DEPARTMENT OF HEALTH & HUMAN SERVICES

Public Health Service

Food and Drug Administration
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Cepheid
Scott Campbell, Ph.D., MBA
Executive Director, Clinical Affairs
904 Caribbean Drive
Sunnyvale CA 94089-1189

November 22, 2014

Re: K142045
Trade/Device Name: Xpert® Flu/RSV XC Assay
Regulation Number: 21 CFR 866.3980
Regulation Name: Respiratory viral panel multiplex nucleic acid assay
Regulatory Class: II
Product Code: OCC, OOI
Dated: October 21, 2014
Received: October 22, 2014

Dear Dr. Campbell:

We have reviewed your Section 510(k) premarket notification of intent to market the device referenced above and have determined the device is substantially equivalent (for the indications for use stated in the enclosure) to legally marketed predicate devices marketed in interstate commerce prior to May 28, 1976, the enactment date of the Medical Device Amendments, or to devices that have been reclassified in accordance with the provisions of the Federal Food, Drug, and Cosmetic Act (Act) that do not require approval of a premarket approval application (PMA). You may, therefore, market the device, subject to the general controls provisions of the Act. The general controls provisions of the Act include requirements for annual registration, listing of devices, good manufacturing practice, labeling, and prohibitions against misbranding and adulteration. Please note: CDRH does not evaluate information related to contract liability warranties. We remind you, however, that device labeling must be truthful and not misleading.

If your device is classified (see above) into either class II (Special Controls) or class III (PMA), it may be subject to additional controls. Existing major regulations affecting your device can be found in the Code of Federal Regulations, Title 21, Parts 800 to 898. In addition, FDA may publish further announcements concerning your device in the Federal Register.

Please be advised that FDA's issuance of a substantial equivalence determination does not mean that FDA has made a determination that your device complies with other requirements of the Act or any Federal statutes and regulations administered by other Federal agencies. You must comply with all the Act's requirements, including, but not limited to: registration and listing (21 CFR Part 807); labeling (21 CFR Parts 801 and 809); medical device reporting (reporting of medical device-related adverse events) (21 CFR 803); good manufacturing practice requirements as set forth in the quality systems (QS) regulation (21 CFR Part 820); and if applicable, the electronic product radiation control provisions (Sections 531-542 of the Act); 21 CFR 1000-1050.

If you desire specific advice for your device on our labeling regulations (21 CFR Parts 801 and 809), please contact the Division of Industry and Consumer Education at its toll-free number (800) 638 2041 or (301) 796-7100 or at its Internet address http://www.fda.gov/MedicalDevices/ResourcesforYou/Industry/default.htm. Also, please note the regulation entitled, "Misbranding by reference to premarket notification" (21 CFR Part 807.97). For questions regarding the reporting of adverse events under the MDR regulation (21 CFR Part 803), please go to http://www.fda.gov/MedicalDevices/Safety/ReportaProblem/default.htm for the CDRH's Office of Surveillance and Biometrics/Division of Postmarket Surveillance.

You may obtain other general information on your responsibilities under the Act from the Division of Industry and Consumer Education at its toll-free number (800) 638-2041 or (301) 796-7100 or at its Internet address http://www.fda.gov/MedicalDevices/ResourcesforYou/Industry/default.htm.

Sincerely yours,

Uwe Scherf -S for

Sally A. Hojvat
Director
Division of Microbiology Devices
Office of In Vitro Diagnostics
and Radiological Health
Center for Devices and Radiological Health

Enclosure

DEPARTMENT OF HEALTH AND HUMAN SERVICES
Food and Drug Administration

Indications for Use

Form Approved: OMB No. 0910-0120
Expiration Date: January 31, 2017
See PRA Statement below.

510(k) Number *(if known)*
K142045

Device Name
Cepheid Xpert® Flu/RSV XC Assay

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

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

Performance characteristics for influenza A were established during the 2013-2014 influenza season. When other novel influenza A viruses are emerging, performance characteristics may vary.

If infection with a novel influenza A virus is suspected based on current clinical and epidemiological screening criteria recommended by public health authorities, specimens should be collected with appropriate infection control precautions for novel virulent influenza viruses and sent to state or local health department for testing. Viral culture should not be attempted in these cases unless a BSL 3+ facility is available to receive and culture specimens.

Type of Use *(Select one or both, as applicable)*

☒ Prescription Use (Part 21 CFR 801 Subpart D) ☐ Over-The-Counter Use (21 CFR 801 Subpart C)

CONTINUE ON A SEPARATE PAGE IF NEEDED.

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5.0 510(k) Summary

As required by 21 CFR Section 807.92(c).

Submitted by:	Cepheid 904 Caribbean Drive Sunnyvale, CA 90489 Phone number: (408) 400-8460 Fax number: (847) 510-0539
Contact:	Kerry J. Flom, Ph.D.
Date of Preparation:	November 17, 2014
Device:	
Trade name:	Xpert® Flu/RSV XC
Common name:	Xpert Flu/RSV XC Assay
Type of Test:	Automated, multiplex real-time reverse transcription-polymerase chain reaction (RT-PCR) assay intended for the *in vitro* qualitative detection and differentiation of influenza A, influenza B, and respiratory syncytial virus.
Regulation number/ Classification name/ Product code:	866.3980/Respiratory viral panel multiplex nucleic acid assay/OCC 866.2570/Instrumentation for clinical multiplex test systems/OOI
Classification Advisory Panel	Class II Microbiology (83)
Prescription Use	Yes
Predicate Devices Assay:	1) *For the detection and differentiation of influenza A, influenza B, and RSV A/B viral RNA in nasopharyngeal swab specimens:* Hologic Prodesse® ProFlu™+ Assay (ProFlu+ Assay) [510(k) #K1109668 and Special 510(k) #K132129]; and, 2) *For the detection and differentiation of influenza A and influenza B viral RNA in nasal aspirate/wash and nasopharyngeal swab specimens:* Cepheid Xpert Flu [510(k) #K123191].
Predicate Devices Ancillary Sample Collection Kit:	Copan Universal Transport Medium (UTM-RT) System [510(k) #K042970]

