur in any patient sample. While there was some homology ≥80% to human genomic DNA, the matches were to different chromosomal regions, and there were no cases where a forward and reverse primer for a specific target matched to the same human genomic DNA fragment.

In silico exclusivity analysis using the five Flu amplicons (Flu A MP, Flu A PB2, Flu A PA, Flu B MP and Flu B NS) against the GenBank database produced no significant matches to non-influenza-related sequences. Similarly, no matches to RSV isolates from other species or to genomic sequences from non-RSV species were observed with the RSV B amplicon. While no matches of the RSV A amplicon to genomic sequences from non-RSV species of ≥80% homology were observed, the RSV A amplicon shared a 95% identity with two Pangolin RSV A isolates.

Therefore, the RSV A primers and probe may cross-react with Pangolin RSV A if the strain is circulating in a human population and present in a sample tested with the Xpert Xpress CoV-2/Flu/RSV *plus* test.

No cross reactivity with non-SARS-CoV-2, non-influenza and non-RSV viruses listed in Table 17 is expected based on the *in silico* analysis.

Table 17. 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	**Viruses**
Human coronavirus OC43	Adenovirus (e.g., C1 Ad. 71)
Human coronavirus HKU1	Cytomegalovirus
Human coronavirus NL63	Enterovirus (e.g., EV68)
SARS-coronavirus	Epstein-Barr virus
MERS-coronavirus	Human Metapneumovirus (hMPV)
Bat coronavirus	Influenza A & B
	Measles
	Mumps
	Parainfluenza virus 1-4
	Parechovirus
	Respiratory syncytial virus
	Rhinovirus
	Bacteria
	Bacillus anthracis

Microorganisms from the Same Genetic Family	High Priority Organisms
	Bordetella pertussis
	Bordetella parapertussis
	Candida albicans
	Chlamydia pneumoniae
	Chlamydia psittaci
	Corynebacterium diphtheriae
	Coxiella burnetii (Q-Fever)
	Escherichia coli
	Fusobacterium necrophorum
	Haemophilus influenzae
	Lactobacillus sp.
	Legionella non-*pneumophila*
	Legionella pneumophila
	Leptospira
	Moraxella catarrhalis
	Mycobacterium tuberculosis
	Mycoplasma genitalium
	Mycoplasma pneumoniae
	Neisseria elongata
	Neisseria meningitidis
	Pneumocystis jirovecii (PJP)
	Pseudomonas aeruginosa
	Staphylococcus aureus
	Staphylococcus epidermidis
	Staphylococcus salivarius
	Streptococcus pneumoniae
	Streptococcus pyogenes
	Fungi
	Aspergillus sp

Wet-Testing

In addition to the *in silico* analysis of the SARS-CoV-2, influenza A, influenza B, and RSV oligonucleotides and amplicons 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 1.2×10^6 IFU/mL and *Lactobacillus reuteri* which was tested at 5×10^7 copies/mL of genomic DNA. Viruses were tested at concentrations of $\geq 1 \times 10^5$ $TCID_{50}$/mL. The analytical specificity was 100%. Results are shown in Table 18.

Table 18. Respiratory Microorganisms and Human Coronavirus Tested, Concentrations and Xpert Xpress CoV-2/Flu/RSV *plus* Test Results

