GeneXpert
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# Xpert® Xpress Flu

REF **XPRSFLU-10**



Instructions for Use
CLIA Complexity: Moderate
**IVD**

Cepheid

*In Vitro* Diagnostic Medical Device

**301-7268, Rev. G**
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**Trademark, Patents and Copyright Statements**



See Section 27, Revision History for a description of changes.

# Xpert® Xpress Flu

℞only

For *In Vitro* Diagnostic Use

## 1 Proprietary Name

Xpert® Xpress Flu

## 2 Common or Usual Name

Xpert Xpress Flu test

## 3 Intended Use

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

Negative results do not preclude influenza 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 2015-2016 influenza season for NP swab specimens and the 2016-2017 influenza season for NS specimens. 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]

# 5 Principle of the Procedure

The Xpert Xpress Flu test is an automated *in vitro* diagnostic test for qualitative detection of influenza A and influenza B viral RNA. The test is performed on Cepheid GeneXpert Instrument Systems.

The GeneXpert Instrument Systems automate and integrate 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 test 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), and influenza B non-structural protein (NS).

The GeneXpert 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 Xpress Flu test includes reagents for the detection and differentiation of influenza A and influenza B viral RNA directly from NP swab or 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 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 test has 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 test, SPC amplification curves will not be seen and their results will not be reported.

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

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 test 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 test provides test results for influenza A and influenza B. It also reports if the test is invalid, has encountered an error or produces no result.

# 6 Reagents and Instruments

## 6.1 Materials Provided

The Xpert Xpress Flu test kit contains sufficient reagents to process 10 specimens or quality control samples. The kits contain the following:

| | |
|---|---|
| **Xpert Xpress Flu test 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** | **1 bag of 12 per kit** |

| | |
|---|---|
| **CD**<br>• Assay Definition Files (ADF)<br>• Instructions to import ADF into GeneXpert software (For use with the GeneXpert Dx and Infinity Systems only. Refer to the GeneXpert Xpress User's Guide for instructions to import ADF into GeneXpert Xpress software)<br>• Instructions for Use (Package Insert) (For use with the GeneXpert Dx and Infinity Systems only) | **1 per kit** |
| **Package Insert**<br>(For use with the GeneXpert Xpress System only) | **1 per kit** |
| **Quick Reference Guide**<br>(For use with the GeneXpert Xpress System only) | **2 per kit** |

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

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

# 7 Storage and Handling

- Store the Xpert Xpress Flu test cartridges and reagents 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 Dx System or GeneXpert Infinity Systems (catalog number varies by configuration): GeneXpert Instrument, computer, barcode scanner, and operator manual.
  - For GeneXpert Dx System: GeneXpert Dx software version 4.7b or higher
  - For GeneXpert Infinity-80 and Infinity-48s Systems: Xpertise software version 6.4b or higher
- Printer. If a printer is required, contact Cepheid Sales Representative to arrange for the purchase of a recommended printer.

# 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 # NATFLUAB-6C (NATtrol Influenza A/B) as an 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 test 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 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.

## 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 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 Test/Reagent

- The test has been validated using Cepheid GeneXpert Dx software version 4.7b or higher, and Cepheid Xpertise software version 6.4b or higher. Cepheid will validate future software versions for use with the Xpert Xpress Flu test.
- Performance may be impacted when using frozen specimens.
- Do not substitute Xpert Xpress Flu test reagents with other reagents.
- Do not open the Xpert Xpress Flu test 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 test 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 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[7,8]

- 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

Specimens can be collected following the user institution's standard procedures and placed into the Xpert Viral Transport Medium or Copan UTM (3 mL tube with transport medium). 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.

# 13 Procedure

## 13.1 Preparing the Cartridge

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

1. Remove a cartridge from the package.
2. Mix specimen by inverting the Xpert Viral Transport Medium or the Copan UTM 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 by expressing the fluid into the large opening in the cartridge (Figure 1).
4. Close the cartridge lid.


**Figure 1. Xpert Xpress Flu Test Cartridge (Top View)**

## 13.2 Starting a New Test

**Important** **Before starting the test, make sure that the Xpert Xpress Flu test Definition File is imported into the software. This section lists the basic steps of running the test. For detailed instructions, see the *GeneXpert Dx System Operator Manual* or the *GeneXpert Infinity System Operator Manual*, depending on the model 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 System:
   - If using the GeneXpert Dx instrument, first turn on the GX Dx instrument and then turn on the computer. The GeneXpert Dx software will launch automatically or may require double-clicking the GeneXpert Dx software shortcut 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 shortcut 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** (Infinity). The **Create Test** window opens.
4. Scan 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 in Sample ID or type 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 Xpress Flu test 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 Xpress Flu test cartridge does not scan, then repeat the test with a new cartridge.

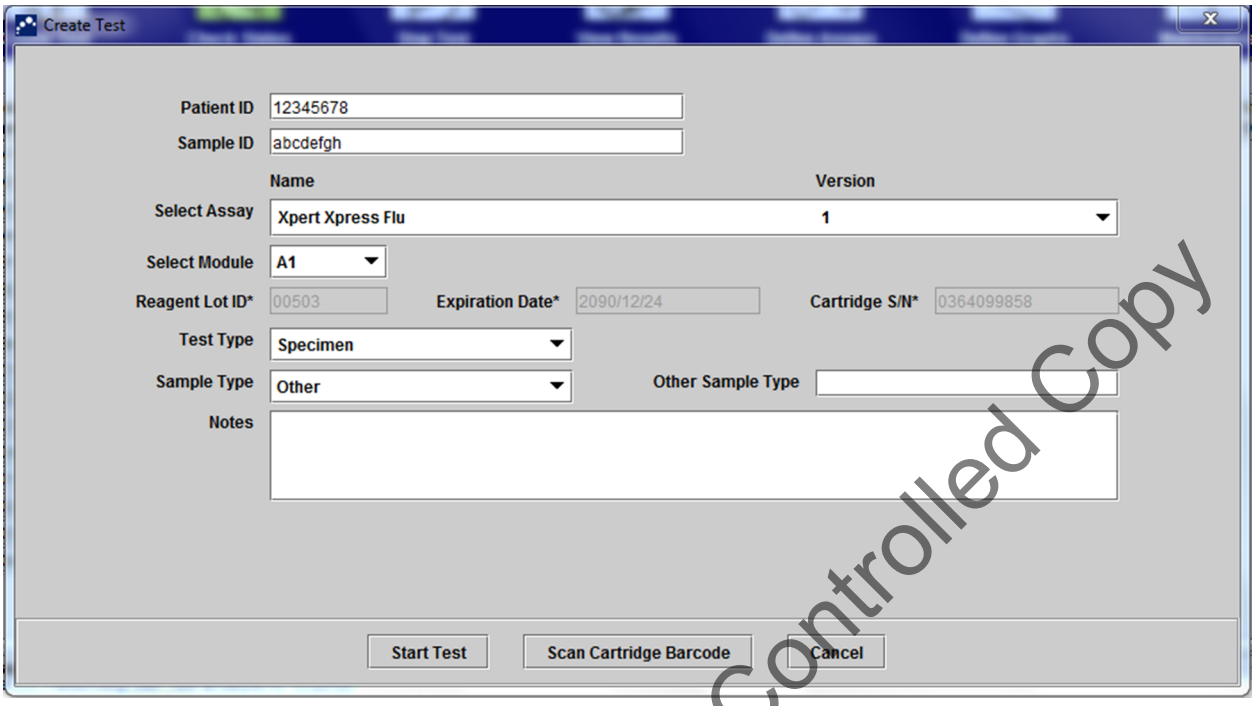


**Figure 2. GX Dx Create Test Window**

7. Click **Start Test** (GeneXpert Dx) or **Submit** (Infinity). Type your password in the dialog box that appears.
8. 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.
9. Dispose of used cartridges in the appropriate specimen waste container 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.

- Click the **View Results** icon to view results.
- 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

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 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.

The test result is **INVALID** if all targets are reported negative and the SPC does not meet the validated acceptance criteria.

