510(k) SUBSTANTIAL EQUIVALENCE DETERMINATION
DECISION SUMMARY

A. 510(k) Number: K123191

B. Purpose for Submission: The submission is for a modification to the previously cleared Xpert® Flu Assay (K103766, K120911). The modification is the addition of a reverse primer that targets the matrix gene segment of the influenza A virus strain A/Victoria/361/2011. The primer was added to improve the Xpert® Flu Assay detection of this virus strain which is predicted to be the dominant circulating H3N2 virus type during the 2012-2013 influenza season. The A/Victoria/361/2011 virus is also included in the trivalent influenza vaccine for the 2012-2013.

C. Measurand: Influenza A and/or influenza B nucleic acid isolated from nasal aspirates/washes or nasopharyngeal swabs and amplified by real-time RT-PCR.

D. Type of Test: The Xpert® Flu Assay is a real-time RT-PCR assay that measures the presence of influenza A and/or influenza B nucleic acid.

E. Applicant: Cepheid

F. Proprietary and Established Names:
Xpert® Flu, Xpert® Flu Assay
GeneXpert® Instrument Systems
GeneXpert® Dx System
GeneXpert® Infinity-48 System
GeneXpert® Infinity-80 System

G. Regulatory Information:

1. Regulation section:

   866.3980, Respiratory viral panel multiplex nucleic acid assay

2. Classification: Class II

3. Product code: OCC, OQW, OOI

4. Panel: Microbiology (83)

H. Intended Use:

1. Intended use(s):
The Cepheid Xpert® Flu Assay, performed on the GeneXpert® Instrument Systems, is an automated, multiplex real-time RT-PCR assay intended for the *in vitro* qualitative

detection and differentiation of influenza A, influenza B and 2009 H1N1 influenza viral RNA. The Xpert Flu Assay uses nasal aspirates/washes and nasopharyngeal swab specimens collected from patients with signs and symptoms of respiratory infection in conjunction with clinical and epidemiological risk factors. The Xpert Flu Assay is intended as an aid in the diagnosis of influenza.

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

Performance characteristics for influenza A were established during the 2009-2010 influenza season when 2009 H1N1 influenza was the predominant influenza A virus in circulation. Performance characteristics for influenza A were confirmed when influenza
A/H3 and influenza A/2009 H1N1 were the predominant influenza A viruses in circulation (2009-2010, 2010-2011 and 2011-2012). 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(s) for use:

   Same as intended use.

3. Special conditions for use statement(s):

   Prescription use only.

4. Special instrument requirements: The Xpert Flu Assay requires the use of either the GeneXpert® Dx System (Software version 4.3 or higher), or the 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 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 minimized.

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 suspected of having influenza. 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 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 PCR for detection and differentiation of influenza A, influenza B and influenza A, subtype 2009 H1N1 in 75 minutes. The GeneXpert® Instrument Systems, which consist of the GeneXpert® Dx System, the GeneXpert® Infinity-48 System, and the GeneXpert® Infinity-80 System, have 1 to 80 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 ICORE® thermocycler for performing real-time PCR and detection.

J. Substantial Equivalence Information:

1. Predicate device name(s): Cepheid Xpert® Flu Assay

2. Predicate 510(k) number(s): K120911

3. Comparison with predicate:

Similarities and Differences		
Item	Device	Predicate
510(K)	K123191	K120911
Regulation	Same	866.3332 and 866.3980
Product Code	Same	OQW, OCC, OOI
Device Class	Same	II
Technology/Detection	Same	Multiplex real time RT/PCR
Intended Use	The Cepheid Xpert® Flu Assay, performed on the GeneXpert® Instrument Systems, is an automated, multiplex real-time RT-PCR assay intended for the *in vitro* qualitative detection and differentiation of influenza A, influenza B and 2009 H1N1 influenza viral RNA. The Xpert® Flu Assay uses nasal aspirates/washes and nasopharyngeal swab specimens collected from patients with signs and symptoms of respiratory infection in conjunction with clinical and epidemiological risk factors. The Xpert® Flu Assay is intended as an aid in the diagnosis of influenza. Negative results do not preclude influenza virus infection and should not be used as the sole basis for treatment or other patient management decisions. Performance characteristics for influenza A were established during the	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

