510(k) SUBSTANTIAL EQUIVALENCE DETERMINATION
DECISION SUMMARY

A. 510(k) Number:

K151226

B. Purpose for Submission:

To obtain a Substantial Equivalence Determination for a new 510(k) application for Xpert® Flu+RSV Xpress Assay performed on the Cepheid GeneXpert Xpress System.

C. Measurand:

This assay uses nasopharyngeal swab specimens from patients with signs and symptoms of respiratory infection. The viral nucleic acid is extracted from the sample and influenza and/or Respiratory Syncytial Virus (RSV) RNA is amplified and detected through real-time reverse transcription polymerase chain reaction (RT-PCR).

D. Type of Test:

This assay is a multiplex nucleic acid assay that detects and differentiates influenza A, influenza B, and RSV through nucleic acid extraction, amplification, and detection using real-time RT-PCR. All steps of the assay are automated, after sample addition, and performed in a single container.

E. Applicant:

Cepheid

F. Proprietary and Established Names:

Xpert® Flu+RSV Xpress Assay

G. Regulatory Information:

1. Regulation section:

21CFR 866.3980 - Respiratory viral panel multiplex nucleic acid assay
21CFR 866.2390 -Transport culture medium

2. Classification:

Class II

3. Product codes:

OCC, JSM, OOI

4. Panel:

Microbiology (83)

H. Intended Use:

1. Intended use(s):
   Assay:

The Cepheid Xpert® Flu+RSV Xpress Assay, performed on the GeneXpert® Xpress System, 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 Xpress Assay uses nasopharyngeal swab specimens collected from patients with signs and symptoms of respiratory infection. The Xpert Flu+RSV Xpress Assay is intended as an aid in the diagnosis of influenza and respiratory syncytial virus 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 2014-2015 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 departments for testing. Viral culture should not be attempted in these cases unless a BSL 3+ facility is available to receive and culture specimens.

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 containing viruses 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 is designed to collect, preserve, and transport nasopharyngeal swab specimens containing viruses from patients with signs and symptoms of respiratory infection prior to analysis with the Xpert Flu+RSV Xpress Assay.

2. Indication(s) for use:

Same as intended use.

3. Special conditions for use statement(s):

This is a prescription only test.

4. Special instrument requirements:

The assay is performed using the GeneXpert® Xpress System.

I. Device Description:

This assay uses nasopharyngeal swab specimens from patients with signs and symptoms of respiratory infection. Viral nucleic acid is extracted from the sample and the influenza and/or RSV RNA is amplified and detected through real-time reverse transcription polymerase chain reaction (RT-PCR). Detection and differentiation of influenza A, influenza B, and RSV is reported to the user.

The assay uses single use disposable cartridge that has a separate section for specimen loading. The cartridge also contains all PCR reagents and is where the PCR reaction takes place. The GeneXpert Xpress System performs all assay steps from clinical sample to reporting assay results automatically. A Sample Processing Control (SPC) and a Probe Check Control (PCC) are also included in the cartridge. 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 GeneXpert Xpress System, comprised of the GeneXpert Dx System GX-I, has one module that is capable of performing separate sample preparation and real-time PCR and RT-PCR tests. This single 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, and a proprietary I-CORE® thermocycler for performing real-time PCR and RT-PCR and detection.

Turnaround time for analysis of a sample is approximately 60 minutes. The assay results are automatically generated at the end of the process and provided in a report that can be viewed and printed.

J. Substantial Equivalence Information:

1. Predicate device name(s):

Cepheid Xpert Flu/RSV XC Assay

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

3. Comparison with predicate:

Comparison of Similarities and Differences
of the Xpert Flu+RSV Xpress Assay with the Predicate Device

Similarities		
Item	**Device**	**Predicate Device**
	Cepheid Xpert Flu+RSV Xpress Assay	**Cepheid Xpert Flu/RSV XC Assay**
Intended Use	The Cepheid Xpert Flu+RSV Xpress Assay, **performed on the GeneXpert Xpress System**, 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 Xpress Assay uses nasopharyngeal swab specimens collected from patients with signs and symptoms of respiratory infection. The Xpert Flu+RSV Xpress Assay is intended as an aid in the diagnosis of influenza and respiratory syncytial virus 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 2014-2015 influenza season. When other novel influenza A viruses are emerging,	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 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. Performance characteristics for influenza A were established during the 2013-2014 influenza season. When other novel influenza A viruses are emerging,

