K103766

510(k) Summary

As required by 21 CFR Section 807.92(c).

APR 2 1 2011

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Contact:	Russel K. Enns, Ph.D.
Date of Preparation:	April 22, 2011
Device:	
Trade name:	Xpert® Flu
Common name:	Xpert Flu Assay
Type of Test:	Automated, multiplex real-time reverse transcription-polymerase chain reaction (rRT-PCR) assay intended for the *in vitro* qualitative detection and differentiation of influenza A, influenza B and 2009 H1N1 influenza viral RNA.
Regulation number/ Classification name:	866.3980/Respiratory viral panel multiplex nucleic acid assay
Product code:	OQW, OCC, OOI
Classification Advisory Panel	Microbiology (83)
Predicate Devices:	K073029: ProFLU+™ Assay, Gen-Probe Prodesse, Inc. K100148: Simplexa™ Influenza A H1N1(2009), Focus Diagnostics, Inc.

Device Description:

The Xpert Flu Assay is a rapid, automated *in vitro* diagnostic test for qualitative detection and differentiation of influenza A, influenza B and influenza A, subtype 2009 H1N1. The assay is performed on the Cepheid GeneXpert Instrument Systems. The GeneXpert Instrument Systems automate and integrate sample purification, nucleic acid amplification, and detection of the target sequence in simple or complex samples using real-time PCR and RT-PCR assays. The systems require the use of single-use disposable cartridges that hold the PCR reagents and host the PCR process. Because the cartridges are self-contained and specimens never come into contact with working parts of the instrument modules, cross-contamination between samples is eliminated.

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 from patients with signs and symptoms of respiratory infection. 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 PCR reaction. The Probe Check Control (PCC) verifies reagent rehydration, PCR tube filling in the cartridge, probe integrity and dye stability.

The liquid specimen (NA/W) or swab specimen (NP) is collected according to the institution's standard procedures and placed into Universal Transport Medium (3mL UTM tubes). Following a brief mixing by inverting the UTM tube five times, the eluted material and one single-use reagent (Reagent 1), that is provided with the assay, are transferred to different, uniquely-labeled chambers of the disposable fluidic cartridge (the Xpert Flu cartridge). The user initiates a test from the system user interface and places the cartridge into the GeneXpert instrument platform, which performs hands-off reverse transcription and real-time, multiplex polymerase chain reaction (PCR) for detection of DNA. In this platform, additional sample preparation, amplification, and real-time detection are all fully-automated and completely integrated.

The single-use, multi-chambered fluidic cartridges are designed to complete sample preparation and real-time RT-PCR for detection and differentiation of influenza A, influenza B and influenza A, subtype 2009 H1N1 in 75 minutes. The GeneXpert Instrument Systems have 1 to 48 randomly accessible modules that are each capable of performing separate sample preparation and real-time PCR tests. Each module contains a syringe drive for dispensing fluids (i.e., the syringe drive activates the plunger that works in concert with the rotary valve in the cartridge to move fluids between chambers), an ultrasonic horn for lysing cells or spores, and a proprietary I-CORE® thermocycler for performing real-time PCR and detection.

Device Intended Use:

The Cepheid® Xpert Flu Assay 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 2009-2010 influenza season when 2009 H1N1 influenza was the predominant influenza A virus in circulation. 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.

Substantial Equivalence:

The Xpert Flu Assay is substantially equivalent to the following predicate assays:

- K073029: ProFLU+™ Assay, Gen-Probe Prodesse, Inc.
- K100148: Simplexa™ Influenza A H1N1(2009), Focus Diagnostics, Inc.

Similarities and differences between the Cepheid Xpert Flu Assay and the predicate devices are shown in Table 5.1.

A clinical study at six sites was conducted to compare Xpert Flu Assay performance to the standard of care, viral culture followed by direct fluorescent assay (DFA). Sequencing for all influenza A positive specimens. For archived specimens, where viral culture was not performed prior to freezing, a FDA cleared molecular assay was performed as the comparator assay followed by sequencing of all influenza A positive specimens.

Table 5.1: Comparison of Similarities and Differences of the Xpert Flu Assay with the Predicate Devices

	Device	**Predicates**	
Item	**Cepheid Xpert Flu**	**Gen-Probe Prodesse, Inc. ProFLU+**	**Focus Simplexa Influenza A H1N1**
510(k) No.	K103766	K073029	K100148
Regulation	866.3332 and 866.3980	866.3980	866.3332
Product Code	OQW, OCC, OOI	OCC	OQW
Device Class	II	II	II
Technology/ Detection	Multiplex real time RT/PCR	Multiplex real time RT/PCR	Multiplex real time RT/PCR
Intended Use	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	A multiplex Real Time RT-PCR *in vitro* diagnostic test for the rapid and qualitative detection and discrimination of Influenza A Virus, Influenza B Virus, and Respiratory Syncytial Virus (RSV) nucleic	For use on the 3M Integrated Cycler as part of the Microfluidic Molecular System for the in vitro qualitative detection and differentiation of influenza A and 2009 H1N1 influenza viral RNA in nasopharyngeal swabs (NPS), nasal swabs (NS),