Similarities and Differences		
Item	Device	Predicate
	2009-2010 influenza season when 2009 H1N1 influenza was the predominant influenza A virus in circulation. Performance characteristics for influenza A were confirmed when influenza A/H3 and influenza A/2009 H1N1 were the predominant influenza A viruses in circulation (2009-2010, 2010-2011 and 2011-2012). 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.	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	Same	Patients with signs and symptoms of respiratory infection in conjunction with clinical and epidemiological risk factors.

Similarities and Differences		
Item	Device	Predicate
Assay Targets	Same	Influenza A, influenza B, and influenza A, subtype 2009 H1N1
Specimen Types	Same	Nasal aspirates/washes (NA/W) and Nasopharyngeal (NP) swabs
Technological Principles	Same	RT-PCR
Nucleic Acid Extraction	Same	Yes
Extraction Methods	Same	Sample preparation integrated in GeneXpert® Cartridge and Gene Xpert® Instrumentation System
Assay Results	Same	Qualitative
Instrument System	Same	Cepheid® GeneXpert® Instrument Systems
Assay Controls	Same	Encapsulated (armored) RNA pseudovirus as a sample processing control. Available but not provided are inactivated virus controls for Flu A/B and Flu A 2009 H1N1 as external positive controls and Coxsackie virus as an external negative control.
Rapid Test Results	Same	Total 75 minutes for sample preparation and real-time RT-PCR
Primers and Probes for influenza A, influenza B and influenza A subtype H1N1	Same plus an additional primer for Influenza A.	Original primers and probes to detect the presence of nucleic acid sequences of influenza A, influenza B, and influenza A, subtype 2009 H1N1
Laboratory Users	Same	CLIA Moderate or High Complexity

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

N/A

L. Test Principle:

The modified Xpert® Flu Assay detects the presence of influenza A and/or influenza B through detection and amplification of nucleic acid. Nucleic acid is isolated from nasal aspirates/washes or nasopharyngeal swabs and amplified by real-time RT-PCR.

M. Performance Characteristics (if/when applicable):

1. Analytical performance:

a. Precision/Reproducibility:

The addition of one primer (oligonucleotide) would not impact the Precision/Reproducibility of this assay. See K120911 for additional information.

b. Linearity/assay reportable range:

The addition of one primer (oligonucleotide) would not impact the Linearity/assay reportable range of this assay. See K120911 for additional information.

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

The addition of one primer (oligonucleotide) would not impact the Traceability, Stability, Expected values (of the controls, calibrators, or methods) of this assay. See K120911 for additional information.

d. Detection limit:

A Limit of Detection study was performed for the following twelve influenza virus strains:

Seasonal influenza A H1N1: A/Brisbane/59/07, A/New Caledonia/20/1999

Seasonal influenza A H3N2: A/Brisbane/10/07, A/Wisconsin/67/05, A/Victoria/361/2011

Influenza A 2009 H1N1: A/SwineNY/01/2009, A/SwineNY/02/2009, A/SwineNY/03/2009, A/SwineCanada/6294, A/WI/629-S1/2009(D00015)

Influenza B: B/Florida/07/04, B/Florida/02/06

Each virus was tested with the modified Xpert® Flu Assay in 20 replicates at four concentrations to obtain the LoD. The LoD was determined to be the lowest concentration at which there were 19/20 or 20/20 positive test results. The LoDs reported in this study were equivalent to or better than those reported for the predicate device.

e. Analytical specificity:

Influenza viruses were diluted in a simulated background matrix of 2.5% porcine mucin and 1% human whole blood in 0.85% sodium chloride in 1X PBS solution with 15% glycerol diluted with UTM at a 1:6 ratio. A representative virus was chosen for each test analyte, influenza A, influenza A 2009 H1N,1 and influenza B. A total of 40 strains of viruses (18), bacteria (21), and yeast (1) were then spiked into the test sample to determine the specificity of the Xpert Flu Assay in the presence of contaminants that are commonly found in the nasopharyngeal flora.

