GeneXpert®
Powered By CEPHEID INNOVATION

Xpert® Xpress Flu/RSV

REF **XPRSFLU/RSV-10**

Instructions for Use

CLIA Complexity: Waived

IVD

Cepheid®

In Vitro Diagnostic Medical Device

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Trademark, Patents and Copyright Statements

See Section 29, Revision History for a description of changes.

Xpert® Xpress Flu/RSV

℞only

For *In Vitro* Diagnostic Use

CLIA Complexity: Waived

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

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

1 Proprietary Name

Xpert® Xpress Flu/RSV

2 Common or Usual Name

Xpert Xpress Flu/RSV Assay

3 Intended Use

The Cepheid Xpert® Xpress Flu/RSV Assay, performed on the GeneXpert® Xpress System, is an automated, multiplex real-time, reverse transcriptase polymerase chain reaction (RT-PCR) assay intended for the in vitro qualitative detection and differentiation of influenza A, influenza B, and respiratory syncytial virus (RSV) viral RNA. The Xpert Xpress Flu/RSV Assay uses nasopharyngeal (NP) swab and nasal swab (NS) specimens collected from patients with signs and symptoms of respiratory infection. The Xpert Xpress Flu/RSV Assay is intended as an aid in the diagnosis of influenza and respiratory syncytial virus infections in conjunction with clinical and epidemiological risk factors.

Negative results do not preclude influenza virus and should not be used as the sole basis for treatment or other patient management decisions.

Performance characteristics for influenza A were established during the 2016-2017 influenza season when influenza A/H3N2 and A/HINI pandemic were the predominant influenza A viruses in circulation. When other novel influenza A viruses are emerging, performance characteristics may vary.

If infection with a novel influenza A virus is suspected based on current clinical and epidemiological screening criteria recommended by public health authorities, specimens should be collected with appropriate infection control precautions for novel virulent influenza viruses and sent to state or local health departments for testing. Viral culture should not be attempted in these cases unless a BSL 3+ facility is available to receive and culture specimens.

4 Summary and Explanation

Influenza, or the flu, is a contagious viral infection of the respiratory tract. Transmission of influenza is primarily airborne (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.[1,2]

Influenza viruses are classified into types A, B, and C, the former two of which cause the most human infections. Influenza A is the most common type of influenza virus in humans, and is generally responsible for seasonal flu epidemics and potentially pandemics. Influenza A viruses can also infect animals such as birds, pigs, and horses. Infections with influenza B virus are generally restricted to humans and are a rare cause of epidemics. Influenza 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 subtypes H1, H2, H3, N1 and N2. In addition to seasonal flu, a novel H1N1 strain was identified in humans in the United States in early 2009.[3]

Respiratory Syncytial Virus (RSV), a member of the *Pneumoviridae* family (formerly *Paramyxoviridae*), consisting of two strains (subgroups A and B) is also a contagious disease that affects primarily infants, the elderly, and other adults that tend to be immunocompromised in some way.[3] The virus can remain infectious for hours on countertops and toys and can cause both upper respiratory infections, such as colds, and lower respiratory infections manifesting as bronchiolitis and pneumonia.[4] 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 reinfected.[3] Symptoms appear four to six days after infection and are usually self-limiting, lasting approximately one to two weeks. 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 mirrors influenza somewhat as infections begin to rise during the fall through early spring.[3,4]

Active surveillance programs in conjunction with infection prevention precautions are important components for preventing transmission of influenza and RSV. The use of assays providing rapid results to identify patients infected with these seasonal viruses is also an important factor for effective control, proper choice of treatment, and prevention of widespread outbreaks.

5 Principle of the Procedure

The Xpert Xpress Flu/RSV Assay is an automated *in vitro* diagnostic test for qualitative detection of influenza A, influenza B, and RSV viral RNA. The assay is performed on the Cepheid GeneXpert Xpress System. With this platform, an untrained operator can run the test by performing three simple steps: 1) transfer liquid sample to the cartridge with a transfer pipette, 2) run the test on the GeneXpert Xpress System, and 3) read the results.

The GeneXpert Xpress System automates and integrates sample extraction, nucleic acid purification and amplification, and detection of target sequences from clinical specimens by using reverse transcription (conversion of RNA templates into DNA) followed by real-time PCR. The primers and probes in the Xpert Xpress Flu/RSV Assay are designed to amplify and detect unique sequences in the genes

that encode the following proteins: 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 System consists of an instrument, computer, and preloaded software for running tests and viewing the results. Each test requires the use of a single-use disposable GeneXpert cartridge that contains target-specific reagents and carries out the RT-PCR and PCR processes. Because the cartridges are self-contained, the risk of cross-contamination between samples is minimized. For a full description of the systems, refer to the appropriate *GeneXpert Xpress System User's Guide*.

The Xpert Xpress Flu/RSV Assay includes reagents for the detection and differentiation of influenza A, influenza B, and RSV viral RNA directly from NP swab and NS specimens from patients with signs and symptoms of respiratory tract infection. A Sample Processing Control (SPC) and a Probe Check Control (PCC) are also included in the cartridge. The SPC is present to control for an adequate extraction and processing of the target sequences and to monitor for the presence of inhibitors in the PCR reaction. The PCC verifies reagent rehydration, PCR tube filling in the cartridge, probe integrity, and dye stability.

The Xpert Xpress Flu/RSV Assay can be run to detect Flu A, Flu B, and RSV by selecting **Xpert Xpress Flu-RSV** from the Select Assay menu; Flu A and Flu B only by selecting **Xpert Xpress_Flu**; or RSV only by selecting **Xpert Xpress_RSV**. Xpert Xpress Flu and Xpert Xpress RSV assays have an Early Assay Termination (EAT) function that enables early result reporting. EAT is activated when the pre-determined threshold for a positive test result is reached before the full 40 PCR cycles have been completed. When Flu A or Flu B viral titers are high enough to generate very early cycle thresholds (Cts) with the Xpert Xpress Flu Assay, SPC amplification curves will not be seen and their results will not be reported. When RSV titers are high enough to generate very early Cts with the Xpert Xpress RSV Assay, SPC amplification curves will not be seen and their results will not be reported.

The specimens for testing (NP swabs or NS) should be collected according to the institution’s standard procedures and placed into the Xpert Nasopharyngeal Sample Collection Kit for Viruses or the Xpert Nasal Sample Collection Kit for Viruses (viral transport tubes containing 3 mL transport medium).

Following brief mixing by inverting the viral transport tube five times, the medium containing the virus suspension is transferred to the sample chamber of the disposable Xpert Xpress Flu/RSV Assay cartridge. The user initiates a test from the system user interface and places the cartridge into the GeneXpert instrument, which performs nucleic acid preparation and real-time, multiplex RT-PCR for detection of viral RNA. On this platform, sample preparation, reverse transcription, amplification, and real-time detection are all fully-automated and completely integrated. Test results are obtained in approximately 30 minutes.

The results are interpreted by the GeneXpert software from measured fluorescent signals and embedded calculation algorithms and are shown in the “View Results” window in tabular and graphic formats. The Xpert Xpress Flu/RSV Assay provides test results for influenza A, influenza B, and RSV. It also reports if the test is indeterminate (i.e., **INSTRUMENT ERROR** or **NO RESULT—REPEAT TEST**).

6 Reagents and Instruments

6.1 Materials Provided

The Xpert Xpress Flu/RSV Assay kit (XPRSFLU/RSV-10) contains sufficient reagents to process 10 specimens or quality control samples. Each kit contains the following:

Xpert Xpress Flu/RSV Assay Cartridges with Integrated Reaction Tubes	**10 per kit**
• Bead 1, Bead 2, and Bead 3 (freeze-dried)	1 of each per cartridge
• Lysis Reagent (Guanidinium thiocyanate)	1.5 mL per cartridge
• Binding Reagent	1.5 mL per cartridge
• Elution Reagent	3.0 mL per cartridge
Disposable 300 µL Transfer Pipettes	**1 bag of 12 per kit**
Instructions for Use (Package Insert) CLIA Complexity: Waived (For use with the GeneXpert Xpress System only)	**1 per kit**
Quick Reference Instructions CLIA Complexity: Waived (For use with the GeneXpert Xpress System–Tablet Configuration only)	**1 per kit**
Quick Reference Instructions CLIA Complexity: Waived (For use with the GeneXpert Xpress System—Hub Configuration only)	**1 per kit**
CD • Assay Definition File (ADF) • Instructions to import ADF into GeneXpert software • Instructions for Use (Package Insert) (For use with the GeneXpert Dx and Infinity Systems only)	**1 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 Flu/RSV Assay cartridges at 2–28 °C until the expiration date provided on the label.
- Do not open a cartridge lid until you are ready to perform testing.
- Do not use cartridges that have passed the expiration date.
- Do not use a cartridge that has leaked.

8 Materials Required but Not Provided

- Nylon flocked swab (Copan P/N 502CS01, 503CS01) or equivalent
- Viral transport medium, 3 mL (Copan P/N 330C) or equivalent
- Nasopharyngeal Sample Collection Kit for Viruses (Cepheid P/N SWAB/B-100, Copan P/N 305C, Copan P/N 3C057N) or equivalent.
- Nasal Sample Collection Kit for Viruses (Cepheid P/N SWAB/F-100, Copan P/N 346C, Copan P/N 3C064N) or equivalent.
- GeneXpert Xpress System (Tablet Configuration): GeneXpert Xpress II and IV Instruments with proprietary GeneXpert Xpress Software Version 5.x, 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 with proprietary GeneXpert Xpress Software Version 6.0 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

- Inactivated virus controls from ZeptoMetrix (Buffalo, NY), catalog #NATCXVA9-6C (Coxsackie virus) as an external negative control, and catalog #NATFLURSV-6C (NATtrol Influenza A/B and RSV) as external positive control.

10 Warnings and Precautions

10.1 General

- For *in vitro* Diagnostic Use
- For prescription use only
- 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 treated with standard precautions.
- Guidelines for specimen handling are available from the U.S. Centers for Disease Control and Prevention[4] and the Clinical and Laboratory Standards Institute.[5,6]
- If infection with a novel influenza A virus is suspected based on current clinical and epidemiological screening criteria recommended by public health authorities, specimens should be collected with appropriate infection control precautions for novel virulent influenza viruses and sent to state or local health departments for testing. Viral culture should not be attempted in these cases unless a BSL 3+ facility is available to receive and culture specimens.

- Performance characteristics of this test have been established with the specimen types listed in the Intended Use Section only. The performance of this assay with other specimen types or samples has not been evaluated.
- Follow your institution's safety procedures for working with chemicals and handling biological samples.
- Do not allow Lysis Reagent, which contains guanidinium thiocyanate, to contact sodium hypochlorite (bleach) solution. This mixture can produce a highly toxic 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 national or regional disposal procedures. If national 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. Consult your institution's environmental waste personnel on proper disposal of used cartridges and unused reagents.

10.2 Specimen

- Specimen collection and handling procedures require specific training and guidance.
- Specimens must be collected and tested before the expiration date of the viral transport medium tube included in the required collection kit.
- Maintain proper storage conditions during specimen transport to ensure the integrity of the specimen (Section 12, Specimen Collection, Transport, and Storage). Specimen stability under shipping conditions other than those recommended has not been evaluated.
- Proper sample collection, storage, and transport are essential for correct results.

10.3 Assay/Reagent

- The assay has been validated using Cepheid GeneXpert Xpress software version 5.0 or higher. Cepheid will validate future software versions for use with the Xpert Xpress Flu/RSV Assay.
- When performing a test in the Xpert Xpress RSV Assay mode, a sample strongly positive for influenza A or influenza B may cause the SPC to fail and a **NO RESULT—REPEAT TEST** result will be reported; if the sample is RSV negative, a valid result (**RSV NEGATIVE**) will be reported, not a **NO RESULT–REPEAT TEST** result.
- Performance may be impacted when using frozen specimens.
- Do not substitute Xpert Xpress Flu/RSV Assay reagents with other reagents.
- Do not open the Xpert Xpress Flu/RSV Assay cartridge lid except when adding sample.
- Do not use a cartridge that has been dropped after removing from the kit or shaken after the cartridge lid has been opened. Shaking or dropping the cartridge after opening the lid may yield false or non-determinate results.
- Do not place the sample ID label on the cartridge lid or on the barcode label.
- Do not use a cartridge that has a damaged reaction tube.
- Each single-use Xpert Xpress Flu/RSV Assay cartridge is used to process one test. Do not reuse cartridges.

- A 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.
- Good laboratory practices, including changing gloves between handling patient specimens, are recommended to avoid contamination of specimens or reagents.
- Wear clean laboratory coats and gloves. In the event of contamination of the work area or equipment with samples or controls, thoroughly clean the contaminated area with a 1:10 dilution of household chlorine bleach and then 70% denatured ethanol. Wipe work surfaces dry completely before proceeding.

11 Chemical Hazards[7,8]

Cartridge and Lysis Reagent (Guanidinium thiocyanate)

- Signal Word: WARNING
- Hazard Class: Acute Oral Toxicity 5
- **CLP/GHS Hazard Statements:**
  - Harmful if swallowed.
  - Causes mild skin irritation.
  - Causes eye irritation.
  - Harmful to aquatic life.
  - Contact with acids or bleach liberates toxic gas.
- **UN GHS Precautionary Statements:**
  - **Prevention**
    - Wash hands thoroughly after handling.
    - Wear protective gloves and eye protection.
    - Avoid release to the environment.
  - **Response**
    - IF ON SKIN: Wash with plenty of soap and water.
    - IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing. If eye irritation occurs, get medical advice/attention.
    - IF SWALLOWED: Rinse mouth. Call a POISON CENTER or physician if you feel unwell.
    - Dispose of contents/container to location in accordance with local and regional/national/international regulations.

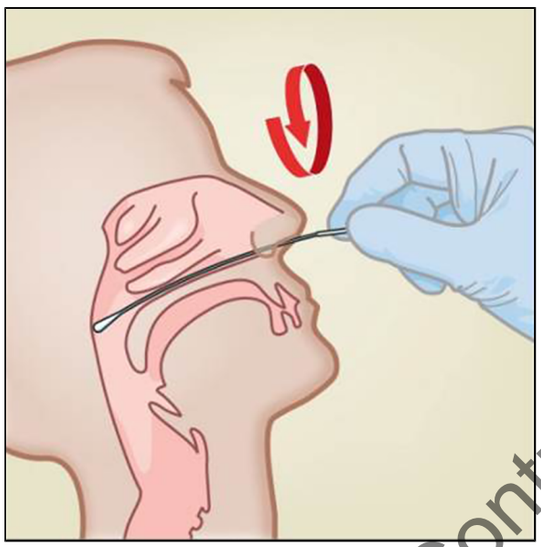
12 Specimen Collection, Transport, and Storage

Specimens can be collected following the user institution's standard procedures and placed into the Xpert Viral Transport Medium or Copan UTM (Universal Transport Medium, 3 mL tube with transport medium). Specimens should be transported at 2–8 °C. Specimens can be stored at room temperature (15–30 °C) for up to 24 hours and refrigerated (2–8 °C) up to seven days until testing is performed on the GeneXpert.

Proper specimen collection, storage, and transport are critical to the performance of this test.

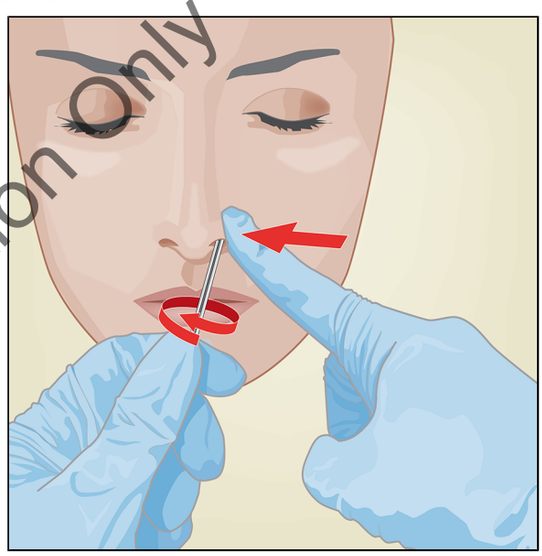
12.1 Nasopharyngeal Swab Collection Procedure

Insert the swab (from the Xpert Nasopharyngeal Sample Collection Kit for Viruses - Cepheid catalog #SWAB/B-100 or equivalent) into either nostril, passing it into the posterior nasopharynx, as shown below. Rotate swab by firmly brushing against the nasopharynx several times. Remove and place the swab into an Xpert Viral Transport Medium tube (from the Xpert Nasopharyngeal Sample Collection Kit for Viruses - Cepheid catalog #SWAB/B-100 or equivalent). Break swab at the indicated break line and cap the tube.



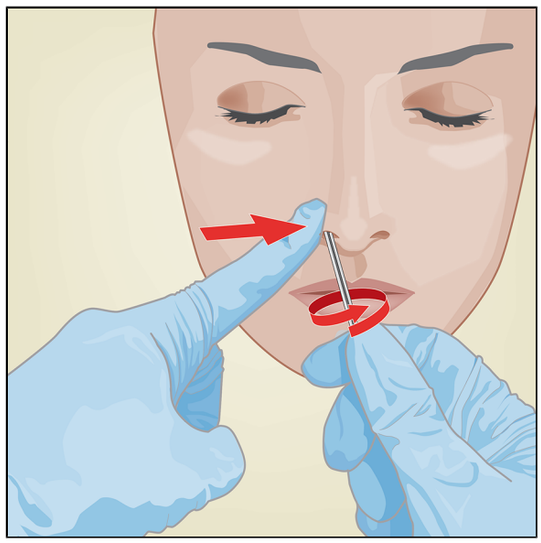
12.2 Nasal Swab Collection Procedure

Insert the swab (from the Xpert Nasal Sample Collection Kit for Viruses - Cepheid catalog #SWAB/F-100 or equivalent) 1–1.5 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.



Repeat on the other nostril with the same swab, using external pressure on the outside of the other nostril.

To avoid specimen contamination, do not touch the swab tip to anything other than the inside of the nostril.



Uncap the tube containing the transport media (from the Xpert Nasal Sample Collection Kit for Viruses - Cepheid catalog #SWAB/F-100 or equivalent). Place the swab into the tube. Break swab at the indicated break line and cap the tube. Cap the tube.

