GeneXpert
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# Xpert® Flu/RSV XC

REF **GXFLU/RSV-CE-10**

Cepheid® *In Vitro* Diagnostic Medical Device CE IVD

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# Xpert® Flu/RSV XC

For *In Vitro* Diagnostic Use Only.

## 1 Proprietary Name

Xpert® Flu/RSV XC

## 2 Common or Usual Name

Xpert Flu/RSV XC Assay

## 3 Intended Use

The Cepheid Xpert Flu/RSV XC Assay, performed on the GeneXpert Instrument Systems, 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 Flu/RSV XC Assay uses nasopharyngeal swab and nasal aspirate/wash specimens collected from patients with signs and symptoms of respiratory infection. The Xpert Flu/RSV XC 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 or respiratory syncytial virus infection 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 2013–2014 influenza season. 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); the peak of transmission usually occurs in the winter months. Symptoms commonly include fever, chills, headache, muscle aches, malaise, cough, and sinus congestion. Gastrointestinal symptoms (i.e., nausea, vomiting, or diarrhea) may also occur, primarily in children, but are less common in adults. Symptoms generally appear within two days of exposure to an infected person. Pneumonia may develop as a complication of 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 most human infections. Influenza A is the most common type of influenza virus in humans, and is generally responsible for seasonal flu epidemics and occasionally for 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 less frequent causes 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, and 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 Paramyxoviridae family consisting of two strains (subgroups A and B), is also the cause of a contagious disease that afflicts primarily infants and the elderly who are immunocompromised, e.g., patients with chronic lung or heart disease or undergoing treatment for conditions that reduces the strength of their immune system.[3] The virus can live for hours on countertops and toys and causes both upper respiratory infections, such as tracheobronchitis and lower respiratory infections manifesting as bronchiolitis and pneumonia.[4] By the age of two, most children have already been infected by RSV, but because only weak immunity develops, both children and adults can become reinfected.[3] Symptoms usually appear four to six days after infection. The disease is typically self-limiting, lasting about one to two weeks in infants. In adults, the infection lasts about five days and presents with symptoms consistent with a cold, such as rhinorrhea, fatigue, headache, and fever. The RSV season overlaps with influenza season somewhat as infections begin to rise during the fall and continues through early spring.[3,4] RSV infections, however, also occur at other times of the year, although rarely.

Active surveillance programs in conjunction with infection control 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 infections is also an important factor for effective control, proper choice of treatment, and prevention of widespread outbreaks.

## 5 Principle of the Procedure

The Xpert Flu/RSV XC Assay is an automated *in vitro* diagnostic test for qualitative detection of influenza A, influenza B, and RSV. The assay is performed on Cepheid GeneXpert Instrument Systems.

The GeneXpert Instrument Systems automate and integrate sample extraction, purification, amplification, and detection of nucleic acid target sequences in clinical specimens by using reverse transcription (conversion of RNA templates into DNA) followed by real-time PCR. The systems consist of an instrument, personal 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 Dx System Operator Manual* or *GeneXpert Infinity System Operator Manual*.

The Xpert Flu/RSV XC Assay includes reagents for the detection and differentiation of influenza A, influenza B, and RSV viral RNA directly from nasal aspirate/wash (NA/W) specimens and nasopharyngeal (NP) swab specimens from patients with signs and symptoms of respiratory 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 adequate extraction and processing of the target sequences and to monitor for the presence of inhibitors in the PCR reaction. The Probe Check Control (PCC) verifies reagent rehydration, PCR tube filling in the cartridge, probe integrity, and dye stability.

The Xpert Flu/RSV XC Assay can be run to detect Flu A and Flu B only by selecting **Xpert Flu XC**; RSV only by selecting **Xpert RSV**; or Flu A, Flu B, and RSV by selecting **Xpert Flu-RSV XC** from the **Select Assay** menu. Xpert Flu XC and Xpert 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 Cts with the Xpert Flu XC Assay, SPC amplification curves will not be seen and its results will not be reported. When RSV titers are high enough to generate very early Cts with the Xpert RSV Assay, SPC amplification curves will not be seen and its results will not be reported.

## 6 Reagents and Instruments

### 6.1 Materials Provided

The Xpert Flu/RSV XC Assay kit contains sufficient reagents to process 10 specimens or quality control samples. The kit contains the following:

| | |
|---|---|
| **Xpert Flu/RSV XC Assay Cartridges with Integrated Reaction Tubes** | **10** |
| • 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** | **2 bags of 12 per kit** |
| **CD** | **1 per kit** |
| • Assay Definition Files (ADF) | |
| • Instructions to import ADF into GeneXpert software | |
| • Instructions for Use (Package Insert) | |

**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 Flu/RSV XC Assay cartridges at 2–28 °C. until the expiration date provided on the package 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

- Specimens must be collected and transported with the Xpert Nasopharyngeal Sample Collection Kit for viruses, Cepheid catalog #SWAB/B-100 or Sample Collection Device catalog #NASL-100N-100.
- GeneXpert Dx Instrument or GeneXpert Infinity Systems (catalog number varies by configuration): GeneXpert instrument, computer, barcode scanner, Operator Manual.
    - For GeneXpert Dx System: GeneXpert Dx software version 4.3 or higher.
- Printer: Contact Cepheid Technical Support to arrange for the purchase of a recommended printer.

## 9 Materials Available but Not Provided

- Inactivated virus controls from ZeptoMetrix, catalog #NATFLUAB-6C and catalog #NATRSV-6C as external positive controls, and catalog #NATCXVA9-6C (Coxsackie virus) as an external negative control.

## 10 Warnings and Precautions

### 10.1 General

- For *In Vitro* Diagnostic 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 using standard precautions. Guidelines for specimen handling are available from the U.S. Centers for Disease Control and Prevention[5] and the Clinical and Laboratory Standards Institute.[6,7]
- 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.
- Biological specimens, transfer devices, and used cartridges should be considered capable of transmitting infectious agents requiring standard precautions. Follow your institution's environmental waste procedures for proper disposal of used cartridges and unused reagents. These materials may exhibit characteristics of chemical hazardous waste requiring specific disposal. If country or regional regulations do not provide clear direction on proper disposal, biological specimens and used cartridges should be disposed per WHO [World Health Organization] medical waste handling and disposal guidelines.

### 10.2 Specimen

- Specimen collection and handling procedures require specific training and guidance.
- For collection and transport of nasopharyngeal swab specimens, use only the Xpert Nasopharyngeal Sample Collection Kit.
- Specimens must be collected and tested before the expiration date of the Xpert Viral Transport Medium tube.
- Maintain proper storage conditions during specimen transport to ensure the integrity of the specimen (see Section 12, Specimen Collection, Transport, and Storage). Specimen stability under shipping conditions other than those recommended has not been evaluated.
- Proper sample collection, storage, and transport are essential for correct results.

### 10.3 Assay/Reagent

- The assay has been validated using Cepheid GeneXpert software version 4.3 or higher. Cepheid will validate future software versions for use with the Xpert Flu/RSV XC Assay.
- Use of the influenza A/influenza B positive control in the Xpert RSV only assay mode may lead to invalid control results.
- Use of the RSV positive control in the Xpert Flu XC only assay mode may lead to invalid control results.
- When performing a test in the Xpert Flu XC only or Xpert RSV only mode, a negative test result does not preclude a positive result for the other targets.
- Sensitivity may be impacted when using frozen, archived specimens.
- Do not open the Xpert Flu/RSV XC Assay cartridge lid except when adding sample.
- Do not use a cartridge that has been dropped after removing it from the packaging.
- Do not shake the cartridge. Shaking or dropping the cartridge after opening the cartridge lid may yield invalid 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 Flu/RSV XC Assay cartridge is used to process one test. Do not reuse spent cartridges, except when diluting NA/W specimens.
- Each single-use disposable pipette is used to transfer one specimen. Do not reuse spent 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 lab 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[8,9]

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

## 12 Specimen Collection, Transport, and Storage

NA/W specimens and NP swab specimens can be collected following the user institution's standard procedures and placed into the Xpert Viral Transport Medium (3 mL tube with transport medium). Specimens should be transported at 2–8 °C.

- Specimens placed in transport medium following collection can be stored for up to 24 hours at 2–30 °C or up to seven days at 2–8 °C prior to testing with the Xpert Flu/RSV XC Assay.

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

# 13 Procedure

## 13.1 Preparing the Cartridge

**Important** **Start the test within 60 minutes of adding the sample to the cartridge.**

**For NP Swab Specimens:**

1. Remove a cartridge from the package.
2. Mix specimen by inverting the Xpert Viral Transport Medium tube five times.
3. Open the cartridge lid. Using a clean 300 µL transfer pipette (supplied), transfer 300 µL (one draw) of the specimen from the transport medium tube to the sample chamber with large opening in the cartridge (Figure 1).
4. Close the cartridge lid.

**For NA/W Specimens:**

1. Using a clean 300 µL transfer pipette (supplied), transfer 600 µL of the sample (two draws, using the same transfer pipette) into the 3 mL Xpert Viral Transport Medium tube and then cap the tube.
2. Mix specimen by inverting the transport medium tube five times.
3. Remove the cartridge from the package.
4. Open the cartridge lid. Using a clean 300 µL transfer pipette (supplied), transfer 300 µL (one draw) of the diluted specimen to the sample chamber with the large opening in the cartridge (Figure 1).
5. Close the cartridge lid.

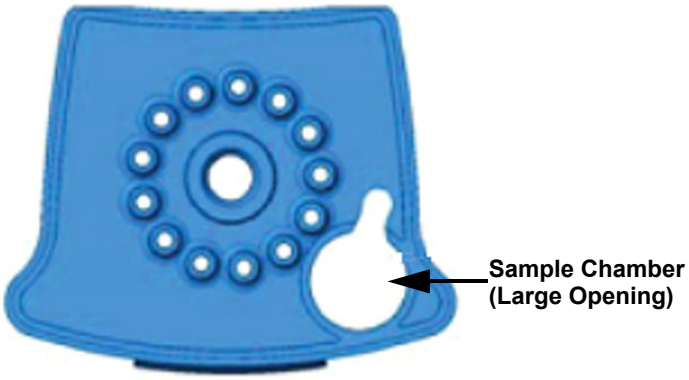


**Figure 1. Xpert Flu/RSV XC Assay Cartridge (Top View)**

## 13.2 Starting the Test

**Important** **Before starting the test, make sure the Xpert Flu/RSV XC Assay definition files (ADFs) are imported into the software.**

This section lists the basic steps for running the test. For detailed instructions, see the *GeneXpert Dx System Operator Manual* or the *GeneXpert Infinity System Operator Manual*, depending on the model that is being used.

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

1. Turn on the GeneXpert instrument:
   - If using the GeneXpert Dx instrument, first turn on the instrument and then turn on the computer. The GeneXpert software will launch automatically or may require double-clicking the GeneXpert Dx software icon on the Windows® desktop.

     or

   - If using the GeneXpert Infinity instrument, power up the instrument. The GeneXpert software will launch automatically or may require double-clicking the Xpertise software icon on the Windows® desktop.
2. Log on to the GeneXpert Instrument System software using your user name and password.

3. In the GeneXpert System window, click **Create Test** (GeneXpert Dx) or click **Orders** and **Order Test** (GeneXpert Infinity). The Create Test window opens.
4. Scan (or type in) the Patient ID (optional). If typing the Patient ID, make sure the Patient ID is typed correctly. The Patient ID is shown on the left side of the View Results window and is associated with the test results.
5. Scan (or type in) the Sample ID. If typing the Sample ID, make sure the Sample ID is typed correctly. The Sample ID is shown on the left side of the View Results window and is associated with the test results.
6. Scan the barcode on the Xpert Flu/RSV XC Assay cartridge. Using the barcode information, the software automatically fills in the boxes for the following fields: Reagent Lot ID, Cartridge SN, and Expiration Date.

**Note** If the barcode on the Xpert Flu/RSV XC Assay cartridge does not scan, then repeat the test with a new cartridge.

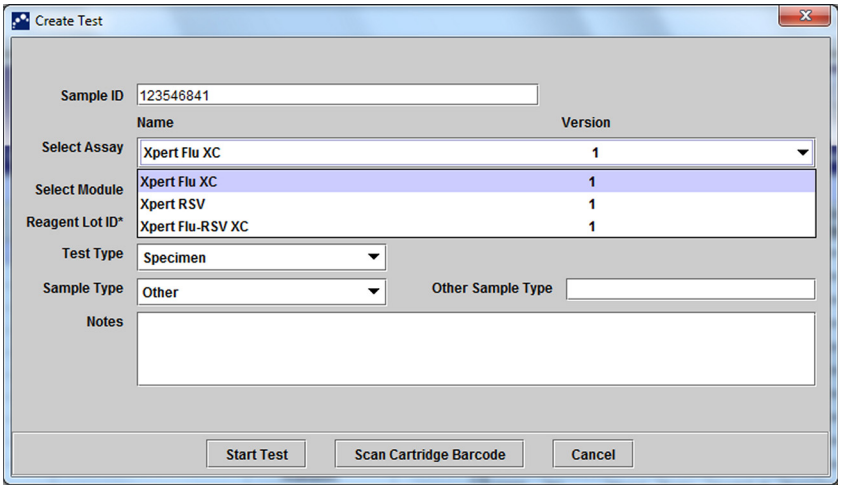


**Figure 2. Create Test Window: Select Assay Menu**

7. Make the appropriate selection from the **Select Assay** menu, as shown in Figure 2.
   - Flu A and Flu B only: Select **Xpert Flu XC**
   - RSV only: Select **Xpert RSV**
   - Flu A, Flu B and RSV: Select **Xpert Flu-RSV XC**

**Note** 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 Flu-RSV XC assay is chosen.

8. Click **Start Test** (GeneXpert Dx) or **Submit** (GeneXpert Infinity). Type your password in the dialog box that appears.
9. For the GeneXpert Infinity System, place the cartridge on the conveyor belt. The cartridge will be automatically loaded, the test will run, and the used cartridge will be placed into the waste container.

   or

   For the GeneXpert Dx Instrument:

   A. Open the instrument module door with the blinking green light and load the cartridge.
   B. Close the door. The test starts and the green light stops blinking. When the test is finished, the light turns off.
   C. Wait until the system releases the door lock before opening the module door and removing the cartridge.
   D. The used cartridges should be disposed in the appropriate specimen waste containers according to your institution's standard practices.

## 14 Viewing and Printing Results

This section lists the basic steps for viewing and printing results. For more detailed instructions on how to view and print the results, see the *GeneXpert Dx System Operator Manual* or the *GeneXpert Infinity System Operator Manual*, depending upon the instrument used.

1. Click the **View Results** icon to view results.
2. Upon completion of the test, click the **Report** button of the View Results window to view and/or generate a PDF report file.

## 15 Quality Control

### 15.1 Built-in Quality Controls

**CONTROL**

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

- **Sample Processing Control (SPC):** Ensures the sample was processed correctly. The SPC is an Armored RNA® control that is included in each cartridge to verify adequate processing of the sample. The SPC verifies that release of RNA from the influenza and RSV viruses 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.

  There are two exceptions in which SPC is ignored and the result is valid:

  - The SPC may be negative in a sample with a high titer of Flu A or Flu B when tested with the Xpert RSV ADF.
  - The SPC may be negative in a sample with a high titer of RSV when tested with the Xpert Flu XC ADF.

- **Probe Check Control (PCC, QC1, QC2):** Before the start of the PCR reaction, the GeneXpert Instrument System measures the fluorescence signal from the first PCC (QC1 and QC2) performed before the reverse transcription step. QC1 checks for the presence of the EZR bead and QC2 checks for the presence of the TSR bead. The second PCC (Flu A 1, Flu A 2, Flu A 3, Flu B, RSV, and SPC) is performed after the reverse transcription step and before PCR begins. The Probe Check Control (PCC, QC1 and QC2) monitors bead rehydration, reaction tube filling, probe integrity, and dye stability. The PCC passes if it meets the validated acceptance criteria.