Count	Strain	Tested Concentration	SARS-CoV-2	Flu A	Flu B	RSV
0	Negative Control	Not Applicable	NEG	NEG	NEG	NEG
00	Positive Control (NATFRC-6C)	Not Applicable	POS	POS	POS	POS
1	Human coronavirus NL63	1.17e5 $TCID_{50}$/mL	NEG	NEG	NEG	NEG
2	MERS-coronavirus	1.17e5 $TCID_{50}$/mL	NEG	NEG	NEG	NEG
3	Human coronavirus 229E	1.21e5 $TCID_{50}$/mL	NEG	NEG	NEG	NEG
4	Human coronavirus OC43	1.02e5 $TCID_{50}$/mL	NEG	NEG	NEG	NEG
5	Human coronavirus HKU1[a]	1.23e6 copies/mL	NEG	NEG	NEG	NEG
6	Adenovirus Type 1	4.07e5 $TCID_{50}$/mL	NEG	NEG	NEG	NEG
7	Adenovirus Type 7	1.15e5 $TCID_{50}$/mL	NEG	NEG	NEG	NEG
8	Cytomegalovirus	1.0e5 $TCID_{50}$/mL	NEG	NEG	NEG	NEG
9	Echovirus	1.14e5 $TCID_{50}$/mL	NEG	NEG	NEG	NEG
10	Enterovirus	2.80e5 $TCID_{50}$/mL	NEG	NEG	NEG	NEG
11	Epstein Barr Virus	5.60e6 $TCID_{50}$/mL	NEG	NEG	NEG	NEG
12	HSV	1.97e5 $TCID_{50}$/mL	NEG	NEG	NEG	NEG
13	Human metapneumovirus	4.07e5 $TCID_{50}$/mL	NEG	NEG	NEG	NEG
14	Human parainfluenza Type 1	1.0e5 $TCID_{50}$/mL	NEG	NEG	NEG	NEG
15	Human parainfluenza Type 2	1.2e5 $TCID_{50}$/mL	NEG	NEG	NEG	NEG
16	Human parainfluenza Type 3	1.2e5 $TCID_{50}$/mL	NEG	NEG	NEG	NEG
17	Human parainfluenza Type 4	1.19e6 $TCID_{50}$/mL	NEG	NEG	NEG	NEG
18	Measles	1.2e5 $TCID_{50}$/mL	NEG	NEG	NEG	NEG
19	Mumps virus	1.2e5 $TCID_{50}$/mL	NEG	NEG	NEG	NEG
20	Rhinovirus Type 1A	1.0e5 $TCID_{50}$/mL	NEG	NEG	NEG	NEG
21	*Acinetobacter baumannii*	1.30e7 CFU/mL	NEG	NEG	NEG	NEG
22	*Bordetella pertussis*	6.40e7 CFU/mL	NEG	NEG	NEG	NEG
23	*Burkholderia cepacia*	1.90e8 CFU/mL	NEG	NEG	NEG	NEG
24	*Candida albicans*	6.30e6 CFU/mL	NEG	NEG	NEG	NEG
25	*Candida parapsilosis*	1.45e6 CFU/mL	NEG	NEG	NEG	NEG
26	*Citrobacter freundii*	1.73e8 CFU/mL	NEG	NEG	NEG	NEG
27	*Corynebacterium sp.*	1.27e7 CFU/mL	NEG	NEG	NEG	NEG
28	*Enterococcus faecalis*	5.87e7CFU/mL	NEG	NEG	NEG	NEG
29	*Escherichia coli*	1.55e8 CFU/mL	NEG	NEG	NEG	NEG
30	*Hemophilus influenzae*	6.62e6 CFU/mL	NEG	NEG	NEG	NEG
31	*Lactobacillus reuteri*[b]	5.0e7 copies/mL	NEG	NEG	NEG	NEG

Count	Strain	Tested Concentration	SARS-CoV-2	Flu A	Flu B	RSV
32	*Legionella pneumophila*	1.42e8 CFU/mL	NEG	NEG	NEG	NEG
33	*Moraxella catarrhalis*	2.46e6 CFU/mL	NEG	NEG	NEG	NEG
34	*Mycoplasma pneumoniae*	2.7e6 CFU/mL	NEG	NEG	NEG	NEG
35	*Neisseria meningitides*	4.2e6 CFU/mL	NEG	NEG	NEG	NEG
36	*Neisseria mucosa*	1.0e8 CFU/mL	NEG	NEG	NEG	NEG
37	*Propionibacterium acnes*	8.25e7 CFU/mL	NEG	NEG	NEG	NEG
38	*Pseudomonas aeruginosa*	1.05e7 CFU/mL	NEG	NEG	NEG	NEG
39	*Staphylococcus haemolyticus*	2.66e6 CFU/mL	NEG	NEG	NEG	NEG
40	*Staphylococcus aureus*	5.87e7 CFU/mL	NEG	NEG	NEG	NEG
41	*Staphylococcus epidermidis*	2.47e7 CFU/mL	NEG	NEG	NEG	NEG
42	*Streptococcus agalactiae*	1.75e7 CFU/mL	NEG	NEG	NEG	NEG
43	*Streptococcus pneumoniae*	2.26e7 CFU/mL	NEG	NEG	NEG	NEG
44	*Streptococcus pyogenes*	9.0e6 CFU/mL	NEG	NEG	NEG	NEG
45	*Streptococcus salivarius*	4.19e6 CFU/mL	NEG	NEG	NEG	NEG
46	*Streptococcus sanguinis*	8.67e6 CFU/mL	NEG	NEG	NEG	NEG
47	*Chlamydia pneumoniae*	1.20e6 CFU/mL	NEG	NEG	NEG	NEG
48	*Mycobacterium tuberculosis (avirulent)*	1.20e6 CFU/mL	NEG	NEG	NEG	NEG

[a] Live virus was not available. Synthetic RNA was used.