Similarities		
Item	**Device**	**Predicate Device**
	Cepheid Xpert Flu+RSV Xpress Assay	**Cepheid Xpert Flu/RSV XC Assay**
Intended Use	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.	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.
Technology Principle of Operation	Same	Multiplex real time RT-PCR
Assay Targets	Same	Influenza A Virus, Influenza B Virus, and RSV viral RNA
Specimen Types	Nasopharyngeal (NP) swab specimens	Nasopharyngeal (NP) swab specimens and Nasal aspirate/wash (NA/W) specimens
Nucleic Acid Extraction	Yes	Yes
Extraction Methods	Sample preparation integrated in GeneXpert Cartridge and **GeneXpert Xpress System**	Sample preparation integrated in GeneXpert Cartridge and GeneXpert Instrument System
Assay Results	Same	Qualitative

Similarities		
Item	**Device**	**Predicate Device**
	Cepheid Xpert Flu+RSV Xpress Assay	**Cepheid Xpert Flu/RSV XC Assay**
Instrument System	**Cepheid GeneXpert Xpress System (instrument model GX-I)**; same Cepheid I-core technology	Cepheid GeneXpert Instrument Systems (various instrument models including instrument model GX-I); Cepheid I-core technology
Assay Controls	Same	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.
Time to obtain test results	Approximately 60 minutes for sample preparation and real-time RT-PCR.	Approximately 60 minutes **or less** for sample preparation and real-time RT-PCR.
Primers and probes	Same	Primers and probes to detect the presence of nucleic acid sequences of influenza A, influenza B, and RSV.
Differences		
Instrument System	Cepheid GeneXpert Xpress System	Cepheid GeneXpert Dx Systems and GeneXpert Infinity Systems
Laboratory Users	Untrained operators with no clinical lab experience in a CLIA-waiver environment.	Operators with no clinical lab experience to experienced clinical laboratory technologists.
Combinatorial Assay Selections	No combinatorial assay selections are available.	Yes, user may select combined assay with all targets or a Flu only assay or a RSV only assay.
Early assay termination function	No early assay termination function is available.	Yes, on Flu only or RSV only assay selections.

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

Class II Special Controls Guidance Document: Respiratory Viral Panel Multiplex Nucleic Acid Assay

L. Test Principle:

The assay detects viral nucleic acids that have been extracted from a patient respiratory sample. A multiplex Real-time RT-PCR reaction is carried out under optimized conditions generating amplicons for influenza A, influenza B, RSV, and the Sample Process Control (SPC). Identification of influenza A, influenza B, RSV, and the SPC occurs by the use of target-specific primers and fluorescent-labeled probes that hybridize to conserved regions in the genomes.

Xpert Flu+RSV Xpress Assay Probe Targets	
Virus	**Target**
Influenza A	Matrix Protein gene, Polymerase B2 Protein gene, Polymerase A Protein gene
Influenza B	Matrix Protein gene, Non-Structrural Protein gene
RSV A	Nucleocapsid Protein gene
RSV B	Nucleocapsid Protein gene

M. Performance Characteristics (if/when applicable):

1. Analytical performance:

a. Precision/Reproducibility:

The reproducibility studies were conducted over 10 days and testing was performed at three clinical sites and one in-house laboratory. There were three operators at each site. Operators were blinded to the sample identity. Testing was conducted following the package insert instructions for use. One kit lot was used to perform the study. One strain of each virus, influenza A, influenza B, and RSV was tested at three different concentrations, 2-3x LoD, 1x LoD, and below LoD for the Moderate Positive, Low Positive, and High Negative samples respectively. The following strains were tested: Flu A/Perth/16/09, Flu B/Wisconsin/01/2011, and RSV-A/2/Australia/61.

Specimen	Concentration	Expected Positivity Rate
Negative	0	0%
Flu A	High negative (below LOD) C_5	20-80%
Flu A	Low positive (~1X LOD) C_{95}	95%
Flu A	Moderate positive (~2-3X LOD)	100%
Flu B	High negative (below LOD) C_5	20-80%
Flu B	Low positive (~1X LOD) C_{95}	95%
Flu B	Moderate positive (~2-3X LOD)	100%
RSV	High negative (below LOD) C_5	20-80%
RSV	Low positive (~1X LOD) C_{95}	95%
RSV	Moderate positive (~2-3X LOD)	100%

The following table presents the summary of results for the reproducibility study. The results show that the assay had appropriate reproducibility at all three sites. The reproducibility for this device is acceptable. The instances where the expected result differed from the actual result are within what is reasonable for C_5 (High negative) and C_{95} (Low positive) samples.