Table 1: Analytical Specificity Determination for Xpert® Flu Assay

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 A2009 H1N1	N/A*	+	+	-
Negative Control Coxsackie virus	N/A*	-	-	-
Adenovirus Type 7A	1.1×10^5 $TCID_{50}$/mL	-	-	-
Adenovirus Type 1	1.0×10^7 $TCID_{50}$/mL	-	-	-
Human Coronavirus 229E	2.5×10^4 $TCID_{50}$/mL	-	-	-
Human Coronavirus OC43	5.6×10^4 $TCID_{50}$/mL	-	-	-
Cytomegalovirus^	4.7×10^7 Copies/mL	-	-	-
Enterovirus Type 71	3.5×10^5 $TCID_{50}$/mL	-	-	-
Epstein-Barr Virus	7.1×10^8 $TCID_{50}$/mL	-	-	-
Parainfluenzavirus Type 1	1.1×10^5 $TCID_{50}$/mL	-	-	-

Parainfluenzavirus Type 2	3.1 x 10^7 $TCID_{50}$/mL	-	-	-
Parainfluenzavirus Type 3	1.9 x 10^6 $TCID_{50}$/mL	-	-	-
Measles Virus	6.3 x 10^4 $TCID_{50}$/mL	-	-	-
Human Metapneumovirus	3.8 x 10^5 $TCID_{50}$/mL	-	-	-
Mumps	6.3 x 10^6 $TCID_{50}$/mL	-	-	-
Respiratory Syncytial Virus A	5.3 x 10^7 $TCID_{50}$/mL	-	-	-
Respiratory Syncytial Virus B	1.2 x 10^7 $TCID_{50}$/mL	-	-	-
HSV	3.1 x 10^6 $TCID_{50}$/mL	-	-	-
Human Rhinovirus	1.2 x 10^5 $TCID_{50}$/mL	-	-	-
Echovirus	3.3 x 10^8 $TCID_{50}$/mL	-	-	-
Bordetella pertussis^	500 ng/mL	-	-	-
Chlamydia pneumonia	5 x 10^6 CFU/mL	-	-	-
Corynebacterium xerosis	1 x 10^6 CFU/mL	-	-	-
Escherichia coli	1 x 10^6 CFU/mL	-	-	-
Proteus vulgaris	1 x 10^6 CFU/mL	-	-	-
Proteus mirabilis	1 x 10^6 CFU/mL	-	-	-
Klebsiella pneumonia	1 x 10^6 CFU/mL	-	-	-
Haemophilus influenza	1 x 10^6 CFU/mL	-	-	-
Lactobacillus crispatus	1 x 10^6 CFU/mL	-	-	-
Legionella pneumophila	1 x 10^6 CFU/mL	-	-	-
Moraxella catarrhalis	1 x 10^6 CFU/mL	-	-	-
Mycobacterium tuberculosis (BCG strain)	1 x 10^6 CFU/mL	-	-	-

Mycoplasm pneumonia	1 x 10^6 CFU/mL	-	-	-
Nisseria meningitides	1 x 10^6 CFU/mL	-	-	-
Nisseria cinerea	1 x 10^6 CFU/mL	-	-	-
Pseudomonas aeruginosa	1 x 10^6 CFU/mL	-	-	-
Staphylococcus aureus	1 x 10^6 CFU/mL	-	-	-
Staphylococcus epidermidis	1 x 10^6 CFU/mL	-	-	-
Streptococcus pneumoniae	1 x 10^6 CFU/mL	-	-	-
Streptococcus pyogenes	1 x 10^6 CFU/mL	-	-	-
Streptococcus salivarius	1 x 10^6 CFU/mL	-	-	-
Candida albicans	1 x 10^6 CFU/mL	-	-	-

*Concentration not available because these are inactivated viruses
^Nucleic acid was tested for *Cytomegalovirus and Bordetella pertussis*