	Device	Predicates	
Item	**Cepheid Xpert Flu**	**Gen-Probe Prodesse, Inc. ProFLU+**	**Focus Simplexa Influenza A H1N1**
	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 2009-2010 influenza season when 2009 H1N1 influenza was the predominant influenza A virus in circulation. 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.	acids isolated and purified from nasopharyngeal (NP) swab specimens obtained from symptomatic patients. This test is intended for use to aid in the differential diagnosis of Influenza A, Influenza B and RSV2 viral infections in humans and is not intended to detect Influenza C. A negative test is presumptive and it is recommended these results be confirmed by cell culture. Negative results do not preclude influenza or RSV virus infection and should not be used as the sole basis for treatment or other management decisions.	and nasopharyngeal aspirates (NPA) from human patients with signs and symptoms of respiratory infection 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 2009-2010 influenza season when 2009 H1N1 influenza was the predominant influenza A virus in circulation. 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

	Device	Predicates	
Item	**Cepheid Xpert Flu**	**Gen-Probe Prodesse, Inc. ProFLU+**	**Focus Simplexa Influenza A H1N1**
Indication for Use	Patients with signs and symptoms of respiratory infection in conjunction with clinical and epidemiological risk factors.	Symptomatic patients	Patients with signs and symptoms of respiratory infection in conjunction with clinical and epidemiological risk factors
Assay Targets	Influenza A, influenza B, and influenza A, subtype 2009 H1N1	Influenza A, influenza B, Respiratory Syncytial Virus Type A and Type B	Influenza A/ 2009 H1N1 influenza
Specimen Types	Nasal aspirates/washes (NA/W) and Nasopharyngeal (NP) swabs	NP swab	NP swab, Nasal Swab (NS) and nasopharyngeal aspirates (NA/W)
Technological Principles	RT/PCR	RT/PCR	RT/PCR
Nucleic Acid Extraction	Yes	Yes	Yes
Extraction Methods	Sample preparation integrated in GeneXpert Cartridge and GeneXpert Instrumentation System	Roche MagNA Pure LC Total NA Isolation Kit	Roche MagNA Pure LC Total NA Isolation Kit, QIAGEN QIAamp Viral RNA mini Kit
Assay Results	Qualitative	Qualitative	Qualitative
Instrument System	Cepheid GeneXpert Instrument Systems	Cepheid Smartcycler® II	3M Integrated cycler
Assay Controls	Encapsulated (armored) RNA pseudovirus as a sample processing control. Available but not provided are inactivated virus controls for Flu A/B and Flu A H1N1 as external positive controls and Coxsackie virus as an external negative control.	Inf A RNA Control, Inf B RNA Control, RSV A RNA Control, RSV B RNA Control and an internal	Armored RNA Internal Control, No Template Control, and H1N1 Positive Control provided
Test results	Total 75 minutes for sample preparation and rRT-PCR	Total 205 minutes (~45 minutes for sample preparation ~2.0 hours for rRT-PCR)	Total 115 minutes (~45 minutes for sample preparation ~70 minutes for rRT-PCR)

	Device	Predicates	
Item	**Cepheid Xpert Flu**	**Gen-Probe Prodesse, Inc. ProFLU+**	**Focus Simplexa Influenza A H1N1**
Laboratory Users	CLIA Moderate to High Complexity	CLIA High Complexity	CLIA High Complexity

Non-Clinical Studies:

Analytical Sensitivity

Analytical Reactivity (Inclusivity)

The analytical reactivity of the Xpert Flu Assay was evaluated against thirty-nine (39) strains of influenza A (H1N1, H3N2, H5N2, H5N1, and H7N3 subtypes), influenza A 2009 H1N1 and influenza B. Of these, influenza A subtype H1N1 (12), influenza A subtype H3N2 (7), influenza A subtype 2009 H1N1 (6), influenza A subtype H5N1 (1), influenza A subtype H5N2 (1), influenza A subtype H7N3 (1) and influenza B (11) were included. Three replicates of each viral strain were tested at 5 – 500 $TCID_{50}$/mL unless noted otherwise. Results are shown in Table 5.2.

Table 5.2: Analytical Reactivity (Inclusivity) of Xpert Flu Assay

Viral Strain	Concentration ($TCID_{50}$/mL)	Influenza A	Influenza A 2009 H1N1	Influenza B
Influenza A/Denver/1/57 (H1N1)	500	+	-	-
Influenza A/NewYork/55/2004 (H1N1)	500	+	-	-
Influenza A/Mal/302/54 (H1N1)	50	+	-	-
Influenza A/New Jersey/8/76 (H1N1)	500	+	-	-
Influenza A/NWS/33 (H1N1)	5	+	-	-
Influenza A/PR/8/34 (H1N1)[a]	500	+	-	-
Influenza A/Taiwan/42/06 (H1N1)[a]	500	+	-	-
Influenza A/WS/33 (H1N1)[a]	5	+	-	-
Influenza A/Swine/1976/31 (Swine H1N1)	500 PFU/mL[a]	+	-	-
Influenza A/Swine/Iowa/15/30 (Swine H1N1)	500 PFU/mL[a]	+	-	-
Influenza A/Brisbane/59/07 (H1N1)[a]	5	+	-	-
Influenza A/NewCalendonia/20/1999 (H1N1)[a]	50	+	-	-
Influenza A/Victoria/3/75 (H3N2)	500	+	-	-