Sample	Site 1			Site 2			Site 3			% Total Agreement[a]
	Op 1	Op 2	Op 3	Op 1	Op 2	Op 3	Op 1	Op 2	Op 3	
Neg	100% (10/10)	100% (10/10)	100% (10/10)	100% (10/10)	100% (10/10)	100% (9/9)[b]	100% (9/9)[b]	100% (10/10)	100% (10/10)	100% (88/88)[b]
Flu A-High Neg	0.0% (0/10)	20.0% (2/10)	60.0% (6/10)	20.0% (2/10)	10.0% (1/10)	50.0% (5/10)	75.0% (6/8)[b]	20.0% (2/10)	20.0% (2/10)	29.5% (26/88)[b]
Flu A-Low Pos	100% (10/10)	100% (10/10)	90.0% (9/10)	100% (10/10)	100% (10/10)	100% (10/10)	100% (10/10)	100% (10/10)	100% (10/10)	98.9% (89/90)
Flu A-Mod Pos	100% (10/10)	100% (10/10)	100% (10/10)	100% (10/10)	100% (10/10)	100% (10/10)	100% (10/10)	100% (10/10)	100% (10/10)	100% (90/90)
Flu B-High Neg	50.0% (5/10)	0.0% (0/10)	50.0% (5/10)	20.0% (2/10)	10.0% (1/10)	55.6% (5/9)[b]	80.0% (8/10)	50.0% (5/10)	20.0% (2/10)	37.1% (33/89)[b]
Flu B-Low Pos	100% (10/10)	100% (10/10)	100% (10/10)	100% (10/10)	100% (10/10)	100% (10/10)	90.0% (9/10)	100% (10/10)	100% (10/10)	98.9% (89/90)
Flu B-Mod Pos	100% (10/10)	100% (10/10)	100% (10/10)	100% (10/10)	100% (10/10)	100% (10/10)	100% (10/10)	100% (10/10)	100% (10/10)	100% (90/90)
RSV-High Neg	60.0% (6/10)	30.0% (3/10)	66.7% (6/9)[b]	50.0% (5/10)	60.0% (6/10)	50.0% (5/10)	90.0% (9/10)	33.3% (3/9)[b]	50.0% (5/10)	54.5% (48/88)[b]
RSV-Low Pos	88.9% (8/9)	100% (10/10)	100% (10/10)	100% (10/10)	100% (10/10)	100% (10/10)	90.0% (9/10)	100% (10/10)	100% (10/10)	97.8% (87/89)[c]
RSV-Mod Pos	100% (10/10)	100% (10/10)	100% (10/10)	100% (10/10)	100% (10/10)	100% (10/10)	100% (10/10)	100% (10/10)	100% (10/10)	100% (90/90)

[a]Percent of samples yielding expected results – negative for Neg and High Neg samples; positive for Low Pos and Mod Pos samples.

[b]Seven samples (2 Neg, 2 Flu A High Neg, 1 Flu B High Neg, and 2 RSV High Neg) were indeterminate upon initial testing and retest.

[c]One RSV Low Pos sample was inadvertently not tested.

All negative samples were correctly classified as negative (88/88).

All moderate positive samples were correctly classified as positive, Flu A (90/90), Flu B (90/90), and RSV (90/90).

The high negative samples were prepared to target a concentration below the LoD. At this concentration we would expect approximately 20-80% of samples to be negative. Overall 29.5% (26/88) of the high negative Flu A samples were classified as negative; 37.1% (33/89) of the high negative Flu B samples were classified as negative; and 54.5% (48/88) of the high negative RSV samples were classified as negative.

The low positive samples were prepared to target a concentration at the limit of detection. At this concentration we would expect approximately 95% of samples to be positive. Overall 98.9% (89/90) of the low positive Flu A samples were classified as positive; 98.9% (89/90) of the low positive Flu B samples were classified as positive; and 97.8% (87/89) of the low positive RSV samples were classified as positive.

b. Limit of Detection:

The limit of detection (LoD) study was conducted with 10% of the Xpert Flu+RSV Xpress Assay cartridges on the GeneXpert Xpress System and 90% with the Xpert Flu/RSV XC Assay cartridges on the Gene Xpert Dx System and the GeneXpert Infinity Systems. The LoD of the Xpert Flu+RSV Xpress Assay, defined as the lowest $TCID_{50}$/mL that is detected ≥95% of the time, was determined by testing influenza A H3N2 strains, influenza A 2009 H1N1 strains, influenza B strains, RSV-A strains, RSV-B strains, and an influenza A H7N9 strain in a background matrix of clinical, pooled swab specimens collected in Copan UTM.

Influenza A pandemic 2009 H1N1 strains
• A/California/7/2009 (H1N1)
• A/Florida/27/2011 (H1N1)

Influenza A H3N2 strains
• A/Perth/16/2009 (H3N2)
• A/Victoria/361/2011 (H3N2)

Influenza B strains
• B/Mass/2/2012
• B/Wisconsin/01/2011

Respiratory Syncytial Virus A strains
• RSV-A/2/Australia/61
• RSV-A/Long/MD/56

Respiratory Syncytial Virus B strains
• RSV-B/Washington/18537/62
• RSV-B/9320/Massachusetts/77

The LoD was estimated using probit regression analysis and confirmed with twenty replicates for each dilution of each virus. The samples were prepared and tested across 3 testing days using one reagent lot. Testing was conducted according to the package insert. The table below shows the LoD determined for each virus type.

