The information previously contained on these webpages were authorized under the 2009 H1N1 Influenza Emergency Use Authorizations (EUAs). As of June 23, 2010, the EUAs have been terminated and this information is no longer current.

Xpert Flu A Panel Assay

Emergency Use Authorization: For *in vitro* Diagnostic Use Only

Proprietary Name

Xpert® Flu A Panel

Common or Usual Name

Xpert Flu A Panel

Intended Use

The Cepheid Xpert® Flu A Panel is an automated, multiplex real-time RT-PCR assay intended for use in laboratories certified under the Clinical Laboratory Improvement Amendments of 1988 (CLIA), 42 U.S.C. §263a, to perform moderate complexity tests and in laboratories certified under CLIA to perform high complexity tests using the Cepheid GeneXpert® Dx System for the *in vitro* qualitative detection and differentiation of 2009 H1N1 influenza viral RNA. The Xpert Flu A Panel 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.

Testing with the Xpert Flu A Panel should not be performed unless the patient meets clinical and epidemiologic criteria for testing suspect specimens. The identification of 2009 H1N1 influenza should be performed along with a clinical and epidemiological assessment.

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

Summary and Explanation

Influenza, or the flu, is a contagious viral infection of the respiratory tract, which often occurs in the winter. Influenza viruses are classified into types A, B, and C. Type A is the most common type of influenza virus in humans, and is 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.[1]

Active surveillance programs in conjunction with infection control precautions are important components for preventing transmission of influenza. The use of assays providing rapid results to identify and differentiate patients infected with seasonal and novel H1N1 flu is also an important factor for effective control, proper choice of treatment, and prevention of widespread outbreaks of influenza.

Principle of the Procedure

The Xpert Flu A Panel is a rapid, automated *in vitro* diagnostic test for qualitative detection and differentiation of 2009 H1N1 influenza viral RNA. The assay is performed on the Cepheid GeneXpert Dx System.

The GeneXpert® Dx System automates and integrates sample purification, nucleic acid amplification, and detection of the target sequence in simple or complex samples using real-time RT-PCR and PCR assays. The system consists of an instrument, personal computer, and preloaded software for running tests and viewing the results. The system requires the use of single-use disposable 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 system, see the GeneXpert Dx System Operator Manual.

The Xpert Flu A Panel includes reagents for the detection and differentiation of 2009 H1N1 influenza viral RNA in nasal aspirates/washes (NA/W) and nasopharyngeal (NP) swab specimens in viral transport media (VTM) or universal transport media (UTM) from patients suspected of having influenza. The assay detects a well-conserved region of the matrix gene from influenza A viruses (Flu A target) and the hemagglutinin gene of 2009 H1N1 influenza virus (2009 H1N1 target). A Sample Processing Control (SPC) and a Probe Check Control (PCC) are also included. The SPC is present to control for adequate processing of the target viruses and to monitor the presence of inhibitors in the RT-PCR and PCR reactions. The Probe Check Control (PCC) verifies reagent rehydration, RT-PCR/PCR tube filling in the cartridge, probe integrity and dye stability.

Reagents and Instruments

Material Provided

The Xpert Flu A Panel kit contains sufficient reagents to process 10 specimens or quality control samples. The kit contains the following:

Xpert Flu A Panel Cartridges with integrated reaction tubes 10

Bead 1 (freeze-dried) 1 per cartridge
- Polymerase
- Salts

Bead 2 (freeze-dried) 1 per cartridge
- Primers
- Probes
- dNTPs
- Salts

Bead 3 (freeze-dried) 1 per cartridge
- Primers
- Probes
- dNTPs
- Salts

Bead 4 (freeze-dried) 1 per cartridge
- Sample Processing Control (SPC) non-infectious sample preparation control armored RNA

Lysis Reagent (Guanidinium Thiocyanate, surfactants) • Guanidinium Thiocyanate, surfactants	0.5 mL per cartridge
Wash Reagent • PEG, MTG, KCl, Tris Buffer, EDTA, surfactants, sodium azide	0.5 mL per cartridge
Elution Reagent • Water, Tris Buffer, Sodium Azide	1.0 mL per cartridge
Xpert Flu A Panel reagent pouch	1 per kit
• Binding Reagent (water, Ethanol)	10 x 1 mL per ampoule
Disposable Transfer Pipettes	12 per kit

Notes:
Material Safety Data Sheets (MSDS) for all reagents provided in this assay are available upon request from Cepheid Technical Support.

Storage and Handling

- Store the Xpert Flu A Panel cartridges and reagents at 2 – 8ºC.
- Do not open a cartridge until you are ready to perform testing.
- The reagents and cartridge are stable up to 7 days after opening the package.
- Do not use any reagents that have become cloudy or discolored.

Materials Required but Not Provided

- GeneXpert Dx System (catalog number varies by configuration): GeneXpert instrument, computer with proprietary software, hand-held barcode scanner and Operator Manual
- Printer (See GeneXpert Dx System Operator Manual for compatibility guidelines)

Materials Available but Not Provided

Zeptometrix catalog #NATFLUAH1N1-STN may be used as a positive control, and a water blank may be used as a ‘no template’ negative control.