Viral Strain	Concentration ($TCID_{50}$/mL)	Influenza A	Influenza A 2009 H1N1	Influenza B
Influenza A/Aichi2/68 (H3N2)	500	+	-	-
Influenza A/Hong Kong/8/68 (H3N2)	50	+	-	-
Influenza A/Hawaii/15/2001 (H3N2)	500	+	-	-
Influenza A/Port Chalmers/1/73 (H3N2)	500	+	-	-
Influenza A/Brisbane/10/07 (H3N2)	10	+	-	-
Influenza A/Wisconsin/67/05 (H3N2)	10	+	-	-
Influenza A/SwineNY/01/2009 (2009 H1N1)	10	+	+	-
Influenza A/SwineNY/02/2009 (2009 H1N1)	100	+	+	-
Influenza A/SwineNY/03/2009 (2009 H1N1)	10	+	+	-
Influenza A/California/4/2009 (2009 H1N1)	5	+	+	-
Influenza A/Canada/6294 (2009 H1N1)	500	+	+	-
Influenza A/WI/929-S1 (2009 H1N1)	100	+	+	-
Influenza A/Mallard/WI/34/75 (H5N2)	3 pg/µL[b]	+	-	-
Influenza A/Anhui/02/2005/PR8-IBCDC-RG5 (H5N1)	0.122 pg/µL[b]	+	-	-
Influenza A/chicken/NJ/15086-3/94 (H7N3)	5 pg/µL[b]	+	-	-
Influenza B/Allen/45	500	-	-	+
Influenza B/Florida/02/06	10	-	-	+
Influenza B/Florida/04/06	500	-	-	+
Influenza B/Florida/07/04	50	-	-	+
Influenza B/GL/1739/54	500	-	-	+
Influenza B/Hong Kong/5/72	500	-	-	+
Influenza B/Lee/40	500	-	-	+
Influenza B/Malaysia/2506/04	500	-	-	+
Influenza B/Maryland/1/59	500	-	-	+

Viral Strain	Concentration ($TCID_{50}$/mL)	Influenza A	Influenza A 2009 H1N1	Influenza B
Influenza B/Panama/45/90	500	-	-	+
Influenza B/Taiwan/2/62	50 $CEID_{50}$/mL[c]	-	-	+

[a]Concentration expressed as PFU/mL
[b]Concentration expressed in picograms/µL[c]Concentration expressed as $CEID_{50}$/mL

Analytical Sensitivity

Limit of Detection

Studies were performed to determine the analytical limit of detection (LoD) of two seasonal influenza A (H1N1), two seasonal influenza A (H3N2), five influenza A 2009 H1N1 and two influenza B strains diluted into a surrogate nasopharyngeal matrix, containing human blood, mucin and sodium chloride. 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 values for each strain tested are summarized in Tables 5.3 – 5.6.

Table 5.3: Confirmed LoD ($TCID_{50}$/mL) – Seasonal Influenza A H1N1

Strain ID – Influenza A subtype H1N1	Confirmed LoD ($TCID_{50}$/mL) (at least 19/20 positive)
Influenza A/H1/Brisbane/59/07	5 (20/20)
Influenza A/H1/New Calendonia/20/1999	25 (20/20)

Table 5.4: Confirmed LoD ($TCID_{50}$/mL) – Seasonal Influenza A H3N2

Strain ID – Influenza A subtype H3N2	Confirmed LOD ($TCID_{50}$/mL) (at least 19/20 positive)
Influenza A/H3/Brisbane/10/07	2.5 (19/20)
Influenza A/H3/Wisconsin/67/05	10 (20/20)

Table 5.5: Confirmed 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)
Influenza A/SwineNY/01/2009	1 (19/20)
Influenza A/SwineNY/02/2009	5 (19/20)
Influenza A/SwineNY/03/2009	3.5 (20/20)
Influenza A/Canada/6294	100 (20/20)
Influenza A/WI/629-S1/2009	20 (20/20)

Table 5.6: Confirmed LoD ($TCID_{50}$/mL) – Influenza B

Strain ID – Influenza B	Confirmed LOD ($TCID_{50}$/mL) (at least 19/20 positive)
Influenza B/Florida/02/06	2 (19/19)
Influenza B/Florida/07/04	75 (20/20)

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 each bacterial and yeast strains were tested at concentrations $\geq 10^6$ CFU/mL. Three replicates of each virus were tested at concentrations $\geq 10^4$ $TCID_{50}$/mL. Purified nucleic acids (genome copies/mL) were tested for one virus strain (Cytomegalovirus) and two bacterial strains (*Bordetella pertussis* and *Haemophilus influenzae*). Positive and negative controls were included in the study. The analytical specificity was 100%. Results are shown in Table 5.7.