The results of this study show that the modified Xpert® Flu Assay has 100% analytical specificity under the test conditions used. This is the same analytical specificity that was reported for the predicate.

f. Analytical reactivity:

All virus strains were tested at either the LoD or the lowest concentration where all three replicates generated appropriate positive results for Flu A, 2009 H1N1, or Flu B. All influenza viruses were diluted in a simulated background matrix of 2.5% porcine mucin and 1% human whole blood in 0.85% sodium chloride in 1X PBS solution with 15% glycerol diluted with UTM at a 1:6 ratio. All dilutions made for testing were prepared on the day of the test and kept on ice prior to testing. The analytical reactivity data for the modified Xpert® Flu Assay is acceptable. All virus strains were detected and the titers tested are appropriate for evaluating reactivity.

Table 2: Summary Table of Analytical Inclusivity Results of the Xpert® Flu (Flu A Add) Assay

Viral Strain (n=3)	Target	Concentration ($TCID_{50}$/mL)	Flu A	2009 H1N1	Flu B
A/Denver/1/57	Seasonal H1N1	250	POS	NEG	NEG
A/Hawaii/15/2001	Seasonal H1N1	50	POS	NEG	NEG
A/Mal/302/54	Seasonal H1N1	50	POS	NEG	NEG
A/New Jersey/8/76	Seasonal H1N1	250	POS	NEG	NEG
A/NWS/33	Seasonal H1N1	5	POS	NEG	NEG
A/PR/8/34	Seasonal H1N1	50	POS	NEG	NEG
A/Taiwan/42/06	Seasonal H1N1	50	POS	NEG	NEG
A/Swine/1976/31 (Swine H1N1)	Seasonal H1N1	250	POS	NEG	NEG
A/Swine/Iowa/15/30 (Swine H1N1)	Seasonal H1N1	50	POS	NEG	NEG
A/Brisbane/59/07*	Seasonal H1N1	1	POS	NEG	NEG
A/NewCaledonia/20/1999*	Seasonal H1N1	5	POS	NEG	NEG
A/Brisbane/10/07*	Seasonal H3N2	1.25	POS	NEG	NEG
A/Victoria/361/2011*	Seasonal H3N2	1.25	POS	NEG	NEG
A/Victoria/3/75	Seasonal H3N2	250	POS	NEG	NEG
A/Aichi2/68	Seasonal H3N2	50	POS	NEG	NEG
A/Hong Kong/8/68	Seasonal H3N2	50	POS	NEG	NEG
A/NewYork/55/2004	Seasonal H3N2	50	POS	NEG	NEG
A/Port Chalmers/1/73	Seasonal H3N2	50	POS	NEG	NEG
A/Wisconsin/67/05*	Seasonal H3N2	10	POS	NEG	NEG
A/Perth/16/2009	Seasonal H3N2	50	POS	NEG	NEG

Viral Strain (n=3)	Target	Concentration ($TCID_{50}$/mL)	Flu A	2009 H1N1	Flu B
A/SwineNY/01/2009*	2009 H1N1	25	POS	POS	NEG
A/SwineNY/02/2009*	2009 H1N1	1.25	POS	POS	NEG
A/SwineNY/03/2009*	2009 H1N1	1.2505	POS	POS	NEG
A/California/7/2009	2009 H1N1	5	POS	POS	NEG
A/Canada/6294*	2009 H1N1	50	POS	POS	NEG
A/Wisconsin/629-S1	2009 H1N1	1	POS	POS	NEG
A/Mallard/WI/34/75	H5N2	3 pg/µL^^	POS	NEG	NEG
A/Anhui/02/2005/PR8-IBCDC-RG5	H5N1	0.122 pg/µL^^	POS	NEG	NEG
A/chicken/NJ/15086-3/94	H7N3	50 pg/µL^^	POS	NEG	NEG
B/Allen/45	Flu B	50	NEG	NEG	POS
B/Florida/02/06*	Flu B	5	NEG	NEG	POS
B/Florida/04/06	Flu B	50	NEG	NEG	POS
B/Florida/07/04*	Flu B	5	NEG	NEG	POS
B/GL/1739/54	Flu B	50	NEG	NEG	POS
B/Hong Kong/5/72	Flu B	50	NEG	NEG	POS
B/Lee/40	Flu B	50	NEG	NEG	POS
B/Malaysia/2506/04	Flu B	50	NEG	NEG	POS
B/Maryland/1/59	Flu B	5	NEG	NEG	POS
B/Panama/45/90	Flu B	250	NEG	NEG	POS
B/Taiwan/2/62	Flu B	50	NEG	NEG	POS

