510(k) SUBSTANTIAL EQUIVALENCE DETERMINATION
DECISION SUMMARY

A. 510(k) Number:

k120911

B. Purpose for Submission:

Change to a previously cleared device – the addition of the GeneXpert Infinity-80 System for use with the Xpert® Flu Assay.

C. Measurand:

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 from nasal aspirates/washes (NA/W) and nasopharyngeal (NP) swab specimens from patients with signs and symptoms of respiratory infection.

D. Type of Test:

Multiplex nucleic acid assay for qualitative detection and differentiation of influenza A, influenza B and influenza A, subtype 2009 H1N1 from nasal aspirates/washes (NA/W) and nasopharyngeal (NP) swab specimens including nucleic acid isolation and multiplex real-time RT-PCR amplification using the Gene Xpert Dx and Infinity Systems.

E. Applicant:

Cepheid

F. Proprietary and Established Names:

Xpert® Flu, Xpert® Flu Assay
GeneXpert Dx Systems
GeneXpert Infinity-48 System
GeneXpert Infinity-80 System

G. Regulatory Information:

1. Regulation section:

   866.3980 - Respiratory viral panel multiplex nucleic acid assay
   866.3332 – Reagents for detection of specific novel influenza A viruses
   862.2570 – Instrumentation for clinical multiplex test systems.

2. Classification:

   Class II

3. Product codes:

   OQW, OCC, OOI

4. Panel:

   Microbiology (83)

H. Intended Use:

1. 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.

2. Indication for use:

   Same as Intended Use

3. Special conditions for use statement:

   For prescription use only

4. Special instrument requirements:

   The Xpert Flu Assay requires use of the GeneXpert Dx (software version 4.3 or higher), GeneXpert Infinity-48 System (software version 4.3 or higher), or the GeneXpert Infinity-80 System (software version 6.0 or higher) from Cepheid.

I. Device Description:

The Xpert Flu Assay is an automated *in vitro* diagnostic test for qualitative detection and differentiation of influenza A, influenza B and influenza A subtype 2009 H1N1 from nasal aspirates/washes (NA/W) and nasopharyngeal (NP) swab specimens from patients with signs and symptoms of respiratory infection. 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 rRT-PCR assays. The GeneXpert Instrument System family comprises the GeneXpert Dx System, the GeneXpert Infinity-48 System, and the GeneXpert Infinity-80 System, a computer, and preloaded software for running tests and viewing the results. The GeneXpert Infinity Instruments also provide robotic features for cartridge handling. Each module contains a syringe drive for dispensing fluids, an ultrasonic horn for lysing cells or spores, a valve drive for sample movement, and an I-CORE® thermocycler for performing real-time PCR and detection.

All systems require the use of the assay-specific single-use disposable cartridges that hold the PCR reagents and host the PCR process. The patented single-use cartridges contain: (1) eleven chambers for holding sample, reagents, or other materials, (2) a valve body composed of a plunger and syringe barrel, (3) a rotary valve system for controlling the movement of fluids between chambers, (4) an area for capturing, concentrating, washing, and lysing cells, (5) dry real-time PCR reagents, (6) an integrated PCR reaction tube that can be automatically filled by the instrument, and (7) liquid reagents. To eliminate test-to-test contamination, all fluids including amplicons, are contained within the disposable cartridge. The instrument never comes into contact with any fluids within the cartridge. Each disposable cartridge is intended to test one sample. Cartridges are not re-usable.

The GeneXpert cartridge is loaded onto the GeneXpert Instrument System platform, which performs hands-off automated sample processing and real-time PCR for detection of RNA or DNA. Summary and detailed test results are obtained in 75 minutes and are displayed in tabular and graphic formats.

A sample processing control (SPC) and a system control (probe check control) are controls utilized by the GeneXpert Instrument System platform. The SPC is pre-loaded into the GeneXpert cartridge provided with the assay. The SPC is an encapsulated RNA made up of recombinant fragments developed so that there is no homology to the influenza genome. The SPC is present to control for adequate processing of the target viruses and to monitor the presence of inhibitors in the PCR reaction to reduce the possibility of false negative results.

