510(k) SUBSTANTIAL EQUIVALENCE DETERMINATION
DECISION SUMMARY

A. 510(k) Number:

K142045

B. Purpose for Submission:

This is a new 510(k) application for a qualitative Real-Time Reverse Transcription Polymerase Chain Reaction (RT-PCR) assay used with the Cepheid Gene Xpert instrument for the *in vitro* qualitative detection and differentiation of influenza A virus, influenza B virus and respiratory syncytial virus (RSV) viral RNA in nasopharyngeal swab (NPS) or nasal wash/aspirate (NA/W) specimens from symptomatic human patients.

C. Measurand:

Influenza A RNA: Flu A Matrix (M), Flu A Basic Polymerase (PB2), Flu A Acidic protein (PA)
Influenza B RNA: Flu B Matrix (M), Flu B Non-Structural protein (NS)
RSV RNA: Nucleoscapsid gene of RSV A and RSV B

D. Type of Test:

Real-Time Reverse Transcription Polymerase Chain Reaction (RT-PCR)

E. Applicant:

Cepheid Inc.

F. Proprietary and Established Names:

Xpert® Flu/RSV XC
Cepheid Xpert Flu/RSV XC Assay

G. Regulatory Information:

1. Regulation section:

21 CFR 866.3980, Respiratory viral panel multiplex nucleic acid assay

2. Classification:

Class II

3. Product code:

OCC - Respiratory virus panel nucleic acid assay system,
OOI - Real time nucleic acid amplification system

4. Panel:

Microbiology (83)

H. Intended Use:

1. Intended use(s):

The Cepheid Xpert Flu/RSV XC Assay is an automated, multiplex real-time, reverse transcriptase polymerase chain reaction (RT-PCR) assay intended for the *in vitro* qualitative detection and differentiation of influenza A, influenza B, and respiratory syncytial virus (RSV) viral RNA. The Xpert Flu/RSV XC Assay uses nasopharyngeal swab and nasal aspirate/wash specimens collected from patients with signs and symptoms of respiratory infection. The Xpert Flu/RSV XC Assay is intended as an aid in the diagnosis of influenza and respiratory syncytial virus infections in conjunction with clinical and epidemiological risk factors.

Negative results do not preclude influenza virus or respiratory syncytial 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 2013-2014 influenza season. When other novel 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.

Ancillary Specimen Collection Kit

Xpert® Nasopharyngeal Sample Collection Kit
The Xpert Nasopharyngeal Sample Collection Kit is designed to collect, preserve and transport nasopharyngeal swab specimens and to preserve and transport nasal aspirate/wash specimens from patients with signs and symptoms of respiratory infection prior to analysis with the Xpert Flu Assay or the Xpert Flu/RSV XC Assay.

The Xpert Nasopharyngeal Sample Collection Kit has only been cleared for use with

the Xpert Flu Assay and Xpert Flu/RSV XC Assays.

2. Indication(s) for use:

Same as intended use

3. Special conditions for use statement(s):

Prescription use only

4. Special instrument requirements:

GeneXpert Instrument Systems

- GeneXpert Dx System
- GeneXpert Infinity-48 System
- GeneXpert Infinity-80 System

I. Device Description:

The Xpert Flu/RSV XC Assay is a rapid, automated *in vitro* diagnostic test for qualitative detection and differentiation of influenza A, influenza B, and respiratory syncytial virus (RSV). The assay is performed on the Cepheid GeneXpert Instrument Systems (GeneXpert Dx systems and GeneXpert Infinity Systems). The GeneXpert Instrument System platform automates and integrates sample purification, nucleic acid amplification, and detection of the target sequence in simple or complex samples using real-time PCR and reverse transcriptase PCR (RT-PCR) assays. The systems require the use of single-use disposable cartridges (the Xpert Flu/RSV XC 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 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 RSV viral RNA in approximately 60 minutes or less. The GeneXpert Instrument Systems, comprised of the GeneXpert Dx Systems and the GeneXpert Infinity Systems, have 1 to 80 randomly accessible modules, depending upon the instrument, that are each capable of performing separate sample preparation and real-time PCR and RT-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 RT-PCR and detection.

Specimens are collected following the user's institution standard procedures for collecting NA/W specimens and NP swab specimens for influenza and RSV testing.

The Cepheid Xpert Nasopharyngeal Sample Collection Kit or Cepheid's Sample Collection Kit are required for the assay but not provided in the kit.

Quality Control

The Xpert Flu/RSV XC Assay includes reagents for the detection and differentiation of influenza A, influenza B, and RSV viral RNA directly from nasopharyngeal (NP) swab and nasal aspirate/wash (NA/W) specimens collected from patients with signs and symptoms of respiratory infection. A Sample Processing Control (SPC) and a Probe Check Control (PCC) are also included in the cartridge. The SPC is an armored RNA pseudovirus present to control for adequate processing of the target viruses and to monitor the presence of inhibitors in the PCR reaction. It is composed of RNA fragments which do not share any homology to assay gene targets. The Probe Check Control (PCC) verifies reagent rehydration, PCR tube filling in the cartridge, probe integrity and dye stability.

Results Interpretation

The Xpert Flu/RSV XC Assay has two channels (Flu A 1 and Flu A 2) to detect most influenza A strains. The primers and probes in the Flu A 1 channel have 100% homology to human influenza A strains. The primers and probes in the Flu A 2 channel have approximately 80% homology to human influenza A strains. All influenza A strains detected by the Xpert Flu/RSV XC Assay are reported as **Flu A POSITIVE**.

The results are interpreted by the GeneXpert Instrument System from measured fluorescent signals and embedded calculation algorithms and will be shown in the View Results window. All possible results are shown in Table 1.

Table 1 – All possible test results with the Xpert Flu/RSV XC Assay

Result Text	Flu A 1	Flu A 2	Flu B	RSV	SPC
Flu A POSITIVE; Flu B NEGATIVE; RSV NEGATIVE	+/-	+/-	-	-	+/-
Flu A NEGATIVE; Flu B POSITIVE; RSV NEGATIVE	-	-	+	-	+/-
Flu A NEGATIVE; Flu B NEGATIVE; RSV POSITIVE	-	-	-	+	+/-
Flu A POSITIVE; Flu B POSITIVE; RSV NEGATIVE	+/-	+/-	+	-	+/-
Flu A POSITIVE; Flu B NEGATIVE; RSV POSITIVE	+/-	+/-	-	+	+/-
Flu A NEGATIVE; Flu B POSITIVE; RSV POSITIVE	-	-	+	+	+/-

Flu A POSITIVE; Flu B POSITIVE; RSV POSITIVE	+/-	+/-	+	+	+/-
Flu A NEGATIVE; Flu B NEGATIVE; RSV NEGATIVE	-	-	-	-	+
INVALID	-	-	-	-	-

J. Substantial Equivalence Information:

1. Predicate device name(s):

   Hologic Prodesse® ProFlu™+ Assay on the Cepheid SmartCycler II System
   Cepheid Xpert Flu on the Cepheid GeneXpert Instrument System

   Copan Universal Transport Medium (UTM-RT) System

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

   K110968
   K123191
   K042970

3. Comparison with predicate:

Table 2a – Assay Comparison with Predicate Devices

Comparison with Predicates			
	Device	**Predicate Devices**	
Item	**Cepheid Xpert Flu/RSV XC**	**Current Cepheid Xpert Flu**	**Hologic Prodesse ProFlu+ Assay**
510(k) Number	K142045	K123191	K110968
Regulation	866.3980	Same	Same
Product Code	OCC, OOI	OQW, OCC, OOI	OCC, OOI
Device Class	II	Same	Same
Technology Principle of Operation	Multiplex real time RT-PCR	Same	Same
Intended Use	The Cepheid Xpert Flu/RSV XC Assay is an automated, multiplex real-time, reverse transcriptase polymerase chain reaction (RT-PCR assay intended for the *in vitro*	The Cepheid Xpert Flu Assay, performed on the GeneXpert Instrument Systems, is an automated, multiplex real-time RT-PCR assay intended for the *in*	The ProFlu™+ Assay is a multiplex Real-Time PCR (RT-PCR) *in vitro* diagnostic test for the rapid and qualitative detection and discrimination of

	qualitative detection and differentiation of influenza A, influenza B, and respiratory syncytial virus (RSV) viral RNA. The Xpert Flu/RSV XC Assay uses nasopharyngeal swab and nasal aspirate/wash specimens collected from patients with signs and symptoms of respiratory infection in conjunction with clinical and epidemiological risk factors. The Xpert Flu/RSV XC Assay is intended as an aid in the diagnosis of influenza and respiratory syncytial virus. Negative results do not preclude influenza virus or respiratory syncytial virus infection and should not be used as the sole basis for treatment or other patient management decisions.	*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.	Influenza A Virus, Influenza B Virus, and Respiratory Syncytial Virus (RSV) nucleic 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 RSV viral infections in humans and is not intended to detect Influenza C. 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. Conversely, positive results do not rule-out bacterial infection or co-infection with other viruses. The agent detected may not be the definite cause of disease. The use of additional laboratory testing and clinical presentation must be considered in order to obtain the final diagnosis of respiratory viral infection.
	Performance characteristics for influenza A were established during the 2013-2014 influenza season. When other novel influenza A viruses are emerging, performance characteristics may vary.	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.	Performance characteristics for Influenza A Virus were established when Influenza A/H3 and A/H1 were the predominant Influenza A viruses in circulation (2006 – 2007 respiratory season). Performance characteristics for Influenza A were confirmed when Influenza A/H1, Influenza A/H3, and Influenza A/2009 H1N1 were the predominant Influenza A viruses in circulation (2008 and 2009). 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	If infection with a novel influenza A virus is suspected based on current clinical and epidemiological screening criteria	If infection with a novel Influenza A virus is suspected based on current clinical and epidemiological screening criteria