## 16 Interpretation of Results

The Xpert Flu/RSV XC Assay has three channels (Flu A 1, Flu A 2, and Flu A 3) to detect most influenza A strains. The primers and probes in the Flu A 1 channel have 100% homology to human influenza A strains. The primers and probes in the Flu A 2 channel have > 95% homology to avian influenza A strains and approximately 80% homology to human influenza A strains. The primers and probes in the Flu A 3 channel detect the hemagglutinin gene segment for the avian influenza A H7N9 strains (subtyping capability). All influenza A strains (human and avian) detected by the Xpert Flu/RSV XC Assay are reported as **Flu A POSITIVE**.

The results are interpreted by the GeneXpert Instrument System from measured fluorescent signals and embedded calculation algorithms and will be shown in the View Results window. A Flu A result in the Xpert Flu/RSV XC Assay requires either the Flu A 1 or Flu A 2 channel to be positive in order for a **Flu A POSITIVE** test result to be reported. A positive in the Flu A 3 channel without a positive Flu A 1 or Flu A 2 result is reported as **INVALID**. Table 1 lists all the possible test results for Flu A.

**Table 1. Possible Test Results for Flu A for Flu A 1, Flu A 2, and Flu A 3 Channels**

| Flu A Test Result | Flu A 1 Channel | Flu A 2 Channel | Flu A 3 Channel |
|---|---|---|---|
| **Flu A POSITIVE** | POS | POS | POS |
| | POS | POS | NEG |
| | POS | NEG | POS |
| | NEG | POS | POS |
| | POS | NEG | NEG |
| | NEG | POS | NEG |
| **INVALID** | NEG | NEG | POS |
| **Flu A NEGATIVE** | NEG | NEG | NEG |

The results are interpreted automatically by the GeneXpert Instrument System from measured fluorescent signals and embedded calculation algorithms and are clearly shown in the View Results window. All the possible results are shown in Table 2.

**Table 2. All Possible Final Test Results for the Xpert Flu-RSV XC Selected Assay**

| Result Text | Flu A 1 | Flu A 2 | Flu A 3 | Flu B | RSV | SPC |
|---|---|---|---|---|---|---|
| **Flu A POSITIVE; Flu B NEGATIVE; RSV NEGATIVE** | +/- | +/- | +/- | - | - | +/- |
| **Flu A NEGATIVE; Flu B POSITIVE; RSV NEGATIVE** | - | - | - | + | - | +/- |
| **Flu A NEGATIVE; Flu B NEGATIVE; RSV POSITIVE** | - | - | - | - | + | +/- |
| **Flu A POSITIVE; Flu B POSITIVE; RSV NEGATIVE** | +/- | +/- | +/- | + | - | +/- |
| **Flu A POSITIVE; Flu B NEGATIVE; RSV POSITIVE** | +/- | +/- | +/- | - | + | +/- |
| **Flu A NEGATIVE; Flu B POSITIVE; RSV POSITIVE** | - | - | - | + | + | +/- |
| **Flu A POSITIVE; Flu B POSITIVE; RSV POSITIVE** | +/- | +/- | +/- | + | + | +/- |
| **Flu A NEGATIVE; Flu B NEGATIVE; RSV NEGATIVE** | - | - | - | - | - | + |
| **INVALID** | - | - | - | - | - | - |

See Figure 3 through Figure 19 for specific examples and Table 3 to interpret test result statements for the Xpert Flu-RSV XC, Xpert Flu XC, and Xpert RSV assays. The format of the test results presented will vary depending on the user's choice to run either an Xpert Flu-RSV XC, Xpert Flu XC, or Xpert RSV selected assay.

**Table 3. Xpert Flu-RSV XC, Xpert Flu XC, and Xpert RSV Assay Results and Interpretations**

| Result | Interpretation |
|---|---|
| **Flu A POSITIVE; Flu B NEGATIVE; RSV NEGATIVE**<br>See Figure 3. | Flu A target RNA is detected; Flu B target RNA is not detected; RSV target RNA is not detected.<br>• The Flu A target has a Ct within the valid range and endpoint above the threshold setting.<br>• SPC: NA (not applicable); SPC is ignored because the Flu A target amplification may compete with this control.<br>• Probe Check: PASS; all probe check results pass. |
| **Flu A NEGATIVE; Flu B POSITIVE; RSV NEGATIVE**<br>See Figure 4. | Flu A target RNA is not detected; Flu B target RNA is detected; RSV target RNA is not detected.<br>• The Flu B target has a Ct within the valid range and endpoint above the threshold setting.<br>• SPC: NA (not applicable); SPC is ignored because the Flu B target amplification may compete with this control.<br>• Probe Check: PASS; all probe check results pass. |
| **Flu A NEGATIVE; Flu B NEGATIVE; RSV POSITIVE**<br>See Figure 5. | Flu A target RNA is not detected; Flu B target RNA is not detected; RSV target RNA is detected.<br>• The RSV target has a Ct within the valid range and endpoint above the threshold setting.<br>• SPC: NA (not applicable); SPC is ignored because the RSV target amplification may compete with this control.<br>• Probe Check: PASS; all probe check results pass. |

**Table 3. Xpert Flu-RSV XC, Xpert Flu XC, and Xpert RSV Assay Results and Interpretations (Continued)**

| Result | Interpretation |
|---|---|
| **Flu A POSITIVE; Flu B POSITIVE; RSV NEGATIVE** See Figure 6. | Flu A target RNA is detected; Flu B target RNA is detected; RSV target RNA is not detected.<br>• The Flu A target has a Ct within the valid range and endpoint above the threshold setting.<br>• The Flu B target has a Ct within the valid range and endpoint above the threshold setting.<br>• SPC: NA (not applicable); SPC is ignored because the Flu A and Flu B target amplification may compete with this control.<br>• Probe Check: PASS; all probe check results pass. |
| **Flu A POSITIVE; Flu B NEGATIVE; RSV POSITIVE** See Figure 7. | Flu A target RNA is detected; Flu B target RNA is not detected; RSV target RNA is detected.<br>• The Flu A target has a Ct within the valid range and endpoint above the threshold setting.<br>• The RSV target has a Ct within the valid range and endpoint above the threshold setting.<br>• SPC: NA (not applicable); SPC is ignored because the Flu A and RSV target amplification may compete with this control.<br>• Probe Check: PASS; all probe check results pass. |
| **Flu A NEGATIVE; Flu B POSITIVE; RSV POSITIVE** See Figure 8. | Flu A target RNA is not detected; Flu B target RNA is detected; RSV target RNA is detected.<br>• The Flu B target has a Ct within the valid range and endpoint above the threshold setting.<br>• The RSV target has a Ct within the valid range and endpoint above the threshold setting.<br>• SPC: NA (not applicable); SPC is ignored because the Flu B and RSV target amplification may compete with this control.<br>• Probe Check: PASS; all probe check results pass. |
| **Flu A POSITIVE; Flu B POSITIVE; RSV POSITIVE** See Figure 9. | Flu A target RNA is detected; Flu B target RNA is detected; RSV target RNA is detected.<br>• The Flu A target has a Ct within the valid range and endpoint above the threshold setting.<br>• The Flu B target has a Ct within the valid range and endpoint above the threshold setting.<br>• The RSV target has a Ct within the valid range and endpoint above the threshold setting.<br>• SPC: NA (not applicable); SPC is ignored because the Flu A, Flu B, and RSV target amplification may compete with this control.<br>• Probe Check: PASS; all probe check results pass. |
| **Flu A NEGATIVE; Flu B NEGATIVE; RSV NEGATIVE** See Figure 10. | Flu A target RNA is not detected; Flu B target RNA is not detected; RSV target RNA is not detected.<br>• Flu A, Flu B, and RSV target RNAs are not detected.<br>• SPC: PASS; SPC has a Ct within the valid range and endpoint above the threshold setting.<br>• Probe Check: PASS; all probe check results pass. |
| **INVALID** See Figure 11. | • SPC does not meet acceptance criteria. Presence or absence of the target RNAs cannot be determined. Repeat test according to the instructions in Section 17.2, Retest Procedure.<br>• SPC meets acceptance criteria. Flu A 1, Flu A 2, Flu B, and/or RSV target RNAs are not detected; FluA3 target RNA is detected. |
| **Flu A POSITIVE; Flu B NEGATIVE** See Figure 12. | Flu A target RNA is detected; Flu B target RNA is not detected.<br>• The Flu A target has a Ct within the valid range and endpoint above the threshold setting.<br>• SPC: NA (not applicable); SPC is ignored because the Flu A target amplification may compete with this control.<br>• Probe Check: PASS; all probe check results pass. |
| **Flu A NEGATIVE; Flu B POSITIVE** See Figure 13. | Flu A target RNA is not detected; Flu B target RNA is detected.<br>• The Flu B target has a Ct within the valid range and endpoint above the threshold setting.<br>• SPC: NA (not applicable); SPC is ignored because the Flu B target amplification may compete with this control.<br>• Probe Check: PASS; all probe check results pass. |

**Table 3. Xpert Flu-RSV XC, Xpert Flu XC, and Xpert RSV Assay Results and Interpretations (Continued)**

| Result | Interpretation |
|---|---|
| **Flu A POSITIVE; Flu B POSITIVE**<br>See Figure 14. | Flu A target RNA is detected; Flu B target RNA is detected.<br>• The Flu A target has a Ct within the valid range and endpoint above the threshold setting.<br>• The Flu B target has a Ct within the valid range and endpoint above the threshold setting.<br>• SPC: NA (not applicable); SPC is ignored because the Flu A and Flu B target amplification may compete with this control.<br>• Probe Check: PASS; all probe check results pass. |
| **Flu A NEGATIVE; Flu B NEGATIVE**<br>See Figure 15 and Figure 16. | Flu A target RNA is not detected; Flu B target RNA is not detected (see Figure 15).<br>• Flu A and Flu B target RNAs are not detected.<br>• SPC: PASS; SPC has a Ct within the valid range and endpoint above the threshold setting.<br>• Probe Check: PASS; all probe check results pass.<br>Or<br>Flu A target RNA is not detected (see Figure 16); Flu B target RNA is not detected (see Figure 16).<br>• Flu A and Flu B target RNAs are not detected.<br>• SPC: NA (not applicable); SPC is ignored because the RSV target amplification may compete with this control.<br>• Probe Check: PASS; all probe check results pass. |
| **RSV POSITIVE**<br>See Figure 17. | RSV target RNA is detected.<br>• The RSV target has a Ct within the valid range and endpoint above the threshold setting.<br>• SPC: PASS; SPC has a Ct within the valid range and endpoint above the threshold setting.<br>• Probe Check: PASS; all probe check results pass. |
| **RSV NEGATIVE**<br>See Figure 18 and Figure 19. | RSV target RNA is not detected (see Figure 18).<br>• RSV target RNA is not detected.<br>• SPC: PASS; SPC has a Ct within the valid range and endpoint above the threshold setting.<br>• Probe Check: PASS; all probe check results pass.<br>Or<br>RSV target RNA is not detected (see Figure 19).<br>• The RSV target RNA is not detected.<br>• SPC: NA (not applicable); SPC is ignored because the Flu A or Flu B target amplification may compete with this control.<br>• Probe Check: PASS; all probe check results pass. |
| **ERROR** | Presence or absence of Flu A, Flu B, and/or RSV target RNA cannot be determined. Repeat test according to the instructions in Section 17.2, Retest Procedure below.<br>• Flu A: NO RESULT<br>• Flu B: NO RESULT<br>• RSV: NO RESULT<br>• SPC: NO RESULT<br>• Probe Check: FAIL*; all or one of the probe check results fail.<br>* If the probe check passed, the error is caused by the maximum pressure limit exceeding the acceptable range or by a system component failure. |

**Table 3. Xpert Flu-RSV XC, Xpert Flu XC, and Xpert RSV Assay Results and Interpretations (Continued)**

| Result | Interpretation |
|---|---|
| **NO RESULT** | Presence or absence of Flu A, Flu B, and/or RSV target RNA cannot be determined. Repeat test according to the instructions in Section 17.2, Retest Procedure below. A **NO RESULT** indicates that insufficient data were collected. For example, the operator stopped a test that was in progress or a power failure occurred.<br>• Flu A: NO RESULT<br>• Flu B: NO RESULT<br>• RSV: NO RESULT<br>• SPC: NO RESULT<br>• Probe Check: NA (not applicable) |