*Strains (12) used in analytical LOD study (D15589) and tested at limit of detection
^^Concentration expressed in picograms/µL

g. Interfering Substances:

Ten potentially interfering substances were tested on the Xpert® Flu Assay platform to look for any significant changes in the reported Ct value. Each potentially interfering substance was added to either a negative control reaction, where change in sample processing control Ct was evaluated, or a simulated clinical sample with A/Victoria/361/2011at a titer of 3.2x LoD, where a change in Flu A Ct was evaluated.

Samples containing blood or mucin and A/Victoria/361/2011 had significantly increased Ct value indicating an inhibitory effect of these two substances. This inhibition is addressed in the Limitations Section of the Package Insert.

Table 3: Effect of Substance on Mean SPC Cts Relative to UTM Control in Flu A Negative Samples

Substance	Concentration Tested	n	Flu A A/Victoria/361/2011 Sample Processing Control Ct (mean)	§P-values
UTM (Control)	**100%(w/v)**	**8**	**28.0**	
Blood	2% (v/v)	8	27.9	1.000
Mucin*	2.5% (w/v)	8	27.4	0.010
Neo-Synephrine® Nasal Drops	15% (v/v)	8	27.7	0.466
Anefrin Nasal Spray	15% (v/v)	8	28.0	1.000
Zicam Nasal gel	5% (v/v)	8	28.0	1.000
Saline Nasal Spray	15% (v/v) of dose	8	27.9	1.000
Lozenges	1.7 mg/mL menthol	8	27.8	0.848
Oseltamivir (TamiFlu)	7.5 mg/mL	8	27.8	0.691
Mupirocin	10 mg/mL	8	28.2	0.520
Tobramycin	4.0µg/mL	8	27.8	0.691

*$p<0.05$ indicates a statistically significant difference; Ct value is 0.6 cycles earlier relative to UTM control

§ Dunnett Simultaneous Test from one-way ANOVA, comparison with UTM control

Substance	Concentration Tested	n	Flu A A/Victoria/361/2011 Sample Processing Control Ct (mean)	§P-values
UTM (Control)	**100%(w/v)**	**8**	**30.6**	
Blood*	2% (v/v)	8	31.6	0.001
Mucin*	2.5% (w/v)	8	31.6	0.002
Neo-Synephrine® Nasal Drops	15% (v/v)	8	31.1	0.295
Anefrin Nasal Spray	15% (v/v)	8	30.7	0.997
Zicam Nasal gel	5% (v/v)	8	31.4	0.012
Saline Nasal Spray	15% (v/v) of dose	8	31.0	.0440
Lozenges	1.7 mg/mL menthol	8	31.2	0.100
Oseltamivir (TamiFlu)	7.5 mg/mL	8	31.1	0.349
Mupirocin	10 mg/mL	8	31.1	0.248
Tobramycin	4.0µg/mL	8	30.8	0.939

*$p<0.05$ indicates a statistically significant difference
§ Dunnett Simultaneous Test from one-way ANOVA, comparison with UTM control

h. Assay cut-off:

The addition of one primer (oligonucleotide) did not impact the Assay cut-off of this assay. This was confirmed in the clinical study for the modified Xpert® Flu Assay.

i. Validation of Internal Controls

Three internal controls were evaluated in testing with the clinical sample results from the Study Protocol 166. The Sample Processing Control (SPC) was reported as "INVALID" in 0.1% of the 689 total runs. A two-sample t-test indicates that false negative and true negatives occur at the same rate, suggesting that the SPC is functioning properly as an internal control.

