GeneXpert®
Powered By CEPHEID INNOVATION

Xpert® Flu

REF GXFLU-CE-10

Cepheid®

In Vitro Diagnostic Medical Device

IVD CE

301-2138, Rev. F July 2020

Trademark, Patents and Copyright Statements

Cepheid®, the Cepheid logo, GeneXpert® and Xpert® are trademarks of Cepheid.
Armored RNA® is a trademark of Asuragen, Inc.
Windows® is a trademark of Microsoft Corporation.
Neo-Synephrine® is a trademark of Bayer HealthCare LLC.
Zicam® is a trademark of Matrixx Initiatives, Inc.
Tamiflu® is a trademark of Genentech USA.

Armored RNA® is a patented technology jointly developed by Asuragen Inc. and Cenetron Diagnostics, LLC under U.S. Patent Nos. 5,677,124, 5,919,625, 5,939,262 and other patents pending.

THE PURCHASE OF THIS PRODUCT CONVEYS TO THE BUYER THE NON-TRANSFERABLE RIGHT TO USE IT IN ACCORDANCE WITH THIS PACKAGE INSERT. NO OTHER RIGHTS ARE CONVEYED EXPRESSLY, BY IMPLICATION OR BY ESTOPPEL. FURTHERMORE, NO RIGHTS FOR RESALE ARE CONFERRED WITH THE PURCHASE OF THIS PRODUCT.

Copyright © Cepheid 2020. All rights reserved.

Cepheid AB

Rontgenvagen 5
SE-171 54 Solna
Sweden

Phone: + 46 8 6843 7000
Fax: + 46 8 6843 7010

Xpert® Flu Assay

For *in vitro* diagnostic use only.

1. Proprietary Name

Xpert® Flu

2. Common or Usual Name

Xpert Flu Assay

3. Intended Use

The Cepheid® Xpert Flu Assay, performed on the GeneXpert® Instrument Systems, is an automated, multiplex real-time RT-PCR assay intended for the *in vitro* qualitative detection and differentiation of influenza A, influenza B and 2009 H1N1 influenza viral RNA. The Xpert Flu Assay uses nasal aspirates/washes and nasopharyngeal swab specimens collected from patients with signs and symptoms of respiratory infection in conjunction with clinical and epidemiological risk factors. The Xpert Flu Assay is intended as an aid in the diagnosis of influenza.

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 2012-2013 influenza season. When other 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 department 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, which often occurs in the winter. Transmission of influenza is primarily airborne (*i.e.,* coughing or sneezing). 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 individual. Secondary bacterial pneumonia may develop as a complication after an influenza infection, causing increased morbidity and mortality in pediatric, elderly and immunocompromised populations.

Influenza viruses are classified into types A, B, and C, the first 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 can cause 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 cause epidemics more rarely. 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 viruses bearing hemagglutinin subtypes H1, H2, or H3, combined with neuraminidase subtypes N1 or N2, e.g., type H3N1. In addition to already circulating seasonal flu viruses, a novel H1N1 strain, which emerged in Mexico, was identified in humans in early 2009.

Active surveillance programs in conjunction with infection control precautions are important components for preventing transmission of influenza.

5. Principle of the Procedure

The Xpert Flu Assay is an automated *in vitro* diagnostic test for the qualitative detection of influenza A, influenza B, and influenza A subtype 2009 H1N1. The assay is performed on Cepheid GeneXpert Instrument Systems.

The GeneXpert Instrument Systems automate and integrate sample processing/lysis, purification, nucleic acid amplification, and detection of the target sequence in simple or complex samples using reverse transcriptase (RT) PCR and real-time PCR assays. The systems consist of an instrument, personal computer, and preloaded software for running tests and viewing the results. The systems require the use of single-use disposable GeneXpert cartridges that hold the RT-PCR and PCR reagents and host the RT-PCR and PCR processes. Because the cartridges are self-contained, 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 Assay includes reagents for the detection and differentiation of influenza A, influenza B, and influenza A subtype 2009 H1N1 directly from nasal aspirates/washes (NA/W) and nasopharyngeal (NP) swab specimens of 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 processing of the target viruses and to monitor 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.

6. Reagents and Instruments

6.1 Material Provided

Xpert Flu Assay kit contains sufficient reagents to process 10 specimens or quality control samples.

The kit contains the following:

Xpert Flu Assay Cartridges with Integrated Reaction Tubes	**10**
• Bead 1, Bead 2 and Bead 3 (freeze-dried)	1 of each per cartridge
• Lysis Reagent • Guanidine Thiocyanate	2.0 mL per cartridge
• Binding Reagent	1.5 mL per cartridge
• Elution Reagent	2.0 mL per cartridge
Disposable 300 µL Transfer Pipettes	2 bags of 12 per kit
CD • Assay Definition File (ADF) • Instructions to import ADF into GeneXpert software • Instructions for Use (Package Insert)	1 per kit

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

Note The bovine serum albumin (BSA) in the beads within this product was produced exclusively from bovine plasma sourced in the United States. The manufacturing of the BSA is also performed 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.

6.2 Storage and Handling

- Store the Xpert Flu Assay cartridges and reagents at 2-28°C until the date provided on the label.
- Do not use any reagents that have become cloudy or discolored.
- Do not use a cartridge that has leaked.

6.3 Materials Required but Not Provided

- 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: If a printer is required, contact Cepheid Technical Support to arrange for the purchase of a validated printer.

6.4 Materials Available but Not Provided

- Nasal Collection Kit, Cepheid catalog #NASL-100N-100 or Xpert Nasopharyngeal Sample Collection Kit, Cepheid catalog #SWAB/B-100.
- Inactivated virus controls from ZeptoMetrix:
    - Catalog Number NATFLUA/B-6MC and NATFLUAH1N1-6MC as external positive controls.
    - Catalog Number. NATCXVA9-6MC (Coxsackie virus) as an external negative control.

7. Warnings and Precautions

- 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 and the Clinical and Laboratory Standards Institute.
- Follow your institution's safety procedures for working with chemicals and handling biological samples.
- Do not substitute Xpert Flu Assay reagents with other reagents.
- Do not open the Xpert Flu Assay cartridge lid except when adding sample.
- Do not use a cartridge that has been dropped or shaken after sample is added.
- Do not use a cartridge that has a damaged reaction tube.

- Each single-use Xpert Flu Assay cartridge is used to process one test. Do not reuse spent cartridges.
- 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.
- Good laboratory practices and changing gloves between handling patient specimens are recommended to avoid contamination of specimens.

- Store the Xpert Flu Assay kit at 2–28 °C.