13 Starting the System

The recommended environmental operating conditions for the GeneXpert Xpress System is 15-30 °C (59-86 °F) and relative humidity 20-80%, non-condensing.

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

Figure 1. 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.
3. The Windows Password screen appears. 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 log in 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 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

Once the GeneXpert Xpress software starts, you can determine your software version based on the appearance of the opening screen (see Figure 2).

Software Version 5.x

Software Version 6.0 or Higher

Figure 2. Xpress Opening Screens and Software Versions

- For Software Version 5.x, see Section 14, GeneXpert Xpress Software Version 5.x.
- For Software Version 6.0 or higher, see Section 15, GeneXpert Xpress Software Version 6.0 or Higher.

14 GeneXpert Xpress Software Version 5.x

1. On the Welcome screen, touch the **TOUCH HERE TO BEGIN** button (Figure 3).

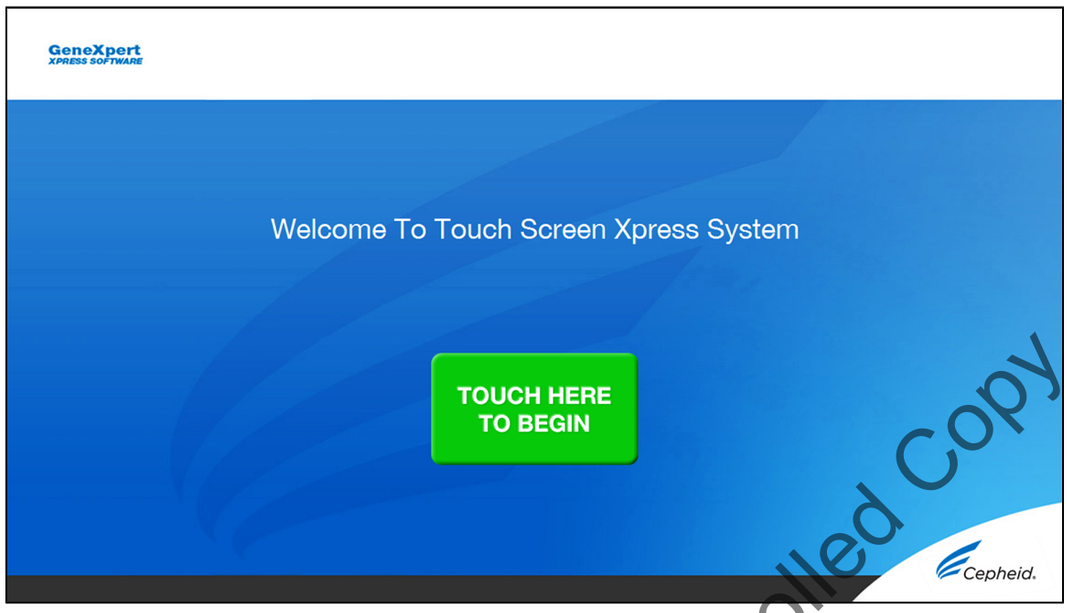


Figure 3. Welcome Screen

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

14.1 Starting a Test

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

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

1. Touch the **RUN NEW TEST** button on the Home screen (Figure 4 and Figure 5).

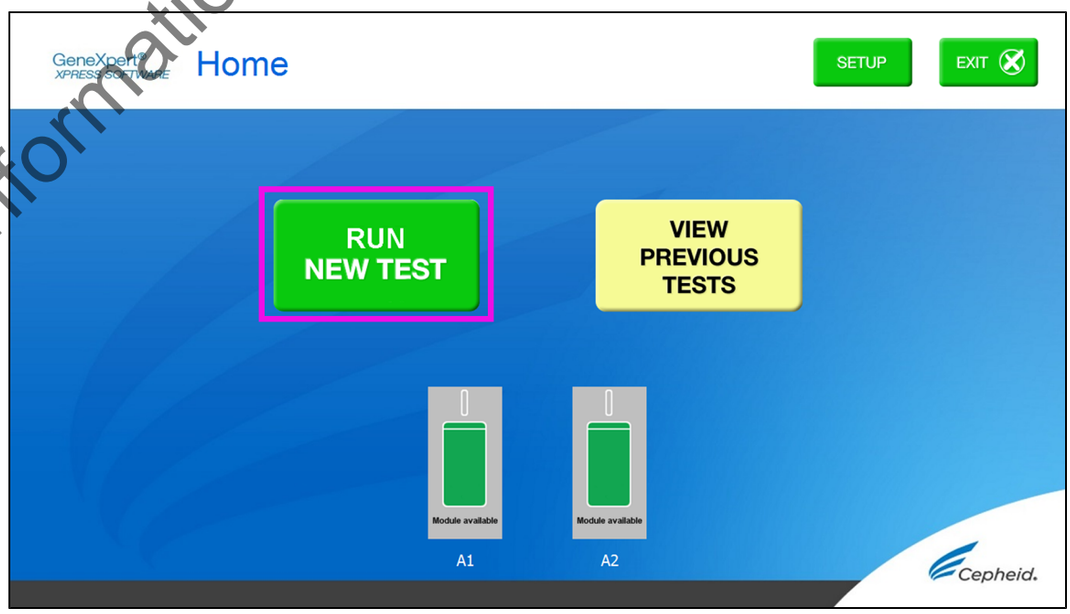


Figure 4. The RUN NEW TEST button on Home Screen (GeneXpert Xpress II)

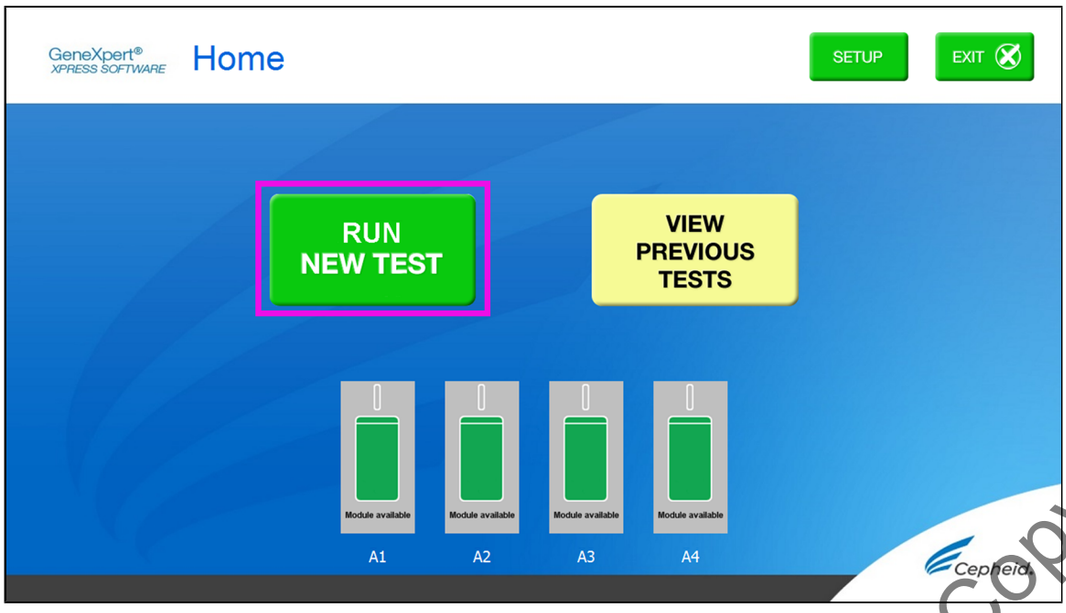


Figure 5. The RUN NEW TEST button on Home Screen (GeneXpert Xpress IV)

2. Check that the transport medium tube cap is closed. Scan with the barcode scanner or manually enter the Sample ID.
3. Touch **YES** if the displayed information is correct.

14.2 Preparing the Specimen and Cartridge

1. Follow the on-screen instructions and video. The following instructions on how to prepare the specimen and the cartridge are also shown in the video.
2. Remove a cartridge and a transfer pipette from the cartridge kit box.
3. Scan the barcode on the cartridge with the scanner.

Note

If the barcode on the Xpert Xpress Flu/RSV test 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.

4. Make the appropriate selection from the Select Assay menu, as shown in Figure 6.
   - Flu A, Flu B and RSV: Select **Xpert Xpress Flu-RSV**
   - Flu A and Flu B only: Select **Xpert Xpress_Flu**
   - RSV only: Select **Xpert Xpress_RSV**

   Only the test result for the assay selected at this step will be collected once the test is started. Flu A, Flu B, and RSV results will only be collected if the **Xpert Xpress Flu-RSV** assay is selected.
5. Confirm the selected test from the Select Assay menu, as shown in Figure 7.

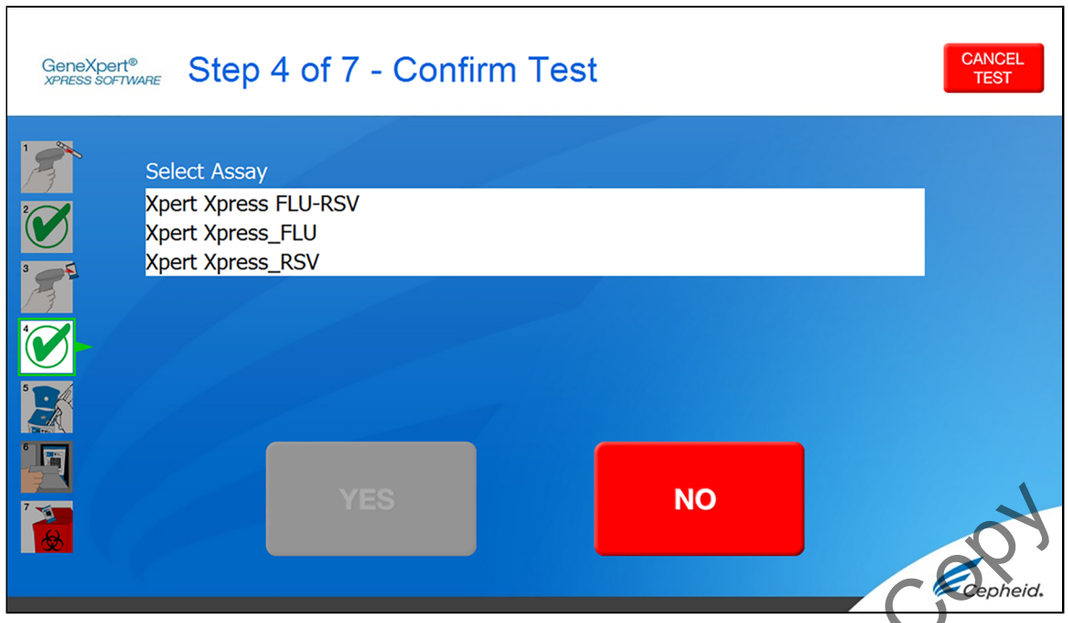


Figure 6. Confirm Test—Select Assay

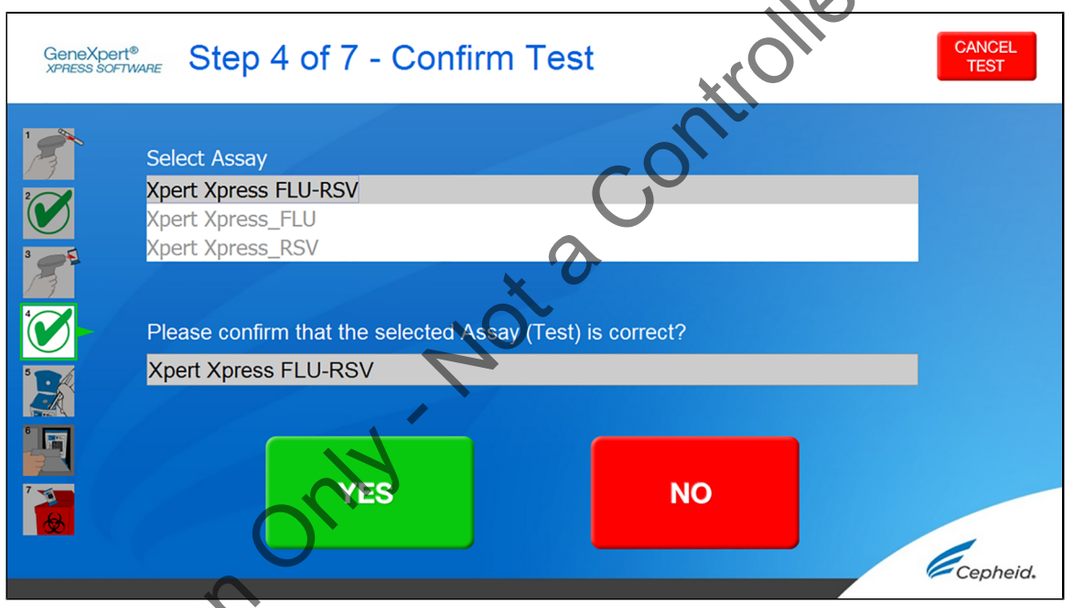


Figure 7. Confirm Test

6. Touch **YES** if the displayed information is correct.
7. Verify the specimen ID on the patient specimen.
8. Confirm the name on the cartridge is Xpert Xpress Flu/RSV. Specimen and cartridge are ready for use.
9. Mix patient sample container by inverting the transport medium tube 5 times.
10. Open the cartridge lid by lifting the front of the cartridge lid.
11. Remove the transfer pipette from the wrapper by opening the end next to the top bulb (Figure 8).

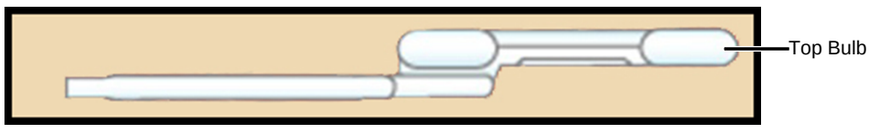


Figure 8. Transfer Pipette

Note Do not place unwrapped pipette on the workbench.

12. Squeeze the top bulb of the transfer pipette **completely** and place the pipette tip in the transport medium tube (Figure 9) containing the patient sample.

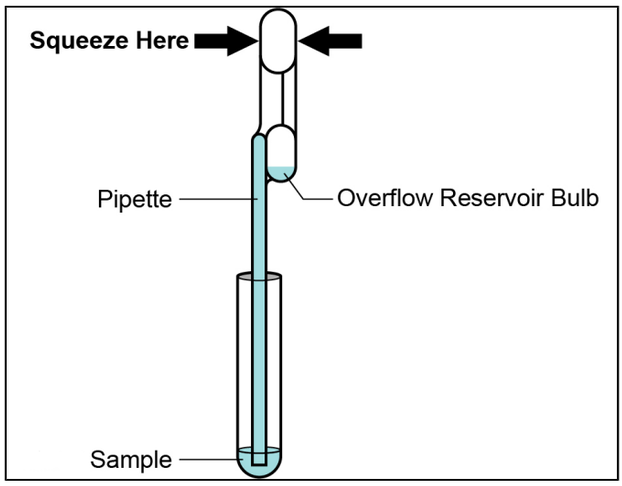


Figure 9. Transfer Pipette and Transport Medium Tube

13. Release the top bulb of the pipette to fill the pipette with the patient sample. Check that the pipette does not contain bubbles.
14. To transfer the patient specimen to the cartridge, squeeze the top bulb of the transfer pipette completely again to empty the contents of the pipette into the large opening (Sample Chamber) of the cartridge shown in Figure 10. It is okay to have excess specimen left in the overflow reservoir of the pipette (Figure 9).

Figure 10. Xpert Xpress Flu/RSV Assay Cartridge (Top View)

15. Close the cartridge lid.
16. Dispose of the pipette in an appropriate waste container after use.

14.3 Loading the Cartridge

1. 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 stops flashing. The Test in Progress screen appears.

   When the test is done (green light goes out), the door automatically unlocks.
4. The Remove Cartridge screen will be displayed. Follow instructions to remove the cartridge. Dispose of the used cartridge and gloves in an appropriate specimen waste container according to your institution's standard practices.

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

5. Touch **CONTINUE** to view the result of the test that has just completed. Touch the **CONTINUE** button again to go back to Home Screen. This completes the procedure for running a test.

Note Refer to Section 14.4, Starting Another Test While a Test is Running for running multiple tests at the same time.

14.4 Starting Another Test While a Test is Running

An additional test may be started after the first is in progress.

1. Touch the **HOME** button. The Home screen will display the module in use as slightly gray and with the notation that data collection is in progress (Figure 11 and Figure 12).