	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.	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.	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 as Xpert Flu/RSV XC Assay	Same - symptomatic patients
Assay Targets	Influenza A Virus M, PA, PB2 genes; Influenza B Virus M, NS genes, and RSV A and RSV B Nucleocapsid genes	Influenza A, Influenza B, and Influenza A, subtype 2009 H1N1	Same as Xpert Flu/RSV XC Assay
Specimen Types	Nasal aspirate/wash (NA/W) specimens and Nasopharyngeal (NP) swab specimens	Same as Xpert Flu/RSV XC Assay	Nasopharyngeal (NP) swab specimens
Nucleic Acid Extraction	Yes	Same	Same
Extraction Methods	Sample preparation integrated in GeneXpert Cartridge and GeneXpert Instrumentation System	Same as Xpert Flu/RSV XC Assay	Extraction and purification with Roche MagNA Pure LC System or bioMérieux NucliSENS easyMAG System
Assay Results	Qualitative	Same	Same
Instrument System	Cepheid GeneXpert Instrument Systems	Same as Xpert Flu/RSV XC Assay	Cepheid SmartCycler II System
Assay Controls	Encapsulated (armored) RNA pseudovirus as a sample processing control. Available but not provided are inactivated virus controls for influenza A/B and RSV as external positive controls, and Coxsackie virus as an external negative control	Same as Xpert Flu/RSV XC Assay	Internal RNA control. Required and provided: influenza A, influenza B, RSV A, RSV B positive RNA transcript controls
Time to obtain test results	Up to 60 minutes for sample preparation and real-time RT-PCR	75 minutes for sample preparation and real-time RT-PCR	Approximately 4 hours for sample preparation and real-time RT-PCR
Primers and	Primers and probes to detect	Primers and probes to detect	Same as Xpert Flu/RSV XC

probes	the presence of nucleic acid sequences of influenza A, influenza B, and RSV	the presence of nucleic acid sequences of influenza A, influenza B, and influenza, subtype H1N1	Assay
Intended Users	Operators in moderate and high complexity labs	Operators in moderate and high complexity labs	Laboratory technologists in CLIA high complexity laboratories

Table 2b – Collection Kit Comparison with Predicate Device

Similarities		
Item	Device	Predicate
Intended Use	The Xpert® Nasopharyngeal Sample Collection Kit is designed to collect, preserve and transport nasopharyngeal swab specimens and to preserve and transport nasal aspirate/wash specimens from patients with signs and symptoms of respiratory infection prior to analysis with the Xpert Flu Assay or the Xpert Flu/RSV XC Assay. The Xpert® Nasopharyngeal Sample Collection Kit has only been cleared for use with the Xpert Flu and Xpert Flu/RSV XC Assays.	Copan Universal Transport Medium (UTM-RT) System is intended for the collection and transport of clinical specimens containing viruses, chlamydiae, mycoplasma or ureaplasma from the collection site to the testing laboratory. UTM-RT can be processed using standard clinical laboratory operating procedures for viral, chlamydial, mycoplasma and ureaplasma culture.
Single-use Device	Yes	Same
Medium Formulation	Hank's Balanced Salt Solution Bovine Serum Albumin L-cysteine Gelatin Sucrose L-glutamic acid HEPES buffer Vancomycin Amphotericin B Colistin Phenol red	Same
pH	7.3 $\pm$ 0.2	Same
Storage Temperature	2 - 25^{o}C (refrigerated and room temperature)	Same
Volume	3 ml	1.5 ml; 3 ml; or 10 ml

Glass Beads	3 x 3 mm	Same
Container	Plastic (medical-grade polypropylene)	Plastic
Product Configuration	Medium Tube in Kit with individually-wrapped sterile swab.	Medium Tubes; Kit with Medium Tubes and Swab Options
Differences		
Item	Device	Predicate
Intended Use (differences)	For collection, preservation and transport of nasopharyngeal swab specimens and to preserve and transport nasal aspirate/wash specimens containing viruses from patients with signs and symptoms of respiratory infection prior to analysis with the Xpert Flu and Xpert Flu/RSV XC Assay.	For collection, transport (and preservation of viability) of swab collected clinical specimens containing viruses, chlamydiae, mycoplasma or ureaplasma. UTM-RT can be processed using standard clinical laboratory operating procedures for viral, chlamydial, mycoplasma and ureaplasma culture.
Swab	Nylon flocked (same collection swab as used with the current Xpert Flu Assay).	Polyester

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

1. FDA Guidance: Establishing the Performance Characteristics of *In Vitro* Diagnostic Devices for the Detection or Detection and Differentiation of Influenza Viruses; issued July 15, 2011.
2. FDA Guidance: Respiratory Viral Panel Multiplex Nucleic Acid Assay; issued Oct 9, 2009.
3. FDA Guidance: In Vitro Diagnostic Devices to Detect Influenza A Viruses: Labeling and Regulatory Path; issued May 1, 2007.
4. FDA Guidance: Format for Traditional and Abbreviated 510(k); issued August 12, 2005.
5. FDA Guidance: Instrumentation for Clinical Multiplex Test Systems; issued March 10, 2005
6. FDA Guidance: Off-The-Shelf Software Use in Medical Devices; issued September 9, 1999.

L. Test Principle:

The Xpert Flu/RSV XC Assay utilizes automated real-time reverse-transcription polymerase
chain reaction (RT-PCR) for unique gene-specific sequence amplification and 5' end cleavage
of hybridized, fluorogenic target-specific probe for the detection of amplified cDNA of the

RNA targets. The primers and probes in the Xpert Flu/RSV XC Assay are designed to amplify and detect unique sequences in the genes which encode for the following proteins: Flu A Matrix (M), Flu A Basic Polymerase (PB2), Flu A Acidic protein (PA), Flu B Matrix (M), Flu B Non-Structural protein (NS), and the RSV A and RSV B Non-Structural proteins. A Sample Processing Control (SPC) verifies adequate lysis of the target virus and sample processing, and detects assay interference. The SPC is mixed with the sample automatically inside the test cartridge to control for adequate sample processing and to monitor the integrity of the RT-PCR assay. Sample organism and SPC are lysed using an ultrasonic device on-board the GeneXpert Instrument. A pump dispenses fluid to and from different cartridge chambers and the PCR and RT-PCR amplification and fluorophore detection occur within the I-CORE module of the instrument.

M. Performance Characteristics (if/when applicable):

1. Analytical performance:

   a. Precision/Reproducibility:

   Reproducibility

   A panel of 10 specimens with varying concentrations of influenza A, influenza B, and RSV was tested on ten different days by two different operators, at each of three sites (10 specimens x 1 time/day x 10 days x 2 operators x 3 sites). Each site used a different GeneXpert Instrument (running the same analytical software) for the analysis. The reproducibility and precision study specimen panel consisted of one seasonal influenza A strain (Flu A/Perth/16/09), one influenza B strain (Flu B/Wisconsin/01/2011), and one respiratory syncytial virus (RSV-A/2/Australia/61) strain. Virus strains were prepared in a simulated matrix: (2.5% (w/v) porcine mucin, 1% (v/v) human whole blood in 0.85% sodium chloride (NaCl) formulated in a 1X PBS solution with 15% glycerol). Each virus strain was prepared at three concentration levels: a "high negative" sample with an analyte concentration below the limit of detection, a "low positive" sample with an analyte concentration approximately 1x the limit of detection, and a "moderate positive" sample with an analyte concentration approximately 2x the limit of detection. Negative samples included in the study were comprised of the simulated background matrix only. One lot of Xpert Flu/RSV XC Assay cartridges was used at each of the 3 testing sites. The Xpert Flu/RSV XC Assay was performed according to the Xpert Flu/RSV XC Assay procedure. For the preparation of the precision panel and reproducibility panels, Cepheid established panel concentration levels and expected results for panel members at those concentrations. This information is summarized in Table 3 below. Reproducibility results are summarized in Table 4.