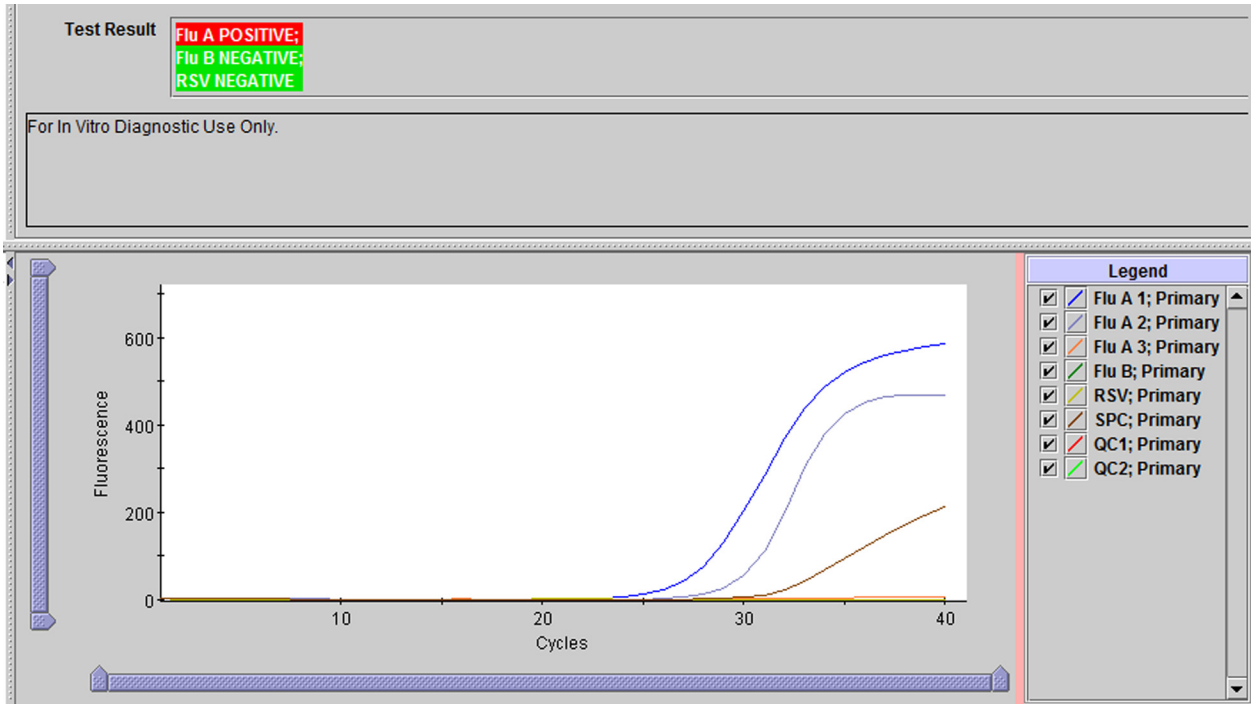


**Figure 3. Xpert Flu-RSV XC: An Example of a Positive Result for Flu A**

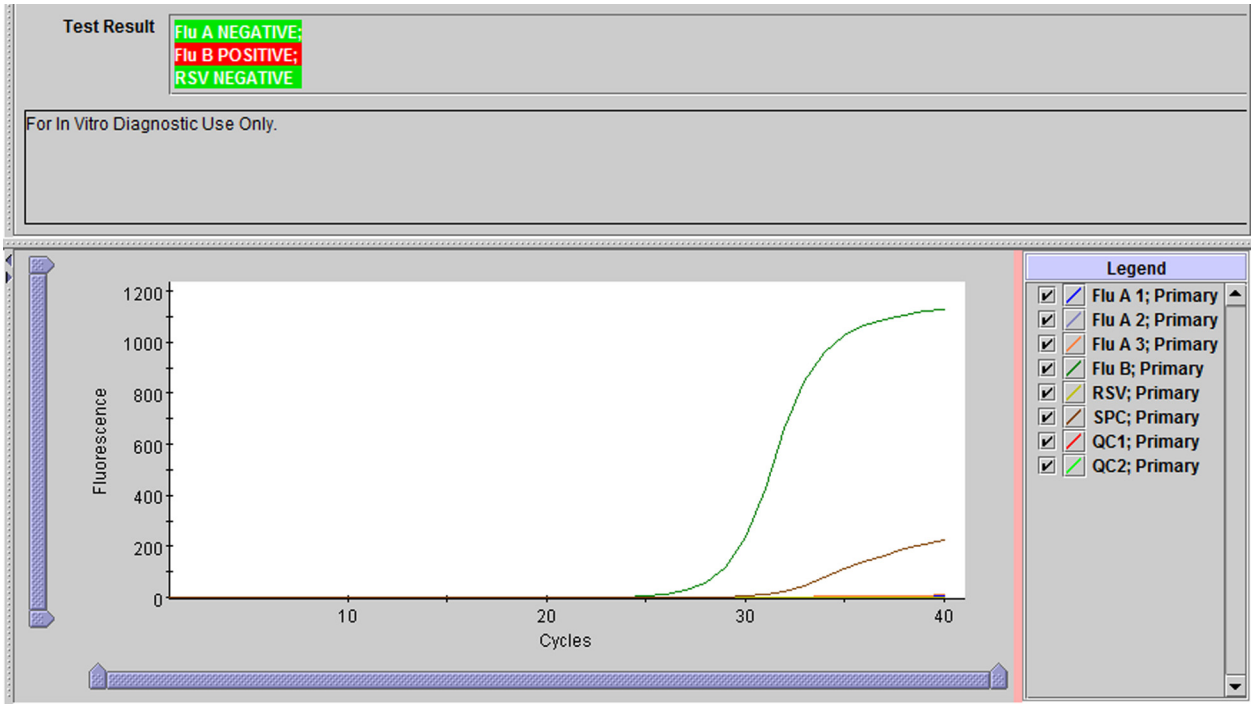


**Figure 4. Xpert Flu-RSV XC: An Example of a Positive Result for Flu B**

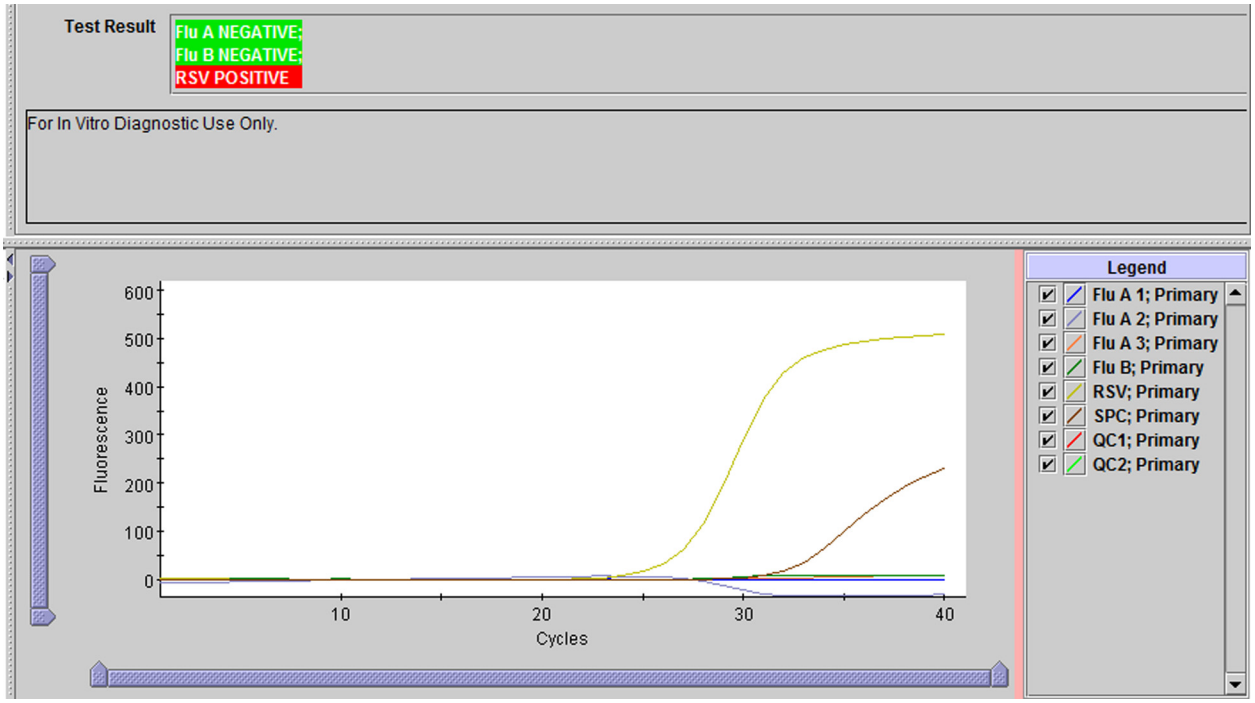


**Figure 5. Xpert Flu-RSV XC: An Example of a Positive Result for RSV**

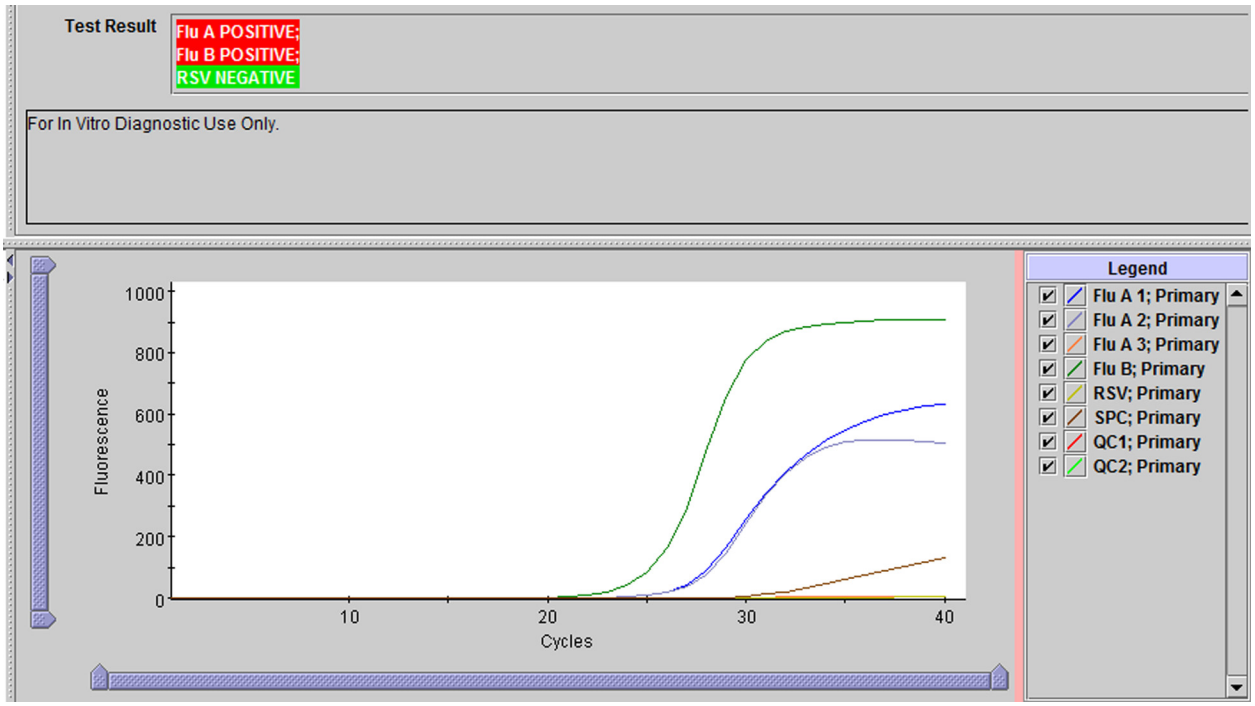


**Figure 6. Xpert Flu-RSV XC: An Example of a Positive Result for Flu A and Flu B**

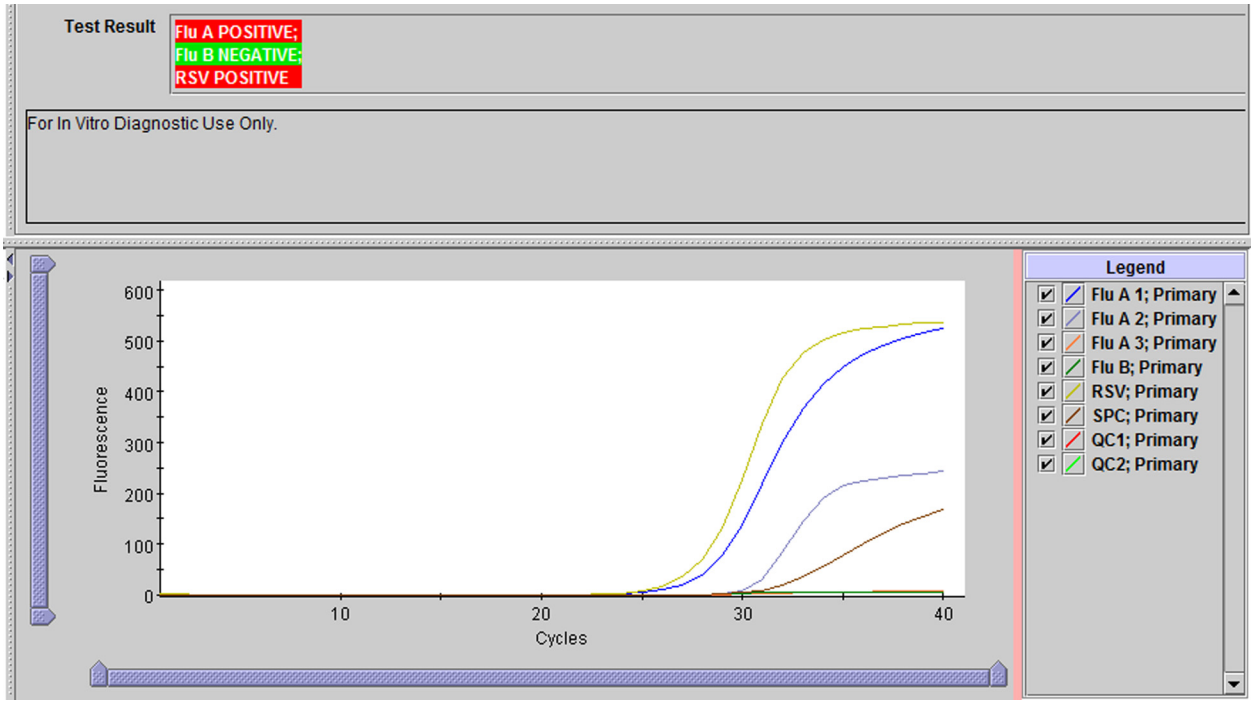


**Figure 7. Xpert Flu-RSV XC: An Example of a Positive Result for Flu A and RSV**

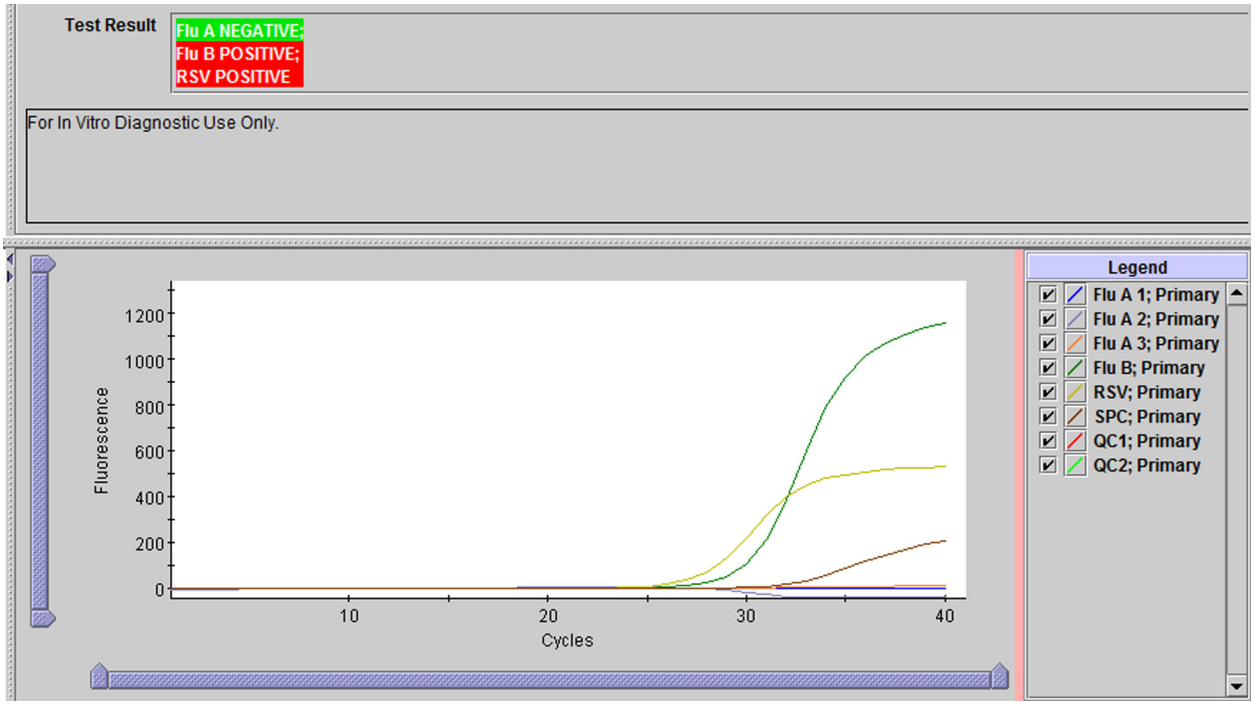


**Figure 8. Xpert Flu-RSV XC: An Example of a Positive Result for Flu B and RSV**

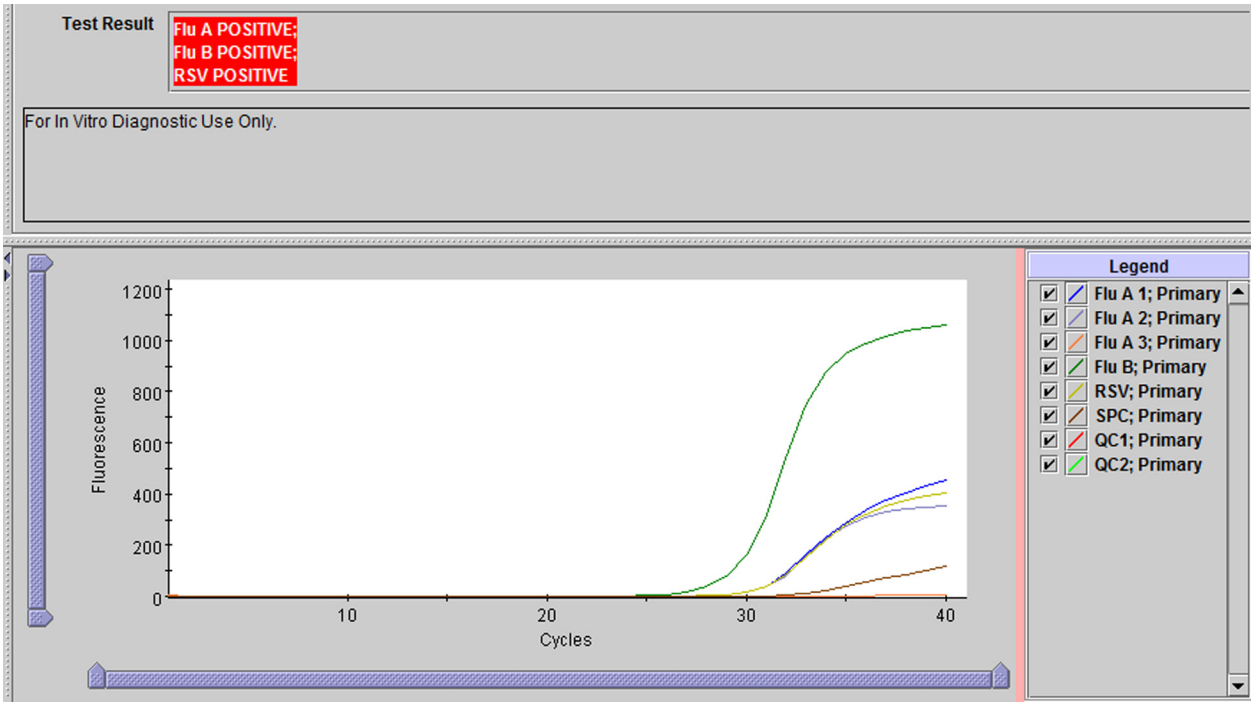


**Figure 9. Xpert Flu-RSV XC: An Example of a Positive Result for Flu A, Flu B, and RSV**

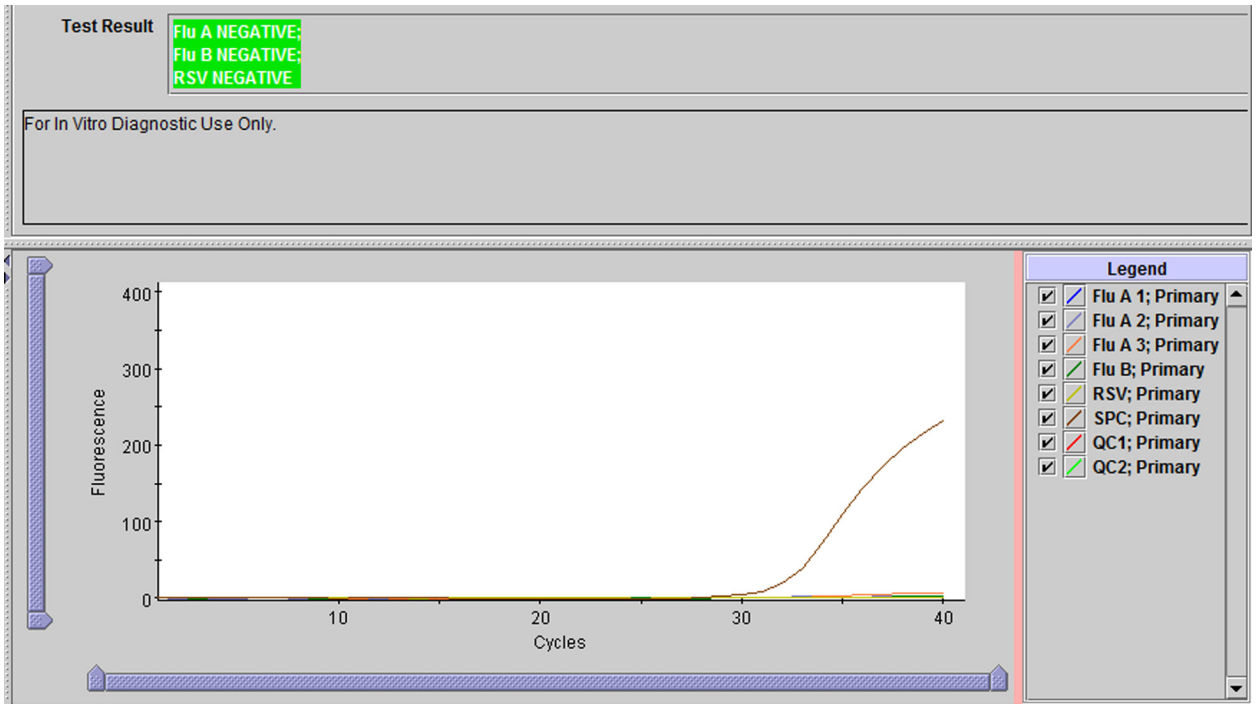