Confirmed LoD, Point Estimates and 95% Confidence Intervals for Influenza and RSV Targets

Influenza A 2009 H1N1	**Confirmed LoD (TCID50/mL) [at least 19/20 positive]**	**LoD Estimate by Probit Regression Analysis (TCID50/mL)**		
		LoD Point Estimate	**Lower 95% CI**	**Upper 95% CI**
A/California/7/2009	**0.3 (20/20)**	**0.1**	0.06	0.11
A/Florida/27/2011	**16.0 (20/20)**	**15.4**	13.77	20.59
Influenza A H3N2	**Confirmed LoD (TCID50/mL) [at least 19/20 positive]**	**LoD Estimate by Probit Regression Analysis (TCID50/mL)**		
		LoD Point Estimate	**Lower 95% CI**	**Upper 95% CI**
A/Perth/16/2009	**0.3 (20/20)**	**0.1**	0.05	0.09
A/Victoria/361/2011	**0.8 (20/20)**	**0.8**	0.65	1.21
Influenza B	**Confirmed LoD (TCID50/mL) [at least 19/20 positive]**	**LoD Estimate by Probit Regression Analysis (TCID50/mL)**		
		LoD Point Estimate	**Lower 95% CI**	**Upper 95% CI**
B/Mass/2/2012	**0.5 (20/20)**	**0. 5**	0.38	0.62
B/Wisconsin/01/2011	**0.6 (20/20)**	**0.5**	0.46	0.74
RSV A	**Confirmed LoD (TCID50/mL) [at least 19/20 positive]**	**LoD Estimate by Probit Regression Analysis (TCID50/mL)**		
		LoD Point Estimate	**Lower 95% CI**	**Upper 95% CI**
RSV A/2/Australia/61	**1.2 (20/20)**	**1.1**	0.96	1.26
RSV A/Long/MD/56	**1.0 (20/20)**	**1.0**	0.84	1.38
RSV B	**Confirmed LoD (TCID50/mL) [at least 19/20 positive]**	**LoD Estimate by Probit Regression Analysis (TCID50/mL)**		
		LoD Point Estimate	**Lower 95% CI**	**Upper 95% CI**
RSV B/Wash/18537/62	**1.8 (20/20)**	**1.6**	1.49	1.95
RSV B/9320/MA/77	**2.0 (20/20)**	**2.1**	1.84	2.64
Influenza A (H7N9)	**Confirmed LoD (TCID50/mL) [at least 19/20 positive]**	**LoD Estimate by Probit Regression Analysis (TCID50/mL)**		
		LoD Point Estimate	**Lower 95% CI**	**Upper 95% CI**
A/Anhui/1/2013 (H7N9)	**0.8 (19/20)**	**0.8**	**0.7**	**0.9**

Analytical Studies: provided in support of K142045 and the current submission

The following study data were originally submitted for the Xpert Flu/RSV XC Assay in K142045 and also included in the current submission. The majority of samples in these studies were tested by the Xpert Flu/RSV XC Assay and a smaller proportion of samples were tested by the Xpert Flu+RSV Xpress Assay. All reagents, buffers, and other components that comprise both assays are exactly the same. The detection method, the signal analysis, and the software algorithm used to calculate the results are also identical.

c. Baseline, Threshold and Cut-off:

The lot specific parameters (LSP) and assay settings (background subtraction, background minimum cycle, background maximum cycle, manual threshold, curve analysis, and valid minimum and maximum Ct values) for the Xpert Flu+RSV XC Assay were determined using pre-clinical and analytical data with the Xpert Flu/RSV XC Assay cleared in K142045. The technology was not significantly changed from the XC Assay to the Xpress Assay; the assay reagents and assay analysis algorithm have not changed. The LSP and assay settings were confirmed for the Xpert Flu +RSV Xpress Assay.

d. Stability:
The Flu/RSV XC Assay was used to determine the stability of reagents in the Xpert Flu+RSV Xpress Assay because all reagents, buffers, and other components that comprise both assays are exactly the same. The detection method, the signal analysis, and algorithm used by the different software to calculate the results are the same.

Kit Stability:
The real-time reagent stability data for the Xpert Flu/RSV XC Assay is currently available for nine months at the claimed storage temperatures of 2°C and 28°C. Results to date show that averaged Ct values are within each assay lot acceptance criteria and testing is ongoing for up to 37 months on three lots.

Specimen Stability:
The study data supports specimen stability at extreme storage temperatures and storage times (7 days at 2°C followed by 24 hours at 30°C followed by testing on day 9) for all three claimed transport media (Becton Dickinson UVTM, Remel M5, and Copan UTM).

e. Expected values (controls, calibrators, or methods):
An internal assay control called the 'sample processing control' (SPC) monitors adequate sample processing and the integrity of the RT-PCR. The SPC is mixed with the sample automatically inside the test cartridge to control for adequate lysis of the target viruses, sample processing, and detection of assay interference.