8. Chemical Hazards[8,9]

- Signal Word: WARNING
- **UN GHS Hazard Statements**
    - May be harmful if swallowed
    - Causes mild skin irritation
    - Causes eye irritation
- **Precautionary Statements**
    - **Prevention**
        - Wash 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.

9. Specimen Collection, Transport and Storage

NA/W or NP swab specimens can be collected following the user institution's standard procedures and placed into Universal Transport Medium (3 mL UTM tubes).

Samples should be transported at 2–8 °C. Samples can be stored for up to 72 hours at 2–8 °C before processing.

10. Procedure

10.1 Preparing the cartridge

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

For NP swab specimens

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

For NA/W specimens

1. Using a clean 300 µL transfer pipette (supplied), transfer 600 µL of the sample (two draws) into the 3 mL UTM tube and then cap the tube.
2. Mix specimen by inverting the 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 chamber with the large sample opening in the cartridge. See Figure 1.
5. Close the cartridge lid. See Figure 1.

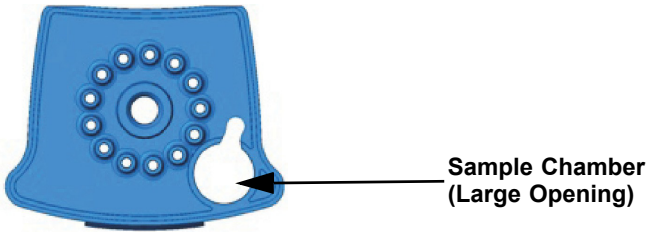

Figure 1. Xpert Flu Assay Cartridge (Top View)

10.2 Starting the Test

Note Before starting the test, make sure the Xpert Flu Assay Definition File is imported into the GeneXpert 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.

1. Turn on the GeneXpert instrument:
   - If using the GeneXpert Dx instrument, first turn on the GX Dx instrument, and then turn on the computer. The GeneXpert 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 **Orders** and **Order Test** (Infinity).

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 result.
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 result.
6. Scan the barcode on the Xpert Flu Assay cartridge. Using the barcode information, the software automatically fills the boxes for the following fields: Reagent Lot ID, Cartridge SN, Expiration Date and Selected Assay.
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.
   D. Dispose of used cartridges in an appropriate specimen waste container according to your institution's standard practices.

11. 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 screen to view and/or generate a pdf report file.

12. Quality Control

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

- **Sample Processing Control (SPC)** – Ensures the sample was correctly processed. The SPC is an Armored RNA® in the form of a dry bead that is included in each cartridge to verify adequate processing of the sample virus. The SPC verifies that lysis of influenza virus has occurred if the organism is present and verifies that the specimen processing is adequate. Additionally this control detects specimen-associated inhibition of the RT-PCR and PCR reactions. The SPC should be positive in a negative sample and can be negative or positive in a positive sample. The SPC passes if it meets the validated acceptance criteria.
- **Probe Check Control (PCC)** – Before the start of the PCR reaction, the GeneXpert Instrument System measures the fluorescence signal from the probes to monitor bead rehydration, reaction tube filling, probe integrity and dye stability. The PCC passes if it meets the validated acceptance criteria.
- **External Controls** – External controls should be used in accordance with local, state, and federal accrediting organizations' requirements as applicable.

13. Interpretation of Results

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. The possible results are shown in Table 1.