Table 3 - Sample Panel Concentration Level and Expected Results

Specimen	Level	Expected Positivity Rate and Acceptable Range
Neg	0	0% (0%-20%)
Target High Neg	High negative (below LoD)	50% (20%-80%)
Target Low Pos	Low positive (~1X LoD)	95% (80%-97.5%)
Target Mod Pos	Moderate positive (~2-3X LoD)	100% (97.5%-100%)

Table 4 - Reproducibility of the Cepheid Xpert Flu/RSV XC Assay

Sample ID	Site 1/GX Dx			Site 2/Infinity-80			Site 3/Infinity-48			% Expected Results[c]
	Op 1	Op 2	Site	Op 1	Op 2	Site	Op 1	Op 2	Site	
Negative	100% (10/10)	100% (10/10)	100% (20/20)	100% (10/10)	100% (10/10)	100% (20/20)	100% (10/10)	100% (10/10)	100% (20/20)	100% (60/60)
Flu A-High Neg	70.0% (7/10)	60.0% (6/10)	65.0% (13/20)	80.0% (8/10)	80.0% (8/10)	80.0% (16/20)	60.0% (6/10)	70.0% (7/10)	65.0% (13/20)	70.0% (42/60)
Flu A-Low Pos	100% (10/10)	90.0% (9/10)	95.0% (19/20)	100% (10/10)	100% (10/10)	100% (20/20)	100% (10/10)	90.0% (9/10)	95.0% (19/20)	96.7% (58/60)
Flu A-Mod Pos	100% (10/10)	90.0% (9/10)	95.0% (19/20)	100% (10/10)	100% (10/10)	100% (20/20)	100% (10/10)	100% (10/10)	100% (20/20)	98.3% (59/60)
Flu B-High Neg	90.0% (9/10)	70.0% (7/10)	80.0% (16/20)	100% (10/10)	70.0% (7/10)	85.0% (17/20)	50.0% (5/10)	80.0% (8/10)	65.0% (13/20)	76.7% (46/60)
Flu B-Low Pos	100% (10/10)	90.0% (9/10)	95.0% (19/20)	90.0% (9/10)	70.0% (7/10)	80.0% (16/20)	100% (10/10)	90.0% (9/10)	95.0% (19/20)	90.0% (54/60)
Flu B-Mod Pos	100% (10/10)	100% (10/10)	100% (20/20)	100% (10/10)	100% (10/10)	100% (20/20)	100% (10/10)	100% (10/10)	100% (20/20)	100% (60/60)
RSV-High Neg	60.0% (6/10)	50.0% (5/10)	55.0% (11/20)	90.0% (9/10)	60.0% (6/10)	75.0% (15/20)	70.0% (7/10)	70.0% (7/10)	70.0% (14/20)	66.7% (40/60)
RSV-Low Pos	77.8%[a] (7/9)	100% (10/10)	89.5% (17/19)	80.0% (8/10)	80.0% (8/10)	80.0% (16/20)	90.0% (9/10)	90.0% (9/10)	90.0% (18/20)	86.4% (51/59)
RSV-Mod Pos	100%[b] (9/9)	100% (10/10)	100% (19/19)	100% (10/10)	100% (10/10)	100% (20/20)	100% (10/10)	100% (10/10)	100% (20/20)	100% (59/59)

[a]One sample indeterminate on initial testing; retest not done.

[b]One sample 2x indeterminate.

[c] 'Negative' expected result = negative. 'High Neg', 'Low Pos' and 'Mod Pos' expected result = positive.

During the panel preparation, it was noted that the final concentration of the Mod Positive panel members were closer to 2x LoD than 3X LoD, but still within the overall targeted range. The reproducibility of the Xpert Flu/RSV XC Assay was also evaluated in terms of the fluorescence signal expressed in Ct values for each target detected. The mean, standard deviation (SD), and coefficient of variation (CV) between-sites, between-days, and between-operators for each panel member are presented in Table 5. One replicate was performed per day per operator, therefore, operator and assay (within-run) precision are confounded.

Table 5 - Summary of Reproducibility Results for the Cepheid Flu/RSV XC Assay

Sample	Assay Channel (Analyte)	N[a]	Mean Ct	Between-Site		Between-Day		Between-Operator + Within-Assay		Total	
				SD	CV (%)	SD	CV (%)	SD	CV (%)	SD	CV (%)
Negative	SPC	60	30.8	0.06	0.2	0	0	0.29	0.9	0.29	0.9
Flu A- High Neg	FluA1	18	38.0	0	0	1.55	4.1	0.85	2.2	1.77	4.6
	FluA2	0	NA	NA	NA	NA	NA	NA	NA	NA	NA
Flu A-Low Pos	FluA1	58	34.9	0.38	1.1	0.10	0.3	1.28	3.7	1.34	3.8
	FluA2	0	NA	NA	NA	NA	NA	NA	NA	NA	NA
Flu A- Mod Pos	FluA1	59	33.5	0.49	1.5	0	0	1.29	3.9	1.38	4.1
	FluA2	10	36.3	NA	NA	NA	NA	NA	NA	NA	NA
Flu B- High Neg	FluB	14	36.6	0.80	1.4	0	0	2.83	7.7	2.94	8.0
Flu B-Low Pos	FluB	54	33.4	0	0	1.07	3.2	1.76	5.3	2.06	6.2
Flu B- Mod Pos	FluB	60	32.1	0	0	0.38	1.2	1.47	4.6	1.51	4.7
RSV-High Neg	RSV	20	37.4	0	0	0.14	0.4	1.68	4.5	1.68	4.5
RSV-Low Pos	RSV	51	36.2	0.22	0.6	0	0	1.75	4.8	1.76	4.9
RSV- Mod Pos	RSV	60	35.1	0	0	0.24	0.9	1.20	3.4	1.24	3.5

[a]Results with non-zero Ct values out of 60.

Instrument System Precision

An in-house precision study was conducted to compare the performance of the GeneXpert Dx and the GeneXpert Infinity instrument systems and to provide supplemental information to the reproducibility of the assay. A panel of 10 specimens with varying concentrations of influenza A, influenza B, and RSV was tested 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 (10 specimens x 2 times/ day x 12 days x 2 operators x 2 instrument systems). Three lots of Xpert Flu/RSV XC Assay cartridges were used for the study. The Xpert Flu/RSV XC Assay was performed according to the Xpert Flu/RSV XC Assay procedure. Results are summarized in Table 6.

Table 6 - Precision of the Cepheid Xpert Flu/RSV XC Assay

Sample	GeneXpert Dx			Infinity			% Total Agreement by Sample
	Op 1	Op 2	Instr	Op 1	Op 2	Instr	
Negative	100% (48/48)	100% (48/48)	100% (96/96)	100% (48/48)	100% (48/48)	100% (96/96)	100% (192/192)
Flu A-High Neg	75.0% (36/48)	77.1% (37/48)	76.0% (73/96)	87.5% (42/48)	75.0% (36/48)	81.3% (78/96)	78.7% (151/192)
Flu A-Low Pos	68.8% (33/48)	97.9% (47/48)	83.3% (80/96)	91.7% (44/48)	93.8% (45/48)	92.7% (89/96)	88.0% (169/192)
Flu A-Mod Pos	97.9% (47/48)	100% (48/48)	99.0% (95/96)	93.8% (45/48)	97.9% (47/48)	95.8% (92/96)	97.4% (187/192)
Flu B-High Neg	81.3% (39/48)	79.2% (38/48)	80.2% (77/96)	89.6% (43/48)	79.2% (38/48)	84.4% (81/96)	82.3% (158/192)
Flu B-Low Pos	89.6% (43/48)	95.8% (46/48)	92.7% (89/96)	89.6% (43/48)	87.5% (42/48)	88.5% (85/96)	90.6% (174/192)
Flu B-Mod Pos	97.9% (47/48)	100% (48/48)	99.0% (95/96)	100% (48/48)	100% (48/48)	100% (96/96)	99.5% (191/192)
RSV-High Neg	89.6% (43/48)	77.1% (37/48)	83.3% (80/96)	87.5% (42/48)	83.3% (40/48)	85.4% (82/96)	84.4% (162/192)
RSV-Low Pos	93.8% (45/48)	93.8% (45/48)	93.8% (90/96)	87.5% (42/48)	89.6% (43/48)	88.5% (85/96)	91.1% (175/192)
RSV-Mod Pos	100% (48/48)	100% (48/48)	100% (96/96)	97.9% (47/48)	100% (48/48)	99.0% (95/96)	99.5% (191/192)

The data demonstrated acceptable reproducibility as it shows ≥ 95% detection of all analytes at the moderate positive level on all three instruments. Detection of low positive specimens (1x LoD) was ≥ 90% for influenza B and RSV and 88.0% for influenza A. Due to the relatively low LoD concentrations for RSV and Flu A, it is believed that small variations in sample aliquots contributed to detection levels below the anticipated 95%.

b. Linearity/assay reportable range:

N/A

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

External Controls

ZeptoMetrix inactivated virus controls: (catalog # NATFLUAB-6C and catalog # NATRSV-6C) are external positive controls required for the Xpert Flu/RSV XC Assay.

ZeptoMetrix (catalog # NATCXVA9-6C) is an inactivated Coxsackie virus external negative control required for the Xpert Flu/RSV XC Assay.

Shipping and Storage Stability

Storage conditions were evaluated for nasopharyngeal (NP) swab specimens and nasal aspirate/wash (NA/W) specimens during the period between collection and processing on the GeneXpert Instrument Systems. A specimen stability study was conducted to establish transport and storage claims for specimens to be analyzed with the Xpert Flu/RSV XC Assay. The following transport media were included in this study: Becton Dickinson Universal Viral Transport Medium (UVTM), Remel M5 Transport Medium, Copan Universal Transport Medium (UTM). Positive and negative specimens were included in the study. Positive specimens consisted of one seasonal Flu A strain (A/H3/Victoria/361/2011), one Flu B strain (B/Wisconsin/01/11), and one RSV strain (RSV-A/Long/MD/56) spiked at 2x the LoD into a simulated matrix. Negative specimens consisted of the simulated matrix only.