Sample Collection Kit, Cepheid catalog #NASL-100N-100.

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 universal precautions. Guidelines for specimen handling are available from the U.S. Centers for Disease Control and Prevention[2,3] and the Clinical and Laboratory Standards Institute (formerly National Committee for Clinical Laboratory Standards). [4]
- Follow your institution's safety procedures for working with chemicals and handling biological samples.
- Do not substitute Xpert Flu A Panel reagents with other reagents.
- Do not open the Xpert Flu A Panel cartridge lid except when adding sample and reagent.
- Do not use a cartridge that has been dropped or shaken after you have added the sample and reagents.
- Do not use a cartridge that has a damaged reaction tube.
- Each single-use Xpert Flu A Panel cartridge is used to process one test. Do not reuse spent cartridges.
- Dispose of used Xpert Flu A Panel cartridges according to your institution's safety guidelines for hazardous material.
- Store the Xpert Flu A Panel cartridges and reagents at 2 – 8ºC.
- Do not open a cartridge package until you are ready to perform testing.

Specimen Collection, Transport and Storage

Nasal aspirate/wash (NA/W) or nasopharyngeal (NP) swab specimens can be collected following the user institution's standard procedures and placed into viral transport media. For transport and storage please follow the recommendations of the WHO.[5]

Procedure

Preparing the Cartridge

Important: Start the test within 30 minutes of adding sample and reagent to the cartridge. To add the sample and reagent to the cartridge:

1. Remove the cartridge and reagents from the package.
2. Open the cartridge lid. Using a clean transfer pipette (supplied), transfer the sample to the "S" chamber of the Xpert cartridge. Perform this step twice to ensure adequate sample is present.
3. Add Binding Reagent into cartridge chamber 1. Squeeze the ampoule until the entire contents are added to the cartridge.
4. Close the cartridge lid.

Figure 1: Xpert Flu cartridge (top view).

1 = Binding Reagent, S = Sample

Starting the Test

Important: Before you start the test, make sure the Xpert Flu A Panel 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.

1. Turn on the computer, and then turn on the GeneXpert Dx instrument.
2. On the Windows® desktop, double-click the GeneXpert Dx shortcut icon.
3. Log on to the GeneXpert Dx System software using your user name and password.
4. In the GeneXpert Dx System window, click **Create Test**. The Scan Cartridge Barcode dialog box appears.
5. Scan the barcode on the Xpert Flu A Panel cartridge. The **Create Test** window appears. Using the barcode information, the software automatically fills the boxes for the following fields: Select Assay, Reagent Lot ID, and Cartridge SN.
6. In the **Sample ID** box, scan or type the sample ID. Be sure to type the correct sample ID. The sample ID is associated with the test results and is shown in the **View Results** window and all reports.
7. Click **Start Test**. In the dialog box that appears, type your password.
8. Open the instrument module door with the blinking green light and load the cartridge.
9. Close the door. The test starts and the green light stops blinking. When the test is finished, the light turns off.
10. Wait until the system releases the door lock before opening the module door and removing the cartridge.
11. The used cartridges should be disposed in the appropriate specimen waste containers according to your institution's standard practices.

Viewing and Printing Results

For detailed instructions on how to view and print the results, see the GeneXpert Dx System Operator Manual.

CONTROL Quality Control

Each test includes a Sample Processing Control (SPC) and 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 A 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 Dx 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, federal accrediting organizations requirements as applicable.

Interpretation of Results

The results are interpreted automatically by the GeneXpert Dx System from measured fluorescent signals and embedded calculation algorithms and are clearly shown in the **View Results** window. The possible results are:

Flu A POSITIVE; 2009 H1N1 DETECTED (Figure 2)
Flu A matrix target RNA detected; 2009 H1N1 RNA detected.

- The 2009 H1N1 target has a Ct within the valid range and endpoint above the minimum setting.
- The Flu A matrix target has a Ct within the valid range and endpoint above the minimum setting.
- SPC – NA (not applicable); SPC is ignored since the 2009 H1N1 and Flu A matrix target amplification may compete with this control.
- Probe Check – PASS; all probe check results pass.

Flu A POSITIVE; 2009 H1N1 NOT DETECTED (Figure 3)
Flu A matrix target RNA detected; 2009 H1N1 RNA not detected.

- The Flu A matrix target has a Ct within the valid range and endpoint above the minimum setting.
- SPC – NA (not applicable); SPC is ignored since the Flu A matrix target amplification may compete with this control.
- Probe Check – PASS; all probe check results pass.

Note: Detection of the Flu A matrix sequence without detection of the 2009 H1N1 sequence (i.e., Flu A POSITIVE result) does not rule out the possibility of 2009 H1N1 influenza.

NEGATIVE (Figure 4)
Target RNAs are not detected. SPC meets acceptance criteria.

- Flu A matrix and 2009 H1N1 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.