Device Description:

The Xpert Flu/RSV XC Assay is a rapid, automated *in vitro* diagnostic test for qualitative detection and differentiation of influenza A, influenza B, and respiratory syncytial virus (RSV). The assay is performed on the Cepheid GeneXpert Instrument Systems (GeneXpert Dx systems and GeneXpert Infinity Systems). The GeneXpert Instrument System platform automates and integrates sample purification, nucleic acid amplification, and detection of the target sequence in simple or complex samples using real-time PCR and reverse transcriptase PCR (RT-PCR) assays. The systems require the use of single-use disposable cartridges (the Xpert Flu/RSV XC cartridges) that hold the PCR reagents and host the PCR process. Because the cartridges are self-contained and specimens never come into contact with working parts of the instrument modules, cross-contamination between samples is minimized.

The Xpert Flu/RSV XC Assay includes reagents for the detection and differentiation of influenza A, influenza B, and RSV viral RNA directly from nasopharyngeal (NP) swab and nasal aspirate/wash (NA/W) specimens collected from patients with signs and symptoms of respiratory infection. A Sample Processing Control (SPC) and a Probe Check Control (PCC) are also included in the cartridge. The SPC is 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 single-use, multi-chambered fluidic cartridges are designed to complete sample preparation and real-time RT-PCR for detection and differentiation of influenza A, influenza B and RSV viral RNA in approximately 60 minutes or less. The GeneXpert Instrument Systems, comprised of the GeneXpert Dx Systems and the GeneXpert Infinity Systems, have 1 to 80 randomly accessible modules, depending upon the instrument, that are each capable of performing separate sample preparation and real-time PCR and RT-PCR tests. Each module contains a syringe drive for dispensing fluids (i.e., the syringe drive activates the plunger that works in concert with the rotary valve in the cartridge to move fluids between chambers), an ultrasonic horn for lysing cells or spores, and a proprietary I-CORE® thermocycler for performing real-time PCR and RT-PCR and detection.

Specimens are collected following the user's institution standard procedures for collecting NA/W specimens and NP swab specimens for influenza and RSV testing. The ancillary Cepheid Xpert Nasopharyngeal Sample Collection Kit (Cepheid catalog #SWAB/B-100) or Cepheid's Sample Collection Kit (Cepheid catalog #NASL-100N-100) are required but not provided for use with the assay. Both kits contain the identical viral transport medium and sterile nylon flocked swab. The NA/W specimen or the NP swab specimen is placed into the Xpert viral transport medium and sent to the GeneXpert® testing area for processing. When stored in the transport medium, the NA/W specimen or NP swab specimen is stable for up to 24 hours at 2–30 °C or up to seven days at 2–8 °C. When ready to test the specimen, the user briefly mixes the specimen by inverting the tube five times, transfers the eluted material to the sample chamber in the

top of the disposable fluidic cartridge. The user initiates a test from the system user interface and places the cartridge into the GeneXpert instrument platform, which performs hands-off real-time, multiplex polymerase chain reaction (PCR) for detection of RNA. The results are automatically generated at the end of the process in a report that can be viewed and printed.

Device Intended Use:

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

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

Performance characteristics for influenza A were established during the 2013-2014 influenza season. When other novel influenza A viruses are emerging, performance characteristics may vary.

If infection with a novel influenza A virus is suspected based on current clinical and epidemiological screening criteria recommended by public health authorities, specimens should be collected with appropriate infection control precautions for novel virulent influenza viruses and sent to state or local health department for testing. Viral culture should not be attempted in these cases unless a BSL 3+ facility is available to receive and culture specimens.

Ancillary Specimen Collection Kit

Xpert® Nasopharyngeal Sample Collection Kit

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

The Xpert Nasopharyngeal Sample Collection Kit has only been cleared for use with the Xpert Flu Assay and Xpert Flu/RSV XC Assays.

Substantial Equivalence**:**

The Xpert Flu/RSV XC Assay is substantially equivalent to the ProFlu+ Assay [510(k) # K110968 and #K132139] and to the current Cepheid Xpert Flu Assay (510(k) #K123191). The ProFlu+ Assay and the Xpert Flu/RSV XC Assay detect influenza A, influenza B, and RSV A/B from NP swab specimens. The Xpert Flu Assay detects influenza A and B from both NP swab specimens and NA/W specimens, and the Xpert Flu/RSV XC Assay detects influenza A, influenza B, and RSV from both NP swab specimens and NA/W specimens. All three assays utilize the same technology by determining the presence of the target organisms through real-time RT-PCR amplification and fluorogenic target-specific hybridization detection. A multi-center clinical study was conducted to determine the performance characteristics of the device relative to the primary predicate device, the ProFlu+ Assay, which was validated for use with NA/W specimens, and FDA cleared for NP swab specimens. Discordant results between the Xpert Flu/RSV XC Assay and the ProFlu+ Assay were analyzed by sequencing using primers different from those used in the Xpert Flu/RSV XC Assay. The study results showed the Xpert Flu/RSV XC Assay is acceptable for its intended use and is substantially equivalent to the predicate devices.

Table 5-1 shows the similarities and differences between the Xpert Flu/RSV XC Assay and the predicate assays.