**Figure 10. Xpert Flu-RSV XC: An Example of a Negative Result for Flu A, Flu B, and RSV**

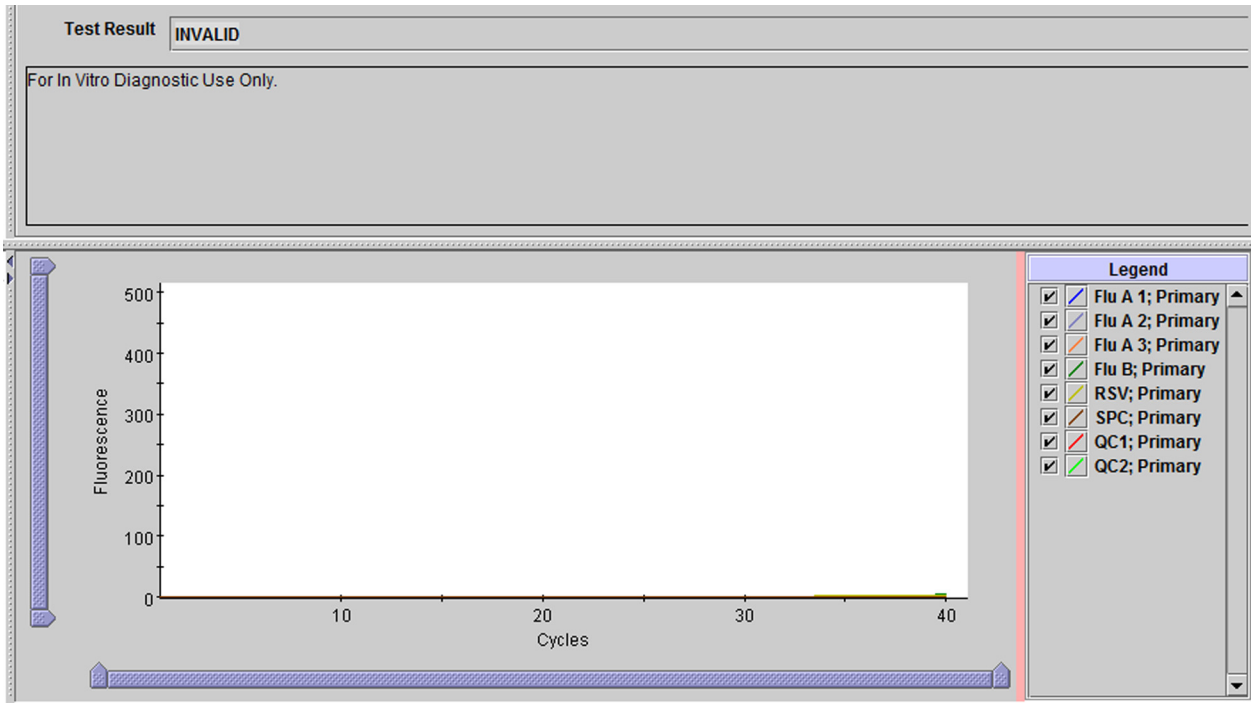


**Figure 11. Xpert Flu-RSV XC: An Example of an Invalid Result (SPC does not meet Acceptance Criteria)**

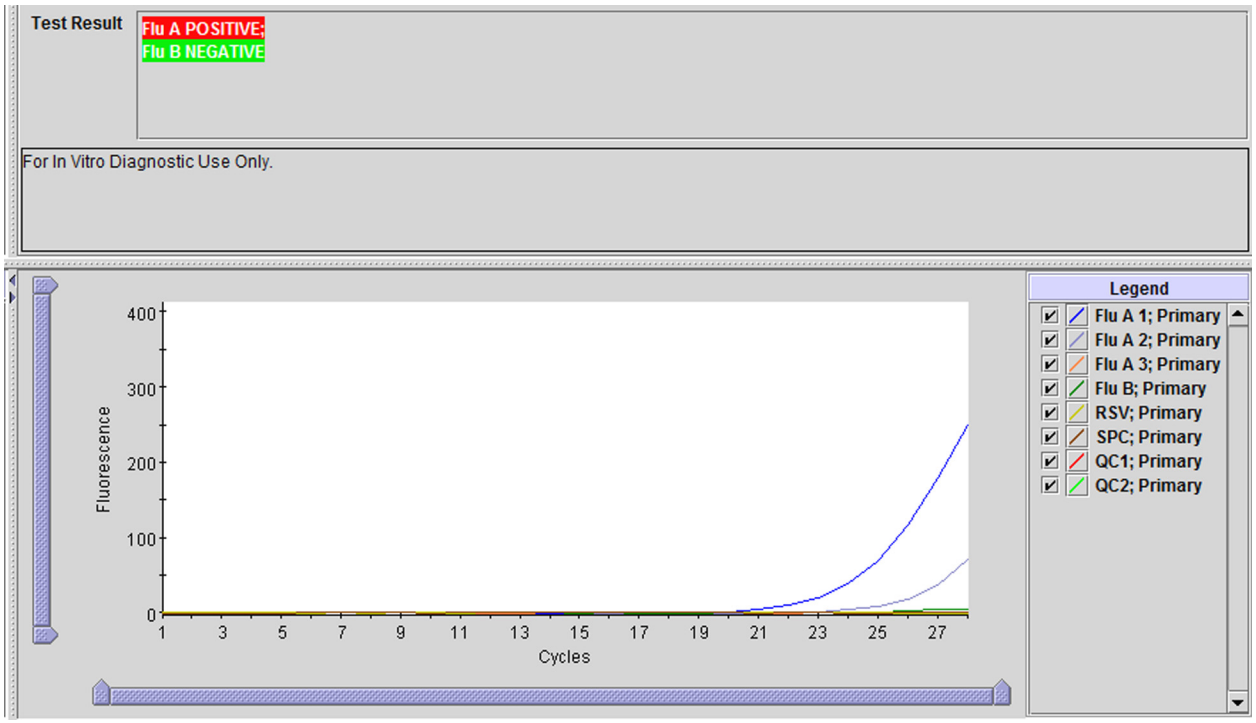


**Figure 12. Xpert Flu XC: An Example of a Positive Result for Flu A**

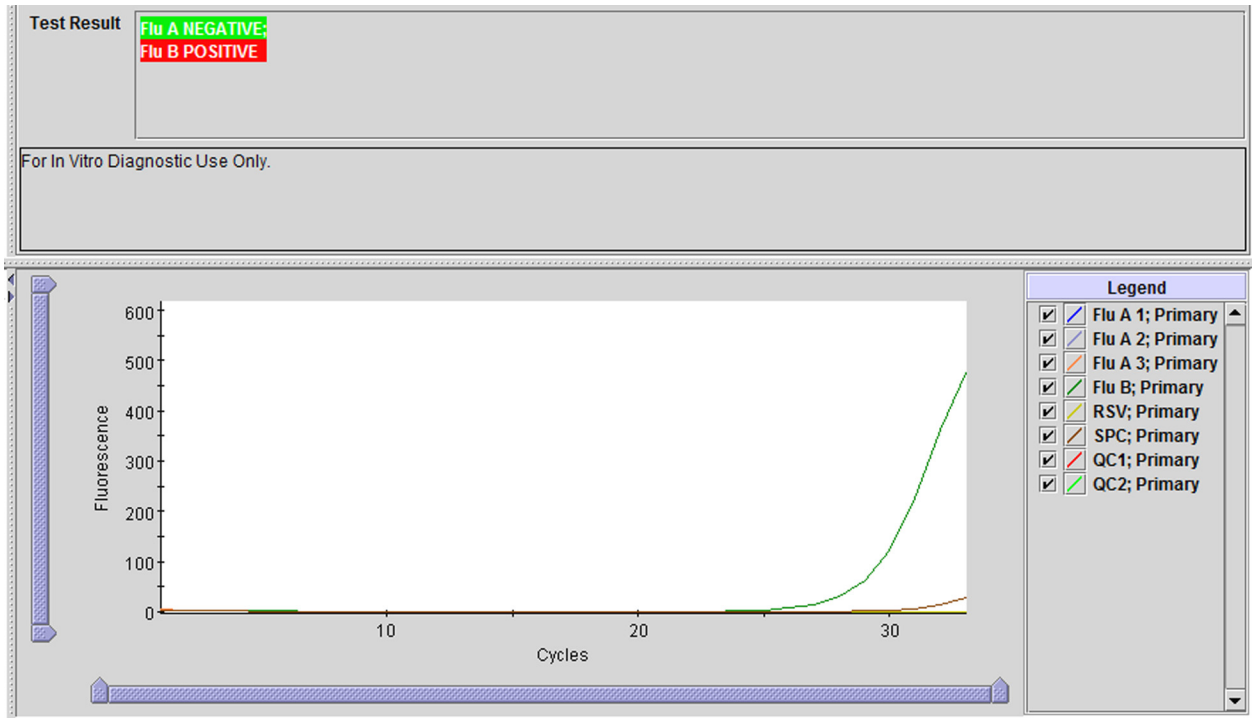


**Figure 13. Xpert Flu XC: An Example of a Positive Result for Flu B**

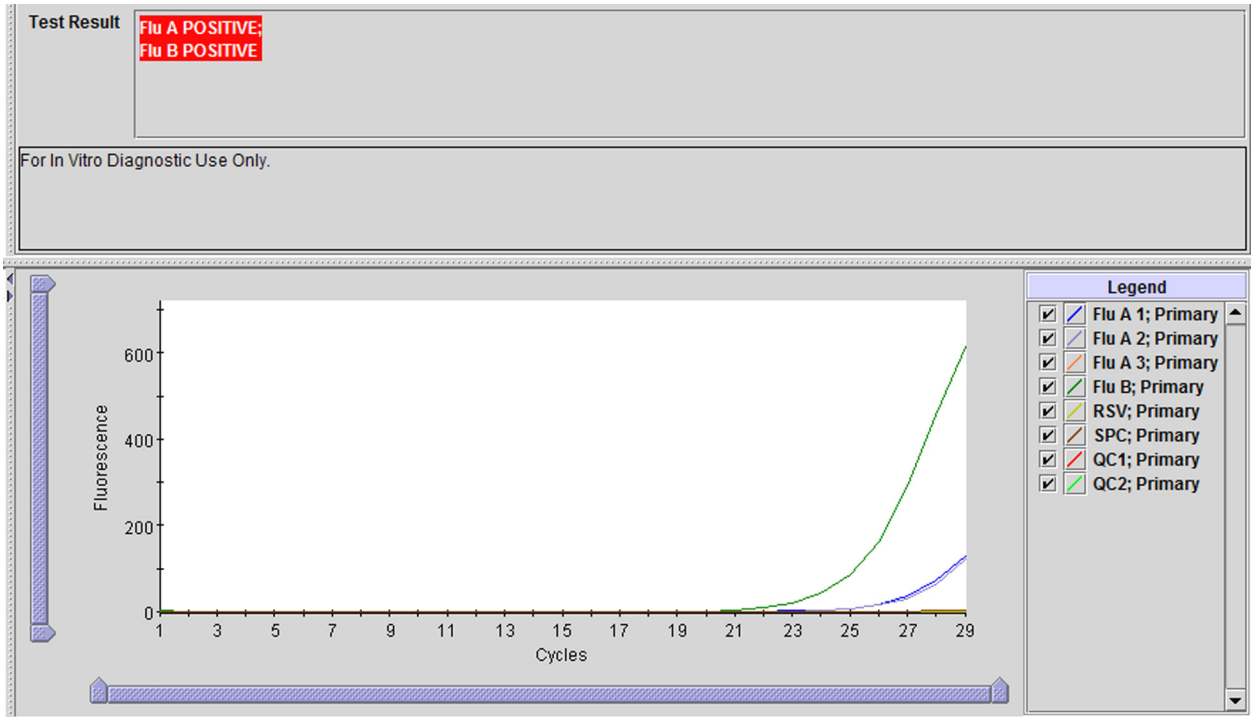


**Figure 14. Xpert Flu XC: An Example of a Positive Result for Flu A and Flu B**

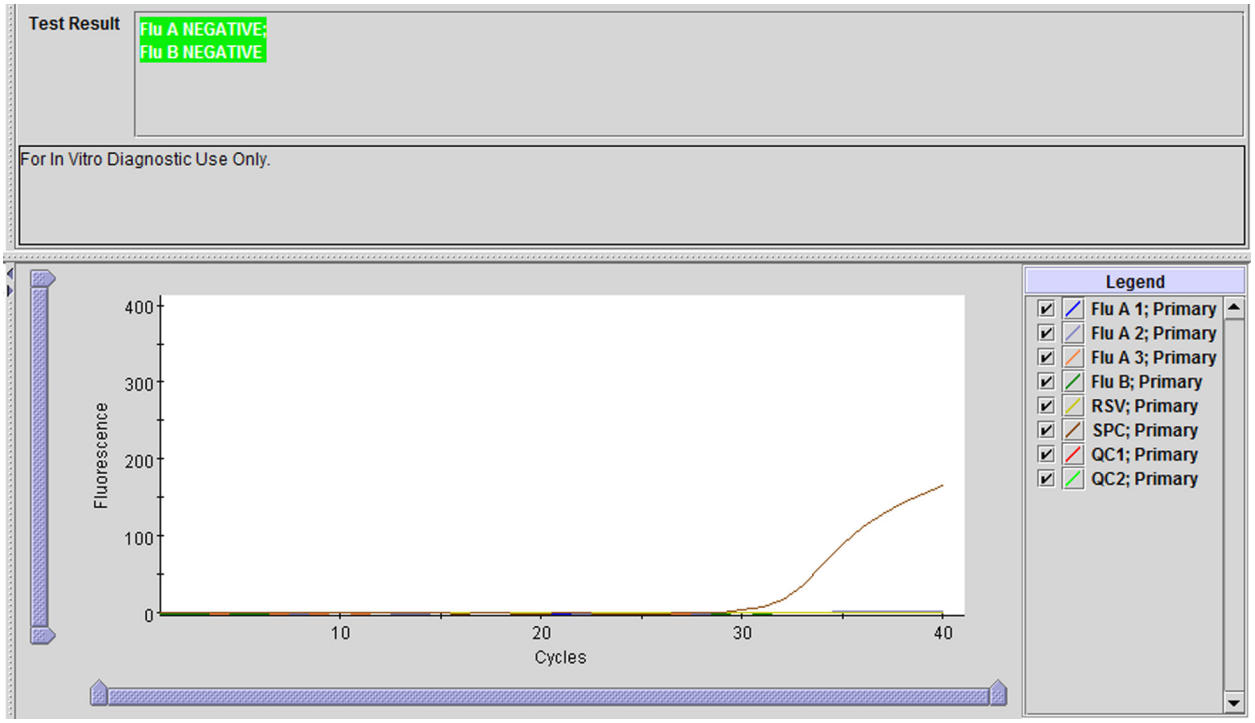


**Figure 15. Xpert Flu XC: An Example of a Negative Result for Flu A and Flu B**

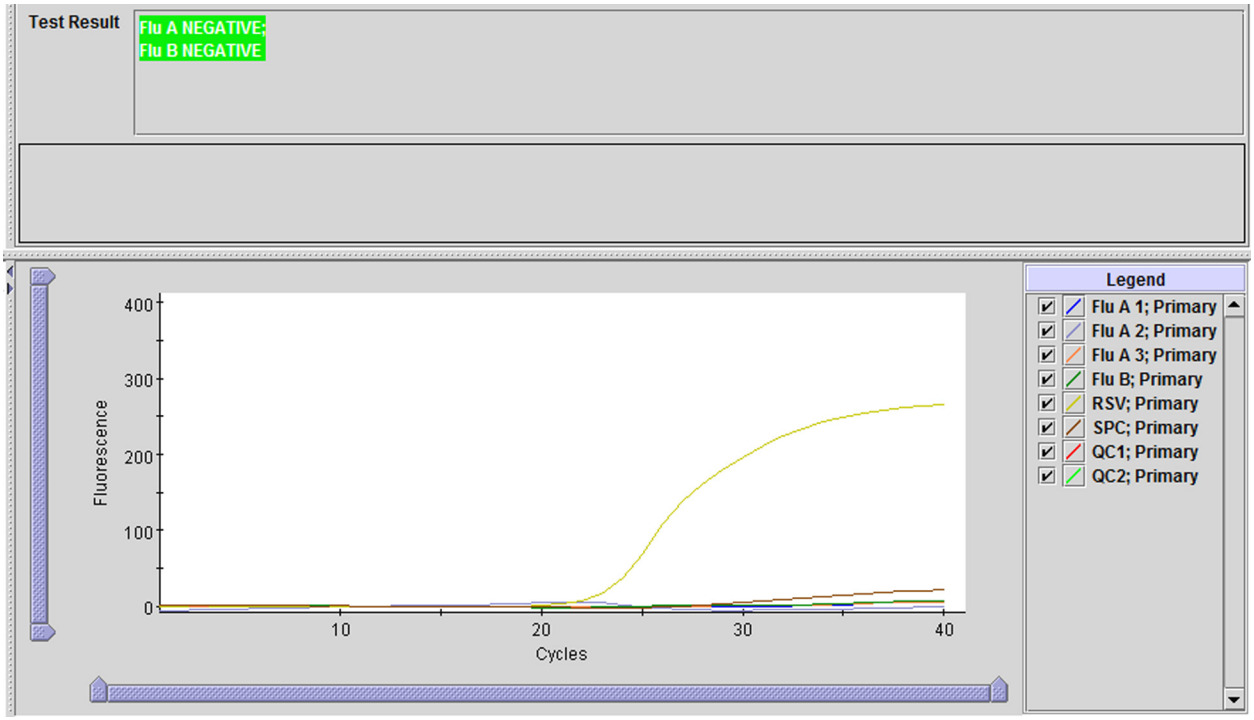


**Figure 16. Xpert Flu XC: An Example of a Negative Result for Flu A and Flu B (Sample Containing RSV Target)**

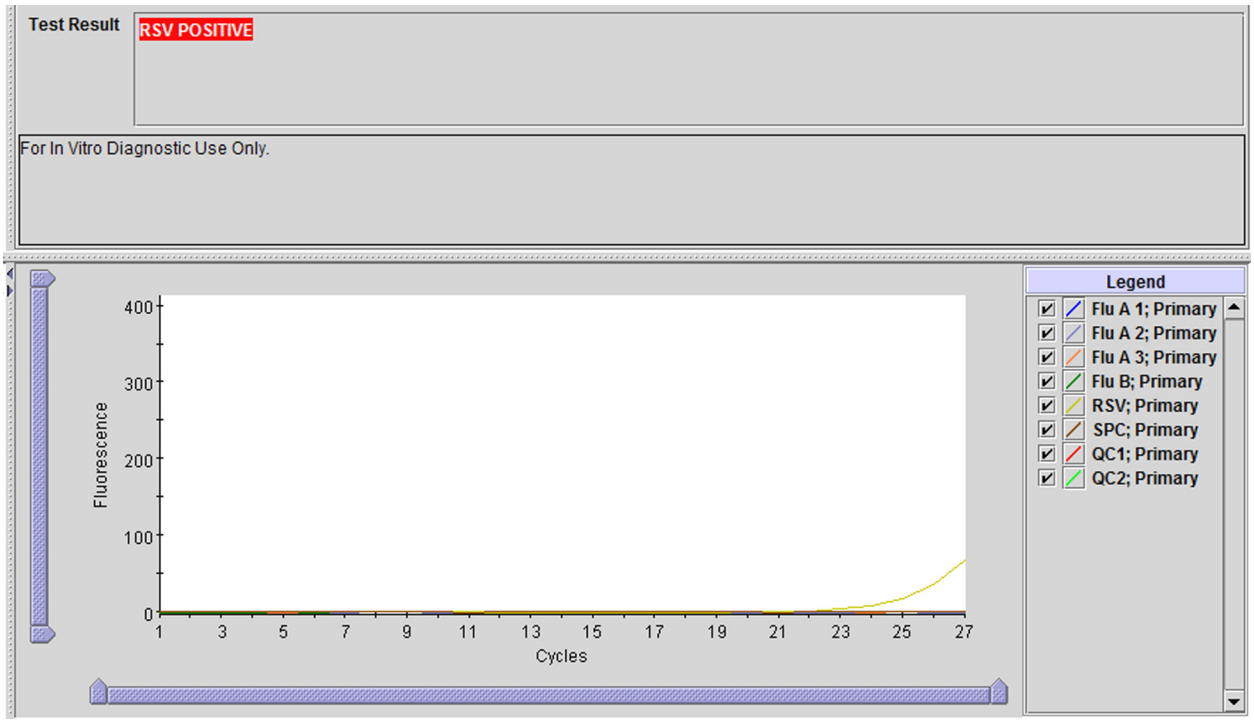


