510(k) SUBSTANTIAL EQUIVALENCE DETERMINATION
DECISION SUMMARY

A. 510(k) Number:

K181289

B. Purpose for Submission:

To obtain 510(k) clearance for the Xpert Xpress Flu Assay performed on the GeneXpert Instrument Systems implementing a new software version and an expanded Intended Use claim that includes nasal swab (NS) specimens as an additional cleared specimen type.

C. Measurand:

Conserved RNA sequences in the genes that encode the following proteins: influenza A matrix protein (M), influenza A polymerase basic protein 2 (PB2), influenza A polymerase acidic protein (PA), influenza B matrix protein (M), and influenza B non-structural proteins (NS1 and NS2).

D. Type of Test:

An automated, multiplex, real-time, reverse transcriptase polymerase chain reaction (RT-PCR) assay for the *in vitro* qualitative detection and differentiation of influenza A and influenza B viral RNA.

E. Applicant:

Cepheid

F. Proprietary and Established Names:

Xpert Xpress Flu
Xpert Xpress Flu Assay

G. Regulatory Information:

1. Regulation Section:
   21CFR 866.3980 - Respiratory viral panel multiplex nucleic acid assay

2. Classification:

   Class II

3. Product code:
   OCC – Respiratory viral panel multiplex nucleic acid assay
   OOI – Instrumentation for clinical multiplex test systems

JSM - Culture media, non-propagating transport

4. Panel:
   Microbiology (83)

H. Intended Use:

1. Intended Use(s):

The Cepheid Xpert Xpress Flu Assay, performed on the GeneXpert Instrument Systems, 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 and influenza B viral RNA. The Xpert Xpress Flu Assay uses nasopharyngeal (NP) swab and nasal swab (NS) specimens collected from patients with signs and symptoms of respiratory infection. The Xpert Xpress Flu Assay is intended as an aid in the diagnosis of influenza infections in conjunction with clinical and epidemiological risk factors.

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

Performance characteristics for influenza A were established during the 2015-2016 influenza season for NP swab specimens and the 2016-2017 influenza season for NS specimens. 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.

Ancillary Nasopharyngeal Swab Specimen Collection Kit for Viruses:

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, Xpert Xpress Flu/RSV Assay or the Xpert Xpress Flu Assay.

Ancillary Nasal Swab Specimen Collection Kit for Viruses:

The Xpert Nasal Sample Collection Kit is designed to collect, preserve, and transport nasal swab specimens containing viruses from patients with signs and symptoms of respiratory infection prior to analysis with the Xpert Xpress Flu Assay.

2. Indication(s) for Use:

   Same as intended use

3. Special Conditions for Use Statement(s):

   For prescription use only

4. Special Instrument Requirements:

   GeneXpert Instrument Systems

   - GeneXpert Dx Systems (GX-I, GX-II, GX-IV, GX-XVI)
   - GeneXpert Infinity Systems (Infinity-48, Infinity-80, Infinity-48s)

I. Device Description:

Overview

The Xpert Xpress Flu Assay, performed on the GeneXpert Instrument (Dx and Infinity) Systems, is a rapid, multiplex nucleic acid amplification test for the qualitative detection and differentiation of influenza A and influenza B viral RNA from nasopharyngeal (NP) swab and nasal swab (NS) specimens.

To perform the Xpert Xpress Flu Assay, NP swab and NS specimens are collected from patients and placed into viral transport medium using the Xpert Xpress Nasopharyngeal Sample Collection Kit for Viruses. With a transfer pipette, eluted NP swab and NS specimens are loaded into the sample chamber of a single-use, self-contained Xpert Xpress Flu Assay cartridge. Each assay cartridge contains separate chambers for sample loading, sample processing and target amplification by real-time RT-PCR, and contains all the reagents necessary to carry out these processes. Because the cartridges are self-contained, and specimens never contact working parts of the instrument modules, cross-contamination between samples is minimized.

The assay cartridge containing the patient sample is inserted into the GeneXpert Instrument System, which performs fully automated and integrated sample preparation and real-time RT-PCR for the Xpert Xpress Flu Assay in approximately 30 minutes or less. The GeneXpert Instrument Systems, comprised of the GeneXpert Dx and GeneXpert Infinity Systems, have one to 80 randomly accessible modules 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 detection.

After completion of the test, the assay results are interpreted by the GeneXpert software

from measured fluorescent signals and embedded calculation algorithms, and are shown in the "View Results" window in tabular and graphic formats.