No external controls are provided with this assay. The recommended external controls are:

- 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.

f. Analytical Inclusivity:

The analytical reactivity of the Xpert Flu+RSV Xpress 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 (H5N2, H6N2, H7N3, and 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. All virus identities and titers were confirmed prior to analysis. Sixty-four (64) strains comprised of 54 influenza viruses and 10 RSV strains were tested in this study with the Xpert Flu+RSV Xpress Assay. Virus dilution and sample preparation was performed with 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%. An aliquot of 300 µL of the final inocula was added to the Xpert Flu+RSV Xpress Assay cartridge and 3 replicates were tested for each strain. Due to the biosafety restrictions on viable viral particles, purified nucleic acids at ≤ 1ρg/mL in simulated background matrix were tested for 9 avian influenza A strains. Three replicates of a no template control were also tested which contained the simulated background matrix only. 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.

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

Virus	Strain	Concentration	Result		
			Flu A	Flu B	RSV
	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
	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)	1 g/ L[b]	POS	NEG	NEG
	A/chicken/Hubei/327/2004 (H5N1)	1 g/ L[b]	POS	NEG	NEG
	A/Anhui/01/2005 (H5N1)	1 g/ L[b]	POS	NEG	NEG
	A/Japanese white eye/HongKong/1038/2006 (H5N1)	1 g/ L[b]	POS	NEG	NEG
	A/mallard/WI/34/75 (H5N2)	1 g/ L[b]	POS	NEG	NEG
	A/chicken/CA431/00 (H6N2)	1 g/ L[b]	POS	NEG	NEG
	A/duck/LTC-10-82743/1943 (H7N2)	1 g/ L[b]	POS	NEG	NEG
	A/chicken/NJ/15086-3/94 (H7N3)	1 g/ L[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)	1 g/ L[b]	POS	NEG	NEG
	A/Mallard/NY/6750/78 (H2N2)	1 g/ L[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

Virus	Strain	Concentration	Result		
			Flu A	Flu B	RSV
	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
	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.

All 64 influenza and RSV strains were correctly identified in the study, 39 as influenza A, 15 as influenza B, and 10 as RSV using the Xpert Flu+RSV Xpress Assay.

g. Analytical Specificity/Cross-Reactivity:

Cross Reactivity:

This study was designed to determine potential cross-reactivity of the assay with viruses, yeast, and bacteria common in the nasopharynx. Bacteria were tested at 1.00E+6 cfu/ml (except one strain which was tested at 1.00E+05 $TCID_{50}$/mL) and viruses were tested at 1.00E+05 $TCID_{50}$/mL. Testing was performed in triplicate. All organisms were diluted in simulated background matrix and testing was performed according to the package insert directions.

Analytical Specificity/Cross-reactivity of the Cepheid Xpert Flu+RSV Xpress 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
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

Organism	Concentration	Influenza A	Influenza B	RSV
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. For *Acinetobacter baumannii,* upon initial testing 1of 3 replicates was positive for Flu A with a Ct of 39.4 (cut-off = 40). An additional 23 replicates were tested at $>1x10_6$ CFU/mL; 23of 23 replicates were correctly reported as "Flu A NEGATIVE; Flu B NEGATIVE; RSV NEGATIVE".

Under the conditions of this study, 43 of the 44 non-influenza isolates tested were correctly reported as "Flu A NEGATIVE; Flu B NEGATIVE; RSV NEGATIVE" by the Xpert Flu+RSV Xpress Assay. For *Acinetobacter baumannii,* 1 of the 3 replicates initially tested was reported as

"Flu A POSITIVE; FLU B NEGATIVE; RSV NEGATIVE" with a Flu A Ct value of 39.4 (cut-off = 40). A Ct value of 39.4 is not typical of positive Cts for Flu A and the false positive result was likely caused by contamination. An additional twenty-three (23) replicates of *Acinetobacter baumannii* were tested; all 23 replicates were correctly reported as "Flu A NEGATIVE; Flu B NEGATIVE; RSV NEGATIVE". Based on the results of this study, the Xpert Flu+RSV Xpress Assay showed no cross-reactivity with any of the organisms tested.

Microbial Interference:

The analytical specificity of the Xpert Flu+RSV Xpress 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.

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*). 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 below. The results demonstrate that the Xpert Flu+RSV Xpress Assay does not cross-react with any of the 44 common respiratory microorganisms tested in this study.

Analytical Specificity/Cross-reactivity of the Cepheid Xpert Flu+RSV Xpress 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

Organism	Concentration	Influenza A	Influenza B	RSV
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
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

Organism	Concentration	Influenza A	Influenza B	RSV
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.

