GeneXpert®
Powered By CEPHEID INNOVATION

Xpert® Xpress CoV-2/Flu/RSV *plus*

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

Instructions for Use

For Use with GeneXpert® Xpress System (point of care system)

IVD

Cepheid®

For *In Vitro* Diagnostic Use

302-8926, Rev. C
2023-10

Trademark, Patents and Copyright Statements

See Section 26, Revision History for a description of changes.

Xpert® Xpress CoV-2/Flu/RSV *plus*

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

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

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

Clinical correlation with patient history and other diagnostic information is necessary to determine patient infection status. The agent detected may not be the definite cause of disease.

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

Testing with the Xpert Xpress CoV-2/Flu/RSV *plus* test is intended for use by trained operators who are proficient in performing tests using GeneXpert Dx, GeneXpert Infinity, and/or GeneXpert Xpress systems.

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.[3,4]

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.[5] Flu A viruses are further divided into subtypes on the basis of two surface proteins: hemagglutinin (H) and neuraminidase (N). Seasonal flu is normally caused by influenza A subtypes H1, H2, H3, N1 and N2.

Respiratory Syncytial Virus (RSV), a member of the *Pneumoviridae* family (formerly *Paramyxoviridae*), consisting of two strains (subgroups A and B) is also the cause of a contagious disease that affects primarily infants, the elderly, and those who are immunocompromised (e.g., patients with chronic lung disease or undergoing treatment for conditions that reduce the strength of their immune system).[6] The virus can cause both upper respiratory infections, such as colds, and lower respiratory infections manifesting as bronchiolitis and pneumonia.[6] 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.[6] RSV remains the leading cause for hospitalizations in infants worldwide.[7] 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.[5,6]

SARS-CoV-2, influenza, and RSV viruses can cause infections that present with very similar symptoms, making clinical differentiation between them very difficult.[8] 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 qualitative detection and differentiation of RNA from Flu A, Flu B, RSV, and SARS-CoV-2. The Xpert Xpress CoV-2/Flu/RSV *plus* test is performed on GeneXpert Xpress Systems. The primers and probes in the Xpert Xpress CoV-2/Flu/RSV *plus* test are designed to amplify and detect unique sequences in the following: nucleocapsid (N) and envelope (E) and RNA-dependent RNA polymerase (RdRP) genes of the SARS-CoV-2 virus genome, influenza A matrix (M), influenza A basic polymerase (PB2), influenza A acidic protein (PA), influenza B matrix (M), influenza B non-structural protein (NS), and the RSV A and RSV B nucleocapsid.

The GeneXpert Xpress Systems automate and integrate sample preparation, nucleic acid extraction and amplification, and detection of the target sequences in simple or complex samples using real-time PCR assays. The systems consist of an instrument, computer, and preloaded software for running tests and viewing the results. The systems require the use of single-use disposable cartridges that hold the RT-PCR reagents and host the RT-PCR process. Because the cartridges are self-contained, cross-contamination between samples is minimized. For a full description of the systems, see the *GeneXpert 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 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 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 Xpress System platform, which performs hands-off, automated sample processing, and real-time RT-PCR for detection of viral RNA.

6 Reagents and Instruments

6.1 Materials Provided

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

Xpert Xpress CoV-2/Flu/RSV *plus* Cartridges with 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	**10-12 per kit**
Flyer	**1 per kit**
• Instructions to locate (and import) the ADF and EUA documentation such as the Product Insert on www.cepheid.com.	
Quick Reference Instructions (For use with the GeneXpert Xpress Systems – Tablet and Hub Configuration)	**2 per kit**

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

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

7 Storage and Handling

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

8 Materials Required but Not Provided

- GeneXpert Xpress System (Tablet configuration): GeneXpert Xpress II and IV instruments with proprietary GeneXpert Xpress Software Version 5.0 and 5.1, tablet computer device with touchscreen, barcode scanner, external CD drive, wireless printer, Getting Started Guide, and GeneXpert Xpress System User's Guide.
- GeneXpert Xpress System (Hub configuration): GeneXpert Xpress IV instrument, GeneXpert Hub with proprietary GeneXpert Xpress Software Version 6.1 or higher, GeneXpert Hub with integrated computer, touchscreen monitor and barcode scanner, external CD drive, Getting Started Guide, and GeneXpert Xpress System User's Guide.

9 Materials Available but Not Provided

External controls in the form of inactivated virus(es) are available from Microbiologics, Inc. (St. Cloud, MN) or ZeptoMetrix (Buffalo, NY).

- 8246 Flu/RSV/SARS-CoV-2 Control Panel (Inactivated Swab)

ZeptoMetrix (Buffalo, NY):

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

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

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

10 Warnings and Precautions

10.1 General

- For *in vitro* diagnostic use.
- Positive results are indicative of presence of Flu A, Flu B, RSV, and/or SARS-CoV-2 RNA.
- 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[9] and the Clinical and Laboratory Standards Institute.[10]
- 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.
- 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.

10.2 Specimens

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

10.3 Assay/Reagent

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

11 Chemical Hazards[11, 12]

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

12 Specimen Collection, Transport, and Storage

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

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

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

Refer to the WHO Laboratory Biosafety Guidance Related to the Coronavirus Disease 2019 (COVID-19).

https://www.who.int/publications-detail/laboratory-biosafety-guidance-related-to-coronavirus-disease-2019-(covid-19)

12.1 Nasopharyngeal Swab Collection Procedure

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

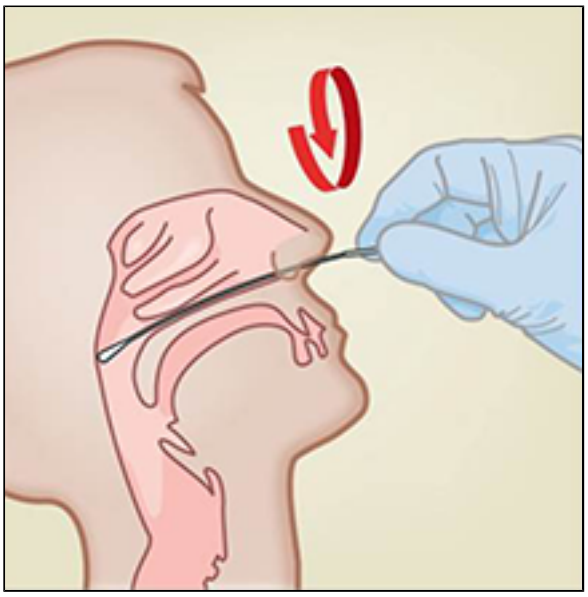


Figure 1. Nasopharyngeal Swab Collection

12.2 Nasal Swab Collection Procedure

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

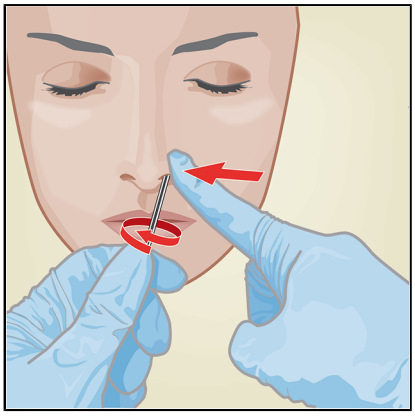


Figure 2. Nasal Swab Collection for First Nostril

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

Figure 3. Nasal Swab Collection for Second Nostril

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

13 Starting the 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.

1. Put on a clean pair of gloves.
2. Determine which system configuration you have (Figure 4).

Figure 4. Tablet and Hub System Configurations

- For the *Tablet* configuration, see Section 13.1, Starting the Tablet Configuration.
- For the *Hub* configuration, see Section 13.2, Starting the Hub Configuration.

13.1 Starting the Tablet Configuration

1. Turn on the GeneXpert Xpress instrument (GeneXpert Xpress II or GeneXpert Xpress IV).
2. Turn on the tablet computer:
   - *Windows ® 7*: The Windows 7 account screen appears. Touch the Cepheid-Admin icon to continue.
   - *Windows ® 10*: The Windows Lock screen appears. Swipe up to continue.

   The Windows Password screen appears.
3. Touch Password to display the keyboard, then type your password.

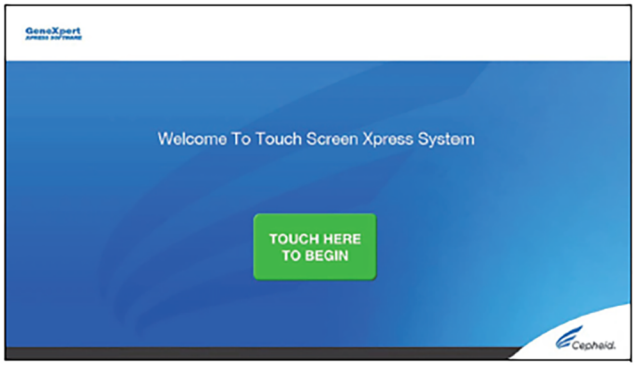
4. Touch the arrow button at the right of the password entry area.
   The GeneXpert Xpress Software starts.

13.2 Starting the Hub Configuration

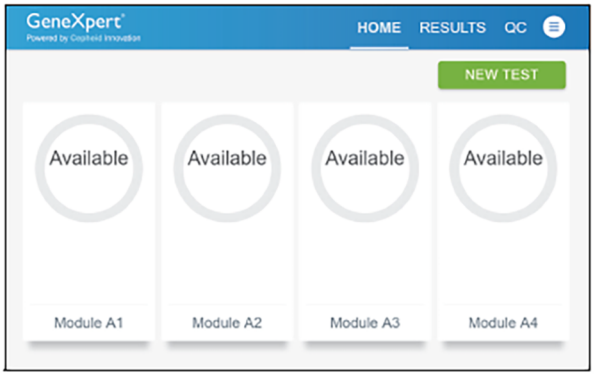
1. Turn on the GeneXpert Xpress IV instrument (in two or four modules configuration).
2. Turn on the Hub computer. The Windows Lock screen appears.
3. Swipe up to continue. The Windows Password screen appears.
4. Touch **Password** to display the keyboard, then type your Windows password.
5. Touch the 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 9. 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. Touch the **LOGIN** button to continue.
9. 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*.

13.3 Determining Your Software Version

When your Xpress opening screen appears, you can determine your software version and the procedure to follow, based on one of the following two screens (see Figure 5).



Software Version 5.0 or Software Version 5.1



Software Version 6.1 or Higher

Figure 5. Xpress Opening Screens and Software Versions

- For Software Version 5.0 or Software Version 5.1, see Section 14.
- For Software Version 6.1 or higher, see Section 16.

14 GeneXpert Xpress Software Version 5.0 or Software Version 5.1

1. On the Welcome screen, touch the **TOUCH HERE TO BEGIN** button (see Figure 6).

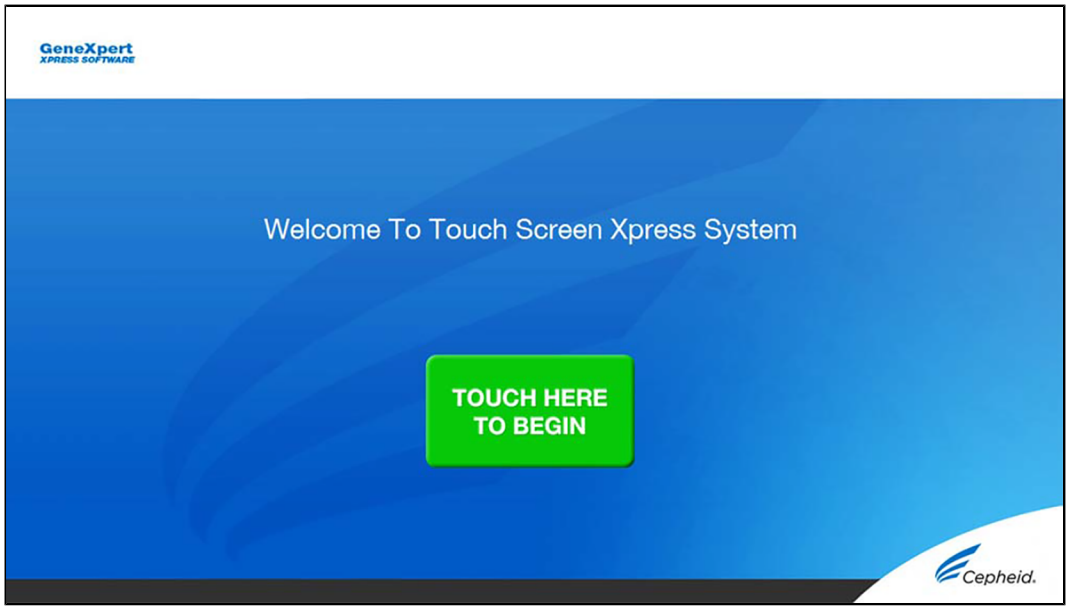


Figure 6. Welcome Screen

2. The **VIEW PREVIOUS TESTS** button appears. The **RUN NEW TEST** button will appear on the Home screen within 3 minutes.