INVALID (Figure 5)
Presence or absence of Flu A matrix and 2009 H1N1 target RNAs cannot be determined. Repeat test according to the instructions in the Retest Procedure section below.

- SPC – FAIL; SPC target result is negative and the SPC Ct is not within valid range and endpoint below minimum setting.
- Probe Check – PASS; all probe check results pass.

ERROR
Presence or absence of Flu A matrix and 2009 H1N1 target RNAs cannot be determined. Repeat test according to the instructions in the Retest Procedure section below.

- 2009 H1N1 – NO RESULT
- Flu A Matrix – NO RESULT
- SPC – NO RESULT
- Probe Check – FAIL*; all or one of the probe check results fail

*If the probe check is NA, the error is caused by the maximum pressure limit exceeding the acceptable range.

NO RESULT
Presence or absence of Flu A matrix and 2009 H1N1 target RNAs cannot be determined. Repeat test according to the instructions in the Retest Procedure section below.

- 2009 H1N1 – NO RESULT
- Flu A Matrix – NO RESULT
- SPC – NO RESULT
- Probe Check – NA (not applicable)

Figure 2 - An Example of Flu A POSITIVE; 2009 H1N1 DETECTED Result.

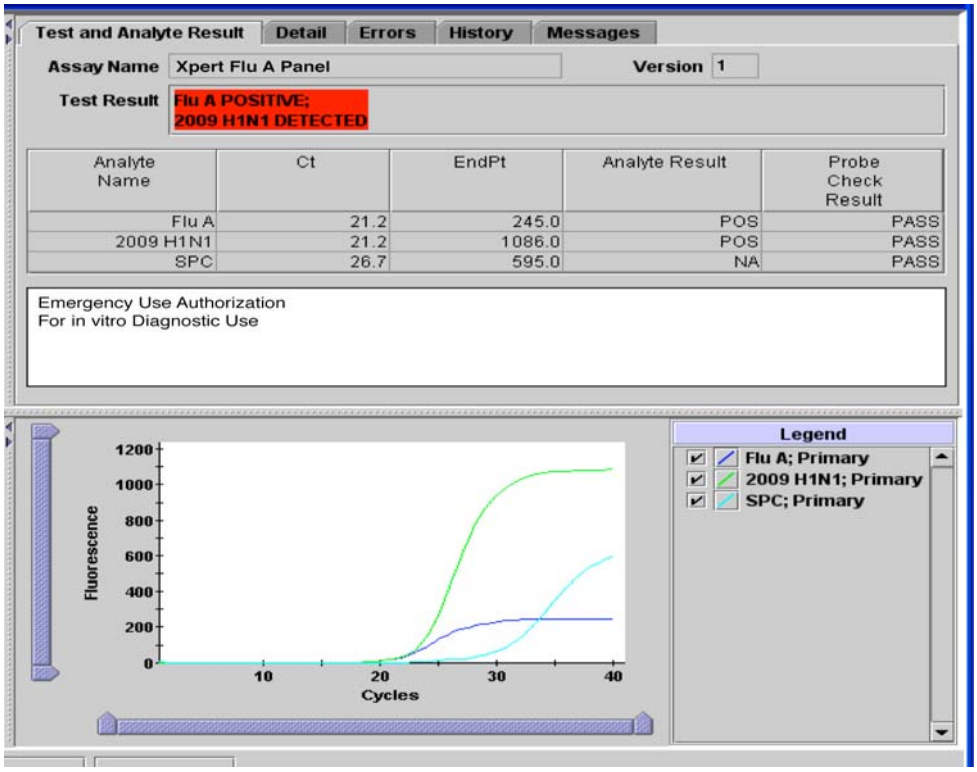


Figure 3 - An Example of Flu A POSITIVE; 2009 H1N1 NOT DETECTED Result.