1. Materials Provided

The Xpert Xpress Flu Assay (Cat # XPRSFLU-10) contains sufficient reagents to process 10 NP swab, NS specimens or quality control samples. The assay kit includes 10 individual cartridges with integrated reaction tubes that hold all the reagents necessary for sample lysis, RNA extraction, and nucleic acid amplification. Additionally, each kit includes a set of 12 transfer pipettes for loading samples into the assay cartridge and a CD containing the Assay Definition Files (ADFs) with instructions for importing ADFs into the GeneXpert software.

2. Materials Required but Not Provided

Validated Specimen Collection and Transport Kit:

- Cepheid Xpert Nasopharyngeal Sample Collection Kit for Viruses (Cat # SWAB/B-100) containing one individually wrapped, sterile nasopharyngeal flocked nylon swab and one Xpert Viral Transport Medium tube with three mL of Xpert Viral Transport Medium.
- Cepheid Xpert Nasal Sample Collection Kit for Viruses (Cat # SWAB/F-100) containing one individually wrapped, sterile nasal flocked nylon swab and one Xpert Viral Transport Medium tube with three mL of Xpert Viral Transport Medium.

GeneXpert Instrument System:

- GeneXpert Dx System (GX-I, GX-II, GX-IV, GX-XVI)
- GeneXpert Infinity System (Infinity-48, Infinity-48s, Infinity-80)

3. Materials Available but Not Provided

External Controls:

- Positive inactivated influenza A and B virus control from ZeptoMetrix (Cat # NATFLUAB-6C)
- Negative inactivated coxsackievirus control from ZeptoMetrix (Cat # NATCXVA9-6C)

4. Quality Control

The Xpert Xpress Flu Assay includes two internal controls: a sample processing control, and a probe check control.

Sample Processing Control:

The sample processing control (SPC) is a non-infectious armored RNA pseudovirus that ensures adequate processing of target viruses, monitors the presence of PCR inhibitors, and verifies the use of proper PCR conditions. The SPC should be POSITIVE in a sample that is

negative for both influenza A and influenza B target analytes, and can be NEGATIVE or POSITIVE in a sample containing detectable levels of one or more of the target analytes.

Probe Check Control:

The probe check control (PCC) is present to control for sufficient reagent rehydration, PCR tube filling, probe integrity, and dye stability. All assay reagents must be present and intact for the PCC to pass the validated acceptance criteria. If any of the PCC conditions fail, the result is reported as an ERROR and the test must be repeated using a new assay cartridge.

5. Results Interpretation

POSITIVE or NEGATIVE test results for influenza A (Flu A) and influenza B (Flu B) viral RNA are automatically generated by the GeneXpert software and presented as text in the "View Results" window of the GeneXpert Instrument Systems following completion of the test. The GeneXpert software is equipped with embedded algorithms to determine the result of the test based on the calculated cycle threshold (Ct) values obtained for each of the RNA target-specific optical curves generated by fluorescent probes during the real-time PCR cycling process. Optical curves for each of the RNA targets are also displayed in the "View Results" window but do not require manual data interpretation or reviewing.

The Xpert Xpress Flu Assay uses two separate channels for the detection of influenza A viral RNA (Flu A 1 and Flu A 2). 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 > 95% homology to avian influenza A strains and approximately 80% homology to human influenza A strains. Detection of influenza A strains in either the Flu A 1 or Flu A 2 channel is reported as Flu A POSITIVE.

The GeneXpert Instrument Systems software also reports if the test is INVALID, encounters an ERROR, or produces NO RESULT.

All possible test results for the Xpert Xpress Flu assay are shown in Table 1.

Table 1: All Possible Final Test Results for the Xpert Xpress Flu Assay

	RNA target			
Result	**Flu A 1**	**Flu A 2**	**Flu B**	**SPC**
Flu A POSITIVE; Flu B NEGATIVE	POS	POS/NEG	NEG	POS/NEG
	POS/NEG	POS		
Flu A POSITIVE; Flu B POSITIVE	POS	POS/NEG	POS	POS/NEG
	POS/NEG	POS		
Flu A NEGATIVE; Flu B POSITIVE	NEG	NEG	POS	POS/NEG
Flu A NEGATIVE; Flu B NEGATIVE	NEG	NEG	NEG	POS
INVALID	NEG	NEG	NEG	NEG
ERROR	NO RESULT	NO RESULT	NO RESULT	NO RESULT
NO RESULT	NO RESULT	NO RESULT	NO RESULT	NO RESULT

If any of the test results mentioned below occur, the test should be repeated using leftover specimen from the original transport medium tube and a new assay cartridge.

- INVALID: indicates that the control SPC failed. The sample was not properly processed, PCR was inhibited, or the sample was not properly collected.
- ERROR: could be a result of PCC failure, or that the maximum pressure limits were exceeded. PCR does not initiate when there is a PCC failure.
- NO RESULT: indicates that insufficient data were collected. For example, the operator stopped a test that was in progress or a power failure occurred.
- Co-infection with two or more viruses: the incidence of co-infection with influenza A and influenza B viruses is low. Specimens should undergo repeat testing if nucleic acids from more than one analyte are detected in a single specimen.

