



**510(k) SUBSTANTIAL EQUIVALENCE DETERMINATION
DECISION SUMMARY
ASSAY AND INSTRUMENT**

I Background Information:

A 510(k) Number

K242071

B Applicant

Cepheid

C Proprietary and Established Names

Xpert Xpress CoV-2/Flu/RSV plus

D Regulatory Information

Product Code(s)	Classification	Regulation Section	Panel
QOF	Class II	21 CFR 866.3981 - Device To Detect And Identify Nucleic Acid Targets In Respiratory Specimens From Microbial Agents That Cause The SARS-Cov-2 Respiratory Infection And Other Microbial Agents When In A Multi-Target Test	MI - Microbiology

II Submission/Device Overview:

A Purpose for Submission:

To obtain 510(k) clearance for the Xpert Xpress Cov-2/Flu/RSV *plus* test for use on the GeneXpert Xpress System.

B Measurand:

Conserved RNA sequences within the genes encoding the nucleocapsid protein (N), envelope protein (E) and RNA-dependent RNA polymerase protein (RdRP) of SARS-CoV-2 viruses; the matrix protein (M), basic polymerase protein 2 (PB2), and polymerase acidic protein (PA) of

influenza A viruses; the matrix protein (M) and non-structural protein (NS) of influenza B viruses; and the nucleocapsid protein of respiratory syncytial virus (RSV) A and B viruses.

C Type of Test:

A multiplexed, real-time reverse transcriptase polymerase chain reaction (RT-PCR) assay for the qualitative detection and differentiation of SARS-CoV-2, influenza A, influenza B, and RSV viral RNA from nasopharyngeal swab (NPS) specimens and anterior nasal swab (ANS) specimens using the GeneXpert Instrument Systems platform.

III Intended Use/Indications for Use:

A Intended Use(s):

See Indications for Use below.

B Indication(s) for Use:

The Xpert Xpress CoV-2/Flu/RSV plus test, performed on the GeneXpert Xpress System, is an automated multiplexed real-time reverse transcriptase polymerase chain reaction (RT-PCR) test intended for use in the simultaneous *in vitro* qualitative detection and differentiation of severe acute respiratory syndrome coronavirus (SARS-CoV-2), influenza A, influenza B, and/or respiratory syncytial virus (RSV) viral RNA in nasopharyngeal swab and anterior nasal swab specimens collected from individuals with signs and symptoms of respiratory tract infection. Clinical signs and symptoms of respiratory tract infection due to SARS-CoV-2, influenza A, influenza B, and RSV can be similar.

The Xpert Xpress CoV-2/Flu/RSV plus is intended for use in the differential detection of SARS-CoV-2, influenza A, influenza B, and/or RSV RNA and aids in the diagnosis of COVID-19, influenza, and/or RSV infections if used in conjunction with other clinical and epidemiological information, and laboratory findings. SARS-CoV-2, influenza A, influenza B, and RSV viral RNA are generally detectable in nasopharyngeal swab and anterior nasal swab specimens during the acute phase of infection.

Positive results are indicative of the presence of the identified virus, but do not rule out bacterial infection or co-infection with other pathogens not detected by the test. The agent(s) detected by the Xpert Xpress CoV-2/Flu/RSV plus test may not be the definite cause of the disease.

Negative results do not preclude SARS-CoV-2, influenza A virus, influenza B virus, and/or RSV infection. The results of this test should not be used as the sole basis for treatment or other patient management decisions.

C Special Conditions for Use Statement(s):

Rx - For Prescription Use Only
For *in vitro* diagnostic use only

D Special Instrument Requirements:

GeneXpert Xpress System

IV Device/System Characteristics:

A Device Description:

The Xpert Xpress CoV-2/Flu/RSV *plus* test, performed on the GeneXpert Xpress System, is an automated *in vitro* diagnostic test for the simultaneous qualitative detection and differentiation of SARS-CoV-2, influenza A, influenza B, and RSV viral RNA in nasopharyngeal swab (NPS) and anterior nasal swab (ANS) specimens collected from individuals showing signs and symptoms of respiratory viral infection. The test is performed with three steps: 1) transfer liquid sample to the test cartridge with the transfer pipette (provided in the test kit), 2) run the test on the GeneXpert Xpress System, and 3) read the results.

The Hub configuration of the GeneXpert Xpress System includes a GeneXpert IV Instrument, an integrated barcode scanner, and a computer with a touchscreen and preloaded GeneXpert Xpress software designed for running tests and viewing results. The system requires the use of single-use disposable cartridges that hold the RT-PCR reagents and host sample purification, nucleic acid amplification, and detection of the target sequences.

The Xpert Xpress CoV-2/Flu/RSV *plus* test cartridge includes all the necessary reagents for the detection of SARS-CoV-2, influenza A, influenza B and RSV viral RNA from NPS and ANS specimens collected with nylon flocked swabs and placed into either 3 mL viral transport medium (VTM)/ universal transport medium (UTM) or 2 mL eNAT. With a supplied transfer pipette, eluted NPS and ANS swab specimens are loaded into the sample chamber of a single-use, self-contained Xpert Xpress CoV-2/Flu/RSV *plus* test cartridge. Each Xpert Xpress CoV-2/Flu/RSV *plus* test cartridge contains separate chambers for sample loading, sample processing, and target amplification by real-time RT-PCR, and includes all the reagents necessary to carry out these processes.