Note If the Home screen does not display **RUN NEW TEST**, the instrument was not powered up or is no longer powered on. Exit the software using the **EXIT** button. The GeneXpert Xpress instrument must first be turned on then turn on the computer. Click on software icon to launch software and enter password.

14.1 Starting a Test

Note 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. Touch the **RUN NEW TEST** button on the Home screen (see Figure 7) to run a patient specimen or an external control.

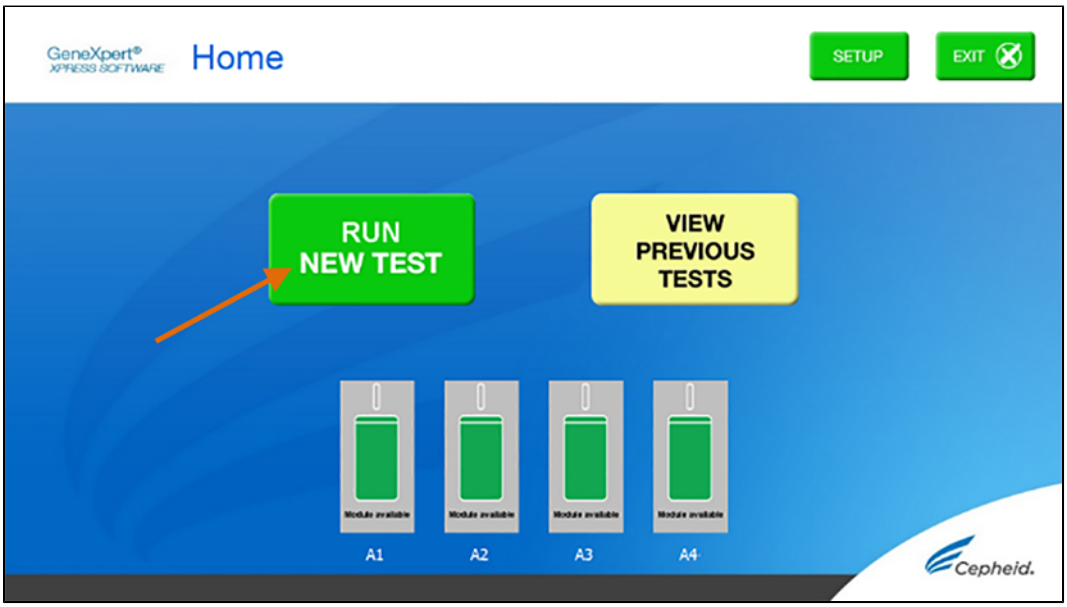


Figure 7. RUN NEW TEST button on Home Screen (GeneXpert Xpress IV screen shown)

2. Check that the specimen transport medium tube cap is closed.
3. If there is a Patient/Sample ID barcode, touch the **YES** button, then scan the Patient/Sample ID with the scanner. If there is no Patient/Sample ID barcode, touch the **NO** button, then manually enter the Patient/Sample ID and touch the **OK** button. For external control, type **Positive Control** or **Negative Control**.
4. Confirm the Patient/Sample ID. Touch **YES** if the Patient/Sample ID is correct.

14.2 Preparing the Specimen or External Control and Cartridge

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. Remove a cartridge and a transfer pipette from the cartridge kit box.
2. Scan the barcode on the cartridge with the scanner.

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 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, contact Cepheid Technical Support.

3. Make the appropriate selection from the Select Assay menu, as shown in Figure 8.
   - SARS-CoV-2, Flu A, Flu B and RSV: Select **Xpress SARS-CoV-2_Flu_RSV plus**
   - SARS-CoV-2 and Flu only: Select **Xpress SARS-CoV-2_Flu plus**
   - SARS-CoV-2 only: Select **Xpress SARS-CoV-2_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 option is selected.

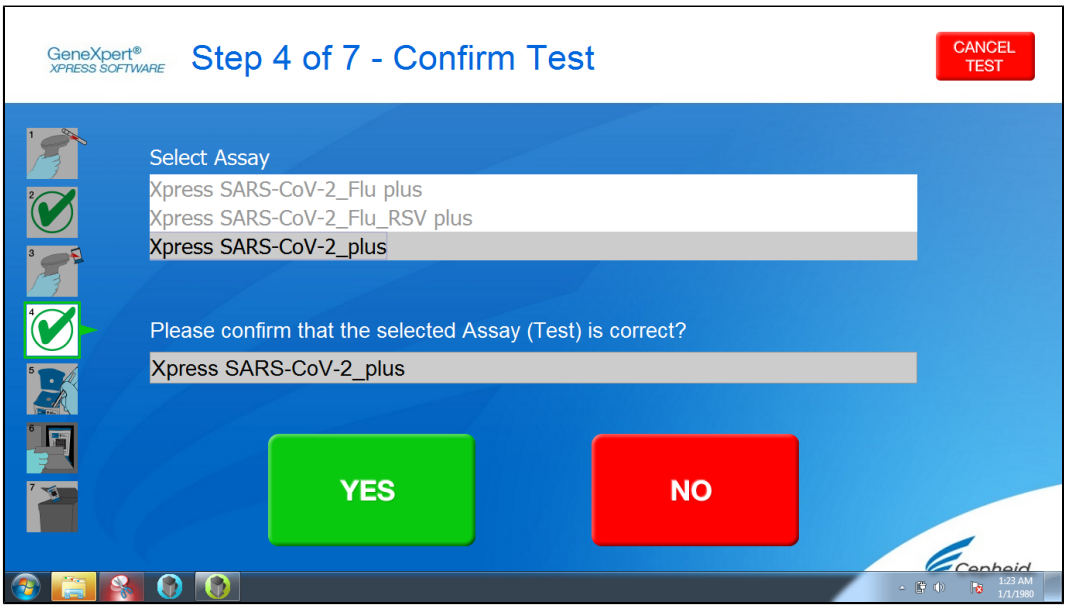


Figure 8. Confirm Test Screen - Select Assay

4. Confirm the selected test from the Select Assay menu (shown in Figure 9 below) and touch **YES** if the displayed information is correct. Enter your user name and password if prompted.

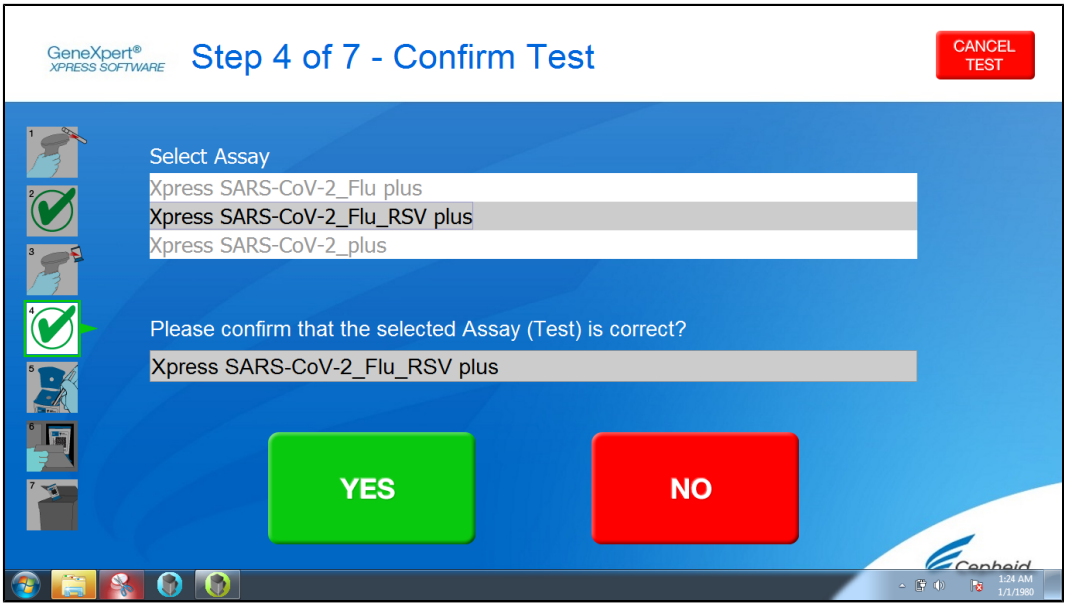


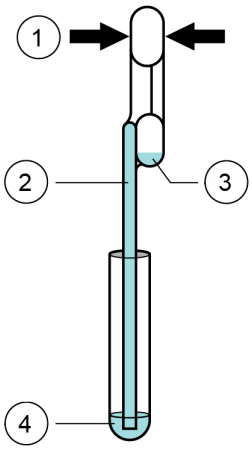
Figure 9. Confirm Test Screen

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.

5. Watch the video before continuing. The video will repeat. Touch the **SKIP VIDEO AND CONTINUE** button to exit video. The **Load Cartridge** screen appears.
6. For external controls, please refer to Microbiologics 8246 Flu/RSV/SARS-CoV-2 Control panel (Inactivated Swab) IFU for swab re-hydration instructions.
7. Mix sample by rapidly inverting the specimen transport tube or external control tube 5 times. Open cap on the specimen transport tube or external control tube.
8. Open the cartridge lid by lifting the front of the cartridge lid.
9. Remove the transfer pipette from the wrapper.

Note Do not place unwrapped pipette on the workbench.

10. 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 10).



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

Figure 10. Transfer Pipette

11. 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 10). Check that the pipette does not contain bubbles.

12. 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) in the cartridge shown in Figure 11. Some liquid may remain in the overflow reservoir. Dispose of the used pipette.

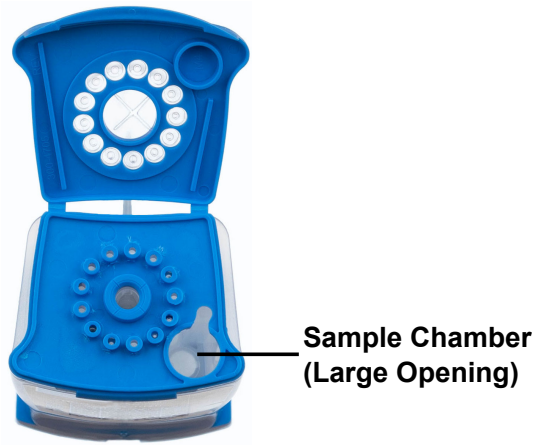


Figure 11. Xpert Xpress CoV-2/Flu/RSV *plus* Cartridge (Top View)

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.

13. Close the cartridge lid.

14.3 Loading the Cartridge

1. Pull open the module door with the flashing green light.
2. Load the cartridge with the barcode facing the operator onto the cartridge bay platform. Do not try to insert the cartridge past the cartridge bay platform.
3. Close the door until it clicks. The green light will stop flashing and the test starts. The **Test in Progress** screen appears. When the test is completed (green light goes out), the door will automatically unlock and the **Remove Cartridge** screen appears.
4. Follow the on-screen instructions to remove the cartridge and to reset the module for a new test.
5. Touch **CONTINUE** to view the result of the test.
6. To print results, touch the **PRINT RESULT** button.
7. Remove cartridge. Dispose of the used cartridge and gloves according to your institution's standard practices.
8. To log out, touch the **SIGN OUT** button.

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

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.

Note If you have scanned the cartridge barcode in the Xpress software and the assay definition file is not available, a screen will appear indicating that the assay definition file is not loaded or that the product code was not found on the system. If this screen appears, contact Cepheid Technical Support.

14.4 Starting a New Test While a Test is Running

1. Put on a clean pair of gloves if performing a new test.
2. Touch the **HOME** button to go to the Home Screen.
3. Touch the **SIGN OUT** button to log out the previous user, if applicable.
4. Start a new test following the steps in Section 14.1, Starting a Test.