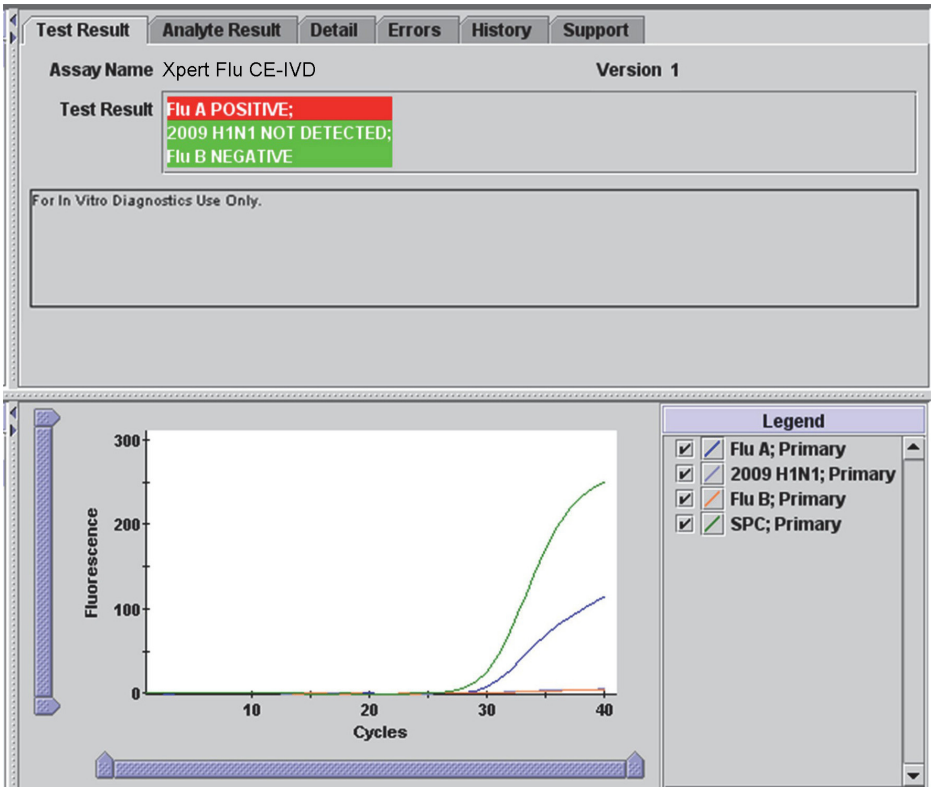

Figure 2. GeneXpert Dx View Results Window: Example of Flu A Positive Result

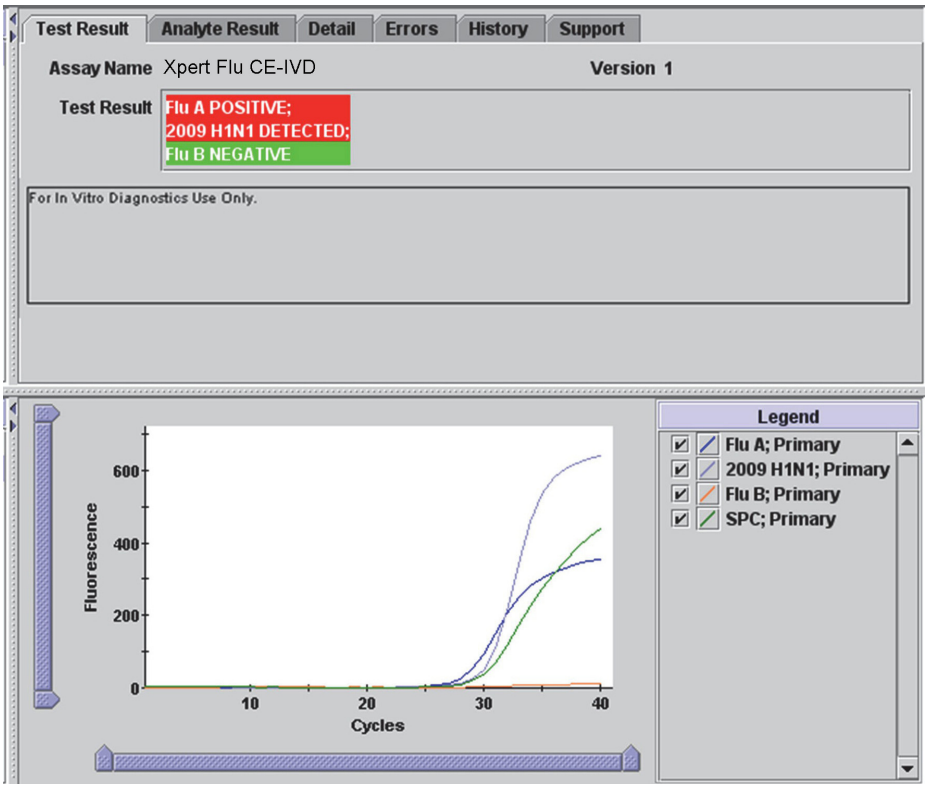

Figure 3. GeneXpert Dx View Results Window: Example of 2009 H1N1 Positive Result

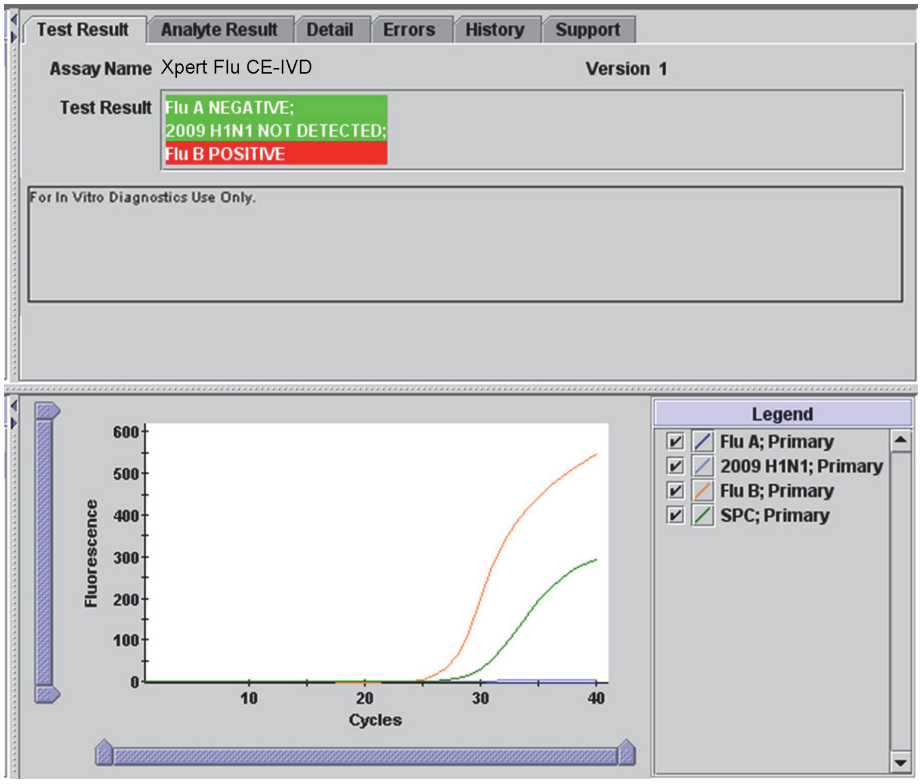

Figure 4. GeneXpert Dx View Results Window: Example of Flu B Positive Result

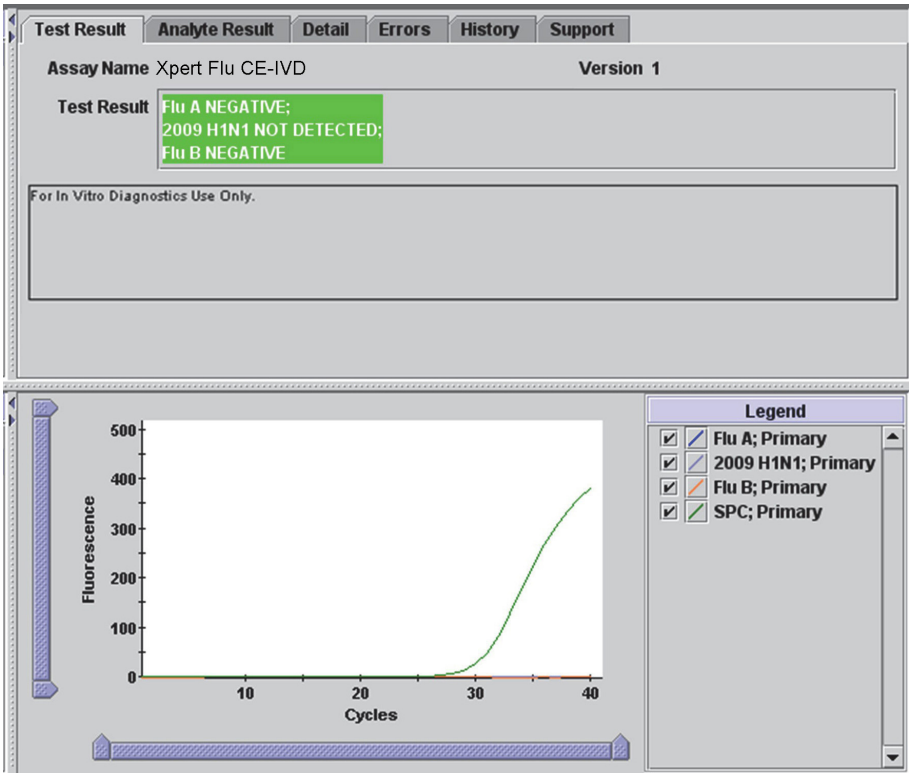

Figure 5. GeneXpert Dx View Results Window: Example of a Negative Result

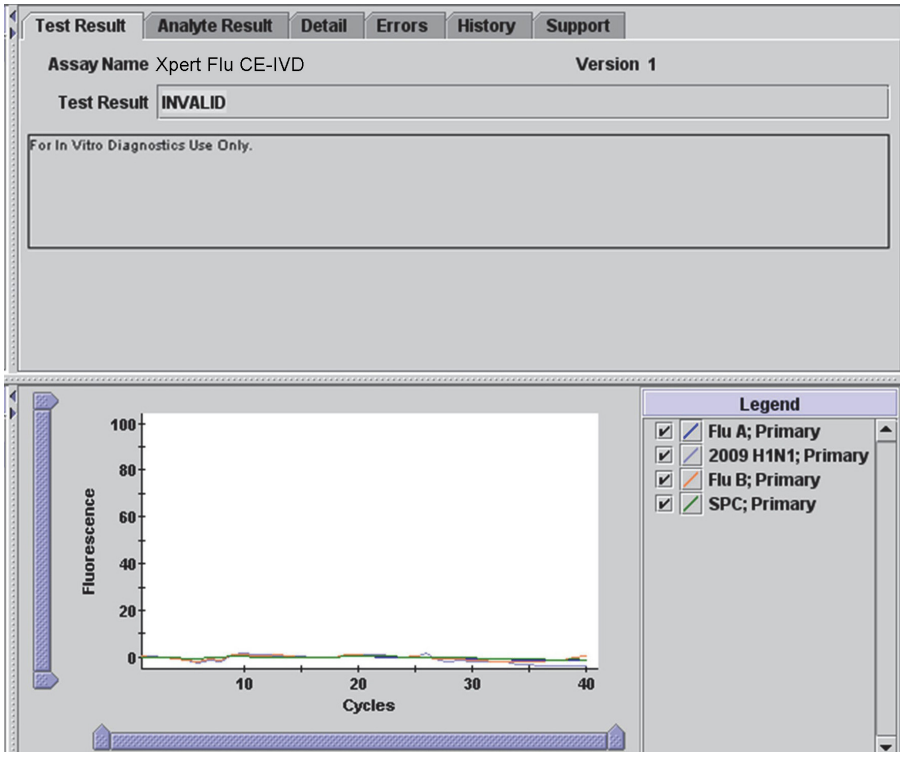

Figure 6. GeneXpert Dx View Results Window: Example of an Invalid Result (SPC does not meet Acceptance Criteria)

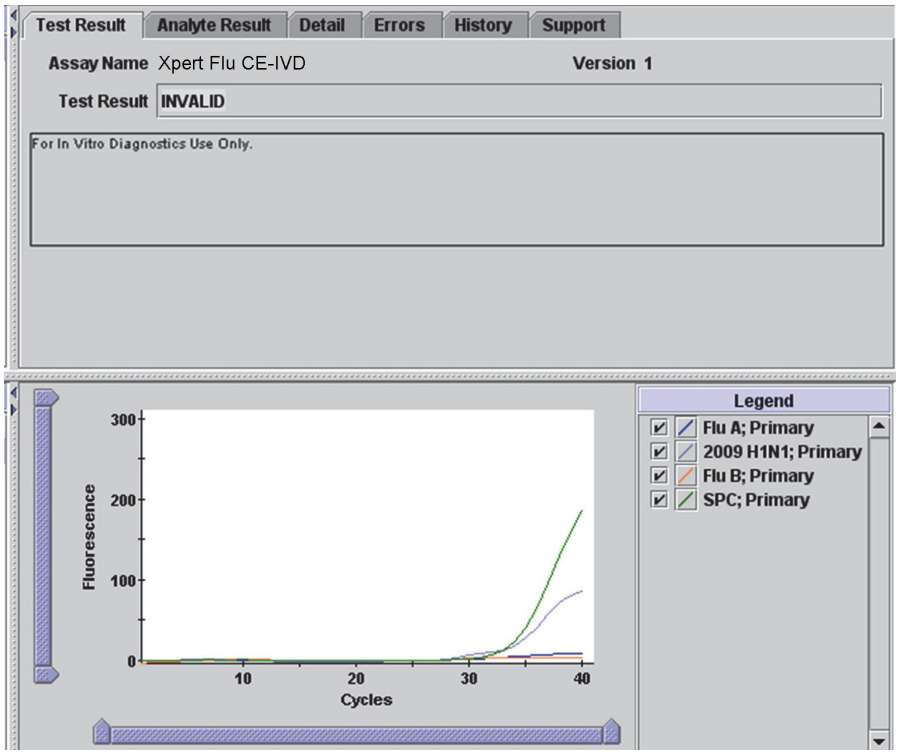

Figure 7. GeneXpert Dx View Results Window: Example of an Invalid Result (Flu A Negative & 2009 H1N1 Positive)

Table 1. Xpert Flu Assay Results and Interpretation

Result	Interpretation
Flu A POSITIVE; 2009 H1N1 NOT DETECTED; Flu B NEGATIVE (Figure 2)	Flu A target RNA is detected; 2009 H1N1 target RNA is not detected; Flu B target RNA is not detected. • The Flu A target has a Ct within the valid range and endpoint above the minimum setting. • SPC – NA (not applicable); SPC is ignored because the Flu A target amplification may compete with this control. • Probe Check – PASS; all probe check results pass.