**Figure 17. Xpert RSV: An Example of a Positive Result for RSV**

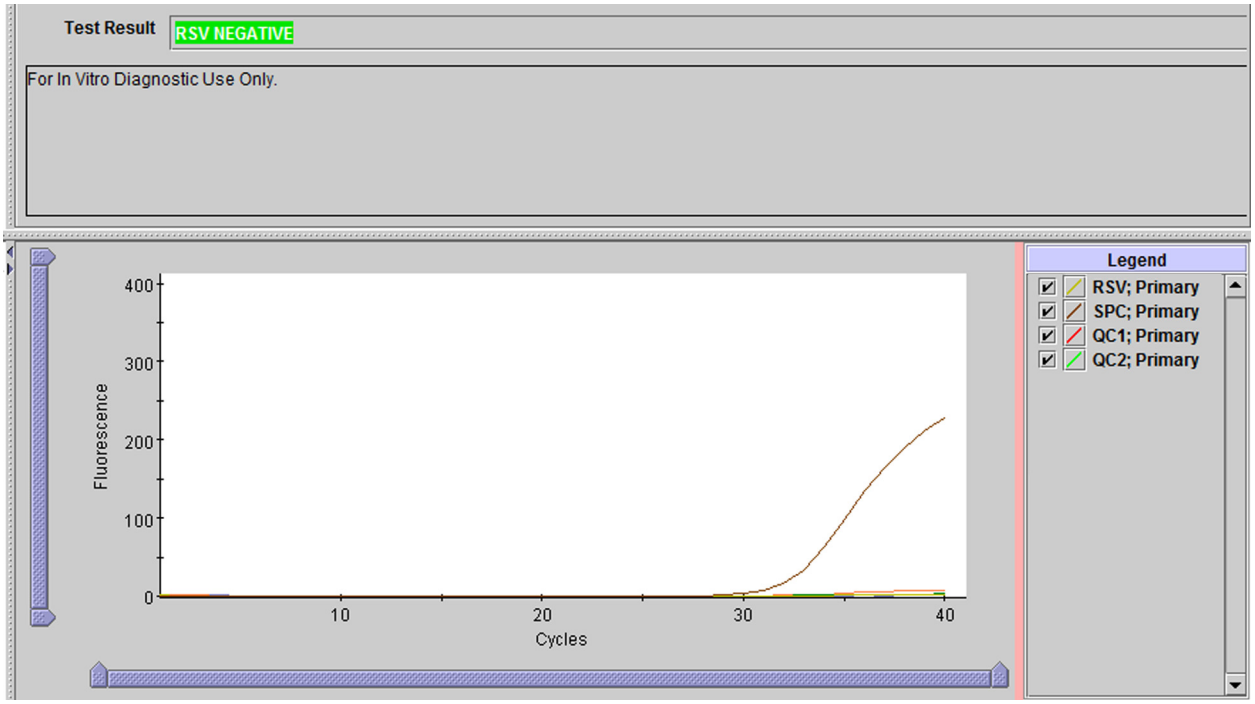


**Figure 18. Xpert RSV: An Example of a Negative Result for RSV**

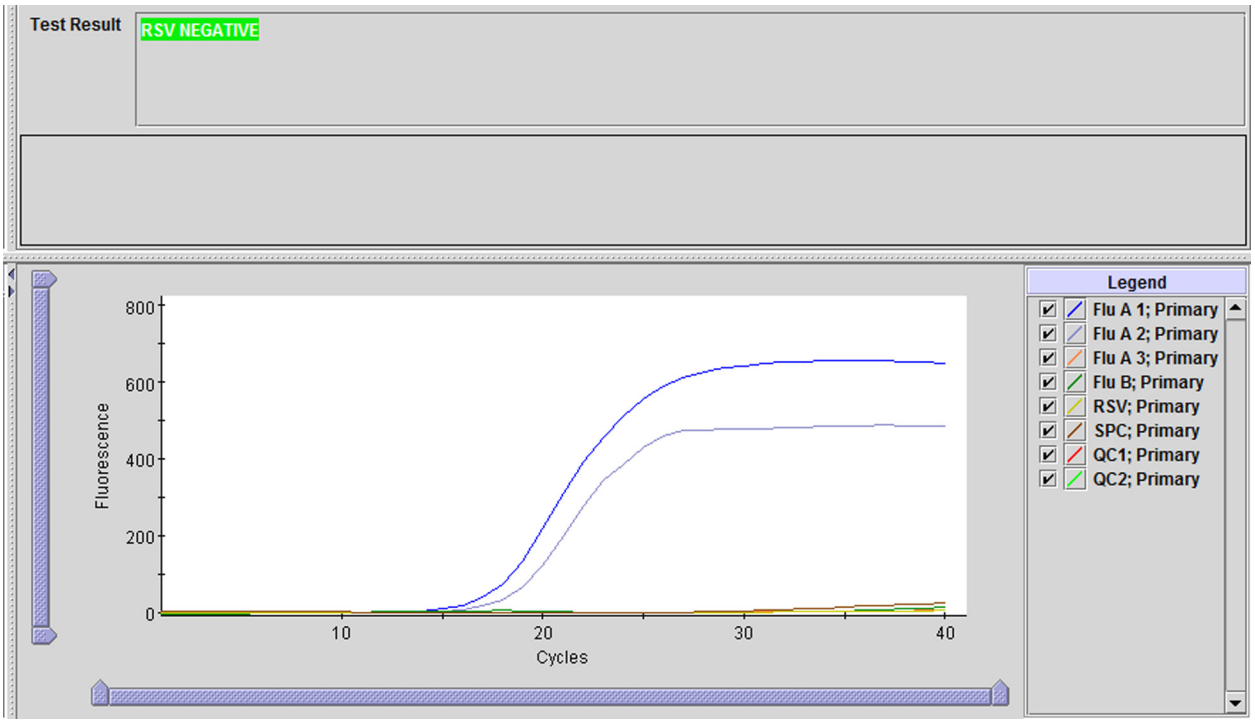


**Figure 19. Xpert RSV: An Example of a Negative Result for RSV (Sample Containing Flu A or Flu B Targets)**

# 17 Retests

## 17.1 Reasons to Repeat the Assay

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

- An **INVALID** result indicates that the control SPC failed or that there was amplification for the Flu A 3 target only. The sample was not properly processed, PCR is inhibited or the sample was not properly collected.
- An **ERROR** result could be due to, but not limited to, Probe Check Control failed or the maximum pressure limits were exceeded.
- A **NO RESULT** indicates that insufficient data were collected. For example, the operator stopped a test that was in progress, or a power failure occurred.