15 View Status of Tests in Progress, Completed Tests, and View Results of Past Tests

15.1 Tests in Progress

1. Touch the **HOME** button to view the status of tests in progress.
2. To view a test in progress, touch the **Test in progress touch for status** button. The time remaining to complete the testing will appear on the progress bar at the bottom of the **Test in Progress** screen.

15.2 Completed Tests

1. When a test is completed, touch the **Test complete, touch to continue** button. The **Remove Cartridge** screen appears.
2. Follow the on-screen instructions to remove the cartridge. Touch the **CONTINUE** button to view the result of the test. To print results, touch the **PRINT RESULT** button.

15.3 Results of Past Tests

1. Touch the **VIEW PREVIOUS TESTS** button on the Home screen shown in Figure 12.

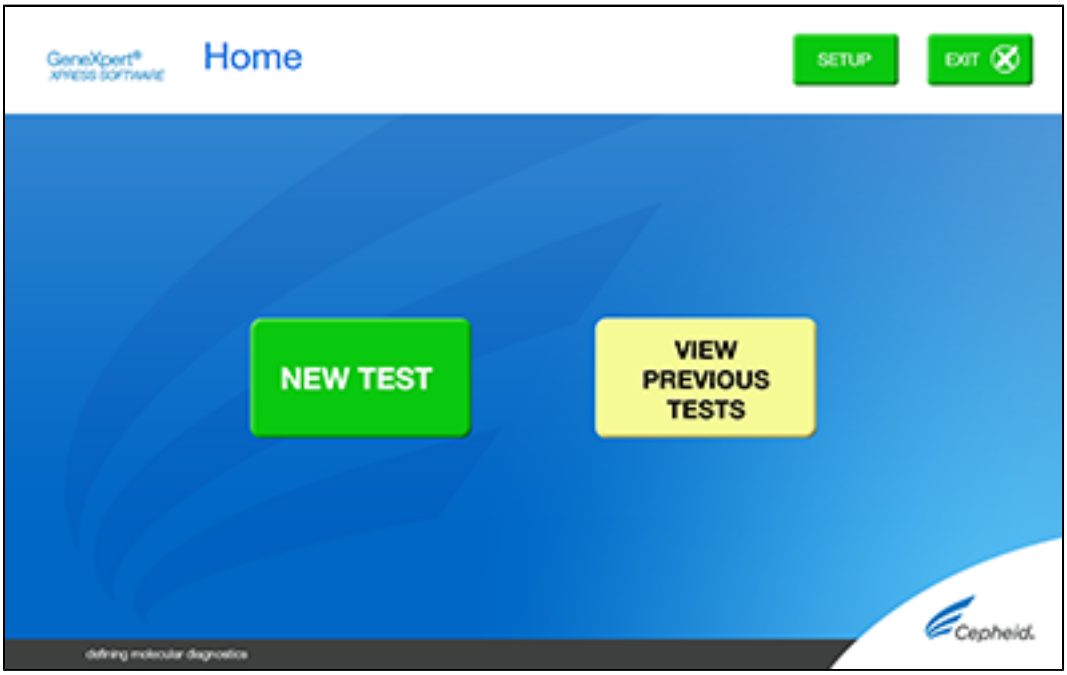


Figure 12. VIEW PREVIOUS TESTS button on Home Screen

2. Select the test by either touching the test name or using the arrows to select the test.
3. Touch the **SELECT** button shown in Figure 13 to view results.
4. To print results, touch the **PRINT RESULT** button.

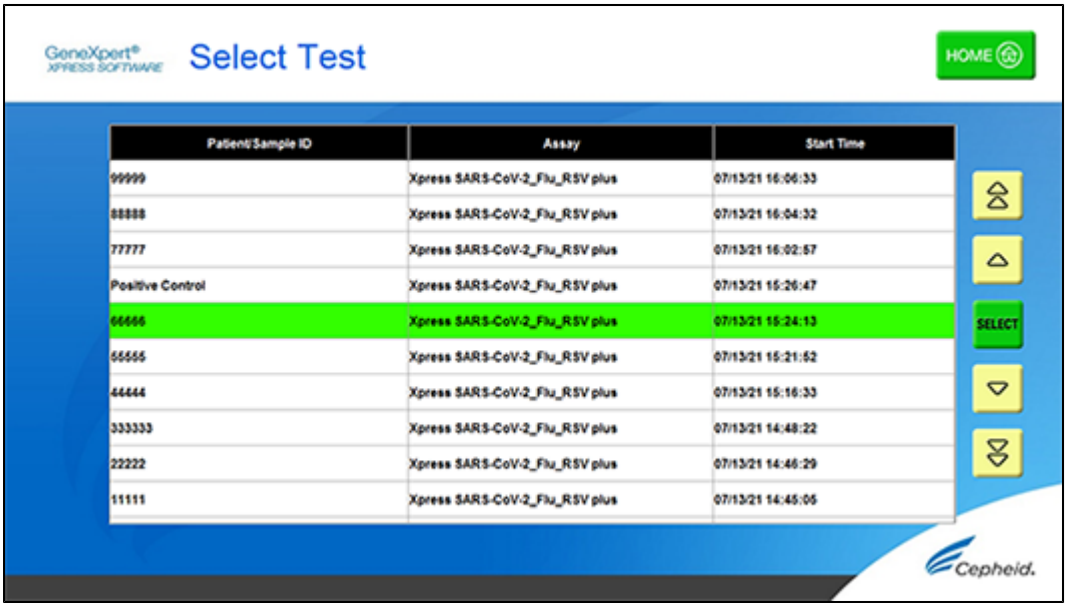


Figure 13. SELECT button

16 GeneXpert Xpress Software Version 6.1 or Higher

16.1 Starting a Test

Note Instructions showing how to prepare the sample and the cartridge are shown on-screen in videos and in the following procedure.

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 14).

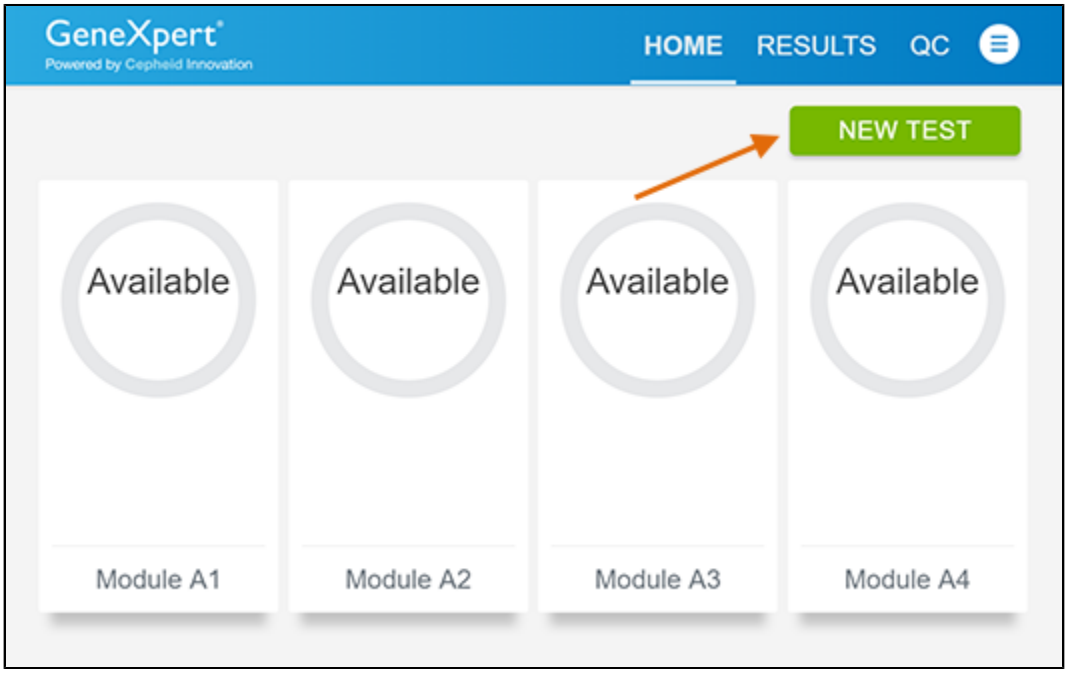


Figure 14. Home Screen

3. Check that the specimen transport medium tube cap is closed.
   If Patient Information is configured by an administrator, then the Patient Information screen appears (see Figure 15). If Patient Information is not configured, the Sample ID screen appears.
4. Skip to Section 16.2 if the Sample ID screen appears.

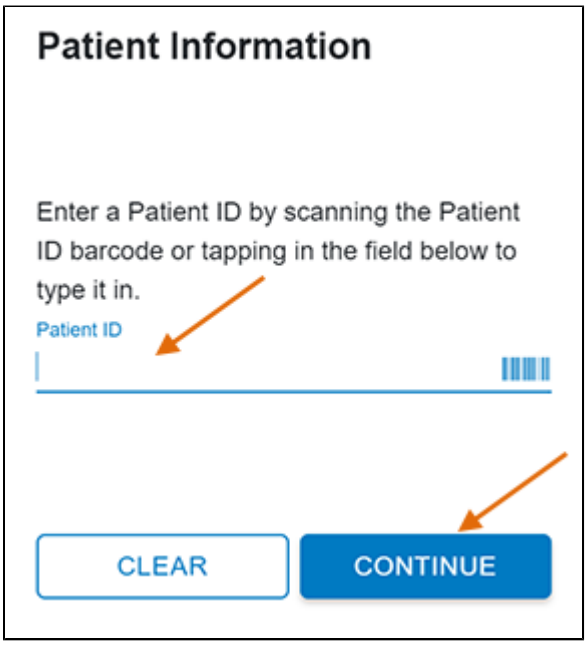


Figure 15. Patient Information Screen

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.

16.2 Preparing the Specimen

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 16).

   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, contact Cepheid Technical Support.

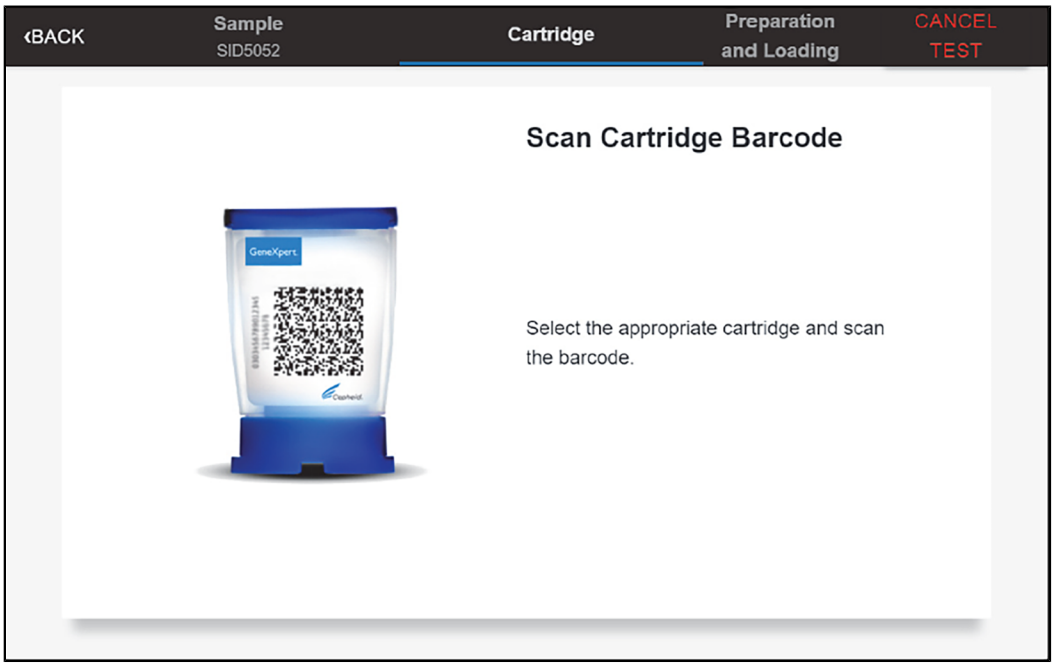


Figure 16. Scan Cartridge Barcode Screen

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 17)
   - SARS-CoV-2, Flu A, Flu B and RSV: Select **Xpress_SARS-CoV-2_Flu_RSV plus**
   - SARS-CoV-2 and Flu only: Select **Xpress_SARS-CoV-2_Flu plus**
   - SARS-CoV-2 only: Select **Xpress_SARS-CoV-2_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.