Flu A POSITIVE; 2009 H1N1 DETECTED; Flu B NEGATIVE (Figure 3)	Flu A target RNA is detected; 2009 H1N1 target RNA is detected; Flu B target RNA is not detected. • The Flu A target has a Ct within the valid range and endpoint above the minimum setting. • The 2009 H1N1 target has a Ct within the valid range and endpoint above the minimum setting. • SPC – NA (not applicable); SPC is ignored because the Flu A and 2009 H1N1 target amplification may compete with this control. • Probe Check – PASS; all probe check results pass.
Flu A NEGATIVE; 2009 H1N1 NOT DETECTED; Flu B POSITIVE (Figure 4)	Flu A target RNA is not detected; 2009 H1N1 target RNA is not detected; Flu B target RNA is detected. • The Flu B target has a Ct within the valid range and endpoint above the minimum setting. • SPC – NA (not applicable); SPC is ignored because the Flu B target amplification may compete with this control. • Probe Check – PASS; all probe check results pass.
Flu A NEGATIVE; 2009 H1N1 NOT DETECTED; Flu B NEGATIVE (Figure 5)	Flu A target RNA is not detected; 2009 H1N1 target RNA is not detected; Flu B target RNA is not detected. SPC meets acceptance criteria. • Flu A, 2009 H1N1 and Flu B target RNAs are not detected. • SPC – PASS; SPC has a Ct within the valid range and endpoint above the minimum setting. • Probe Check – PASS; all probe check results pass.