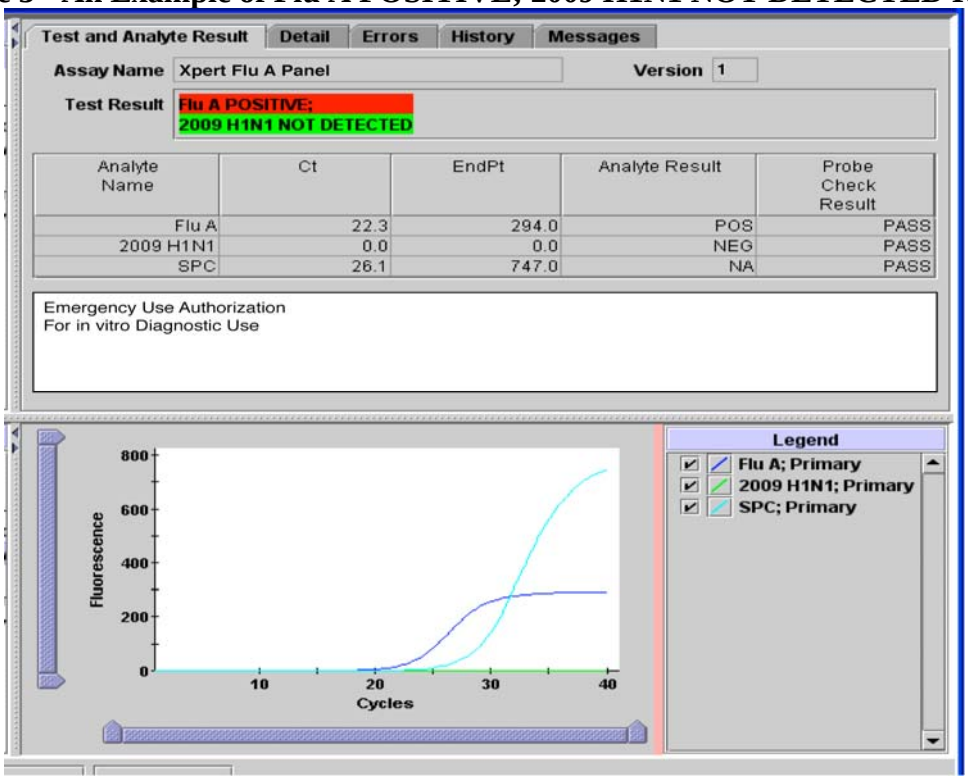


Figure 4 - An Example of NEGATIVE Result

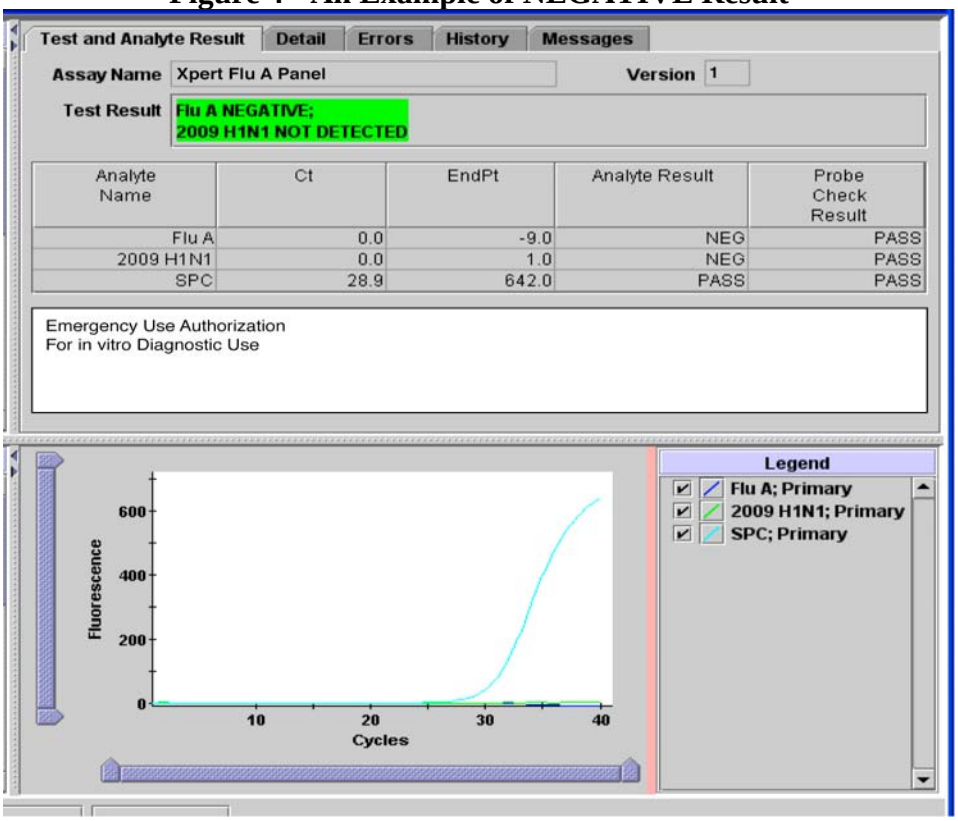
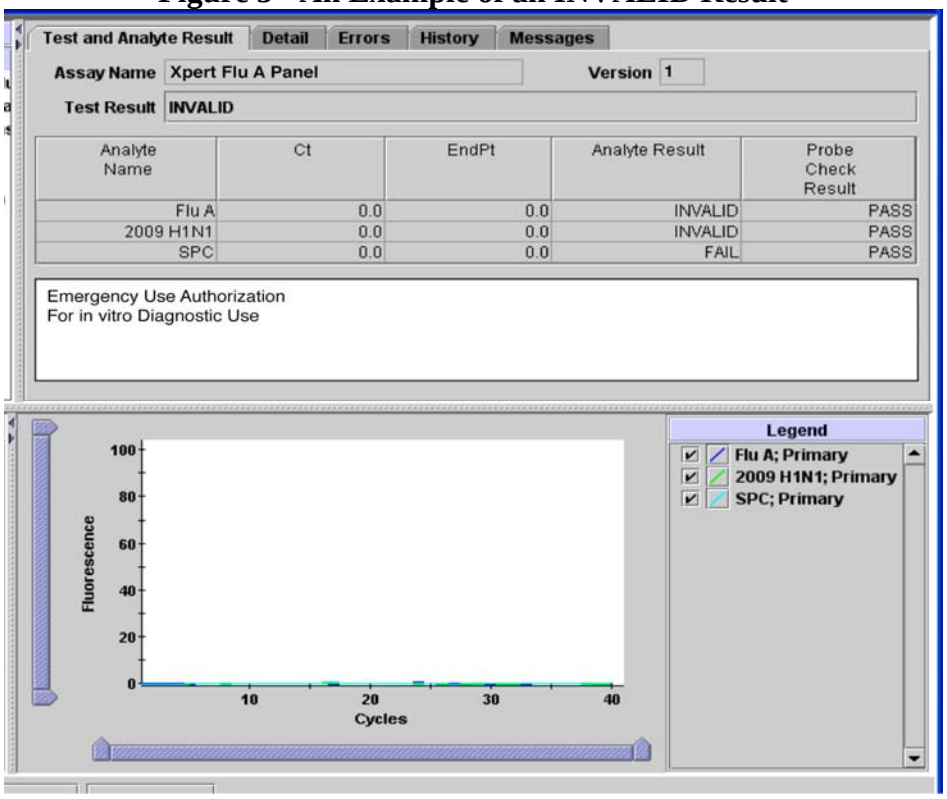