Table 5.7: Analytical Specificity of Xpert Flu Assay[a]

Strain	Concentration (per Cartridge)	Influenza A	Influenza A 2009 H1N1	Influenza B
Positive Control 1 – Influenza A/influenza B	N/A	+	-	+
Positive Control 2 – Influenza A 2009 H1N1	N/A	+	+	-
Negative Control	N/A	-	-	-
Adenovirus Type 7A	$1x10^6$ $TCID_{50}$/mL	-	-	-
Adenovirus Type 1	$1x10^6$ $TCID_{50}$/mL	-	-	-
Human Coronavirus 229E	$1x10^6$ $TCID_{50}$/mL	-	-	-
Human Coronavirus OC43	$1x10^4$ $TCID_{50}$/mL	-	-	-
Cytomegalovirus[b]	$1x10^5$ Copies /mL	-	-	-
Enterovirus Type 71	$1x10^6$ $TCID_{50}$/mL	-	-	-
Epstein-Barr Virus	$1x10^5$ $TCID_{50}$/mL	-	-	-

Strain	Concentration (per Cartridge)	Influenza A	Influenza A 2009 H1N1	Influenza B
Parainfluenzavirus Type 1	$1x10^5$ $TCID_{50}$/mL	-	-	-
Parainfluenzavirus Type 2	$1x10^6$ $TCID_{50}$/mL	-	-	-
Parainfluenzavirus Type 3	$1x10^6$ $TCID_{50}$/mL	-	-	-
Measles Virus	$1x10^6$ $TCID_{50}$/mL	-	-	-
Human Metapneumovirus	$1x10^6$ $TCID_{50}$/mL	-	-	-
Mumps Virus	$1x10^6$ $TCID_{50}$/mL	-	-	-
Respiratory Syncytial Virus A	$1x10^6$ $TCID_{50}$/mL	-	-	-
Respiratory Syncytial Virus B	$1x10^6$ $TCID_{50}$/mL	-	-	-
Human HSV Type 1	$1x10^6$ $TCID_{50}$/mL	-	-	-
Human Rhinovirus Type 4	$1x10^6$ $TCID_{50}$/mL	-	-	-
Echovirus 11	$1x10^6$ $TCID_{50}$/mL	-	-	-
Bordetella pertussis[b]	$1x10^6$ Copies/mL	-	-	-
Chlamydia pneumoniae	$1x10^6$ 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[b]	$1x10^6$ Copies/mL	-	-	-
Lactobacillus crispatus	$1x10^6$ CFU/mL	-	-	-
Legionella pneumophila	$1x10^6$ CFU/mL	-	-	-
Moraxella catarrhalis	$1x10^6$ CFU/mL	-	-	-
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 was not evaluated.
bNucleic acid was tested for Cytomegalovirus, *Bordetella pertussis,* and *Haemophilus influenzae.*

Interfering Substances

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 exogenous 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. These substances are listed in Table 5.8 with active ingredients and concentrations tested shown. Highly viscous samples, resulting from the addition of 1.5% (w/v) and 2.5% (w/v) mucin yielded false negative test results from the Xpert Flu Assay. Inhibition of the Xpert Flu Assay was also observed from the addition of 1% (w/v) mucin, resulting in delayed detection of influenza A, influenza A subtype 2009 H1N1 and influenza B.

Table 5.8: Potentially Interfering Substances in Xpert Flu Assay.

Substance	Description/Active Ingredient	Concentration Tested
Blood (human)	N/A	2% (v/v)
Mucin	Purified mucin protein (Bovine or porcine submaxillary gland)	2.5%, 1.5%, 1% and 0.5% (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	5% (v/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
Throat lozenges, oral anesthetic and analgesic	Menthol	1.7 mg/mL menthol

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) or influenza B sample (approximately 10^6 $TCID_{50}$/test). This testing scheme was repeated 20 times on four GeneXpert modules for a total of 88 runs resulting in 40 positive and 48 negative specimens. All 40 positive samples were correctly reported as influenza A 2009 H1N1 positive or influenza B positive. All 48 negative samples were correctly reported as Flu A negative, 2009 H1N1 not detected and Flu B negative.

Linearity

A study was conducted to define the reportable range of the Xpert Flu Assay and demonstrate a linear relationship between target input and assay output. Linearity was evaluated using two seasonal influenza A H1N1, two seasonal influenza A H3N2, two influenza A 2009 H1N1, and two influenza B strains diluted over four to five logs and processed using the Xpert Flu Assay. Replicates of 4 were tested.

Under the conditions of the study, the Xpert Flu Assay responds linearly over six logs for seasonal influenza A H1N1, seasonal influenza A H3N2 and influenza A 2009 H1N1; and over 5 logs for influenza B strains tested.

Reproducibility

A panel of 10 specimens with varying concentrations of influenza A, influenza B, and influenza A subtype 2009 H1N1 were tested in duplicate on 10 different days at each of three sites (10 specimens x 2 times/ day x 10 days x 3 sites). One lot of Xpert Flu Assay was used at each of the 3 testing sites. Xpert Flu Assays were performed according to the Xpert Flu Assay procedure. Results are summarized in Table 5.9.