- **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 B, and SPC) is performed after the reverse transcription step and before PCR begins. The PCC monitors bead rehydration, reaction tube filling, probe integrity, and dye stability. The PCC passes if it meets the validated acceptance criteria. If any of the PCC criteria fail, the test results in an **ERROR**.
- **External Controls**—External controls may be used in accordance with local, state and federal accrediting organizations as applicable.

# 16 Interpretation of Results

The Xpert Xpress Flu test has two channels (Flu A 1 and Flu A 2) to detect most influenza A strains. All influenza A strains detected by the Xpert Xpress Flu test are reported as **Flu A POSITIVE**. The Xpert Xpress Flu test 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. Table 1 below lists all the possible test results for Flu A.

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

| Flu A Test Result | Flu A 1 Channel | Flu A 2 Channel |
|---|---|---|
| Flu A POSITIVE | POS | POS/NEG |
| | POS/NEG | POS |
| Flu A NEGATIVE | NEG | NEG |

The results reported from testing with the Xpert Xpress Flu test 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 Xpress Flu test**

| Result Text | Flu A 1 | Flu A 2 | Flu B | SPC |
|---|---|---|---|---|
| **Flu A POSITIVE; Flu B NEGATIVE** | POS | POS/NEG | NEG | POS/NEG |
| | POS/NEG | POS | | |
| **Flu A POSITIVE; Flu B POSITIVE** | POS | POS/NEG | POS | POS/NEG |
| | POS/NEG | POS | | |
| **Flu A NEGATIVE; Flu B POSITIVE** | NEG | NEG | POS | POS/NEG |
| **Flu A NEGATIVE; Flu B NEGATIVE** | NEG | NEG | NEG | POS |
| **INVALID** | NEG | NEG | NEG | NEG |
| **ERROR** | NO RESULT | NO RESULT | NO RESULT | NO RESULT |
| **NO RESULT** | NO RESULT | NO RESULT | NO RESULT | NO RESULT |

See Table 3 and Figure 3 through Figure 9 for specific examples and to interpret test result statements for the Xpert Xpress Flu test.

**Table 3. Xpert Xpress Flu test Results and Interpretation**

| Result | Interpretation |
|---|---|
| **Flu A POSITIVE;**<br>**Flu B NEGATIVE**<br>See Figure 3. | 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;**<br>**Flu B POSITIVE**<br>See Figure 4. | 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. |
| **Flu A POSITIVE;**<br>**Flu B POSITIVE**[a]<br>See Figure 5. | 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;**<br>**Flu B NEGATIVE**<br>See Figure 6. | Flu A target RNA is not detected; Flu B target RNA is not detected.<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. |
| **INVALID**<br>See Figure 7. | 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. |
| **ERROR**<br>See Figure 8. | Presence or absence of Flu A and/or Flu B target RNA cannot be determined. Repeat test according to the instructions in Section 17.2, Retest Procedure.<br>● Flu A – NO RESULT<br>● Flu B – 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. |
| **NO RESULT**<br>See Figure 9. | Presence or absence of Flu A and/or Flu B target RNA cannot be determined. Repeat test according to the instructions in Section 17.2, Retest Procedure. 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>● SPC – NO RESULT<br>● Probe Check – NA (not applicable) |

[a] Note: Because the incidence of co-infection with Influenza A and Influenza B viruses is low, it is recommended that specimens undergo repeat testing if nucleic acids from both analytes are detected in a single specimen. Repeat test according to the instructions in Section 17.2, Retest Procedure.