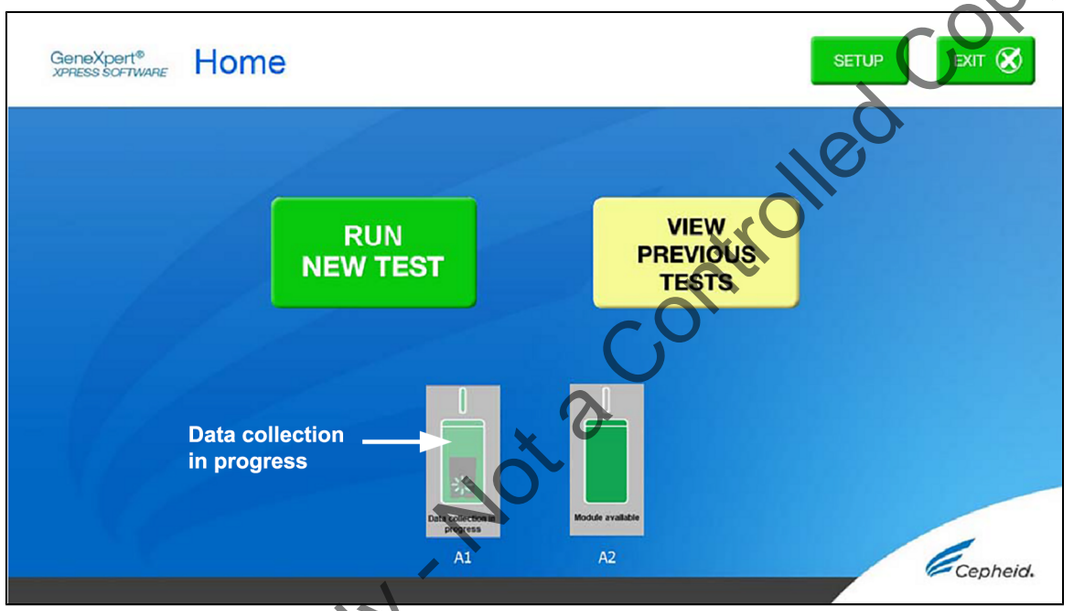


Figure 11. Home Screen with One Test in Progress (GeneXpert Xpress II)

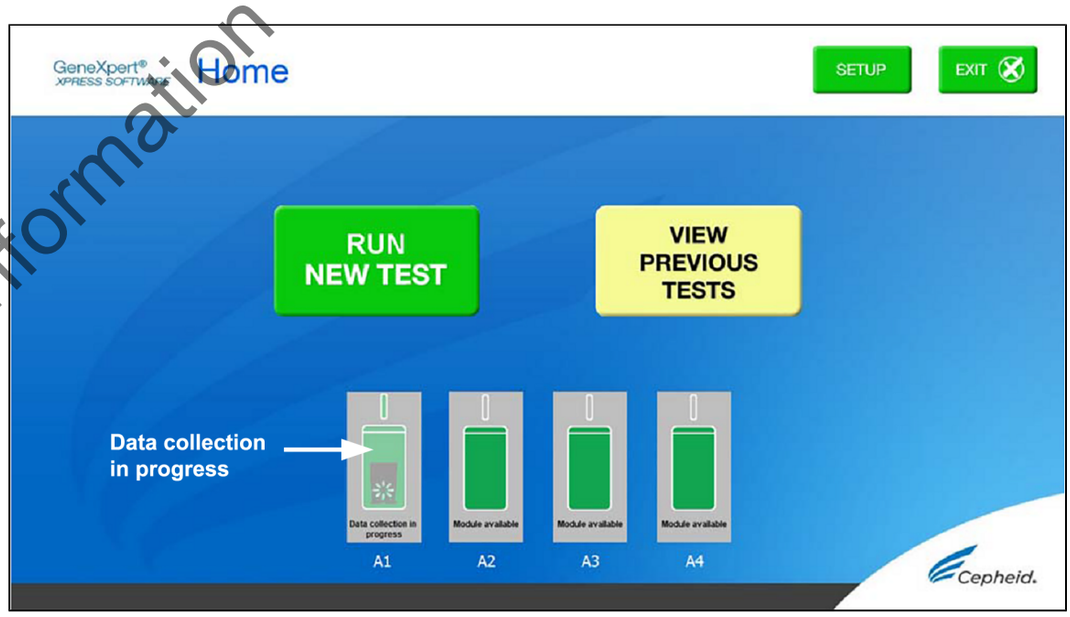


Figure 12. Home Screen with One Test in Progress (GeneXpert Xpress IV)

2. Touch **RUN NEW TEST** button and proceed with the new test following the steps in Section 14.1, Starting a Test.
3. After the second test is in progress, touch the **HOME** button. The status of both tests is displayed. When a test is complete, the icon text will change to Data

collection complete and will show a check mark on the icon (Figure 13 and Figure 14).

4. Touch the **Data collection complete** icon to show the Remove Cartridge screen (Figure 13 and Figure 14). Follow the instructions on the screen to remove the cartridge (Figure 15 and Figure 16).

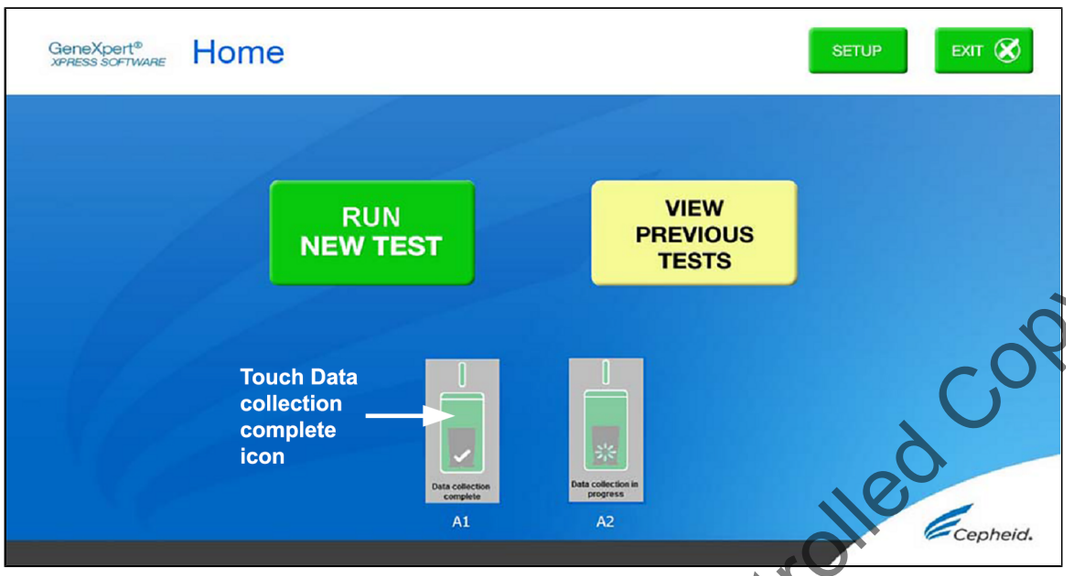


Figure 13. Home Screen with One Test Completed (GeneXpert Xpress II)

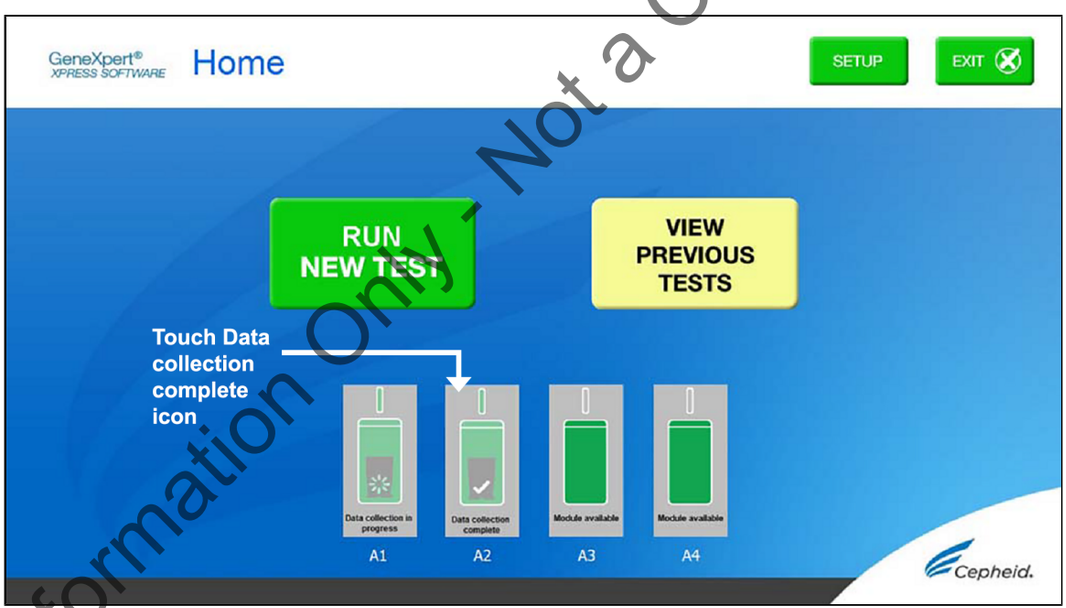


Figure 14. Home Screen with One Test Completed (GeneXpert Xpress IV)

Figure 15. Remove Cartridge Screen (GeneXpert Xpress II)

Figure 16. Remove Cartridge Screen (GeneXpert Xpress IV)

14.5 Viewing Results

1. Touch the **VIEW PREVIOUS TESTS** button on the Home screen shown in Figure 17 and Figure 18.

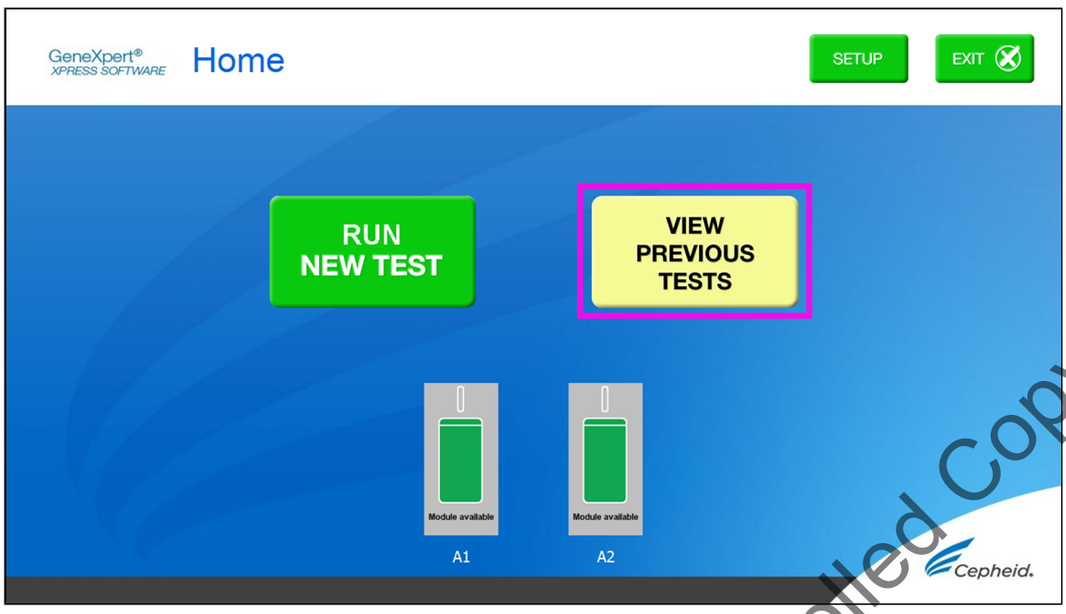


Figure 17. VIEW PREVIOUS TESTS Button on Home Screen (GeneXpert Xpress II)

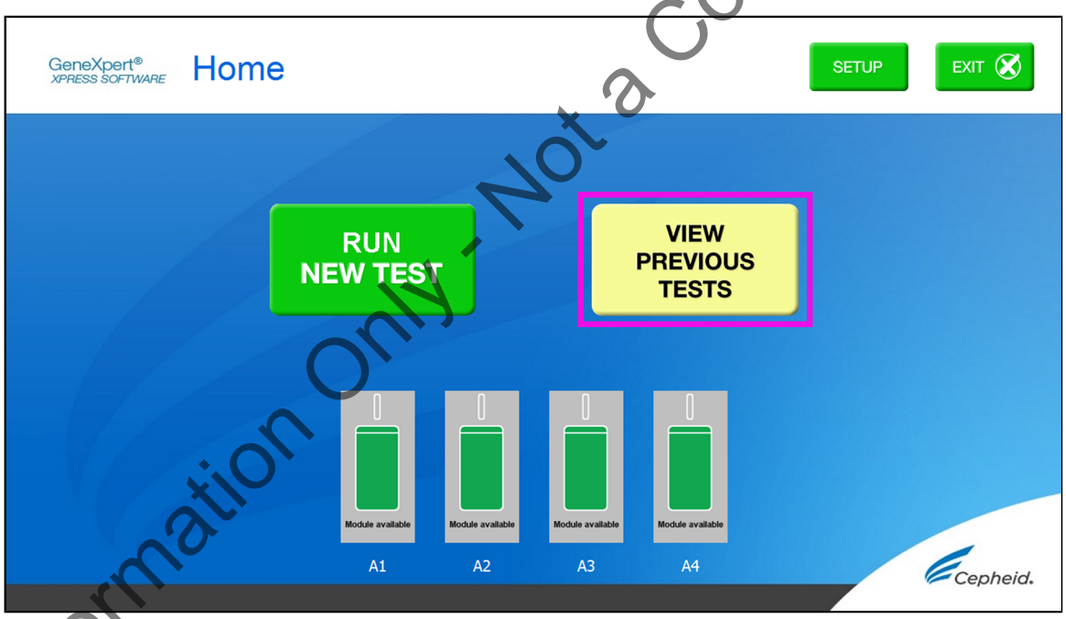


Figure 18. VIEW PREVIOUS TESTS Button on Home Screen (GeneXpert Xpress IV)

2. On the Select Test Screen, select the test by either touching the test name or using the arrows to select the test (Figure 19).

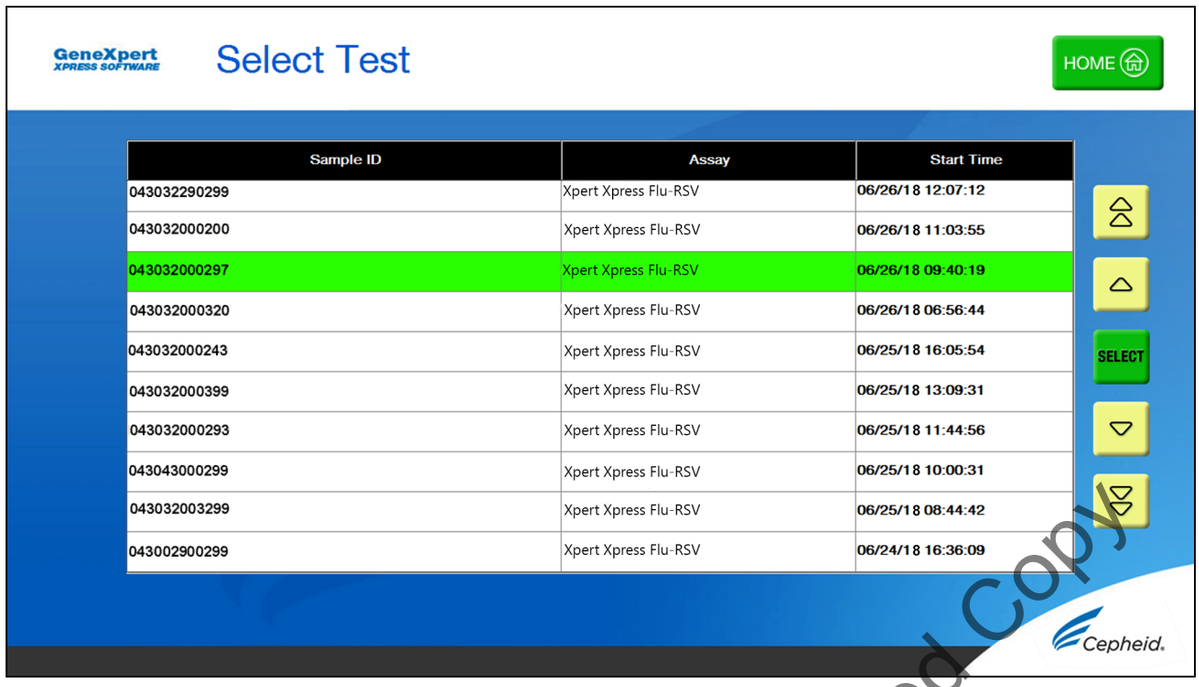


Figure 19. SELECT button on Select Test Screen

15 GeneXpert Xpress Software Version 6.0 or Higher

15.1 Starting a Test

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

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

1. Touch the **NEW TEST** button on the Home screen (see Figure 20).

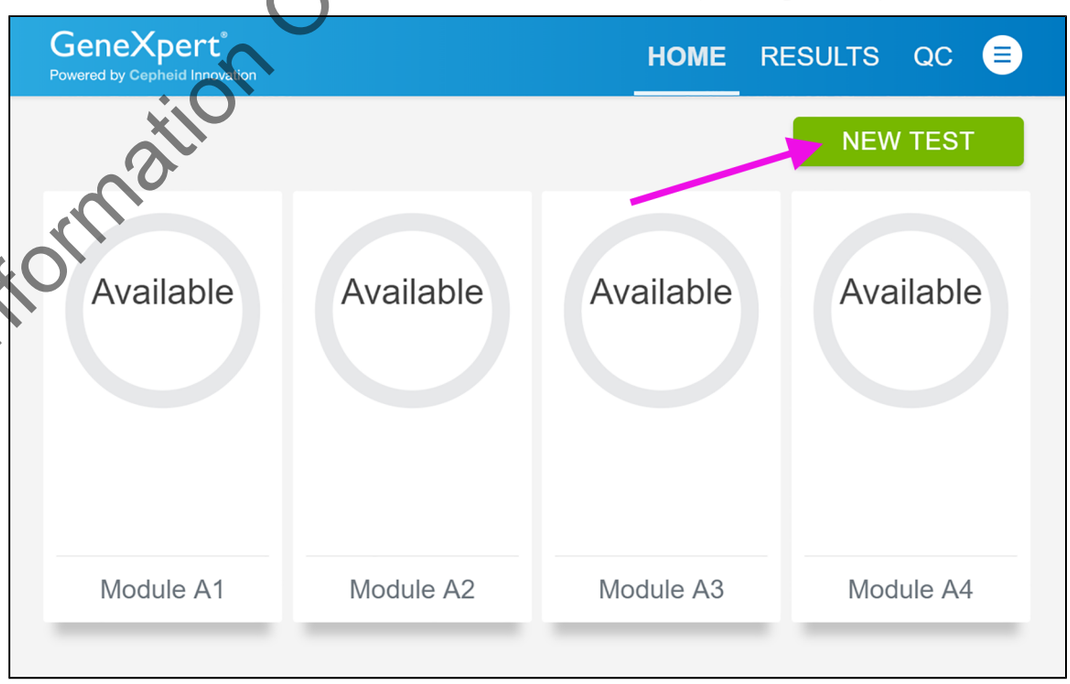


Figure 20. The Home Screen

If Patient Information is configured by an administrator, then the Patient Information screen appears (see Figure 21). If Patient Information is not configured, the Sample ID screen appears. Skip to Section 15.2 if the Sample ID screen appears.