Note The following two outcomes, while possible, are rare mixed infections.

Result	Interpretation
Flu A POSITIVE; 2009 H1N1 NOT DETECTED; Flu B POSITIVE	Flu A target RNA is detected; 2009 H1N1 target RNA is not detected; Flu B target RNA is detected. • The Flu A target has a Ct within the valid range and endpoint above the minimum setting. • The Flu B target has a Ct within the valid range and endpoint above the minimum setting. • SPC – NA (not applicable); SPC is ignored because the Flu A and 2009 H1N1 target amplification may compete with this control. • Probe Check – PASS; all probe check results pass.
Flu A POSITIVE; 2009 H1N1 DETECTED; Flu B POSITIVE	Flu A target RNA is detected; 2009 H1N1 target RNA is detected; Flu B target RNA is detected. • The Flu A target has a Ct within the valid range and endpoint above the minimum setting. • The 2009 H1N1 target has a Ct within the valid range and endpoint above the minimum setting. • The Flu B target has a Ct within the valid range and endpoint above the minimum setting. • SPC – NA (not applicable); SPC is ignored because the Flu A and Flu B target amplification may compete with this control. • Probe Check – PASS; all probe check results pass.

Result	Interpretation
INVALID (Figure 6 and Figure 7)	1. SPC does not meet acceptance criteria. Presence or absence of the target RNAs cannot be determined (Figure 6). Repeat test according to the instructions in Section 14.2, Retest Procedure. • SPC – FAIL; SPC result is negative and the SPC Ct is not within valid range and the endpoint is below the minimum setting. • Probe Check – PASS; all probe check results pass. Or 2. Presence or absence of 2009 H1N1 target RNA cannot be determined (Figure 7). Repeat test according to the instructions in Section 14.2, Retest Procedure. • Flu A and Flu B target RNA are not detected and 2009 H1N1 target RNA is detected. • SPC – NA (not applicable); SPC is ignored because a target is amplified. • Probe Check – PASS; all probe check results pass.
ERROR	Presence or absence of Flu A, 2009 H1N1, and Flu B target RNAs cannot be determined. Repeat test according to the instructions in Section 14.2, Retest Procedure. • 2009 H1N1 – NO RESULT • Flu A – NO RESULT • Flu B – NO RESULT • SPC – NO RESULT • Probe Check – FAIL[a]; all or one of the probe check results fail.
NO RESULT	Presence or absence of Flu A, 2009 H1N1, and Flu B target RNAs cannot be determined. Repeat test according to the instructions in Section 14.2, Retest Procedure. A NO RESULT indicates that insufficient data were collected. For example, the operator stopped a test that was in progress. • 2009 H1N1 – NO RESULT • Flu A – NO RESULT • Flu B – NO RESULT • SPC – NO RESULT • Probe Check – NA (not applicable).

a. If the probe check passed, the error is caused by the maximum pressure limit exceeding the acceptable range or by a system component failure.

14. Retests

14.1 Reasons to Repeat the Assay

If any of the test results mentioned below occur, repeat the test according to instructions in Section 14.2, Retest Procedure.

- An **INVALID** result indicates one or more of the following:
  - The control SPC failed;
  - Flu A target RNA is not detected and 2009 H1N1 target RNA is detected;
  - The sample was not properly processed or PCR was inhibited.
- An **ERROR** result indicates that the assay was aborted. Possible causes include: the reaction tube was filled improperly; a reagent probe integrity problem was detected; or the maximum pressure limit was 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.

14.2 Retest Procedure

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

For NP swabs use 300 µL of the left over specimen from original UTM tube.

For NA/W use 300 µL of the left over diluted specimen from the 3 mL UTM tube.

1. Remove a new cartridge from the kit.
2. Mix the specimen by inverting the tube five times.
3. Open the cartridge lid. Using a clean 300 µL transfer pipette (supplied), transfer 300 µL (one draw) of the diluted specimen to the chamber with large Sample opening in the cartridge (see Figure 1).
4. Close the cartridge lid.

15. Limitations

- The performance of the Xpert Flu Assay was validated using the procedures provided in this package insert only. Modifications to these procedures may alter the performance of the test.
- Good laboratory practices and changing gloves between handling patient specimens are recommended to avoid contamination of specimens or reagents.
- Results from the Xpert Flu 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 infection and should not be used as the sole basis for treatment or other patient management decisions.
- Results from the Xpert Flu 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 may not be detected or may be detected less predictably.
- 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 product 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. Interference by substances other than those described can lead to erroneous results.
- Cross-reactivity with respiratory tract organisms other than those contained herein can lead to erroneous results.
- This assay has not been evaluated for patients receiving intranasal administered influenza vaccine.
- This assay has not been evaluated for immunocompromised individuals.

16. Performance Characteristics

16.1 Clinical Performance

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

Subjects included individuals whose routine care called for collection of NA/W or NP swab specimens for influenza testing. For eligible subjects, aliquots of leftover sample were obtained for testing with the Xpert Flu Assay and reference testing, and patient management continued at the site per the standard practice.

The Xpert Flu Assay (for purposes of this Clinical Performance section, referred to as the New Xpert Flu Assay) performance was compared to the Xpert Flu Assay currently marketed in the U.S. (for purposes of this Clinical Performance section, referred to as the Xpert Flu Assay). Bi-directional sequencing was performed to resolve any discrepancies between assays.

16.2 Overall Results

A total of 482 specimens (255 NP swab and 227 NA/W) were tested by both Xpert Flu Assays.

Relative to the Xpert Flu Assay, the New Xpert Flu Assay demonstrated a positive and negative agreement for detection of influenza A in NP swabs of 100% and 98.6%, respectively (Table 2). The New Xpert Flu Assay positive and negative agreement for influenza A subtype 2009 H1N1 in NP swabs was 100% and 99.6% (Table 3). The New Xpert Flu Assay positive and negative agreement for influenza B in NP swabs were 100% and 95.7%, respectively (see Table 4).

Table 2. New Xpert Flu Assay Performance NP Swab Specimens: Influenza A

New Xpert Flu	Xpert Flu		
	Pos	Neg	Total
Pos	48	3[a]	51
Neg	0	204	204
Total	48	207	255
Positive Agreement:	100% (95% CI: 94.7-100)		
Negative Agreement:	98.6% (95% CI: 95.8-99.5)		

a. Discrepant testing results by sequencing: 3 of 3 Flu A positive (1 of the 3 was also H1N1 positive.