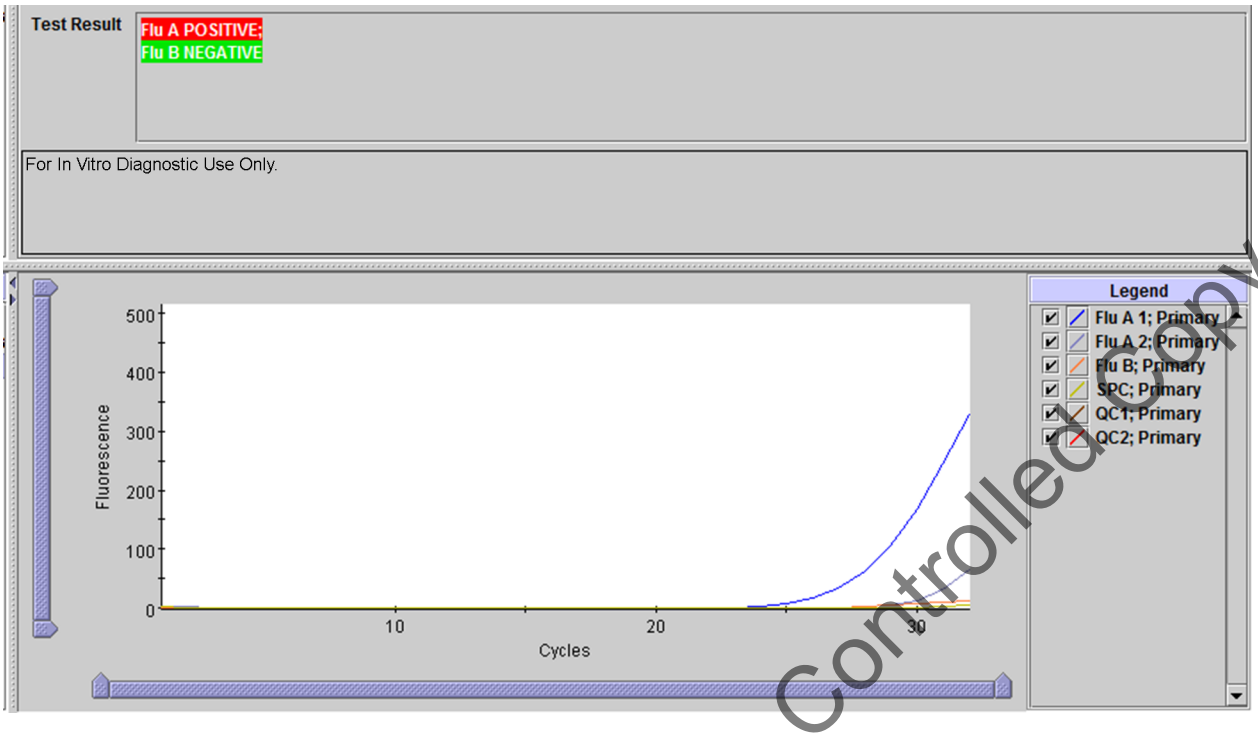


**Figure 3. Xpert Xpress Flu US-IVD: An Example of a Positive Result for Flu A**

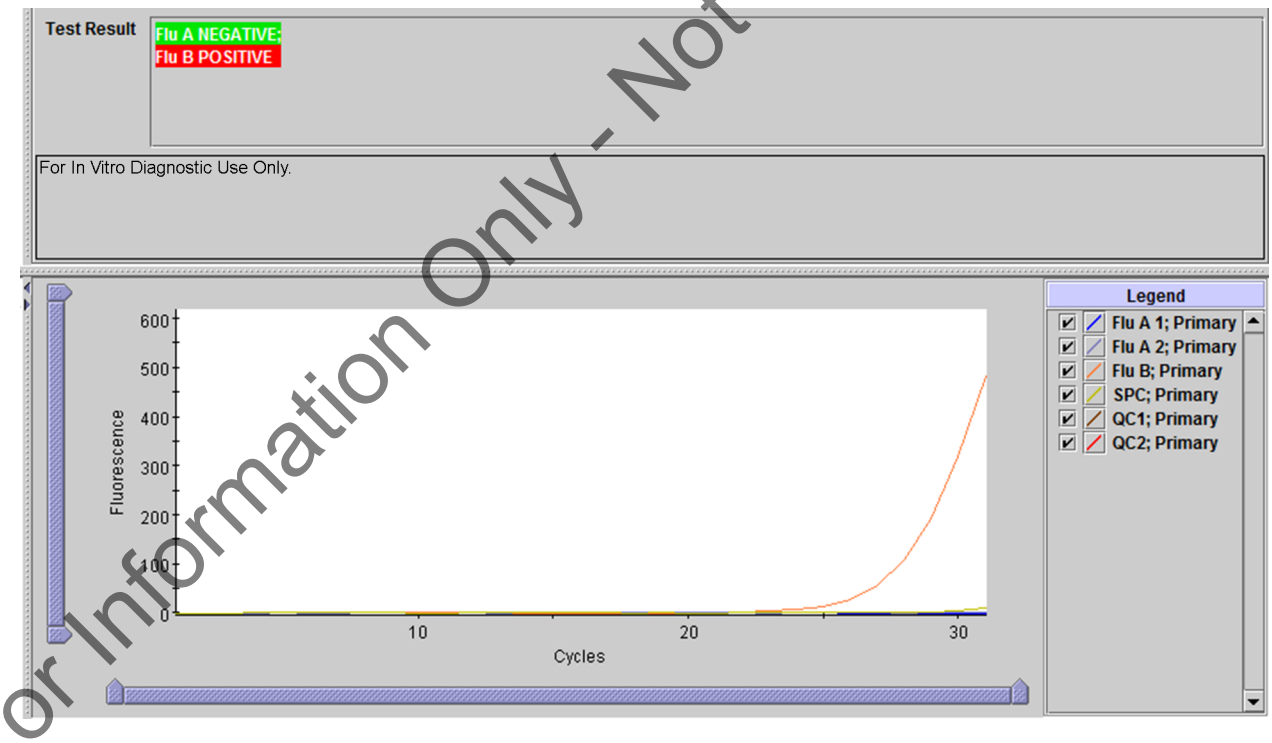


**Figure 4. Xpert Xpress Flu US-IVD: An Example of a Positive Result for Flu B**

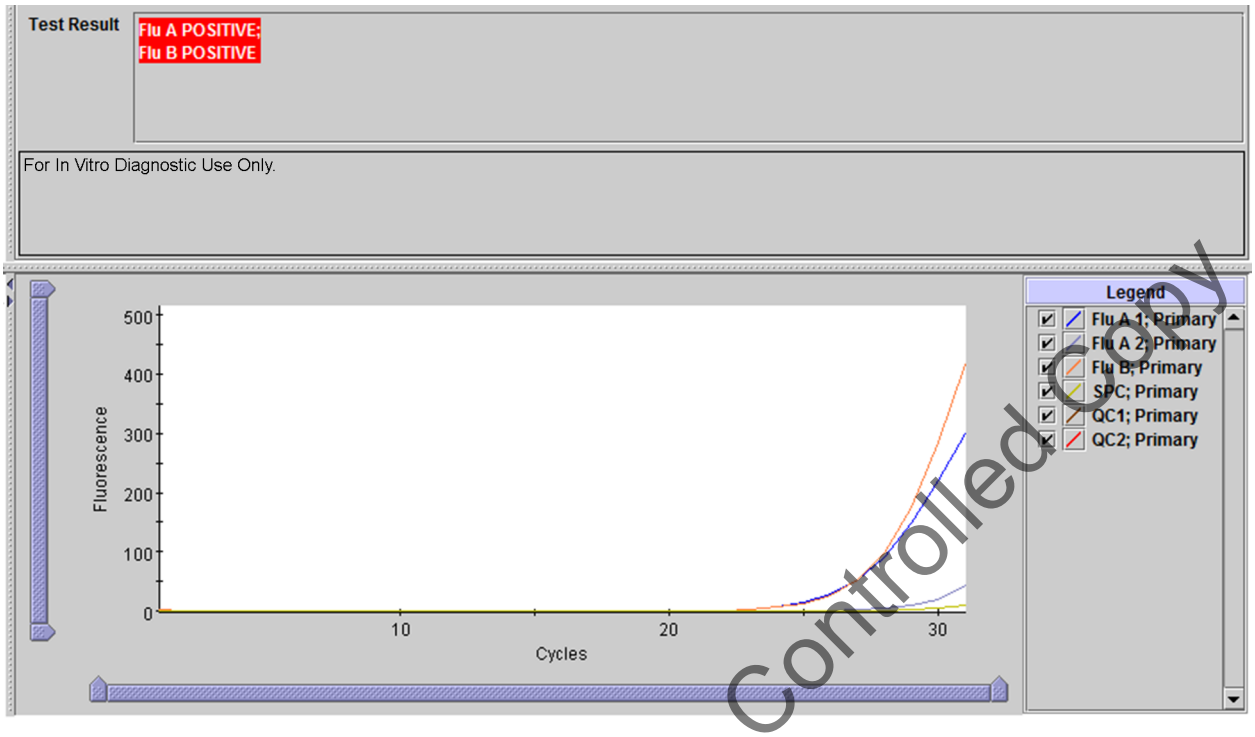


**Figure 5. Xpert Xpress Flu US-IVD: An Example of a Positive Result for Flu A and Flu B**

Because the incidence of co-infection with Influenza A and Influenza B viruses is low, it is recommended that specimens undergo repeat testing if nucleic acids from both analytes are detected in a single specimen. Repeat test according to the instructions in Section 17.2, Retest Procedure.