## 17.2 Retest Procedure

For retest of an indeterminate result, use a new cartridge (do not re-use the cartridge).

For NP swab specimens, use 300 µL of the leftover specimen from original transport medium tube.

For NA/W specimens, use 300 µL of the leftover diluted specimen from the 3 mL transport medium tube.

1. Remove a new cartridge from the kit.
2. Mix the specimen by inverting the Xpert Viral Transport Medium tube five times.
3. Open the cartridge lid. Use a clean 300 µL transfer pipette (supplied) to transfer 300 µL (one draw) of the specimen from the transport medium tube to the sample chamber with large opening in the cartridge (Figure 1).
4. Close the cartridge lid.
5. Follow the procedure in Section 13.2, Starting the Test.

# 18 Limitations

- The performance of the Xpert Flu/RSV XC 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 Flu/RSV XC 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.
- Negative results do not preclude influenza virus or RSV infection and should not be used as the sole basis for treatment or other patient management decisions.
- Results from the Xpert Flu/RSV XC 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 in the target region, influenza virus and RSV 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 2013–2014 influenza season. The performance may vary depending on the prevalence 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 or RSV 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 dual positive results.
- Remel M4 and Remel M4RT transport media are not recommended for use with the Xpert Flu/RSV XC Assay.

# 19 Performance Characteristics

## 19.1 Clinical Performance

Performance characteristics of the Xpert Flu/RSV XC Assay were evaluated at six institutions in the U.S. Due to the low prevalence of influenza viruses and the difficulty in obtaining fresh influenza and RSV-positive specimens, the specimen population for this study was supplemented with frozen archived specimens.

Subjects included individuals with signs and symptoms of respiratory infection and whose routine care called for collection of nasal aspirate/wash (NA/W) specimens or nasopharyngeal (NP) swab specimens for influenza and/or RSV testing. For eligible subjects, aliquots of leftover specimens were obtained for testing with the Xpert Flu/RSV XC Assay and reference testing, and patient management continued at the site per their standard practice.

The Xpert Flu/RSV XC Assay performance was compared to a FDA-cleared comparator assay. Bi-directional sequencing was performed on specimens where the Xpert Flu/RSV XC Assay and the comparator assay were discrepant, and is provided for informational purposes only.

## 19.2 Overall Results

### NA/W Specimens

A total of 657 NA/W specimens were tested for influenza A, influenza B and RSV by the Xpert Flu/RSV XC Assay and the reference assay. Of the 657 NA/W specimens, 581 were fresh, prospectively collected and 76 were frozen, archived specimens.

Overall, with NA/W specimens the Xpert Flu/RSV XC Assay demonstrated positive percent agreement (PPA), negative percent agreement (NPA), and overall percent agreement (OPA) for detection of influenza A of 98.6%, 100% and 99.8%, respectively relative to the reference assay (Table 4). The Xpert Flu/RSV XC Assay PPA, NPA, and OPA for influenza B were 99.2%, 100%, and 99.8%, respectively (Table 4). The Xpert Flu/RSV XC Assay PPA, NPA, and OPA for RSV were 97.2%, 99.6%, and 99.1%, respectively (Table 4).

On fresh, prospectively collected NA/W specimens, the Xpert Flu/RSV XC Assay demonstrated a PPA, NPA, and OPA for detection of influenza A of 100%, 100%, and 100% respectively, relative to the reference assay (Table 4). The Xpert Flu/RSV XC Assay PPA, NPA, and OPA for influenza B were 99.2%, 100%, and 99.8%, respectively (Table 4). The Xpert Flu/RSV XC Assay PPA, NPA, and OPA for RSV were 98.5%, 99.6%, and 99.3%, respectively (Table 4).

On frozen, archived NA/W specimens, the Xpert Flu/RSV XC Assay demonstrated a PPA, NPA, and OPA for detection of influenza A of 97.1%, 100%, and 98.7%, respectively, relative to the reference assay (Table 4). The Xpert Flu/RSV XC Assay PPA, NPA, and OPA for influenza B were 100%, 100%, and 100%, respectively (Table 4). The Xpert Flu/RSV XC Assay PPA, NPA, and OPA for RSV were 84.6%, 100%, and 97.4%, respectively (Table 4).

**Table 4. Xpert Flu/RSV XC Assay Performance on NA/W Specimens**

| Specimen Type | Target | n | TP | FP | TN | FN | PPA % (95 CI) | NPA % (95 CI) | OPA % (95 CI) |
|---|---|---|---|---|---|---|---|---|---|
| Fresh | Flu A | 581 | 35 | 0 | 546 | 0 | 100 (90.0–100) | 100 (99.3–100) | 100 (99.4–100) |
| | Flu B | 581 | 126 | 0 | 454 | 1[a] | 99.2 (95.7–100) | 100 (99.2–100) | 99.8 (99.0–100) |
| | RSV | 581 | 128 | 2[b] | 449 | 2[c] | 98.5 (94.6–99.8) | 99.6 (98.4–99.9) | 99.3 (98.2–99.8) |
| Frozen | Flu A | 76 | 34 | 0 | 41 | 1[d] | 97.1 (85.1–99.9) | 100 (91.4–100) | 98.7 (92.9–100) |
| | Flu B | 76 | 1 | 0 | 75 | 0 | 100 (2.5–100) | 100 (95.2–100) | 100 (95.3–100) |
| | RSV | 76 | 11 | 0 | 63 | 2[e] | 84.6 (54.6–98.1) | 100 (94.3–100) | 97.4 (90.8–99.7) |
| All NA/W Specimens | Flu A | 657 | 69 | 0 | 587 | 1[f] | 98.6 (92.3–100) | 100 (99.4–100) | 99.8 (99.2–100) |
| | Flu B | 657 | 127 | 0 | 529 | 1[g] | 99.2 (95.7–100) | 100 (99.3–100) | 99.8 (99.2–100) |
| | RSV | 657 | 139 | 2[h] | 512 | 4[i] | 97.2 (93.0–99.2) | 99.6 (98.6–100) | 99.1 (98.0–99.7) |

a Testing results by sequencing: NA; sample not sequenced.
b Testing results by sequencing: 2 of 2 were RSV Positive.
c Testing results by sequencing: 1 of 2 was RSV Positive; 1 of 2 was RSV Negative.
d Testing results by sequencing: 1 of 1 was Flu A Negative.
e Testing results by sequencing: 1 of 2 was RSV Positive; 1 of 2 was RSV Negative.
f Testing results by sequencing: 1 of 1 was Flu A Negative.
g Testing results by sequencing: NA; sample not sequenced.
h Testing results by sequencing: 2 of 2 were RSV Positive.
i Testing results by sequencing: 2 of 4 were RSV Positive; 2 of 4 were RSV Negative.

### NP Swab Specimens

A total of 593 NP swab specimens were tested for influenza A, influenza B and RSV by the Xpert Flu/RSV XC Assay and the reference assay. Of the 593 NP swab specimens, 190 were fresh, prospectively collected and 403 were frozen, archived specimens.

Overall, with NP swab specimens the Xpert Flu/RSV XC Assay demonstrated a PPA, NPA and OPA for detection of influenza A of 98.1%, 95.1%, and 95.6%, respectively, relative to the reference assay (Table 5). The Xpert Flu/RSV XC Assay PPA, NPA, and OPA for influenza B were 98.9%, 100%, and 99.8%, respectively (Table 5). The Xpert Flu/RSV XC Assay PPA, NPA, and OPA for RSV were 91.9%, 99.4%, and 98.7%, respectively (Table 5).

On fresh, prospectively collected NP swab specimens, the Xpert Flu/RSV XC Assay demonstrated a PPA, NPA, and OPA for detection of influenza A of 85.7%, 98.9%, and 98.4%, respectively, relative to the reference assay (Table 5). The Xpert Flu/RSV XC Assay PPA, NPA, and OPA for influenza B were 100%, 100%, and 100%, respectively (Table 5). The Xpert Flu/RSV XC Assay PPA, NPA, and OPA for RSV were 100%, 100%, and 100%, respectively (Table 5).

On frozen, archived NP swab specimens, the Xpert Flu/RSV XC Assay demonstrated a PPA, NPA, and OPA for detection of influenza A of 99.0%, 92.8%, and 94.3%, respectively, relative to the reference assay (Table 5). The Xpert Flu/RSV XC Assay PPA, NPA, and OPA for influenza B were 98.8%, 100%, and 99.8%, respectively (Table 5). The Xpert Flu/RSV XC Assay PPA, NPA, and OPA for RSV were 90.4%, 99.1%, and 98.0%, respectively (Table 5).

**Table 5. Xpert Flu/RSV XC Assay Performance on NP Swab Specimens**

| Specimen Type | Target | n | TP | FP | TN | FN | PPA % (95 CI) | NPA % (95 CI) | OPA % (95 CI) |
|---|---|---|---|---|---|---|---|---|---|
| Fresh | Flu A | 190 | 6 | 2[a] | 181 | 1[b] | 85.7 (42.1–99.6) | 98.9 (96.1–99.9) | 98.4 (95.5–99.7) |
| | Flu B | 190 | 3 | 0 | 187 | 0 | 100 (29.2–100) | 100 (98.0–100) | 100 (98.1–100) |
| | RSV | 190 | 10 | 0 | 180 | 0 | 100 (69.2–100) | 100 (98.0–100) | 100 (98.1–100) |
| Frozen | Flu A | 403 | 96 | 22[c] | 284 | 1[d] | 99.0 (94.4–100) | 92.8 (89.3–95.4) | 94.3 (91.6–96.3) |
| | Flu B | 403 | 85 | 0 | 317 | 1[e] | 98.8 (93.7–100) | 100 (98.8–100) | 99.8 (98.6–100) |
| | RSV | 403 | 47 | 3[f] | 348 | 5[g] | 90.4 (79.0–96.8) | 99.1 (97.5–99.8) | 98.0 (96.1–99.1) |
| All NP Swabs | Flu A | 593 | 102 | 24[h] | 465 | 2[i] | 98.1 (93.2–99.8) | 95.1 (92.8–96.8) | 95.6 (93.6–97.1) |
| | Flu B | 593 | 88 | 0 | 504 | 1[j] | 98.9 (93.9–100) | 100 (99.3–100) | 99.8 (99.1–100) |
| | RSV | 593 | 57 | 3[k] | 528 | 5[l] | 91.9 (82.2–97.3) | 99.4 (98.4–99.9) | 98.7 (97.4–99.4) |

a Testing results by sequencing: 2 of 2 were Flu A Positive.
b Testing results by sequencing: 1 of 1 was Flu A Negative.
c Testing results by sequencing: 17 of 22 were Flu A Positive; 5 of 22 were Flu A Negative.
d Testing results by sequencing: 1 of 1 was Flu A Negative.
e Testing results by sequencing: 1 of 1 was Flu B Negative.
f Testing results by sequencing: 2 of 3 were RSV Positive; 1 of 3 was RSV Negative.
g Testing results by sequencing: 1 of 5 was RSV Positive; 4 of 5 were RSV Negative.
h Testing results by sequencing: 19 of 24 were Flu A Positive; 5 of 24 were Flu A Negative.
i Testing results by sequencing: 2 of 2 were Flu A Negative.
j Testing results by sequencing: 1 of 1 was Flu B Negative.
k Testing results by sequencing: 2 of 3 were RSV Positive; 1 of 3 was RSV Negative.
l Testing results by sequencing: 1 of 5 was RSV Positive; 4 of 5 were RSV Negative.

Of the Xpert Flu/RSV XC Assay runs performed with eligible specimens, 98.6% (1236/1254) of these specimens were successful on the first attempt. The remaining 18 gave indeterminate results on the first attempt (11 **ERROR**, 3 **INVALID** and 4 **NO RESULT**). Seventeen of the 18 specimens were retested, of which 14 yielded valid results after a single retest. There were four NA/W specimens with indeterminate results upon retest which were excluded in the analyses.

# 20 Analytical Performance

## 20.1 Analytical Sensitivity (Limit of Detection)

Studies were performed to determine the analytical limit of detection (LoD) of the Xpert Flu/RSV XC 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, two respiratory syncytial virus B (RSV B) strains, and one influenza A H7N9 strain diluted into a negative pooled clinical matrix. 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.

The LoD was determined empirically as the first concentration that had 19/20 or 20/20 positive results. The LoD point values for each strain tested are summarized in Table 6 to Table 11.

**Table 6. Confirmed LoD ($TCID_{50}$/mL): Influenza A 2009 H1N1**

| Strain ID | Confirmed LoD ($TCID_{50}$/mL) (at least 19/20 positive) |
|---|---|
| Influenza A/California/7/2009 | 0.3 (20/20) |
| Influenza A/Florida/27/2011 | 16 (19/20) |

**Table 7. Confirmed LoD ($TCID_{50}$/mL): Influenza A H3N2**

| Strain ID | Confirmed LoD ($TCID_{50}$/mL) (at least 19/20 positive) |
|---|---|
| Influenza A/Perth/16/2009 | 0.3 (20/20) |
| Influenza A/Victoria/361/2011 | 0.8 (20/20) |

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

| Strain ID | Confirmed LoD ($TCID_{50}$/mL) (at least 19/20 positive) |
|---|---|
| Influenza B/Massachusetts/2/2012 | 0.5(20/20) |
| Influenza B/Wisconsin/01/2010 | 0.6 (20/20) |

**Table 9. Confirmed LoD ($TCID_{50}$/mL): Respiratory Syncytial Virus A**

| Strain ID | Confirmed LoD ($TCID_{50}$/mL) (at least 19/20 positive) |
|---|---|
| RSV A/2/Australia/61 | 1.2 (20/20) |
| RSV A/Long/MD/56 | 1.0 (19/20) |

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

| Strain ID | Confirmed LoD ($TCID_{50}$/mL) (at least 19/20 positive) |
|---|---|
| RSV B/Washington/18537/62 | 1.8 (20/20) |
| RSV B/9320/Massachusetts/77 | 2.0 (19/20) |

**Table 11. Confirmed LoD ($TCID_{50}$/mL): Influenza A H7N9**

| Strain ID | Confirmed LoD ($TCID_{50}$/mL) (at least 19/20 positive) |
|---|---|
| Influenza A/Anhui/1/2013 | 21.0 (19/20) |

Although this test has been shown to detect the novel avian influenza A(H7N9) cultured material, the performance characteristics of this device with clinical specimens that are positive for the novel avian influenza A(H7N9) virus have not been established. The Xpert Flu/RSV XC Assay can distinguish between influenza A and B viruses, but it cannot differentiate influenza subtypes.

### 20.2 Analytical Specificity (Exclusivity)

The analytical specificity of the Xpert Flu/RSV XC 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 nasopharynx. Three replicates of all bacterial and yeast strains were tested at concentrations of $\geq 10^6$ CFU/mL with the exception of one strain which was tested at $10^5$ CFU/mL (*Chlamydia pneumoniae*). Three replicates of all viruses were tested at concentrations of $\geq 10^5$ $TCID_{50}$/mL. The analytical specificity was 100%. Results are shown in Table 12.