Figure 5 - An Example of an INVALID Result



Reasons to Repeat the Assay

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

An INVALID result indicates that the controls SPC failed. The sample was not properly processed or PCR was inhibited.

An ERROR result indicates that the Probe Check control failed and 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.

Retest Procedure

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

1. Using a clean transfer pipette (supplied), transfer the sample to the "S" chamber of a new Xpert Flu A Panel cartridge (twice).
2. Add new Binding Reagent to Chamber 1.
3. Close the lid and start new test.

Limitations

- The performance of the Xpert Flu A Panel was evaluated using the procedures provided in this package insert only. Modifications to these procedures may alter the performance of the test.
- Results from the Xpert Flu A Panel should be interpreted in conjunction with other laboratory and clinical data available to the clinician.
- Detection of the Flu A matrix sequence without detection of the 2009 H1N1 sequence (*i.e.*, Flu A POSITIVE result) does not rule out the possibility of 2009 H1N1 influenza.

Performance Characteristics

Clinical Performance – Method Comparison

Performance characteristics of the Xpert Flu A Panel were determined in a multi-site investigation study at 3 US institutions by comparing the Xpert Flu A Panel to the EUA Focus Influenza A H1N1 (2009) Real-Time RT-PCR test.

Subjects included individuals whose routine care called for influenza testing. Samples included leftover (fresh or frozen) unlinked nasal aspirates/washes (NA/W) and nasopharyngeal (NP) swab specimens. Excess specimen was used for testing with the Xpert Flu A Panel and the Focus H1N1 Assay.

Performance of the Xpert Flu A Panel was calculated relative to the results of the Focus H1N1 Assay for Influenza A and 2009 H1N1 detection. The Xpert Flu A Panel results were not reported back to health care providers.

Overall Results

The performance of the Xpert Flu A Panel relative to the Focus H1N1 Assay for detection of 2009 H1N1 and Flu A marker is shown in Table 1.

Of the Xpert Flu A Panel assays run on eligible specimens, 90.9% (241/265) were successful on the first attempt. The remaining 24 gave indeterminate results on the first attempt (14 INVALID, and 10 ERROR). Two of the indeterminate specimens could not be retested due to insufficient sample available to perform the retest. Of the 22 indeterminate on the first attempt with sufficient sample for retest, 77.3% (17/22) gave a result on the second attempt; five were indeterminate on the second attempt. This resulted in 258 specimens available for method comparison analyses and seven with indeterminate results (five indeterminate after two attempts and two indeterminate on the first attempt with insufficient volume for retest).

Table 1: Xpert Flu A Panel Performance vs. Focus H1N1 Assay: 2009 H1N1

		Focus H1N1		
Xpert Flu A Panel		Pos	Neg	Total
	Pos	58	0	58
	Neg	5[a]	195	200
	Total	63	195	258
	% Positive Agreement:	92.1% (95% CI: 82.4-97.4)		
	% Negative Agreement:	100% (95% CI: 98.1-100)		
	PPV:	100% (95% CI: 93.8-100)		
	NPV:	97.5% (95% CI: 94.3-99.2)		
	Prevalence:	24.4% (95% CI: 19.3-30.1)		

[a]None of the 5 discordant results tested positive for the Flu A matrix sequence by the Xpert Flu A Panel.

Analytical Specificity

Cross-Reactivity of the Xpert Flu A Panel was evaluated using viruses and bacteria that represent respiratory pathogens or flora commonly found in the nasopharynx. Twenty-seven bacterial, 13 viral, and one yeast strains were included. A list of the strains and genome copies/cartridge or concentration tested is shown in Table 2.