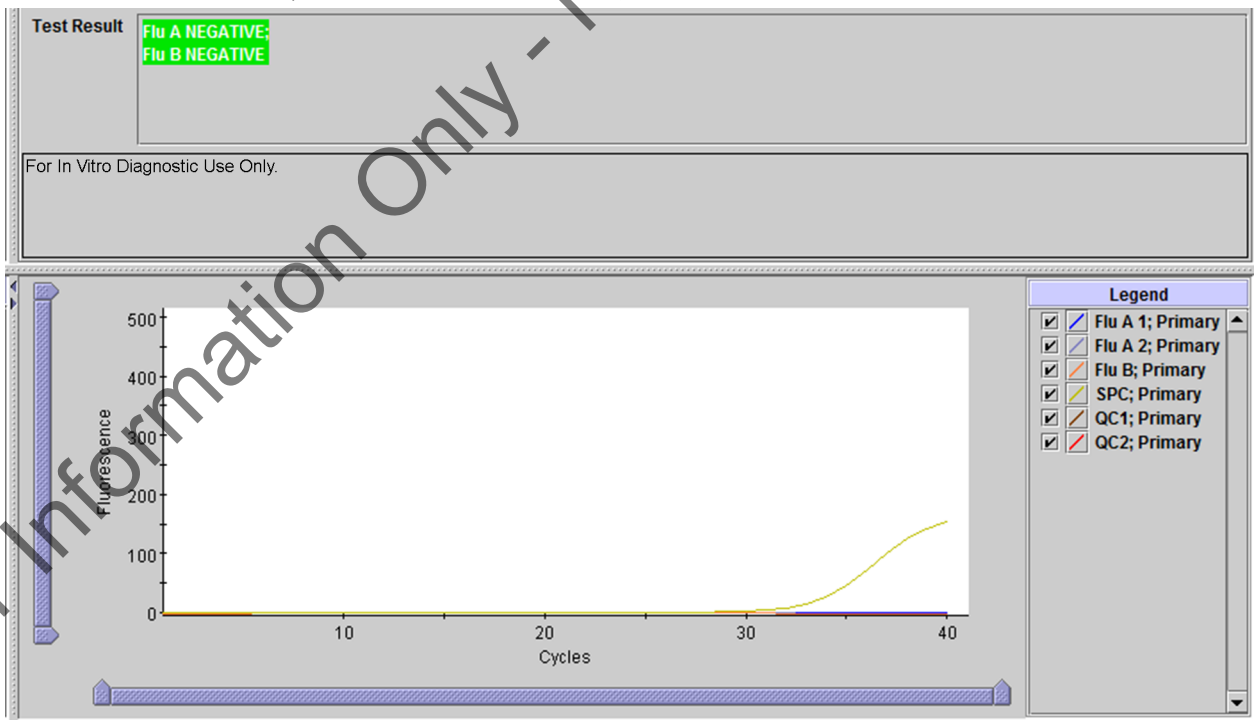


**Figure 6. Xpert Xpress Flu US-IVD: An Example of a Negative Result for Flu A and Flu B**

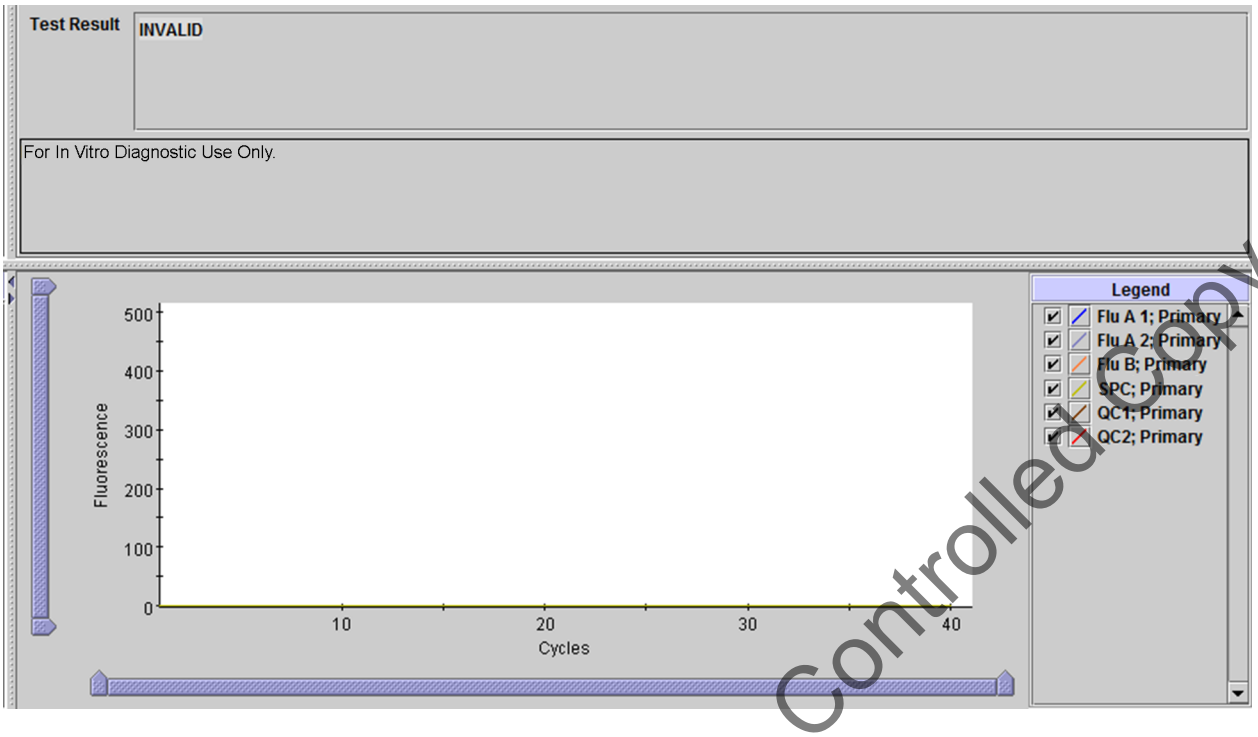


**Figure 7. Xpert Xpress Flu US-IVD: An Example of an Invalid Result**

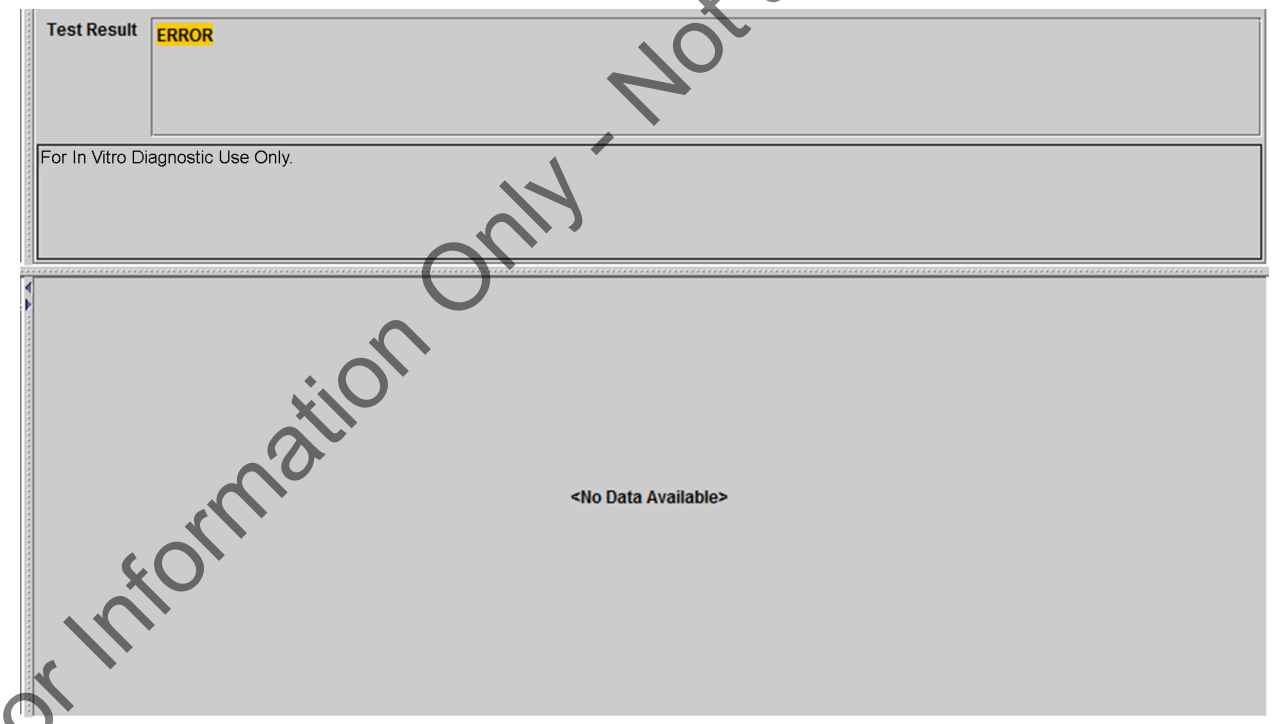


**Figure 8. Xpert Xpress Flu US-IVD: An Example of an Error**

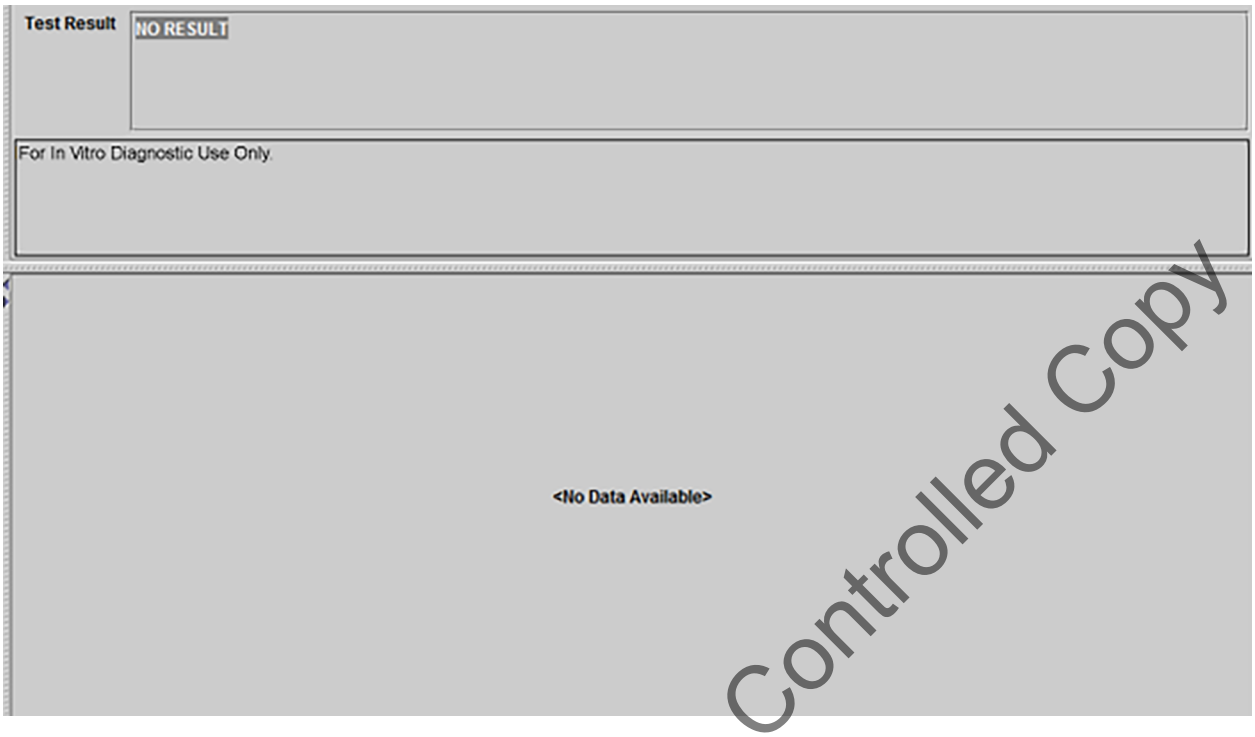


**Figure 9. Xpert Xpress Flu US-IVD: An Example of a No Result**

# 17 Retests

## 17.1 Reasons to Repeat the Test

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

- Because the incidence of co-infection with Influenza A and Influenza B viruses is low, it is recommended that specimens undergo repeat testing if nucleic acids from both analytes are detected in a single specimen. Repeat test according to the instructions in Section 17.2, Retest Procedure.
- An **INVALID** result indicates that the control SPC failed. The sample was not properly processed, PCR is inhibited, or the sample was not properly collected.
- An **ERROR** result could be due to, but not limited to, Probe Check Control 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 or a result indicating co-infection, use a new cartridge (do not re-use the cartridge). Use 300 µL of the left over specimen from the original transport medium tube.

1. Remove a new cartridge from the kit box.
2. Mix the specimen by inverting the Xpert Viral Transport Medium or the Copan UTM tube five times.
3. Open the cartridge lid. Use a clean 300 µL transfer pipette (supplied) to transfer 300 µL of the sample to the chamber by expressing the fluid into the large opening in the cartridge (Figure 1).
4. Close the cartridge lid.
5. Start the test according to instructions in Section 13.2, Starting a New Test.

# 18 Limitations

- The performance of the Xpert Xpress Flu test 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 test 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 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 both influenza A and influenza B viruses present. However, numerous studies have shown that infections with combinations of only these specific viruses (Flu A and Flu B) occur in <1.6% of patients.[9,10,11]
- The Xpert Xpress Flu test uses EAT. In the event of a mixed Flu A and Flu B infection, the target with the higher titer of the two infections may be reported as **POSITIVE** and the lower titer target may be reported as **NEGATIVE**.
- Results from the Xpert Xpress Flu test should be correlated with the clinical history, epidemiological data, and other data available to the clinician evaluating the patient.
- Viral nucleic acid may persist *in vivo*, independent of virus 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 virus may not be detected, or may be detected less predictably.
- Positive and negative predictive values are highly dependent on prevalence. The test performance was established during the 2015-2016 influenza season for NP swab specimens and during the 2016-2017 influenza season for NS specimens. 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 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.
- 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 test 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 Influenza A subtypes or Influenza B lineages. If differentiation of specific influenza subtypes and strains is needed, additional testing, in consultation with state or local public health departments, is required.

# 19 Expected Values

The Xpert Xpress Flu NP swab clinical study included a total of 1139 prospectively collected fresh specimens and 912 consecutively collected, frozen specimens. The number and percentage of cases positive for one or more of influenza A and influenza B, as determined by the Xpert Xpress Flu test, are shown by age category in Table 4.

**Table 4. Number and Percent of Specimens by Age Range**

| Age Group | Number of Patients | % of Total | Flu A | | Flu B | |
|---|---|---|---|---|---|---|
| | | | Number of Positives | Positivity | Number of Positives | Positivity |
| ≤5 years | 360 | 17.6% | 25 | 7.0% | 17 | 4.7% |
| 6-21 years | 225 | 11.0% | 18 | 8.0% | 30 | 13.3% |
| 22-59 years | 729 | 35.5% | 52 | 7.1% | 26 | 3.6% |
| ≥60 years | 736 | 35.9% | 32 | 4.3% | 22 | 3.0% |