Table 3. New Xpert Flu Assay Performance on NP Swab Specimens: Influenza A, 2009 H1N1

New Xpert Flu	Xpert Flu		
	Pos	Neg	Total
Pos	21	1[a]	22
Neg	0	233	233
Total	21	234	255
Positive Agreement:	100% (95% CI: 88.6-100)		
Negative Agreement:	99.6% (95% CI: 97.6-99.9)		

a. Discrepant testing results by sequencing: 1 of 1 H1N1 positive.

Table 4. New Xpert Flu Assay Performance on NP Swab Specimens: Influenza B

New Xpert Flu	Xpert Flu		
	Pos	Neg	Total
Pos	67	8[a]	75
Neg	0	180	180
Total	67	188	255
Positive Agreement:	100% (95% CI: 96.1-100)		
Negative Agreement:	95.7% (95% CI: 91.8-97.8)		

a. Discrepant testing results by sequencing: 8 of 8 Flu B positive.

Relative to the Xpert Flu Assay, the New Xpert Flu Assay demonstrated a positive and negative agreement for detection of influenza A in NA/W specimens of 100% and 96.0%, respectively (Table 5). The New Xpert Flu Assay positive and negative agreement for influenza A subtype 2009 H1N1 with NA/W specimens were 100% and 99.5% (Table 6). The New Xpert Flu Assay positive and negative agreement for influenza B with NA/W specimens were 100% and 98.9%, respectively (Table 7).

Table 5. New Xpert Flu Assay Performance on NA/W Specimens: Influenza A

		Xpert Flu		
		Pos	Neg	Total
New Xpert Flu	Pos	101	5[a]	106
	Neg	0	121	121
	Total	101	126	227
		Positive Agreement:	100% (95% CI: 97.4-100)	
		Negative Agreement:	96.0% (95% CI: 91.1-98.3)	

a. Discrepant testing results by sequencing: 5 of 5 Flu A positive (1 of the 5 was also H1N1 positive).

Table 6. New Xpert Flu Assay Performance on NA/W Specimens: Influenza A, 2009 H1N1

		Xpert Flu		
		Pos	Neg	Total
New Xpert Flu	Pos	20	1[a]	21
	Neg	0	206	206
	Total	20	207	227
		Positive Agreement:	100% (95% CI: 88.1-100)	
		Negative Agreement:	99.5% (95% CI: 97.3-99.9)	

a. Discrepant testing results by sequencing: 1 of 1 H1N1 positive.

Table 7. New Xpert Flu Assay Performance on NA/W Specimens: Influenza B

		Xpert Flu		
		Pos	Neg	Total
New Xpert Flu	Pos	47	2[a]	49
	Neg	0	178	178
	Total	47	180	227
		Positive Agreement:	100% (95% CI: 94.6-100)	
		Negative Agreement:	98.9% (95% CI: 96.0-99.7)	

a. Discrepant testing results by sequencing: 2 of 2 Flu B positive.

17. Analytical Performance

17.1 Analytical Sensitivity (Limit of Detection)

Studies were performed to determine the analytical Limit of Detection (LoD) of 2 seasonal influenza A (H1N1), 2 seasonal influenza A (H3N2), 2 influenza A 2009 H1N1 and 2 influenza B strains diluted into a surrogate nasopharyngeal 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 8 through Table 11.

Table 8. LoD ($TCID_{50}$/mL) – Seasonal Influenza A H1N1

Strain ID - Influenza A subtype H1N1	Confirmed LoD ($TCID_{50}$/mL) [at least 19/20 positive]	Probit Regression ($TCID_{50}$/mL)		
		LoD Point Estimate	Lower 95% CI	Upper 95% CI
A/Brisbane/59/07	0.2 (19/20)	0.2	0.14	0.23
A/New Caledonia/20/1999	30 (20/20)	12.7	10.4	17.01

Table 9. LoD ($TCID_{50}$/mL) – Seasonal Influenza A H3N2

Strain ID - Influenza A subtype H3N2	Confirmed LoD ($TCID_{50}$/mL) [at least 19/20 positive]	Probit Regression ($TCID_{50}$/mL)		
		LoD Point Estimate	Lower 95% CI	Upper 95% CI
A/Perth/16/2009	1 (20/20)	0.2	0.1	0.3
A/Victoria/361/2011	0.5 (20/20)	0.4	0.3	0.6

Table 10. LoD ($TCID_{50}$/mL) – Influenza A 2009 H1N1

Strain ID - Influenza A subtype 2009 H1N1	Confirmed LoD ($TCID_{50}$/mL) [at least 19/20 positive]	Probit Regression ($TCID_{50}$/mL)		
		LoD Point Estimate	Lower 95% CI	Upper 95% CI
A/SwineNY/01/2009	0.5 (20/20)	0.4	0.3	0.6
A/SwineCanada/6294	100 (20/20)	93.3	82.5	113.3

Table 11. LoD ($TCID_{50}$/mL) – Influenza B

Strain ID - Influenza B	Confirmed LoD ($TCID_{50}$/mL) [at least 19/20 positive]	Probit Regression ($TCID_{50}$/mL)		
		LoD Point Estimate	Lower 95% CI	Upper 95% CI
B/Florida/07/04	0.9 (20/20)	0.4	0.3	0.5
B/Wisconsin/01/10	25 (19/20)	18.1	14.2	26.9

17.2 Analytical Specificity (Exclusivity)

The analytical specificity of the Xpert Flu Assay was evaluated by testing a panel of 40 cultures consisting of 18 viral, 21 bacterial, and one yeast representing common respiratory pathogens or those potentially encountered in the nasopharynx. Three replicates of all bacterial and yeast strains were tested at concentrations $\geq 10^6$ CFU/mL. Three replicates of all viruses were tested at concentrations $\geq 10^4$ $TCID_{50}$/mL. Purified nucleic acids (copies/mL) were tested for one virus strain (Cytomegalovirus) and one bacterial strain (*Bordetella pertussis*). The analytical specificity was 100%. Results are shown in Table 12.