The Probe Check Control (PCC) is designed to verify, reagent rehydration, PCR tube filling, probe integrity and dye stability. Eleven test results reported and "ERROR" due to PCC failure (1.6%). This is an adequate control and an acceptable level of error.

The Maximum Pressure (Max PSI) detects fluidic movement within the cartridge and aborts any run that generates a pressure of over 120PSI which may result in failure of the cartridge integrity. There were no Max PSI errors reported during any runs.

The internal controls for the modified Xpert ® Flu Assay are appropriate and adequate to control for the various steps of sample processing, PCR reaction and detection.

2. Comparison studies:

a. Method comparison with predicate device:

The modified Xpert ® Flu Assay was compared to the currently cleared Xpert ® Flu Assay.

b. Matrix comparison:

The matrix comparison study was not needed since device modification did not affect the matrices.

3. Clinical studies:

a. Retrospective clinical study:

The modified Xpert ® Flu Assay was compared to the current Xpert ® Flu Assay. The modified assay detected more positive samples than the current assay, detecting not only additional A/Victoria/361-like viruses, but also A/Perth/16/2009-like viruses. The positive and negative percent agreement of based on testing 166 samples are listed in the table below. The modified Xpert ® Flu Assay with the additional A/Victoria/361 primer demonstrated an improved ability of the test to detect this virus. In addition to testing archived clinical samples, ten contrived specimens (5 NP swab and 5 NA/W background matrices) spiked at LoD with A/Victoria/361 were tested. These specimens were tested blinded as part of the clinical specimens and all were reported as Flu A Positive; 2009 H1N1 NOT Detected; Flu B negative.

Table 4: Positive and Negative Agreement of the Modified Xpert ® Flu Assay.

Analyte	Sample Type	Positive Agreement	Negative Agreement
Influenza A	Archived NP Swab	94.8% (95% CI: 90.4-97.6)	100% (95% CI: 97.9-100)
Influenza A, 2009 H1N1	Archived NP Swab	98.5% (95% CI: 92.1-100)	99.6% (95% CI: 98.1-100)
Influenza B	Archived NP Swab	90.0% (95%	100% (95% CI:

		CI: 73.5-97.9)	98.9-100)
Influenza A	Archived NA/W	98.0% (95% CI: 94.2-99.6)	100% (95% CI: 97.6-100)
Influenza A, 2009 H1N1	Archived NA/W	98.5% (95% CI: 92.0-100)	99.1% (95% CI: 97.0-99.9)
Influenza B	Archived NA/W	95.7% (95% CI: 78.1-99.9)	100% (95% CI: 98.7-100)

4. Clinical cut-off**:**

The clinical cut-off is reported as Ct value of 37 for the sample processing control and a Ct of 40 for Influenza A, Influenza A, 2009 H1N1, and Influenza B analytes.

5. Expected values/Reference range:

In the prospective Cepheid Xpert Flu clinical study there was a total of 639 samples collected. Of the 639 samples, 348 were collected from male subjects and 291 from female subjects. The age distribution among study subjects was: 59.3% (379/639) < 5 years; 16.1% (103/639) 6-21 years; 16.9% (108/639) 22-59 years; and 7.7% (49/639) ≥60 years. 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. These samples were collected during the 2010 influenza season at three clinical laboratories in Australia from August through October of 2010 and from a U.S. clinical site from May to mid-August. The number and percentage of influenza A, influenza A subtype 2009 H1N1, and influenza B prospectively collected positive cases as determined positive by the Xpert Flu Assay are: For NA/W prospective samples influenza A is 2.6% (9/342), influenza A subtype 2009 H1N1 is 2.3% (8/342), and influenza B is 2.6% (9/342). For NP swab prospective samples influenza A is 4.0% (12/297), influenza A subtype 2009 H1N1 is 2.7% (8/297), and influenza B is 2.7% (8/297).

N. Proposed Labeling:

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

O. Conclusion:

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