| Unknown | 1 | <0.1% | 0 | 0 | 0 | 0 |
| Total | 2051 | 100% | 127 | 6.2% | 95 | 4.6% |

The Xpert Xpress Flu NS clinical study included a total of 1598 prospectively collected fresh specimens for evaluation of influenza A and influenza B detection.

The number and percentage of cases positive for one or more of influenza A and influenza B in NS specimens as determined by the Xpert Xpress Flu test are shown by age category in Table 5.

**Table 5. Age Group Flu A and Flu B Positive by Xpert Xpress Flu test – NS Specimens**

| Age Group (years) | Number of Patients | % of Total | Flu A | | Flu B | |
|---|---|---|---|---|---|---|
| | | | Number of Positives | Positivity Rate | Number of Positives | Positivity Rate |
| ≤5 | 604 | 37.8% | 67 | 11.1% | 26 | 4.3% |
| 6-21 | 273 | 17.1% | 65 | 23.8% | 26 | 9.5% |
| 22-59 | 554 | 34.7% | 58 | 10.5% | 19 | 3.4% |
| ≥60 | 167 | 10.5% | 30 | 18.0% | 3 | 1.8% |
| Total | 1598 | 100% | 220 | 14.0% | 74 | 4.6% |

# 20 Performance Characteristics

## 20.1 Clinical Performance

Performance characteristics of the Xpert Xpress Flu test were evaluated at eleven institutions in the U.S. during the 2015-2016 influenza season for NP swab specimens and at fourteen institutions in the U.S. during the 2016-2017 influenza season for NS specimens. Due to the low prevalence of influenza viruses and the difficulty in obtaining fresh influenza positive specimens, the NP swab specimen population for this study was supplemented with consecutively collected, frozen specimens.

Specimens were collected from the following:

- Individuals exhibiting signs and symptoms of respiratory infection who provided informed consent for the collection of a NP or NS swab specimen.
- Individuals with signs and symptoms of respiratory infection and whose routine care called for collection of NP swabs or NS specimens for influenza testing. For eligible subjects, aliquots of leftover specimens were obtained for testing with the Xpert Xpress Flu test and reference testing, and patient management continued at the site per their standard practice.

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

## 20.2 Overall Results – NP Swab Specimens

A total of 2051 NP swab specimens were tested for influenza A and influenza B by the Xpert Xpress Flu test and the comparator assay. Of the 2051 NP swab specimens, 1139 were fresh, prospectively collected and 912 were consecutively collected frozen specimens.

For the fresh, prospectively collected NP swab specimens, the Xpert Xpress Flu test demonstrated a PPA and NPA of 94.6% and 99.4%, detection of influenza A respectively; and 100.0% and 99.3% for influenza B, respectively (Table 6).

For the consecutively collected, frozen NP swab specimens, the Xpert Xpress Flu test demonstrated a PPA and NPA of 100.0% and 98.0% for the detection of influenza A, respectively; 100.0% and 99.0% for influenza B, respectively (Table 6).

For the combined dataset, the Xpert Xpress Flu test demonstrated a PPA and NPA of 98.1% and 98.8% for the detection of influenza A, respectively; 100.0% and 99.1% for influenza B respectively (Table 6).

The NP clinical study was conducted using the Xpert Xpress Flu/RSV ADF v1. The data shown in Table 4 in Section 19 and Table 6 in Section 20 represent a re-analysis of the same data using Xpert Xpress Flu ADF v3. The re-analysis did not result in any significant changes to the clinical study results.

**Table 6. Xpert Xpress Flu test Performance on NP Swab Specimens**

| Specimen Type | Target | n | TP | FN | TN | FP | PPA (95% CI) | NPA (95% CI) |
|---|---|---|---|---|---|---|---|---|
| Fresh | Flu A | 1139 | 35 | 2[a] | 1095 | 7[b] | 94.6% (82.3 - 98.5) | 99.4% (98.7 - 99.7) |
| | Flu B | 1139 | 42 | 0 | 1089 | 8[c] | 100.0% (91.6 - 100.0) | 99.3% (98.6 - 99.6) |
| Frozen Consecutively Collected | Flu A | 912 | 68 | 0 | 827 | 17[d] | 100.0% (94.7 - 100.0) | 98.0% (96.8 - 98.7) |
| | Flu B | 912 | 36 | 0 | 867 | 9[e] | 100.0% (90.4 - 100.0) | 99.0% (98.1 - 99.5) |
| Combined | Flu A | 2051 | 103 | 2[a] | 1922 | 24[f] | 98.1% (93.3 - 99.5) | 98.8% (98.2 - 99.2) |
| | Flu B | 2051 | 78 | 0 | 1956 | 17[g] | 100.0% (95.3 - 100.0) | 99.1% (98.6 - 99.5) |

[a] Testing results by sequencing: 2 of 2 were Flu A Negative.