Table 12. Analytical Specificity of Xpert Flu Assay[a]

Strain	Concentration (per Cartridge)	Influenza A	Influenza A 2009 H1N1	Influenza B
Adenovirus Type 7A	1.1 x10^6 $TCID_{50}$/mL	-	-	-
Adenovirus Type 1	1.1 x10^7 $TCID_{50}$/mL	-	-	-
Human Coronavirus 229E	2.0x10^4 $TCID_{50}$/mL	-	-	-
Human Coronavirus OC43	5.6 x10^4 $TCID_{50}$/mL	-	-	-
Cytomegalovirus[b]	4.7x10^7 Copies /mL	-	-	-
Enterovirus Type 71	3.5 x10^5 $TCID_{50}$/mL	-	-	-
Epstein-Barr Virus	7.1x10^8 $TCID_{50}$/mL	-	-	-
Parainfluenzavirus Type 1	1.1 x10^6 $TCID_{50}$/mL	-	-	-
Parainfluenzavirus Type 2	3.1 x10^7 $TCID_{50}$/mL	-	-	-
Parainfluenzavirus Type 3	1.9 x10^6 $TCID_{50}$/mL	-	-	-
Measles Virus	6.3 x10^4 $TCID_{50}$/mL	-	-	-
Human Metapneumovirus	3.8 x10^5 $TCID_{50}$/mL	-	-	-
Mumps Virus	6.3 x10^6 $TCID_{50}$/mL	-	-	-
Respiratory Syncytial Virus A	5.3 x10^7 $TCID_{50}$/mL	-	-	-
Respiratory Syncytial Virus B	1.2 x10^7 $TCID_{50}$/mL	-	-	-
Human HSV Type 1	8.9 x10^6 $TCID_{50}$/mL	-	-	-
Human Rhinovirus Type 4	1.2 x10^6 $TCID_{50}$/mL	-	-	-
Echovirus 11	3.3 x10^9 $TCID_{50}$/mL	-	-	-
Bordetella pertussis[c]	5000 ng/mL	-	-	-
Chlamydia pneumoniae	5 x10^7 CFU/mL	-	-	-
Corynebacterium xerosis	1x10^6 CFU/mL	-	-	-
Escherichia coli	1x10^6 CFU/mL	-	-	-
Proteus vulgaris	1x10^6 CFU/mL	-	-	-
Proteus mirabilis	1x10^6 CFU/mL	-	-	-
Klebsiella pneumoniae	1x10^6 CFU/mL	-	-	-
Haemophilus influenzae	1x10^6 CFU/mL	-	-	-
Lactobacillus crispatus	1x10^6 CFU/mL	-	-	-
Legionella pneumophila	1x10^6 CFU/mL	-	-	-
Moraxella catarrhalis	1x10^6 CFU/mL	-	-	-

Table 12. Analytical Specificity of Xpert Flu Assay[a] (Continued)

Strain	Concentration (per Cartridge)	Influenza A	Influenza A 2009 H1N1	Influenza B
Mycobacterium tuberculosis (BCG strain)	$1x10^6$ CFU/mL	-	-	-
Mycoplasma pneumoniae	$1x10^6$ CFU/mL	-	-	-
Neisseria meningitides	$1x10^6$ CFU/mL	-	-	-
Neisseria cinneria	$1x10^6$ CFU/mL	-	-	-
Pseudomonas aeruginosa	$1x10^6$ CFU/mL	-	-	-
Staphylococcus aureus	$1x10^6$ CFU/mL	-	-	-
Staphylococcus epidermidis	$1x10^6$ CFU/mL	-	-	-
Streptococcus pneumoniae	$1x10^6$ CFU/mL	-	-	-
Streptococcus pyogenes	$1x10^6$ CFU/mL	-	-	-
Streptococcus salivarius	$1x10^6$ CFU/mL	-	-	-
Candida albicans	$1x10^6$ CFU/mL	-	-	-

a. Cross reactivity with other swine origin strains has not been tested.
b. Nucleic acid was tested for Cytomegalovirus.
c. Nucleic acid was tested for *Bordetella pertussis.*

17.3 Analytical Reactivity (Inclusivity)

The analytical reactivity of the Xpert Flu Assay was evaluated against forty one (41) strains of influenza A (H1N1, H3N2, H5N2, H5N1 and H7N3 subtypes), influenza A 2009 H1N1 and influenza B. Of these, influenza A subtype H1N1 (10), influenza A subtype H3N2 (8), influenza A subtype H3N2v (2), influenza A subtype 2009 H1N1 (6), influenza A subtype H5N1 (1), influenza A subtype H5N2 (1), influenza A subtype H7N3 (1), and influenza B (12) were included. Eight of the forty-one influenza strains evaluated in this study were tested at the LoD concentration while all remaining strains were tested using viral stocks at 5-250 $TCID_{50}$/mL. Three (3) replicates were tested for each strain. Results are shown in Table 13.

Table 13. Analytical Reactivity (Inclusivity) of Xpert Flu Assay

Strain	$TCID_{50}$/mL	Influenza A	Influenza A 2009 H1N1	Influenza B
A/Swine/Iowa/15/30 (Swine H1N1)	50	+	-	-
A/Mal/302/54 (H1N1)	50	+	-	-
A/New Jersey/8/76 (H1N1)	250	+	-	-
A/NewYork/55/2004 (H1N1)	50	+	-	-
A/PR/8/34 (H1N1)	100	+	-	-
A/Denver/1/57 (H1N1)	250	+	-	-
A/Brisbane/59/07[a] (H1N1)	0.2	+	-	-
A/NewCalendonia/20/1999[a] (H1N1)	30	+	-	-
A/WS/33 (H1N1)	5	+	-	-
A/Taiwan/42/06 (H1N1)	50	+	-	-
A/Aichi/2/68 (H3N2)	100	+	-	-
A/Hawaii/15/2001 (H3N2)	50	+	-	-
A/Hong Kong/8/68 (H3N2)	50	+	-	-
A/Port Chalmers/1/73 (H3N2)	50	+	-	-

Table 13. Analytical Reactivity (Inclusivity) of Xpert Flu Assay (Continued)

Strain	$TCID_{50}$/mL	Influenza A	Influenza A 2009 H1N1	Influenza B
A/Wisconsin/67/05 (H3N2)	50	+	-	-
A/Perth/16/2009[a] (H3N2)	1	+	-	-
A/Victoria/361/2011[a] (H3N2)	0.5	+	-	-
A/Brisbane/10/07 (H3N2)	25	+	-	-
A/Indiana/08/2011 (H3N2v)	5	+	-	-
A/Minnesota/11/2010 (H3N2v)	250	+	-	-
A/California/7/2009 (09 H1N1)	0.5	+	+	-
A/SwineNY/03/2009 (09 H1N1)	250	+	+	-
A/WI/929-S1 (09 H1N1)	50	+	+	-
A/Canada/6294[a] (09 H1N1)	100	+	+	-
A/SwineNY/01/2009[a] (09 H1N1)	0.5	+	+	-
A/SwineNY/02/2009 (09 H1N1)	100	+	+	-
A/Anhui/02/2005/PR8-IBCDC-RG5 (H5N1)[b]	1.2e-4[b]	+	-	-
A/chicken/NJ/15086-3/94 (H7N3)[b]	1.2e-4[b]	+	-	-
A/Mallard/WI/34/75 (H5N2)[b]	3.9e-4[b]	+	-	-
B/Allen/45	50	-	-	+
B/Florida/04/06	50	-	-	+
B/Florida/02/06	25	-	-	+
B/GL/1739/54	50	-	-	+
B/Hong Kong/5/72	250	-	-	+
B/Lee/40	50	-	-	+
B/Malaysia/2506/04	50	-	-	+
B/Taiwan/2/62	50	-	-	+
B/Maryland/1/59	5	-	-	+
B/Panama/45/90	5	-	-	+
B/Florida/07/04[a]	0.9	-	-	+
B/Wisconsin/01/2010[a]	25	-	-	+

a. Strains (n=8) used in analytical LoD study (D16266) and tested at limit of detection concentration.
b. Concentration expressed in picograms/µL.