[b] Live organism was not available. Genomic DNA was used.

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 potentially interfering 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 $1x10^5$ $TCID_{50}$/mL), *Hemophilus influenzae* (seeded at $1x10^6$ CFU/mL), *Staphylococcus aureus* or *Staphylococcus epidermidis* (each seeded at $1x10^7$ CFU/mL).

Eight (8) replicates of the 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 replicates of the positive samples were correctly identified using the Xpert Xpress CoV-2/Flu/RSV *plus* test. No microbial interference by the 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 $TCID_{50}$/mL for Flu A/Idaho/072018, 38.7 $CEID_{50}$/mL for Flu B/Washington/2/2019; 0.99 $TCID_{50}$/mL for RSV A/2/Australia/61), and 1.11 $TCID_{50}$/mL for RSV B/9320/MA/77. The competitive strains were evaluated at 10^5 or higher titer units (copies/mL, $TCID_{50}$/mL, $CEID_{50}$/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. The results for competitive interference study are presented in Table 19 through Table 23 for high concentration of Flu A, Flu B, RSV A, RSV B and SARS-CoV-2, respectively.

Table 19. 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 20. 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 21. 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 22. 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 23. 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

Test Viruses at 3X LoD	Interferent Virus	Correct Calls (n/3)	
		at 1e6 RNA copies/mL	at 1e5 RNA copies/mL
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 19). In addition, SARS-CoV-2 at concentrations above 1e5 RNA copies/mL inhibited detection of Flu B at 3x LoD (Table 23). 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 are normally found in or may be introduced into clinical NPS or NS matrix that could 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, two influenza A H1N1, two influenza A H3N2, two influenza B and two RSV (RSV A and RSV B) strains. The substances, with active ingredients, that were evaluated are listed in Table 24.

Table 24. Potentially Interfering Substances Tested

Substance ID	Substance/Class	Substance/ Active Ingredient	Concentrations Tested
No substance	Control	Simulated NPS/ NS Matrix	100% (v/v)
Albuterol Sulfate	Beta-adrenergic bronchodilator	Albuterol Sulfate (5mg/mL)	0.83 mg/mL (equivalent to 1 dose per day)
Afrin	Nasal Spray	Oxymetazoline, 0.05%	15% (v/v)
BD Universal Transport Medium	Transport Media	N/A	100% (v/v)
Blood	Blood	Blood (Human)	2% (v/v)
Copan Swab M	Transport Media	N/A	100% (v/v)
FluMist Quadrivalent	Vaccine	Live attenuated influenza viruses	6.7e-4% (v/v)
			6.7e-6% (v/v)
			6.7e-7% (v/v)
Fluticasone Propionate Nasal Spray	Nasal corticosteroid	Fluticasone Propionate	5 µg/mL
Human peripheral blood mononuclear cells	Human cells	PBMC	1 x 10^6 cells/mL
			0.5 x 10^6 cells/mL
			0.25 x 10^6 cells/mL
Ibuprofen	Nonsteroidal anti-inflammatory drug	Ibuprofen 200 mg/tablet	5% w/v

Substance ID	Substance/Class	Substance/ Active Ingredient	Concentrations Tested
Menthol	Throat lozenges, oral anesthetic and analgesic	Benzocaine, Menthol	1.7 mg/mL
Mucin	Mucin	Purified Mucin protein (Bovine or porcine submaxillary gland)	0.1 (w/v)
Mupirocin	Antibiotic, nasal ointment	Mupirocin (20 mg/g = 2%)	10 mg/mL
PHNY	Nasal Drops	Phenylephrine, 1%	15% (v/v)
Remel M4RT	Transport Media	N/A	100% (v/v)
Remel M5	Transport Media	N/A	100% (v/v)
Saline	Saline Nasal Spray	Sodium Chloride (0.65%)	15% (v/v)
Snuff	Tobacco product	Nicotine	1% (w/v)
			0.5% (w/v)
			0.25% (w/v)
			0.1% (w/v)
Tamiflu	Anti-viral drugs	Zanamivir	7.5 mg/mL
Tobramycin	Antibacterial, systemic	Tobramycin	4 µg/mL
Zicam	Nasal Gel	Luffa opperculata, Galphimia glauca, Histaminum hydrochloricum Sulfur (0.05%)	15% (w/v)
			7.5% (w/v)
Zinc	Zinc supplement	Zinc Gluconate	0.1 µg/mL