Table 5.9: Summary of Reproducibility Results

Sample ID	Site 1	Site 2	Site 3	% Total Agreement by Sample
Negative	100% (20/20)	100% (20/20)	100% (20/20)	100% (60/60)
Flu A moderate positive	100% (20/20)	100% (19/19)[a]	100% (20/20)	100% (59/59)
Flu A low positive	100% (20/20)	100% (20/20)	100% (20/20)	100% (60/60)
Flu A high negative	100% (20/20)	95.0% (19/20)	90.0% (18/20)	95.0% (57/60)
2009 H1N1 moderate positive	100% (20/20)	100% (20/20)	100% (20/20)	100% (60/60)
2009 H1N1 low positive	100% (20/20)	100% (19/19)[b]	100% (20/20)	100% (59/59)

Sample ID	Site 1	Site 2	Site 3	% Total Agreement by Sample
2009 H1N1 high negative	94.7% (18/19)[a]	100% (20/20)	89.5% (17/19)[a]	94.8% (55/58)[c]
Flu B moderate positive	100% (20/20)	100% (20/20)	100% (20/20)	100% (60/60)
Flu B low positive	100% (20/20)	100% (20/20)	100% (20/20)	100% (60/60)
Flu B high negative	90.0% (18/20)	95.0% (19/20)	55.0% (11/20)	80.0% (48/60)
% Total Agreement	98.5%% (196/199)	99.0% (196/198)	93.5% (186/199)	97.0% (578/596)

[a]n=19 because repeat yielded indeterminate result.
[b]n=19 because one sample was indeterminate and not retested.
[c]9/55 samples negative for 2009 H1N1 resulted in a valid Flu A positive call, as the Flu A signal was detected. A valid 2009 H1N1 positive call requires detection of both the Flu A and 2009 H1N1 signals.

Instrument System Reproducibility

An in-house reproducibility study was conducted to compare the performance of the GeneXpert Dx and the Infinity instrument systems. A panel of 10 specimens with varying concentrations of influenza A, influenza B, and influenza A subtype 2009 H1N1 were tested in duplicate on 12 different days by two operators. Each operator conducted four runs of each panel specimen per day on each of the two instrument systems (11 specimens x 2 times/ day x 12 days x 2 operators x 2 instrument systems). One lot of Xpert Flu Assay was used for the study. Xpert Flu Assays were performed according to the Xpert Flu Assay procedure. Results are summarized in Table 5.10.

Table 5.10: Summary of Instrument System Reproducibility Results

Sample ID	GeneXpert Dx	Infinity	% Total Agreement by Sample
Negative	100.0% (96/96)	100.0% (96/96)	100.0% (192/192)
Flu A moderate positive	100.0% (96/96)	100.0% (96/96)	100.0% (192/192)
Flu A low positive	100.0% (96/96)	100.0% (96/96)	100.0% (192/192)
Flu A high negative	89.6% (86/96)	86.5% (83/96)	88.0% (169/192)
2009 H1N1 moderate positive	100.0% (96/96)	100.0% (96/96)	100.0% (192/192)
2009 H1N1 low positive	100.0% (96/96)	100.0% (96/96)	100.0% (192/192)
2009 H1N1 high negative	84.4% (81/96)	92.6% (88/95)[a]	88.5% (169/191)
Flu B moderate positive	100.0% (96/96)	100.0% (96/96)	100.0% (192/192)

Sample ID	GeneXpert Dx	Infinity	% Total Agreement by Sample
Flu B low positive	100.0% (96/96)	100.0% (96/96)	100.0% (192/192)
Flu B high negative	87.5% (84/96)	82.3% (79/96)	84.9% (163/192)
% Total Agreement	96.1% (923/960)	96.1% (922/959)	96.1% (1845/1919)

[a]n=95 because repeat yielded indeterminate result.

Clinical Performance Characteristics

Clinical Performance Study

Performance characteristics of the Xpert Flu Assay on prospective and archived specimens were evaluated at six institutions in the U.S. and Australia. 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 performance was compared to viral culture followed by direct fluorescent assay (DFA). Sequencing was performed for all influenza A positive specimens. For archived specimens, where viral culture was not performed prior to freezing, a FDA cleared molecular assay was performed as the comparator assay. Samples included nasal NA/W and NP swab specimens collected for routine testing from patients suspected of influenza infection.

Overall Results

Prospective Specimens

A total of 342 prospective NA/W specimens were tested for influenza A, influenza A subtype 2009 H1N1 and influenza B by the Xpert Flu Assay and viral culture plus DFA. A total of 297 prospectively collected NP swab specimens were tested for influenza A, influenza A subtype 2009 H1N1 and influenza B by the Xpert Flu Assay and viral culture plus DFA. All influenza A positive specimens, identified by viral culture/DFA, were sequenced to differentiate influenza A subtype 2009 H1N1 from other influenza A subtypes

On fresh, prospective NA/W specimens, the Xpert Flu Assay demonstrated a sensitivity and specificity for detection of influenza A of 85.7% and 99.1%, respectively, relative to viral culture plus DFA, with sequence confirmation of all influenza A positive viral isolates, or specimens in transport medium if isolates were not available (Table 5.11).

The Xpert Flu Assay sensitivity and specificity for influenza A subtype 2009 H1N1 with NA/W specimens were 100% and 98.8%, respectively (Table 5.12). The Xpert Flu Assay sensitivity and specificity for influenza B with NA/W specimens were 100% and 99.4%, respectively (Table 5.13).