Table 2: Analytical Specificity – Strains and Concentrations Tested

Species	Genome Copies/Concentration (per Cartridge)
Adenovirus Type 7	$1x10^4$ Copies
Human Coronavirus 229E	$1x10^4$ Copies
Human Coronavirus OC43	$1x10^4$ Copies
Coxsackievirus A9	$>2x10^2$ $TCID_{50}$
Cytomegalovirus	$1x10^4$ Copies
Enterovirus Type 70	$>3x10^1$ $TCID_{50}$
Epstein-Barr Virus	$1x10^4$ Copies
Parainfluenzavirus Type 1	$1x10^4$ Copies
Measles Virus	$>2x10^5$ $TCID_{50}$
Human Metapneumovirus	$1x10^4$ Copies
Mumps Virus	$>1x10^7$ Copies
Respiratory Syncytial Virus A	$>1x10^7$ Copies
Respiratory Syncytial Virus B	$>1x10^7$ Copies
Bordetella parapertussis	$>1x10^6$ Copies
Bordetella pertussis	$>1x10^6$ Copies
Candida albicans	$>1x10^5$ Copies
Chlamydia pneumoniae	$>1x10^6$ Copies
Chlamydia trachomatis	$>8x10^4$ Copies
Corynebacterium amycolatum	$>1x10^6$ Copies
Corynebacterium jeikeium	$>1x10^6$ Copies
Corynebacterium xerosis	$>1x10^6$ Copies
Escherichia coli	$>9x10^5$ Copies
Haemophilus influenzae	$>1x10^6$ Copies
Klebsiella oxytoca	$>8x10^5$ Copies
Klebsiella pneumoniae	$>8x10^5$ Copies
Lactobacillus delbreuckii	$>1x10^6$ Copies
Legionella pneumophila	$>1x10^6$ Copies
Moraxella catarrhalis	$>1x10^6$ Copies
Mycobacterium sp. BCG	$>1x10^6$ Copies
Mycoplasma pneumoniae	$>5x10^6$ Copies
Neisseria meningitides	$>1x10^6$ Copies
Neisseria gonorrhea	$>1x10^6$ Copies
Neisseria mucosa	$>1x10^6$ Copies
Proteus mirabilis	$>8x10^5$ Copies
Proteus vulgaris	$>1x10^6$ Copies
Pseudomonas aeruginosa	$>7x10^5$ Copies
Staphylococcus aureus	$>1x10^6$ Copies
Staphylococcus epidermidis	$>1x10^6$ Copies
Streptococcus pneumoniae	$>1x10^6$ Copies
Streptococcus pyogenes	$>1x10^6$ Copies
Streptococcus salivarius	$>1x10^6$ Copies

Each strain was tested in triplicate at concentrations indicated above. Positive and negative controls were included in the study. Under the conditions of the study, all isolates were reported “Flu A NEGATIVE; 2009 H1N1 NOT DETECTED”. The analytical specificity was 100%. Additional cross-reactivity tests with whole organisms were performed and did not show any cross-reactivity with the Xpert Flu A Panel.

Analytical Reactivity

The analytical reactivity of the Xpert Flu A Panel was evaluated against nine strains of Flu A H1 Seasonal, six strains of Flu A H3 Seasonal, and one strain of Flu A 2009 H1N1 at levels near the limit of detection (LoD). Of the tested species, all replicates of the nine Flu A H1 strains were detected and correctly reported as “Flu A POSITIVE; 2009 H1N1 NOT DETECTED”. All replicates of the six Flu A H3 strains were correctly reported as “Flu A POSITIVE; All replicates of the single Flu A 2009 H1N1 strain were detected and correctly reported as “Flu A POSITIVE; 2009 H1N1 DETECTED”. Results are shown in Table 3.

Table 3: Summary Table of Analytical Inclusivity Results of the Xpert Flu A Panel

Viral Strain	Target	Concentration	Flu A	2009 H1N1
A/PR/8/34	Seasonal H1	2.81×10^2 $CEID_{50}$/mL	+	-
A/New Caledonia/20/99	Seasonal H1	9.55×10^1 $TCID_{50}$/mL	+	-
A/Taiwan/42/06 H1N1	Seasonal H1	3.39×10^2 $TCID_{50}$/mL	+	-
A/Brisbane/59/07	Seasonal H1	7.24×10^0 $TCID_{50}$/mL	+	-
A/Solomon Islands/3/2006	Seasonal H1	1.41×10^1 $TCID_{50}$/mL	+	-
A/Mal/302/54	Seasonal H1	8.89×10^3 $CEID_{50}$/mL	+	-
A/WS/33	Seasonal H1	1.58×10^2 $CEID_{50}$/mL	+	-
A/Denver/1/57	Seasonal H1	8.89×10^3 $CEID_{50}$/mL	+	-
A/FM/1/47	Seasonal H1	1.58×10^3 $CEID_{50}$/mL	+	-
A/Brisbane/10/07	Seasonal H3	2.45×10^1 $TCID_{50}$/mL	+	-
A/Wisconsin/67/05	Seasonal H3	1.15×10^1 $TCID_{50}$/mL	+	-
A/Hong Kong/8/68	Seasonal H3	0.89×10^0 $CEID_{50}$/mL	+	-
A/Aichi/2/68	Seasonal H3	1.58×10^1 $CEID_{50}$/mL	+	-
A/Victoria/3/75	Seasonal H3	8.89×10^2 $CEID_{50}$/mL	+	-
A/Port Chambers/1/73	Seasonal H3	1.58×10^2 $CEID_{50}$/mL	+	-
A/WI/629-S11 (D-00015)/2009*	2009 H1N1	1.12×10^0 $TCID_{50}$/mL	+	+