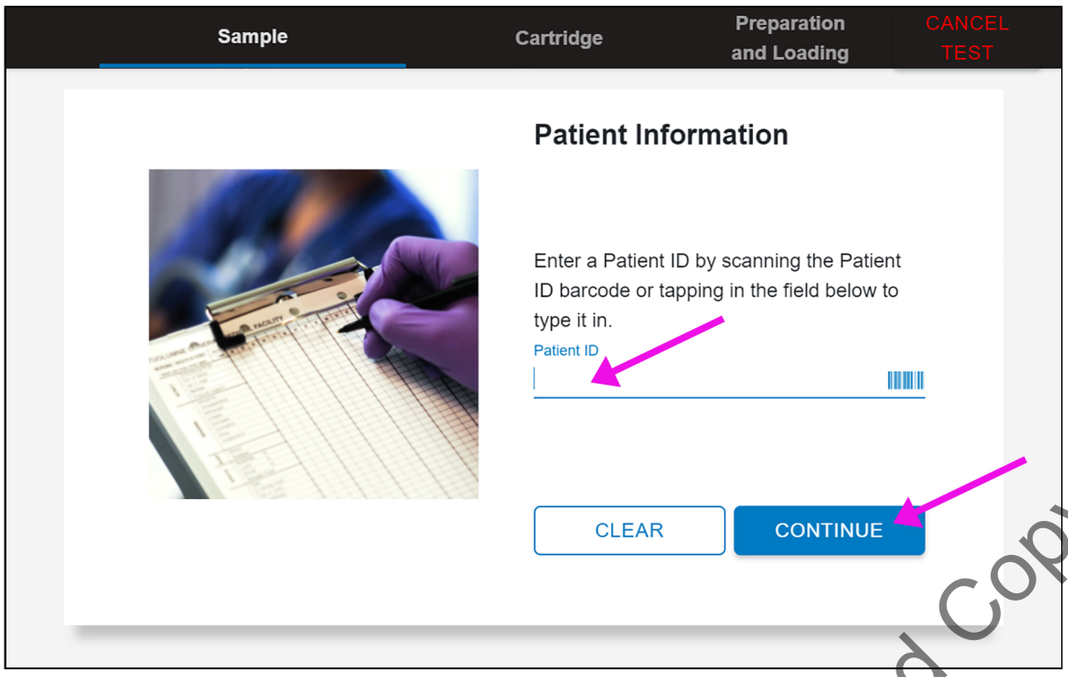


Figure 21. The Patient Information Screen

2. Scan the Patient ID barcode or manually enter the Patient ID.
3. Touch **CONTINUE**. The Confirm Patient Information screen appears.
4. Verify the Patient ID and touch **CONFIRM**. The Sample ID screen appears.

15.2 Preparing the Specimen and Cartridge

1. Remove a cartridge and a transfer pipette from the cartridge kit box.
2. Check that the transport medium tube cap is closed. Scan Sample ID barcode or manually enter the Sample ID.
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 22).

Biological Risks

In the following steps, cartridges should be kept 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 Flu/RSV test 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.

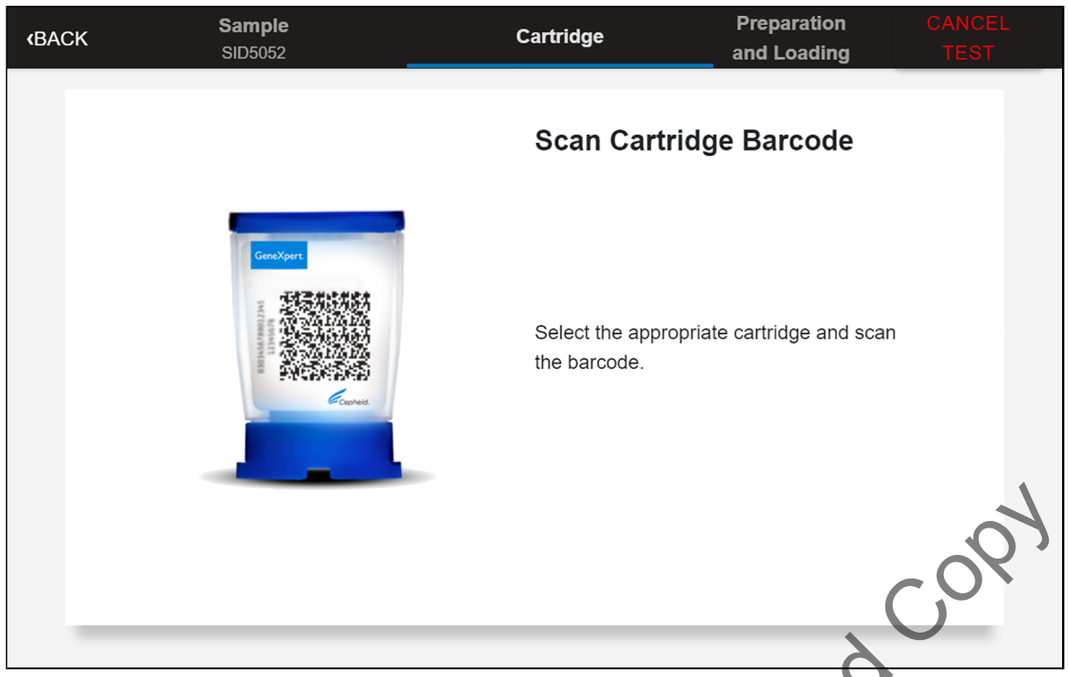


Figure 22. Scan Cartridge Barcode Screen

5. Select the appropriate cartridge with the sample and scan the cartridge barcode. After scanning, the Select Test screen appears (Figure 23).
6. Select the test to run:
   - Flu A, Flu B and RSV: **Xpert Xpress Flu-RSV**
   - Flu A and Flu B only: **Xpert Xpress_Flu**
   - RSV only: **Xpert Xpress_RSV**

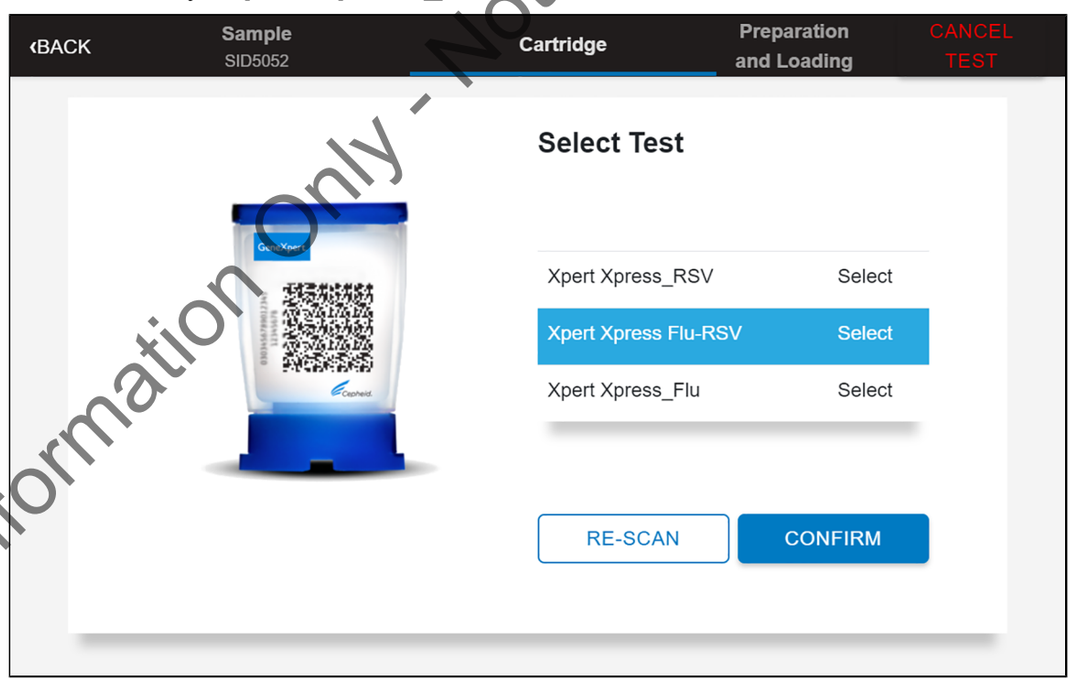


Figure 23. Select Test Screen

Only the test result for the assay selected at this step will be collected once the test is started. Flu A, Flu B, and RSV results will only be collected if the Xpert Xpress Flu-RSV assay is selected.

7. Touch **CONFIRM**. The Confirm Test Information screen appears (see Figure 24).

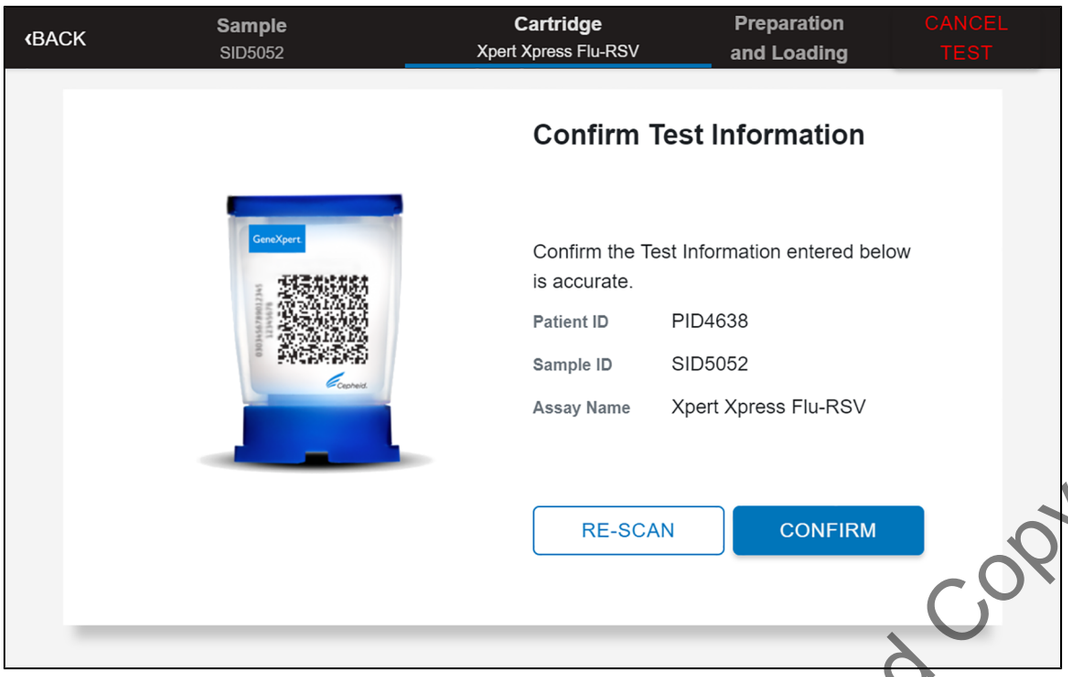


Figure 24. Confirm Test Information Screen

8. Touch **CONFIRM** if the displayed information is correct.
9. Depending on your configuration, the Enter Credentials to Continue screen may appear (see Figure 25). If enabled, you may log in by scanning your institutional ID. Otherwise, manually enter your User Name and Password and touch **LOGIN** to continue.

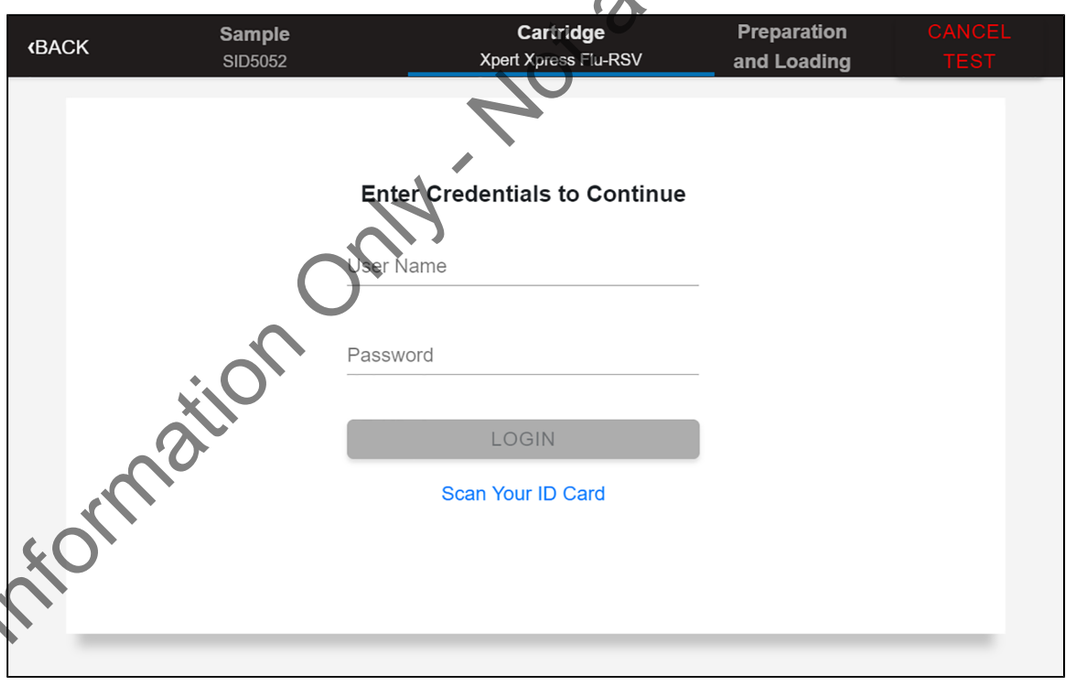


Figure 25. Enter Credentials to Continue Screen

The Cartridge Preparation screen appears (see Figure 26).

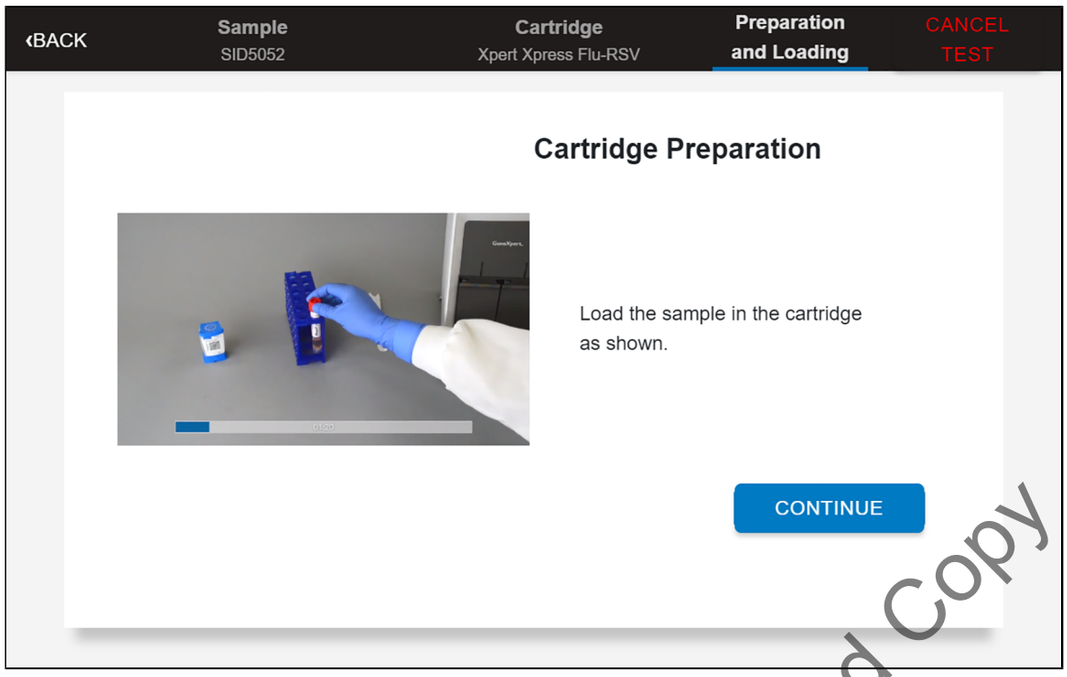


Figure 26. Cartridge Preparation Screen

10. A video clip shows the cartridge preparation steps. Prepare the cartridge according to the directions below, which are also shown in the video. Once complete, the video restarts from the beginning automatically. Touch the **CONTINUE** button to exit video.
11. Verify the specimen ID on the patient specimen.
12. Confirm the name on the cartridge is Xpert Xpress Flu/RSV.
13. Specimen and cartridge are ready for use.
14. Mix patient sample container by inverting the transport medium tube 5 times.
15. Open the cartridge lid by lifting the front of the cartridge lid.
16. Remove the transfer pipette from the wrapper by opening the end next to the top bulb (Figure 27).

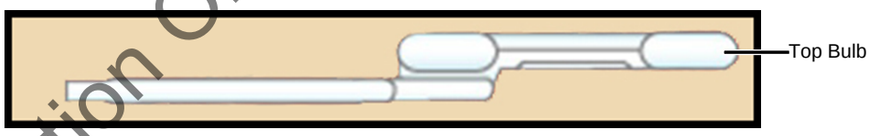


Figure 27. Transfer Pipette

Note Do not place unwrapped pipette on the workbench.

17. Squeeze the top bulb of the transfer pipette **completely** and place the pipette tip in the transport medium tube (Figure 28) containing the patient sample.

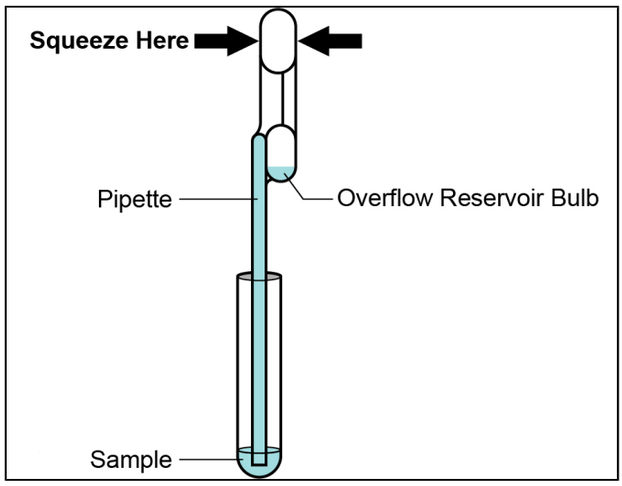


Figure 28. Transfer Pipette and Transport Medium Tube

18. Release the top bulb of the pipette to fill the pipette with the patient sample. Check that the pipette does not contain bubbles.
19. To transfer the patient specimen to the cartridge, squeeze the top bulb of the transfer pipette completely again to empty the contents of the pipette into the large opening (Sample Chamber) of the cartridge shown in Figure 29. It is okay to have excess specimen left in the overflow reservoir of the pipette (Figure 28).

Figure 29. Xpert Xpress Flu/RSV Assay Cartridge (Top View)

20. Close the cartridge lid.
21. Dispose of the pipette in an appropriate waste container after use.

15.3 Loading the Cartridge

1. Touch the **CONTINUE** button on the Cartridge Preparation screen (see Figure 26). The Load Cartridge into Module screen appears (see Figure 30).
2. Open the module door with the flashing green light.