J. Substantial Equivalence Information:

1. Predicate device name(s):
   Cepheid Xpert Flu/RSV XC

2. Predicate 510(k) number(s):
   K142045

3. Comparison with predicate:

Table 2. Differences between device and predicate

Differences		
Item	**Device** **Cepheid Xpert Xpress Flu**	**Predicate** **Cepheid Xpert Flu/RSV XC K142045**
Assay Targets	Influenza A and Influenza B viral RNA	Influenza A, Influenza B, and RSV viral RNA
Specimen Types	Nasopharyngeal (NP) swab and nasal swab (NS) specimens	Nasal aspirate/wash (NA/W) specimens and Nasopharyngeal (NP) swab specimens
Assay Controls	Encapsulated (armored) RNA pseudovirus as a sample processing control. Available but not provided are inactivated virus controls for influenza A/B as external positive controls, and Coxsackie virus as an external negative control.	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 30 minutes or less for sample preparation and RT-PCR	Approximately 60 minutes or less for sample preparation and RT-PCR
Combinatorial Assay Selections	Not applicable	Yes, user may select combined assay with all targets or a Flu only assay or a RSV only assay.

Table 3. Similarities between device and predicate

Similarities		
	Device	**Predicate**
Item	**Cepheid Xpert Xpress Flu**	**Cepheid Xpert Flu/RSV XC K142045**
Regulation	866.3980	Same
Product Code	OCC, OOI	Same
Device Class	II	Same
Technology Principle of Operation	Multiplex real time RT-PCR	Same
Intended Use	The Cepheid Xpert Xpress Flu Assay, performed on the GeneXpert Instrument Systems, is an automated,	The Cepheid Xpert Flu/RSV XC Assay is an automated, multiplex real-time, reverse transcriptase polymerase chain reaction

Similarities		
	Device	**Predicate**
Item	**Cepheid Xpert Xpress Flu**	**Cepheid Xpert Flu/RSV XC K142045**
	multiplex real-time, reverse transcriptase polymerase chain reaction (RT-PCR) assay intended for the *in vitro* qualitative detection and differentiation of influenza A and influenza B viral RNA. The Xpert Xpress Flu Assay uses nasopharyngeal (NP) swab and nasal swab (NS) specimens collected from patients with signs and symptoms of respiratory infection. The Xpert Xpress Flu Assay is intended as an aid in the diagnosis of influenza infections in conjunction with clinical and epidemiological risk factors. Negative results do not preclude influenza virus infection and should not be used as the sole basis for treatment or other patient management decisions. Performance characteristics for influenza A were established during the 2015-2016 influenza season for NP swab specimens and the 2016-2017 influenza season for NS specimens. 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	(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.

Similarities		
	Device	**Predicate**
Item	**Cepheid Xpert Xpress Flu**	**Cepheid Xpert Flu/RSV XC K142045**
	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
Nucleic Acid Extraction	Yes	Same
Extraction Methods	Sample preparation integrated in GeneXpert Cartridge and GeneXpert Instrumentation System.	Same
Assay Results	Qualitative	Same
Instrument System	Cepheid GeneXpert Instrument Systems; same Cepheid I-core technology	Same
Primers and probes	Primers and probes to detect the presence of nucleic acid sequences of influenza A, influenza B, and RSV. Only results for influenza A and influenza B are reported.	Primers and probes to detect the presence of influenza A, influenza A subtype H7N9, influenza B, and RSV. Results for influenza A, influenza B and RSV analytes are reported.
Laboratory Users	Laboratory users in moderate complexity laboratory settings.	Same
Sample Preparation	Self-contained and automated after mixed specimen is added to cartridge. All other reagents are contained in the cartridge.	Same

Similarities		
	Device	**Predicate**
Item	**Cepheid Xpert Xpress Flu**	**Cepheid Xpert Flu/RSV XC K142045**
Primers and probes for influenza A, influenza B	Primers and probes to detect the presence of nucleic acid sequences of influenza A, influenza B, and RSV A/B. The Xpert Xpress Flu Assay contains primers and probes to detect additional RNA segments in order to protect the assay sensitivity and specificity from mutations in the influenza genome due to antigenic drifts and shifts. Only results for influenza A and influenza B are reported.	Primers and probes to detect the presence of nucleic acid sequences of influenza A, influenza B, and RSV A/B. The Xpert Flu+RSV Xpress Assay contains primers and probes to detect additional RNA segments in order to protect the assay sensitivity and specificity from mutations in the influenza genome due to antigenic drifts and shifts. Results for influenza A, influenza B and RSV analytes are reported.
Target Sequences	Influenza A: Matrix protein (M), basic polymerase (PB2), and acidic protein (PA) Influenza B: Matrix protein (M) and Non-structural proteins (NS1 and NS2) RSV A and RSV B: Nucleocapsid protein Only results for influenza A and influenza B are reported.	Influenza A: Matrix protein (M), basic polymerase (PB2), and acidic protein (PA) Influenza B: Matrix protein (M) and Non-structural proteins (NS1 and NS2) RSV A and RSV B: Nucleocapsid protein
Internal Controls	Sample processing control (SPC) and probe check control (PCC).	Same
Early Assay termination function	Yes	Yes