The primers and probes in the Xpert Xpress CoV-2/Flu/RSV *plus* test are designed to amplify and detect gene sequences for the following:

- SARS-CoV-2 (envelope small membrane protein [E], a sequence in the N nucleocapsid protein [N2] and RNA-dependent RNA polymerase [RdRP])
- influenza A (matrix protein gene [MP], basic polymerase protein [PB2] and acidic protein [PA] genes from the viral polymerase complex)
- influenza B (Matrix protein gene and non-structural protein [NS] gene)
- RSV A and B (Nucleocapsid gene segment) viruses

A Sample Processing Control (SPC) and a Probe Check Control (PCC) are also included in the cartridge utilized by the GeneXpert instrument. The SPC is present to control for adequate processing of the sample and to monitor for the presence of potential inhibitor(s) in the RT-PCR reaction. The SPC also ensures that the RT-PCR reaction conditions (temperature and time) are appropriate for the amplification reaction and that the RT-PCR reagents are functioning as expected. The PCC verifies reagent rehydration, PCR tube filling, and confirms that all reaction components are present in the cartridge including monitoring for probe integrity and dye stability. Because the cartridges are self-contained, and specimens never contact working parts of the instrument modules, cross-contamination between samples is minimized. The test

cartridge containing the patient sample is loaded onto one of four randomly accessible GeneXpert instrument modules, which performs fully automated and integrated sample preparation and real-time RT-PCR for the Xpert Xpress CoV-2/Flu/RSV *plus* test in approximately 36 minutes.

The Xpert Xpress CoV-2/Flu/RSV *plus* test can be run in one of five different modes, varying in the number of target analyte(s) tested for and reported. The five modes are: SARS-CoV-2 only; SARS-CoV-2 and Flu; SARS-CoV-2/Flu/RSV; Flu only; or Flu and RSV. For the SARS-CoV-2 only and Flu only modes, there is an option to activate an Early Assay Termination (EAT) function, which will provide earlier time to results in high titer specimens if the signal from the target nucleic acid reaches a predetermined threshold before the assay has finished running. When EAT is activated, the earliest time to a positive result is approximately 25 minutes. 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.

B Principle of Operation:

The Xpert Xpress CoV-2/Flu/RSV *plus* test, performed on the GeneXpert Xpress System, is a nucleic acid-based test using real-time RT-PCR. After addition of the specimen to the Sample Chamber of the Xpert Xpress CoV-2/Flu/RSV *plus* test cartridge, the cartridge is loaded onto the GeneXpert Xpress System platform. The instrument then performs automated sample processing including RNA extraction, followed by reverse transcription, amplification, detection, and reporting of results. The results are interpreted automatically by the GeneXpert Xpress System and are shown in the View Results window.

C Instrument Description Information:

1. Instrument Name:

GeneXpert Xpress System

2. Specimen Identification:

Sample ID is an identifier that links the sample being processed to the patient that provided the specimen. The Sample ID can be entered into the GeneXpert Xpress System either by scanning a barcode, entering the Sample ID manually using the virtual keyboard or having the system assign a random Sample ID.

To perform a test, the user selects “NEW TEST” icon from the Home Screen. Either patient information must be entered if configured by an administrator or the Sample ID screen appears. If the Patient Information screen appears, manually enter or scan the Patient ID barcode. If the Sample ID screen appears, scan the Sample ID barcode or manually enter the Sample ID for the patient specimen. The user is then instructed to scan the cartridge barcode and select the test to run. The prepared test cartridge is loaded into an available instrument module with the flashing green light to initiate the test.

3. Specimen Sampling and Handling:

The Xpert Xpress CoV-2/Flu/RSV *plus* test cartridge includes reagents for the detection of SARS-CoV-2, influenza A, influenza B and RSV viral RNA in either nasopharyngeal swab (NPS) or anterior nasal swab (ANS) specimens. The NPS or NS specimen is collected using nylon flocked swabs and eluted into a transport tube containing 3 mL of viral transport medium (VTM)/Universal Transport Medium (UTM) or 2 mL of eNAT. At the test facility, the operator mixes the specimen by rapidly inverting the collection tube 5 times. A disposable transfer pipette supplied with the Xpert Xpress CoV-2/Flu/RSV *plus* is used to transfer an aliquot of the specimen into the Sample Chamber of the Xpert Xpress CoV-2/Flu/RSV *plus* test cartridge. After closing the cartridge lid, the GeneXpert cartridge is loaded onto the GeneXpert Xpress instrument, which automates and integrates sample preparation, nucleic acid extraction and amplification, and detection of target sequences in simple or complex samples using real-time PCR and RT-PCR assays.