Table 5.11: Xpert Flu Assay Performance on Prospective NA/W Specimens: Influenza A

		Culture/DFA		
Xpert Flu Assay		Pos	Neg	Total
	Pos	6	3[a]	9
	Neg	1[b]	332	333
	Total	7	335	342
		Sensitivity:	85.7% (95% CI: 42.1-99.6)	
		Specificity:	99.1% (95% CI: 97.4-99.8)	

[a]Testing results by sequencing: 3 of 3 were H1N1.
[b]Testing results by sequencing: Flu A

Table 5.12: Xpert Flu Assay Performance on Prospective NA/W Specimens: Influenza A, 2009 H1N1

		Culture/DFA & Sequencing		
Xpert Flu Assay		Pos	Neg	Total
	Pos	4	4[a]	8
	Neg	0	334	334
	Total	4	338	342
		Sensitivity:	100% (95% CI: 39.8-100)	
		Specificity:	98.8% (95% CI: 97.0-99.7)	

[a]Testing results by sequencing: 3 of 4 were H1N1; 1 of 4 was Flu A.

Table 5.13: Xpert Flu Assay Performance on Prospective NA/W Specimens: Influenza B

		Culture/DFA		
Xpert Flu Assay		Pos	Neg	Total
	Pos	7	2[a]	9
	Neg	0	333	333
	Total	7	335	342
		Sensitivity:	100% (95% CI: 65.2-100)	
		Specificity:	99.4% (95% CI: 98.1-99.9)	

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

On prospectively collected NP swabs, the Xpert Flu Assay demonstrated a sensitivity and specificity for detection of influenza A of 100% and 98.3%, respectively, relative to viral culture plus DFA, with sequence confirmation of all influenza A positive viral isolates, or specimens in transport medium if isolates were not available (Table 5.14). The Xpert Flu Assay sensitivity and specificity for influenza A subtype 2009 H1N1 with NP swabs were 100% and 99.0%, respectively (Table 5.15). The Xpert Flu Assay sensitivity and specificity for influenza B with NP swabs were 87.5% and 99.7%, respectively (Table 5.16).

Table 5.14: Xpert Flu Assay Performance on Prospective NP Swab Specimens: Influenza A

		Culture/DFA		
Xpert Flu Assay		Pos	Neg	Total
	Pos	7	5[a]	12
	Neg	0	285	285
	Total	7	290	297
		Sensitivity:	100% (95% CI: 59.0-100)	
		Specificity:	98.3% (95% CI: 96.0-99.4)	

[a]Testing results by sequencing: 3 of 5 were H1N1; 2 of 5 were Flu A.

Table 5.15: Xpert Flu Assay Performance on Prospective NP Swab Specimens: Influenza A, 2009 H1N1

		Culture/DFA & Sequencing		
Xpert Flu Assay		Pos	Neg	Total
	Pos	5	3[a]	8
	Neg	0	289	289
	Total	5	292	297
		Sensitivity:	100% (95% CI: 47.8-100)	
		Specificity:	99.0% (95% CI: 97.0-99.8)	

[a]Testing results by sequencing: 2 of 3 were H1N1; 1 of 3 was Flu A.

Table 5.16: Xpert Flu Assay Performance on Prospective NP Swab Specimens: Influenza B

		Culture/DFA		
Xpert Flu Assay		Pos	Neg	Total
	Pos	7	1[a]	8
	Neg	1[b]	288	289
	Total	8	289	297
		Sensitivity:	87.5% (95% CI: 47.3-99.7)	
		Specificity:	99.7% (95% CI: 98.1-100)	

[a]Testing results by sequencing: Flu B.
[b]Testing results by sequencing: Flu A.

Archived Specimens

A total of 425 archived NA/W specimens were tested for influenza A, influenza A subtype 2009 H1N1 and influenza B by the Xpert Flu Assay and a FDA cleared molecular comparator device. A total of 150 archived NP swab specimens were tested for influenza A, influenza A subtype 2009 H1N1 and influenza B by the Xpert Flu Assay and viral culture plus DFA; 177 archived NP swab specimens did not have viral culture results available and were tested by the FDA cleared molecular comparator device. All influenza A positive specimens indentified by viral culture/DFA or cleared molecular comparator device were sequenced to differentiate influenza A subtype 2009 H1N1 from other influenza A subtypes.

On archived NA/W specimens, the Xpert Flu Assay demonstrated a positive and negative agreement for detection of influenza A of 99.4% and 100%, respectively, relative to viral culture plus DFA, with sequence confirmation of all influenza A positive viral isolates, or

specimens in transport medium if isolates were not available (Table 5.17). The Xpert Flu Assay positive and negative agreement for influenza A subtype 2009 H1N1 with NA/W specimens were 98.4% and 99.7% (Table 5.18). The Xpert Flu Assay positive and negative agreement for influenza B with NA/W specimens were 100% and 100%, respectively (Table 5.19)

Table 5.17: Xpert Flu Assay Performance on Archived NA/W Specimens: Influenza A

		FDA Cleared Molecular Comparator		
Xpert Flu Assay		Pos	Neg	Total
	Pos	159	0	159
	Neg	1[a]	265	266
	Total	160	265	425
		Positive Agreement:	99.4% (95% CI: 96.6-100)	
		Negative Agreement:	100% (95% CI: 98.6-100)	

[a]Testing by sequencing: no sequence match for Flu A, H1N1 or Flu B

Table 5.18: Xpert Flu Assay Performance on Archived NA/W Specimens: Influenza A, 2009 H1N1

		FDA Cleared Molecular Comparator & Sequencing		
Xpert Flu Assay		Pos	Neg	Total
	Pos	124	1[a]	125
	Neg	2[b]	295	297
	Total	126	296	422[c]
		Positive Agreement:	98.4% (95% CI: 94.4-99.8)	
		Negative Agreement:	99.7% (95% CI: 98.1-100)	

[a]Testing results by sequencing: Flu A, not H1N1.
[b]Testing results by sequencing: 2 of 2 H1N1.
[c]3 samples excluded due to PHRED score <20.