The SPC also ensures the PCR reaction conditions (temperature and time) are appropriate for the amplification reaction and that the PCR reagents are functional. The Probe Check Control verifies reagent rehydration, PCR tube filling in the cartridge, probe integrity and dye stability.

Commercially-available external controls may also be run in accordance with local, state, and federal accrediting organizations, as applicable.

The Xpert Flu Assay includes reagents for the simultaneous detection of the target viruses. The primers and probes in the Xpert Flu Assay detect the presence of nucleic acid sequences for Influenza A (Flu A), Influenza B (Flu B) and Influenza A sub-type 2009 H1N1 (2009 H1N1). The assay for detection of Influenza A uses primers and probes designed against a region in segment 8 of the influenza genome that encodes the matrix gene. The assay for differentiation of 2009 H1N1 targets a region in segment 4 that encodes the hemagglutinin gene. The assay for detection of RNA from Influenza B uses primers and probes designed against segment 4 in a region that encodes the hemagglutinin gene.

Nasal aspirates/washes (NA/W) and nasopharyngeal (NP) swab specimens from patients suspected of having influenza are collected in Universal Transport Medium (UTM) and transported to the GeneXpert area.

The specimen is briefly mixed by inverting the collection tube 5 times. For NP swab specimens, using the supplied 300 µL transfer pipette, 300 µL of the sample are transferred to the sample chamber (large opening) of the Xpert Flu Assay Cartridge. For NA/W specimens, the specimen is first diluted by using the supplied 300 µL transfer pipette and transferring 300 µL of the specimen 2 times (600 µL total) into a fresh 3 mL (3000 µL) UTM tube. The diluted specimen is mixed by inverting the tube 5 times. Using a clean supplied 300 µL transfer pipette, 300 µL of the diluted specimen are transferred to the chamber with the large opening of the Xpert Flu Assay Cartridge.

Reagent 1 (Binding Reagent) is dispensed by squeezing the entire contents into the chamber with the small opening of the Xpert Flu Assay Cartridge. The GeneXpert Instrument system performs sample preparation by mixing the sample with the sample processing control and treatment reagents, capturing the nucleic acids on a cellulose column. The column is washed to remove contaminants and the purified nucleic acids are eluted and then mixed with dry real-time RT-PCR (rRT-PCR) reagents and transferred into the PCR tube for rRT-PCR and detection of Flu RNA. The complete process takes about 75 minutes.

The results are interpolated by the GeneXpert Instrument Systems software from measured fluorescent signals and embedded calculation algorithms. All possible final test results are described below.

The Xpert Flu Assay provides test results for influenza A, influenza B and influenza A, subtype 2009 H1N1, according to the following algorithms:

Flu A:

Target	Test Result
Influenza A	
NEG	Flu A NEGATIVE*
POS	Flu A POSITIVE

*If SPC is INVALID, overall test result is INVALID; if 2009 H1N1 is positive and FLU A is negative, overall test result is INVALID

Flu B:

Target	Test Result
Influenza B	
NEG	Flu B NEGATIVE*
POS	Flu B POSITIVE

*If SPC is INVALID, overall test result is INVALID

2009 H1N1:

Target	Test Result
Influenza A, 2009 H1N1	
NEG	2009 H1N1 NOT DETECTED*
POS	2009 H1N1 DETECTED**

*If SPC is INVALID, overall test result is INVALID
**If Flu A is NEGATIVE, overall test result is INVALID

The possible results of the GeneXpert Flu Assay are:

Flu A POSITIVE; 2009 H1N1 NOT DETECTED; Flu B NEGATIVE
Flu A target RNA detected; 2009 H1N1 target RNA not detected; Flu B target RNA 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
Flu A target RNA detected; 2009 H1N1 target RNA detected; Flu B target RNA 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
Flu B target RNA detected; Flu A target RNA not detected; 2009 H1N1 target RNA not 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
Flu A target RNA not detected; 2009 H1N1 target RNA not detected; Flu B target RNA 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.