The results from the study (Table 25) 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. In the presence of FluMist at 6.7e-4% (v/v), interfering effects were observed when testing SARS-CoV-2, RSV A and RSV B strains. Inhibitory effects were not observed when testing these viruses in the presence of FluMist at 6.7e-6% (v/v) except for RSV A/Long/MD/56. For RSV A/Long/MD/56, the inhibitory effect was not observed when FluMist concentration was further reduced to 6.7e-7% (v/v). In the presence of human PBMC at 1 x 10^6 cells/mL, interfering effects were observed when testing Flu B/Washington /2/2019. Inhibitory effects were not observed when the PBMC concentration was reduced to 2.5 x 10^5 cells/mL. In the presence of snuff at 1% (w/v), interfering effects were observed when testing Flu A /California/07/2009 and Flu B/Washington/2/2019. Inhibitory effects were not observed when testing the viruses at a snuff concentration of 0.1% (w/v). In the presence of Zicam at 15% (w/v), interfering effects were observed when testing Flu A, Flu B and RSV A strains. Inhibitory effects were not observed when testing the viruses in the presence of Zicam at 7.5% (w/v).

Table 25. Number of Correct Results 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 for Each Virus and the No Virus Control					
		No Virus Control	SARS-CoV-2/USA-WA-1	Flu A / California/7/2009	Flu A /Idaho/07/2018	Flu A / Hong Kong /45/2019	Flu A / Victoria/361/2011
Control Simulated NPS/NS Matrix (No substance)	100% (v/v)	32/32[a]	24/24	24/24	16/16	16/16	24/24[b]
Albuterol Sulfate	0.83 mg/mL	16/16	8/8	8/8	8/8	8/8	8/8

Substance	Concentration Tested	Number of Correct Results/Number Tested for Each Virus and the No Virus Control					
		No Virus Control	SARS-CoV-2/USA-WA-1	Flu A / California/7/2009	Flu A /Idaho/07/2018	Flu A / Hong Kong /45/2019	Flu A / Victoria/361/2011
Afrin	15% (v/v)	16/16	8/8	8/8[b]	8/8	8/8	8/8
BD Universal Transport Medium	100% v/v	16/16	8/8	8/8	8/8	8/8	8/8[b]
Blood	2% (v/v)	16/16	8/8	8/8	8/8	8/8	8/8
Copan Swab M	100% (v/v)	16/16	8/8	8/8	8/8	8/8	8/8
FluMist	6.7% (v/v)	8/8	N/A	N/A	N/A	N/A	N/A
	6.7e-4% (v/v)	N/A	**7/8**	N/A	N/A	N/A	N/A
	6.7e-6% (v/v)	N/A	8/8	N/A	N/A	N/A	N/A
	6.7e-7% (v/v)	N/A	N/A	N/A	N/A	N/A	N/A
Fluticasone Propionate Nasal Spray	5 µg/mL	16/16	8/8	8/8	8/8	8/8	8/8
Human peripheral blood mononuclear cells	1e6 cells/mL	8/8	8/8	8/8[b]	8/8[b]	8/8	8/8
	0.5e6 cells/mL	N/A	N/A	N/A	N/A	N/A	N/A
	0.25e6 cells/mL	N/A	N/A	N/A	N/A	N/A	N/A
Ibuprofen	5% (w/v)	8/8	8/8	8/8	8/8	8/8	8/8
Menthol	1.7 mg/mL	16/16[a]	8/8	8/8	8/8	8/8[a]	8/8
Mucin	0.1% (w/v)	16/16	8/8	8/8	8/8	8/8	8/8
Mupirocin	10 mg/mL	16/16	8/8	8/8	8/8	8/8	8/8
PHNY	15% (v/v)	16/16	8/8	8/8	8/8	8/8	8/8
Remel M4RT	100% (v/v)	16/16[a]	8/8	8/8	8/8	8/8	8/8
Remel M5	100% (v/v)	16/16	8/8	8/8	8/8	8/8	8/8
Saline	15% (v/v)	16/16	8/8	8/8[a]	8/8	8/8	8/8
Snuff	1% (w/v)	8/8	8/8	**6/8**	8/8	8/8[b]	8/8
	0.5% (w/v)	N/A	N/A	**7/8**	N/A	N/A	N/A
	0.25 % (w/v)	N/A	N/A	8/8	N/A	N/A	N/A
	0.1 % (w/v)	N/A	N/A	N/A	N/A	N/A	N/A
Tamiflu	7.5 mg/mL	16/16[a]	8/8	8/8	8/8	8/8	8/8
Tobramycin	4 µg/mL	16/16	8/8	8/8	8/8	8/8	8/8
Zicam	15% (w/v)	16/16	8/8	**7/8**	8/8	8/8	8/8
	7.5% (w/v)	N/A	N/A	8/8	N/A	N/A	N/A
Zinc	0.1µg/mL	16/16	8/8	8/8	8/8	8/8	8/8