Aliquots of each positive and negative specimen were stored at 2°C and 8°C to represent the limits of the recommended refrigerated storage temperature range and at 15°C and 30°C to represent the limits of the recommended room temperature storage temperature range. Replicates of 8 positive specimens and replicates of 4 negative specimens were tested at T=0, 1 day, 2 days, 3 days, 4 days, 5 days, 6 days, 7 days, and 8 days for 2°C and 8°C. Replicates of 8 positive specimens and replicates of 4 negative specimens were also tested at T=0, 4 hours, 8 hours, 24 hours, and 48 hours for 15°C and 30°C. For extreme storage conditions, aliquots of each positive and negative specimen were stored refrigerated at 2°C for 7 days. Specimens were then transferred to 30°C and stored for 24 hours and then tested on day 9 with the Xpert Flu/RSV XC Assay. These conditions represent the extremes of all recommended storage temperatures and storage times. Replicates of 8 positive specimens and replicates of 4 negative specimens were tested at T=0 and 9 days.

One replicate each of three external controls (one Flu A/B positive, one RSV positive and one negative) was tested with the Xpert Flu/RSV XC Assay on each day of the study. All positive and negative specimens at all storage conditions and temperatures tested were correctly identified using the Xpert Flu/RSV XC Assay. The study supports the recommended specimen storage conditions at refrigerated (2-8°C) temperatures for up to seven days and at room temperature (15-30°C) for up to 24 hours until testing is performed on the GeneXpert Instrument Systems for all three transport media types: Becton Dickinson UVTM, Remel M5, and Copan UTM. The study also supports specimen stability at an extreme storage temperature and storage time (7 days at 2°C followed by 24 hours at 30°C, and tested on day 9) for all three transport media.

Specimen Stability – Cartridge Hold Time Study

Xpert Flu/RSV XC samples that are prepared for testing on a GeneXpert Infinity System may wait up to four hours for a GeneXpert module to become available after the cartridge is loaded onto the System. During this wait time, the target viral particles may degrade or become unstable such that a low positive signal is rendered "NEGATIVE" by the Xpert Flu/RSV Assay. A Cartridge Hold Time Study was performed to determine an acceptable maximum hold time for the Xpert Flu/RSV XC Assay between sample addition and cartridge processing.

One lot of the Xpert Flu/RSV XC Assay was subjected to a sample hold time study where the sample was added to cartridges to be held at three storage conditions (ambient, 25°C/75% relative humidity, and 35°C) and processed at scheduled hourly time intervals ranging from 0-5 hours. "Ambient" storage condition refers to the controlled temperature of the open laboratory set to 72°F (22.2°C) ±5°F. Chambers set to 25°C with 75% relative humidity and 35°C were monitored continuously as part of Cepheid's automated temperature monitoring and access control system. Positive and negative samples were tested in replicates of eight. Negative samples contained the simulated matrix only.

Table 7 - Cartridge Hold Time Study conditions tested

T = 0*	Environmental Condition	1 Hour*	2 Hours*	3 Hours*	4 Hours*	5 Hours*	Total
8+ 8+ 8+ 8-	Room Temp and ambient humidity	8+ 8+ 8+ 8-	8+ 8+ 8+ 8-	8+ 8+ 8+ 8-	8+ 8+ 8+ 8-	8+ 8+ 8+ 8-	512
	25 °C, 75% RH	8+ 8+ 8+ 8-	8+ 8+ 8+ 8-	8+ 8+ 8+ 8-	8+ 8+ 8+ 8-	8+ 8+ 8+ 8-	
	35 °C	8+ 8+ 8+ 8-	8+ 8+ 8+ 8-	8+ 8+ 8+ 8-	8+ 8+ 8+ 8-	8+ 8+ 8+ 8-	

* Flu A (8+), Flu B (8+), and RSV (8+) 8 replicates tested each; (8-) 8 negative samples tested.

Under the conditions of this study, positive and negative viral samples were stable up to five hours between sample addition and cartridge processing under all storage conditions tested. The data supports the claim to begin the test within 60 minutes of adding the specimen to the cartridge.

d. Detection limit:

Limit of Detection (LoD)

LoD was established using two influenza A H3N2 strains, two influenza A 2009 H1N1 strains, two influenza B strains, two respiratory syncytial virus A (RSV A) strains, two respiratory syncytial virus B (RSV B) strains, and one influenza A H7N9 strain diluted into a negative pooled clinical matrix. The LoD is defined as the lowest concentration (tissue culture infective dose, $TCID_{50}$/mL) per sample that can be reproducibly distinguished from negative samples with 95% confidence or the lowest concentration at which 19 of 20 replicates were positive. Each strain was initially tested in a range finding study at 5 different concentrations in replicates of 20 per concentration of virus. Testing was performed with two lots of reagents across three testing days. The higher LoD observed per strain and per lot was selected for verification. Verification of the estimated LoD claim was performed on one reagent lot across a minimum of three testing days using 20 replicates per strain.

The LoD was determined empirically as the lowest concentration that had 19/20 or 20/20 positive results. The LoD point values for each strain tested are summarized in Table 8.

Table 8 – LoD Results on the Cepheid Xpert Flu/RSV XC Assay

Virus Strain	Confirmed LoD ($TCID_{50}$/mL)
Influenza A/California/7/2009	0.3 (20/20)
Influenza A/Florida/27/2011	16 (19/20)
Influenza A/Perth/16/2009	0.3 (20/20)
Influenza A/Victoria/361/2011	0.8 (20/20)
Influenza B/Massachusetts/2/2012	0.5(20/20)
Influenza B/Wisconsin/01/2011	0.6 (20/20)
RSV A/2/Australia/61	1.2 (20/20)
RSV A/Long/MD/56	1.0 (19/20)
RSV B/Washington/18537/62	1.8 (20/20)
RSV B/9320/Massachusetts/77	2.0 (19/20)
Influenza A/Anhui/1/2013	21.0 (19/20)

e. Analytical reactivity:

Analytical Reactivity

The analytical reactivity of the Xpert Flu/RSV XC Assay was evaluated against multiple strains of influenza A H1N1 (seasonal pre-2009), influenza A H1N1 (pandemic 2009), influenza A H3N2 (seasonal), avian influenza A (H5N1, H5N2, H6N2, H7N2, H7N3, H2N2, H7N9, and H9N2), influenza B (representing strains from both Victoria and Yamagata lineages), and respiratory syncytial virus subgroups A and B (RSV A and RSV B) at levels near the analytical LoD. A total of 64 strains including 54 influenza viruses and 10 RSV strains were tested in this study with the Xpert Flu/RSV XC Assay. Three replicates were tested for each strain. Results are shown in Table 9.

Table 9 – Reactivity Results for the Cepheid Xpert Flu/RSV XC Assay

Virus	Strain	Concentration	Result		
			Flu A	Flu B	RSV
No Template Control			NEG	NEG	NEG
Influenza A H1N1 (pre-2009)	A/swine/Iowa/15/30	32.0 $TCID_{50}$/mL	POS	NEG	NEG
	A/WS/33	32.0 $TCID_{50}$/mL	POS	NEG	NEG
	A/PR/8/34	32.0 $TCID_{50}$/mL	POS	NEG	NEG
	A/Mal/302/54	32.0 $TCID_{50}$/mL	POS	NEG	NEG
	A/Denver/1/57	32.0 $TCID_{50}$/mL	POS	NEG	NEG
	A/New Jersey/8/76	32.0 $TCID_{50}$/mL	POS	NEG	NEG
	A/New Caledonia/20/1999	32.0 $TCID_{50}$/mL	POS	NEG	NEG
	A/New York/55/2004	32.0 $TCID_{50}$/mL	POS	NEG	NEG
	A/Soloman Island/3/2006	32.0 $TCID_{50}$/mL	POS	NEG	NEG
	A/Taiwan/42/06	32.0 $TCID_{50}$/mL	POS	NEG	NEG
	A/Brisbane/59/2007	32.0 $TCID_{50}$/mL	POS	NEG	NEG
Influenza A H1N1 (pdm2009)	A/California/7/2009	32.0 $TCID_{50}$/mL	POS	NEG	NEG
	A/swine/NY/02/2009	32.0 $TCID_{50}$/mL	POS	NEG	NEG
	A/Florida/27/2011	32.0 $TCID_{50}$/mL	POS	NEG	NEG
	A/Colorado/14/2012	32.0 $TCID_{50}$/mL	POS	NEG	NEG
	A/Washington/24/2012	80.0[a] $TCID_{50}$/mL	POS	NEG	NEG
Influenza A H3N2 (Seasonal)	A/Aichi/2/68	1.6 $TCID_{50}$/mL	POS	NEG	NEG
	A/HongKong/8/68	1.6 $TCID_{50}$/mL	POS	NEG	NEG
	A/Port Chalmers/1/73	1.6 $TCID_{50}$/mL	POS	NEG	NEG
	A/Hawaii/15/2001	1.6 $TCID_{50}$/mL	POS	NEG	NEG
	A/Wisconsin/67/05	1.6 $TCID_{50}$/mL	POS	NEG	NEG
	A/Brisbane/10/2007	1.6 $TCID_{50}$/mL	POS	NEG	NEG