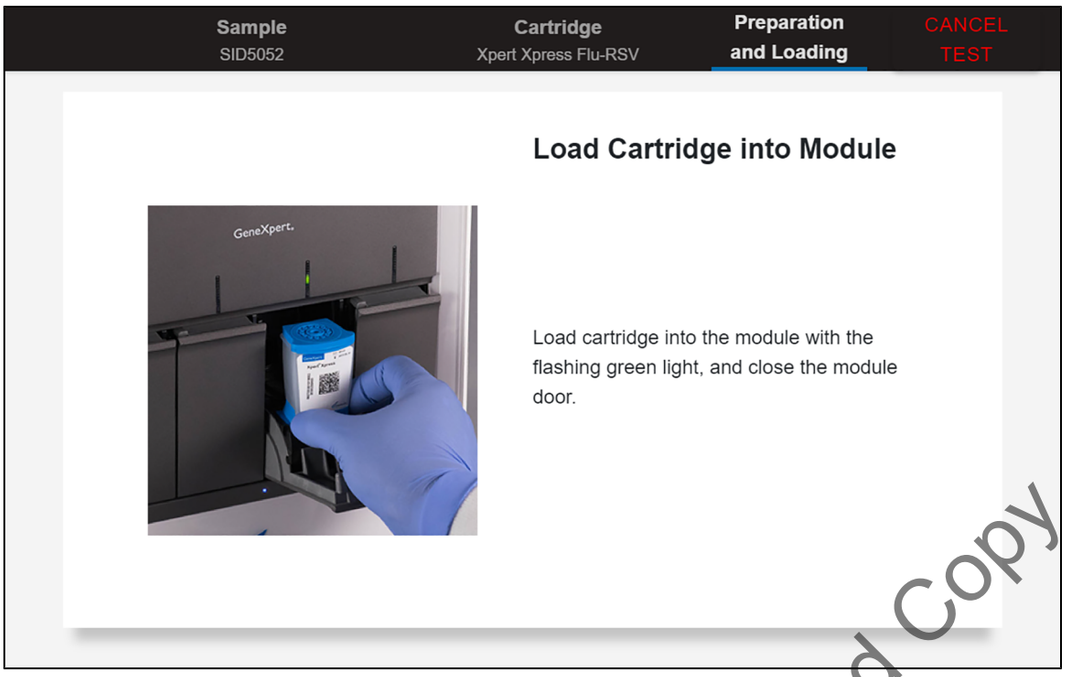


Figure 30. Load Cartridge into Module Screen

3. 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 (see Figure 31).

Figure 31. Cartridge Inserted into Module

4. Close the door. The green light stops flashing and the test starts.

   When the cartridge is loaded, the Test Loading screen appears, followed by the Test Running screen showing that the test is running (see Figure 32).

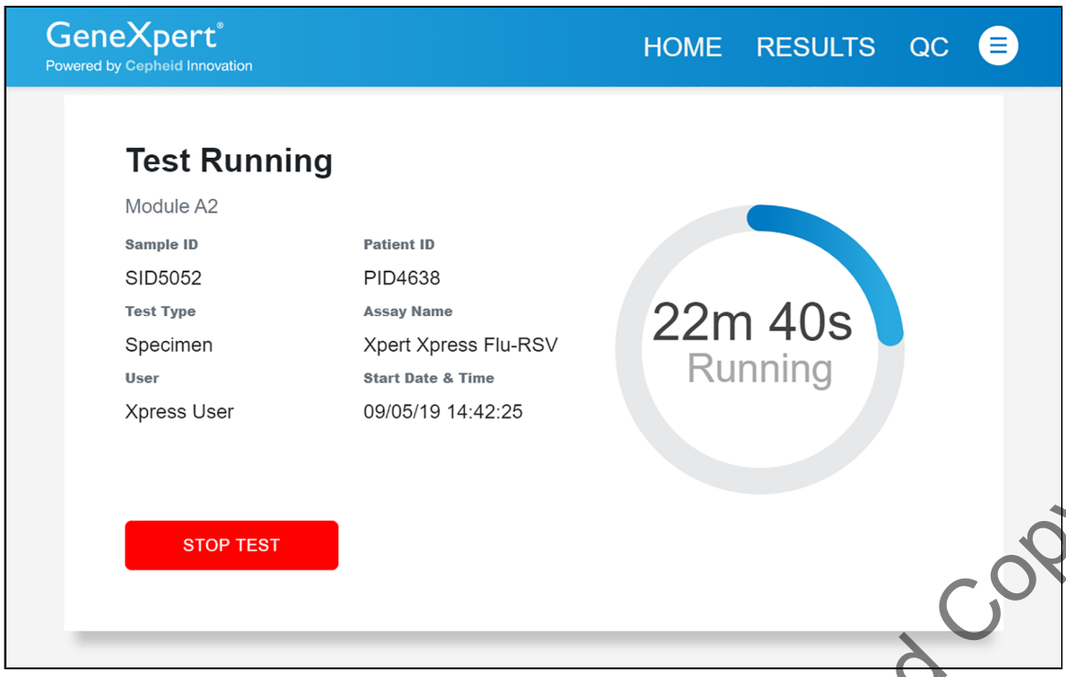


Figure 32. Test Running Screen showing Test Time Remaining

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 15.4, Starting Another 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 the Hub stops the test. If necessary, touch the **STOP TEST** button to cancel a test while it is loading or running.

5. When the test is done, the green light goes out and the door automatically unlocks. The screen text change to Test Completed (see Figure 33).

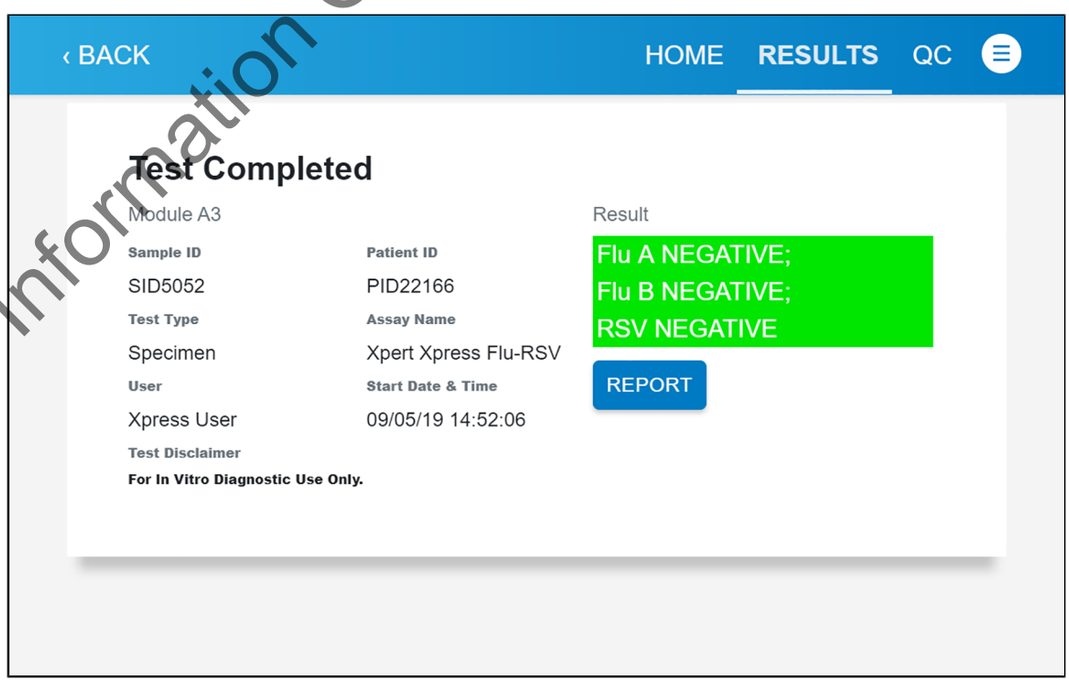


Figure 33. Test Completed Screen

The Test Completed screen provides the results for the test just completed.

6. Open the module door, remove the used cartridge, and properly dispose of the cartridge according to your institution's hazardous waste disposal policies.

7. Touch **REPORT** to view the result of the test that has just completed. Touch **HOME** to go back to the Home screen. This completes the procedure for running a test.
8. To log out, touch the **User Menu** icon, then select **Logout**.

15.4 Starting Another Test While a Test is Running

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

1. Put on a new pair of gloves if performing a new test.
2. Touch the **HOME** button on the Test Running screen (see Figure 32).
3. For a new user login, touch the **User Menu** icon to log in.
4. Repeat the steps in Section 15, GeneXpert Xpress Software Version 6.0 or Higher through Section 15.3, Loading the Cartridge.
5. 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 34).

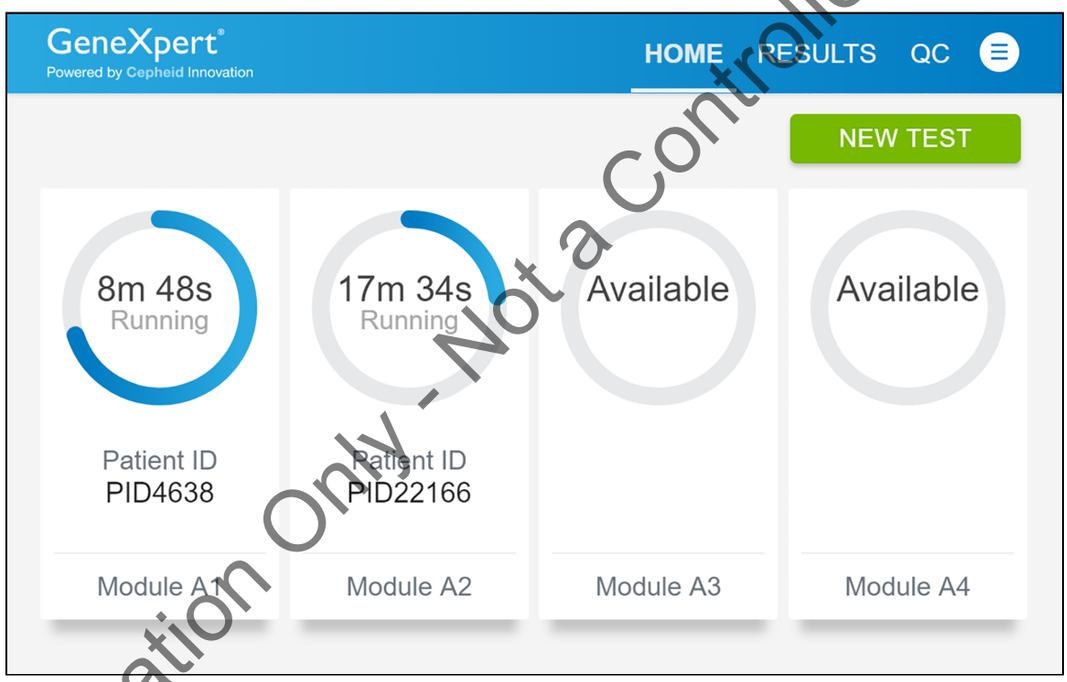


Figure 34. Home Screen Showing Two Tests Running

After a test has completed, the module icon text changes to **Complete** (see Figure 35).

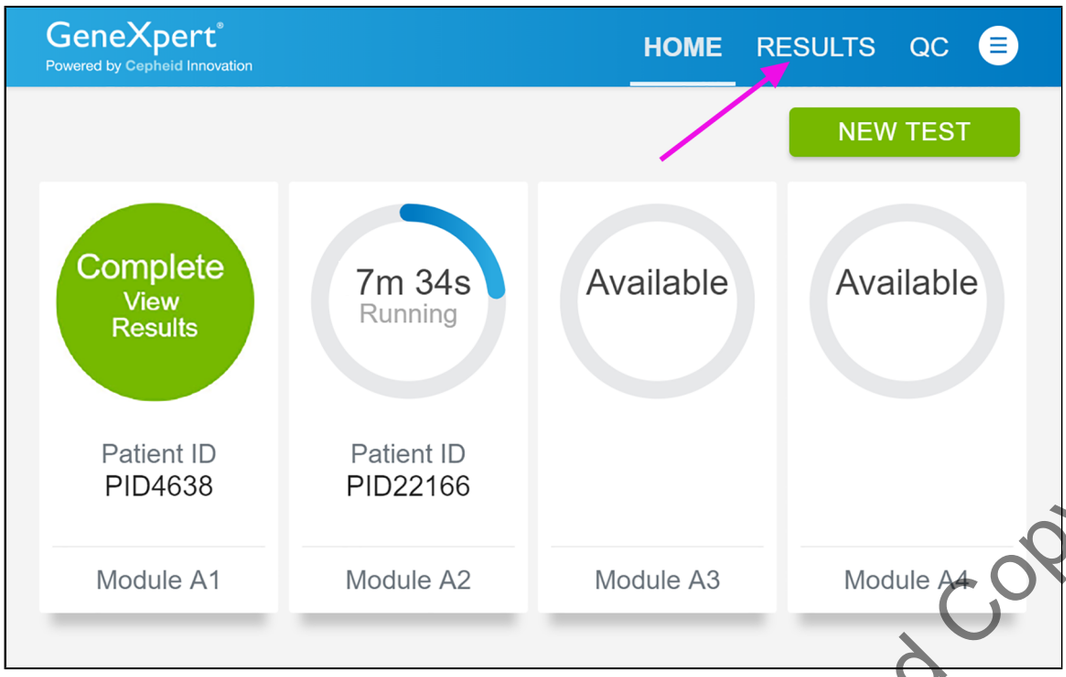


Figure 35. Home Screen With One of Two Tests Completed

15.5 Viewing Test Results

1. Touch the **RESULTS** button located on the panel at the top of the screen (see Figure 35). The Results screen appears (see Figure 36).

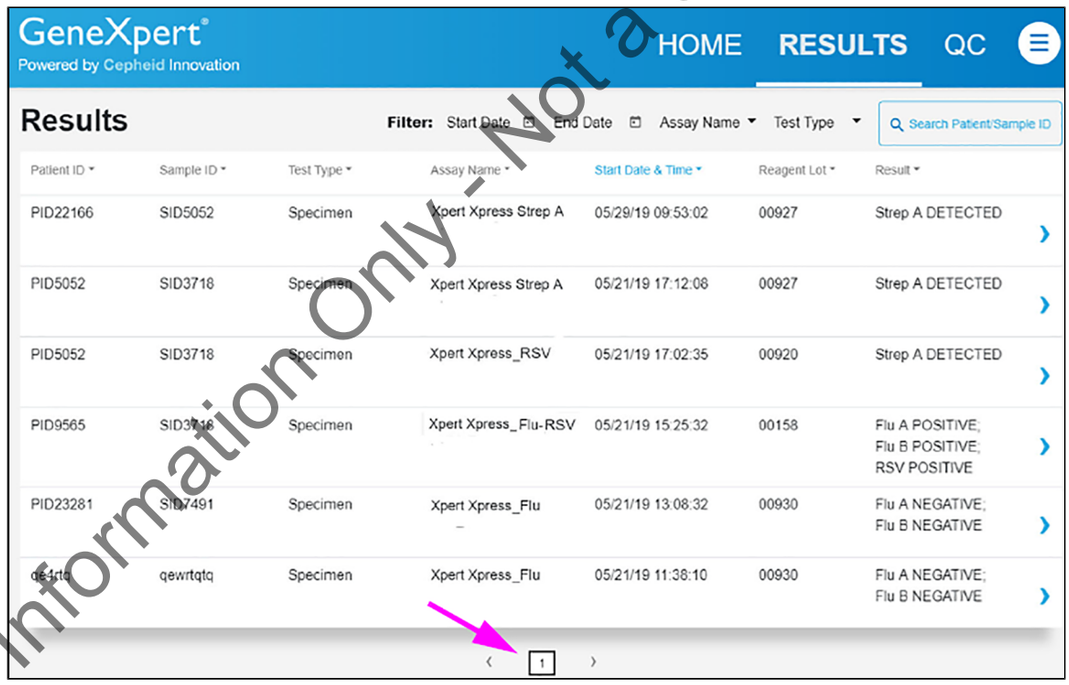


Figure 36. Results Screen

Test results are, by default, in order by 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.

2. Touch the desired result to open the Test Result screen (see Figure 37).

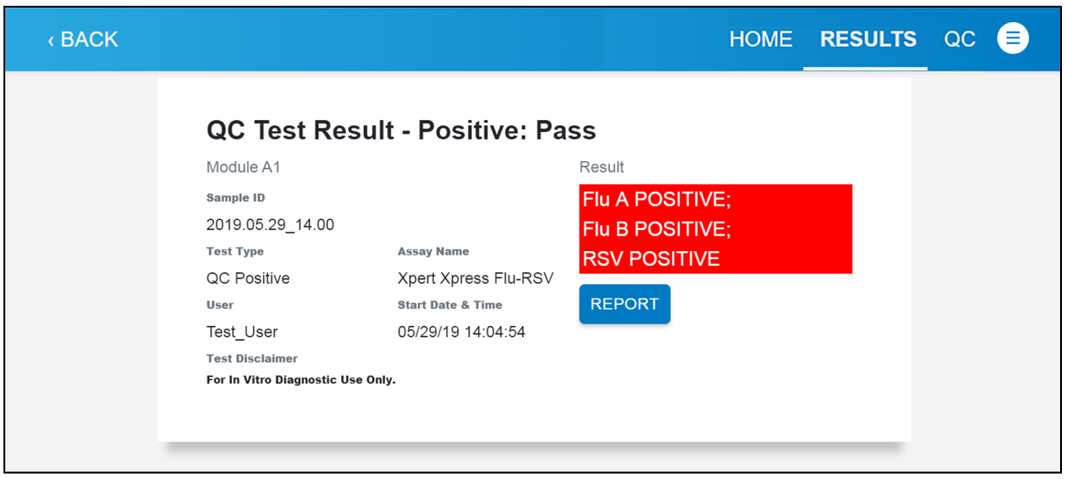


Figure 37. Test Result Screen (Example)

16 Quality Control

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

- **Sample Processing Control (SPC)** – Ensures the sample was processed correctly. The SPC is an Armored RNA® that is included in each cartridge to verify adequate processing of the sample. The SPC verifies that release of RNA from the Flu and RSV virus has occurred if the organism is present and verifies that the specimen processing is adequate. Additionally this control detects specimen-associated inhibition of the RT-PCR and PCR reactions. 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.