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

1. The 510(k) Program: Evaluating Substantial Equivalence in Premarket Notifications [510(k)]-Guidance for Industry and Food and Drug Administration Staff. Document issued on July 28, 2014.
2. Class II Special Controls Guidance Document: Instrumentation for Clinical Multiplex Test Systems-Guidance for Industry and Staff. Document issued on March10, 2005.
3. Guidance for the Content of Premarket Submissions for Software Contained in Medical Devices. Document issued on May 11, 2005.
4. EP17-A2: Protocols for Determination of Limit of Detection and Limits of Quantitation;

Approved Guideline-Second Edition 2006.

5. Class II Special Controls Guidance Document: Testing for Detection and Differentiation of Influenza A Virus Subtypes Using Multiplex Nucleic Acid Assays. Document issued on October 9, 2009.
6. Class II Special Controls Guidance Document: Respiratory Viral Panel Multiplex Nucleic Acid Assay. Document issued October 9, 2009.

L. Test Principle:

The Xpert Xpress Flu Assay performed on the GeneXpert Instrument platform utilizes automated real-time RT-PCR for unique gene-specific sequence amplification and 5' end cleavage of hybridized, fluorogenic target-specific probe for the detection of influenza A and influenza B viral RNA. Specific gene targets of the Xpert Xpress Flu Assay primers and probes include the matrix protein (M), basic polymerase protein 2 (PB2), and polymerase acidic protein (PA) of influenza A viruses and the matrix protein (M) and non-structural proteins (NS1 and NS2) of influenza B viruses. Additional assay primers generate amplicons for the SPC.

The GeneXpert Instrument Systems automates sample preparation, amplification and real-time detection of target-specific cDNA from clinical specimens. Each system utilizes a syringe pump drive to dispense fluids to and from the different cartridge chambers and ultrasonic lysis to release nucleic acids from both the target organisms and integrated SPC. During the amplification process, the I-CORE module contained within the GeneXpert Instrument systems heats and cools the reaction tube contents, and detects emitted fluorescence generated in the presence of target sequences. Embedded data analysis algorithms within the GeneXpert Instrument software generate test results based on the calculated cycle threshold (Ct) values obtained for each of the RNA target-specific optical curves generated by fluorescent probes during the real-time RT-PCR cycling process. The time to results is 30 minutes or less.

M. Performance Characteristics:

1. Analytical performance

a. Precision/Reproducibility:

Precision

Not Applicable.

Reproducibility

A multi-center, blinded study using a five-member specimen panel consisting of negative samples, and simulated nasal matrix spiked with low positive samples containing influenza A or influenza B analyte at 1X the respective LoDs, and moderate positive samples containing influenza A or influenza B at 2-3X the respective LoDs. Testing was performed

at three sites (one internal, two external), by two operators per site, on either the GeneXpert Dx system, the Infinity-48 system or the Infinity-80 system (each of the three sites used a different instrument). Each operator tested one panel in duplicate, two times per day over six, not necessarily consecutive days. Three lots of Xpert cartridges were used, with each lot representing approximately two days of testing.

Original testing was performed on the GeneXpert Dx Instrument System with system software version 4.6a or higher as part of K162456. Re-analysis of the original data was conducted for this submission using the Xpert Xpress Flu Assay ADF version 3 in order to determine reproducibility of the newly modified assay.

The expected results for each of the panel members are shown in Table 4 below. Overall percent agreement between the expected results and the re-analyzed reproducibility study results is presented in Table 5. Analysis of variance (CV) between sites, days, lots, and operators for each reproducibility panel member is presented in Table 6.

Re-analysis of K162456 reproducibility data resulted in no changes to the percent agreement for the negative, influenza A (Flu A) moderate positive, or influenza B (Flu B) low positive sample types. For influenza A low positive samples, the percent agreement decreased from 93.7% to 93.6% using ADF version 3. For influenza B moderate positive samples, the percent agreement increased from 99.3% to 100%.