Virus	Strain	Concentration	Result		
			Flu A	Flu B	RSV
	A/Perth/16/2009	1.6 $TCID_{50}$/mL	POS	NEG	NEG
	A/Minnesota/11/2010 (H3N2)v	1.6 $TCID_{50}$/mL	POS	NEG	NEG
	A/Indiana/08/2011 (H3N2)v	1.6 $TCID_{50}$/mL	POS	NEG	NEG
	A/Victoria/361/2011	1.6 $TCID_{50}$/mL	POS	NEG	NEG
	A/Texas/50/2012	1.6 $TCID_{50}$/mL	POS	NEG	NEG
Avian influenza A	A/duck/Hunan/795/2002 (H5N1)	≤ 1ρg/μL[b]	POS	NEG	NEG
	A/chicken/Hubei/327/2004 (H5N1)	≤ 1ρg/μL[b]	POS	NEG	NEG
	A/Anhui/01/2005 (H5N1)	≤ 1ρg/μL[b]	POS	NEG	NEG
	A/Japanese white eye/HongKong/1038/2006 (H5N1)	≤ 1ρg/μL[b]	POS	NEG	NEG
	A/mallard/WI/34/75 (H5N2)	≤ 1ρg/μL[b]	POS	NEG	NEG
	A/chicken/CA431/00 (H6N2)	≤ 1ρg/μL[b]	POS	NEG	NEG
	A/duck/LTC-10-82743/1943 (H7N2)	≤ 1ρg/μL[b]	POS	NEG	NEG
	A/chicken/NJ/15086-3/94 (H7N3)	≤ 1ρg/μL[b]	POS	NEG	NEG
	A/Anhui/1/2013 (H7N9)	N/A[c]	POS	NEG	NEG
	A/Shanghai/1/2013 (H7N9)	N/A[c]	POS	NEG	NEG
	A/chicken/Korea/38349-p96323/ 1996 (H9N2)	≤ 1ρg/μL[b]	POS	NEG	NEG
	A/Mallard/NY/6750/78 (H2N2)	≤ 1ρg/μL[b]	POS	NEG	NEG
Influenza B	B/Lee/40	1.2 $TCID_{50}$/mL	NEG	POS	NEG
	B/Allen/45	1.2 $TCID_{50}$/mL	NEG	POS	NEG
	B/GL/1739/54	1.2 $TCID_{50}$/mL	NEG	POS	NEG
	B/Maryland/1/59	1.2 $TCID_{50}$/mL	NEG	POS	NEG
	B/Panama/45/90[d]	3.0 $TCID_{50}$/mL[e]	NEG	POS	NEG
	B/Florida/07/2004[f]	1.2 $TCID_{50}$/mL	NEG	POS	NEG
	B/Florida/02/06[d]	1.2 $TCID_{50}$/mL	NEG	POS	NEG
	B/Florida/04/06[f]	1.2 $TCID_{50}$/mL	NEG	POS	NEG
	B/Wisconsin/01/2011[d]	1.2 $TCID_{50}$/mL	NEG	POS	NEG
	B/Massachusetts/2/2012[f]	1.2 $TCID_{50}$/mL	NEG	POS	NEG
	B/Hong Kong/5/72	1.2 $TCID_{50}$/mL	NEG	POS	NEG
	B/Wisconsin/01/2010[f]	1.2 $TCID_{50}$/mL	NEG	POS	NEG
	B/Malaysia/2506/04[d]	1.2 $TCID_{50}$/mL	NEG	POS	NEG
	B/Taiwan/2/62	1.2 $TCID_{50}$/mL	NEG	POS	NEG
	B/Brisbane/60/2008[d]	1.2 $TCID_{50}$/mL	NEG	POS	NEG
RSV A	RSV-A/Long/MD/56	2.4 $TCID_{50}$/mL	NEG	NEG	POS
	RSV-A/2/Australia/61	2.4 $TCID_{50}$/mL	NEG	NEG	POS
	RSV-A/NY (Clinical unknown)	2.4 $TCID_{50}$/mL	NEG	NEG	POS
	RSV-A/WI/629-8-2/2007	2.4 $TCID_{50}$/mL	NEG	NEG	POS
	RSV-A/WI/629-11-1/2008	2.4 $TCID_{50}$/mL	NEG	NEG	POS
RSV B	RSV-B/Wash/18537/62	4.0 $TCID_{50}$/mL	NEG	NEG	POS

Virus	Strain	Concentration	Result		
			Flu A	Flu B	RSV
	RSV-B/9320/MA/77	4.0 $TCID_{50}$/mL	NEG	NEG	POS
	RSV-B/WV14617/85	4.0 $TCID_{50}$/mL	NEG	NEG	POS
	RSV-B/CH93(18)-18	20.0 $TCID_{50}$/mL[g]	NEG	NEG	POS
	RSV-B/WI/629-5B/0607	4.0 $TCID_{50}$/mL	NEG	NEG	POS

[a]Influenza A/Washington/24/2012 was tested at 5x LoD (80.0 $TCID_{50}$/mL) to obtain 3 of 3 Flu A POSITIVE result calls.
[b]Purified viral RNA in simulated background matrix was used for avian influenza A viruses due to biosafety regulations.
[c]Inactivated avian influenza A (H7N9) viruses without viral titer was diluted 100,000 fold in simulated background matrix and tested due to biosafety regulations.
[d]Known Victoria lineage.
[e]Influenza B/Panama/45/90 was tested at 5x LoD (3.0 $TCID_{50}$/mL) to obtain 3/3 Flu B POSITIVE result calls.
[f]Known Yamagata lineage.
[g]RSV-B/CH93(18)-18 was tested at 10x LoD (20.0 $TCID_{50}$/mL) to obtain 3/3 RSV POSITIVE result calls.

Although this test has been shown to detect the novel avian influenza A (H7N9) cultured material, the performance characteristics of this device with clinical specimens that are positive for the novel avian influenza A (H7N9) virus have not been established. The Xpert Flu/RSV XC Assay can distinguish between influenza A and B viruses, but it cannot differentiate influenza subtypes.

f. Analytical Specificity:

The analytical specificity of the Xpert Flu/RSV XC Assay was evaluated by testing a panel of forty-four (44) microorganisms consisting of 16 viral, 26 bacterial, and 2 yeast strains representing common respiratory pathogens or those potentially encountered in the nasopharynx. One replicate each of three external controls (one positive control for Flu A/B, one positive control for RSV, and one negative control) was tested with the Xpert Flu/RSV XC Assay on each day of the study.

Bacterial stock cultures were prepared by suspending the bacterial growth from an agar plate in PBS buffer containing 15% glycerol. All bacterial strains were tested in triplicate at concentrations of $\geq 10^6$ CFU/mL with the exception of one strain which was tested at 10^5 CFU/mL (Chlamydia pneumoniae). Viral stock cultures were propagated using recommended tissue culture cell lines and hemagglutination assays were used to establish titers. All viral strains were tested in triplicate at concentrations of $\geq 10^5$ $TCID_{50}$/mL. Three replicates of a no template control (background matrix diluted in background matrix) were also tested.

All the strains were diluted into a simulated background matrix. The simulated background matrix consisted of 2.5% (w/v) porcine mucin, 1% (v/v) human whole blood in 0.85% sodium chloride (NaCl) formulated in 1X PBS solution with 15% glycerol, which was then diluted in UTM to a final concentration of 16.7%. Negative specimens consisted of the diluted simulated background matrix only. Dilutions were prepared daily and kept on ice prior to testing.

Results are summarized in Table 10. The results demonstrate that the Xpert Flu/RSV XC Assay does not cross-react with any of the 44 common respiratory microorganisms tested in this study.