Table 5-1: Comparison of Similarities and Differences of the Xpert Flu/RSV XC Assay with the Predicate Devices

Similarities			
	Device	**Predicate Devices**	
Item	**Cepheid Xpert Flu/RSV XC**	**Current Cepheid Xpert Flu**	**Hologic Prodesse ProFlu+ Assay**
510(k) Number	#K142045	#K123191	#K110968 and #K132129 (special)
Regulation	866.3980	Same	Same
Product Code	OCC, OOI	OQW, OCC, OOI	OCC, OOI
Device Class	II	Same	Same
Technology Principle of Operation	Multiplex real time RT-PCR	Same	Same

Similarities			
	Device	**Predicate Devices**	
Item	**Cepheid Xpert Flu/RSV XC**	**Current Cepheid Xpert Flu**	**Hologic Prodesse ProFlu+ Assay**
Intended Use	The Cepheid Xpert Flu/RSV XC 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, influenza B, and respiratory syncytial virus (RSV) viral RNA. The Xpert Flu/RSV XC Assay uses nasopharyn-geal 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.	The Cepheid Xpert Flu Assay, performed on the GeneXpert Instrument Systems, is an automated, multiplex real-time RT-PCR assay intended for the *in vitro* qualitative detection and differentiation of influenza A, influenza B and 2009 H1N1 influenza viral RNA. The Xpert Flu Assay uses nasal aspirates/washes and nasopharyngeal swab specimens collected from patients with signs and symptoms of respiratory infection in conjunction with clinical and epidemiological risk factors. The Xpert Flu Assay is intended as an aid in the diagnosis of influenza. Negative results do not preclude influenza virus infection and should not be used as the sole basis for treatment or other patient management decisions.	The ProFlu™+ Assay is a multiplex Real-Time PCR (RT-PCR) *in vitro* diagnostic test for the rapid and qualitative detection and discrimination of Influenza A Virus, Influenza B Virus, and Respiratory Syncytial Virus (RSV) nucleic acids isolated and purified from nasopharyngeal (NP) swab specimens obtained from symptomatic patients. This test is intended for use to aid in the differential diagnosis of Influenza A, Influenza B and RSV viral infections in humans and is not intended to detect Influenza C. Negative results do not preclude influenza or RSV virus infection and should not be used as the sole basis for treatment or other management decisions. Conversely, positive results do not rule-out bacterial infection or

Similarities			
	Device	Predicate Devices	
Item	Cepheid Xpert Flu/RSV XC	Current Cepheid Xpert Flu	Hologic Prodesse ProFlu+ Assay
			co-infection with other viruses. The agent detected may not be the definite cause of disease. The use of additional laboratory testing and clinical presentation must be considered in order to obtain the final diagnosis of respiratory viral infection.
	Performance characteristics for influenza A were established during the 2013-2014 influenza season. When other novel influenza A viruses are emerging, performance characteristics may vary.	Performance characteristics for influenza A were established during the 2009-2010 influenza season when 2009 H1N1 influenza was the predominant influenza A virus in circulation. Performance characteristics for influenza A were confirmed when influenza A/H3 and influenza A/2009 H1N1 were the predominant influenza A viruses in circulation (2009-2010, 2010-2011 and 2011-2012). When other influenza A viruses are emerging, performance characteristics may vary.	Performance characteristics for Influenza A Virus were established when Influenza A/H3 and A/H1 were the predominant Influenza A viruses in circulation (2006 – 2007 respiratory season). Performance characteristics for Influenza A were confirmed when Influenza A/H1, Influenza A/H3, and Influenza A/2009 H1N1 were the predominant Influenza A viruses in circulation (2008 and 2009). When other Influenza A viruses are emerging, performance characteristics may vary.

Similarities			
	Device	**Predicate Devices**	
Item	**Cepheid Xpert Flu/RSV XC**	**Current Cepheid Xpert Flu**	**Hologic Prodesse ProFlu+ Assay**
	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.	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.	If infection with a novel Influenza A virus is suspected based on current clinical and epidemiological screening criteria recommended by public health authorities, specimens should be collected with appropriate infection control precautions for novel virulent Influenza viruses and sent to state or local health department for testing. Viral culture should not be attempted in these cases unless a BSL 3+ facility is available to receive and culture specimens.
Indication for Use	Patients with signs and symptoms of respiratory infection in conjunction with clinical and epidemiological risk factors	Same as Xpert Flu/RSV XC Assay	Same - symptomatic patients
Assay Targets	Influenza A Virus, Influenza B Virus, and RSV viral RNA	Influenza A, influenza B, and influenza A, subtype 2009 H1N1	Same as Xpert Flu/RSV XC Assay
Specimen Types	Nasal aspirate/wash (NA/W) specimens and Nasopharyngeal (NP) swab specimens	Same as Xpert Flu/RSV XC Assay	Nasopharyngeal (NP) swab specimens; use of ProFlu+ Assay with NA/W specimens was validated.

Similarities			
	Device	**Predicate Devices**	
Item	**Cepheid Xpert Flu/RSV XC**	**Current Cepheid Xpert Flu**	**Hologic Prodesse ProFlu+ Assay**
Nucleic Acid Extraction	Yes	Same	Same
Extraction Methods	Sample preparation integrated in GeneXpert Cartridge and GeneXpert Instrumentation System	Same as Xpert Flu/RSV XC Assay	Extraction and purification with Roche MagNA Pure LC System or bioMérieux NucliSENS easyMAG System
Assay Results	Qualitative	Same	Same
Instrument System	Cepheid GeneXpert Instrument Systems; same Cepheid I-core technology	Same as Xpert Flu/RSV XC Assay	Cepheid SmartCycler II System; same Cepheid I-core technology
Assay Controls	Encapsulated (armored) RNA pseudovirus as a sample processing control. Available but not provided are inactivated virus controls for influenza A/B and RSV as external positive controls, and Coxsackie virus as an external negative control.	Same as Xpert Flu/RSV XC Assay	Internal RNA control. Required and provided: influenza A, influenza B, RSV A, RSV B positive RNA transcript controls
Time to obtain test results	Approximately 60 minutes or less for sample preparation and real-time RT-PCR.	75 minutes for sample preparation and real-time RT-PCR	Approximately 4 hours for sample preparation and real-time RT-PCR
Primers and probes	Primers and probes to detect the presence of nucleic acid sequences of influenza A, influenza B, and RSV	Primers and probes to detect the presence of nucleic acid sequences of influenza A, influenza B, and influenza, subtype H1N1	Same as Xpert Flu/RSV XC Assay