Table 4. Reproducibility sample panel

Panel Sample (Strain Name)	Expected Level	Expected Positivity Rate	Concentration ($TCID_{50}$/mL)
Negative	0	0%	N/A
Flu A (A/Victoria/361/2011)	Low positive (1X LoD)	~95%	0.75
Flu A (A/Victoria/361/2011)	Moderate positive (~2-3X LoD)	100%	1.5
Flu B (B/Mass/2/2012)	Low positive (1X LOD)	~95%	0.2
Flu B (B/Mass/2/2012)	Moderate positive (~2-3X LoD)	100%	0.4

Table 5. Summary of Reproducibility Results - % Agreement-Re-analysis of K162456 Data Using Assay Definition File Version 3 (ADF v3)

Sample ID	Site 1/Infinity-80			Site 2/Dx			Site 3/Infinity-48			% Total Agreement by Sample[a]
	Op 1	Op 2	Site	Op 1	Op 2	Site	Op 1	Op 2	Site	
Negative	100% (24/24)	100% (24/24)	100% (48/48)	100% (24/24)	100% (24/24)	100% (48/48)	100% (24/24)	100% (24/24)	100% (48/48)	100% (144/144)
Flu A Low Pos	87% (20/23)	95.8% (23/24)	91.5% (43/47)	95.7% (22/23)	91.7% (22/24)	93.6% (44/47)	100% (23/23)	91.3% (21/23)	95.7% (44/46)	93.6% (131/140)[b]
Flu A Mod Pos	100% (24/24)	100% (24/24)	100% (48/48)	100% (23/23)	100% (23/23)	100% (46/46)	100% (24/24)	100% (24/24)	100% (48/48)	100% (142/142)[b]
Flu B Low Pos	95.8% (23/24)	95.8% (23/24)	95.8% (46/48)	95.8% (23/24)	95.8% (23/24)	95.8% (46/48)	95.8% (23/24)	91.7% (22/24)	93.8% (45/48)	95.1% (137/144)
Flu B Mod Pos	100% (23/23)	100% (24/24)	100% (47/47)	100% (24/24)	100% (24/24)	100% (48/48)	100% (24/24)	100% (23/23)	100% (47/47)	100% (142/142)[b]

[a]Agreement calculated based on expected result: Negative for Negative (targeted positivity: 0%); Positive for Low Pos (targeted positivity: 95%) and Mod Pos (targeted positivity: 100%) samples.

[b]Eight samples were indeterminate [Flu A Low Pos (4); Flu A Mod Pos (2); Flu B Mod Pos (2)]

Table 6. Summary of Reproducibility Data-Re-analysis of K162456 Data Using Assay Definition File Version 3 (ADF v3)

Sample	Assay Channel (Analyte)	N[a]	Mean Ct	Between-Site		Between-Lot		Between-Day		Between-Operator		Within-Assay		Total	
				SD	CV (%)	SD	CV (%)	SD	CV (%)	SD	CV (%)	SD	CV (%)	SD	CV (%)
Negative	SPC	144	32.3	0	0	0.7	2.1	0.1	0.4	0	0	0.6	1.9	0.9	2.8
Flu A-Low Pos	FluA1	131	35.3	0	0	0.6	1.6	0	0	0	0	1.1	3.0	1.2	3.4
Flu A-Mod Pos	FluA1	142	33.1	0	0	0.0	0.1	0.2	0.6	0	0	0.6	1.8	0.6	1.9
Flu B-Low Pos	FluB	137	34.6	0	0	0	0	0.5	1.3	0.4	1.2	1.3	3.9	1.5	4.2
Flu B-Mod Pos	FluB	142	32.3	0.1	0.3	0.3	0.8	0	0	0.3	0.8	0.8	2.4	0.9	2.7

[a]Results with non-zero values out of 144

All negative samples produced a negative result (144/144).

All moderate positive samples produced a positive result [Flu A (142/142) and Flu B (142/142)].

The differences observed upon re-analysis of reproducibility data using the updated ADF v3 are minimal, and are summarized in Table 7 below. Based on these data, the ADF changes do not appear to impact the overall assay reproducibility.

Table 7. Xpert Xpress Flu Assay Performance Comparison

Sample Type	% Total Agreement by Sample	
	Reference ADF	Candidate ADF
Negative	100% (144/144)	100% (144/144)
Flu A – Low Pos	93.7% (134/143)	93.6% (131/140)*
Flu A – Mod Pos	100% (142/142)	100% (142/142)
Flu B – Low Pos	95.1% (137/144)	95.1% (137/144)
Flu B – Mod Pos	99.3% (143/144)	100% (142/142)*

*Fewer samples are reported for the re-analysis due to an increase in the number of invalid results when using Assay Definition File version 3.