[a] One replicate reported **NO RESULT**. The run was successfully repeated to obtain the required number of valid replicates.

[b] One replicate reported **ERROR**. The run was successfully repeated to obtain the required number of valid replicates.

BOLD: False negative or INVALID results indicating interference from the substance

Table 26. Number of Correct Results 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 for Each Virus and the No Virus Control					
		Flu B Wisconsin/10/2016	Flu B Washington/02/2019	RSV A 2/ Australia/61	RSV A Long/MD/56	RSV B 9320/MA/77	RSV B WA/18537/62
Control Simulated NPS/NS Matrix (No substance)	100% (v/v)	24/24	32/32	32/32[a]	32/32	24/24	24/24
Albuterol Sulfate	0.83 mg/mL	8/8	8/8	8/8	8/8	8/8	8/8
Afrin	15% (v/v)	8/8	8/9[b]	8/8	8/8	8/8	8/8
BD Universal Transport Medium	100% v/v	8/8	8/8	8/8	8/8	8/8	8/8
Blood	2% (v/v)	8/8[c]	8/8	8/8[a]	8/8[a]	8/8	8/8
Copan Swab M	100% (v/v)	8/8	8/8	8/8	8/8	8/8	8/8
FluMist	6.7% (v/v)	N/A	N/A	N/A	N/A	N/A	N/A
	6.7e-4% (v/v)	N/A	N/A	**0/8**	**0/8**	**2/8**	**0/8**
	6.7e-6% (v/v)	N/A	N/A	8/8	**7/8**	8/8[a]	8/8
	6.7e-7% (v/v)	N/A	N/A	N/A	8/8[c]	N/A	N/A
Fluticasone Propionate Nasal Spray	5 µg/mL	8/8[ac]	8/8	8/8[d]	8/8	8/8[c]	8/8
Human peripheral blood mononuclear cells	1e6 cells/mL	8/8	**6/8**	8/8[a]	8/8	8/8[a]	8/8[a]
	0.5e6 cells/mL	N/A	**7/8**	N/A	N/A	N/A	N/A
	0.25e6 cells/mL	N/A	8/8	N/A	N/A	N/A	N/A
Ibuprofen	5% (w/v)	8/8	8/8	8/8	8/8	8/8	8/8
Menthol	1.7 mg/mL	8/8[a]	8/8	8/8	8/8[a]	8/8	8/8
Mucin	0.1% (w/v)	8/8	8/8	8/8	8/8[ac]	8/8	8/8
Mupirocin	10 mg/mL	8/8	8/8	8/8	8/8	8/8	8/8
PHNY	15% (v/v)	8/8	8/8	8/8	8/8	8/8	8/8
Remel M4RT	100% (v/v)	8/8	8/8	8/8	8/8	8/8	8/8
Remel M5	100% (v/v)	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[c]
Snuff	1% (w/v)	8/8	**4/8**[a]	8/8	8/8	8/8	8/8[e]
	0.5% (w/v)	N/A	**3/8**	N/A	N/A	N/A	N/A
	0.25 % (w/v)	N/A	**7/8**	N/A	N/A	N/A	N/A
	0.1 % (w/v)	N/A	8/8	N/A	N/A	N/A	N/A
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[c]	**5/8**	**7/8**	8/8	8/8	8/8
	7.5% (w/v)	N/A	8/8	8/8	N/A	N/A	N/A
Zinc	0.1µg/mL	8/8	8/8	8/8	8/8	8/8	8/8

[a] One replicate reported **ERROR**. The run was successfully repeated to obtain the required number of valid replicates.