Similarities			
	Device	**Predicate Devices**	
Item	**Cepheid Xpert Flu/RSV XC**	**Current Cepheid Xpert Flu**	**Hologic Prodesse ProFlu+ Assay**
Laboratory Users	Operators in moderate and high complexity labs	Same as Xpert Flu/RSV XC Assay; categorized as CLIA Moderate Complexity.	CLIA High Complexity

Primary Differences (Differences are also Captured in the Similarities Table above)			
	New Device	**Predicate Devices**	
Item	**Cepheid Xpert Flu/RSV XC**	**Current Cepheid Xpert Flu**	**Prodesse® ProFlu™+ Assay**
Intended Use Differences	Detects and differentiates between influenza A, influenza B, and RSV viral RNA.	Detects and differentiates between influenza A, influenza B, and 2009 H1N1 influenza viral RNA. Does not detect RSV viral RNA.	Same as Xpert Flu/RSV XC Assay
Specimen Types	Nasal aspirate/wash (NA/W) specimens and Nasopharyngeal (NP) swab specimens	Same as Xpert Flu/RSV XC Assay	Nasopharyngeal (NP) swab specimens; not FDA cleared for NA/W specimens, but specimen type was validated for use in the clinical study.
Test Cartridge	Disposable single-use, multi-chambered fluidic cartridge with all reagents contained in the cartridge.	Disposable single-use, multi-chambered fluidic cartridge. One reagent must be added to the cartridge.	Disposable single-use PCR tube. All reagents and controls must be added to the PCR tube.
Instrument System	Cepheid GeneXpert Dx Systems and GeneXpert Infinity Systems	Same as Xpert Flu/RSV XC Assay	Cepheid SmartCycler

Primary Differences (Differences are also Captured in the Similarities Table above)			
	New Device	**Predicate Devices**	
Item	**Cepheid Xpert Flu/RSV XC**	**Current Cepheid Xpert Flu**	**Prodesse® ProFlu™+ Assay**
Sample Preparation	Self-contained and automated after mixed specimen is added to cartridge. All other reagents are contained in the cartridge.	Self-contained and automated after mixed specimen and one single-dose reagent are added to cartridge.	Manual
Primers and probes for influenza A, influenza B, and influenza A subtype H1N1	Primers and probes to detect the presence of nucleic acid sequences of influenza A, influenza B, and RSV A/B. The Xpert Flu/RSV Assay contains additional RNA segments in order to protect the assay sensitivity from mutations in the influenza genome due to antigenic drifts and shifts as compared to the Xpert Flu Assay.	Primers and probes to detect the presence of nucleic acid sequences of influenza A, influenza B, and influenza, subtype H1N1. The current Xpert Flu Assay does not detect RSV	Primers and probes to detect the presence of nucleic acid sequences of influenza A, influenza B, and RSV A/B.
Target Sequence	Influenza A: Matrix protein (MP), Polymerase (PB 2 and PA), and Hemagglutinin (HA for H7N9) Influenza B: Matrix protein (MP) and Non-structural proteins (NS 1 and NS 2) RSV A and RSV B: Nucleocapsid protein	Influenza A: Matrix protein and Hemagglutinin (HA for H1N1) Influenza B: Hemagglutinin (HA) None	Influenza A: Matrix protein (MP) Influenza B: Non-structural protein (NS 1 and NS 2) RSV A and RSV B: Polymerase
Internal Controls	Sample processing control (SPC) and probe check control (PCC).	Same as Xpert Flu/RSV XC Assay	Internal RNA control

Primary Differences (Differences are also Captured in the Similarities Table above)			
	New Device	Predicate Devices	
Item	**Cepheid Xpert Flu/RSV XC**	**Current Cepheid Xpert Flu**	**Prodesse® ProFlu™+ Assay**
Laboratory Users	Operators with no clinical lab experience to experienced clinical laboratory technologists.	Operators with no clinical lab experience to experienced clinical laboratory technologists; categorized as CLIA Moderate Complexity.	CLIA High Complexity
Time to obtain test results	Approximately 60 minutes or less for sample preparation and RT-PCR	75 minutes for sample preparation and RT-PCR	Approximately 4 hours for sample preparation and RT-PCR
Combinatorial Assay Selections	Yes, user may select combined assay with all targets or a Flu only assay or a RSV only assay.	No	No
Early assay termination function	On Flu only or RSV only assay selections	No	No

The Xpert Flu/RSV XC Assay has the same intended use as the primary predicate device and the same technological characteristics as both predicate devices. The clinical study demonstrates that the Xpert Flu/RSV XC Assay is acceptable for its intended use and is substantially equivalent to the predicate devices.

The predicate device for the ancillary specimen collection kit, the Xpert® Nasopharyngeal Sample Collection Kit, is the Copan Universal Transport Medium (UTM-RT) System, [510(k) # K042970]. The similarities and differences are shown in Table 5-2.

Table 5-2: Comparison of Similarities and Differences of the Xpert Nasopharyngeal Sample Collection Kit with the Predicate Device

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

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

Both devices have the same general intended use and use the same technology to collect, store and transport clinical specimens, including viruses, to the laboratory for further testing. The prospective component of the multi-center clinical study of the Xpert Flu/RSV XC was conducted using Copan-manufactured UTM-RT and sterile nylon flocked swab demonstrating that the Xpert Nasopharyngeal Sample Collection Kit is acceptable for its intended use and substantially equivalent to the predicate device.