Flu A POSITIVE; 2009 H1N1 NOT DETECTED; Flu B POSITIVE
Flu A target RNA detected; 2009 H1N1 target RNA not detected; Flu B target RNA 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 detected; 2009 H1N1 target RNA detected; Flu B target RNA 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 2009 H1N1 target amplification may compete with this control.
- Probe Check – PASS; all probe check results pass

INVALID
SPC does not meet acceptance criteria. Presence or absence of the target RNAs cannot be determined. Repeat test according to the instructions in the Retest Procedure section below.

- 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.

INVALID
Presence or absence of 2009 H1N1 target RNA cannot be determined. Repeat test according to the instructions in the Retest Procedure section below.

- Flu A target RNA not detected and 2009 H1N1 target RNA detected.
- SPC – NA (not applicable); SPC is ignored because a target amplified.
- Probe Check – PASS; all probe check results pass.

J. Substantial Equivalence Information:

1. Predicate device name:

   Xpert® Flu Assay

2. Predicate 510(k) number:

   k103766

3. Comparison with predicate:

	Device	Predicate
Item	**Cepheid Xpert Flu**	**Cepheid Xpert Flu**
510(k) No.	k120911	k103766
Regulation	866.3332, 866.3980, 862.2570	Same
Product Code	OQW, OCC, OOI	Same
Device Class	II	II
Technology/ Detection	Multiplex real time RT/PCR	Same
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 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	Same

	Device	Predicate
Item	**Cepheid Xpert Flu**	**Cepheid Xpert Flu**
	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.	
Indication for Use	Patients with signs and symptoms of respiratory infection in conjunction with clinical and epidemiological risk factors.	Same
Assay Targets	Influenza A, Influenza B, and Influenza A subtype 2009 H1N1	Same
Specimen Types	Nasal aspirates/washes (NA/W) and Nasopharyngeal (NP) swabs	Same
Technological Principles	RT/PCR	RT/PCR

	Device	Predicate
Item	**Cepheid Xpert Flu**	**Cepheid Xpert Flu**
Nucleic Acid Extraction	Yes	Yes
Extraction Methods	Sample preparation integrated in GeneXpert Cartridge and GeneXpert Instrumentation System	Same
Assay Results	Qualitative	Qualitative
Instrument Systems	GeneXpert Dx Systems (I, IV, XVI) GeneXpert Infinity-48 System GeneXpert Infinity-80 System	GeneXpert Dx Systems (I, IV, XVI) GeneXpert Infinity-48 System
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.	Same
Test results	Total 75 minutes for sample preparation and rRT-PCR	Same
Laboratory Users	CLIA Moderate Complexity	CLIA Moderate Complexity

K. Standard/Guidance Document Referenced (if applicable):

Not applicable.

L. Test Principle:

The primers and probes in the Xpert Flu Assay detect the presence of proprietary sequences for influenza A (Flu A), influenza B (Flu B) and influenza A sub-type 2009 H1N1 (2009 H1N1). Nasopharyngeal (NP) swabs and nasal aspirate/wash (NA/W) samples are collected following the user institution's standard procedures and placed into Universal Transport Medium (3 mL UTM tubes) prior to being transported to the GeneXpert System area. For nasopharyngeal (NP) swab specimens, the sample is mixed briefly by inverting the UTM

tube 5 times. Using a supplied 300 µL transfer pipette, 300 µL of the mixed sample is transferred to the chamber with the large opening of the Xpert Flu Assay Cartridge. For nasal aspirate/wash (NA/W) samples, the sample is diluted by placing 600 µL of the NAW sample (using a 300 µL transfer pipetter 2 times) into a 3 mL UTM tube. The contents of the tube are mixed by inverting the tube 5 times. Following the mixing step, 300 µL of the diluted, mixed sample is transferred to the chamber with the large opening of the Xpert Flu Assay Cartridge.

After the sample is added, Reagent 1, a Binding Reagent, is transferred by squeezing the entire contents of the Reagent 1 tube into the chamber with the small opening of the Xpert Flu Assay Cartridge.