Competitive Interference:

The purpose of this study is to determine whether competitive interference exists between analytes when more than one analyte is present in a clinical sample. Competitive interference caused by clinically relevant co-infections of the targets in the Xpert Flu+RSV Xpress Assay 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 Xpress 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 the following four tables.
Note: The average Ct values for each strain tested at LoD without a competitive analyte are taken from the Limit of Detection study.

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

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

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

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

h. 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 Xpress 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 spiked at 2x the analytical LoD determined for each of the following six influenza viruses, two 2009 H1N1 strains (A/California/7/2009 and A/Florida/27/2011), two Flu A H3N2 strains (H3/Perth/16/09 and H3/Victoria/361/2011, and two Flu B stains (Flu B/Wisconsin/01/11 and Flu B/Mass/02/2012), and four RSV viruses, two RSV A strains (RSV-A/Long/MD/56 and RSV-A/2/Australia/61) and two RSV B strains (RSV-B/Wash/18537/62 and RSV-B/9320/MA/77). All results were compared to positive and negative Universal Transport Medium (UTM) controls. The evaluated substances are below 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 Xpress 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.

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, Galphimia glauca, histanium hydrochloricum	15% (w/v)
FluMist	Live vaccine	Live influenza virus	6.7% (v/v)
Fluticasone Propionate Nasal Spray	Nasal corticosteroid	Fluticasone Propionate	5 µg/mL

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

* The FluMist vaccine was tested as part of the interfering substance study. FluMist contains three live attenuated influenza vaccine virus strains: one influenza A (H1N1) strain, one influenza A (H3N2) strain, and one influenza B

strain. Negative samples of this substance cannot be tested to observe its impact on SPC Ct. The FluMist vaccine samples tested with the Xpert Flu+RSV Xpress Assay were correctly reported as "Flu A POSITIVE; Flu B POSITIVE; RSV NEGATIVE" as expected. Samples containing FluMist may cause false positives. This is addressed in the Limitations Section of the Package Insert.

i. Carry-Over/Cross-Contamination:

The purpose of this study was to demonstrate whether single use, self-contained GeneXpert cartridges prevent specimen and amplicon carry-over contamination into negative samples when these are run following very high positive samples in the same GeneXpert module. GeneXpert cartridges are designed such that patient specimens and all assay reagents are confined within the self-contained GeneXpert cartridge and do not come into contact with the instrument and/or its moving parts.

This study consisted of a negative sample (16.7% simulated background matrix only) processed in the same module of the GeneXpert Xpress System (GX-I) immediately following a high positive sample comprised of a Flu A strain (A/Canada/6294/2009, $1x10^6$ $TCID_{50}$/mL) or a RSV A virus (A/2/Australia/61, $1x10^4$ $TCID_{50}$/mL) spiked into a 16.7% simulated background matrix. This process was repeated 20 times in a single GeneXpert module for a total of 41 runs resulting in 20 positives and 21 negatives. 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%.

All of the 42 negative replicates were correctly reported as "Flu A NEGATIVE; Flu B NEGATIVE; RSV NEGATIVE". All the negative sample Cts were zero.

All 20 high Flu A positive replicates were correctly reported as "Flu A POSITIVE; Flu B NEGATIVE; RSV NEGATIVE". All 20 high RSV positive replicates were correctly reported as "Flu A NEGATIVE; Flu B NEGATIVE; RSV POSITIVE".

Of the total 121 runs, two runs provided indeterminate GeneXpert results (2 ERROR). Both runs were repeated such that 21 negative and 20 positive replicates were tested for each virus strain. Under the conditions of this study, no evidence of specimen or amplicon carry-over contamination was observed in the GeneXpert Dx GX-IV module.

2. Comparison studies:

a. Method comparison with predicate device:

N/A

b. Matrix comparison:

Clinical vs. simulated matrix comparison data was originally submitted for the Xpert Flu/RSV XC Assay in K142045.

c. Fresh vs. Frozen sample comparison:

Fresh vs. Frozen study data was originally submitted for the Xpert Flu/RSV XC Assay in K142045. The Flu/RSV XC Assay was used to determine the stability of the Xpert Flu+RSV Xpress Assay because all reagents, buffers and other components that comprise both assays are exactly the same. The detection method, the signal analysis, and algorithm used by the different software to calculate the results are the same.

3. Clinical studies:

A total of 12 sites, 10 CLIA waived and 2 High Complexity CLIA laboratories participated in the study. Five sites were emergency departments, five sites were walk-in clinics or student health centers, and two sites were hospital laboratories. The study was conducted from September 2014 to March 2015.