Non-Clinical Studies:

Analytical Sensitivity (Limit of Detection)

Studies were performed to determine the analytical limit of detection (LoD) of the Xpert Flu/RSV XC Assay with two lots of reagents across three testing days. The higher LoD observed per strain and per lot was selected for verification. Verification of the estimated LoD claim was performed on one reagent lot across a minimum of three testing days. LoD was established using two influenza A H3N2 strains, two influenza A 2009 H1N1 strains, two influenza B strains, two respiratory syncytial virus A (RSV A) strains, two respiratory syncytial virus B (RSV B) strains, and one influenza A H7N9 strain diluted into a negative pooled clinical matrix. The LoD is

defined as the lowest concentration (tissue culture infective dose, $TCID_{50}$/mL) per sample that can be reproducibly distinguished from negative samples with 95% confidence or the lowest concentration at which 19 of 20 replicates were positive. Each strain was tested in replicates of 20 per concentration of virus.

The LoD was determined empirically as the first concentration that had 19/20 or 20/20 positive results. The LoD point values for each strain tested are summarized in 5-3 to Table 5-8.

Table 5-3: Confirmed LoD ($TCID_{50}$/mL): Influenza A 2009 H1N1

Strain ID	Confirmed LOD ($TCID_{50}$/mL) (at least 19/20 positive)
Influenza A/California/7/2009	0.3 (20/20)
Influenza A/Florida/27/2011	16 (19/20)

Table 5-4: Confirmed LoD ($TCID_{50}$/mL): Influenza A H3N2

Strain ID	Confirmed LOD ($TCID_{50}$/mL) (at least 19/20 positive)
Influenza A/Perth/16/2009	0.3 (20/20)
Influenza A/Victoria/361/2011	0.8 (20/20)

Table 5-5: Confirmed LoD ($TCID_{50}$/mL): Influenza B

Strain ID	Confirmed LOD ($TCID_{50}$/mL) (at least 19/20 positive)
Influenza B/Massachusetts/2/2012	0.5(20/20)
Influenza B/Wisconsin/01/2011	0.6 (20/20)

Table 5-6: Confirmed LoD ($TCID_{50}$/mL): Respiratory Syncytial Virus A

Strain ID	Confirmed LOD ($TCID_{50}$/mL) (at least 19/20 positive)
RSV A/2/Australia/61	1.2 (20/20)
RSV A/Long/MD/56	1.0 (19/20)

Table 5-7: Confirmed LoD ($TCID_{50}$/mL): Respiratory Syncytial Virus B

Strain ID	Confirmed LOD ($TCID_{50}$/mL) (at least 19/20 positive)
RSV B/Washington/18537/62	1.8 (20/20)
RSV B/9320/Massachusetts/77	2.0 (19/20)

Table 5-8: Confirmed LoD ($TCID_{50}$/mL): Influenza A H7N9

Strain ID	Confirmed LOD ($TCID_{50}$/mL) (at least 19/20 positive)
Influenza A/Anhui/1/2013	21.0 (19/20)

Analytical Specificity (Exclusivity)

The analytical specificity of the Xpert Flu/RSV XC Assay was evaluated by testing a panel of 44 cultures consisting of 16 viral, 26 bacterial, and two yeast strains representing common respiratory pathogens or those potentially encountered in the nasopharynx. Three replicates of all bacterial and yeast strains were tested at concentrations of $\geq 10^6$ CFU/mL with the exception of one strain which was tested at 10^5 CFU/mL (*Chlamydia pneumoniae*). Three replicates of all viruses were tested at concentrations of $\geq 10^5$ $TCID_{50}$/mL. The analytical specificity was 100%. Results are shown in Table 5-9.

Table 5-9: Analytical Specificity of Xpert Flu/RSV XC Assay

Organism	Concentration	Influenza A	Influenza B	RSV
No Template Control		NEG	NEG	NEG
Adenovirus Type 1	$1.12x10^7$ $TCID_{50}$/mL	NEG	NEG	NEG
Adenovirus Type 7	$1.87x10^5$ $TCID_{50}$/mL	NEG	NEG	NEG
Human coronavirus OC43	$2.85x10^5$ $TCID_{50}$/mL	NEG	NEG	NEG
Human coronavirus 229E	$1x10^5$ $TCID_{50}$/mL	NEG	NEG	NEG
Cytomegalovirus	$7.24x10^5$ $TCID_{50}$/mL	NEG	NEG	NEG
Echovirus	$3.31x10^7$ TCID50/mL	NEG	NEG	NEG
Enterovirus	$1x10^5$ $TCID_{50}$/mL	NEG	NEG	NEG
Epstein Barr Virus	$7.16x10^7$ $TCID_{50}$/mL	NEG	NEG	NEG
HSV	$8.9x10^6$ $TCID_{50}$/mL	NEG	NEG	NEG
Measles	$6.3x10^5$ $TCID_{50}$/mL	NEG	NEG	NEG
Human metapneumovirus	$3.8x10^5$ $TCID_{50}$/mL	NEG	NEG	NEG
Mumps virus	$6.31x10^6$ $TCID_{50}$/mL	NEG	NEG	NEG
Human parainfluenza Type 1	$1.15x10^6$ $TCID_{50}$/mL	NEG	NEG	NEG
Human parainfluenza Type 2	$1x10^5$ $TCID_{50}$/mL	NEG	NEG	NEG
Human parainfluenza Type 3	$3.55x10^7$ $TCID_{50}$/mL	NEG	NEG	NEG
Rhinovirus Type 1A	$1.26x10^5$ $TCID_{50}$/mL	NEG	NEG	NEG
Acinetobacter baumannii	$>1x10^6$ CFU/mL	NEG[a]	NEG	NEG
Burkholderia cepacia	$>1x10^6$ CFU/mL	NEG	NEG	NEG
Candida albicans	$>1x10^6$ CFU/mL	NEG	NEG	NEG
Candida parapsilosis	$>1x10^6$ CFU/mL	NEG	NEG	NEG
Bordetella pertussis	$1x10^8$ CFU/mL	NEG	NEG	NEG

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

Analytical Reactivity (Inclusivity)

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

Three replicates were tested for each strain. Results are shown in Table 5-10.