Table 5.19: Xpert Flu Assay Performance on Archived NA/W Specimens: Influenza B

		FDA Cleared Molecular Comparator		
Xpert Flu Assay		Pos	Neg	Total
	Pos	40	0	40
	Neg	0	385	385
	Total	40	385	425
	Positive Agreement:		100% (95% CI: 91.2-100)	
	Negative Agreement:		100% (95% CI: 99.0-100)	

On archived NP swabs, the Xpert Flu Assay demonstrated a positive and negative agreement for detection of influenza A of 97.5% and 100%, respectively, relative to viral culture plus DFA, with sequence confirmation of all influenza A positive viral isolates, or specimens in transport medium if isolates were not available (Table 5.20). The Xpert Flu Assay positive and negative agreement for influenza A subtype 2009 H1N1 with NP swabs were 100% and 100%, respectively (Table 5.21). The Xpert Flu Assay positive and negative for influenza B with NP swabs were 93.8% and 99.2%, respectively (Table 5.22).

Table 5.20: Xpert Flu Assay Performance vs. Comparator Method with NP Swab Specimens: Influenza A

		Culture/DFA		
Xpert Flu Assay		Pos	Neg	Total
	Pos	115	0	115
	Neg	3[a]	32	35
	Total	118	32	150
	Positive Agreement:		97.5% (95% CI: 92.7-99.5)	
	Negative Agreement:		100% (95% CI: 89.1-100)	

[a]Testing results by sequencing: 2 of 3 were no sequence match for Flu A, H1N1, or Flu B; 1 of 3 was Flu B.

Table 5.21: Xpert Flu Assay Performance vs. Comparator Method with NP Swab Specimens: 2009 H1N1

		Culture/DFA & Sequencing		
Xpert Flu Assay		Pos	Neg	Total
	Pos	84	0	84
	Neg	0	65	65
	Total	84	65	149[a]
		Positive Agreement:	100% (95% CI: 95.7-100)	
		Negative Agreement:	100% (95% CI: 94.5-100)	

[a]One sample excluded due to PHRED score <20.

Table 5.22: Xpert Flu Assay Performance vs. Comparator Method with NP Swab-Specimens: Influenza B

		Culture/DFA		
Xpert Flu Assay		Pos	Neg	Total
	Pos	30	1[a]	31
	Neg	2[b]	117	119
	Total	32	118	150
		Positive Agreement:	93.8% (95% CI: 79.2-99.2)	
		Negative Agreement:	99.2% (95% CI: 95.4-100)	

[a]Testing results by sequencing: Flu B.
[b]Testing results by sequencing: no sequence match for Flu A, H1N1 or Flu B.

On archived NP swabs, the Xpert Flu Assay demonstrated a positive agreement and negative agreement for detection of influenza A of 98.1% and 99.2%, respectively, relative to the comparator assay, with sequence confirmation of all influenza A positive viral isolates, or specimens in transport medium if isolates were not available (Table 5.23). The Xpert Flu Assay positive and negative agreement for influenza A subtype 2009 H1N1 with NP swabs were 100% and 99.3%, respectively (Table 5.24). The Xpert Flu Assay positive and negative agreements for influenza B with NP swabs were 93.8% and 100%, respectively (Table 5.25).

Table 5.23: Xpert Flu Assay Performance on Archived NP Swab Specimens: Influenza A

		FDA Cleared Molecular Comparator		
Xpert Flu Assay		Pos	Neg	Total
	Pos	51	1[a]	52
	Neg	1[b]	120	121
	Total	52	121	173
		Positive Agreement:	98.1% (95% CI: 89.7-100)	
		Negative Agreement:	99.2% (95% CI: 95.5-100)	

[a]No test results by sequencing available.
[b]Testing results by sequencing: Flu A

Table 5.24: Xpert Flu Assay Performance vs. Comparator Method with NP Swab Specimens: 2009 H1N1

		FDA Cleared Molecular Comparator & Sequencing		
Xpert Flu Assay		Pos	Neg	Total
	Pos	29	1[a]	30
	Neg	0	142	142
	Total	29	143	172[b]
		Positive Agreement:	100% (95% CI: 88.1-100)	
		Negative Agreement:	99.3% (95% CI: 96.2-100)	

[a]No test results available.
[b]Sequence confirmation not available for one sample.