One nasopharyngeal swab was collected from each subject and placed into UTM after collection. This sample was used for immediate testing with Xpert Flu+ RSV Xpress Assay and the Comparator NAAT. Due to the low prevalence of Flu B and RSV during the sampling time frame of this study, supplementation with pre-selected archived NP swab specimens known to be positive for Flu B or RSV was required to meet the sample size for these targets. A total of 2435 NP swab specimens were tested for influenza A, influenza B, and RSV by the Xpert Flu+RSV Xpress Assay and the comparator assay. Of the 2435 NP swab specimens 2176 were fresh, prospectively collected and 259 were pre-selected frozen, archived specimens.

Of the Xpert Flu+RSV Xpress Assay runs performed with eligible specimens, 95.0% (2335/2459) of these specimens were successful on the first attempt. The initial invalid rate was 5.0% (95% CI 4.2-6.0%). Invalid results were returned on the first attempt for 124 specimens (121 NO RESULT-REPEAT TEST and 3 INSTRUMENT ERROR). One-hundred eighteen of the 124 specimens were retested, of which 107 yielded valid results after a single retest. There were 17 NP swab specimens with invalid results upon retest which were excluded from the analyses.

The tables below show the performance of the Xpert Flu+RSV Xpress Assay, the data are stratified by analyte, Influenza A, Influenza B, and RSV and by sample collection, prospective or retrospective.

Xpert Flu+RSV Xpress Assay Performance on NP Swab Specimens

Specimen Type	Target	n	TP	FP	TN	FN	PPA % (95 CI)	NPA % (95 CI)
Fresh	Flu A	2176	250	101[a]	1825	0	100 (98.5-100)	94.8 (93.7-95.7)
	Flu B	2176	63	10[b]	2103	0	100 (94.3-100)	99.5 (99.1-99.8)
	RSV	2176	125	8[c]	2039	4[d]	96.9 (92.3-99.1)	99.6 (99.2-99.8)
Pre-selected Frozen	Flu A	259	0	4[e]	255	0	NA	98.5 (96.1-99.6)
	Flu B	259	100	2[f]	157	0	100	98.7

							(96.4-100)	(95.5-99.8)
	RSV	259	40	1[g]	217	1[h]	97.6 (87.1-99.9)	99.5 (97.5-100)

a. Testing results by sequencing: 92 of 101 were Flu A positive; 8 of 101 failed to sequence; 1 of 101had insufficient remaining volume for sequencing.
b. Testing results by sequencing: 9 of 10 were Flu B positive; 1 of 10 had insufficient remaining volume for sequencing.
c. Testing results by sequencing: 7 of 8 were RSV positive; 1 of 8 was RSV negative.
d. Testing results by sequencing: 3 of 4 were RSV positive; 1 of 4 was RSV negative.
e. Testing results by sequencing: 4 of 4 had insufficient remaining volume for sequencing.
f. Testing results by sequencing: 2 of 2 had insufficient remaining volume for sequencing.
g. Testing results by sequencing: 1 of 1had insufficient remaining volume for sequencing.
h. Testing results by sequencing: 1 of 1 had insufficient remaining volume for sequencing.

4. Clinical cut-off**:**

Please refer to the "Baseline, Threshold, Cut-off" sections of this document.

5. Expected values/Reference range:

The Xpert Flu+RSV Xpress clinical study included a total of 2176 prospectively collected fresh NP swab specimens. The number and percentage of cases positive for one or more viruses of influenza A, influenza B, and RSV, as determined by the Xpert Flu+RSV Xpress Assay are shown by age category below.

Age Group Flu A, Flu B and RSV Positive by Xpert Flu+RSV Xpress Assay – NP Swabs[a]

Age Group	Number of Patients	Flu A		Flu B		RSV	
		Number of Positives	Positivity	Number of Positives	Positivity	Number of Positives	Positivity
≤5 years	347	44	12.7%	4	1.2%	73	21.0%
6-21 years	382	90	23.6%	8	2.1%	15	3.9%
22-59 years	1222	175	14.3%	53	4.3%	33	2.7%
≥60 years	225	42	18.7%	8	3.6%	12	5.3%
Total	2176	351	16.1%	73	3.4%	133	6.1%

a. Five subjects had dual infections (Flu A & RSV) by Xpert Flu+RSV Xpress Assay (all were RSV POS only by comparator assay) and are therefore counted twice in this table.

N. Instrument Name:

GeneXpert Xpress System using Cepheid GeneXpert Xpress software version 4.4 or higher.

O. System Descriptions:

The GeneXpert Xpress System automates and integrates sample purification, nucleic acid amplification, and detection of the target sequences in simple or complex samples using real-time PCR and RT-PCR. The system consists of a GeneXpert GX-I instrument (also referred to as GeneXpert I), touchscreen computer, and preloaded software for running the tests and viewing the results. The GeneXpert Xpress System requires the use of single-use disposable assay-specific cartridges (the Xpert Flu+RSV Xpress Assay cartridge) that hold the PCR reagents and host the PCR process. In this platform, sample preparation, amplification, and real-time detection are fully-automated and completely integrated.