[b] Testing results by sequencing: 3 of 7 were Flu A Positive; 3 of 7 were Flu A Negative; 1 of 7 insufficient specimen for sequencing.

[c] Testing results by sequencing: 6 of 8 were Flu B Positive; 1 of 8 were Flu B Negative; 1 of 8 insufficient specimen for sequencing.

[d] Testing results by sequencing: 7 of 17 were Flu A Positive; 7 of 17 were Flu A Negative; 3 of 17 insufficient specimen for sequencing.

[e] Testing results by sequencing: 7 of 9 were Flu B Positive; 0 of 9 were Flu B Negative; 2 of 9 insufficient specimen for sequencing.

[f] Testing results by sequencing: 10 of 24 were Flu A Positive; 10 of 24 were Flu A Negative; 4 of 24 insufficient specimen for sequencing.

[g] Testing results by sequencing: 13 of 17 were Flu B Positive; 1 of 17 were Flu B Negative; 3 of 17 insufficient specimen for sequencing.

In addition, there were 98 pre-selected frozen NP swab specimens that were tested. The results of this testing were analyzed separately and are as follows: the Xpert Xpress Flu test demonstrated a PPA and NPA of 100% and 97.8%, for influenza A, respectively; and 100% and 96.6% for influenza B, respectively.

## 20.3 Overall Results – NS Specimens

A total of 1598 prospectively collected fresh NS specimens were tested for influenza A and influenza B by the Xpert Xpress Flu test and the comparator assay.

The Xpert Xpress Flu test demonstrated a PPA and NPA relative to the reference method of 98.9% and 97.6%, for detection of Flu A, respectively; and 98.4% and 99.3% for Flu B, respectively (Table 6).

The NS clinical study was conducted using the Xpert Xpress Flu/RSV ADF v1. The data shown in Table 5 in Section 19 and Table 7 in Section 20 represent a re-analysis of the same data using Xpert Xpress Flu ADF v3. The re-analysis did not result in any significant changes to the clinical study results.

**Table 7. Xpert Xpress Flu test Performance on NS Specimens**

| Specimen Type | Target | n | TP | FN | TN | FP | PPA (95% CI) | NPA (95% CI) |
|---|---|---|---|---|---|---|---|---|
| NS | Flu A | 1598 | 186 | 2[a] | 1376 | 34[b] | 98.9% (96.2-99.7) | 97.6% (96.6-98.3) |
| | Flu B | 1598 | 63 | 1[c] | 1523 | 11[d] | 98.4% (91.7-99.7) | 99.3% (98.7-99.6) |

[a] Discrepant Testing: 1 of 2 Flu A NEG; 1 of 2 Flu A POS.

[b] Discrepant Testing: 16 of 34 Flu A NEG; 11 of 34 Flu A POS; 7 of 34 inconclusive.

[c] Discrepant Testing: 1 of 1 inconclusive.

[d] Discrepant Testing: 5 of 11 Flu B POS; 6 of 11 inconclusive.

Of the Xpert Xpress Flu test runs performed with eligible NP swab and NS specimens, 97.8% (3594/3674) of these specimens were successful on the first attempt. The remaining 80 specimens gave indeterminate results on the first attempt (39 **ERROR**, 32 **INVALID**, and 9 **NO RESULT**). The initial indeterminate rate was 2.2% (80/3674) with the 95% CI 1.8-2.7%. Sixty of the 80 indeterminate cases were retested, of which 54 yielded valid results upon repeat testing; 20 specimens were not retested. The overall rate of test success was 99.3% (3648/3674). The overall indeterminate rate after retesting was 0.7% (26/3674) with 95% CI 0.5-1.0%.

# 21 Analytical Performance

## 21.1 Analytical Sensitivity (Limit of Detection)

Studies were performed to determine the analytical limit of detection (LoD) of the Xpert Xpress Flu test with two lots of reagents across three testing days. The higher LoD observed per strain and per lot as determined by probit analysis 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 and two influenza B strains. Viruses were diluted into negative pooled NP swab and NS clinical matrix for testing. The LoD is defined as the lowest concentration (tissue culture infective dose, $TCID_{50}$/mL) 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 matrix. The LoD values for each strain tested are summarized in Table 8, Table 9, and Table 10.

The LoD study was originally conducted using the Xpert Xpress Flu/RSV ADF v1. The data shown in Table 8, Table 9, and Table 10 represent a re-analysis of the same data using Xpert Xpress Flu ADF v3. The re-analysis had no effect on the test LoD for any of the influenza strains tested.

**Table 8. 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 | 0.02 | 0.02 |
| Influenza A/Florida/27/2011 | 0.04 | 0.04 |

**Table 9. 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 | 0.01 | 0.01 |
| Influenza A/Victoria/361/2011 | 0.75 | 0.21 |

**Table 10. 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 | 0.40 | 0.07 |
| Influenza B/Wisconsin/01/2011 | 0.19 | 0.17 |

## 21.2 Analytical Specificity (Exclusivity)

The analytical specificity of the Xpert Xpress Flu test 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 each bacterial and yeast strain 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 each virus were tested at concentrations of ≥ 1 x $10^5$ $TCID_{50}$/mL. The analytical specificity was 100%. Results are shown in Table 11.

**Table 11. Analytical Specificity of the Xpert Xpress Flu test**

| Organism | Concentration | Result | |
|---|---|---|---|
| | | Influenza A | Influenza B |
| *No Template Control* | N/A | NEG | NEG |
| Adenovirus Type 1 | 1.12E+06 $TCID_{50}$/mL | NEG | NEG |
| Adenovirus Type 7 | 1.87E+05 $TCID_{50}$/mL | NEG | NEG |
| Human coronavirus OC43 | 2.85E+05 $TCID_{50}$/mL | NEG | NEG |
| Human coronavirus 229E | 1.00E+05 $TCID_{50}$/mL | NEG | NEG |
| Cytomegalovirus | 1.00E+05 $TCID_{50}$/mL | NEG | NEG |
| Echovirus | 3.31E+07 $TCID_{50}$/mL | NEG | NEG |
| Enterovirus | 3.55E+05 $TCID_{50}$/mL | NEG | NEG |
| Epstein Barr Virus | 7.16E+07 $TCID_{50}$/mL | NEG | NEG |
| HSV | 8.90E+05 $TCID_{50}$/mL | NEG | NEG |
| Measles | 6.31E+05 $TCID_{50}$/mL | NEG | NEG |
| Human metapneumovirus | 1.00E+05 $TCID_{50}$/mL | NEG | NEG |
| Mumps virus | 6.31E+06 $TCID_{50}$/mL | NEG | NEG |
| Human parainfluenza Type 1 | 1.15E+06 $TCID_{50}$/mL | NEG | NEG |
| Human parainfluenza Type 2 | 6.31E+05 $TCID_{50}$/mL | NEG | NEG |
| Human parainfluenza Type 3 | 3.55E+06 $TCID_{50}$/mL | NEG | NEG |
| Rhinovirus Type 1A | 1.26E+05 $TCID_{50}$/mL | NEG | NEG |
| *Acinetobacter baumannii* | 1.00E+06 CFU/mL | NEG | NEG |