Table 10 - Analytical Specificity/Cross-reactivity of the Cepheid Xpert Flu/RSV XC Assay

Organism	Concentration	Influenza A	Influenza B	RSV
No Template Control		0/3	0/3	0/3
Adenovirus Type 1	$1.12x10^7$ $TCID_{50}$/mL	0/3	0/3	0/3
Adenovirus Type 7	$1.87x10^5$ $TCID_{50}$/mL	0/3	0/3	0/3
Human coronavirus OC43	$2.85x10^5$ $TCID_{50}$/mL	0/3	0/3	0/3
Human coronavirus 229E	$1x10^5$ $TCID_{50}$/mL	0/3	0/3	0/3
Cytomegalovirus	$7.24x10^5$ $TCID_{50}$/mL	0/3	0/3	0/3
Echovirus	$3.31x10^7$ TCID50/mL	0/3	0/3	0/3
Enterovirus	$1x10^5$ $TCID_{50}$/mL	0/3	0/3	0/3
Epstein Barr Virus	$7.16x10^7$ $TCID_{50}$/mL	0/3	0/3	0/3
HSV	$8.9x10^6$ $TCID_{50}$/mL	0/3	0/3	0/3
Measles	$6.3x10^5$ $TCID_{50}$/mL	0/3	0/3	0/3
Human metapneumovirus	$3.8x10^5$ $TCID_{50}$/mL	0/3	0/3	0/3
Mumps virus	$6.31x10^6$ $TCID_{50}$/mL	0/3	0/3	0/3
Human parainfluenza Type 1	$1.15x10^6$ $TCID_{50}$/mL	0/3	0/3	0/3
Human parainfluenza Type 2	$1x10^5$ $TCID_{50}$/mL	0/3	0/3	0/3
Human parainfluenza Type 3	$3.55x10^7$ $TCID_{50}$/mL	0/3	0/3	0/3
Rhinovirus Type 1A	$1.26x10^5$ $TCID_{50}$/mL	0/3	0/3	0/3
Acinetobacter baumannii	$>1x10^6$ CFU/mL	1/26[a]	0/26	0/26
Burkholderia cepacia	$>1x10^6$ CFU/mL	0/3	0/3	0/3

Organism	Concentration	Influenza A	Influenza B	RSV
Candida albicans	>1x10^6 CFU/mL	0/3	0/3	0/3
Candida parapsilosis	>1x10^6 CFU/mL	0/3	0/3	0/3
Bordetella pertussis	1x10^8 CFU/mL	0/3	0/3	0/3
Chlamydia pneumoniae	3.16x10^5 CFU/mL	0/3	0/3	0/3
Citrobacter freundii	>1x10^6 CFU/mL	0/3	0/3	0/3
Corynebacterium sp.	>1x10^6 CFU/mL	0/3	0/3	0/3
Escherichia coli	>1x10^6 CFU/mL	0/3	0/3	0/3
Enterococcus faecalis	>1x10^6 CFU/mL	0/3	0/3	0/3
Hemophilus influenzae	1x10^6 CFU/mL	0/3	0/3	0/3
Lactobacillus sp.	1x10^6 CFU/mL	0/3	0/3	0/3
Legionella spp.	1x10^8 CFU/mL	0/3	0/3	0/3
Moraxella catarrhalis	>1x10^6 CFU/mL	0/3	0/3	0/3
Mycobacterium tuberculosis (avirulent)	1.15x10^6 CFU/mL	0/3	0/3	0/3
Mycoplasma pneumoniae	1x10^7 CFU/mL	0/3	0/3	0/3
Neisseria meningitides	>1x10^6 CFU/mL	0/3	0/3	0/3
Neisseria mucosa	>1x10^6 CFU/mL	0/3	0/3	0/3
Propionibacterium acnes	>1x10^6 CFU/mL	0/3	0/3	0/3
Pseudomonas aeruginosa	>1x10^6 CFU/mL	0/3	0/3	0/3
Staphylococcus aureus (protein A producer)	>1x10^6 CFU/mL	0/3	0/3	0/3
Staphylococcus epidermidis	>1x10^6 CFU/mL	0/3	0/3	0/3
Staphyloccus haemolyticus	>1x10^6 CFU/mL	0/3	0/3	0/3
Streptococcus agalactiae	>1x10^6 CFU/mL	0/3	0/3	0/3
Streptococcus pneumoniae	>1x10^6 CFU/mL	0/3	0/3	0/3
Streptococcus pyogenes	>1x10^6 CFU/mL	0/3	0/3	0/3
Streptococcus salivarius	>1x10^6 CFU/mL	0/3	0/3	0/3
Streptococcus sanguinis	>1x10^6 CFU/mL	0/3	0/3	0/3

[a]1 out of the 3 original replicates tested as "Flu A Positive". The Ct score associated with the false positive was 39.4; near the cut-off of 40.0 for a positive call. An additional 23 replicates were tested and all 23 were negative for all three analyte channels.

g. Potentially Interfering Substances:

In a non-clinical study, potentially interfering substances that may be present in the nasopharynx were evaluated relative to the performance of the Xpert Flu/RSV XC Assay. Negative samples (n = 8) were tested per each substance to determine the effect on the performance of the sample processing control (SPC). Positive samples (n = 8) were tested per substance with six influenza (four influenza A and two influenza B) and four RSV (two RSV A and two RSV B) strains spiked at 2x the analytical LoD determined for each strain. All results were compared to positive and negative Universal Transport Medium (UTM) controls. The evaluated substances are listed in Table 11 with active ingredients and concentrations tested shown. There was no assay interference in the presence of the substances at the concentrations tested in this study. All positive and negative replicates were correctly identified using the Xpert Flu/RSV XC Assay.

FluMist vaccine samples were correctly reported as **Flu A POSITIVE**; **FLU B POSITIVE**; **RSV NEGATIVE** as expected. Samples containing FluMist may cause false positive results.

Table 11 - List of Interfering Substances Tested

Substance ID	Substance/Class	Substance/Active Ingredient	Concentration Tested
C	Control	UTM	100% (v/v)
Albuterol Sulfate	Beta-adrenergic bronchodilator	Albuterol Sulfate	0.83 mg/mL
Blood	Blood	Blood (human)	2% (v/v)
BD	Transport Media	n/a	100% (v/v)
M4	Transport Media	n/a	100% (v/v)
M4RT	Transport Media	n/a	100% (v/v)
M5	Transport Media	n/a	100% (v/v)
Menthol	Oral anesthetic and analgesic	Benzocaine, Menthol	1.7 mg/mL
Mucin	Mucin	Purified Mucin protein	2.5% (w/v)
Mupirocin	Antibiotic nasal ointment	Mupirocin	10 mg/mL
Saline	Saline Nasal Spray	Sodium Chloride (0.65%)	15% (v/v)
Anefrin	Nasal Spray	Oxymetazoline (0.05%)	15% (v/v)
PHNY	Nasal Drops	Phenylephrine (0.5%)	15% (v/v)
Tamiflu	Anti-viral drug	Zanamivir	7.5 mg/mL
Tobramycin	Antibacterial, systemic	Tobramycin	4 µg/mL
Zicam	Nasal Gel	Luffa Opperculata,	15% (w/v)

		Galphimia glauca, histanium hydrochloricum	
FluMist	Live vaccine	Live influenza virus	6.7% (v/v)
Fluticasone Propionate Nasal Spray	Nasal corticosteroid	Fluticasone Propionate	5 µg/mL

Table 12 – Interfering Substances: Agreement with Expected Results

Substance	Negative	Flu A H3N2		Flu A 2009 H1N1		Flu B		RSV A		RSV B	
		Strain 1	Strain 2	Strain 1	Strain 2	Strain 1	Strain 2	Strain 1	Strain 2	Strain 1	Strain 2
UTM	8/8	8/8	8/8	8/8	8/8	8/8	8/8	8/8	8/8	8/8	8/8
Albuterol	8/8	8/8	8/8	8/8	8/8	8/8	8/8	8/8	8/8	8/8	8/8
Blood	8/8	8/8	8/8	8/8	8/8	8/8	8/8	8/8	8/8	8/8	8/8
BD UVT transport media	8/8	8/8	8/8	8/8	8/8	8/8	8/8	8/8	8/8	8/8	8/8
M4 transport media	8/8	8/8	8/8	8/8	8/8	8/8	8/8	8/8	8/8	8/8	8/8
M4RT transport media	8/8	8/8	8/8	8/8	8/8	8/8	8/8	8/8	8/8	8/8	8/8
M5 transport media	8/8	8/8	8/8	8/8	8/8	8/8	8/8	8/8	8/8	8/8	8/8
Menthol	8/8	8/8	8/8	8/8	8/8	8/8	8/8	8/8	8/8	8/8	8/8
Mucin	8/8	8/8	8/8	8/8	8/8	8/8	8/8	8/8	8/8	8/8	8/8
Mupirocin	8/8	8/8	8/8	8/8	8/8	8/8	8/8	8/8	8/8	8/8	8/8
NaCl	8/8	8/8	8/8	8/8	8/8	8/8	8/8	8/8	8/8	8/8	8/8
Oxymetazoline	8/8	8/8	8/8	8/8	8/8	8/8	8/8	8/8	8/8	8/8	8/8
Phenylephrine	8/8	8/8	8/8	8/8	8/8	8/8	8/8	8/8	8/8	8/8	8/8
Tamiflu	8/8	8/8	8/8	8/8	8/8	8/8	8/8	8/8	8/8	8/8	8/8
Tobramycin	8/8	8/8	8/8	8/8	8/8	8/8	8/8	8/8	8/8	8/8	8/8
Zicam	8/8	8/8	8/8	8/8	8/8	8/8	8/8	8/8	8/8	8/8	8/8
FluMist*	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
UTM control for Fluticasone Propionate	8/8	8/8	8/8	8/8	8/8	8/8	8/8	8/8	8/8	8/8	8/8
Fluticasone Proprionate	8/8	8/8	8/8	8/8	8/8	8/8	8/8	8/8	8/8	8/8	8/8

*FluMist contains live, attenuated influenza strains comprised of influenza A H3N2, influenza A H1N1, and influenza B. FluMist tested positive for Flu A and Flu B and negative for RSV using the Xpert Flu A/B & RSV XC Assay as expected. The strains listed in Table 12 were not tested in the presence of FluMist. Negative samples were not tested in the presence of FluMist due to an inability to assess the effect of FluMist on the SPC controls as compared to a positive sample.