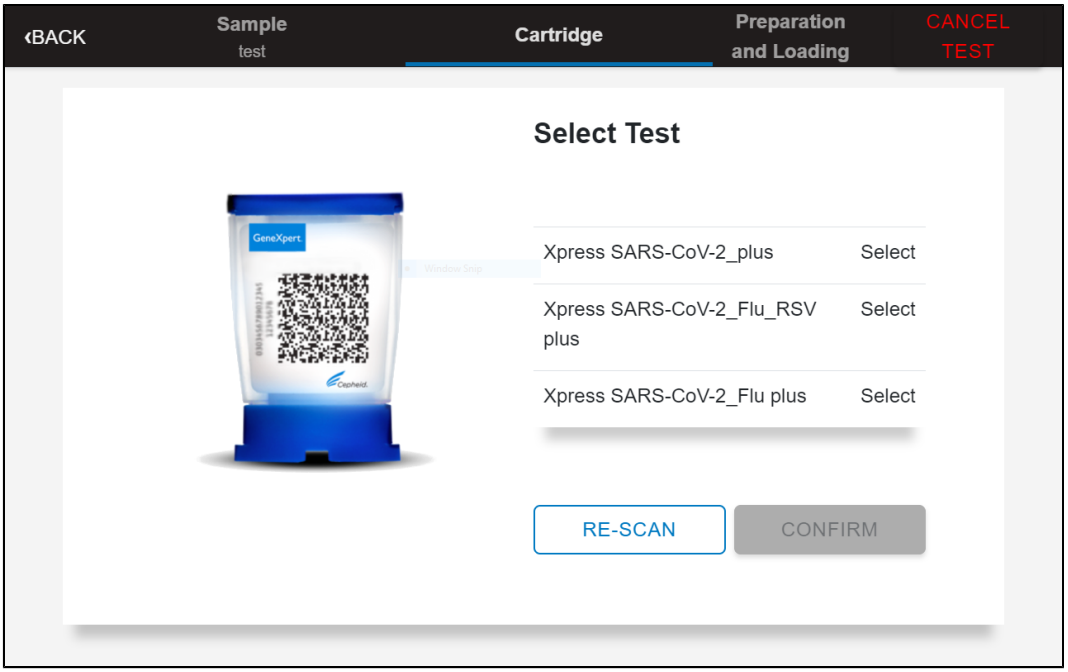


Figure 17. Select Test Screen

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 18).

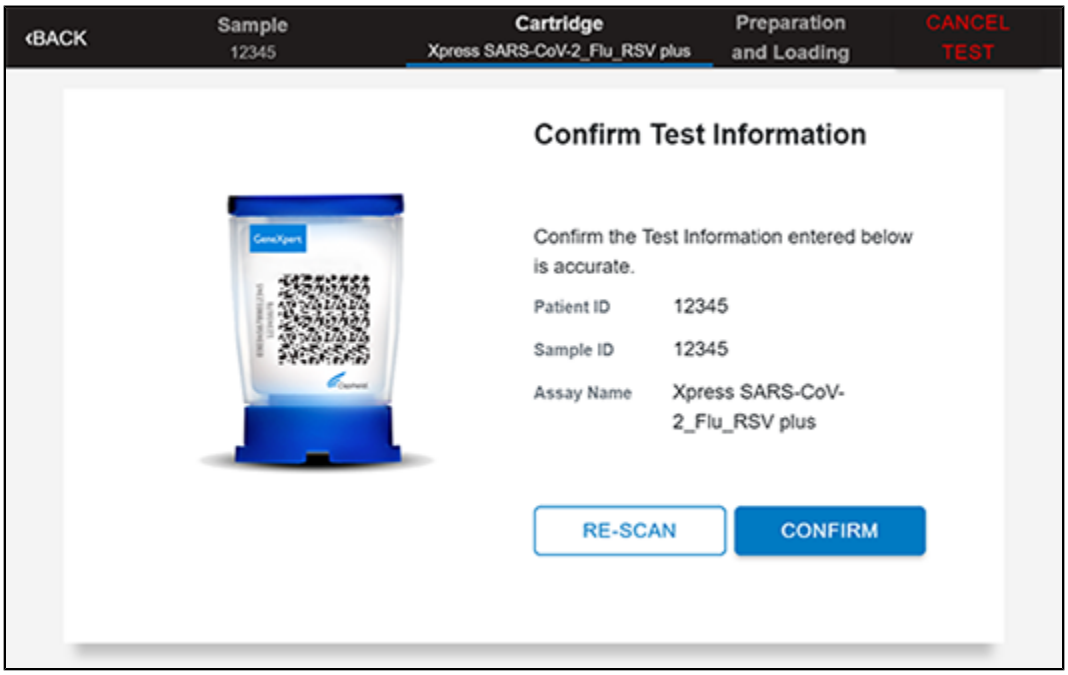


Figure 18. Confirm Test Information Screen

8. Touch **CONFIRM** if the displayed information is correct.
9. Depending on your configuration, the Enter Credentials to Continue screen may appear (see Figure 19). If enabled, you may log in by scanning your institutional ID. Otherwise, manually enter your User Name and Password and touch **LOGIN** to continue.

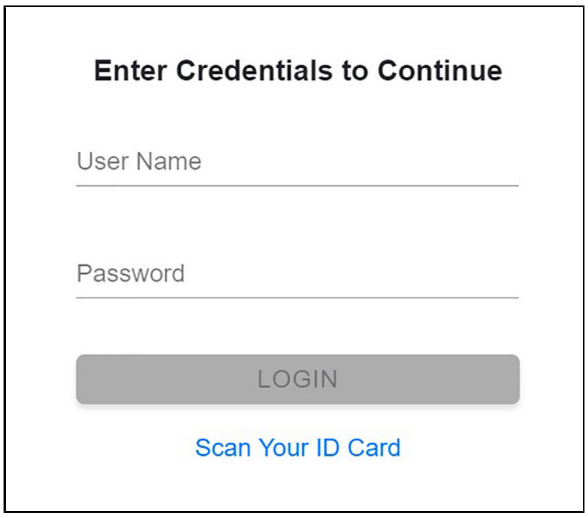


Figure 19. Enter Credentials to Continue Screen

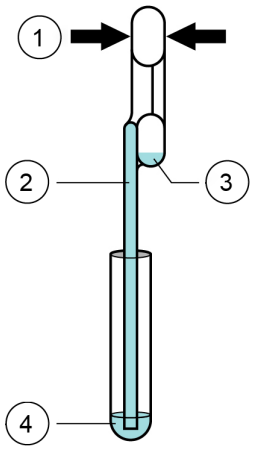
10. The Cartridge Preparation screen appears (see Figure 20).

Figure 20. 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 21).



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

Figure 21. 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 21). 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 22. Some liquid may remain in the overflow reservoir. Dispose of the used pipette.

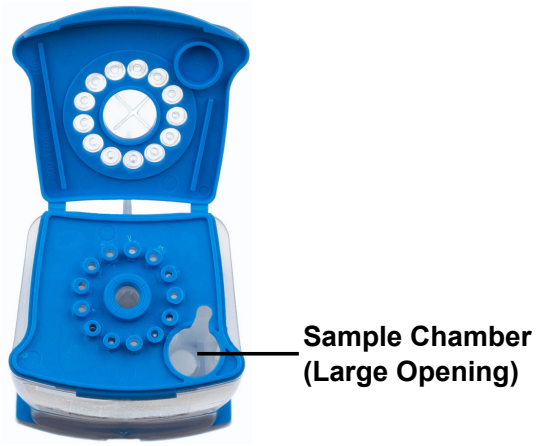


Figure 22. Xpert Xpress CoV-2/Flu/RSV *plus* Cartridge (Top View)

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 16.4, Loading the Cartridge.

16.3 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 23).

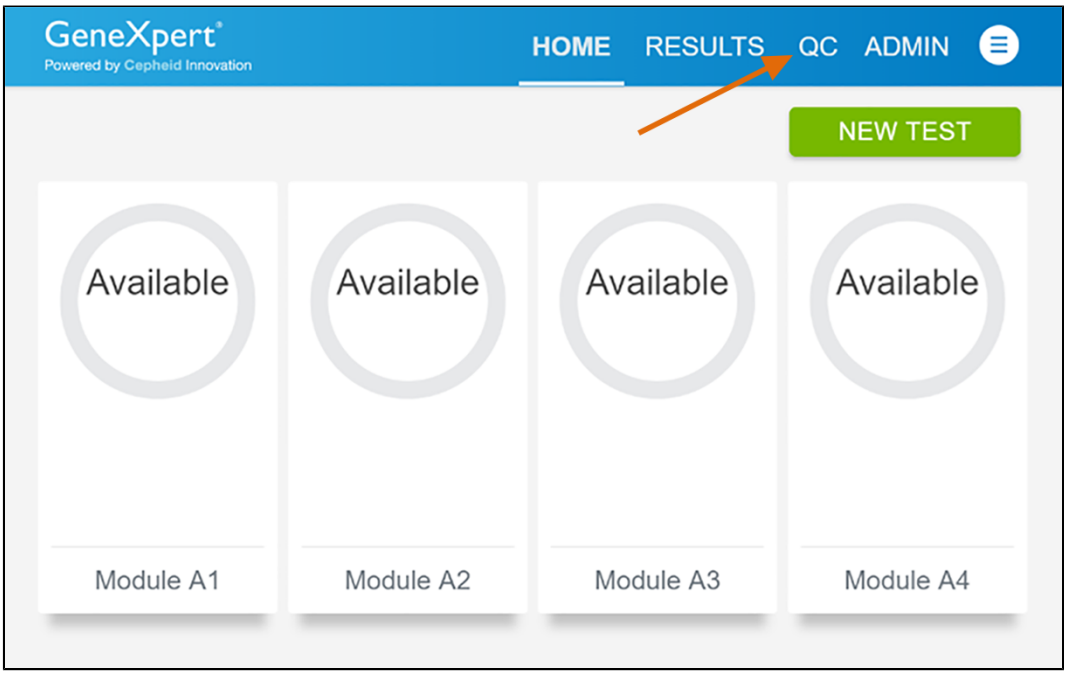


Figure 23. Home Screen

2. The Quality Control screen appears. Touch **RUN QC POSITIVE Test**, **RUN QC NEGATIVE TEST**, or **RUN PROFICIENCY TEST** option (Figure 24).

Figure 24. 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 25).

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, contact Cepheid Technical Support.

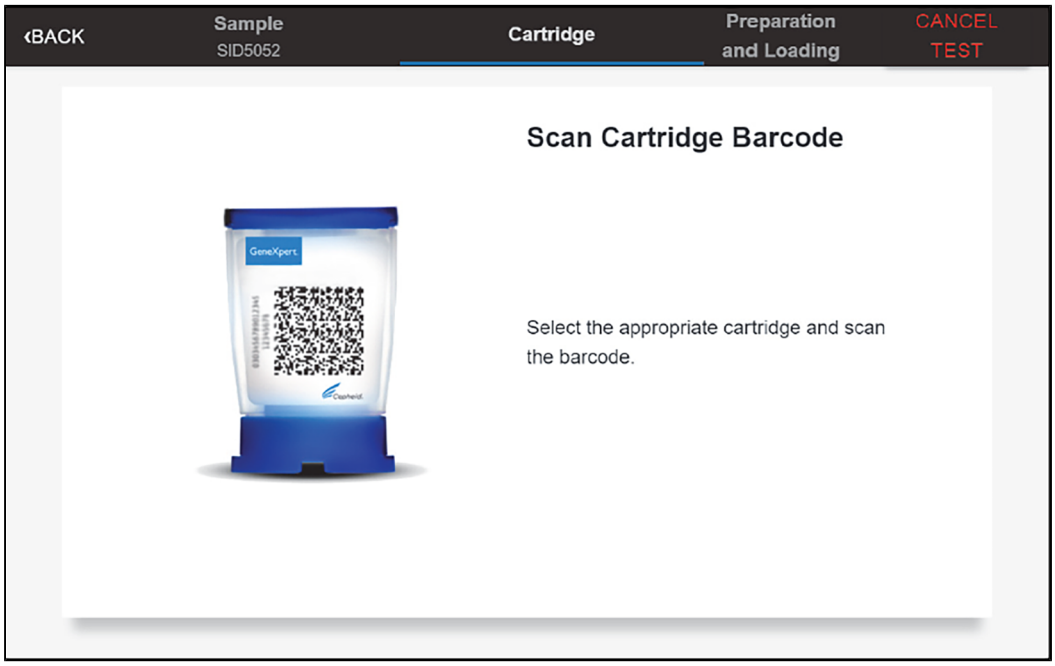


Figure 25. Scan Cartridge Barcode Screen

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 26).