  If the sample is negative for Flu and RSV viruses and the SPC fails, the result will be NO RESULT–REPEAT TEST. See Section 18, Retests.

  When performing a test in the Xpert Xpress RSV Assay mode, a sample strongly positive for influenza A or influenza B may cause the SPC to fail; if the sample is RSV negative, a valid result (**RSV NEGATIVE**) will be reported instead of **NO RESULT–REPEAT TEST**.
- **Probe Check Control (PCC)** – Before the start of the PCR reaction, the GeneXpert Xpress 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.

  If PCC fails, the result will be NO RESULT–REPEAT TEST. See Section 18, Retests.

16.1 Testing Quality Control Sample

Note If the QC Lockout feature is enabled, follow the QC Lockout instructions detailed in the *GeneXpert Xpress System User's Guide*.

Note

Cepheid recommends that external controls be tested at the frequency noted below:

- Each time a new lot of Xpert Xpress Flu/RSV test reagents is received.
- Each time a new shipment of Xpert Xpress Flu/RSV test reagents 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 QC procedures.

1. Put on a clean pair of gloves.
2. Have a new Xpert Xpress Flu/RSV test cartridge, a quality control tube, and a transfer pipette ready.
3. For GeneXpert Xpress Software Version 5.x:
   a) On the Home screen, touch **RUN NEW TEST** (see Figure 4).
   b) Touch **NO** for the sample barcode on the Sample ID screen if there is no sample barcode.
   c) Touch the sample ID field to display the keyboard.
   d) Type **Negative Control** for the Negative Control or **Positive Control** for the Positive Control.
   e) Touch the **OK** button.
   f) Proceed to Step 5.
4. For GeneXpert Xpress Software Version 6.0 or higher:
   a) On the Home screen, touch **QC** (see Figure 38).

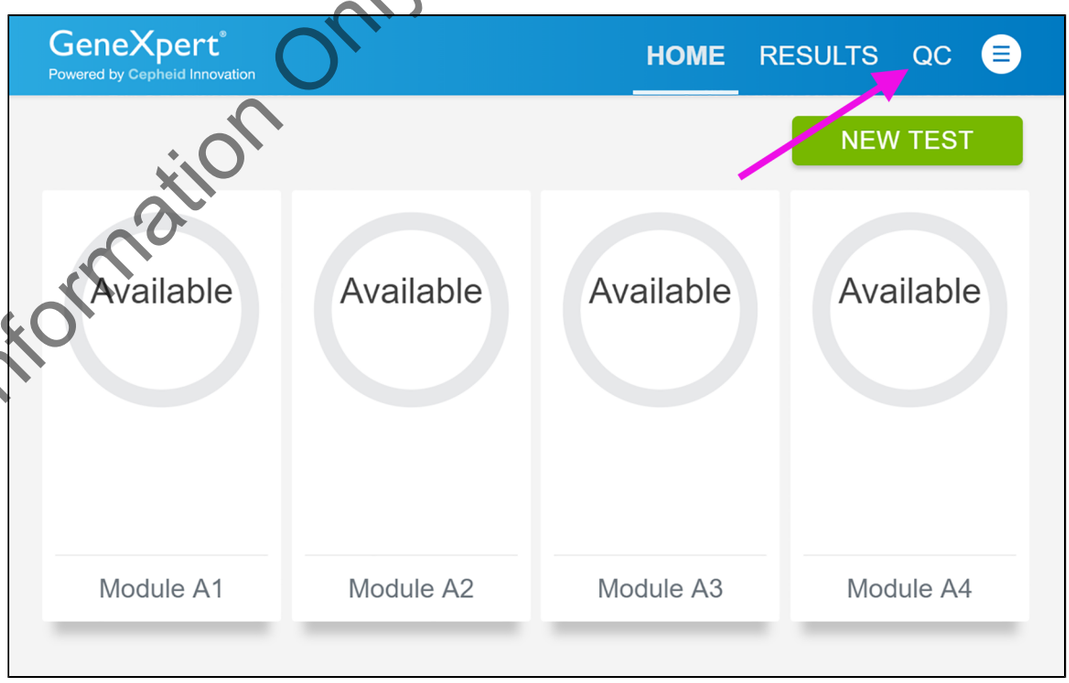


Figure 38. Home Screen

   b) The Quality Control screen appears. Touch **Run QC Positive Test**, **Run QC Negative Test**, or **Run Proficiency Test** (see Figure 39).

Figure 39. Quality Control Screen

c) The Sample ID screen appears.

d) Manually enter **Negative Control** for the Negative Control or **Positive Control** for the Positive Control, or scan the sample ID barcode, if enabled.

e) Touch **CONTINUE**.

5. Scan the cartridge barcode with the attached barcode scanner, and touch **YES** to verify the information displayed is correct.

Note If the barcode on the Xpert Xpress Flu/RSV test 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.

6. Select **Xpert Xpress Flu/RSV** from the Select Assay menu.

7. Touch **YES** to confirm the selection.

8. If applicable, enter your user name and password.

9. Watch the video before continuing. The video will repeat. Touch the **CONTINUE** button to exit video.

10. Mix the control by inverting the control tube 5 times.

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

12. Remove the transfer pipette from the wrapper by opening the end next to the top bulb.

13. Squeeze the top bulb of the pipette completely then put the pipette tip into the control tube.

14. Release the top bulb of the pipette to fill the pipette with the sample from the control tube.

15. To transfer the control sample to the cartridge, squeeze the top bulb of the pipette completely to empty the pipette into the large opening (Sample Chamber) in the cartridge. It is okay to have excess sample left in the overflow reservoir of the pipette.

16. Close the cartridge lid.

17. Dispose of the used quality control tube and pipette in an appropriate specimen waste container according to your institution's standard practices.

18. Open the module door with the flashing green light.

19. 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.
20. Close the door until it clicks. The flashing green light will stop flashing. The Test in Progress screen appears.

    When the test is done (green light goes out), the door automatically unlocks. The Remove Cartridge screen appears (Software Version 5.x) or the screen text changes to Test Completed (Software Version 6.0 or higher).
21. Open the module door, remove the used cartridge, and properly dispose of the cartridge according to your institution's hazardous waste disposal policies.
22. Touch **CONTINUE** (Software Version 5.x) or **REPORT** (Software Version 6.0 or higher) to view the result of the test.
23. Repeat the above steps with the second control tube before testing patient samples.

    If you encounter an instrument error, touch **CLEAR ERROR** and follow the onscreen instructions. When the Home screen appears, test a new quality control sample using a new cartridge.

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 an unexpected result occurs, test a new Quality Control sample using a new cartridge. If an unexpected result occurs upon retest, contact Cepheid Technical Support.

17 Interpretation of Results

The results are interpreted automatically by the GeneXpert Xpress System and are clearly shown in the View Results window. The possible results are shown in Table 1.

Table 1. Xpert Xpress Flu/RSV Assay Results and Interpretation

Result	Interpretation
Flu A POSITIVE; **Flu B NEGATIVE**; **RSV NEGATIVE**	Flu A target RNA is detected; Flu B target RNA is not detected; RSV target RNA is not detected.
Flu A POSITIVE; **Flu B POSITIVE**; **RSV NEGATIVE****	Flu A target RNA is detected; Flu B target RNA is detected; RSV target RNA is not detected. Repeat test according to the instructions in Section 18.2, Retest Procedure.
Flu A POSITIVE; **Flu B NEGATIVE**; **RSV POSITIVE****	Flu A target RNA is detected; Flu B target RNA is not detected; RSV target RNA is detected. Repeat test according to the instructions in Section 18.2, Retest Procedure.
Flu A POSITIVE; **Flu B POSITIVE**; **RSV POSITIVE****	Flu A target RNA is detected; Flu B target RNA is detected; RSV target RNA is detected. Repeat test according to the instructions in Section 18.2, Retest Procedure.

Result	Interpretation
Flu A NEGATIVE; **Flu B POSITIVE**; **RSV NEGATIVE**	Flu A target RNA is not detected; Flu B target RNA is detected; RSV target RNA is not detected.
Flu A NEGATIVE; **Flu B NEGATIVE**; **RSV POSITIVE**	Flu A target RNA is not detected; Flu B target RNA is not detected; RSV target RNA is detected.
Flu A NEGATIVE; **Flu B POSITIVE**; **RSV POSITIVE****	Flu A target RNA is not detected; Flu B target RNA is detected; RSV target RNA is detected. Repeat test according to the instructions in Section 18.2, Retest Procedure.
Flu A NEGATIVE; **Flu B NEGATIVE**; **RSV NEGATIVE**	Flu A target RNA is not detected; Flu B target RNA is not detected; RSV target RNA is not detected.
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.
NO RESULT—REPEAT TEST	If result is **NO RESULT—REPEAT TEST**, retest with a new cartridge. If retest is **NO RESULT—REPEAT TEST**, call for assistance at 888-838-3222.

Note ** Because the incidence of co-infection with two or more viruses (Influenza A, Influenza B, or RSV) within a single specimen is low, repeat the test according to the instructions in Section 18.2, Retest Procedure and use the results from the repeat test.

Table 2. Xpert Xpress Flu Assay Results and Interpretation

Result	Interpretation
Flu A POSITIVE; **Flu B NEGATIVE**	Flu A target RNA is detected; Flu B target RNA is not detected.
Flu A NEGATIVE; **Flu B POSITIVE**	Flu A target RNA is not detected; Flu B target RNA is detected.
Flu A POSITIVE; **Flu B POSITIVE****	Flu A target RNA is detected; Flu B target RNA is detected. Repeat test according to the instructions in Section 18.2, Retest Procedure.
Flu A NEGATIVE; **Flu B NEGATIVE**	Flu A target RNA is not detected; Flu B target RNA is not detected.
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.

Result	Interpretation
NO RESULT—REPEAT TEST	If result is **NO RESULT—REPEAT TEST**, retest with a new cartridge. If retest is **NO RESULT—REPEAT TEST**, call for assistance at 888-838-3222.

Note ** Because the incidence of co-infection with two or more viruses (Influenza A and Influenza B) within a single specimen is low, repeat the test according to the instructions in Section 18.2, Retest Procedure and use the results from the repeat test.

Table 3. Xpert Xpress RSV Assay Results and Interpretation

Result	Interpretation
RSV POSITIVE	RSV target RNA is detected.
RSV NEGATIVE	RSV target RNA is not detected.
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.
NO RESULT—REPEAT TEST	If result is **NO RESULT—REPEAT TEST**, retest with a new cartridge. If retest is **NO RESULT—REPEAT TEST**, call for assistance at 888-838-3222.

18 Retests

18.1 Reasons to Repeat the Assay

If any of the test results mentioned below occur, repeat the test once according to instructions in the Retest Procedure section (Section 18.2, Retest Procedure).

- Two or more positive virus results in a single specimen. Because the incidence of co-infection with two or more viruses (Influenza A and Influenza B) within a single specimen is low, it is recommended that repeat testing is performed according to the instructions in Section 18.2, Retest Procedure.
- An **INSTRUMENT ERROR** result could be due to, but not limited to, the maximum pressure limits were exceeded.
- A **NO RESULT—REPEAT TEST** indicates that insufficient data were collected. For example, Probe Check control failed or a power failure occurred.

18.2 Retest Procedure

To retest a **NO RESULT—REPEAT TEST** or **INSTRUMENT ERROR** result (nondeterminate result), use a new cartridge (do not re-use the original cartridge).

Do not perform more than one retest of the original specimen.

Use the leftover specimen from the original swab transport medium tube.

1. Remove a new cartridge from the kit box.
2. Mix the specimen by inverting the Xpert Viral Transport or the Copan UTM tube 5 times.

3. Open the cartridge lid. Using a clean transfer pipette (supplied), transfer specimen (one draw) to the sample chamber with the large opening in the cartridge.
4. Close the cartridge lid.
5. Start the test according to instructions in Section 14.1, Starting a Test (Software Version 5.x) or Section 15.1, Starting a Test (Software Version 6.0 or higher).

19 Limitations

- The performance of the Xpert Xpress Flu/RSV Assay was validated using the procedures provided in this package insert only. Modifications to these procedures may alter the performance of the test.
- Results from the Xpert Xpress Flu/RSV Assay should be interpreted with other laboratory and clinical data available to the clinician.
- Erroneous test results might occur from improper specimen collection; failure to follow the recommended sample collection, handling, and storage procedures; technical error; sample mix-up; or because the number of organisms in the specimen is too low to be detected by the test. 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 influenza or RSV virus infection and should not be used as the sole basis for treatment or other patient management decisions.
- Results from analytical studies show potential for competitive inhibition in specimens with two different viruses.
- When using the Xpert Xpress Flu/RSV Assay in the Flu Only mode, in the event of a mixed infection one of the two infections may be reported as **NEGATIVE** because of differences in the software.
- Results from the Xpert Xpress Flu/RSV Assay 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 viability. 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.
- If the virus mutates or there are other sequence changes in the target region, influenza and/or RSV virus may not be detected, or may be detected less predictably.
- Positive and negative predictive values are highly dependent on prevalence. The assay performance was established during the 2016–2017 influenza season. The performance may vary depending on the prevalence of the different viruses and population tested.
- 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 influenza or RSV infection.
- This test has not been evaluated for monitoring treatment of influenza infection.
- This test has not been evaluated for screening of blood or blood products for the presence of influenza or RSV.
- This test cannot rule out diseases caused by other bacterial or viral pathogens.

- 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.
- Cross-reactivity with respiratory tract organisms other than those described herein can lead to erroneous results.
- This assay has not been evaluated for immunocompromised individuals.
- Recent patient exposure to FluMist® or other live attenuated influenza vaccines may cause inaccurate positive results.
- Although this test has been shown to detect A/H1N1 (pre-2009 pandemic), A/H7N9 (detected in China in 2013) and A/H3N2v viruses cultured from positive human respiratory specimens, the performance characteristics of this device with clinical specimens that are positive for the A/H1N1 (pre-2009 pandemic), A/H7N9 (detected in China in 2013) and A/H3N2v viruses have not been established.
- 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.

20 Expected Values

The Xpert Xpress Flu/RSV clinical study included a total of 3265 prospectively collected nasal swab (NS) and nasopharyngeal (NP) swab specimens to evaluate performance for influenza A and influenza B detection. The number and percentage of cases positive for one or more of influenza A and influenza B as determined by the Xpert Xpress Flu/RSV test are shown by age range in Table 4.

Table 4. Number and Percent of Specimens by Age Range—Flu A and Flu B[a]

Age Group (years)	Number of Patients	% of Total	Flu A		Flu B	
			Number of Positives	Positivity Rate	Number of Positives	Positivity Rate
≤5	1284	39.3%	137	10.7%	57	4.4%
6-21	516	15.8%	132	25.6%	53	10.3%
22-59	1141	34.9%	122	10.7%	37	3.2%
≥60	324	9.9%	56	17.3%	5	1.5%
Total	3265	100%	447	13.7%	152	4.7%

[a] Five subjects had multi-infections by the Xpert Xpress Flu/RSV test and are therefore counted more than once in this table. Of the 5 subjects with Xpert multi-infections, 1 sample was Flu A and Flu B POS by comparator method; 1 sample was Flu A POS by comparator method; 1 sample was Flu B POS by comparator method; 2 samples were NEG for both targets by comparator method.

The Xpert Xpress Flu/RSV clinical study included a total of 3103 prospectively collected NS and NP swab specimens to evaluate performance for RSV detection. The number and percentage of cases positive for RSV as determined by the Xpert Xpress Flu/RSV test are shown by age range in Table 5.

Table 5. Number and Percent of Specimens by Age Range—RSV

Age Group (years)	Number of Patients	% of Total	RSV	
			Number of Positives	Positivity Rate
≤5	1212	39.1%	483	39.9%
6-21	483	15.6%	21	4.3%
22-59	1090	35.1%	39	3.6%
≥60	318	10.2%	32	10.1%
Total	3103	100%	575	18.5%

21 Performance Characteristics

21.1 Clinical Performance

Performance characteristics of the Xpert Xpress Flu/RSV test were evaluated at fourteen institutions in the U.S. during the 2016-2017 influenza season.

Specimens were collected from the following:

- Individuals exhibiting signs and symptoms of respiratory infection who provided informed consent for the collection of a NS or NP swab specimen.

The Xpert Xpress Flu/RSV test performance was compared to FDA-cleared molecular comparator assays. Bi-directional sequencing was performed on specimens where the Xpert Xpress Flu/RSV test and the comparator method were discrepant, and is provided for informational purposes only.

21.2 Overall Results

A total of 3265 specimens (1598 NS and 1667 NP swab) were tested for influenza A and influenza B by the Xpert Xpress Flu/RSV test and the comparator method. A total of 3103 specimens (1543 NS and 1560 NP swab) were tested for RSV by Xpert Xpress Flu/RSV test and the comparator method.

For NS specimens, the Xpert Xpress Flu/RSV test demonstrated a positive percent agreement (PPA) and a negative percent agreement (NPA) relative to the comparator method of 98.9% and 97.5% for the detection of influenza A, 98.4% and 99.3% for influenza B, and 98.2% and 99.1% for the detection of RSV, respectively (Table 6).

For NP swab specimens, the Xpert Xpress Flu/RSV test demonstrated a PPA and NPA relative to the comparator method of 97.6% and 98.2% for the detection of influenza A, 97.3% and 99.6% for influenza B, and 98.2% and 98.5% for the detection of RSV, respectively (Table 6).

For the combined dataset, the Xpert Xpress Flu/RSV test demonstrated a PPA and NPA relative to the comparator method of 98.2% and 97.9% for the detection of influenza A, 97.8% and 99.4% for influenza B, and 98.2% and 98.8% for the detection of RSV, respectively (Table 6).