Competitive interference

Competitive interference caused by clinically relevant co-infections of the targets in the Xpert Flu/RSV XC was evaluated by testing individual influenza and RSV

strains at LoD and spiking in different influenza or RSV strains at a higher concentration in a simulated background matrix. The concentration of each strain at LoD ranged from 0.57 TCID50/mL to 2.0 TCID50/mL and the concentration of the competitive strains ranged from 10^3 TCID50/mL to 10^4 TCID50/mL. Analytical competitive interference was assessed using one (1) seasonal Flu A H3 strain (H3/Victoria/361/2011), one (1) Flu B strain (Flu B/Mass/2/2012), one (1) RSV A strain (RSV-A/2/Australia/61), and one (1) RSV B strain (RSV-B/Wash/18537/62).

Replicates of 20 were tested for each target strain and each competitive strain combination. The normal binomial distribution with 20 replicate samples at LoD is between 17 and 20 positive results based on the binomial distribution with N=20, p=.95 (X~Bin(20,0.95)). Therefore, sets of 20 with 16 or less positives would be rare and an indication of a competitive inhibitory effect due to high levels of a competing analyte. One replicate each of three external controls (one Flu A/B positive, one RSV positive and one negative) was tested with the Xpert Flu/RSV XC Assay on each day of this study.

Under the conditions of the study using the normal binomial distribution with 20 replicate samples at LoD of between 17 and 20 positive results, no competitive inhibitory effects were observed at the analytical LoD for each of the strains tested in the presence of another analyte. The results for each analyte at LoD in combination with another competitive analyte are shown in Tables 13 through 16. The average Ct values for each strain tested at LoD without a competitive analyte are taken from the Limit of Detection study.

Table 13 – Competitive Interference for Flu A/Victoria/361/2011 at LoD

Target Strain Titer ($TCID_{50}$/mL)	Competitive Strain	Competitive Strain Titer ($TCID_{50}$/mL)	Positives/ 20 replicates	Average Ct			
				Flu A1	Flu B	RSV	SPC
0.8	n/a	n/a	20/20	33.3	-	-	30.8
1.0 (Upper 95% CI of LoD)	B/Wisconsin/01/11	1.00E+03	18/20	36.2	22.7	-	31.2
	RSV-A/Long/MD/56	1.00E+04	18/20	36.3	-	19.4	31.2
	RSV-B/Wash/18537/62	1.00E+04	19/20	35.0	-	20.3	31.0

Table 14 – Competitive Interference for Flu B/Mass/2/2012 at LoD

Target Strain Titer ($TCID_{50}$/mL)	Competitive Strain	Competitive Strain Titer ($TCID_{50}$/mL)	Positives/ 20 replicates	Average Ct			
				Flu A1	Flu B	RSV	SPC
0.5	n/a	n/a	20/20	-	32.4	-	30.7
0.57 (Upper 95% CI of LoD)	H3/Victoria/361/2011	1.00E+03	17/20	23.4	37.1	-	31.4
	RSV-A/Long/MD/56	1.00E+03	19/20	-	34.9	23.1	31.4
	RSV-B/Wash/18537/62	1.00E+03	19/20	-	33.5	24.0	31.3

Table 15 – Competitive Interference for RSV-A/2/Australia/61 at LoD

Target Strain Titer ($TCID_{50}$/mL)	Competitive Strain	Competitive Strain Titer ($TCID_{50}$/mL)	Positives/ 20 replicates	Average Ct			
				Flu A1	Flu B	RSV	SPC
1.2	n/a	n/a	20/20	-	-	35.4	30.7
1.3 (Upper 95% CI of LoD)	H3/Victoria/361/2011	1.00E+03	18/20	23.7	-	37.2	31.7
	B/Wisconsin/01/11	1.00E+03	18/20	-	22.9	36.4	31.5

Table 16 – Competitive Interference for RSV-B/Wash/18537/62 at LoD

Target Strain Titer ($TCID_{50}$/mL)	Competitive Strain	Competitive Strain Titer ($TCID_{50}$/mL)	Positives/ 20 replicates	Average Ct			
				Flu A1	Flu B	RSV	SPC
1.8	n/a	n/a	20/20	-	-	34.1	30.8
2.0 (Upper 95% CI of LoD)	H3/Victoria/361/2011	1.00E+03	19/20	23.3	-	35.4	31.6
	B/Wisconsin/01/11	1.00E+03	19/20	-	22.9	34.6	31.5

f. Assay cut-off:

The Xpert Flu/RSV XC Assay detects Flu A, Flu B, and RSV targets. For Flu A, Flu B and RSV, the valid cycle threshold (Ct) range is 12.0 to 40.0. A Flu A 1, Flu A 2 cycle threshold (Ct) outside the maximum valid range is reported "Flu A NEGATIVE". A Flu B cycle threshold (Ct) outside the maximum valid range is reported "Flu B NEGATIVE". A RSV cycle threshold (Ct) outside the maximum valid range is reported "RSV NEGATIVE". The Ct cutoffs are included as automatic calculations in the assay definition file (ADF) provided with the Xpert Flu/RSV XC Assay.

2. Comparison studies:

a. Method comparison with predicate device:

N/A

b. Matrix comparison:

To demonstrate that the Xpert Flu/RSV XC Assay performs equivalently in either mid-turbinate (MT) swab clinical matrix or simulated matrix, an equivalency study was performed. The MT swab specimens were collected from healthy individuals and pooled to obtain a "clinical background matrix" for the equivalency study. The simulated background matrix consisted of 2.5% (w/v) porcine mucin, 1% (v/v) human whole blood in 0.85% sodium chloride (NaCl) formulated in 1X PBS solution with 15% glycerol, which was then diluted in Copan UTM to a final concentration of 16.7%. This simulated background matrix represents both swab specimens and nasal aspirate/wash (NA/W) specimens. Both matrices were

confirmed to be negative with the Xpert Flu/RSV XC. Assay before being used to prepare positive samples.

Table 17 - Study Design for MT Swab and Simulated Matrix Equivalency Study

Target	Replicates Tested for Each Matrix Type			
	2x LoD	5x LoD	10x LoD	100x LoD
A/Victoria/361/2011 (H3N2)	N= 40	N= 10	N=10	N=5
A/Florida/27/2011 (H1N1)	N= 40	N= 10	N= 10	N=5
B/Mass2012	N= 40	N= 10	N= 10	N=5
RSV-A/Long/MD/56	N= 40	N= 10	N= 10	N=5
RSV-B/9320/MA/77	N= 40	N= 10	N= 10	N=5

Sensitivity (positive agreement) for all strains in both MT swab clinical matrix and simulated matrix was 100% (Lower 95% CI = 94.5%) using the Fisher Exact Method (all 65 positive samples for each strain in MT swab clinical matrix and simulated matrix background were correctly reported positive for all strains).

c. Fresh versus frozen equivalency

Fresh and frozen specimen equivalency in the Xpert Flu/RSV XC Assay was evaluated by testing individual influenza and RSV strains at three different concentrations representing low positives (2x LoD), moderate positives (5x LoD), and high positives (10x LoD) in simulated background matrix. Negative samples consisted of simulated background matrix only. Fresh and frozen specimen equivalency was determined using one seasonal Flu A H3N2 strain (A/Victoria/361/2011), one Flu B strain B/Wisconsin/01/11), one RSV A strain (RSV A/Long/MD/56), and one RSV B strain (RSV B/9320/MA/77). Replicates of 20 were tested for each specimen type and concentration. All positive and negative specimens were tested fresh, after one freeze-thaw cycle, and after two freeze-thaw cycles. There was no statistically significant effect in the performance of the Xpert Flu/RSV XC Assay between fresh virus dilutions and two sequential freeze thaw cycles for positive and negative samples. All positive and negative replicates were correctly identified using the Xpert Flu/RSV XC Assay.

3. Clinical studies:

a. Clinical Sensitivity and Specificity

A total of 1275 subject specimens were collected in the clinical study. Of the 1275 eligible specimens, 1254 were included in the study (16 specimens were delayed for shipment for reference testing, 3 specimens leaked during transit or were mislabeled, 1 specimen was not tested on the subject device within 24

hours, and one specimen was unresolved on the comparator assay). There were 18 indeterminate results on first attempt. One of the indeterminate results was not retested according to protocol. Of the remaining 17 indeterminate results, 14 yielded valid results upon repeat testing resulting in 1250 results (657 NA/W and 593 NP swab specimens) included in the final dataset used for the analyses. Three results were invalid and therefore not included in the final results. Of the 1250 specimens included in the data analyses, 637 were collected from female subjects and 613 from male subjects. Collection and testing was performed at 6 geographically diverse locations representing intended use sites.

Age distribution of the patients from whom the specimens were obtained was as follows: 332 (26.6%) of the specimens 5 years or younger; 258 (20.6%) from ages 6 to 21; 413 (33.0%) from ages 22 to 59; 235 (18.8%) from ages 60 or older; and 12 (1.0%) were from patients of unknown age.