| Organism | Concentration | Result | |
|---|---|---|---|
| | | Influenza A | Influenza B |
| *Burkholderia cepacia* | 3.30E+06 CFU/mL | NEG | NEG |
| *Candida albicans* | 3.20E+06 CFU/mL | NEG | NEG |
| *Candida parapsilosis* | 3.00E+06 CFU/mL | NEG | NEG |
| *Bordetella pertussis* | 3.30E+06 CFU/mL | NEG | NEG |
| *Chlamydia pneumoniae* | 1.00E+05 CFU/mL | NEG | NEG |
| *Citrobacter freundii* | 3.30E+06 CFU/mL | NEG | NEG |
| *Corynebacterium sp.* | 3.30E+06 CFU/mL | NEG | NEG |
| *Escherichia coli* | 1.00E+07 CFU/mL | NEG | NEG |
| *Enterococcus faecalis* | 1.30E+06 CFU/mL | NEG | NEG |
| *Haemophilus influenzae* | 1.00E+06 CFU/mL | NEG | NEG |
| *Lactobacillus reuteri* | 1.00E+06 CFU/mL | NEG | NEG |
| *Legionella spp.* | 1.00E+06 CFU/mL | NEG | NEG |
| *Moraxella catarrhalis* | 1.00E+07 CFU/mL | NEG | NEG |
| *Mycobacterium tuberculosis (avirulent)* | 1.00E+06 CFU/mL | NEG | NEG |
| *Mycoplasma pneumoniae* | 1.00E+06 CFU/mL | NEG | NEG |
| *Neisseria meningitidis* | 2.15E+06 CFU/mL | NEG | NEG |
| *Neisseria mucosa* | 1.00E+07 CFU/mL | NEG | NEG |
| *Propionibacterium acnes* | 2.40E+07 CFU/mL | NEG | NEG |
| *Pseudomonas aeruginosa* | 3.70E+06 CFU/mL | NEG | NEG |
| *Staphylococcus aureus* (protein A producer) | 2.20E+06 CFU/mL | NEG | NEG |
| *Staphylococcus epidermidis* | 3.40E+06 CFU/mL | NEG | NEG |
| *Staphylococcus haemolyticus* | 4.00E+06 CFU/mL | NEG | NEG |
| *Streptococcus agalactiae* | 3.50E+06 CFU/mL | NEG | NEG |
| *Streptococcus pneumoniae* | 1.00E+06 CFU/mL | NEG | NEG |
| *Streptococcus pyogenes* | 1.00E+07 CFU/mL | NEG | NEG |
| *Streptococcus salivarius* | 1.00E+07 CFU/mL | NEG | NEG |
| *Streptococcus sanguinis* | 3.10E+06 CFU/mL | NEG | NEG |

## 21.3 Analytical Reactivity (Inclusivity)

The analytical reactivity of the Xpert Xpress Flu test 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) and influenza B (representing strains from both Victoria and Yamagata lineages) at levels near the analytical LoD. A total of 48 strains comprised of 35 influenza A viruses and 13 influenza B strains were tested in this study with the Xpert Xpress Flu test. Three replicates were tested for each strain. All Flu 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 12.

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

**Table 12. Analytical Reactivity (Inclusivity) of the Xpert Xpress Flu test**

| Virus | Strain | Target Concentration | Result | |
|---|---|---|---|---|
| | | | Flu A | Flu B |
| *No Template Control* | | n/a | NEG | NEG |
| **Influenza A H1N1 (pre-2009)** | A/swine/Iowa/15/30 | 0.1 $TCID_{50}$/mL | POS | NEG |
| | A/WS/33 | 0.1 $TCID_{50}$/mL | POS | NEG |
| | A/PR/8/34 | 0.1 $TCID_{50}$/mL | POS | NEG |
| | A/Mal/302/54 | 0.1 $TCID_{50}$/mL | POS | NEG |
| | A/Denver/1/57 | 0.1 $TCID_{50}$/mL | POS | NEG |
| | A/New Jersey/8/76 | 0.1 $TCID_{50}$/mL | POS | NEG |
| | A/New Caledonia/20/1999 | 0.1 $TCID_{50}$/mL | POS | NEG |
| | A/New York/55/2004 | 0.1 $TCID_{50}$/mL | POS | NEG |
| | A/Soloman Island/3/2006 | 0.1 $TCID_{50}$/mL | POS | NEG |
| | A/Taiwan/42/06 | 0.1 $TCID_{50}$/mL | POS | NEG |
| | A/Brisbane/59/2007 | 0.1 $TCID_{50}$/mL | POS | NEG |
| **Influenza A H1N1 (pdm2009)** | A/swine/NY/02/2009 | 0.1 $TCID_{50}$/mL | POS | NEG |
| | A/Colorado/14/2012 | 0.1 $TCID_{50}$/mL | POS | NEG |
| | A/Washington/24/2012 | 0.1 $TCID_{50}$/mL | POS | NEG |
| **Influenza A H3N2 (Seasonal)** | A/Aichi/2/68 | 2.0 $TCID_{50}$/mL | POS | NEG |
| | A/Hong Kong/8/68 | 2.0 $TCID_{50}$/mL | POS | NEG |
| | A/Port Chalmers/1/73 | 2.0 $TCID_{50}$/mL | POS | NEG |
| | A/Hawaii/15/2001 | 2.0 $TCID_{50}$/mL | POS | NEG |
| | A/Wisconsin/67/05 | 2.0 $TCID_{50}$/mL | POS | NEG |
| | A/Brisbane/10/2007 | 2.0 $TCID_{50}$/mL | POS | NEG |
| | A/Minnesota/11/2010 (H3N2)v | 2.0 $TCID_{50}$/mL | POS | NEG |
| | A/Indiana/08/2011 (H3N2)v | 2.0 $TCID_{50}$/mL | POS | NEG |
| | A/Texas/50/2012 | 2.0 $TCID_{50}$/mL | POS | NEG |
| **Avian influenza A** | A/duck/ Hunan/795/2002 (H5N1) | ≤ 1ρg/μL[a] | POS | NEG |

| Virus | Strain | Target Concentration | Result | |
|---|---|---|---|---|
| | | | Flu A | Flu B |
| | A/chicken/ Hubei/327/2004 (H5N1) | ≤ 1pg/µL | POS | NEG |
| | A/Anhui/01/2005 (H5N1) | ≤ 1pg/µL | POS | NEG |
| | A/Japanesewhite eye/ HongKong/ 1038/2006 (H5N1) | ≤ 1pg/µL | POS | NEG |
| | A/mallard/WI/34/75 (H5N2) | ≤ 1pg/µL | POS | NEG |
| | A/chicken/CA431/00 (H6N2) | ≤ 1pg/µL | POS | NEG |
| | A/duck/ LTC-10-82743/1943 (H7N2) | ≤ 1pg/µL | POS | NEG |
| | A/chicken/ NJ/15086-3/94 (H7N3) | ≤ 1pg/µL | POS | NEG |
| | A/Anhui/1/2013 (H7N9) | N/A[b] | POS | NEG |
| | A/Shanghai/1/2013 (H7N9) | N/A[b] | POS | NEG |
| | A/chicken/Korea/38349-p96323/ 1996 (H9N2) | ≤ 1pg/µL | POS | NEG |
| | A/Mallard/NY/6750/78 (H2N2) | ≤ 1pg/µL | POS | NEG |
| **Influenza B** | B/Lee/40 | 1.0 $TCID_{50}$/mL | NEG | POS |
| | B/Allen/45 | 1.0 $TCID_{50}$/mL | NEG | POS |
| | B/GL/1739/54 | 1.0 $TCID_{50}$/mL | NEG | POS |
| | B/Maryland/1/59 | 1.0 $TCID_{50}$/mL | NEG | POS |
| | B/Panama/45/90[c] | 1.0 $TCID_{50}$/mL | NEG | POS |
| | B/Florida/07/2004[d] | 1.0 $TCID_{50}$/mL | NEG | POS |
| | B/Florida/02/06[c] | 1.0 $TCID_{50}$/mL | NEG | POS |
| | B/Florida/04/06[d] | 1.0 $TCID_{50}$/mL | NEG | POS |
| | B/Hong Kong/5/72 | 1.0 $TCID_{50}$/mL | NEG | POS |
| | B/Wisconsin/01/2010[d] | 1.0 $TCID_{50}$/mL | NEG | POS |
| | B/Malaysia/2506/04[c] | 1.0 $TCID_{50}$/mL | NEG | POS |
| | B/Taiwan/2/62 | 1.0 $TCID_{50}$/mL | NEG | POS |
| | B/Brisbane/60/2008[c] | 1.0 $TCID_{50}$/mL | 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.