*Dr. Nathan Ledeboer, Dynacare Laboratories, Medical College of Wisconsin, USA

Analytical Sensitivity

Studies were performed to determine the analytical LoD of three strains of influenza virus diluted into universal transport medium (UTM) that can be detected by the Xpert Flu A Panel. The LoD was defined as the lowest detectable concentration of influenza virus at which approximately 95% of all replicates test positive.

The LoD for each strain was confirmed by running a total of twenty (20) replicates at the estimated LoD concentration, and negatives. Results are shown in Table 4.

Table 4: Estimated Limit of Detection ($TCID_{50}$/mL) of Flu A

Strain ID	$LoD_{95\%}$ ($TCID_{50}$/mL)
A/Solomon Islands/2/2006 H1 strain [a]	1.13×10^0
A/Brisbane/10/07 H3N2 strain [a]	1.0×10^0
A/W1/629-D00015/2009 strain [b]	0.2×10^0

Strain Source:
(a) Zeptometrix, Buffalo, NY, USA
(b) Dr. Nathan Ledeboer, Dynacare Laboratories, Medical College of Wisconsin, USA

Under the conditions of the study, the Xpert Flu A Panel demonstrated a higher LoD with the Flu A 2009 H1N1 strain compared to the Focus Diagnostic assay, with an LoD of 0.2 x 10^0 $TCID_{50}$/mL (see Table 5).

Table 5: Xpert Flu A Panel vs. Focus Diagnostics: Limit of Detection ($TCID_{50}$/mL) of Flu A Strains Tested

Strain ID	$TCID_{50}$ /mL	Xpert Flu A Panel	Focus Diagnostics
A/Solomon Islands/2/2006 H1 strain	2.26×10^0	19/20	17/20[a]
	1.13×10^0	19/20	14/20[b]
A/Brisbane/10/07 H3N2 strain	1.0×10^0	20/20	3/20[c]
	0.5×10^0	18/20	2/20[d]
A/W1/629-D00015/2009 strain	0.4×10^0	20/20	20/20
	0.2×10^0	20/20	20/20
	0.1×10^0	15/20	20/20
Negative	NA	19/20[e]	15/20[f]

[a] 3 samples were indeterminate by the Focus Diagnostic Assay
[b] 6 samples were indeterminate by the Focus Diagnostic Assay
[c] 16 samples were indeterminate and 1 sample was Not Detected by the Focus Diagnostic Assay
[d] 16 samples were indeterminate and 2 samples were Not Detected by the Focus Diagnostic Assay
[e] 1 sample was indeterminate by the Xpert Flu A Panel Assay
[f] 5 samples were indeterminate by the Focus Diagnostic Assay

The dynamic range of the Xpert Flu A Panel was determined by performing 10-fold serial dilutions of virus titer stocks in UTM. For seasonal influenza A and 2009 H1N1 virus strains the dynamic range was determined to be 10^4 $TCID_{50}$/mL to 10^0 $TCID_{50}$/mL. The lowest concentration with uniform positivity per analyte was used to estimate the LoD. Results of the dynamic range dilutions are shown in Table 6.