The user initiates a test from the system user interface, the instrument signals the user where to place the cartridge, and the cartridge is placed into the indicated module in the GeneXpert Dx System instrument, or onto a conveyor belt on the GeneXpert Infinity System, which then transports the cartridge to the appropriate GeneXpert module or to the holding area for later transport to the appropriate GeneXpert module. Fluidic movements under control of the instrument move the sample and reagents to and from different chambers within the Xpert Flu Assay cartridge. The GeneXpert instrument systems perform sample preparation by first mixing the sample with the Sample Processing Control (encapsulated RNA) in the form of a lyophilized bead within the cartridge. Lysis of the viral particles with Lysis Reagent is followed by mixing with Binding Reagent which allows capture of the nucleic acids on the cellulose column. The column is then washed to remove contaminants and finally, the purified nucleic acids are eluted with an elution reagent. The nucleic acid solution is mixed with dry rRT-PCR reagents and transferred into the PCR tube for real-time RT-PCR and detection of Flu RNA. The Xpert Flu Assay completes sample preparation and real-time RT-PCR in 75 minutes. Internal controls in the Xpert Flu Assay check key automated steps.

M. Performance Characteristics (if/when applicable):

The performance characteristics of the Xpert Flu Assay were established under k103766. The Intended Use of the Xpert Flu Assay has not changed.

1. Analytical performance:

   a. Precision/Reproducibility:

   See k103766

   b. Linearity/assay reportable range:

   See k103766

   c. Traceability, Stability, Expected values (controls, calibrators, or methods):

See k103766

d. Detection limit:

See k103766

e. Analytical specificity:

See k103766

f. Assay cut-off:

See k103766

g. Potentially Interfering Substances

See k103766

h. Fresh vs. Frozen Equivalency Study

See k103766

2. Comparison studies:

a. Method comparison with predicate device:

Not Applicable - See k103766

b. Matrix comparison:

Not Applicable - See k103766

3. Clinical studies:

a. Clinical Sensitivity:

See k103766

b. Clinical specificity:

See k103766

4. Clinical cut-off:

Not Applicable - See k103766

5. Expected values/Reference range:

   See k103766

N. Instrument Name:

GeneXpert Infinity-80 System, (software version 6.0).

O. System Descriptions:

1. Modes of Operation:

   The GeneXpert Infinity-80 System is an extension of the GeneXpert instrument systems which consists of the GeneXpert Dx Instruments (I, IV, and XVI), and GeneXpert Infinity-48 systems.

   The GeneXpert Infinity-80 System is a fully-automated, high-throughput, on-demand, random-access closed system that integrates the processes required for real-time PCR-based molecular diagnostic testing with minimal hands-on time.

   The system combines sample preparation with the amplification and detection process. It has the following smart technology built-in:
   - modules that include a six-channel optics system capable of exciting and detecting multiple fluorescent dyes in the same reaction tube
   - a fluid master scheduler prioritizes test runs to meet dynamic workflow needs
   - smart alerts that keep the user informed
   - bi-directional connectivity with user LIS and HIS networks providing communication flow of all incoming and outgoing test orders and results
   - RemoteXpert web based SSL application for remote access support from Cepheid Technical Support Representatives

2. Software:

   FDA has reviewed applicant's Hazard Analysis and software development processes for this line of product types:

   Yes ____X___ or No _____

3. Specimen Identification:

   Specimen identification is performed with a barcode scanner or by manually entering the information into GeneXpert system software.

4. Specimen Sampling and Handling:

   Specimen handling (once loaded test cartridge) is performed in either an Automation Mode or in a Manual Mode. In the Automation Mode, test cartridges are placed on a conveyor belt, picked up by a gantry, and introduced into an available module for processing.

5. Calibration:

   Cepheid recommends the GeneXpert Infinity-80 System be recalibrated after 1 year of use or at 2000 tests per instrument module, whichever comes first. Calibration is performed by Cepheid.

6. Quality Control:

   Follow Xpert Flu Assay procedures for quality control.