17.4 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 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. These substances are listed in Table 11 with active ingredients and concentrations tested shown.

Negative samples (n=8) were tested in each substance to determine the effect on the performance of the sample processing control (SPC). Positive samples (n=8) were tested per substance with 2 seasonal Flu A H1N1 strains (A/Brisbane/59/07 and A/New Calendonia/20/1999), 2 seasonal Flu A H3N2 strains (A/Perth/16/09 and A/Victoria/361/2011), 2 Flu A 2009 H1N1 strains (A/SwineNY/01/2009 and A/SwineNY/02/2009) and 2 Flu B strains (B/Wisconsin/01/2011 and B/Florida/07/04) spiked near the analytical LoD determined for each isolate.

All results were compared to positive and negative controls prepared in Universal Transport Medium (UTM). All positive and negative specimens were correctly reported using the Xpert Flu Assay.

None of the potentially interfering substances resulted in a Ct difference of ≥1 cycle relative to the buffer controls and no false-negative results were reported.

None of the potentially interfering substances had a statistically significant inhibitory effect on SPC performance in negative samples (p-value = >0.05).

All of the positive Flu A specimens were correctly reported **Flu A POSITIVE; 2009 H1N1 NOT DETECTED; Flu B NEGATIVE** using the Xpert Flu Assay.

All of the positive Flu A 2009 H1N1 specimens were correctly reported **Flu A POSITIVE; 2009 H1N1 DETECTED; Flu B NEGATIVE** using the Xpert Flu Assay.

All of the positive Flu B specimens were correctly reported **Flu A NEGATIVE; 2009 H1N1 NOT DETECTED; Flu B POSITIVE** using the Xpert Flu Assay.

Table 14. Potentially Interfering Substances in Xpert Flu Assay

Substance	Description/Active Ingredient	Concentration Tested
Blood (human)	N/A	1% (v/v)
Mucin	Purified mucin protein (Bovine or porcine submaxillary gland)	2.5% (w/v)
Neo-Synephrine® Nasal Drops	Phenylephrine HCl	15% (v/v)
Anefrin Nasal Spray	Oxymetazoline Hydrochloride	15% (v/v)
Zicam® Nasal Gel	Luffa opperculata, Galphimia glauca, Histaminum hydrochloricum Sulfur	15% (w/v)
Saline Nasal Spray	Sodium Chloride with preservatives	15% (v/v)
Antibiotic, nasal ointment	Mupirocin	10 mg/mL
Antibacterial, systemic	Tobramycin	4.0 µg/mL
Antiviral	Oseltamivir Phosphate (TamiFlu®)	7.5 mg/mL
Throat lozenges, oral anesthetic and analgesic	Menthol	1.7 mg/mL

17.5 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 following 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 subtype 2009 H1N1 sample (approximately 10^6 $TCID_{50}$/test) and influenza B sample (approximately 10^6 $TCID_{50}$/test). This testing scheme was repeated 20 times on a single GeneXpert module for a total of 41 runs resulting in 20 positive and 21 negative specimens. All 20 positive samples were correctly reported **Flu A POSITIVE; 2009 H1N1 DETECTED; Flu B POSITIVE**. All 21 negative samples were correctly reported **Flu A NEGATIVE; 2009 H1N1 NOT DETECTED; Flu B NEGATIVE**.

18. References

1. Petric M, Comanor L, Petti CA. Role of the laboratory in diagnosis of influenza during seasonal epidemics and potential pandemics. J Infect Dis. 2006;194:S98-110.
2. Schweiger B, Zadow I, Heckler R, et al. Application of a fluorogenic PCR assay for typing and subtyping of influenza viruses in respiratory samples. J Clin Micro. 2000;38:1552-1558.
3. Center for Disease Control and Prevention, Seasonal Influenza. http://www.cdc.gov
4. Accessed on September 19, 2012.
5. Centers for Disease Control and Prevention. Biosafety in Microbiological and Biomedical laboratories. Richmond JY and McKinney RW (eds) (1993). HHS Publication number (CDC) 93-8395.
6. Interim Biosafety Guidance for All Individuals Handling Clinical Specimens or Isolates Containing 2009-H1N1 influenza A Virus (Novel H1N1), including Vaccine Strains, August 15, 2009; (http://www.cdc.gov/h1n1flu/guidelines_labworkers.htm).
7. Clinical and Laboratory Standards Institute. Protection of Laboratory Workers from Occupationally Acquired Infections; Approved Guideline. Document M29 (refer to latest edition.
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) No 1907/2007).
9. Occupational Safety and Health Standards, Hazard Communication, Toxic and Hazard Substances (March 26, 2012) (29 C.F.R., pt. 1910, subpt. Z).

19 Cepheid Headquarters Locations

Corporate Headquarters

Cepheid
904 Caribbean Drive
Sunnyvale, CA 94089
United States

Telephone: + 1 408 541 4191

Fax: + 1 408 541 4192

www.cepheid.com

European Headquarters

Cepheid Europe SAS
Vira Solelh
81470 Maurens-Scopont
France

Telephone: + 33 563 825 300

Fax: + 33 563 825 301

www.cepheidinternational.com

20 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

Telephone: + 1 888 838 3222

Email: techsupport@cepheid.com

France

Telephone: + 33 563 825 319

Email: support@cepheideurope.com

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

21. Table of Symbols

Symbol	Meaning
	Catalog number
	*In vitro* diagnostic medical device
	CE marking – European Conformity
	Do not reuse
	Batch code
	Consult instructions for use
	Caution
	Manufacturer
	Country of manufacture
	Contains sufficient for <n> tests
	Control
	Expiration date
	Temperature limitation
	Biological risks
	Warning

Cepheid AB

Rontgenvagen 5
SE-171 54 Solna
Sweden
Product of Sweden