Table 5-10: Analytical Reactivity (Inclusivity) of Xpert Flu/RSV XC Assay

Virus	Strain	Concentration	Result		
			Flu A	Flu B	RSV
No Template Control			NEG	NEG	NEG
Influenza A H1N1 (pre-2009)	A/swine/Iowa/15/30	32.0 $TCID_{50}$/mL	POS	NEG	NEG
	A/WS/33	32.0 $TCID_{50}$/mL	POS	NEG	NEG
	A/PR/8/34	32.0 $TCID_{50}$/mL	POS	NEG	NEG
	A/Mal/302/54	32.0 $TCID_{50}$/mL	POS	NEG	NEG
	A/Denver/1/57	32.0 $TCID_{50}$/mL	POS	NEG	NEG
	A/New Jersey/8/76	32.0 $TCID_{50}$/mL	POS	NEG	NEG
	A/New Caledonia/20/1999	32.0 $TCID_{50}$/mL	POS	NEG	NEG
	A/New York/55/2004	32.0 $TCID_{50}$/mL	POS	NEG	NEG
	A/Soloman Island/3/2006	32.0 $TCID_{50}$/mL	POS	NEG	NEG
	A/Taiwan/42/06	32.0 $TCID_{50}$/mL	POS	NEG	NEG
	A/Brisbane/59/2007	32.0 $TCID_{50}$/mL	POS	NEG	NEG
Influenza A H1N1 (pdm2009)	A/California/7/2009	32.0 $TCID_{50}$/mL	POS	NEG	NEG
	A/swine/NY/02/2009	32.0 $TCID_{50}$/mL	POS	NEG	NEG
	A/Florida/27/2011	32.0 $TCID_{50}$/mL	POS	NEG	NEG
	A/Colorado/14/2012	32.0 $TCID_{50}$/mL	POS	NEG	NEG
	A/Washington/24/2012	80.0[a] $TCID_{50}$/mL	POS	NEG	NEG
Influenza A H3N2 (Seasonal)	A/Aichi/2/68	1.6 $TCID_{50}$/mL	POS	NEG	NEG
	A/HongKong/8/68	1.6 $TCID_{50}$/mL	POS	NEG	NEG
	A/Port Chalmers/1/73	1.6 $TCID_{50}$/mL	POS	NEG	NEG
	A/Hawaii/15/2001	1.6 $TCID_{50}$/mL	POS	NEG	NEG
	A/Wisconsin/67/05	1.6 $TCID_{50}$/mL	POS	NEG	NEG
	A/Brisbane/10/2007	1.6 $TCID_{50}$/mL	POS	NEG	NEG
	A/Perth/16/2009	1.6 $TCID_{50}$/mL	POS	NEG	NEG

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

Virus	Strain	Concentration	Result		
			Flu A	Flu B	RSV
	B/Wisconsin/01/2010[f]	1.2 $TCID_{50}$/mL	NEG	POS	NEG
	B/Malaysia/2506/04[d]	1.2 $TCID_{50}$/mL	NEG	POS	NEG
	B/Taiwan/2/62	1.2 $TCID_{50}$/mL	NEG	POS	NEG
	B/Brisbane/60/2008[d]	1.2 $TCID_{50}$/mL	NEG	POS	NEG
RSV A	RSV-A/Long/MD/56	2.4 $TCID_{50}$/mL	NEG	NEG	POS
	RSV-A/2/Australia/61	2.4 $TCID_{50}$/mL	NEG	NEG	POS
	RSV-A/NY (Clinical unknown)	2.4 $TCID_{50}$/mL	NEG	NEG	POS
	RSV-A/WI/629-8-2/2007	2.4 $TCID_{50}$/mL	NEG	NEG	POS
	RSV-A/WI/629-11-1/2008	2.4 $TCID_{50}$/mL	NEG	NEG	POS
RSV B	RSV-B/Wash/18537/62	4.0 $TCID_{50}$/mL	NEG	NEG	POS
	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 TCID50/mL) to obtain 3/3 RSV POSITIVE result calls.

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

Potentially Interfering Substances

In a non-clinical study, potentially interfering substances that may be present in the nasopharynx were evaluated directly relative to the performance of the Xpert Flu/RSV XC Assay. Potentially interfering substances in the nasopharynx may include, but are not limited to: blood, nasal secretions or mucus, and nasal and throat medications used to relieve congestion, nasal dryness, irritation, or asthma and allergy symptoms, as well as antibiotics and antivirals. Negative samples (n = 8) were tested per each substance

to determine the effect on the performance of the sample processing control (SPC). Positive samples (n = 8) were tested per substance with six influenza (four influenza A and two influenza B) and four RSV (two RSV A and two RSV B) strains spiked at 2X the analytical LoD determined for each strain. All results were compared to positive and negative Universal Transport Medium (UTM) controls.

These evaluated substances are listed in Table 5-11 with active ingredients and concentrations tested shown. There was no assay interference in the presence of the substances at the concentrations tested in this study. All positive and negative replicates were correctly identified using the Xpert Flu/RSV XC Assay.

FluMist vaccine samples were correctly reported as **Flu A POSITIVE**; **FLU B POSITIVE**; **RSV NEGATIVE** as expected. Samples containing FluMist may cause false positive results. This is addressed in Section 17, Limitations.