**Table 12. Analytical Specificity of Xpert Flu/RSV XC Assay**

| Organism | Concentration | Result | | |
|---|---|---|---|---|
| | | Flu A | Flu B | RSV |
| *No Template Control* | N/A | NEG | NEG | NEG |
| Adenovirus Type 1 | $1.12 \times 10^7$ $TCID_{50}$/mL | NEG | NEG | NEG |
| Adenovirus Type 7 | $1.87 \times 10^5$ $TCID_{50}$/mL | NEG | NEG | NEG |
| Human coronavirus OC43 | $2.85 \times 10^5$ $TCID_{50}$/mL | NEG | NEG | NEG |
| Human coronavirus 229E | $1 \times 10^5$ $TCID_{50}$/mL | NEG | NEG | NEG |
| Cytomegalovirus | $7.24 \times 10^5$ $TCID_{50}$/mL | NEG | NEG | NEG |
| Echovirus | $3.31 \times 10^7$ $TCID_{50}$/mL | NEG | NEG | NEG |
| Enterovirus | $1 \times 10^5$ $TCID_{50}$/mL | NEG | NEG | NEG |
| Epstein Barr Virus | $7.16 \times 10^7$ $TCID_{50}$/mL | NEG | NEG | NEG |
| HSV | $8.9 \times 10^6$ $TCID_{50}$/mL | NEG | NEG | NEG |
| Measles | $6.3 \times 10^5$ $TCID_{50}$/mL | NEG | NEG | NEG |
| Human metapneumovirus | $3.8 \times 10^5$ $TCID_{50}$/mL | NEG | NEG | NEG |
| Mumps virus | $6.31 \times 10^6$ $TCID_{50}$/mL | NEG | NEG | NEG |
| Human parainfluenza Type 1 | $1.15 \times 10^6$ $TCID_{50}$/mL | NEG | NEG | NEG |
| Human parainfluenza Type 2 | $1 \times 10^5$ $TCID_{50}$/mL | NEG | NEG | NEG |
| Human parainfluenza Type 3 | $3.55 \times 10^7$ $TCID_{50}$/mL | NEG | NEG | NEG |
| Rhinovirus Type 1A | $1.26 \times 10^5$ $TCID_{50}$/mL | NEG | NEG | NEG |

**Table 12. Analytical Specificity of Xpert Flu/RSV XC Assay (Continued)**

| Organism | Concentration | Result | | |
|---|---|---|---|---|
| | | Flu A | Flu B | RSV |
| *Acinetobacter baumannii* | > $1 \times 10^6$ CFU/mL | NEG[a] | NEG | NEG |
| *Burkholderia cepacia* | > $1 \times 10^6$ CFU/mL | NEG | NEG | NEG |
| *Candida albicans* | > $1 \times 10^6$ CFU/mL | NEG | NEG | NEG |
| *Candida parapsilosis* | > $1 \times 10^6$ CFU/mL | NEG | NEG | NEG |
| *Bordetella pertussis* | $1 \times 10^8$ CFU/mL | NEG | NEG | NEG |
| *Chlamydia pneumoniae* | $3.16 \times 10^5$ CFU/mL | NEG | NEG | NEG |
| *Citrobacter freundii* | > $1 \times 10^6$ CFU/mL | NEG | NEG | NEG |
| *Corynebacterium sp.* | > $1 \times 10^6$ CFU/mL | NEG | NEG | NEG |
| *Escherichia coli* | > $1 \times 10^6$ CFU/mL | NEG | NEG | NEG |
| *Streptococcus faecalis* | > $1 \times 10^6$ CFU/mL | NEG | NEG | NEG |
| *Hemophilus influenzae* | $1 \times 10^6$ CFU/mL | NEG | NEG | NEG |
| *Lactobacillus sp.* | $1 \times 10^6$ CFU/mL | NEG | NEG | NEG |
| *Legionella spp.* | $1 \times 10^8$ CFU/mL | NEG | NEG | NEG |
| *Moraxella catarrhalis* | > $1 \times 10^6$ CFU/mL | NEG | NEG | NEG |
| *Mycobacterium tuberculosis (avirulent)* | $1.15 \times 10^6$ CFU/mL | NEG | NEG | NEG |
| *Mycoplasma pneumoniae* | $1 \times 10^7$ CFU/mL | NEG | NEG | NEG |
| *Neisseria meningitidis* | > $1 \times 10^6$ CFU/mL | NEG | NEG | NEG |
| *Neisseria mucosa* | > $1 \times 10^6$ CFU/mL | NEG | NEG | NEG |
| *Propionibacterium acnes* | > $1 \times 10^6$ CFU/mL | NEG | NEG | NEG |
| *Pseudomonas aeruginosa* | > $1 \times 10^6$ CFU/mL | NEG | NEG | NEG |
| *Staphylococcus aureus* | > $1 \times 10^6$ CFU/mL | NEG | NEG | NEG |
| *Staphylococcus epidermidis* | > $1 \times 10^6$ CFU/mL | NEG | NEG | NEG |
| *Staphylococcus haemolyticus* | > $1 \times 10^6$ CFU/mL | NEG | NEG | NEG |
| *Streptococcus agalactiae* | > $1 \times 10^6$ CFU/mL | NEG | NEG | NEG |
| *Streptococcus pneumoniae* | > $1 \times 10^6$ CFU/mL | NEG | NEG | NEG |
| *Streptococcus pyogenes* | > $1 \times 10^6$ CFU/mL | NEG | NEG | NEG |
| *Streptococcus salivarius* | > $1 \times 10^6$ CFU/mL | NEG | NEG | NEG |
| *Streptococcus sanguinis* | > $1 \times 10^6$ CFU/mL | NEG | NEG | NEG |

a For *Acinetobacter baumannii* upon initial testing 1 of 3 replicates was positive for Flu A with a Ct of 39.2 (cut-off = 40). An additional 23 replicates were tested at > $1 \times 10^6$ CFU/mL; 23 of 23 replicates were correctly reported as **Flu A NEGATIVE; Flu B NEGATIVE; RSV NEGATIVE**.

### 20.3 Analytical Reactivity (Inclusivity)

The analytical reactivity of the Xpert Flu/RSV XC 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 64 strains including 54 influenza viruses and 10 RSV strains were tested in this study with the Xpert Flu/RSV XC Assay.

Three replicates were tested for each strain. Results are shown in Table 13.

**Table 13. Analytical Reactivity (Inclusivity) of Xpert Flu/RSV XC Assay**

| Virus | Strain | 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 | 32.0 $TCID_{50}$/mL | POS | NEG | NEG |
| | A/WS/33 | 32.0 $TCID_{50}$/mL | POS | NEG | NEG |
| | A/PR/8/34 | 32.0 $TCID_{50}$/mL | POS | NEG | NEG |
| | A/Mal/302/54 | 32.0 $TCID_{50}$/mL | POS | NEG | NEG |
| | A/Denver/1/57 | 32.0 $TCID_{50}$/mL | POS | NEG | NEG |
| | A/New Jersey/8/76 | 32.0 $TCID_{50}$/mL | POS | NEG | NEG |
| | A/New Caledonia/20/1999 | 32.0 $TCID_{50}$/mL | POS | NEG | NEG |
| | A/New York/55/2004 | 32.0 $TCID_{50}$/mL | POS | NEG | NEG |
| | A/Solomon Island/3/2006 | 32.0 $TCID_{50}$/mL | POS | NEG | NEG |
| | A/Taiwan/42/06 | 32.0 $TCID_{50}$/mL | POS | NEG | NEG |
| | A/Brisbane/59/2007 | 32.0 $TCID_{50}$/mL | POS | NEG | NEG |
| Influenza A H1N1 (pdm2009) | A/California/7/2009 | 32.0 $TCID_{50}$/mL | POS | NEG | NEG |
| | A/swine/NY/02/2009 | 32.0 $TCID_{50}$/mL | POS | NEG | NEG |
| | A/Florida/27/2011 | 32.0 $TCID_{50}$/mL | POS | NEG | NEG |
| | A/Colorado/14/2012 | 32.0 $TCID_{50}$/mL | POS | NEG | NEG |
| | A/Washington/24/2012 | 80.0[a] $TCID_{50}$/mL | POS | NEG | NEG |
| Influenza A H3N2 (Seasonal) | A/Aichi/2/68 | 1.6 $TCID_{50}$/mL | POS | NEG | NEG |
| | A/Hong Kong/8/68 | 1.6 $TCID_{50}$/mL | POS | NEG | NEG |
| | A/Port Chalmers/1/73 | 1.6 $TCID_{50}$/mL | POS | NEG | NEG |
| | A/Hawaii/15/2001 | 1.6 $TCID_{50}$/mL | POS | NEG | NEG |
| | A/Wisconsin/67/05 | 1.6 $TCID_{50}$/mL | POS | NEG | NEG |
| | A/Brisbane/10/2007 | 1.6 $TCID_{50}$/mL | POS | NEG | NEG |
| | A/Perth/16/2009 | 1.6 $TCID_{50}$/mL | POS | NEG | NEG |
| | A/Minnesota/11/2010 (H3N2)v | 1.6 $TCID_{50}$/mL | POS | NEG | NEG |
| | A/Indiana/08/2011 (H3N2)v | 1.6 $TCID_{50}$/mL | POS | NEG | NEG |
| | A/Victoria/361/2011 | 1.6 $TCID_{50}$/mL | POS | NEG | NEG |
| | A/Texas/50/2012 | 1.6 $TCID_{50}$/mL | POS | NEG | NEG |

**Table 13. Analytical Reactivity (Inclusivity) of Xpert Flu/RSV XC Assay (Continued)**

| Virus | Strain | Concentration | Result | | |
|---|---|---|---|---|---|
| | | | Flu A | Flu B | RSV |
| Avian influenza A | A/duck/Hunan/795/2002 (H5N1) | ≤ 1pg/µL[b] | POS | NEG | NEG |
| | A/chicken/Hubei/327/2004 (H5N1) | ≤ 1pg/µL[b] | POS | NEG | NEG |
| | A/Anhui/01/2005 (H5N1) | ≤ 1pg/µL[b] | POS | NEG | NEG |
| | A/Japanese white eye/Hong Kong/ 1038/2006 (H5N1) | ≤ 1pg/µL[b] | POS | NEG | NEG |
| | A/mallard/WI/34/75 (H5N2) | ≤ 1pg/µL[b] | POS | NEG | NEG |
| | A/chicken/CA431/00 (H6N2) | ≤ 1pg/µL[b] | POS | NEG | NEG |
| | A/duck/LTC-10-82743/1943 (H7N2) | ≤ 1pg/µL[b] | POS | NEG | NEG |
| | A/chicken/NJ/15086-3/94 (H7N3) | ≤ 1pg/µL[b] | POS | NEG | NEG |
| | A/Anhui/1/2013 (H7N9) | N/A[c] | POS | NEG | NEG |
| | A/Shanghai/1/2013 (H7N9) | N/A[c] | POS | NEG | NEG |
| | A/chicken/Korea/38349-p96323/ 1996 (H9N2) | ≤ 1pg/µL[b] | POS | NEG | NEG |
| | A/Mallard/NY/6750/78 (H2N2) | ≤ 1pg/µL[b] | POS | NEG | NEG |
| Influenza B | B/Lee/40 | 1.2 $TCID_{50}$/mL | NEG | POS | NEG |
| | B/Allen/45 | 1.2 $TCID_{50}$/mL | NEG | POS | NEG |
| | B/GL/1739/54 | 1.2 $TCID_{50}$/mL | NEG | POS | NEG |
| | B/Maryland/1/59 | 1.2 $TCID_{50}$/mL | NEG | POS | NEG |
| | B/Panama/45/90[d] | 3.0 $TCID_{50}$/mL[e] | NEG | POS | NEG |
| | B/Florida/07/2004[f] | 1.2 $TCID_{50}$/mL | NEG | POS | NEG |
| | B/Florida/02/06[d] | 1.2 $TCID_{50}$/mL | NEG | POS | NEG |
| | B/Florida/04/06[f] | 1.2 $TCID_{50}$/mL | NEG | POS | NEG |
| | B/Wisconsin/01/2011[d] | 1.2 $TCID_{50}$/mL | NEG | POS | NEG |
| | B/Massachusetts/2/2012[f] | 1.2 $TCID_{50}$/mL | NEG | POS | NEG |
| | B/Hong Kong/5/72 | 1.2 $TCID_{50}$/mL | NEG | POS | NEG |
| | B/Wisconsin/01/2010[f] | 1.2 $TCID_{50}$/mL | NEG | POS | NEG |
| | B/Malaysia/2506/04[d] | 1.2 $TCID_{50}$/mL | NEG | POS | NEG |
| | B/Taiwan/2/62 | 1.2 $TCID_{50}$/mL | NEG | POS | NEG |
| | B/Brisbane/60/2008[d] | 1.2 $TCID_{50}$/mL | NEG | POS | NEG |
| RSV A | RSV-A/Long/MD/56 | 2.4 $TCID_{50}$/mL | NEG | NEG | POS |
| | RSV-A/2/Australia/61 | 2.4 $TCID_{50}$/mL | NEG | NEG | POS |
| | RSV-A/NY (Clinical unknown) | 2.4 $TCID_{50}$/mL | NEG | NEG | POS |
| | RSV-A/WI/629-8-2/2007 | 2.4 $TCID_{50}$/mL | NEG | NEG | POS |
| | RSV-A/WI/629-11-1/2008 | 2.4 $TCID_{50}$/mL | NEG | NEG | POS |

**Table 13. Analytical Reactivity (Inclusivity) of Xpert Flu/RSV XC Assay (Continued)**

| Virus | Strain | Concentration | Result | | |
|---|---|---|---|---|---|
| | | | Flu A | Flu B | RSV |
| RSV B | RSV-B/Wash/18537/62 | 4.0 $TCID_{50}$/mL | NEG | NEG | POS |
| | RSV-B/9320/MA/77 | 4.0 $TCID_{50}$/mL | NEG | NEG | POS |
| | RSV-B/WV14617/85 | 4.0 $TCID_{50}$/mL | NEG | NEG | POS |
| | RSV-B/CH93(18)-18 | 20.0 $TCID_{50}$/mL[g] | NEG | NEG | POS |
| | RSV-B/WI/629-5B/0607 | 4.0 $TCID_{50}$/mL | NEG | NEG | POS |

a Influenza A/Washington/24/2012 was tested at 5X LoD (80.0 $TCID_{50}$/mL) to obtain 3 of 3 **Flu A POSITIVE** result calls.
b Purified viral RNA in simulated background matrix was used for avian influenza A viruses due to biosafety regulations.
c Inactivated avian influenza A (H7N9) viruses without viral titer was diluted 100,000 fold in simulated background matrix and tested due to biosafety regulations.
d Known Victoria lineage.
e Influenza B/Panama/45/90 was tested at 5X LoD (3.0 $TCID_{50}$/mL) to obtain 3 of 3 **Flu B POSITIVE** result calls.
f Known Yamagata lineage.
g RSV-B/CH93(18)-18 was tested at 10X LoD (20.0 $TCID_{50}$/mL) to obtain 3 of 3 **RSV POSITIVE** result calls.

### 20.4 Interfering Substances Study

In a non-clinical study, potentially interfering substances that may be present in the nasopharynx were evaluated directly relative to the performance of the Xpert Flu/RSV XC Assay. Potentially interfering substances in the 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 2X the analytical LoD determined for each strain. All results were compared to positive and negative Universal Transport Medium (UTM) controls.

These evaluated substances are listed in Table 14 with active ingredients and concentrations tested shown. There was no assay interference in the presence of the substances at the concentrations tested in this study. All positive and negative replicates were correctly identified using the Xpert Flu/RSV XC Assay.

FluMist vaccine samples were correctly reported as **Flu A POSITIVE**; **FLU B POSITIVE**; **RSV NEGATIVE** as expected. Samples containing FluMist may cause false positive results. This is addressed in Section 18, Limitations.

**Table 14. Potentially Interfering Substances in Xpert Flu/RSV XC Assay**

| Substance/Class | Description/Active Ingredient | Concentration Tested |
|---|---|---|
| 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) |
| 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) |
| 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) |
| FluMist® | Live intranasal influenza virus vaccine | 6.7% (v/v) |
| Nasal corticosteroid | Fluticasone Propionate | 5 µg/mL |

### 20.5 Carry-over Contamination Study

A study was conducted to demonstrate that single-use, self-contained GeneXpert cartridges prevent carry-over contamination in negative samples run followed by very high positive samples in the same GeneXpert module. The study consisted of a negative sample processed in the same GeneXpert module immediately followed by a very high influenza A sample (approximately $10^6$ $TCID_{50}$/test) or a very high RSV A sample (approximately $10^6$ $TCID_{50}$/test). 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 for each virus type. 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**.