Re-analysis of reproducibility data originally obtained during studies conducted for K171552, which used the Xpert Xpress Flu Assay on the GeneXpert Xpress System, was also performed and reported in this submission. Because the re-analysis resulted in no changes to the percent agreement for any of the sample types tested, data are not shown. Please refer to K171552 for results.

b. Linearity/assay reportable range:

Not Applicable.

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

Traceability:
Not Applicable.

Calibrator
Not Applicable.

Controls
Please refer to submission K162456.

Stability:
Stability studies have been performed to support the following claims:

Sample Stability:
The following specimen stability claims are supported by study data from K162456:

- 15-30°C for up to 24 hours
- 2-8°C for up to seven days

Kit Stability:

Additional study data to support stability claims at 16 months were provided in this submission.

- 2-28°C for up to 16 months.

Refer to K162456 and K171552 for all other stability information.

Cartridge Hold Time:

The following stability claim for prepared samples waiting on the GeneXpert Xpress GX II or IV are supported by study data from K162456:

- Up to 4.5 hours at room temperature

Carry-over:

A carry-over study was conducted to demonstrate that single-use, self-contained GeneXpert cartridges prevent carry-over contamination of negative samples if preceded by very high positive samples in the same GeneXpert module. The study consisted of a negative sample processed in the same GeneXpert module (GeneXpert Dx System; GX-IV instrument) immediately following a very high positive influenza A sample (A/Victoria/361/2011, $2x10^7$ $TCID_{50}$/mL) spiked into a simulated background matrix. The 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%.

The testing was repeated 20 times on two GeneXpert modules for a total of 41 runs resulting in 20 positive and 21 negative specimens for each virus type. All 20 positive samples were correctly reported as Flu A POSITIVE; Flu B NEGATIVE. All 21 negative samples were correctly reported as Flu A NEGATIVE; Flu B NEGATIVE. Therefore, under the conditions of the study, no evidence of specimen or amplicon carry-over contamination was observed in the GeneXpert Dx modules.

d. Detection limit:

The following influenza strains were used in the Limit of Detection (LoD) study:

- 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/Massachusetts/2/2012
  - B/Wisconsin/01/2011

The LoD studies for the NP and NS specimens were originally conducted as part of submissions K162456 (using GeneXpert Instrument systems; NP samples) and

K171552 (using GeneXpert Xpress systems; NS samples). These data were re-analyzed for this submission using a new Assay Definition File version 3 (ADF v3) and presented in Table 8. There was no change in LoD, using ADF v3, for any of the strains tested when compared to previous submissions K162456 and K171552.

Table 8. Confirmed LoD ($TCID_{50}$/mL) for Influenza A and Influenza B Strains - Re-analysis of K162456 (NP data) and K171552 (NS data) Using Assay Definition File Version 3(ADF v3)

Sample Type	Influenza Strain	LOD ($TCID_{50}$/mL)
NP	Influenza A/California/7/2009 (H1N1)	0.02
NP	Influenza A/Florida/27/2011 (H1N1)	0.04
NP	Influenza A/Perth/16/2009 (H3N2)	0.01
NP	Influenza A/Victoria/361/2011 (H3N2)	0.75
NP	Influenza B/Massachusetts/2/2012	0.40
NP	Influenza B/Wisconsin/01/2011	0.19
NS	Influenza A/California/7/2009 (H1N1)	0.02
NS	Influenza A/Florida/27/2011 (H1N1)	0.04
NS	Influenza A/Perth/16/2009 (H3N2)	0.01
NS	Influenza A/Victoria/361/2011 (H3N2)	0.21
NS	Influenza B/Massachusetts/2/2012	0.07
NS	Influenza B/Wisconsin/01/2011	0.17

e. Analytical specificity:

Interfering Substances
Please refer to submission K162456.

Analytical Reactivity (inclusivity)
Please refer to submission K162456.

Competitive Interference
Please refer to submission K162456.

Cross-Reactivity
Please refer to submission K162456.

f. Assay cut-off:

The Xpert Xpress Flu Assay detects Flu A and Flu B targets. The Assay has two channels (Flu A 1 and Flu A 2) to detect influenza A strains. The Assay has one channel (Flu B) to detect influenza B strains.

For each of the analytes (Flu A 1, Flu A 2, and Flu B) the valid cycle threshold (Ct) range to report a positive result is Ct 10.0 to 40.0. For the Sample Processing Control (SPC) the valid Ct range to report a positive result is Ct 26 to 40. The Ct cutoffs are included as automatic calculations in the ADF that is provided with the Xpert Xpress Flu Assay.