P. Other Supportive Instrument Performance Characteristics Data Not Covered In The "Performance Characteristics" Section above:

Cepheid conducted a comparative precision evaluation of the Xpert Flu Assay, run blinded and in-house, on the GeneXpert Dx and Infinity-80 Systems, using a precision panel consisting of 10 panel members. The total number of tests for each panel member in the study, excluding controls and any required repeats, was 192 [**1** lot * **1** site * **2** instrument systems (1 GeneXpert system; 1 Infinity system) * **2** operators * **12** days * **4** runs]. The study was also conducted under challenging conditions to validate the potential hold times of the samples on the Infinity-80 system at the maximum hold time of one hour for the Xpert Flu Assay.

The performance results are summarized in the table below:

		GeneXpert Dx			GeneXpert Infinity-80			Overall
Specimen ID		**Op. 1 GX**	**Op. 2 GX**	**% Total Agreement**	**Op. 1 I-80**	**Op. 2 I 80**	**% Total Agreement**	**% Total Agreement**
Negative	**%NEG**	100.0% (48/48)	100.0% (48/48)	100.0% (96/96)	100.0% (48/48)	100.0% (48/48)	100.0% (96/96)	100.0% (192/192)
Flu A mod positive	**%POS**	100.0% (48/48)	100.0% (48/48)	100.0% (96/96)	100.0% (48/48)	100.0% (48/48)	100.0% (96/96)	100.0% (192/192)
Flu A low positive	**%POS**	97.9% (47/48)	97.9% (47/48)	97.9% (94/96)	97.9% (47/48)	100.0% (48/48)	99.0% (95/96)	98.4% (189/192)
Flu A high negative	**%NEG**	93.8% (45/48)	93.8% (45/48)	93.8% (90/96)	83.3% (40/48)	91.7% (44/48)	87.5% (84/96)	90.6% (174/192)
2009 H1N1 mod positive	**%POS**	100.0% (48/48)	100.0% (48/48)	100.0% (96/96)	100.0% (48/48)	100.0% (48/48)	100.0% (96/96)	100.0% (192/192)
2009 H1N1 low positive	**%POS**	97.9% (47/48)	97.9% (47/48)	97.9% (94/96)	97.9% (47/48)	100.0% (48/48)	99.0% (95/96)	98.4% (189/192)
2009 H1N1 high negative	**%NEG**	60.4% (29/48)	47.9% (23/48)	54.2% (52/96)	32.6% (15/46)	44.6% (21/47)[b]	38.7% (36/93)[c]	46.6% (88/189)
Flu B mod positive	**%POS**	97.9% (47/48)	97.9% (47/48)	97.9% (94/96)	100.0% (48/48)	100.0% (48/48)	100.0% (96/96)	99.0% (190/192)
Flu B low positive	**%POS**	81.3% (39/48)	81.3% (39/48)	81.3% (78/96)	89.6% (43/48)	87.5% (42/48)	88.5% (85/96)	84.9% (163/192)
Flu B high negative	**%NEG**	89.6% (43/48)	81.3% (39/48)	85.4% (82/96)	89.6% (43/48)	77.1% (37/48)	83.3% (80/96)	84.4% (162/192)
%Total Agreement		91.9% (441/480)	89.8% (431/480)	90.8% (872/960)	89.3% (427/478)	90.2% (432/479)	89.8% (859/957)	90.3% (1731/1917)

[a] 2 specimens yielded indeterminate results on both the first and second attempt
[b] 1 specimen yielded indeterminate results on both the first and second attempt
[c] 3/96 specimens yielded indeterminate results on both the first and second attempt.
[a,b] A valid 2009 H1N1 positive call requires detection of both the Flu A and 2009 H1N1 signals. The 3 invalids were due to a Flu A NEGATIVE; 2009 H1N1 DETECTED; Flu B NEGATIVE result.

Q. Proposed Labeling:

The labeling is sufficient and it satisfies the requirements of 21 CFR Part 809.10.

R. Conclusion:

The submitted information in this premarket notification is complete and supports a substantial equivalence decision.