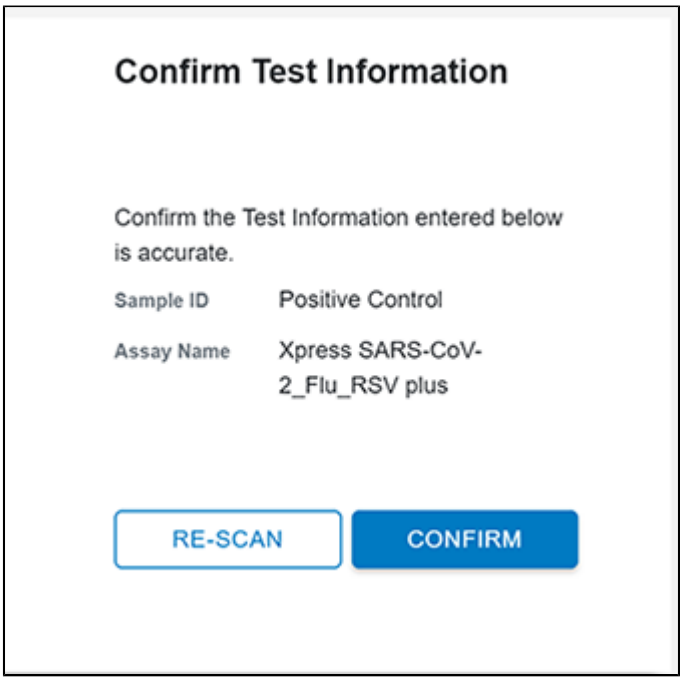
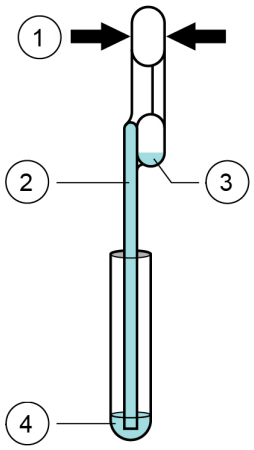


Figure 26. Confirm Test Information

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 27).



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

Figure 27. 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 27). 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 28. Dispose of the used pipette.

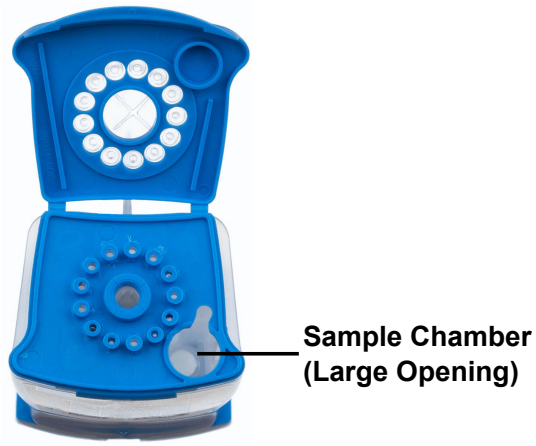


Figure 28. Xpert Xpress CoV-2/Flu/RSV *plus* Cartridge (Top View)

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 16.4, Loading the Cartridge.

16.4 Loading the Cartridge

1. Touch the **CONTINUE** button on the Cartridge Preparation screen. The Load Cartridge into Module screen appears (see Figure 29).
2. Open the module door with the flashing green light.

Figure 29. 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 16.5, 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 or Hub 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**.

16.5 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 16.1, Starting a Test, Section 16.2, Preparing the Specimen, and Section 16.4, 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 30.

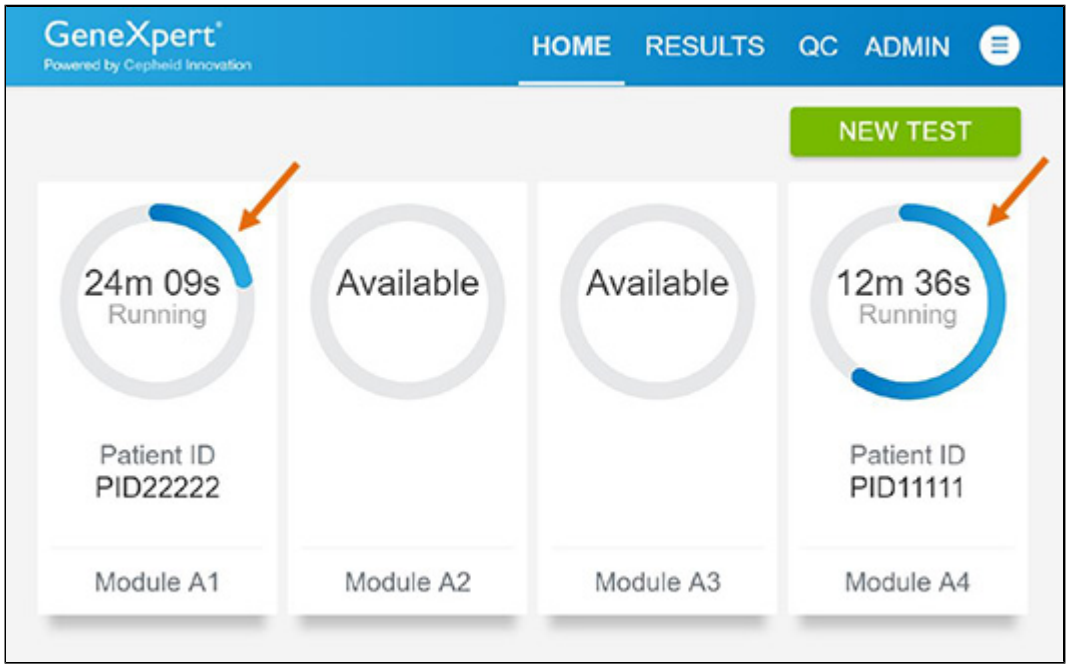


Figure 30. Home Screen showing Two Tests Running

5. After a test has completed, the module icon text changes to Complete (see Figure 31). Touch **Complete View Result** to view test results.

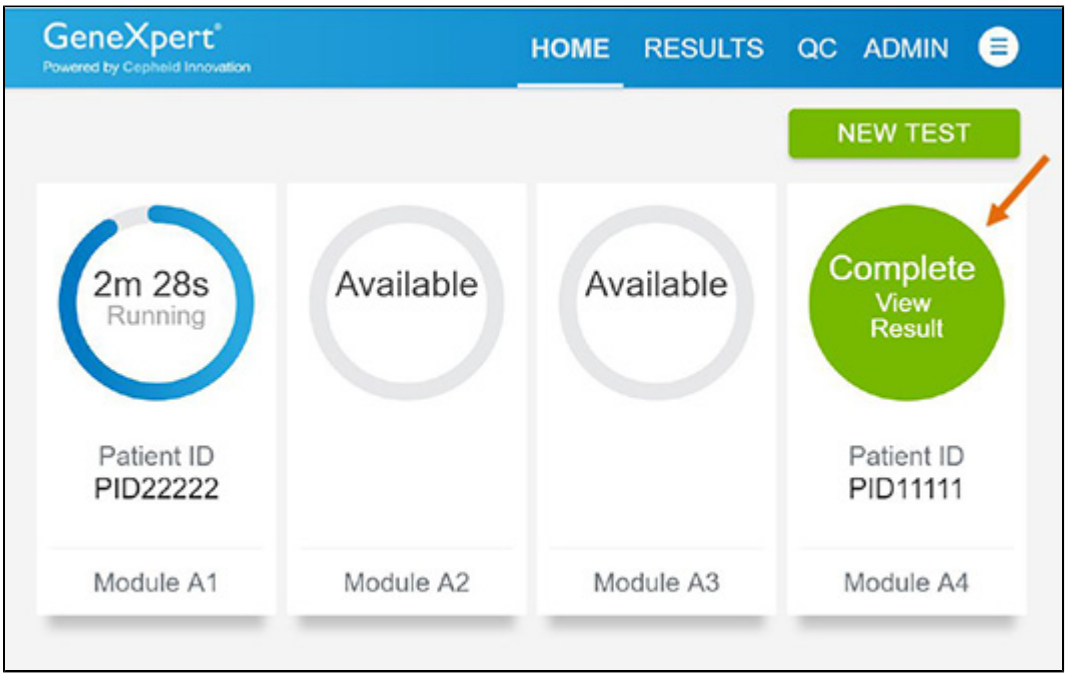


Figure 31. Home Screen with One of Two Tests Completed

16.6 Viewing Test Results

1. Touch the **RESULTS** button located on the panel at the top of the screen (see Figure 31). The Results screen appears (see Figure 32). 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.

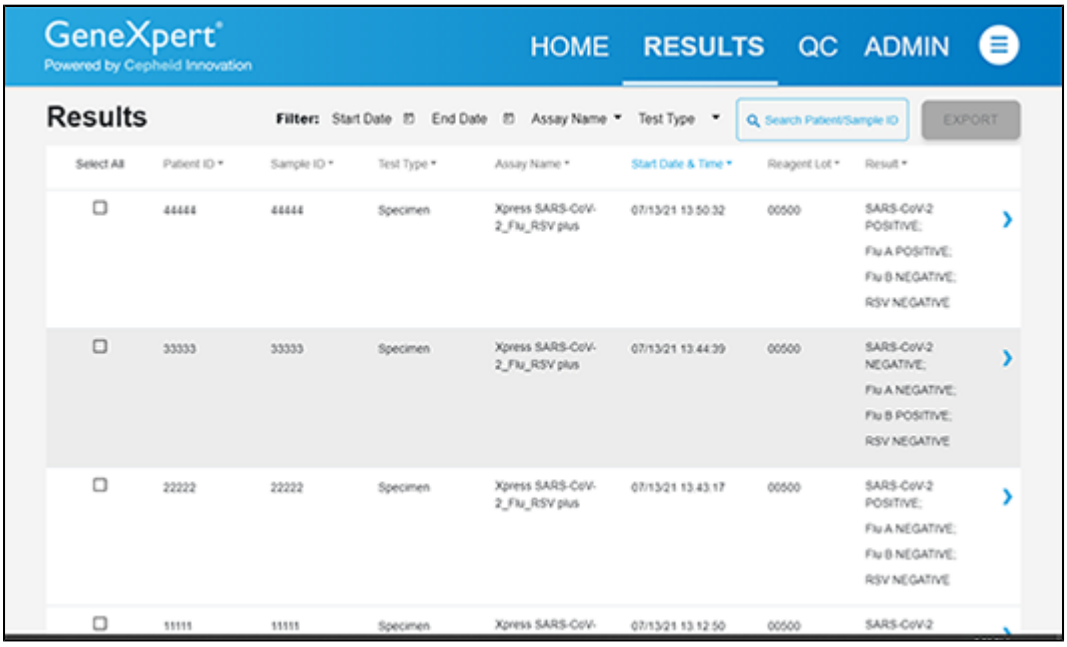


Figure 32. Results Screen

2. Touch the desired result to open the Test Result screen (see Figure 33).
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 33. 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.

17 Quality Control

17.1 Internal Controls

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

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

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

17.2 External Controls

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

18 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 either an Xpress SARS-CoV-2_Flu_RSV plus, Xpress SARS-CoV-2_Flu plus or Xpress SARS-CoV-2_plus test.