Table 6. Xpert Xpress Flu/RSV Assay Performance

Target[a]	Specimen Type	Number of Patients	True Positive (TP)	False Negative (FN)	True Negative (TN)	False Positive (FP)	Positive Percent Agreement (PPA) (95% CI)	Negative Percent Agreement (NPA) (95% CI)
Flu A	NS	1598	186	2[b]	1375	35[c]	98.9% (96.2-99.7)	97.5% (96.6-98.2)
	NP	1667	200	5[d]	1436	26[e]	97.6% (94.4-99.0)	98.2% (97.4-98.8)
	Overall	3265	386	7[f]	2811	61[g]	98.2% (96.4-99.1)	97.9% (97.3-98.3)
Flu B	NS	1598	63	1[h]	1523	11[i]	98.4% (91.7-99.7)	99.3% (98.7-99.6)
	NP	1667	71	2[j]	1587	7[k]	97.3% (90.6-99.2)	99.6% (99.1-99.8)
	Overall	3265	134	3[l]	3110	18[m]	97.8% (93.8-99.3)	99.4% (99.1-99.6)
RSV	NS	1543	269	5[n]	1257	12[o]	98.2% (95.8-99.2)	99.0% (98.4-99.5)
	NP	1560	275	5[p]	1261	19[q]	98.2% (95.9-99.2)	98.5% (97.7-99.0)
	Overall	3103	544	10[r]	2518	31[s]	98.2% (96.7-99.0)	98.8% (98.3-99.1)

a Five specimens were positive for both Flu A and Flu B.
b Discrepant Testing: 1 of 2 Flu A NEG; 1 of 2 Flu A POS.
c Discrepant Testing: 17 of 35 Flu A NEG; 11 of 35 Flu A POS; 7 of 35 inconclusive
d Discrepant Testing: 3 of 5 Flu A NEG; 2 of 5 Flu A POS.
e Discrepant Testing: 11 of 26 Flu A NEG; 9 of 26 Flu A POS; 6 of 26 inconclusive.
f Discrepant Testing: 4 of 7 Flu A NEG; 3 of 7 Flu A POS.
g Discrepant Testing: 26 of 61 Flu A NEG; 22 of 61 Flu A POS; 13 of 61 inconclusive
h Discrepant Testing: 1 of 1 inconclusive.
i Discrepant Testing: 5 of 11 Flu B POS; 6 of 11 inconclusive.
j Discrepant Testing: 1 of 2 Flu B POS; 1 of 2 inconclusive
k Discrepant Testing: 1 of 7 Flu B NEG; 5 of 7 Flu B POS; 1 of 7 inconclusive.
l Discrepant Testing: 1 of 3 Flu B POS; 2 of 3 inconclusive.
m Discrepant Testing: 1 of 18 Flu B NEG; 10 of 18 Flu B POS; 7 of 18 inconclusive.
n Discrepant Testing: 3 of 5 RSV NEG; 1 of 5 inconclusive; 1 of 5 not done.
o Discrepant Testing: 5 of 12 RSV NEG; 3 of 12 RSV POS, 4 of 12 inconclusive.
p Discrepant Testing: 2 of 5 RSV NEG; 2 of 5 inconclusive; 1 of 5 not done.
q Discrepant Testing: 6 of 19 RSV NEG; 2 of 19 RSV POS, 6 of 19 inconclusive; 5 of 19 not done.
r Discrepant Testing: 5 of 10 RSV NEG; 3 of 10 inconclusive; 2 of 10 not done.
s Discrepant Testing: 11 of 31 RSV NEG; 5 of 31 RSV POS; 10 of 31 inconclusive; 5 of 31 not done.

Of the Xpert Xpress Flu/RSV test runs performed with eligible specimens, 98.0% (3212/3279) of these specimens were successful on the first attempt. The remaining 67 gave indeterminate results on the first attempt (38 **NO RESULT—REPEAT TEST** results and 29 **INSTRUMENT ERROR**). Fifty-eight of the 67 indeterminate cases were retested, of which 53 yielded valid results upon repeat testing; nine specimens were not retested. The overall rate of assay success was 99.6% (3265/3279). The overall indeterminate rate was 0.4%.

22 Analytical Performance

22.1 Analytical Sensitivity (Limit of Detection)

Studies were performed to determine the analytical limit of detection (LoD) of the Xpert Xpress Flu/RSV Assay with two lots of reagents across three testing days. The higher LoD observed per strain and per lot was selected for verification. Verification of the estimated LoD claim was performed on one reagent lot across a minimum of three testing days. LoD was established using two influenza A H3N2 strains, two influenza A 2009 H1N1 strains, two influenza B strains, two respiratory syncytial virus A (RSV A) strains and two respiratory syncytial virus B (RSV B) strains. Viruses were diluted into negative pooled NP swab and NS clinical matrices for testing. The LoD is defined as the lowest concentration (tissue culture infective dose, $TCID_{50}$/mL) per sample that can be reproducibly distinguished from negative samples with 95% confidence or the lowest concentration at which 19 of 20 replicates were positive. Each strain was tested in replicates of 20 per concentration of virus in NP swab and NS clinical matrices. The LoD values for each strain tested are summarized in Table 7 to Table 11.

Table 7. Confirmed LoD ($TCID_{50}$/mL): Influenza A 2009 H1N1

Virus Strain	Confirmed LoD Probit ($TCID_{50}$/mL)	
	NP Swab Matrix	NS Matrix
Influenza A/ California/7/2009	0.020	0.018
Influenza A/ Florida/27/2011	0.040	0.04

Table 8. Confirmed LoD ($TCID_{50}$/mL): Influenza A H3N2

Virus Strain	Confirmed LoD Probit ($TCID_{50}$/mL)	
	NP Swab Matrix	NS Matrix
Influenza A/ Perth/16/2009	0.013	0.006
Influenza A/ Victoria/361/2011	0.750	0.21

Table 9. Confirmed LoD ($TCID_{50}$/mL): Influenza B

Virus Strain	Confirmed LoD Probit ($TCID_{50}$/mL)	
	NP Swab Matrix	NS Matrix
Influenza B/ Mass/2/2012	0.400	0.07
Influenza B/ Wisconsin/01/2011	0.190	0.17

Table 10. Confirmed LoD ($TCID_{50}$/mL): Respiratory Syncytial Virus A

Virus Strain	Confirmed LoD Probit ($TCID_{50}$/mL)	
	NP Swab Matrix	NS Matrix
RSV A/2/Australia/61	0.870	0.32
RSV A/Long/MD/56	1.100	0.45

Table 11. Confirmed LoD ($TCID_{50}$/mL): Respiratory Syncytial Virus B

Virus Strain	Confirmed LoD Probit ($TCID_{50}$/mL)	
	NP Swab Matrix	NS Matrix
RSV B/ Wash/18537/62	0.790	0.29
RSV B/9320/MA/77	2.300	0.35

22.2 Analytical Specificity (Exclusivity)

The analytical specificity of the Xpert Xpress Flu/RSV Assay was evaluated by testing a panel of 44 cultures consisting of 16 viral, 26 bacterial, and two yeast strains representing common respiratory pathogens or those potentially encountered in the nasal passage and nasopharynx. Three replicates of all bacterial and yeast strains were tested at concentrations of ≥ 1 x 10^6 CFU/mL with the exception of one strain that was tested at 1 x 10^5 CFU/mL (*Chlamydia pneumoniae*). Three replicates of all viruses were tested at concentrations of ≥ 1 x 10^5 $TCID_{50}$/mL. The analytical specificity was 100%. Results are shown in Table 12.

Predicted analytical reactivity from in silico analyses showed 100% sequence homology for additional pH1N1 strains.

Table 12. Analytical Specificity of the Xpert Xpress Flu/RSV Assay

Organism	Concentration	Result		
		Influenza A	Influenza B	RSV
No Template Control	N/A	NEG	NEG	NEG
Adenovirus Type 1	1.12E+06 $TCID_{50}$/mL	NEG	NEG	NEG
Adenovirus Type 7	1.87E+05 $TCID_{50}$/mL	NEG	NEG	NEG
Human coronavirus OC43	2.85E+05 $TCID_{50}$/mL	NEG	NEG	NEG
Human coronavirus 229E	1.00E+05 $TCID_{50}$/mL	NEG	NEG	NEG
Cytomegalovirus	1.00E+05 $TCID_{50}$/mL	NEG	NEG	NEG

Organism	Concentration	Result		
		Influenza A	Influenza B	RSV
Echovirus	3.31E+07 $TCID_{50}$/mL	NEG	NEG	NEG
Enterovirus	3.55E+05 $TCID_{50}$/mL	NEG	NEG	NEG
Epstein Barr Virus	7.16E+07 $TCID_{50}$/mL	NEG	NEG	NEG
HSV	8.90E+05 $TCID_{50}$/mL	NEG	NEG	NEG
Measles	6.31E+05 $TCID_{50}$/mL	NEG	NEG	NEG
Human metapneumovirus	1.00E+05 $TCID_{50}$/mL	NEG	NEG	NEG
Mumps virus	6.31E+06 $TCID_{50}$/mL	NEG	NEG	NEG
Human parainfluenza Type 1	1.15E+06 $TCID_{50}$/mL	NEG	NEG	NEG
Human parainfluenza Type 2	6.31E+05 $TCID_{50}$/mL	NEG	NEG	NEG
Human parainfluenza Type 3	3.55E+06 $TCID_{50}$/mL	NEG	NEG	NEG
Rhinovirus Type 1A	1.26E+05 $TCID_{50}$/mL	NEG	NEG	NEG
Acinetobacter baumannii	1.00E+06 CFU/mL	NEG	NEG	NEG
Burkholderia cepacia	3.30E+06 CFU/mL	NEG	NEG	NEG
Candida albicans	3.20E+06 CFU/mL	NEG	NEG	NEG
Candida parapsilosis	3.00E+06 CFU/mL	NEG	NEG	NEG
Bordetella pertussis	3.30E+06 CFU/mL	NEG	NEG	NEG
Chlamydia pneumoniae	1.00E+05 CFU/mL	NEG	NEG	NEG
Citrobacter freundii	3.30E+06 CFU/mL	NEG	NEG	NEG
Corynebacterium sp.	3.30E+06 CFU/mL	NEG	NEG	NEG

Organism	Concentration	Result		
		Influenza A	Influenza B	RSV
Escherichia coli	1.00E+07 CFU/mL	NEG	NEG	NEG
Enterococcus faecalis	1.30E+06 CFU/mL	NEG	NEG	NEG
Haemophilus influenzae	1.00E+06 CFU/mL	NEG	NEG	NEG
Lactobacillus reuteri	1.00E+06 CFU/mL	NEG	NEG	NEG
Legionella spp.	1.00E+06 CFU/mL	NEG	NEG	NEG
Moraxella catarrhalis	1.00E+07 CFU/mL	NEG	NEG	NEG
Mycobacterium tuberculosis (avirulent)	1.00E+06 CFU/mL	NEG	NEG	NEG
Mycoplasma pneumoniae	1.00E+06 CFU/mL	NEG	NEG	NEG
Neisseria meningitidis	2.15E+06 CFU/mL	NEG	NEG	NEG
Neisseria mucosa	1.00E+07 CFU/mL	NEG	NEG	NEG
Propionibacterium acnes	2.40E+07 CFU/mL	NEG	NEG	NEG
Pseudomonas aeruginosa	3.70E+06 CFU/mL	NEG	NEG	NEG
Staphylococcus aureus (protein A producer)	2.20E+06 CFU/mL	NEG	NEG	NEG
Staphylococcus epidermidis	3.40E+06 CFU/mL	NEG	NEG	NEG
Staphylococcus haemolyticus	4.00E+06 CFU/mL	NEG	NEG	NEG
Streptococcus agalactiae	3.50E+06 CFU/mL	NEG	NEG	NEG
Streptococcus pneumoniae	1.00E+06 CFU/mL	NEG	NEG	NEG
Streptococcus pyogenes	1.00E+07 CFU/mL	NEG	NEG	NEG
Streptococcus salivarius	1.00E+07 CFU/mL	NEG	NEG	NEG
Streptococcus sanguinis	3.10E+06 CFU/mL	NEG	NEG	NEG

22.3 Analytical Reactivity (Inclusivity)

The analytical reactivity of the Xpert Xpress Flu/RSV Assay was evaluated against multiple strains of 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 53 strains comprised of 35 influenza A viruses, 13 influenza B strains and 5 RSV strains were tested in this study with the Xpert Xpress Flu/RSV Assay. Three replicates were tested for each strain. All Flu and RSV strains tested positive in all three replicates, except for one Flu A H1N1 strain (A/New Jersey/8/76), which tested positive in 2 of 3 replicates at 0.1 $TCID_{50}$/mL. Results are shown in Table 13.

Predicted analytical reactivity from in silico analyses showed 100% sequence homology for additional pH1N1 strains.

Table 13. Analytical Reactivity (Inclusivity) of the Xpert Xpress Flu/RSV Assay

		Target Concentration	Result		
			Flu A	Flu B	RSV
No Template Control		n/a	NEG	NEG	NEG
Influenza A H1N1 (pre-2009)	A/swine/ Iowa/15/30	0.1 $TCID_{50}$/mL	POS	NEG	NEG
	A/WS/33	0.1 $TCID_{50}$/mL	POS	NEG	NEG
	A/PR/8/34	0.1 $TCID_{50}$/mL	POS	NEG	NEG
	A/Mal/302/54	0.1 $TCID_{50}$/mL	POS	NEG	NEG
	A/Denver/1/57	0.1 $TCID_{50}$/mL	POS	NEG	NEG
	A/New Jersey/8/76	0.1 $TCID_{50}$/mL	POS	NEG	NEG
	A/New Caledonia/20/1999	0.1 $TCID_{50}$/mL	POS	NEG	NEG
	A/New York/55/2004	0.1 $TCID_{50}$/mL	POS	NEG	NEG
	A/Soloman Island/3/2006	0.1 $TCID_{50}$/mL	POS	NEG	NEG
	A/Taiwan/42/06	0.1 $TCID_{50}$/mL	POS	NEG	NEG
	A/ Brisbane/59/2007	0.1 $TCID_{50}$/mL	POS	NEG	NEG
Influenza A H1N1 (pdm2009)	A/swine/ NY/02/2009	0.1 $TCID_{50}$/mL	POS	NEG	NEG
	A/ Colorado/14/2012	0.1 $TCID_{50}$/mL	POS	NEG	NEG
	A/ Washington/24/2012	0.1 $TCID_{50}$/mL	POS	NEG	NEG

		Target Concentration	Result		
			Flu A	Flu B	RSV
Influenza AH3N2 (Seasonal)	A/Aichi/2/68	2.0 $TCID_{50}$/mL	POS	NEG	NEG
	A/Hong Kong/8/68	2.0 $TCID_{50}$/mL	POS	NEG	NEG
	A/Port Chalmers/1/73	2.0 $TCID_{50}$/mL	POS	NEG	NEG
	A/Hawaii/15/2001	2.0 $TCID_{50}$/mL	POS	NEG	NEG
	A/Wisconsin/67/05	2.0 $TCID_{50}$/mL	POS	NEG	NEG
	A/ Brisbane/10/2007	2.0 $TCID_{50}$/mL	POS	NEG	NEG
	A/ Minnesota/11/2010 (H3N2)v	2.0 $TCID_{50}$/mL	POS	NEG	NEG
	A/Indiana/08/2011 (H3N2)v	2.0 $TCID_{50}$/mL	POS	NEG	NEG
	A/Texas/50/2012	2.0 $TCID_{50}$/mL	POS	NEG	NEG
Avian influenza A	A/duck/ Hunan/795/2002 (H5N1)	≤ 1pg/µL[a]	POS	NEG	NEG
	A/chicken/ Hubei/327/2004 (H5N1)	≤ 1pg/µL[a]	POS	NEG	NEG
	A/Anhui/01/2005 (H5N1)	≤ 1pg/µL[a]	POS	NEG	NEG
	A/Japanese white eye/ Hong Kong/ 1038/2006 (H5N1)	≤ 1pg/µL[a]	POS	NEG	NEG
	A/mallard/ WI/34/75 (H5N2)	≤ 1pg/µL[a]	POS	NEG	NEG
	A/chicken/ CA431/00 (H6N2)	≤ 1pg/µL[a]	POS	NEG	NEG
	A/duck/ LTC-10-82743/1943 (H7N2)	≤ 1pg/µL[a]	POS	NEG	NEG
	A/chicken/ NJ/15086-3/94 (H7N3)	≤ 1pg/µL[a]	POS	NEG	NEG
	A/Anhui/1/2013 (H7N9)	N/A[b]	POS	NEG	NEG
	A/Shanghai/1/2013 (H7N9)	N/A[b]	POS	NEG	NEG
	A/chicken/ Korea/38349-p96323/ 1996 (H9N2)	≤ 1pg/µL[a]	POS	NEG	NEG
	A/Mallard/ NY/6750/78 (H2N2)	≤ 1pg/µL[a]	POS	NEG	NEG

		Target Concentration	Result		
			Flu A	Flu B	RSV
Influenza B	B/Lee/40	1.0 $TCID_{50}$/mL	NEG	POS	NEG
	B/Allen/45	1.0 $TCID_{50}$/mL	NEG	POS	NEG
	B/GL/1739/54	1.0 $TCID_{50}$/mL	NEG	POS	NEG
	B/Maryland/1/59	1.0 $TCID_{50}$/mL	NEG	POS	NEG
	B/Panama/45/90[c]	1.0 $TCID_{50}$/mL	NEG	POS	NEG
	B/Florida/07/2004[d]	1.0 $TCID_{50}$/mL	NEG	POS	NEG
	B/Florida/02/06[c]	1.0 $TCID_{50}$/mL	NEG	POS	NEG
	B/Florida/04/06[d]	1.0 $TCID_{50}$/mL	NEG	POS	NEG
	B/Hong Kong/5/72	1.0 $TCID_{50}$/mL	NEG	POS	NEG
	B/ Wisconsin/01/2010[d]	1.0 $TCID_{50}$/mL	NEG	POS	NEG
	B/ Malaysia/2506/04[c]	1.0 $TCID_{50}$/mL	NEG	POS	NEG
	B/Taiwan/2/62	1.0 $TCID_{50}$/mL	NEG	POS	NEG
	B/ Brisbane/60/2008[c]	1.0 $TCID_{50}$/mL	NEG	POS	NEG
RSV A	RSV-A/NY (Clinical unknown)	3.0 $TCID_{50}$/mL	NEG	NEG	POS
	RSV-A/ WI/629-8-2/2007	3.0 $TCID_{50}$/mL	NEG	NEG	POS
	RSV-A/ WI/629-11-1/2008	3.0 $TCID_{50}$/mL	NEG	NEG	POS
RSV B	RSV-B/ WV14617/85	7.0 $TCID_{50}$/mL	NEG	NEG	POS
	RSV-B/ CH93(18)-18	7.0 $TCID_{50}$/mL	NEG	NEG	POS

[a] Purified viral RNA in simulated background matrix was used for avian influenza A viruses due to biosafety regulations.
[b] Inactivated avian influenza A (H7N9) viruses without viral titer was diluted 100,000 fold in simulated background matrix and tested due to biosafety regulations.
[c] Known Victoria lineage.
[d] Known Yamagata lineage.