Table 5-11. Potentially Interfering Substances in Xpert Flu/RSV XC Assay

Substance/Class	Description/Active Ingredient	Concentration Tested
Beta-adrenergic bronchodilator	Albuterol Sulfate	0.83 mg/mL (equivalent to 1 dose per day)
Blood	Blood (Human)	2% (v/v)
BD™ Universal Viral Transport System	Transport Media	100% (v/v)
Remel M4®	Transport Media	100% (v/v)
Remel M4RT®	Transport Media	100% (v/v)
Remel M5®	Transport Media	100% (v/v)
Throat lozenges, oral anesthetic and analgesic	Benzocaine, Menthol	1.7 mg/mL
Mucin	Purified Mucin protein (Bovine or porcine submaxillary gland)	2.5% (w/v)
Antibiotic, nasal ointment	Mupirocin	10 mg/mL
Saline Nasal Spray	Sodium Chloride (0.65%)	15% (v/v)
Anefrin Nasal Spray	Oxymetazoline, 0.05%	15% (v/v)
Nasal Drops	Phenylephrine, 0.5%	15% (v/v)
Tamiflu®/Anti-viral drugs	Zanamivir	7.5 mg/mL
Antibacterial, systemic	Tobramycin	4 µg/mL

Substance/Class	Description/Active Ingredient	Concentration Tested
Zicam®/Nasal Gel	Luffa opperculata, Galphimia glauca, Histaminum hydrochloricum Sulfur	15% (w/v)
FluMist®	Live intranasal influenza virus vaccine	6.7% (v/v)
Nasal corticosteroid	Fluticasone Propionate	5 µg/mL

Carry-Over Contamination

A study was conducted to demonstrate that single-use, self-contained GeneXpert cartridges prevent carry-over contamination in negative samples run followed by very high positive samples in the same GeneXpert module. The study consisted of a negative sample processed in the same GeneXpert module immediately followed by a very high influenza A sample (approximately 10^6 $TCID_{50}$/test) or a very high RSV A sample (approximately 10^6 $TCID_{50}$/test). This testing scheme was repeated 20 times on two GeneXpert modules for a total of 82 runs resulting in 40 positive and 42 negative specimens for each virus type. All 40 positive samples were correctly reported as **Flu A POSITIVE; Flu B NEGATIVE; RSV NEGATIVE** or **Flu A NEGATIVE; Flu B NEGATIVE; RSV POSITIVE**. All 42 negative samples were correctly reported as **Flu A NEGATIVE; Flu B NEGATIVE; RSV NEGATIVE**.

Fresh vs. Frozen Sample Equivalency Study

Fresh and frozen specimen equivalency in the Xpert Flu/RSV XC Assay was evaluated by testing individual influenza and RSV strains at three different concentrations representing low positives (2X LoD), moderate positives (5X LoD), and high positives (10X LoD) in simulated background matrix. Negative samples consisted of simulated background matrix only. Fresh and frozen specimen equivalency was determined using one seasonal Flu A H3N2 strain (A/Victoria/361/2011), one Flu B strain (B/Wisconsin/01/11), one RSV A strain (RSV A/Long/MD/56), and one RSV B strain (RSV B/9320/MA/77). Replicates of 20 were tested for each specimen type and concentration. All positive and negative specimens were tested fresh, after one freeze-thaw cycle, and after two freeze-thaw cycles.

There was no statistically significant effect in the performance of the Xpert Flu/RSV XC Assay between fresh virus dilutions and two sequential freeze thaw cycles for positive and negative samples. All positive and negative replicates were correctly identified using the Xpert Flu/RSV XC Assay.

Linearity

Not applicable, the Xpert Flu/RSV XC Assay is a qualitative assay.

Clinical Studies

Clinical Comparison Study

Performance characteristics of the Xpert Flu/RSV XC Assay were evaluated at six institutions in the U.S during the 2013-2014 influenza season. Due to the low prevalence of influenza viruses and the difficulty in obtaining fresh influenza and RSV-positive specimens, the specimen population for this study was supplemented with frozen prospective, and frozen pre-selected archived specimens.

Subjects included individuals with signs and symptoms of respiratory infection and whose routine care called for collection of nasal aspirate/wash (NA/W) specimens or nasopharyngeal (NP) swab specimens for influenza and/or RSV testing. For eligible subjects, aliquots of leftover specimens were obtained for testing with the Xpert Flu/RSV XC Assay and reference testing, and patient management continued at the site per their standard practice.

The Xpert Flu/RSV XC Assay performance was compared to a FDA-cleared comparator assay. Bi-directional sequencing was performed on specimens where the Xpert Flu/RSV XC Assay and the comparator assay were discrepant, and is provided for informational purposes only.

Overall Results

NA/W Specimens

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

On fresh, prospectively collected NA/W specimens, the Xpert Flu/RSV XC Assay demonstrated a PPA and NPA for detection of influenza A of 100% and 100%, respectively, relative to the comparator assay (Table 5-12). The Xpert Flu/RSV XC Assay PPA and NPA for influenza B were 99.2% and 100%, respectively (Table 5-12). The Xpert Flu/RSV XC Assay PPA and NPA for RSV were 98.5% and 99.6%, respectively (Table 5-12).

On frozen, archived NA/W specimens, the Xpert Flu/RSV XC Assay demonstrated a PPA and NPA for detection of influenza A of 97.1% and 100%, respectively, relative to the comparator assay (Table 5-12). The Xpert Flu/RSV XC Assay PPA and NPA for influenza B were 100% and 100%, respectively (Table 5-12). The Xpert Flu/RSV XC Assay PPA and NPA for RSV were 84.6% and 100%, respectively (Table 5-12).

Table 5-12: Xpert Flu/RSV XC Assay Performance on NA/W Specimens

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

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

NP Swab Specimens

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

On fresh, prospectively collected NP swab specimens, the Xpert Flu/RSV XC Assay demonstrated a PPA and NPA for detection of influenza A of 85.7% and 98.9%, respectively, relative to the comparator assay (Table 5-13). The Xpert Flu/RSV XC Assay PPA and NPA for influenza B were 100% and 100%, respectively (Table 5-13). The Xpert Flu/RSV XC Assay PPA and NPA for RSV were 100% and 100%, respectively (Table 5-13).