## 21.4 Interfering Substances Study

In a non-clinical study, potentially interfering substances that may be present in the nasal passage and the nasopharynx were evaluated directly relative to the performance of the Xpert Xpress Flu test. 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) strains spiked at 3X the analytical LoD determined for each strain. All results were compared to positive and negative simulated nasal matrix controls. The simulated nasal 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 13 with active ingredients and concentrations tested shown. None of the substances caused interference of the test at the concentrations tested in this study. All positive and negative replicates were identified correctly using the Xpert Xpress Flu test.

**Table 13. Potentially Interfering Substances in the Xpert Xpress Flu test**

| Substance/Class | Description/Active Ingredient | Concentration Tested |
|---|---|---|
| Control | Simulated nasal 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) |
| Nasal corticosteroid | Fluticasone Propionate | 5 µg/mL |

## 21.5 Carry-over Contamination Study

A study was conducted to demonstrate that single-use, self-contained GeneXpert cartridges prevent carry-over contamination of negative samples when 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) spiked into a simulated nasal matrix. This testing scheme was repeated 20 times for a total of 41 runs resulting in 20 positive and 21 negative specimens. All 20 positive samples were correctly reported as **Flu A POSITIVE**; **Flu B NEGATIVE**. All 21 negative samples were correctly reported as **Flu A NEGATIVE**; **Flu B NEGATIVE**.

## 21.6 Fresh vs Frozen Sample Equivalency Study

Fresh and frozen specimen equivalency in the Xpert Xpress Flu test was evaluated by testing individual influenza strains at three different concentrations representing low positives (2X LoD), moderate positives (5X LoD), and high positives (10X LoD) in pooled negative NP swab or pooled negative NS clinical matrix. Negative samples consisted of pooled negative NP swab or pooled negative NS clinical matrix only. Fresh and frozen specimen equivalency was determined using one seasonal Flu A H3N2 strain (A/Victoria/361/2011) and one Flu B strain (B/Mass/2/2012). 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 difference in the performance of the Xpert Xpress Flu test 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 Xpress Flu test.

## 21.7 Competitive Interference Study

Competitive interference of the test caused by the presence of two targets in the Xpert Xpress Flu test was evaluated by testing individual influenza strains near the LoD in the presence of different influenza strains at a higher concentration in a simulated background matrix. Analytical competitive interference was assessed using one (1) seasonal Flu A H3 strain (H3/Victoria/361/2011) at 0.8 $TCID_{50}$/mL and one (1) Flu B strain (B/Mass/2/2012) at 0.45 $TCID_{50}$/mL; the strains were tested in the presence of competing strains at either 1 x $10^2$ $TCID_{50}$/mL or 1 x $10^3$ $TCID_{50}$/mL. 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=0.95 (X~Bin(20,0.95)). Therefore, sets of 20 replicates 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.

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.

Under the conditions of this study, internal competitive inhibitory effects were observed on the Flu B target in the presence of Flu A for the Xpert Xpress Flu test. The competitive inhibitory effect on the Xpert Xpress Flu targets is addressed in the Limitations section of this package insert.

# 22 Reproducibility

Reproducibility was established in a multi-center, blinded study using a five-member specimen panel consisting of a negative con- trol and two each of simulated nasal matrix spiked with influenza A, or influenza B at 1X (low pos) and 2-3X (mod pos) the respective LoDs. Testing was performed at three sites (one internal, two external) using the GeneXpert Dx system, the Infinity-48 system, and the Infinity-80 system. Two operators at each site tested one panel in duplicate two times per day (equivalent to four replicates per day) over six (not necessarily consecutive) days. Three lots of Xpert Xpress Flu cartridges were used, with each lot representing approximately two days of testing. This study was conducted using the Xpert Flu/RSV ADF v1. The data presented in Table 14 represent a re-analysis of the same data using Xpert Xpress Flu ADF v3. The re-analysis did not result in any significant changes to the reproducibility study results. Results are summarized in Table 14.

**Table 14. Summary of Reproducibility Results**

| Sample ID | Site 1/Infinity-80 | | | Site 2/DX | | | Site 3/Infinity-48 | | | % Total Agreement by Sample[a] |
|---|---|---|---|---|---|---|---|---|---|---|
| | Op 1 | Op 2 | Site | Op 1 | Op 2 | Site | Op 1 | Op 2 | Site | |
| **Negative** | 100% (24/24) | 100% (24/24) | 100% (48/48) | 100% (24/24) | 100% (24/24) | 100% (48/48) | 100% (24/24) | 100% (24/24) | 100% (48/48) | 100% (144/144) |
| **Flu A-Low Pos** | 87.0% (20/23) | 95.8% (23/24) | 91.5% (43/47) | 95.7% (22/23) | 91.7% (22/24) | 93.6% (44/47) | 100% (23/23) | 91.3% (21/23) | 95.7% (44/46) | 93.6% (131/140)[b] |
| **Flu A-Mod Pos** | 100% (24/24) | 100% (24/24) | 100% (48/48) | 100% (23/23) | 100% (23/23) | 100% (46/46) | 100% (24/24) | 100% (24/24) | 100% (48/48) | 100% (142/142)[b] |
| **Flu B-Low Pos** | 95.8% (23/24) | 95.8% (23/24) | 95.8% (46/48) | 95.8% (23/24) | 95.8% (23/24) | 95.8% (46/48) | 95.8% (23/24) | 91.7% (22/24) | 93.8% (45/48) | 95.1% (137/144) |
| **Flu B-Mod Pos** | 100% (23/23) | 100% (24/24) | 100% (47/47) | 100% (24/24) | 100% (24/24) | 100% (48/48) | 100% (24/24) | 100% (23/23) | 100% (47/47) | 100% (142/142)[b] |

[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] Eight samples indeterminate [Flu A Low Pos (4); Flu A Mod Pos (2); Flu B Mod Pos (2)]

The reproducibility of the Xpert Xpress Flu 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 15.

**Table 15. Summary of Reproducibility Data**

| Sample | Test Channel (Analyte) | N[a] | Mean Ct | Between-Site | | Between-Lot | | Between-Day | | Between-Operator | | Within-Test | | Total | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | SD | CV (%) | SD | CV (%) | SD | CV (%) | SD | CV (%) | SD | CV (%) | SD | CV (%) |
| **Negative** | SPC | 144 | 32.3 | 0 | 0 | 0.7 | 2.1 | 0.1 | 0.4 | 0 | 0 | 0.6 | 1.9 | 0.9 | 2.8 |
| **Flu A-Low Pos** | FluA1 | 131 | 35.3 | 0 | 0 | 0.6 | 1.6 | 0 | 0 | 0 | 0 | 1.1 | 3.0 | 1.2 | 3.4 |
| **Flu A-Mod Pos** | FluA1 | 142 | 33.1 | 0 | 0 | 0 | 0.1 | 0.2 | 0.6 | 0 | 0 | 0.6 | 1.8 | 0.6 | 1.9 |
| **Flu B-Low Pos** | FluB | 137 | 34.6 | 0 | 0 | 0 | 0 | 0.5 | 1.3 | 0.4 | 1.2 | 1.3 | 3.9 | 1.5 | 4.2 |
| **Flu B-Mod Pos** | FluB | 142 | 32.3 | 0.1 | 0.3 | 0.3 | 0.8 | 0 | 0 | 0.3 | 0.8 | 0.8 | 2.4 | 0.9 | 2.7 |

[a] Results with non-zero Ct values out of 144.

# 23 References


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# 24 Cepheid Headquarters Locations

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# 25 Technical Assistance

Before contacting Cepheid Technical Support, collect the following information:

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

### United States Technical Support

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

### France Technical Support

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

# 26 Table of Symbols

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

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IVD

# 27 Revision History

**Description of Changes**: 301-7268 Rev. F to Rev. G

**Purpose**: Updates to the Instructions for Use

| Section | Description of Change |
|---|---|
| Trademarks, Patents and Copyright Statements | Updated to current legal standards. |
| 8 | Updated the Materials Required but Not Provided section. |
| 10.2 | Updated the Warnings in the Specimen section. |
| 24 | Updated to standard content and layout. |
| 25 | Updated to standard content and layout. |
| 27 | Added the Revision History section and table. |
| Throughout | Formatting updates, minor document usability improvements (cross references), and removal of margin icons. Instances of "assay" used as a brand name changed to "test". |