[b] One of 8 replicates reported a **Flu B NEGATIVE** result. The Flu B Probe check signals were reduced in this sample suggesting an issue with the EZR bead. The test was repeated and gave a **Flu B positive** result.
[c] One replicate reported **NO RESULT**. The run was successfully repeated to obtain the required number of valid replicates.
[d] One of 8 replicates reported **INVALID**. The run was successfully repeated to obtain 8 valid replicates.
[e] Two of 8 replicates reported **ERROR**. The 2 runs were successfully repeated to obtain 8 valid replicates.

BOLD: False negative or INVALID results indicating interference from the substance

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 $TCID_{50}$/mL and inactivated SARS-CoV-2 USA-WA1/2020 at 1e4 copies/mL) seeded into simulated 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.

20.9 Reproducibility

The reproducibility of the Xpert Xpress CoV-2/Flu/RSV *plus* test was established at 3 external sites using a 10-member panel including 2 negative, 4 low positive (~1.5x LoD) and 4 moderate positive (~3x LoD) samples. The negative samples consisted of simulated matrix without target microorganism or target RNA. The positive samples were contrived using inactivated NATtrol SARS-CoV-2 (ZeptoMetrix), cultured viruses Influenza A/ Idaho/07/2018, Influenza B/Wisconsin/10/2016, and RSV B/Wash/18537/62 in a simulated NPS/NS matrix. Testing was conducted over 5 days, using 1 lot of Xpert Xpress CoV-2/Flu/RSV *plus* cartridges at 3 participating sites, each with 3 operators to yield a total of 90 observations per panel member (3 Sites x 3 Operators x 1 Lot x 5 Days x 1 Run x 2 Replicates = 90 observations/panel member). The results from the study are summarized in Table 27.

The percent agreement of the correct results compared to the expected results analyzed by each of the 3 operators and each site is shown in Table 27. In addition, the overall percent agreement for each sample (% total agreement) and the two-sided Wilson Score confidence intervals (CI) are presented in the last column.

Table 27. Summary of Reproducibility Results – % Agreement

Sample	Site 1				Site 2				Site 3				% Total Agreement [95% CI]
	Op 1	Op 2	Op 3	Site	Op 1	Op 2	Op 3	Site	Op 1	Op 2	Op 3	Site	
Negative – 1	100% 10/10	100% 10/10	100% 10/10	100% 30/30	100% 10/10	100% 10/10	100% 10/10	100% 30/30	100% 10/10	100% 10/10	100% 10/10	100% 30/30	100% (90/90) [95.9-100.0]
Negative – 2	100% 10/10	100% 10/10	100% 10/10	100% 30/30	100% 10/10	100% 10/10	100% 10/10	100% 30/30	100% 10/10	100% 10/10	100% 10/10	100% 30/30	100% (90/90) [95.9-100.0]
SARS-CoV-2 Low Pos	100% 10/10	100% 10/10	100% 10/10	100% 30/30	100% 10/10	100% 10/10	100% 10/10	100% 30/30	100% 10/10	100% 10/10	100% 10/10	100% 30/30	100% (90/90) [95.9-100.0]
SARS-CoV-2 Mod Pos	100% 10/10	100% 10/10	100% 10/10	100% 30/30	100% 10/10	100% 10/10	100% 10/10	100% 30/30	100% 9/9	100% 10/10	100% 10/10	100% 29/29	100% (89/89)[a] [95.9-100.0]