The GeneXpert Xpress System platform is the same platform as the GeneXpert Dx System (model GX-I) that is used with Cepheid's previously cleared Xpert assays (including K142025). The detection method, the signal analysis, and algorithm used by the systems to calculate the results are the same. The differences between the systems lie in the user interface of the system and the reporting of results: 1) the Xpress system has an ATM-like user interface and is simplified to utilize pictures and videos to guide the user through ordering and running a test, and 2) the test results for INVALID and ERROR results are simplified.

1. Modes of Operation:

The Xpert Flu+RSV Xpress Assay can be used with GeneXpert Xpress software version 4.4.2 and GeneXpert Dx software version 4.4. Directions for operating the system are included in the package insert.

2. Software:

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

Yes ___X_____ or No _____

Level of Concern:
Moderate

Software Description:
The GeneXpert Xpress software utilizes the same GeneXpert module control (optics, thermal, fluidics, etc.), data collection and data analysis as the GeneXpert Dx software using the GeneXpert GX-I instrument (identical instrument as the GeneXpert Dx GX-I instrument). Two major differences are: 1) Xpress software user interface is simplified to utilize pictures and videos to guide the user through ordering and running a test, and 2) the test result interpretations for INVALID and ERROR results are simplified. The following table outlines the similarities and differences:

Comparison of GeneXpert Xpress Versions 4.4.2 and GeneXpert Dx 4.4 Software Component or Feature For Use With the GeneXpert Dx GX-I

Component or Feature	GeneXpert Xpress version 4.4.2	GeneXpert Dx version 4.4
Communication with Module	Yes - 1 module (for use only with the **GeneXpert Dx GX-I**)	Same (and for use with the GeneXpert Dx family of instruments)
Graphic User Interface (GUI)	Yes – **Simplified user interface** (touchpad and keyboard integrated laptop and touchscreen) adopted for use in a CLIA waived environment by untrained users	Yes – Interface (keyboard and mouse with laptop or desktop PC or keyboard integrated laptop) adopted for use in a CLIA moderate complexity environment by untrained or experienced users
Test Results	Same except the test results for INVALID and ERROR results are simplified (**NO RESULT – REPEAT TEST** or **INSTRUMENT ERROR**)	Same standard test results including **INVALID**, **ERROR**, and **NO RESULT**.
Reports	No	Yes (Individual, Patient, Specimen, and External Control)
Detailed Raw Data Results	No	Yes, according to user access privileges (Basic or Administrator user). Cycle threshold data is only visible to the user with Administrator privileges and access to data files.
Data Management	No	Yes (Archive of data and back up data)
Supports computer resolution	Screen resolution of 1366 x 768	Screen resolution of 1366 x 768 or 1024 X 768
Operating System	Windows 7	Windows XP or Windows 7
2D Barcode Scanner Configuration	Yes (hand held)	Same
Assay Definition File (ADF)	Supports qualitative ADFs	Same
Handling of Lot Specific Parameters embedded in barcode	Yes	Same

Cartridge handling including loading, staging, removal and disposal	Manual	Same
Fluidics handling	Yes	Same
Ultrasonic handling	Yes	Same
Thermal control	Yes	Same
Optical Detection - 6-color	Yes	Same
Data Acquisition and Analyses	Yes	Same
Data Storage	MS SQL 2005 database	Same
Internal Control data analysis	Yes	Same
External Control data analysis	Yes	Same
Viewing Test Results	Yes	Same
Laboratory Information System connectivity: uploading results	Yes	Same
Provide alerts for low memory and for modules nearing re-calibration	Yes	Same

3. Specimen Identification:

Specimen identification is described in the "Test Principle" and "Baseline, Threshold, Cut-off" sections of this document.

4. Calibration:

N/A

6. Quality Control:

- Sample Processing Control (SPC): Ensures the sample was processed correctly.

  The SPC is an Armored RNA® that is included in each cartridge to verify adequate processing of the sample. The SPC verifies that release of RNA from the Flu or RSV virus has occurred if the organism is present and verifies that the specimen processing is adequate. Additionally, this control detects specimen-associated inhibition of the RT-PCR and PCR reactions. The SPC should be positive in a negative sample and can be negative or positive in a positive sample. The SPC passes if it meets the validated acceptance criteria.

- Probe Check Control (PCC): Before the start of the PCR reaction, the GeneXpert Xpress System measures the fluorescence signal from the probes to monitor bead rehydration, reaction tube filling, probe integrity, and dye stability. The PCC passes if it meets the validated acceptance criteria.

P. Other Supportive Instrument Performance Characteristics Data Not Covered in the "Performance Characteristics" Section Above:

N/A

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.