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

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

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

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

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

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

Result	Interpretation
SARS-CoV-2 POSITIVE	The SARS-CoV-2 target RNA is detected. ● The SARS-CoV-2 signal has a Ct within the valid range and endpoint above the minimum setting ● SPC: NA (not applicable); SPC is ignored because SARS-CoV-2 target amplification occurred ● Probe Check: PASS; all probe check results pass
Flu A POSITIVE	The Flu A target RNA is detected. ● The Flu A signal for either the Flu A1 RNA target or the Flu A2 RNA target or signals for both RNA targets has a Ct within the valid range and endpoint above the threshold setting ● SPC – NA; SPC is ignored because the Flu A target amplification occurred ● Probe Check – PASS; all probe check results pass
Flu B POSITIVE	The Flu B target RNA is detected. ● The Flu B signal has a Ct within the valid range and endpoint above the minimum setting ● SPC: NA; SPC is ignored because Flu B target amplification occurred ● Probe Check: PASS; all probe check results pass.
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 the 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 Retest Procedure of the IFU.
If the SPC is negative and the results for any of the targets are positive, the results for all targets are considered valid.	

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

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

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

Result	Interpretation
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 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 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 Retest Procedure of the IFU.

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

19 Retests

19.1 Reasons to Repeat the Test

If any of the test results mentioned below occur, repeat the test once according to instructions in Section 19.2, 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.

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

19.2 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.

20 Limitations

- Performance of the Xpert Xpress CoV-2/Flu/RSV *plus* has only been established in nasopharyngeal swab specimens. Use of the Xpert Xpress CoV-2/Flu/RSV *plus* test with other specimen types has not been assessed and performance characteristics are unknown.
- The performance of this test was established based on the evaluation of a limited number of clinical specimens. Clinical performance has not been established with all circulating variants but is anticipated to be reflective of the prevalent variants in circulation at the time and location of the clinical evaluation. Performance at the time of testing may vary depending on the variants circulating, including newly emerging strains of SARS-CoV-2 and their prevalence, which change over time.
- The performance of this device has not been assessed in a population vaccinated against COVID-19.
- As with any molecular test, mutations within the target regions of Xpert Xpress CoV-2/Flu/RSV *plus* could affect primer and/or probe binding resulting in failure to detect the presence of virus.
- As with any molecular test, mutations within the target regions of the Xpert Xpress CoV-2/Flu/RSV *plus* test could affect primer and/or probe binding resulting in failure to detect the presence of virus or the virus being detected less predictably.
- This test cannot rule out diseases caused by other bacterial or viral pathogens.
- The performance of this test was validated using the procedures provided in this package insert only. Modifications to these procedures may alter the performance of the test.
- Erroneous test results might occur from improper specimen collection; failure to follow the recommended sample collection, handling, and storage procedures; technical error; or sample mix-up. Careful compliance with the instructions in this insert is necessary to avoid erroneous results.
- False negative results may occur if virus is present at levels below the analytical limit of detection.
- Negative results do not preclude SARS-CoV-2, influenza or RSV infection and should not be used as the sole basis for treatment or other patient management decisions.
- Results from the Xpert Xpress CoV-2/Flu/RSV *plus* test should be correlated with the clinical history, epidemiological data, and other data available to the clinician evaluating the patient.
- Viral nucleic acid may persist *in vivo*, independent of virus infectivity. Detection of analyte target(s) does not imply that the corresponding virus(es) are infectious or are the causative agents for clinical symptoms.
- This test has been evaluated for use with human specimen material only.
- This test is a qualitative test and does not provide the quantitative value of detected organism present.
- This test has not been evaluated for patients without signs and symptoms of respiratory tract infection.
- This test has not been evaluated for monitoring treatment of infection.
- This test has not been evaluated for screening of blood or blood products for the presence of SARS-CoV-2, influenza, or RSV.
- The effect of interfering substances has only been evaluated for those listed within the labeling. Interference by substances other than those described can lead to erroneous results.
- Results from analytical studies with contrived co-infected samples showed potential for competitive interference of influenza B or RSV A at low concentrations (~3X LoD) when influenza A concentration is >1.7e5 RNA copies/mL or 1.7e6 RNA copies/mL, respectively. In addition, there is potential for competitive interference of influenza B at low concentration (~3X LoD) when SARS-CoV-2 concentration is >1e5 RNA copies/mL.
- Cross-reactivity with respiratory tract organisms other than those described herein can lead to erroneous results.
- Recent patient exposure to FluMist® or other live attenuated influenza vaccines may cause inaccurate positive results.
- Zicam at 15% (w/v) may interfere with the detection of low levels of influenza B and RSV A.
- As the Xpert Xpress CoV-2/Flu/RSV *plus* test does not differentiate between the N2, RdRP and E gene targets, the presence of other coronaviruses in the B lineage, Betacoronavirus genus, including SARS-CoV may cause a false positive result. None of these other coronaviruses is known to currently circulate in the human population.
- This test is not intended to differentiate RSV subgroups, influenza A subtypes or influenza B lineages. If differentiation of specific RSV or influenza subtypes and strains is needed, additional testing, in consultation with state or local public health departments, is required.
- Performance has not been established with media containing guanidine thiocyanate (GTC) other than eNAT.

21 Performance Characteristics

21.1 Clinical Evaluation

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

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

Xpert Xpress CoV-2/Flu/RSV *plus* demonstrated a PPA and NPA of 100.0% and 100.0% for SARS-CoV-2, respectively; 100.0% and 100.0% for Flu A, respectively; 100.0% and 100.0% for Flu B, respectively; 100.0% and 100.0% for RSV, respectively (Table 4). The initial non-determinate rate for the Xpert Xpress CoV-2/Flu/RSV *plus* test was 0.7% (2/279). On repeat testing, both (2) specimens yielded valid results. The final non-determinate rate for the Xpert Xpress CoV-2/Flu/RSV *plus* test was 0.0% (0/279).

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

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

For the NS specimens, Xpert Xpress CoV-2/Flu/RSV *plus* demonstrated a PPA and NPA of 100.0% and 100.0% for SARS-CoV-2, respectively; 100.0% and 99.5% for Flu A, respectively; 100.0% and 100.0% for Flu B, respectively; 100.0% and 100.0% for RSV, respectively (Table 5). The initial non-determinate rate for the Xpert Xpress CoV-2/Flu/RSV *plus* test was 1.3% (3/240). Two (2) of the three (3) specimens gave valid results upon retest. One specimen was not re-tested due to insufficient volume. The final non-determinate rate for the Xpert Xpress CoV-2/Flu/RSV *plus* test was 0.4% (1/240).

Table 5. Xpert Xpress CoV-2/Flu/RSV *plus* Performance Results Using NS Specimens

Target	Number of Specimens	TP	FP	TN	FN	PPA (95% CI)	NPA (95% CI)
SARS-CoV-2	239	47	0	192	0	100.0% (92.4% - 100.0%)	100.0% (98.0% - 100.0%)
Flu A	239	48	1	191	0	100.0% (92.6% - 100.0%)	99.5% (97.1% - 99.9%)
Flu B	239	48	0	191	0	100.0% (92.6% - 100.0%)	100.0% (98.0% - 100.0%)
RSV	239	47	0	192	0	100.0% (92.4% - 100.0%)	100.0% (98.0% - 100.0%)
TP: True Positive; FP: False Positive; TN: True Negative; FN: False Negative; CI: Confidence Interval							

21.2 Analytical Sensitivity (Limit of Detection)

The analytical sensitivity of the Xpert Xpress CoV-2/Flu/RSV *plus* test was first estimated using two reagent lots by testing limiting dilutions of seven respiratory viruses (NATtrol SARS-CoV-2, Flu A H1, Flu A H3, Flu B Victoria lineage, Flu B Yamagata lineage, RSV A and RSV B) into pooled negative clinical NP swab matrix, following the guidance in Clinical and Laboratory Standards Institute (CLSI) document EP17-A2. The estimated LoD values as determined by Probit regression analysis were verified using two lots of Xpert Xpress CoV-2/Flu/RSV *plus* reagents. The verified LoD values for the viruses tested are summarized in Table 6.

Table 6. Xpert Xpress CoV-2/Flu/RSV *plus* Limit of Detection

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

21.3 Analytical Reactivity (Inclusivity)

The inclusivity of Xpert Xpress CoV-2/Flu/RSV *plus* was evaluated on September 27, 2021 using *in silico* analysis of the assay amplicons in relation to 2,685,478 SARS-CoV-2 sequences available in the GISAID gene database for three targets, E, N2 and RdRP.

For analysis of the E target, 3,818 sequences were excluded due to ambiguous nucleotides, which reduced the total to 2,681,660 sequences. Of the 2,681,660 GISAID sequences, 2,667,594 (99.48%) were an exact match to the SARS-CoV-2 E target amplicon generated in the Xpert Xpress CoV-2/Flu/RSV *plus* test. Single nucleotide mismatches were observed for 13,990 sequences and two mismatches or more were observed for 76 sequences. Of the 76 sequences with two or more mismatches, 43 sequences contained 2 or 3 mismatches in the forward primer region; one sequence contained 3 mismatches in the reverse primer region; and one sequence contained 2 mismatches in the forward primer and 2 mismatches in the reverse primer. These double and triple mismatches could have a negative impact on the performance of the assay.

For analysis of the N2 target, 4,110 sequences were excluded due to ambiguous nucleotides, which reduced the total used in the evaluation to 2,681,368 sequences. Of the 2,681,368 GISAID sequences, 2,608,487 (97.3%) were an exact match to the SARS-CoV-2 N2 target amplicon generated in the Xpert Xpress CoV-2/Flu/RSV *plus* test. Single nucleotide mismatches

were observed for 70,212 sequences. Two or three mismatches were observed for 2,669 sequences. Of the 31 sequences with three variant positions, 5 sequences have two of the mismatched nucleotides in the probe region and 5 of the sequences have two of the mismatched nucleotides in the reverse primer region. These double mismatches could have an impact on probe or reverse primer binding. None of the other mismatches are predicted to have a negative impact on the performance of the assay.

The RdRP is amplified using a semi-nested primer/probe set; only the inner amplicon is used for the *in silico* analysis. For analysis of the RdRP target, 1,374 sequences were excluded due to ambiguous nucleotides, which reduced the total to 2,684,104 sequences. Of the 2,684,104 GISAID sequences, 2,657,136 (99.0%) were an exact match to the SARS-CoV-2 RdRP target amplicon generated in the Xpert Xpress CoV-2/Flu/RSV *plus* test. Single nucleotide mismatches were observed for 26,864 sequences and two or more mismatches were observed for 77 sequences. Two sequences have 5 mismatches, three located in the probe region and two in the reverse primer region; 20 sequences have two nucleotide mismatches in the forward primer or probe region. These mismatches could have an impact on probe or reverse primer binding. None of the other mismatches are predicted to have a negative impact on the performance of the assay.

In addition to the *in silico* analysis of the SARS-CoV-2 primers and probes for inclusivity, the inclusivity of the Xpert Xpress CoV-2/Flu/RSV *plus* test was evaluated by bench testing against multiple strains of SARS-CoV-2, influenza A H1N1 (seasonal pre-2009), influenza A H1N1 (pandemic 2009), influenza A H3N2 (seasonal), avian influenza A (H5N1, H5N2, H6N2, H7N2, H7N3, H2N2, H7N9, and H9N2), influenza B (representing strains from both Victoria and Yamagata lineages), and respiratory syncytial virus subgroups A and B (RSV A and RSV B) at levels near the analytical LoD. A total of 84 strains comprised of 5 SARS-CoV-2 virus strains, 4 SARS-CoV-2 in vitro RNA transcripts representing variant strains, 69 influenza viruses (48 influenza A and 21 influenza B) and 6 RSV strains (4 RSVA and 2 RSV B) were tested in this study with the Xpert Xpress CoV-2/Flu/RSV *plus* test. Three replicates were tested for each strain. All SARS-CoV-2, Flu and RSV strains tested positive in all three replicates. Results are shown in Table 7.