Sample	Site 1				Site 2				Site 3				% Total Agreement [95% CI]
	Op 1	Op 2	Op 3	Site	Op 1	Op 2	Op 3	Site	Op 1	Op 2	Op 3	Site	
Flu A Low Pos	100% 10/10	100% 10/10	100% 10/10	100% 30/30	90% 9/10	90% 9/10	100% 10/10	93.3% 28/30	100% 10/10	100% 10/10	100% 10/10	100% 30/30	97.8% (88/90) [92.3-99.4]
Flu A Mod Pos	100% 10/10	100% 10/10	100% 10/10	100% 30/30	100% 10/10	100% 10/10	100% 10/10	100% 30/30	100% 10/10	100% 10/10	100% 10/10	100% 30/30	100% (90/90) [95.9-100.0]
Flu B Low Pos	100% 10/10	90% 9/10	100% 10/10	96.7% 29/30	100% 10/10	100% 10/10	90% 9/10	96.7% 29/30	100% 10/10	100% 10/10	100% 10/10	100% 30/30	97.8% (88/90) 92.3-99.4)
Flu B Mod Pos	100% 10/10	100% 10/10	100% 10/10	100% 30/30	100% 10/10	100% 10/10	100% 10/10	100% 30/30	100% 10/10	100% 10/10	100% 10/10	100% 30/30	100% (90/90) [95.9-100.0]
RSV Low Pos	100% 10/10	100% 10/10	100% 10/10	100% 30/30	100% 10/10	100% 10/10	100% 10/10	100% 30/30	100% 10/10	100% 10/10	100% 10/10	100% 30/30	100% (90/90) [95.9-100.0]
RSV Mod Pos	100% 10/10	100% 10/10	100% 10/10	100% 30/30	100% 10/10	100% 10/10	100% 10/10	100% 30/30	100% 10/10	100% 10/10	100% 10/10	100% 30/30	100% (90/90) [95.9-100.0]

[a] One replicate was excluded due to a repeat non-determinate test result.

The evaluation of reproducibility of the underlying analyte Ct values for the Xpert Xpress CoV-2/Flu/RSV *plus* test was analyzed using nested Analysis of Variance (ANOVA). The mean Ct, standard deviation (SD), and coefficient of variation (CV; %) between-sites, between-operators, between-days, and within-run for each panel member are presented in Table 28.

Table 28. Summary of Nested ANOVA by Coefficient Variation

Sample	Analyte	N	Mean Ct	Variance Source									
				Site		Operator		Day		Within-run		Total	
				SD	CV (%)	SD	CV (%)	SD	CV (%)	SD	CV (%)	SD	CV (%)
Negative – 1	SPC	90	28.54	0.00	0.0	0.10	0.3	0.23	0.8	0.87	3.0	0.90	3.2
Negative – 2	SPC	90	28.51	0.10	0.3	0.00	0.0	0.00	0.0	0.36	1.3	0.37	1.3
SARS-CoV-2 Low Pos	SARS-CoV-2	90	36.86	0.09	0.2	0.00	0.0	0.00	0.0	0.60	1.6	0.61	1.7
SARS-CoV-2 Mod Pos	SARS-CoV-2	89[a]	35.74	0.00	0.0	0.12	0.3	0.00	0.0	0.30	0.8	0.33	0.9
Flu A Low Pos	Flu A1	90	35.88	0.24	0.7	0.00	0.0	0.23	0.6	1.11	3.1	1.16	3.2
	Flu A2	72[b]	38.34	0.00	0.0	0.32	0.8	0.00	0.0	1.34	3.5	1.38	3.6
Flu A Mod Pos	Flu A1	90	34.64	0.00	0.0	0.1	0.3	0.00	0.0	0.40	1.2	0.41	1.2
	Flu A2	90	36.75	0.26	0.7	0.34	0.9	0.00	0.0	0.90	2.4	1.00	2.7
Flu B Low Pos	Flu B	90	36.05	0.00	0.0	0.00	0.0	0.48	1.3	1.12	3.1	1.21	3.4
Flu B Mod Pos	Flu B	90	35.12	0.09	0.2	0.08	0.2	0.00	0.0	0.61	1.7	0.62	1.8
RSV Low Pos	RSV	90	35.92	0.00	0.0	0.16	0.5	0.00	0.0	0.75	2.1	0.77	2.1

Sample	Analyte	N	Mean Ct	Variance Source									
				Site		Operator		Day		Within-run		Total	
				SD	CV (%)	SD	CV (%)	SD	CV (%)	SD	CV (%)	SD	CV (%)
RSV Mod Pos	RSV	90	35.07	0.00	0.0	0.2	0.6	0.00	0.0	0.57	1.6	0.60	1.7

[a] One replicate was excluded due to a repeat non-determinate test result.

[b] Eighteen replicates were excluded due to zero Flu A2 Ct values.