22.4 Interfering Substances Study

In a non-clinical study, potentially interfering substances that may be present in the nasal passage and nasopharynx were evaluated directly relative to the performance of the Xpert Xpress Flu/RSV Assay. 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. Negative samples (n = 8) were tested per each substance to determine the effect on the performance of the sample processing control (SPC). Positive samples (n = 8) were tested per substance with six influenza (four influenza A

and two influenza B) and four RSV (two RSV A and two RSV B) strains spiked at 3X the analytical LoD determined for each strain. All results were compared to positive and negative simulated background matrix controls. The simulated background matrix consisted of 2.5% (w/v) porcine mucin, 1% (v/v) human whole blood in 0.85% sodium chloride (NaCl) formulated in 1x PBS solution with 15% glycerol, which was then diluted 1:5 in UTM. The evaluated substances are listed in Table 14 with active ingredients and concentrations tested shown. None of the substances caused interference of the assay at the concentrations tested in this study. All positive and negative replicates were identified correctly using the Xpert Xpress Flu/RSV Assay.

Table 14. Potentially Interfering Substances in the Xpert Xpress Flu/RSV Assay

Substance/Class	Description/ Active Ingredient	Concentration Tested
Control	Simulated background matrix	100% (v/v)
Beta-adrenergic bronchodilator	Albuterol Sulfate	0.83 mg/mL (equivalent to 1 dose per day)
Blood	Blood (Human)	2% (v/v)
BD™ Universal Viral Transport System	Transport Media	100% (v/v)
Remel M4®	Transport Media	100% (v/v)
Remel M4RT®	Transport Media	100% (v/v)
Remel M5®	Transport Media	100% (v/v)
Remel M6®	Transport Media	100% (v/v)
Throat lozenges, oral anesthetic and analgesic	Benzocaine, Menthol	1.7 mg/mL
Mucin	Purified Mucin protein (Bovine or porcine submaxillary gland)	2.5% (w/v)
Antibiotic, nasal ointment	Mupirocin	10 mg/mL
Saline Nasal Spray	Sodium Chloride (0.65%)	15% (v/v)
Anefrin Nasal Spray	Oxymetazoline, 0.05%	15% (v/v)
PHNY Nasal Drops	Phenylephrine, 0.5%	15% (v/v)
Tamiflu Anti-viral drugs	Zanamivir	7.5 mg/mL
Antibacterial, systemic	Tobramycin	4 µg/mL
Zicam Nasal Gel	Luffa opperculata, Galphimia glauca, Histaminum hydrochloricum Sulfur	15% (w/v)

Substance/Class	Description/ Active Ingredient	Concentration Tested
Nasal corticosteroid	Fluticasone Propionate	5 µg/mL

22.5 Carry-over Contamination Study

A study was conducted to demonstrate that single-use, self-contained GeneXpert cartridges prevent carry-over contamination of negative samples if preceded by very high positive samples in the same GeneXpert module. The study consisted of a negative sample processed in the same GeneXpert module immediately following a very high influenza A sample (A/Victoria/361/2011, $2x10^7$ $TCID_{50}$/mL) or a very high RSV A sample (A/Long/MD/26, $1x10^4$ $TCID_{50}$/mL) spiked into a simulated background matrix. This testing scheme was repeated 20 times on two GeneXpert modules for a total of 82 runs resulting in 40 positive and 42 negative specimens. All 40 positive samples were correctly reported as **Flu A POSITIVE**; **Flu B NEGATIVE**; **RSV NEGATIVE** or **Flu A NEGATIVE**; **Flu B NEGATIVE**; **RSV POSITIVE**. All 42 negative samples were correctly reported as **Flu A NEGATIVE**; **Flu B NEGATIVE**; **RSV NEGATIVE**.

22.6 Competitive Interference Study

Competitive interference of the assay caused by the presence of two targets in the Xpert Xpress Flu/RSV Assay was evaluated by testing individual influenza and RSV strains near the LoD in the presence of different influenza or RSV strains at a higher concentration in a simulated background matrix. The concentration of each strain at LoD ranged from 0.45 $TCID_{50}$/mL to 1.6 $TCID_{50}$/ mL and the concentration of the competitive strains ranged from 101 $TCID_{50}$/mL to 10^4 $TCID_{50}$/mL.

Analytical competitive interference was assessed using one (1) seasonal Flu A H3 strain (H3/Victoria/361/2011), one (1) Flu B strain (B/Mass/2/2012), one (1) RSV A strain (RSV-A/2/Australia/61), and one (1) RSV B strain (RSV-B/Wash/18537/62). Replicates of 20 were tested for each target strain and each competitive strain combination. The normal binomial distribution with 20 replicate samples at LoD is between 17 and 20 positive results based on the binomial distribution with N=20, p=.95 (X~Bin(20,0.95)). Therefore, sets of 20 with 16 or less positives would be rare and an indication of a competitive inhibitory effect due to high levels of a competing analyte.

With Flu A/Victoria/361/2011 at a concentration of 0.8 $TCID_{50}$/mL no competitive inhibitory effects were observed in the presence of $1x10^3$ $TCID_{50}$/mL of Flu B/Mass/2/2012; $1x10^3$ $TCID_{50}$/mL of RSV-A/2/Australia/6; or $1x10^4$ $TCID_{50}$/mL of RSV-B/Wash/18537/62.

With Flu B/Mass/2/2012 at a concentration of 0.45 $TCID_{50}$/mL competitive inhibitory effects were observed in the presence of $1x10^3$ $TCID_{50}$/mL of Flu A/Victoria/361/2011. No competitive inhibitory effects were observed in the presence of $1x10^2$ $TCID_{50}$/mL of Flu A/Victoria/361/2011; $1x10^3$ $TCID_{50}$/mL of RSV-A/2/Australia/6; or $1x10^3$ $TCID_{50}$/mL of RSV-B/Wash/18537/62.

With RSV-A/2/Australia/6 at a concentration of 1.1 $TCID_{50}$/mL competitive inhibitory effects were observed in the presence of $1x10^3$ $TCID_{50}$/mL of Flu A/Victoria/361/2011. No competitive inhibitory effects were observed in the presence of $1x10^2$ $TCID_{50}$/mL of Flu A/Victoria/361/2011; or $1x10^3$ $TCID_{50}$/mL of Flu B/Mass/2/2012.

With RSV-B/Wash/18537/62 at a concentration of 0.9 $TCID_{50}$/mL competitive inhibitory effects were observed in the presence of $1x10^2$ $TCID_{50}$/mL of Flu A/Victoria/361/2011 or $1x10^3$ $TCID_{50}$/mL of Flu B/Mass/2/2012. No competitive inhibitory effects were observed in the presence of 10 $TCID_{50}$/mL of Flu A/Victoria/361/2011; or $1x10^2$ $TCID_{50}$/mL of Flu B/Mass/2/2012. When the concentration of RSV-B/Wash/18537/62 was increased to 1.6 $TCID_{50}$/mL, no competitive inhibitory effects were observed in the presence of $1x10^2$ $TCID_{50}$/mL of Flu A/Victoria/361/2011; or $1x10^3$ $TCID_{50}$/mL of Flu B/Mass/2/2012.

Under the conditions of this study, internal competitive inhibitory effects were observed on the targets (Flu A, Flu B, and RSV) in the presence of two targets for the Xpert Xpress Flu/RSV Assay. The competitive inhibitory effect on the Xpert Xpress Flu/RSV targets is addressed in the Limitations section of this package insert.

23 Reproducibility

Reproducibility was established in a multi-center, blinded study using a 7-member specimen panel. Testing was performed at three sites using the GeneXpert Xpress System using the Xpert Xpress Flu/RSV ADF v3. Re-analysis of the data was performed using the Xpert Xpress Flu/RSV ADF v4, no changes resulted from the new ADF version.

Testing was conducted for five (not necessarily consecutive) days, with one lot of Xpert Xpress Flu/RSV cartridges. Each site had three operators, who tested each panel twice each day. Results are summarized in Table 15.

Table 15. Summary of Reproducibility Results

Sample	Titer of Virus ($TCID_{50}$/mL)	Site 1				Site 2				Site 3				%Total Agreement by Sample[a]
		Op 1	Op 2	Op 3	Site	Op 1	Op 2	Op 3	Site	Op 1	Op 2	Op 3	Site	
Neg	0	100% (10/10)	100% (10/10)	100% (10/10)	100% (30/30)	100% (10/10)	100% (10/10)	100% (10/10)	100% (30/30)	100% (10/10)	100% (10/10)	100% (10/10)	100% (30/30)	100% (90/90)
Flu A Low Pos	0.75	100% (10/10)	100% (10/10)	90.0% (9/10)	96.7% (29/30)	70.0% (7/10)	100% (10/10)	100% (10/10)	90.0% (27/30)	70.0% (7/10)	100% (10/10)	88.9% (8/9)[b]	86.2% (25/29)	91.0% (81/89)
Flu A Mod Pos	1.5	100% (10/10)	100% (10/10)	100% (10/10)	100% (30/30)	100% (10/10)	100% (10/10)	100% (10/10)	100% (30/30)	100% (10/10)	100% (10/10)	100% (10/10)	100% (30/30)	100% (90/90)
Flu B Low Pos	0.2	90.0% (9/10)	100% (10/10)	90.0% (9/10)	93.3% (28/30)	100% (10/10)	100% (10/10)	90.0% (9/10)	96.7% (29/30)	100% (10/10)	70.0% (7/10)	100% (10/10)	90.0% (27/30)	93.3% (84/90)
Flu B Mod Pos	0.4	100% (10/10)	100% (10/10)	100% (10/10)	100% (30/30)	100% (10/10)	100% (10/10)	100% (10/10)	100% (30/30)	100% (10/10)	100% (10/10)	100% (10/10)	100% (30/30)	100% (90/90)
RSV Low Pos	1.1	80.0% (8/10)	90.0% (9/10)	100% (10/10)	90.0% (27/30)	100% (10/10)	80.0% (8/10)	100% (10/10)	93.3% (28/30)	90.0% (9/10)	80.0% (8/10)	100% (10/10)	90.0% (27/30)	91.0% (82/90)
RSV Mod Pos	2.2	100% (10/10)	100% (10/10)	100% (10/10)	100% (30/30)	100% (10/10)	100% (10/10)	100% (10/10)	100% (30/30)	100% (10/10)	100% (10/10)	100% (10/10)	100% (30/30)	100% (90/90)

[a] Agreement calculated based on expected result: Negative for Negative (targeted positivity: 0%); Positive for Low Pos (targeted positivity: 95%) and Mod Pos (targeted positivity: 100%) samples.

[b] One sample 2x indeterminate (Flu A Low Pos).

The reproducibility of the Xpert Xpress Flu/RSV test was also evaluated in terms of the fluorescence signal expressed in Ct values for each target detected. The mean, standard deviation (SD), and coefficient of variation (CV) between-sites, between-days, between-lots and between-operators for each panel member are presented in Table 16.

Table 16. Summary of Reproducibility Data

Sample	Assay Channel (Analyte)	N[a]	Mean Ct	Between-Site		Between-Day		Between-Operator		Within-Assay		Total	
				SD	CV (%)	SD	CV (%)	SD	CV (%)	SD	CV (%)	SD	CV (%)
Neg	SPC	90	32.2	0.2	0.6	0.2	0.6	0.2	0.7	0.4	1.4	0.6	1.8
Flu A Low Pos	A	80	36.4	0.1	0.4	0	0	0	0	1.8	4.9	1.8	4.9
Flu A Mod Pos	A	90	33.7	0.1	0.2	0	0	0	0	0.6	1.7	0.6	1.8
Flu B Low Pos	B	84	35.8	0	0	0	0	0.6	1.8	1.5	4.1	1.6	4.5
Flu B Mod Pos	B	90	33.7	0	0.1	0.1	0.4	0	0	0.5	1.6	0.6	1.7
RSV Low Pos	RSV	82	36.8	0.7	2.0	0.1	0.4	0	0	1.1	2.9	1.3	3.6
RSV Mod Pos	RSV	90	33.1	0	0.1	0.2	0.6	0	0	0.5	1.4	0.5	1.5

[a] **Results with non-zero Ct values of 90.**

24 CLIA Waiver Studies

The accuracy of the Xpert Xpress Flu/RSV test was evaluated when it was used by operators who had no laboratory experience and who were representative of CLIA waived testing sites (intended users). The study was conducted at 14 CLIA waived sites with 35 intended users participating. No training on the use of the test was provided to the operators. Testing included prospectively collected NS and NP swab specimens, described above in the section titled “Performance Characteristics”. Testing was performed on the GeneXpert Xpress System using the Xpert Xpress Flu/RSV ADF v3. The data shown below represents a re-analysis of the original data using the Xpert Xpress Flu/RSV ADF v4. The new analysis showed minimal impact on clinical results. As a result of the re-analysis, 7 samples that previously had valid results changed to invalid.

The Xpert Xpress Flu/RSV test results generated by intended users at CLIA waived sites were compared with results obtained by an FDA cleared molecular assay for the detection of influenza A, influenza B and RSV as the comparator methods for this study. The positive percent agreement (PPA) and the negative percent agreement (NPA) between the Xpert Xpress Flu/RSV test results and the comparator method test results, are presented in the tables below, including the 95% confidence intervals (95% CI). There were fourteen samples that generated indeterminate results that are not included in the calculations presented below. The percent of indeterminate results was 0.4% (14/3279) with 95% CI: 0.3-0.7%.

Table 17. Performance of Xpert Xpress Flu/RSV test vs. Comparator (Influenza A)—NS Specimens

Total Number of Samples	PPA	95% Confidence Interval	NPA	95% Confidence Interval
1598	98.9% (186/188)	96.2-99.7	97.5% (1375/1410)	96.6-98.2

Table 18. Performance of Xpert Xpress Flu/RSV test vs. Comparator (Influenza B)—NS Specimens

Total Number of Samples	PPA	95% Confidence Interval	NPA	95% Confidence Interval
1598	98.4% (63/64)	91.7-99.7	99.3% (1523/1534)	98.7-99.6

Table 19. Performance of Xpert Xpress Flu/RSV test vs. Comparator (RSV)—NS Specimens

Total Number of Specimens	PPA	95% Confidence Interval	NPA	95% Confidence Interval
1543	98.2% (269/274)	95.8-99.2	99.1% (1257/1269)	98.4-99.5

Table 20. Performance of Xpert Xpress Flu/RSV test vs. Comparator (Influenza A)—NP Swab Specimens

Total Number of Samples	PPA	95% Confidence Interval	NPA	95% Confidence Interval
1667	97.6% (200/205)	94.4-99.0	98.2% (1436/1462)	97.4-98.8

Table 21. Performance of Xpert Xpress Flu/RSV test vs. Comparator (Influenza B)—NP Swab Specimens

Total Number of Samples	PPA	95% Confidence Interval	NPA	95% Confidence Interval
1667	97.3% (71/73)	90.5-99.2	99.6% (1587/1594)	99.1-99.8

Table 22. Performance of Xpert Xpress Flu/RSV Assay vs. Comparator NP Swabs (RSV)—NP Swab Specimens

Total Number of Specimens	PPA	95% Confidence Interval	NPA	95% Confidence Interval
1560	98.2% (275/280)	95.9-99.2	98.5% (1261/1280)	97.7-99.0

A study was conducted to evaluate the performance of the Xpert Xpress Flu/RSV test with weakly reactive samples when used by untrained users. Randomized blind-coded panels, containing negative and low positive (at the limit of detection {LoD} or assay cutoff) influenza A, influenza B and RSV specimens, were tested with the Xpert Xpress Flu/RSV Assay by nine untrained users at 3 sites (90 tests in total per sample type). The panel testing was conducted over a minimum of five days at each site. Testing was performed using the GeneXpert Xpress System using the Xpert Xpress Flu/RSV ADF v3. Re-analysis of the data was performed using the Xpert Xpress Flu/RSV ADF v4, no changes resulted from the new ADF version.

Table 23 shows the performance of the test with samples near the cutoff of the assay for influenza A and influenza B and RSV in the hands of untrained users.

Table 23. Performance of the Xpert Xpress Flu/RSV Assay with Samples Near the Cutoff of the Assay for Influenza A, Influenza B, and RSV by Untrained Intended Operators

	Untrained Operators		
Sample Type	**Titer of Virus ($TCID_{50}$/mL)**	**Percent Detection**	**95% Confidence Interval**
Negative	0	100.0% (90/90)	95.9–100.0
Influenza A Low Positive (at LoD)[a]	0.75	91.0% (81/89)	83.3–95.4
Influenza B Low Positive (at LoD)	0.2	93.3% (84/90)	86.2–96.9
RSV Low Positive (at LoD)	1.1	91.1% (82/90)	83.4–95.4

[a] One sample (Flu A Low Pos) was indeterminate upon initial and repeat test.

Flex Studies

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

25 References

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8. Occupational Safety and Health Standards, Hazard Communication, Toxic and Hazard Substances (March 26, 2012) (29 C.F.R., pt. 1910, subpt. Z).

26 Cepheid Headquarters Locations

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27 Technical Assistance

Before contacting Cepheid Technical Support, collect the following information:

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

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

28 Table of Symbols

Symbol	Meaning
REF	Catalog number
IVD	*In vitro* diagnostic medical device
	Do not reuse
LOT	Batch code
	Consult instructions for use
	Manufacturer
CC	Country of manufacture
Σ	Contains sufficient for *n* tests
CONTROL	Control
	Expiration date
	Temperature limitation
	Biological risks
	Caution
	Warning
Rx only	For prescription use only

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IVD

29 Revision History

Description of Changes: 302-2296, Rev. A to Rev. B

Purpose: Updates to the Instructions for Use

Section	Description of Change
Trademark, Patents and Copyright Statements	Updated to current legal standards.
8	Updates to the Materials Required but Not Provided section.
10.2	Updates to the Warnings in the Specimen section.
12	Updates to the Specimen Collection, Transport and Storage section.
29	Addition of the Revision History section and table.
Throughout	Updates to the formatting and design of the IFU.