Table 6: Serial Dilutions for LoD Dynamic Range Determination

Virus Strain Tested	Analyte Tested	Stock Virus Titer	Serial 10-Fold Dilution Factor	$TCID_{50}$/mL Dilution Tested	Call Rate	Run 1 Ct	Run 2 Ct	Run 3 Ct	Run 4 Ct	Ave. Ct (n=4)	Lowest Concentration with Uniform Positivity per Analyte
A/Solomon Islands/2/2006 H1	Flu A	$1x10^{5.15}$ $TCID_{50}$/mL	10e1	1.41×10^{4}	4/4	17.6	16.9	18.5	17.3	17.9	1.41×10^{0} $TCID_{50}$/mL
			10e2	1.41×10^{3}	4/4	20.2	20.7	20.7	20.7	20.6	
			10e3	1.41×10^{2}	4/4	24.2	23.9	24.2	24.0	24.1	
			10e4	1.41×10^{1}	4/4	26.9	27.2	26.7	26.7	26.9	
			10e5	1.41×10^{0}	4/4	29.2	29.3	30.4	29.4	29.6	
			10e6	0.14×10^{0}	4/4	33.5	33.2	33.9	33.9	33.6	
A/Brisbane/10/07 H3N2	Flu A	$1x10^{5.39}$ $TCID_{50}$/mL	10e1	2.45×10^{4}	4/4	18.5	18.9	18.6	19.3	18.8	2.45×10^{0} $TCID_{50}$/mL
			10e2	2.45×10^{3}	4/4	22.2	22.1	22.3	22.5	22.3	
			10e3	2.45×10^{2}	4/4	25.4	25.7	25.5	25.4	25.5	
			10e4	2.45×10^{1}	3/3	28.4	28.6	NA*	28.5	28.5	
			10e5	2.45×10^{0}	4/4	31.5	31.7	31.5	31.6	31.6	
			10e6	0.25×10^{0}	3/4	35.4	34.8	39.0	36.2	36.4	
A/W1/629-D00015/2009	Flu A	$1x10^{6.05}$ $TCID_{50}$/mL	10e2	1.12×10^{4}	4/4	16.4	16.5	16.4	16.5	16.5	1.12×10^{0} $TCID_{50}$/mL
			10e3	1.12×10^{3}	4/4	20.2	19.9	19.9	19.9	20.0	
			10e4	1.12×10^{2}	4/4	23.3	23.3	23.6	23.6	23.5	
			10e5	1.12×10^{1}	4/4	26.3	26.5	26.3	26.3	26.4	
			10e6	1.12×10^{0}	4/4	29.6	29.5	29.3	29.4	29.5	
			10e7	1.12×10^{-1}	4/4	32.4	32.5	32.5	32.7	32.5	
A/W1/629-D00015/2009	2009 H1N1	$1x10^{6.05}$ $TCID_{50}$/mL	10e2	1.12×10^{4}	4/4	16.6	16.5	16.7	16.3	16.5	1.12×10^{0} $TCID_{50}$/mL
			10e3	1.12×10^{3}	4/4	20.2	20.0	20.2	20.3	20.2	
			10e4	1.12×10^{2}	4/4	23.6	23.5	24.0	24.1	23.8	
			10e5	1.12×10^{1}	4/4	26.6	26.8	26.6	26.3	26.6	
			10e6	1.12×10^{0}	4/4	29.8	30.0	29.6	29.4	29.7	
			10e7	1.12×10^{-1}	3/4	35.9	34.5	34.3	0	36.2	

* Cartridge aborted due to exceeding the pressure limit

Reproducibility

A panel of four samples comprised of three viral stock samples at three levels each (positive, low positive and high negative) and one negative template were tested on three different days by six different operators (three with prior PCR experience and three without). One lot of Xpert Flu A Panel was used. Xpert Flu A Panel assays were performed according to the Xpert Flu A Panel procedure. Results are summarized, by operator, in Table 7. Note that the three "high negative" samples are not included in Table 7, as each has more than one expected result.

Table 7: Summary of Reproducibility Results by Operator; Percent Agreement[a]

Sample ID	Op 1 (Inexp)	Op 2 (Inexp)	Op 3 (Inexp)	Op 4 (Exp)	Op 5 (Exp)	Op 6 (Exp)	% Total Agreement by Sample
Negative	100% (9/9)	100% (9/9)	100% (9/9)	100% (9/9)	100% (9/9)	100% (9/9)	100% (54/54)
2009 H1N1 positive	100% (9/9)	100% (9/9)	100% (9/9)	100% (9/9)	100% (9/9)	100% (9/9)	100% (54/54)
2009 H1N1 low positive	100% (9/9)	100% (9/9)	100% (9/9)	100% (9/9)	100% (9/9)	100% (9/9)	100% (54/54)
% Total Agreement by Operator	100% (27/27)	100% (27/27)	100% (27/27)	100% (27/27)	100% (27/27)	100% (27/27)	100% (162/162)

[a]The high negative samples are not included in this analysis, as there was more than one expected result for each of the high negative samples. Overall 48 of the 54 high negative samples tested positive by the Xpert Flu A Panel.

References

1. www.cdc.gov, Accessed on December 21, 2009.
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4. Clinical and Laboratory Standards Institute (formerly National Committee for Clinical Laboratory Standards). Protection of Laboratory Workers from Occupationally Acquired Infections; Approved Guideline. Document M29 (refer to latest edition).
5. World Health Organization. Instructions for Storage and Transport of Suspected or Confirmed Human and Animal Specimens and Virus Isolates of Pandemic (H1N1) 2009. Published 16 July 2009. http://www.who.int/csr/resources/publications/swineflu/storage_transport/en/print.html.

Table of Symbols

Symbol	Meaning
	Consult instructions for use
	Date of manufacture
	Do not reuse
	Caution, consult accompanying document
	Manufacturer
	Contains sufficient for <n> tests
	Control
	Temperature limitation
	Biological hazard

 Manufacturer

Cepheid
904 Caribbean Drive
Sunnyvale, CA 94089-1189
USA
Phone: +1.408.541.4191 Fax: +1.408.541.4192

For assistance, contact Cepheid using one of the following contact details. Make sure you provide the instrument serial number and reagent lot ID when you call or email.
North America
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Email: techsupport@cepheid.com
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