2. Comparison studies:

a. Method comparison with predicate device:
Not Applicable.

b. Matrix comparison:
An equivalency study for clinical and simulated nasal matrix was described in K162456.

An equivalence study for NP swab matrix, NS matrix and simulated matrix was described in K171552.

3. Clinical studies:

a. Clinical Sensitivity and Specificity:

Performance of the Xpert Xpress Flu Assay was previously evaluated against FDA-cleared nucleic acid reference tests during two separate prospective clinical studies (K162456 and K171552). For the K162456 study, data was collected during the 2015-2016 influenza season and used to support clearance of the Xpert Xpress Assay on the GeneXpert Instrument systems (K162456) with nasopharyngeal (NP) swab specimens. For the K171552 study, data was collected during the 2016-2017 influenza season and used to support clearance of the Xpert Xpress Flu Assay on the GeneXpert Xpress Instrument Systems (K171552) with both NP and nasal swab (NS) specimens. Details for the two clinical studies can be found in the respective decision summaries.

To determine the clinical performance of the newly modified Xpert Xpress Flu Assay (ADF v3) on the GeneXpert Instrument Systems, data from the previously conducted studies were re-analyzed using the new software version. Results from the reanalysis are summarized in the following tables.

Table 9. Number and Percent of NP Specimens by Age Range-from K162456 Study

Age Group (years)	Number of Patients	% of Total	Flu A		Flu B	
			Number of Positives	Positivity Rate	Number of Positives	Positivity Rate
≤5 years	360	17.6%	25	7.0%	17	4.7%
6-21 years	224	11.0%	18	8.0%	30	13.3%
22-59 years	729	35.5%	52	7.1%	26	3.6%
≥60 years	36	35.9%	32	4.3%	22	3.0%
Unknown	1	<0.1%	0	0	0	0
Total	2051	100%	127	6.2%	95	4.6%

Table 10. Xpert Xpress Flu Assay Performance with NP Swab Specimens-Re-analysis of K162456 Data Using Assay Definition File Version 3 (ADF v3)

Specimen Type	Target	N	TP	FN	TN	FP	PPA (95% CI)	NPA (95% CI)
Fresh, prospective	Flu A	1139	35	2[a]	1095	7[b]	94.6% (82.3-98.5)	99.4% (98.7-99.7)
	Flu B	1139	42	0	1089	8[c]	100.0% (91.6-100.0)	99.3% (98.6-99.6)
Frozen, Consecutively Collected	Flu A	912	68	0	827	17[d]	100.0% (94.7-100.0)	98.0% (96.8-98.7)
	Flu B	912	36	0	867	9[e]	100.0% (90.4-100.0)	99.0% (98.1-99.5)
Combined	Flu A	2051	103	2[a]	1922	24[f]	98.1% (93.3-99.5)	98.8% (98.2-99.2)
	Flu B	2051	78	0	1956	17[g]	100.0% (95.3-100.0)	99.1% (98.6-99.5)

[a]. Testing results by sequencing: 2 of 2 were Flu A Negative.
[b]. Testing results by sequencing: 3 of 7 were Flu A Positive; 3 of 7 were Flu A Negative; 1 of 7 insufficient specimen for sequencing.
[c]. Testing results by sequencing: 6 of 8 were Flu B Positive; 1 of 8 were Flu B Negative; 1 of 8 insufficient specimen for sequencing.
[d]. Testing results by sequencing: 7 of 17 were Flu A Positive; 7 of 17 were Flu A Negative; 3 of 17 insufficient specimen for sequencing.

[e]. Testing results by sequencing: 7 of 9 were Flu B Positive; 0 of 9 were Flu B Negative; 2 of 9 insufficient specimen for sequencing.
f. Testing results by sequencing: 10 of 24 were Flu A Positive; 10 of 24 were Flu A Negative; 4 of 24 insufficient specimen for sequencing.
[g]. Testing results by sequencing: 13 of 17 were Flu B Positive; 1 of 17 were Flu B Negative; 3 of 17 insufficient specimen for sequencing.

Implementation of ADF v3 resulted in a 0.1% increase in NPA for influenza A and influenza B for prospectively collected, fresh specimens when compared to the previous ADF version. In addition, there was a 0.8% increase in NPA for influenza A and influenza B for consecutively collected, frozen specimens when compared to the previous ADF version. Therefore, ADF v3 has minimal impact on assay performance using clinical NP swab specimens.