Table 7. Analytical Reactivity (Inclusivity) of the Xpert Xpress CoV-2/Flu/RSV *plus* Test

Virus	Strain	Tested Titer	SARS-CoV-2	Flu A	Flu B	RSV
SARS-CoV-2	NATtrol SARS-CoV-2 USA-WA1/2020	412 copies/mL	POS	NEG	NEG	NEG
	SARS-CoV-2/Hong Kong/VM20001061/2020	0.5 $TCID_{50}$/mL	POS	NEG	NEG	NEG
	SARS-CoV-2/Italy-INMI1	4 $TCID_{50}$/mL	POS	NEG	NEG	NEG
	SARS-CoV-2/South_Africa/ KRISP-K005325/2020	0.2 $TCID_{50}$/mL	POS	NEG	NEG	NEG
	SARS-CoV-2/England/ 204820464/2020	0.5 $TCID_{50}$/mL	POS	NEG	NEG	NEG
	SARS-CoV-2 RNA USA/WA2/2020(C09)[a]	100 copies/mL	POS	NEG	NEG	NEG
	SARS-CoV-2RNA/ England/205041766/ 2020(C14)[a]	100 copies/mL	POS	NEG	NEG	NEG
	SARS-CoV-2 RNA /England/ MILK-9E05B3/2020 (C15)[a]	200 copies/mL	POS	NEG	NEG	NEG
	SARS-CoV-2 RNA /Japan (Brazil)/IC-0564/2021 (C17)[a]	100 copies/mL	POS	NEG	NEG	NEG
Influenza A H1N1 (pre-2009)	A/swine/Iowa/15/30	30 $TCID_{50}$/mL	NEG	POS	NEG	NEG
	A/WS/33	5.0 $CEID_{50}$/mL	NEG	POS	NEG	NEG
	A/PR/8/34	20 $CEID_{50}$/mL	NEG	POS	NEG	NEG
	A/Mal/302/54	0.156 $CEID_{50}$/mL	NEG	POS	NEG	NEG
	A/Denver/1/57	10 $CEID_{50}$/mL	NEG	POS	NEG	NEG

Virus	Strain	Tested Titer	SARS-CoV-2	Flu A	Flu B	RSV
	A/New Jersey/8/76	5.0 $CEID_{50}$/mL	NEG	POS	NEG	NEG
	A/New Caledonia/20/1999	0.10 $TCID_{50}$/mL	NEG	POS	NEG	NEG
	A/New York/55/2004	30 $TCID_{50}$/mL	NEG	POS	NEG	NEG
	A/Solomon Island/3/2006	0.0159 $TCID_{50}$/mL	NEG	POS	NEG	NEG
	A/Taiwan/42/06	0.0159 $TCID_{50}$/mL	NEG	POS	NEG	NEG
	A/Brisbane/59/2007	0.060 $TCID_{50}$/mL	NEG	POS	NEG	NEG
	A/Swine/NY/02/2009	20 $TCID_{50}$/mL	NEG	POS	NEG	NEG
Influenza A H1N1 (pdm2009)	A/Colorado/14/2012	0.13 $TCID_{50}$/mL	NEG	POS	NEG	NEG
	A/Michigan/45/2015	100 $CEID_{50}$/mL	NEG	POS	NEG	NEG
	A/Iowa/53/2015	100 $CEID_{50}$/mL	NEG	POS	NEG	NEG
	A/Michigan/272/2017	1.0 $TCID_{50}$/mL	NEG	POS	NEG	NEG
	A/Idaho/07/2018	0.0159 $TCID_{50}$/mL	NEG	POS	NEG	NEG
	A/Wisconsin/505/2018	0.25 $TCID_{50}$/mL	NEG	POS	NEG	NEG
	A/Hawaii/66/2019	100 $CEID_{50}$/mL	NEG	POS	NEG	NEG
	A/Indiana/02/2020	NA[b]	NEG	POS	NEG	NEG
Influenza A H3N2 (Seasonal)	A/Aichi/2/68	2.0 $CEID_{50}$/mL	NEG	POS	NEG	NEG
	A/Hong Kong/8/68	2.0 $CEID_{50}$/mL	NEG	POS	NEG	NEG
	A/Port Chalmers/1/73	100 $CEID_{50}$/mL	NEG	POS	NEG	NEG
	A/Hawaii/15/2001	100 $CEID_{50}$/mL	NEG	POS	NEG	NEG
	A/Wisconsin/67/05[c]	0.22 $TCID_{50}$/mL	NEG	POS	NEG	NEG
	A/Brisbane/10/2007	0.025 $TCID_{50}$/mL	NEG	POS	NEG	NEG
	A/Minnesota/11/2010	30 $CEID_{50}$/mL	NEG	POS	NEG	NEG
	A/Indiana/08/2011	0.25 $TCID_{50}$/mL	NEG	POS	NEG	NEG
	A/Texas/50/2012	0.050 $TCID_{50}$/mL	NEG	POS	NEG	NEG
	A/Alaska/232/2015	20 $CEID_{50}$/mL	NEG	POS	NEG	NEG
	A/Singapore/ INFIMH-16-0019/2016	20 $CEID_{50}$/mL	NEG	POS	NEG	NEG
	A/Texas/71/2017	1.0 FFU/mL	NEG	POS	NEG	NEG
	A/Kansas/14/2017	1.0 FFU/mL	NEG	POS	NEG	NEG
	A/Wisconsin/04/2018	1.0 FFU/mL	NEG	POS	NEG	NEG
	A/Arizona/45/2018	2.0 FFU/mL	NEG	POS	NEG	NEG
	A/Hong Kong/45/2019	2.0 FFU/mL	NEG	POS	NEG	NEG
Avian influenza A[d]	A/Mallard/NY/6750/78 (H2N2)	<1 pg/µL	NEG	POS	NEG	NEG
	A/duck/Hunan/ 795/2002 (H5N1)	<1 pg/µL	NEG	POS	NEG	NEG

Virus	Strain	Tested Titer	SARS-CoV-2	Flu A	Flu B	RSV
	A/Vietnam/1194/2004 (H5N1)	<1 pg/µL	NEG	POS	NEG	NEG
	A/Anhui/01/2005 (H5N1)	<1 pg/µL	NEG	POS	NEG	NEG
	A/Japanese white eye/Hong Kong/1038/2006 (H5N1)	<1 pg/µL	NEG	POS	NEG	NEG
	A/mallard/WI/34/75 (H5N2)	<1 pg/µL	NEG	POS	NEG	NEG
	A/chicken/CA431/00 (H6N2)	<1 pg/µL	NEG	POS	NEG	NEG
	A/duck/LTC-10-82743 (H7N2)	<1 pg/µL	NEG	POS	NEG	NEG
	A/chicken/New Jersey/15086/3 (H7N3)	<1 pg/µL	NEG	POS	NEG	NEG
	A/Anhui/1/2013 (H7N9)	0.612 ng/µL	NEG	POS	NEG	NEG
	A/Shanghai/1/2013 (H7N9)	NA[e]	NEG	POS	NEG	NEG
	A/chicken/Korea/38349-p96323/1996 (H9N2)	<1 pg/µL	NEG	POS	NEG	NEG
Influenza B	B/Lee/40	1.0 PFU/mL	NEG	NEG	POS	NEG
	B/Allen/45	0.25 $CEID_{50}$/mL	NEG	NEG	POS	NEG
	B/GL/1739/54	0.50 $CEID_{50}$/mL	NEG	NEG	POS	NEG
	B/Maryland/1/59	1.0 $CEID_{50}$/mL	NEG	NEG	POS	NEG
	B/Taiwan/2/62	1.0 $CEID_{50}$/mL	NEG	NEG	POS	NEG
	B/Hong Kong/5/72	1.0 $CEID_{50}$/mL	NEG	NEG	POS	NEG
Influenza B Victoria Lineage	B/Panama/45/90	1.0 $TCID_{50}$/mL	NEG	NEG	POS	NEG
	B/Malaysia/2506/04	0.025 $TCID_{50}$/mL	NEG	NEG	POS	NEG
	B/Florida/02/06	0.025 $TCID_{50}$/mL	NEG	NEG	POS	NEG
	B/Brisbane/60/2008	0.05 $TCID_{50}$/mL	NEG	NEG	POS	NEG
	B/Maryland/15/2016	0.25 $TCID_{50}$/mL	NEG	NEG	POS	NEG
	B/Colorado/6/2017	0.25 $TCID_{50}$/mL	NEG	NEG	POS	NEG
	B/Hawaii/01/2018	8.0 $TCID_{50}$/mL	NEG	NEG	POS	NEG
	B/Missouri/12/2018(NA D197E)	10 $TCID_{50}$/mL	NEG	NEG	POS	NEG
	B/Washington/02/2019	60 $TCID_{50}$/mL	NEG	NEG	POS	NEG
Influenza B Yamagata Lineage	B/Florida/07/2004	0.50 $TCID_{50}$/mL	NEG	NEG	POS	NEG
	B/Florida/04/06	0.25 $TCID_{50}$/mL	NEG	NEG	POS	NEG
	B/Wisconsin/01/2010	0.50 $CEID_{50}$/mL	NEG	NEG	POS	NEG
	B/Wisconsin/10/2016	20 $TCID_{50}$/mL	NEG	NEG	POS	NEG
	B/Indiana/17/2017	10 $TCID_{50}$/mL	NEG	NEG	POS	NEG
	B/Oklahoma/10/2018	10 $TCID_{50}$/mL	NEG	NEG	POS	NEG

Virus	Strain	Tested Titer	SARS-CoV-2	Flu A	Flu B	RSV
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	0.25 $TCID_{50}$/mL	NEG	NEG	NEG	POS
RSV B	RSV-B/WV14617/85	0.10 $TCID_{50}$/mL	NEG	NEG	NEG	POS
	RSV-B-CH93(18)-18-01	0.10 $TCID_{50}$/mL	NEG	NEG	NEG	POS

[a] *in vitro* RNA transcripts

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

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

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

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

21.4 Analytical Specificity (Exclusivity)

An *in silico* analysis for possible cross-reactions with all the organisms listed in Table 8 was conducted by mapping the SARS-CoV-2 primers and probes in the Xpert Xpress CoV-2/Flu/RSV *plus* test individually to the sequences downloaded from the GISAID database. E primers and probes are not specific for SARS-CoV-2 and will detect Human and Bat SARS-coronavirus. No potential unintended cross reactivity with other organisms listed in Table 8 is expected based on the *in silico* analysis.

Table 8. Microorganisms Analyzed in the in silico Analysis for the SARS-CoV-2 Target

Microorganisms from the Same Genetic Family	High Priority Organisms
Human coronavirus 229E	Adenovirus (e.g. C1 Ad. 71)
Human coronavirus OC43	Human metapneumovirus (hMPV)
Human coronavirus HKU1	Parainfluenza viruses 1-4
Human coronavirus NL63	Influenza A
SARS-coronavirus	Influenza B
MERS-coronavirus	Influenza C
Bat coronavirus	Enterovirus (e.g. EV68)
	Respiratory syncytial virus
	Rhinovirus
	Chlamydia pneumoniae
	Haemophilus influenzae
	Legionella pneumophila
	Mycobacterium tuberculosis
	Streptococcus pneumoniae
	Streptococcus pyogenes
	Bordetella pertussis
	Mycoplasma pneumoniae

Microorganisms from the Same Genetic Family	High Priority Organisms
	Pneumocystis jirovecii (PJP)
	Parechovirus
	Candida albicans
	Corynebacterium diphtheriae
	Legionella non-*pneumophila*
	Bacillus anthracis (Anthrax)
	Moraxella catarrhalis
	Neisseria elongata and *N. meningitidis*
	Pseudomonas aeruginosa
	Staphylococcus epidermidis
	Streptococcus salivarius
	Leptospira
	Chlamydia psittaci
	Coxiella burnetii (Q-Fever)
	Staphylococcus aureus

In addition to the *in silico* analysis of the SARS-CoV-2 primers and probes for cross-reactivity, the analytical specificity of the Xpert Xpress CoV-2/Flu/RSV *plus* test was evaluated by bench-testing a panel of 48 microorganisms comprising 4 human coronaviruses, 1 MERS coronavirus and 43 common respiratory pathogens or those potentially encountered in the nasopharynx. The panel was tested in different pools of microorganisms; if a pool produced a positive result, then each member of the pool would have been tested individually. Three replicates of each pool were tested. A sample was considered negative if all three replicates were negative. The bacterial and yeast strains were tested at concentrations of $\geq 1 \times 10^6$ CFU/mL with the exception of *Chlamydia pneumoniae* which was tested at 1.2×10^6 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 9.