### 20.6 Fresh vs Frozen Sample Equivalency Study

Fresh and frozen specimen equivalency in the Xpert Flu/RSV XC Assay was evaluated by testing individual influenza and RSV strains at three different concentrations representing low positives (2X LoD), moderate positives (5X LoD), and high positives (10X LoD) in simulated background matrix. Negative samples consisted of simulated background matrix only. Fresh and frozen specimen equivalency was determined using one seasonal Flu A H3N2 strain (A/Victoria/361/2011), one Flu B strain (B/Wisconsin/01/11), one RSV A strain (RSV A/Long/MD/56), and one RSV B strain (RSV B/9320/MA/77). Replicates of 20 were tested for each specimen type and concentration. All positive and negative specimens were tested fresh, after one freeze-thaw cycle, and after two freeze-thaw cycles.

There was no statistically significant effect in the performance of the Xpert Flu/RSV XC Assay between fresh virus dilutions and two sequential freeze thaw cycles for positive and negative samples. All positive and negative replicates were correctly identified using the Xpert Flu/RSV XC Assay.

## 21 Reproducibility

A panel of 10 specimens with varying concentrations of influenza A, influenza B, and RSV was tested on ten different days by two different operators, at each of three sites (10 specimens × 1 time/day × 10 days × 2 operators × 3 sites). One lot of Xpert Flu/RSV XC Assay cartridges was used at each of the 3 testing sites. The Xpert Flu/RSV XC Assay was performed according to the Xpert Flu/RSV XC Assay procedure. Results are summarized in Table 15.

**Table 15. Summary of Reproducibility Results**

| Sample ID | Site 1/GX Dx | | | Site 2/Infinity-80 | | | Site 3/Infinity-48 | | | % Total Agreement by Sample |
|---|---|---|---|---|---|---|---|---|---|---|
| | Op 1 | Op 2 | Site | Op 1 | Op 2 | Site | Op 1 | Op 2 | Site | |
| **Negative** | 100% (10/10) | 100% (10/10) | 100% (20/20) | 100% (10/10) | 100% (10/10) | 100% (20/20) | 100% (10/10) | 100% (10/10) | 100% (20/20) | 100% (60/60) |
| **Flu A - High Neg** | 70.0% (7/10) | 60.0% (6/10) | 65.0% (13/20) | 80.0% (8/10) | 80.0% (8/10) | 80.0% (16/20) | 60.0% (6/10) | 70.0% (7/10) | 65.0% (13/20) | 70.0% (42/60) |
| **Flu A - Low Pos** | 100% (10/10) | 90.0% (9/10) | 95.0% (19/20) | 100% (10/10) | 100% (10/10) | 100% (20/20) | 100% (10/10) | 90.0% (9/10) | 95.0% (19/20) | 96.7% (58/60) |
| **Flu A - Mod Pos** | 100% (10/10) | 90.0% (9/10) | 95.0% (19/20) | 100% (10/10) | 100% (10/10) | 100% (20/20) | 100% (10/10) | 100% (10/10) | 100% (20/20) | 98.3% (59/60) |
| **Flu B - High Neg** | 90.0% (9/10) | 70.0% (7/10) | 80.0% (16/20) | 100% (10/10) | 70.0% (7/10) | 85.0% (17/20) | 50.0% (5/10) | 80.0% (8/10) | 65.0% (13/20) | 76.7% (46/60) |
| **Flu B - Low Pos** | 100% (10/10) | 90.0% (9/10) | 95.0% (19/20) | 90.0% (9/10) | 70.0% (7/10) | 80.0% (16/20) | 100% (10/10) | 90.0% (9/10) | 95.0% (19/20) | 90.0% (54/60) |
| **Flu B - Mod Pos** | 100% (10/10) | 100% (10/10) | 100% (20/20) | 100% (10/10) | 100% (10/10) | 100% (20/20) | 100% (10/10) | 100% (10/10) | 100% (20/20) | 100% (60/60) |
| **RSV- High Neg** | 60.0% (6/10) | 50.0% (5/10) | 55.0% (11/20) | 90.0% (9/10) | 60.0% (6/10) | 75.0% (15/20) | 70.0% (7/10) | 70.0% (7/10) | 70.0% (14/20) | 66.7% (40/60) |
| **RSV - Low Pos** | 77.8%[a] (7/9) | 100% (10/10) | 89.5% (17/19) | 80.0% (8/10) | 80.0% (8/10) | 80.0% (16/20) | 90.0% (9/10) | 90.0% (9/10) | 90.0% (18/20) | 86.4% (51/59) |
| **RSV - Mod Pos** | 100%[b] (9/9) | 100% (10/10) | 100% (19/19) | 100% (10/10) | 100% (10/10) | 100% (20/20) | 100% (10/10) | 100% (10/10) | 100% (20/20) | 100% (59/59) |

a One sample indeterminate on initial testing; retest not done.

b One sample 2x indeterminate.

The reproducibility of the Xpert Flu/RSV XC Assay 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 and between-operators for each panel member are presented in Table 16. One replicate was performed per day per operator; therefore, operator and assay (within-run) precision are confounded.

**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 (%) |
| **Negative** | SPC | 60 | 30.8 | 0.06 | 0.2 | 0 | 0 | 0.29 | 0.9 | 0.29 | 0.9 |
| **Flu A - High Neg** | FluA1 | 18 | 38.0 | 0 | 0 | 1.55 | 4.1 | 0.85 | 2.2 | 1.77 | 4.6 |
| | FluA2 | 0 | NA | NA | NA | NA | NA | NA | NA | NA | NA |
| | FluA3 | 0 | NA | NA | NA | NA | NA | NA | NA | NA | NA |
| **Flu A - Low Pos** | FluA1 | 58 | 34.9 | 0.38 | 1.1 | 0.10 | 0.3 | 1.28 | 3.7 | 1.34 | 3.8 |
| | FluA2 | 0 | NA | NA | NA | NA | NA | NA | NA | NA | NA |
| | FluA3 | 0 | NA | NA | NA | NA | NA | NA | NA | NA | NA |
| **Flu A - Mod Pos** | FluA1 | 59 | 33.5 | 0.49 | 1.5 | 0 | 0 | 1.29 | 3.9 | 1.38 | 4.1 |
| | FluA2 | 10 | 36.3 | NA | NA | NA | NA | NA | NA | NA | NA |
| | FluA3 | 0 | NA | NA | NA | NA | NA | NA | NA | NA | NA |
| **Flu B - High Neg** | FluB | 14 | 36.6 | 0.80 | 1.4 | 0 | 0 | 2.83 | 7.7 | 2.94 | 8.0 |
| **Flu B - Low Pos** | FluB | 54 | 33.4 | 0 | 0 | 1.07 | 3.2 | 1.76 | 5.3 | 2.06 | 6.2 |
| **Flu B - Mod Pos** | FluB | 60 | 32.1 | 0 | 0 | 0.38 | 1.2 | 1.47 | 4.6 | 1.51 | 4.7 |
| **RSV - High Neg** | RSV | 20 | 37.4 | 0 | 0 | 0.14 | 0.4 | 1.68 | 4.5 | 1.68 | 4.5 |
| **RSV - Low Pos** | RSV | 51 | 36.2 | 0.22 | 0.6 | 0 | 0 | 1.75 | 4.8 | 1.76 | 4.9 |
| **RSV - Mod Pos** | RSV | 60 | 35.1 | 0 | 0 | 0.24 | 0.9 | 1.20 | 3.4 | 1.24 | 3.5 |

a Results with non-zero Ct values out of 60.

## 22 Instrument System Precision

An in-house precision study was conducted to compare the performance of the GeneXpert Dx and the GeneXpert Infinity instrument systems. A panel of 10 specimens with varying concentrations of influenza A, influenza B, and RSV was tested on 12 different days by two operators. Each operator conducted four runs of each panel specimen per day on each of the two instrument systems (10 specimens × 2 times/ day × 12 days × 2 operators × 2 instrument systems). Three lots of Xpert Flu/RSV XC Assay cartridges were used for the study. The Xpert Flu/RSV XC Assay was performed according to the Xpert Flu/RSV XC Assay procedure. Results are summarized in Table 17.

**Table 17. Summary of Instrument System Precision Results (Dx vs. Infinity)**

| Sample | GeneXpert Dx | | | Infinity | | | % Total Agreement by Sample |
|---|---|---|---|---|---|---|---|
| | Op 1 | Op 2 | Inst | Op 1 | Op 2 | Inst | |
| **Negative** | 100% (48/48) | 100% (48/48) | 100% (96/96) | 100% (48/48) | 100% (48/48) | 100% (96/96) | 100% (192/192) |
| **Flu A - High Neg** | 75.0% (36/48) | 77.1% (37/48) | 76.0% (73/96) | 87.5% (42/48) | 75.0% (36/48) | 81.3% (78/96) | 78.7% (151/192) |
| **Flu A - Low Pos** | 68.8% (33/48) | 97.9% (47/48) | 83.3% (80/96) | 91.7% (44/48) | 93.8% (45/48) | 92.7% (89/96) | 88.0% (169/192) |
| **Flu A - Mod Pos** | 97.9% (47/48) | 100% (48/48) | 99.0% (95/96) | 93.8% (45/48) | 97.9% (47/48) | 95.8% (92/96) | 97.4% (187/192) |
| **Flu B - High Neg** | 81.3% (39/48) | 79.2% (38/48) | 80.2% (77/96) | 89.6% (43/48) | 79.2% (38/48) | 84.4% (81/96) | 82.3% (158/192) |

**Table 17. Summary of Instrument System Precision Results (Dx vs. Infinity) (Continued)**

| Sample | GeneXpert Dx | | | Infinity | | | % Total Agreement by Sample |
|---|---|---|---|---|---|---|---|
| | Op 1 | Op 2 | Inst | Op 1 | Op 2 | Inst | |
| **Flu B - Low Pos** | 89.6% (43/48) | 95.8% (46/48) | 92.7% (89/96) | 89.6% (43/48) | 87.5% (42/48) | 88.5% (85/96) | 90.6% (174/192) |
| **Flu B - Mod Pos** | 97.9% (47/48) | 100% (48/48) | 99.0% (95/96) | 100% (48/48) | 100% (48/48) | 100% (96/96) | 99.5% (191/192) |
| **RSV- High Neg** | 89.6% (43/48) | 77.1% (37/48) | 83.3% (80/96) | 87.5% (42/48) | 83.3% (40/48) | 85.4% (82/96) | 84.4% (162/192) |
| **RSV - Low Pos** | 93.8% (45/48) | 93.8% (45/48) | 93.8% (90/96) | 87.5% (42/48) | 89.6% (43/48) | 88.5% (85/96) | 91.1% (175/192) |
| **RSV - Mod Pos** | 100% (48/48) | 100% (48/48) | 100% (96/96) | 97.9% (47/48) | 100% (48/48) | 99.0% (95/96) | 99.5% (191/192) |

The precision of the Xpert Flu/RSV XC Assay 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-instruments, between-lots, between-days, between-operators, and within-assays for each panel member are presented in Table 18.

**Table 18. Summary of Precision Data**

| Sample | Assay Channel (Analyte) | N[a] | Mean Ct | Between-Instrument | | Between-Lot | | Between-Day | | Between-Operator | | Within-Assay | | Total | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | SD | CV (%) | SD | CV (%) | SD | CV (%) | SD | CV (%) | SD | CV (%) | SD | CV (%) |
| **Negative** | SPC | 192 | 30.6 | 0 | 0 | 0.19 | 0.6 | 0.06 | 0.2 | 0.02 | 0.1 | 0.36 | 1.2 | 0.41 | 1.3 |
| **Flu A - High Neg** | FluA1 | 41 | NA | NA | NA | NA | NA | NA | NA | NA | NA | NA | NA | NA | NA |
| | FluA2 | 0 | NA | NA | NA | NA | NA | NA | NA | NA | NA | NA | NA | NA | NA |
| | FluA3 | 0 | NA | NA | NA | NA | NA | NA | NA | NA | NA | NA | NA | NA | NA |
| **Flu A - Low Pos** | FluA1 | 169 | 35.6 | 0 | 0 | 0.42 | 1.2 | 0.93 | 2.6 | 0.28 | 0.8 | 1.61 | 4.5 | 1.93 | 5.4 |
| | FluA2 | 1 | NA | NA | NA | NA | NA | NA | NA | NA | NA | NA | NA | NA | NA |
| | FluA3 | 0 | NA | NA | NA | NA | NA | NA | NA | NA | NA | NA | NA | NA | NA |
| **Flu A - Mod Pos** | FluA1 | 187 | 34.1 | 0 | 0 | 0.41 | 1.2 | 0.95 | 2.8 | 0 | 0 | 1.54 | 4.5 | 1.86 | 5.5 |
| | FluA2 | 14 | NA | NA | NA | NA | NA | NA | NA | NA | NA | NA | NA | NA | NA |
| | FluA3 | 0 | NA | NA | NA | NA | NA | NA | NA | NA | NA | NA | NA | NA | NA |
| **Flu B- High Neg** | FluB | 34 | NA | NA | NA | NA | NA | NA | NA | NA | NA | NA | NA | NA | NA |
| **Flu A - Low Pos** | FluB | 174 | 33.2 | 0 | 0 | 0.47 | 1.4 | 0 | 0 | 0.66 | 2.0 | 2.03 | 6.1 | 2.18 | 6.6 |
| **Flu A - Mod Pos** | FluB | 191 | 32.1 | 0 | 0 | 0.17 | 0.5 | 0.25 | 0.8 | 0 | 0 | 1.73 | 5.4 | 1.75 | 5.5 |
| **RSV - High Neg** | RSV | 30 | NA | NA | NA | NA | NA | NA | NA | NA | NA | NA | NA | NA | NA |
| **RSV - Low Pos** | RSV | 175 | 36.0 | 0 | 0 | 0.75 | 2.1 | 0 | 0 | 0.36 | 1.0 | 1.47 | 4.1 | 1.69 | 4.7 |
| **RSV - Mod Pos** | RSV | 191 | 34.7 | 0 | 0 | 0.57 | 1.7 | 0.16 | 0.5 | 0 | 0 | 1.23 | 3.6 | 1.37 | 3.9 |

a Results with non-zero Ct values out of 192.

## 23 References


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8. REGULATION (EC) No 1272/2008 OF THE EUROPEAN PARLIAMENT AND OF THE COUNCIL of 16 December. 2008 on the classification labeling and packaging of substances and mixtures amending and repealing, List of Precautionary Statements, Directives 67/548/EEC and 1999/45/EC (amending Regulation (EC).
9. Occupational Safety and Health Standards, Hazard Communication, Toxic and Hazard Substances (March 26, 2012) (29 C.F.R., pt. 1910, subpt. Z).

## 24 Cepheid Headquarters Locations

| Corporate Headquarters | European Headquarters |
|---|---|
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| www.cepheid.com | www.cepheidinternational.com/ |

## 25 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

**Contact Information**

| | |
|---|---|
| United States | France |
| Telephone: + 1 888 838 3222 | Telephone: + 33 563 825 319 |
| Email: techsupport@cepheid.com | Email: support@cepheideurope.com |

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

## 26 Table of Symbols

| Symbol | Meaning |
|---|---|
| REF | Catalog number |
| IVD | *In vitro* diagnostic medical device |
| | Do not reuse |
| LOT | Batch code |
| | Consult instructions for use |
| | Caution |
| | Manufacturer |
| | Country of manufacture |
| | Contains sufficient for <n> tests |
| CONTROL | Control |
| | Expiration date |
| CE | CE marking – European Conformity |
| EC REP | Authorized Representative in the European Community |
| | Temperature limitation |
| | Biological risks |
| | Warning |

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