The study included prospectively collected NP swab or NA/W specimens from subjects with signs and symptoms of respiratory infection. Inclusion criteria required leftover specimens with volumes ≥ 2mL in order to allow for sufficient aliquots for testing (0.6 mL to 0.8 mL for Xpert Flu/RSV XC testing, 1.0 mL for reference method testing, and 0.2 mL available for sequencing in the event of a discrepancy). Due to the low prevalence of some influenza strains during the study season, the study population was supplemented with frozen specimens. Frozen specimens consisted of archived positive specimens originally collected for standard of care influenza testing between November 28, 2012 and May 3, 2014. A fresh vs. frozen equivalency study was performed prior to including the frozen specimens in the clinical study. Only those samples meeting the inclusion and exclusion criteria were included. Specimens were tested on the Xpert Flu/RSV XC Assay and an FDA-cleared molecular assay as the comparator method. Specimens were tested with the Xpert Flu/RSV XC Assay within 24 hours of collection (fresh) or 24 hours of thawing (frozen). Discordant results between the Xpert Flu/RSV XC Assay and the FDA-cleared molecular comparator assay were further analyzed by bi-directional sequencing using primers different from those used in the Xpert Flu/RSV XC Assay.

A total of 657 NA/W specimens were tested for influenza A, influenza B and RSV by the Xpert Flu/RSV XC Assay and the comparator assay. Of the 657 NA/W specimens, 581 were fresh, prospectively collected and 76 were frozen, archived specimens.

When testing fresh, prospectively collected NA/W specimens, the Xpert Flu/RSV XC Assay demonstrated a PPA and NPA for detection of influenza A of 100% and 100%, respectively, relative to the comparator assay. The Xpert Flu/RSV XC Assay PPA and NPA for influenza B were 99.2% and 100%, respectively. The Xpert Flu/RSV XC Assay PPA and NPA for RSV were 98.5% and 99.6%, respectively.

When testing frozen, archived NA/W specimens, the Xpert Flu/RSV XC Assay demonstrated a PPA and NPA for detection of influenza A of 97.1% and 100%, respectively, relative to the comparator assay. The Xpert Flu/RSV XC Assay PPA and NPA for influenza B were 100% and 100%, respectively. The Xpert Flu/RSV XC Assay PPA and NPA for RSV were 84.6% and 100%, respectively. Results are summarized in Table 18.

Table 18 - Xpert Flu/RSV XC Assay Performance on NA/W Specimens

Specimen Type	Target	n	TP	FP	TN	FN	PPA % (95 CI)	NPA % (95 CI)
Fresh	Flu A	581	35	0	546	0	100 (90.0-100)	100 (99.3-100)
	Flu B	581	126	0	454	1[a]	99.2 (95.7-100)	100 (99.2-100)
	RSV	581	128	2[b]	449	2[c]	98.5 (94.6-99.8)	99.6 (98.4-99.9)
Frozen	Flu A	76	34	0	41	1[d]	97.1 (85.1-99.9)	100 (91.4-100)
	Flu B	76	1	0	75	0	100 (2.5-100)	100 (95.2-100)
	RSV	76	11	0	63	2[e]	84.6 (54.6-98.1)	100 (94.3-100)

[a]Testing results by sequencing: NA; sample not sequenced.
[b]Testing results by sequencing: 2 of 2 were RSV positive.
[c]Testing results by sequencing: 1 of 2 was RSV positive; 1 of 2 was RSV negative.
[d]Testing results by sequencing: 1 of 1 was Flu A negative.
[e]Testing results by sequencing: 1 of 2 was RSV positive; 1 of 2 was RSV negative.

A total of 593 NP swab specimens were tested for influenza A, influenza B and RSV by the Xpert Flu/RSV XC Assay and the FDA-cleared molecular comparator assay. Of the 593 NP swab specimens, 190 were fresh, prospectively collected and 403 were frozen, archived specimens.

When testing fresh, prospectively collected NP swab specimens, the Xpert Flu/RSV XC Assay demonstrated a PPA and NPA for detection of influenza A of 85.7% and 98.9%, respectively, relative to the comparator assay. The Xpert Flu/RSV XC Assay PPA and NPA for influenza B were 100% and 100%, respectively. The Xpert Flu/RSV XC Assay PPA and NPA for RSV were 100% and 100%, respectively.

When testing frozen, archived NP swab specimens, the Xpert Flu/RSV XC Assay demonstrated a PPA and NPA for detection of influenza A of 99.0% and 92.8%, respectively, relative to the comparator assay. The Xpert Flu/RSV XC Assay PPA and NPA for influenza B were 98.8% and 100%, respectively. The Xpert Flu/RSV XC Assay PPA and NPA for RSV were 90.4% and 99.1%, respectively. Results are summarized in Table 19.

Table 19 - Xpert Flu/RSV XC Assay Performance on NP Swab Specimens

Specimen Type	Target	n	TP	FP	TN	FN	PPA % (95 CI)	NPA % (95 CI)
Fresh	Flu A	190	6	2[a]	181	1[b]	85.7 (42.1-99.6)	98.9 (96.1-99.9)
	Flu B	190	3	0	187	0	100 (29.2-100)	100 (98.0-100)
	RSV	190	10	0	180	0	100 (69.2-100)	100 (98.0-100)
Frozen	Flu A	403	96	22[c]	284	1[d]	99.0 (94.4-100)	92.8 (89.3-95.4)
	Flu B	403	85	0	317	1[e]	98.8 (93.7-100)	100 (98.8-100)
	RSV	403	47	3[f]	348	5[g]	90.4 (79.0-96.8)	99.1 (97.5-99.8)

[a]Testing results by sequencing: 2 of 2 were Flu A positive.
[b]Testing results by sequencing: 1 of 1 was Flu A negative.
[c]Testing results by sequencing: 17 of 22 were Flu A positive; 5 of 22 were Flu A negative.
[d]Testing results by sequencing: 1 of 1 was Flu A negative.
[e]Testing results by sequencing: 1 of 1 was Flu B negative.
[f]Testing results by sequencing: 2 of 3 were RSV positive; 1 of 3 was RSV negative.
[g]Testing results by sequencing: 1 of 5 was RSV positive; 4 of 5 were RSV negative.

4. Clinical cut-off**:**

The assay cut-off was determined by analysis of pre-clinical data derived from a beta study using the comparator assay. The valid minimum cycle threshold setting for Flu A 1, Flu A 2, Flu B, and RSV was set at 12 Ct (Cycle threshold) because a Ct cannot be calculated before the end of the minimum background subtraction range, which is 10 cycles. The earliest Flu A 1, Flu A 2, Flu B, and RSV Ct reported in true influenza A positive, influenza B positive, and RSV positive results during pre-clinical testing was greater than 12 Ct. Pre-clinical testing of Flu A Ct in NP and NA/W specimens indicated a Mean Ct of 23.55 with a standard deviation of 4.98 Ct. Testing of Flu B NA/W specimens indicated a Mean Ct of 24.52 with a standard deviation of 4.20 Ct. Testing of RSV NP and NA/W specimens indicated a mean Ct of 24.24 with a standard deviation of 4.60 Ct. The valid maximum cycle threshold setting for Flu A 1, Flu A 2, Flu B, and RSV was set at 40.0 to account for true positives with low viral concentrations.

5. Expected values/Reference range:

The Xpert Flu/RSV XC clinical study included a total of 771 prospectively collected fresh specimens and 479 prospectively collected frozen specimens. The number and percentage of cases positive for one or more of influenza A, influenza B, and RSV, as determined by the Xpert Flu/RSV XC Assay are shown by age category and specimen type (NA/W or NP swab specimen) in Tables 20 and 21:

Table 20 - Expected Values for Flu A, Flu B, and RSV by Xpert Flu/RSV XC Assay in NP Swabs[a]

Age Group	# of Patients	Flu A		Flu B		RSV	
		# of Pos	Positivity	# of Pos	Positivity	# of Pos	Positivity
≤5 years	34	4	11.8%	3	8.8%	20	58.8%
6-21 years	60	13	21.7%	19	31.7%	3	5.0%
22-59 years	322	82	23.5%	55	17.1%	13	4.0%
≥60 years	165	27	16.4%	11	6.7%	21	12.7%
Unknown	12	0	0	0	0	3	25.0%
Total	593	126	21.2%	88	14.8%	60	10.1%

[a]Four subjects had dual infections and are therefore counted twice in this table: Flu A & RSV POS (2); Flu A & Flu B POS (1); and Flu B & RSV POS (1).

Table 21 - Expected Values for Flu A, Flu B, and RSV by Xpert Flu/RSV XC Assay in NA/W

Age Group	# of Patients	Flu A		Flu B		RSV	
		# of Pos	Positivity	# of Pos	Positivity	# of Pos	Positivity
≤5 years	298	24	8.1%	55	18.5%	114	38.3%
6-21 years	198	33	16.7%	66	33.3%	21	10.6%
22-59 years	91	9	9.9%	5	5.5%	2	2.2%
≥60 years	70	3	4.3%	1	1.4%	4	5.7%
Unknown	0	0	0	0	0	0	0
Total	657	69	10.5%	127	19.3%	141	21.5%

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.