Table 9. Respiratory Microorganisms and Human Coronavirus Tested, Concentrations and Xpert Xpress CoV-2/Flu/RSV *plus* Test Results

Strain	Tested Concentration	SARS-CoV-2	Flu A	Flu B	RSV
Negative Control	NA	NEG	NEG	NEG	NEG
Positive Control	NA	POS	POS	POS	POS
Human coronavirus NL63	1.17e5 $TCID_{50}$/mL	NEG	NEG	NEG	NEG
MERS-coronavirus	1.17e5 $TCID_{50}$/mL	NEG	NEG	NEG	NEG
Human coronavirus 229E	1.21e5 $TCID_{50}$/mL	NEG	NEG	NEG	NEG
Human coronavirus OC43	1.02e5 $TCID_{50}$/mL	NEG	NEG	NEG	NEG
Human coronavirus HKU1	1.23e6 copies/mL	NEG	NEG	NEG	NEG
Adenovirus Type 1	4.07e5 $TCID_{50}$/mL	NEG	NEG	NEG	NEG
Adenovirus Type 7	1.14e5 $TCID_{50}$/mL	NEG	NEG	NEG	NEG
Cytomegalovirus	1.0e5 $TCID_{50}$/mL	NEG	NEG	NEG	NEG
Echovirus	1.14e5 $TCID_{50}$/mL	NEG	NEG	NEG	NEG

Strain	Tested Concentration	SARS-CoV-2	Flu A	Flu B	RSV
Enterovirus	2.80e5 $TCID_{50}$/mL	NEG	NEG	NEG	NEG
Epstein Barr Virus	5.60e6 $TCID_{50}$/mL	NEG	NEG	NEG	NEG
HSV	1.97e5 $TCID_{50}$/mL	NEG	NEG	NEG	NEG
Human metapneumovirus	4.07e5 $TCID_{50}$/mL	NEG	NEG	NEG	NEG
Human parainfluenza Type 1	1.0e5 $TCID_{50}$/mL	NEG	NEG	NEG	NEG
Human parainfluenza Type 2	1.2e5 $TCID_{50}$/mL	NEG	NEG	NEG	NEG
Human parainfluenza Type 3	1.2e5 $TCID_{50}$/mL	NEG	NEG	NEG	NEG
Human parainfluenza Type 4	1.19e6 $TCID_{50}$/mL	NEG	NEG	NEG	NEG
Measles	1.2e5 $TCID_{50}$/mL	NEG	NEG	NEG	NEG
Mumps virus	1.2e5 $TCID_{50}$/mL	NEG	NEG	NEG	NEG
Rhinovirus Type 1A	1.0e5 $TCID_{50}$/mL	NEG	NEG	NEG	NEG
Acinetobacter baumannii	1.30e7 CFU/mL	NEG	NEG	NEG	NEG
Bordetella pertussis	6.40e7 CFU/mL	NEG	NEG	NEG	NEG
Burkholderia cepacia	1.90e8 CFU/mL	NEG	NEG	NEG	NEG
Candida albicans	6.30e6 CFU/mL	NEG	NEG	NEG	NEG
Candida parapsilosis	1.45e6 CFU/mL	NEG	NEG	NEG	NEG
Citrobacter freundii	1.73e8 CFU/mL	NEG	NEG	NEG	NEG
Corynebacterium sp.	1.27e7 CFU/mL	NEG	NEG	NEG	NEG
Enterococcus faecalis	5.87e7 CFU/mL	NEG	NEG	NEG	NEG
Escherichia coli	1.55e8 CFU/mL	NEG	NEG	NEG	NEG
Hemophilus influenzae	6.62e6 CFU/mL	NEG	NEG	NEG	NEG
Lactobacillus reuteri	5.0e7 copies/mL	NEG	NEG	NEG	NEG
Legionella spp.	1.42e8 CFU/mL	NEG	NEG	NEG	NEG
Moraxella catarrhalis	2.46e6 CFU/mL	NEG	NEG	NEG	NEG
Mycoplasma pneumoniae	2.7e6 CFU/mL	NEG	NEG	NEG	NEG
Neisseria meningitides	4.2e6 CFU/mL	NEG	NEG	NEG	NEG
Neisseria mucosa	1.0e8 CFU/mL	NEG	NEG	NEG	NEG
Propionibacterium acnes	8.25e7 CFU/mL	NEG	NEG	NEG	NEG
Pseudomonas aeruginosa	1.05e7 CFU/mL	NEG	NEG	NEG	NEG
Staphylococcus haemolyticus	2.66e6 CFU/mL	NEG	NEG	NEG	NEG
Staphylococcus aureus	5.87e7 CFU/mL	NEG	NEG	NEG	NEG
Staphylococcus epidermidis	2.47e7 CFU/mL	NEG	NEG	NEG	NEG
Streptococcus agalactiae	1.75e7 CFU/mL	NEG	NEG	NEG	NEG
Streptococcus pneumoniae	2.26e7 CFU/mL	NEG	NEG	NEG	NEG
Streptococcus pyogenes	9.0e6 CFU/mL	NEG	NEG	NEG	NEG

Strain	Tested Concentration	SARS-CoV-2	Flu A	Flu B	RSV
Streptococcus salivarius	4.19e6 CFU/mL	NEG	NEG	NEG	NEG
Streptococcus sanguinis	8.67e6 CFU/mL	NEG	NEG	NEG	NEG
Chlamydia pneumoniae	1.20e6 CFU/mL	NEG	NEG	NEG	NEG
Mycobacterium tuberculosis (avirulent)	1.20e6 CFU/mL	NEG	NEG	NEG	NEG

21.5 Microbial Interference

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

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

21.6 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. Below is a summary of the results:

Table 10. 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 11. 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 12. 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 13. 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 14. Summary of Competitive Interference Study with SARS-CoV-2 at High Concentration

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

The study showed that Flu A/Idaho/07/2018 at concentrations above 1.7e5 RNA copies/mL inhibited detection of Flu B at 3X LoD, and at concentrations above 1.7e6 RNA copies/mL inhibited detection of RSV A at 3X LoD (Table 10). In addition, SARS-CoV-2 at concentrations above 1e5 RNA copies/mL inhibited detection of Flu B at 3X LoD (Table 14). No other competitive interference was observed for the potential co-infections tested in the study at the concentrations tested.

21.7 Potentially Interfering Substances

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

Potentially interfering substances in the nasal passage and nasopharynx may include, but are not limited to: blood, nasal secretions or mucus, and nasal and throat medications used to relieve congestion, nasal dryness, irritation, or asthma and allergy symptoms, as well as antibiotics and antivirals. Positive and negative samples were prepared in simulated nasopharyngeal swab (NPS)/ nasal swab (NS) matrix. Negative samples (N = 8) were tested in the presence of each substance to determine the effect on the performance of the sample processing control (SPC). Positive samples (N = 8) were tested per substance with viruses spiked at 3x the LoD determined for each strain. Positive samples tested with the Xpert Xpress CoV-2/Flu/RSV *plus* included one SARS-CoV-2, one influenza A H1N1, one influenza A H3N2, one influenza B and two RSV (RSV A and RSV B) strains. The substances, with active ingredients, that were evaluated are listed in Table 15.

Table 15. Potentially Interfering Substances Tested

Substance ID	Substance/Class	Substance/Active Ingredient
No substance	Control	Copan Universal Transport Medium (UTM)
Albuterol Sulfate	Beta-adrenergic bronchodilator	Albuterol Sulfate (5mg/mL)
Afrin	Nasal Spray	Oxymetazoline, 0.05%
BD Universal Transport Medium	Transport Media	N/A
Copan 3U045N.PH (Cepheid Swab/M)	Transport Media	N/A
Blood	Blood	Blood (Human)
Fluticasone Propionate Nasal Spray	Nasal corticosteroid	Fluticasone Propionate
Menthol	Throat lozenges, oral anesthetic and analgesic	Benzocaine, Menthol
Mucin	Mucin	Purified Mucin protein (Bovine or porcine submaxillary gland)
Mupirocin	Antibiotic, nasal ointment	Mupirocin (20 mg/g=2%)
PHNY	Nasal Drops	Phenylephrine, 1%
Saline	Saline Nasal Spray	Sodium Chloride (0.65%)
Remel M4RT	Transport Media	N/A
Remel M5	Transport Media	N/A
Tamiflu	Anti-viral drugs	Zanamivir
Tobramycin	Antibacterial, systemic	Tobramycin
Zicam	Nasal Gel	Luffa opperculata, Galphimia glauca, Histaminum hydrochloricum Sulfur (0.05%)
Zinc	Zinc supplement	Zinc Gluconate

The results from the study (Table 16) show that for most cases, 8 out of 8 replicates reported positive results for each combination of virus and substance tested and no interference was observed. When Zicam was initially tested at 15% w/v, interference was observed in the detection of Flu B and RSV A. However, when Zicam was tested at 7.5% w/v, no interference was observed.

Table 16. Mean Ct values for Xpert Xpress CoV-2/Flu/RSV *plus* Targets Tested in the Presence of Potentially Interfering Substances

Substance	Concentration Tested	Number of Correct Results/Number Tested					
		SARS-CoV-2/ USA-WA-1	Influenza A/Idaho/07/ 2018	H3N2 Flu A/ Hong Kong/ 45/2019	Flu B/ Washington /02/2019	RSV A/2/ Australia/61	RSV B/9320/ MA/77
Control Simulated NPS/NS Matrix (No substance)	100% (v/v)	8/8	8/8	8/8	8/8	8/8	8/8
Afrin	15% (v/v)	8/8	8/8	8/8	8/8	8/8	8/8
Albuterol Sulfate	0.83 mg/mL	8/8	8/8	8/8	8/8	8/8	8/8
BD Universal Transport Medium	N/A	8/8	8/8	8/8	8/8	8/8	8/8
Blood	2% (v/v)	8/8	8/8	8/8	8/8	8/8	8/8
Copan Swab M	N/A	8/8	8/8	8/8	8/8	8/8	8/8
Fluticasone Propionate Nasal Spray	5 µg/mL	8/8	8/8	8/8	8/8	8/8	8/8
Menthol	1.7 mg/mL	8/8	8/8	8/8	8/8	8/8	8/8
Mucin	0.1% (w/v)	8/8	8/8	8/8	8/8	8/8	8/8
Mupirocin	10 mg/mL	8/8	8/8	8/8	8/8	8/8	8/8
PHNY	15% (v/v)	8/8	8/8	8/8	8/8	8/8	8/8
Remel M4RT	N/A	8/8	8/8	8/8	8/8	8/8	8/8
Remel M5	N/A	8/8	8/8	8/8	8/8	8/8	8/8
Saline	15% (v/v)	8/8	8/8	8/8	8/8	8/8	8/8
Tamiflu	7.5 mg/mL	8/8	8/8	8/8	8/8	8/8	8/8
Tobramycin	4 µg/mL	8/8	8/8	8/8	8/8	8/8	8/8
Zicam	15% (w/v)	8/8	8/8	8/8	5/8[a]	7/8[b]	8/8
Zinc	0.1 µg/mL	8/8	8/8	8/8	8/8	8/8	8/8

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

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

21.8 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 negative NPS/NS matrix. The negative sample was tested in a GeneXpert module at the start of the study. Following the initial testing of the negative sample, the high positive sample was processed in the same GeneXpert module immediately followed by another negative sample. This was repeated 20 times in the same module, resulting in 20 positives and 21 negatives for the module. The study was repeated using a second GeneXpert module for a total of 40 positive and 42 negative samples. All 40 positive samples were correctly reported as **SARS-CoV-2 POSITIVE**; **Flu A NEGATIVE**; **Flu B POSITIVE**; **RSV NEGATIVE**. All 42 negative samples were correctly reported as **SARS-CoV-2 NEGATIVE**; **Flu A NEGATIVE**; **Flu B NEGATIVE**; **RSV NEGATIVE** with the Xpert Xpress CoV-2/Flu/RSV *plus* test. No specimen or amplicon carry-over contamination was observed in this study.

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23 Cepheid Headquarters Locations

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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
	Expiration date
	Temperature limitation
	Biological risks

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26 Revision History

Description of Changes: 302-8926, Rev. B to Rev. C

Purpose: Updates to the Instructions for Use

Section	Description of Change
9	Update with ZeptoMetrix external controls.
18	Interpretation of Results: Tables 1 and 2 updated to align the results interpretation with change in ADF Algorithm.
21.1	Specified initial non-determinate rate and added final non-determinate rate.
26	Updated revision history section.