Table 5.25: Xpert Flu Assay Performance vs. Comparator Method with NP Swab Specimens: Influenza B

		FDA Cleared Molecular Comparator		
Xpert Flu Assay		Pos	Neg	Total
	Pos	15	0	15
	Neg	1[a]	157	158
	Total	16	157	173
		Positive Agreement:	93.8% (95% CI: 69.8-99.8)	
		Negative Agreement:	100% (95% CI: 97.7-100)	

[a]No test results by sequencing available.

Of the Xpert Flu Assays runs performed with eligible specimens, 97.1% (1351/1391) of these specimens were successful on the first attempt. The remaining 40 gave indeterminate results on the first attempt (26 ERROR, 10 INVALID and 4 NO RESULT). Thirty-six of the 40 specimens yielded valid results after a single retest; four of the specimens were indeterminate on the second attempt. The assay success rate was equivalent for archived [96.8% (727/751)] and fresh [97.5% (624/640)] specimens.

Conclusions

The results of the nonclinical analytical and clinical performance studies summarized above demonstrate that the Xpert Flu Assay is as safe and effective as the reference method, and therefore is substantially equivalent to the predicate devices.

DEPARTMENT OF HEALTH & HUMAN SERVICES

Public Health Service

Food and Drug Administration
10903 New Hampshire Avenue
Silver Spring, MD 20993

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c/o Kerry J. Flom, Ph.D.
Senior Vice President,
Clinical Affairs and Regulatory Submissions
904 Caribbean Drive
Sunnyvale, California 94089-1189

APR 2 1 2011

Re: K103766
Trade/Device Name: Xpert Flu Assay
Regulation Number: 21 CFR§ 866.3980
Regulation Name: Respiratory viral panel multiplex nucleic acid assay
Regulatory Class: Class II
Product Code: OQW,OCC, OOI
Dated: March 04, 2011
Received: March 07, 2011

Dear Dr. Flom:

We have reviewed your Section 510(k) premarket notification of intent to market the device referenced above and have determined the device is substantially equivalent (for the indications for use stated in the enclosure) to legally marketed predicate devices marketed in interstate commerce prior to May 28, 1976, the enactment date of the Medical Device Amendments, or to devices that have been reclassified in accordance with the provisions of the Federal Food, Drug, and Cosmetic Act (Act) that do not require approval of a premarket approval application (PMA). You may, therefore, market the device, subject to the general controls provisions of the Act. The general controls provisions of the Act include requirements for annual registration, listing of devices, good manufacturing practice, labeling, and prohibitions against misbranding and adulteration.

If your device is classified (see above) into class II (Special Controls), it may be subject to such additional controls. Existing major regulations affecting your device can be found in Title 21, Code of Federal Regulations (CFR), Parts 800 to 895. In addition, FDA may publish further announcements concerning your device in the Federal Register.

Please be advised that FDA's issuance of a substantial equivalence determination does not mean that FDA has made a determination that your device complies with other requirements of the Act or any Federal statutes and regulations administered by other Federal agencies. You must comply with all the Act's requirements, including, but not limited to: registration and listing (21 CFR Part 807); labeling (21 CFR Parts 801 and 809); medical device reporting (reporting of medical device-related adverse events) (21 CFR 803); and good manufacturing practice requirements as set forth in the quality systems (QS) regulation (21 CFR Part 820). This letter

will allow you to begin marketing your device as described in your Section 510(k) premarket notification. The FDA finding of substantial equivalence of your device to a legally marketed predicate device results in a classification for your device and thus, permits your device to proceed to the market.

If you desire specific advice for your device on our labeling regulation (21 CFR Parts 801 and 809), please contact the Office of *In Vitro* Diagnostic Device Evaluation and Safety at (301) 796-5450. Also, please note the regulation entitled, "Misbranding by reference to premarket notification" (21 CFR Part 807.97). For questions regarding the reporting of adverse events under the MDR regulation (21 CFR Part 803), please go to http://www.fda.gov/MedicalDevices/Safety/ReportaProblem/default.htm for the CDRH's Office of Surveillance and Biometrics/Division of Postmarket Surveillance.

You may obtain other general information on your responsibilities under the Act from the Division of Small Manufacturers, International and Consumer Assistance at its toll-free number (800) 638-2041 or (301) 796-7100 or at its Internet address http://www.fda.gov/cdrh/industry/support/index.html.

Sincerely yours,

Sally A. Hojvat, M.Sc., Ph.D.
Director
Division of Microbiology Devices
Office of *In Vitro* Diagnostic Device
Evaluation and Safety
Center for Devices and Radiological Health

Enclosure

4.0 Indications for Use Form

510(k) Number (if known): K103766

Device Name: Xpert® Flu Assay

Indications for Use:

The Cepheid® Xpert Flu Assay 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 2009-2010 influenza season when 2009 H1N1 influenza was the predominant influenza A virus in circulation. 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.

Prescription Use ___X____ (Part 21 CFR 801 Subpart D)	AND/OR	Over-The-Counter Use _____ (21 CFR 801 Subpart C)

(PLEASE DO NOT WRITE BELOW THIS LINE-CONTINUE ON ANOTHER PAGE OF NEEDED)

Concurrence of CDRH, Office of In Vitro Diagnostic Devices (OIVD)

Division Sign-Off
Office of In Vitro Diagnostic
Device Evaluation and Safety

510(k) K103766