Table 11. Number and Percent of NS Specimens by Age Range-from K171552 Study

Age Group (years)	Number of Patients	% of Total	Flu A		Flu B	
			Number of Positives	Positivity Rate	Number of Positives	Positivity Rate
≤5 years	604	37.8%	67	11.1%	26	4.3%
6-21 years	273	17.1%	65	23.8%	26	9.5%
22-59 years	554	34.7%	58	10.5%	19	3.4%
≥60 years	167	10.5%	30	18.0%	3	1.8%
Total	1598	100%	220	14.0%	74	4.6%

Table 12. Xpert Xpress Flu Assay Performance with NS Specimens - Re-analysis of K171552 Data Using Assay Definition File Version 3 (ADF v3)

Specimen Type	Target	N	TP	FN	TN	FP	PPA (95% CI)	NPA (95% CI)
Fresh, prospective	Flu A	1598	186	2[a]	1376	34[b]	98.9% (96.2-99.7)	97.6% (96.6-98.3)
	Flu B	1598	63	1[c]	1523	11[d]	98.4% (91.7-99.7)	99.3% (98.7-99.6)

[a.] Testing results by sequencing: 1 of 2 Flu A NEG; 1 of 2 Flu A POS.
[b.] Testing results by sequencing: 16 of 34 Flu A NEG; 11 of 34 Flu A POS; 7 of 34 inconclusive.
[c.] Testing results by sequencing: 1 of 1 inconclusive.
[d.] Testing results by sequencing: 5 of 11 Flu B POS; 6 of 11 inconclusive.

Implementation of ADF v3 resulted in a 0.3% increase in NPA for influenza A and a 0.1% increase in NPA for influenza B when compared to the previous ADF version. Therefore, ADF v3 has minimal impact on assay performance using clinical NS specimens.

4. Clinical cut-off**:**

   N/A

5. Expected values/Reference range:

   Expected prevalence values for influenza A and influenza B infections were calculated using the data acquired during the NP and NS clinical studies conducted under K162456 and K171552, respectively. The number and percentage of cases positive for influenza A and influenza B, as reported in Table 13, were determined following re-analysis of the original study data with the Xpert Xpress Flu Assay ADF v3.

   Table 13. Expected Values for Influenza A and Influenza B by Xpert Xpress Flu Assay (All swabs; NP and NS)

Age Group (years)	Number of Patients	% of Total	Flu A		Flu B	
			Number of Positives	Positivity Rate	Number of Positives	Positivity Rate
≤5 years	964	26.4%	92	9.5%	43	4.5%
6-21 years	498	13.6%	83	16.7%	56	11.2%
22-59 years	1283	35.2%	110	8.6%	45	3.5%
≥60 years	903	24.7%	62	6.9%	25	2.8%
Unknown	1	<0.1%	0	0	0	0
Total	3649	100%	347	9.5%	169	4.6%

N. Instrument/System Description:

1. Instrument Name:

   GeneXpert Dx Systems (GX-I, GX-II, GX-IV, GX-XVI) with GeneXpert Dx software version 4.7b or higher

   GeneXpert Infinity-48 System with Xpertise software version 6.4b

   GeneXpert Infinity-80 and Infinity-48s Systems with Xpertise software version 6.4b or higher

2. System Description:

The GeneXpert Instrument System family (GeneXpert Dx and Infinity Systems) automates and integrates sample purification, nucleic acid amplification and detection of target sequences within compatible, assay-specific, single-use cartridges. The instrument systems each contain a computer and preloaded software for running tests and viewing the results.

3. Software:

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

Yes _____X_____ or No _____

4. Level of Concern

Moderate

5. Software Description

The GeneXpert Instrument Systems are provided with a computer, preloaded with software for running tests and viewing results. Each instrument (Dx and Infinity) contains random access, closed-system, computer-based software and embedded firmware which run dedicated microprocessor-controlled modules to integrate sample preparation, amplification, and real-time detection in a single system.

The GeneXpert Infinity modules contain extra robotic features for cartridge handling. The Xpertise software utilized by the Infinity Systems is the user interface and provides the ordering of tests as well as automates loading and unloading of cartridges into GeneXpert modules within the system. The Xpertise user interface builds upon the existing core software functionality for handling GeneXpert modules for cartridge fluidics control, temperature control, optics control, and data analysis by the addition of automation handling for the robotic arm.

6. Specimen Identification

Specimens are manually loaded into the Xpert Xpress Flu Assay cartridge by the user. The user can then either scan or type the sample and patient ID into the system. Prior to placing the cartridge into the GeneXpert Instrument System, the barcode on the Xpert Xpress Flu Assay cartridge is scanned. The information contained in the assay barcode is utilized by the software to run the appropriate assay definition file (ADF). If an assay is being run that does not already exist in the GeneXpert database, the user must import the ADF before starting the test.

7. Calibration

   Not required.

8. Quality Control

   Please refer to Section I-4 of this document.

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

Not Applicable

Q. Proposed Labeling:

The labeling supports the finding of substantial equivalence for this device.

R. Conclusion:

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