On frozen, archived NP swab specimens, the Xpert Flu/RSV XC Assay demonstrated a PPA and NPA for detection of influenza A of 99.0% and 92.8%, respectively, relative to the comparator assay (Table 5-13). The Xpert Flu/RSV XC Assay PPA and NPA for influenza B were 98.8% and 100%, respectively (Table 5-13). The Xpert Flu/RSV XC Assay PPA and NPA for RSV were 90.4% and 99.1%, respectively (Table 5-13).

Table 5-13: Xpert Flu/RSV XC Assay Performance on NP Swab Specimens

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

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

Of the Xpert Flu/RSV XC Assay runs performed with eligible specimens, 98.6% (1236/1254) of these specimens were successful on the first attempt. The initial indeterminate rate was 1.4% (95% CI 0.9-2.3%). The remaining 18 gave indeterminate results on the first attempt (11 ERROR, 3 INVALID and 4 NO RESULT). Seventeen of the 18 specimens were retested, of which 14 yielded valid results after a single retest. There were four NA/W specimens with indeterminate results upon retest which were excluded in the analyses.

Reproducibility Study

A panel of 10 specimens with varying concentrations of influenza A, influenza B, and RSV was tested on ten different days by two different operators, at each of three sites (10 specimens x 1 time/day x 10 days x 2 operators x 3 sites). One lot of Xpert Flu/RSV XC Assay cartridges was used at each of the 3 testing sites. The Xpert Flu/RSV XC Assay was performed according to the Xpert Flu/RSV XC Assay procedure. Results are summarized in Table 5-14.

Table 5-14: Summary of Reproducibility Results

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

[a]Agreement calculated based on expected result: Negative for Negative (targeted positivity: 0%) and High Neg (targeted positivity: 20-80%) samples; Positive for Low Pos (targeted positivity: 95%) and Mod Pos (targeted positivity: 100%) samples.
[b]One sample indeterminate on initial testing; retest not done.
[c]One sample 2x indeterminate.

The reproducibility of the Xpert Flu/RSV XC Assay was also evaluated in terms of the fluorescence signal expressed in Ct values for each target detected. The mean, standard deviation (SD), and coefficient of variation (CV) between-sites, between-days, and between-operators for each panel member are presented in Table 5-15. One replicate was performed per day per operator, therefore, operator and assay (within-run) precision are confounded.

Table 5-15: Summary of Reproducibility Data

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

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

Instrument System Precision

An in-house precision study was conducted to compare the performance of the GeneXpert Dx and the GeneXpert Infinity instrument systems. A panel of 10 specimens with varying concentrations of influenza A, influenza B, and RSV was tested on 12 different days by two operators. Each operator conducted four runs of each panel specimen per day on each of the two instrument systems (10 specimens x 2 times/ day x 12 days x 2 operators x 2 instrument systems). Three lots of Xpert Flu/RSV XC Assay cartridges were used for the study. The Xpert Flu/RSV XC Assay was performed according to the Xpert Flu/RSV XC Assay procedure. Results are summarized in Table 5-16.

Table 5-16: Summary of Instrument System Precision Results (Dx vs. Infinity)

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

[a]Agreement calculated based on expected result: Negative for Negative (targeted positivity: 0%) and High Neg (targeted positivity: 20-80%) samples; Positive for Low Pos (targeted positivity: 95%) and Mod Pos (targeted positivity: 100%) samples.

The precision of the Xpert Flu/RSV XC Assay was also evaluated in terms of the fluorescence signal expressed in Ct values for each target detected. The mean, standard deviation (SD), and coefficient of variation (CV) between-instruments, between-lots, between-days, between-operators, and within-assays for each panel member are presented in Table 5-17.

Table 5-17. Summary of Precision Data

Sample	Assay Channel (Analyte)	N[a]	Mean Ct	Between-Instrument		Between-Lot		Between-Day		Between-Operator		Within-Assay		Total	
				SD	CV (%)	SD	CV (%)	SD	CV (%)	SD	CV (%)	SD	CV (%)	SD	CV (%)
Negative	SPC	192	30.6	0	0	0.19	0.6	0.06	0.2	0.02	0.1	0.36	1.2	0.41	1.3
Flu A-High Neg	FluA1	41	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
	FluA2	0	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
Flu A-Low Pos	FluA1	169	35.6	0	0	0.42	1.2	0.93	2.6	0.28	0.8	1.61	4.5	1.93	5.4
	FluA2	1	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
Flu A-Mod Pos	FluA1	187	34.1	0	0	0.41	1.2	0.95	2.8	0	0	1.54	4.5	1.86	5.5
	FluA2	14	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
Flu B-High Neg	FluB	34	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
Flu B-Low Pos	FluB	174	33.2	0	0	0.47	1.4	0	0	0.66	2.0	2.03	6.1	2.18	6.6
Flu B-Mod Pos	FluB	191	32.1	0	0	0.17	0.5	0.25	0.8	0	0	1.73	5.4	1.75	5.5
RSV-High Neg	RSV	30	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
RSV-Low Pos	RSV	175	36.0	0	0	0.75	2.1	0	0	0.36	1.0	1.47	4.1	1.69	4.7
RSV-Mod Pos	RSV	191	34.7	0	0	0.57	1.7	0.16	0.5	0	0	1.23	3.6	1.37	3.9

[a]Results with non-zero Ct values out of 192.

Conclusions

The results of the nonclinical analytical and clinical performance studies summarized above demonstrate that the Xpert Flu/RSV XC Assay is substantially equivalent to the predicate devices.