21 CLIA Waiver Studies

The accuracy of the Xpert Xpress CoV-2/Flu/RSV *plus* test was evaluated in an observational, method comparison study with untrained operators at 22 geographically diverse CLIA-waived sites in the United States. Operators performing Xpert Xpress CoV-2/Flu/RSV *plus* testing were representative of the intended "waived users" with limited or no hands-on experience in conducting CLIA moderate/high complexity laboratory testing. No training on the use of the Xpert Xpress CoV-2/Flu/RSV *plus* test was provided to the participating operators. A total of 4561 (N=2300 NPS; 2261 NS) specimens were tested and eligible for inclusion in the Xpert Xpress CoV-2/Flu/RSV *plus* clinical study. The performance of Xpert Xpress CoV-2/Flu/RSV *plus* was established in 3147 (N=1565 NPS; 1582 NS) specimens for SARS-CoV-2 analyte and in 4310 specimens (2175 NPS; 2135 NS) specimens for Flu A, Flu B, and RSV analytes relative to the comparators and the results are shown in Section 20.1, Table 9 and Table 10, for NPS and NS specimens, respectively.

Flex Studies

Using risk analysis as a guide, flex studies were conducted on Xpert Xpress CoV-2/Flu/RSV *plus* for use with the GeneXpert Xpress System. The testing evaluated numerous sources of potential human errors that could affect the accuracy of results, including those related to sample handling, reagent handling, and the operation of the GeneXpert Xpress System. The studies demonstrated that the Xpert Xpress CoV-2/Flu/RSV *plus* test and the GeneXpert Xpress System are robust to the usage variation that may be encountered.

22 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. Morris DE, Cleary DW, Clarke SC. Secondary Bacterial Infections Associated with Influenza Pandemics. Front Microbiol. 2017 Jun 23;8:1041. doi: 10.3389/fmicb.2017.01041. PMCID 28690590; PMCID: PMC5481322.
4. http://www.cdc.gov/flu/about/viruses/types.htm. Accessed on May 19, 2016.
5. http://www.cdc.gov/RSV/index.html. Accessed on March 14, 2013.
6. 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.
7. Solomon, D. A., Sherman, A. C., & Kanjilal, S. (2020). Influenza in the COVID-19 Era. Jama, 324(13), 1342-1343.
8. Centers for Disease Control and Prevention. Biosafety in Microbiological and Biomedical laboratories (refer to latest edition). http://www.cdc.gov/biosafety/publications/
9. Clinical and Laboratory Standards Institute. Protection of Laboratory Workers from Occupationally Acquired Infections; Approved Guideline. Document M29 (refer to latest edition).
10. 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).
11. Occupational Safety and Health Standards, Hazard Communication, Toxic and Hazard Substances (March 26, 2012) (29 C.F.R., pt. 1910, subpt. Z).

23 Cepheid Headquarters Location

Corporate Headquarters

Cepheid
904 Caribbean Drive
Sunnyvale, CA 94089
USA

Telephone: + 1 408 541 4191
Fax: + 1 408 541 4192
www.cepheid.com

24 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

United States Technical Support

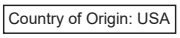
Telephone: + 1 888 838 3222
Email: techsupport@cepheid.com

France Technical Support

Telephone: + 33 563 825 319
Email: support@cepheideurope.com

Contact information for all Cepheid Technical Support offices is available on our website: www.cepheid.com/en/support/contact-us.

25 Table of Symbols

Symbol	Meaning
	Catalog number
	*In vitro* diagnostic medical device
	Do not reuse
	Batch code
	Consult instructions for use
	Caution
	Manufacturer
	Country of manufacture
	Contains sufficient for *n* tests
	Control
	Use-by-date
	Temperature limitation
	Biological risks
	For prescription use only
	Country of Origin: Sweden
	Country of Origin: United States of America

Cepheid
904 Caribbean Drive
Sunnyvale, CA 94089
USA

Phone: + 1 408 541 4191

Fax: + 1 408 541 4192

IVD

26 Revision History

Description of Changes: 302-8058, Rev. A to Rev. B

Section	Description of Change
Intended Use	Added “diagnosis” and removed “virus” in the Intended Use
Materials Available but Not Provided	Removed optional use of controls
Warnings and Precautions	Updated link to CDC Interim Guidelines for Collecting and Handling of Clinical Specimens for COVID-19 Testing
Quality Control	Updated for clarity
External Controls	Removed optional use of controls
Clinical Evaluation	Table 9, RSV Overall NPA corrected to 99.9%
Cepheid Headquarters Location	Removed EU headquarters since Sunnyvale, CA is the official global headquarters
Table of Symbols	Updated to for EN ISO 15223-1:2021 Compliance