4. Calibration:

Calibration of the GeneXpert Xpress instrument is performed at the factory, and recalibration in the field at customer sites is not required during the initial system startup. A GeneXpert operator or Field Service Engineer with Administrator user permissions can perform calibration checks during annual maintenance.

5. Quality Control:

Internal Controls

The GeneXpert Xpress System relies on the built-in internal controls included in each Xpert Xpress CoV-2/Flu/RSV *plus* test cartridge to ensure the test system is performing as expected. These internal controls consist of Sample Processing Control (SPC) and Probe Check Control (PCC).

The Sample Processing Control (SPC) is a non-infectious armored RNA pseudovirus that ensures adequate processing of the sample and monitors the presence of potential inhibitors in the RT-PCR reaction. The SPC should be POSITIVE in a sample that is negative for all four target analytes (SARS-CoV-2, influenza A, influenza B and RSV), and can be NEGATIVE or POSITIVE in a sample containing detectable levels of one or more of the target analytes.

The 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 fails, the result is reported as NO RESULT – REPEAT TEST and the test must be repeated using a new test cartridge.

External Controls

External controls (ECs) are not required for the end users to obtain a valid Xpert Xpress CoV-2/Flu/RSV *plus* test result. External controls are not provided with the Xpert Xpress CoV-2/Flu/RSV *plus* test. Optional external quality control materials are available from Zephetrix. Specifically, the following external controls have been validated for use with the Xpert Xpress CoV-2/Flu/RSV *plus* test, performed on the GeneXpert Xpress System:

- External Positive Control: NATtrol Flu/RSV/SARS-CoV-2; Cat # NATFRC-6C-IVD
- External Negative Control: Coxsackievirus A9; Cat # NATCV9-6C-IVD

All external controls must be used in accordance with local, state, and federal accrediting organizations, as applicable.

It is recommended that external controls be tested following the manufacturer's instruction at the frequency noted below.

- Each time a new lot of Xpert Xpress CoV-2/Flu/RSV *plus* test kits is received.
- Each time a new shipment of Xpert Xpress CoV-2/Flu/RSV *plus* test kits is received even if it is the same lot previously received.
- Each time a new operator is performing the test (i.e., operator who has not performed the test before).
- When problems (storage, operator, instrument, or other) are suspected or identified.
- If otherwise required by your institution's standard Quality Control (QC) procedures.

V Substantial Equivalence Information:

A Predicate Device Name(s):

Xpert Xpress CoV-2/Flu/RSV *plus*, performed on the GeneXpert Instrument System

B Predicate 510(k) Number(s):

K231481

C Comparison with Predicate(s):

Device & Predicate Device(s):	<u>K242071</u>	<u>K231481</u>
Device Trade Name	Xpert Xpress CoV-2/Flu/RSV <i>plus</i> , Performed on the GeneXpert Xpress System	Xpert Xpress CoV 2/Flu/RSV <i>plus</i> , Performed on the GeneXpert Instrument Systems
Regulation	Same	21 CFR 866.3981 Devices to detect and identify nucleic acid targets in respiratory samples from microbial agents that cause the SARS- CoV-2 respiratory infection and other microbial agents when in a multi-analyte test
Product Code	Same	QOF Multi-target respiratory specimen nucleic acid test including SARS-CoV-2 and other microbial agents
Technology/ Detection	Same	Real-time reverse transcription polymerase chain reaction (RT-qPCR)
General Device Characteristics		
Intended Use/Indications for Use	Xpert Xpress CoV-2/Flu/RSV <i>plus</i> test, performed on the GeneXpert Xpress Systems, is an automated multiplexed real- time reverse	Xpert Xpress CoV-2/Flu/RSV <i>plus</i> test, performed on the GeneXpert Dx and GeneXpert Infinity Systems, is an automated multiplexed real-time

	<p>transcriptase polymerase chain reaction (RT-PCR) test intended for use in the simultaneous <i>in vitro</i> qualitative detection and differentiation of severe acute respiratory syndrome coronavirus (SARS-CoV-2), influenza A, influenza B, and/or respiratory syncytial virus (RSV) viral RNA in nasopharyngeal swab and anterior nasal swab specimens collected from individuals with signs and symptoms of respiratory tract infection. Clinical signs and symptoms of respiratory tract infection due to SARS-CoV-2, influenza A, influenza B, and RSV can be similar.</p> <p>The Xpert Xpress CoV-2/Flu/RSV <i>plus</i> is intended for use in the differential detection of SARS-CoV-2, influenza A, influenza B and/or RSV RNA and aids in the diagnosis of COVID-19, influenza and/or RSV infections if used in conjunction with other clinical and epidemiological information, and laboratory findings. SARS-CoV-2, influenza A, influenza B, and RSV viral RNA are generally detectable in nasopharyngeal swab and anterior nasal swab specimens during the acute phase of infection.</p> <p>Positive results are indicative of the presence of the identified virus, but do not rule out bacterial infection or co-infection with other pathogens not detected by the test. The agent(s) detected by the Xpert Xpress CoV-2/Flu/RSV <i>plus</i> test may not be the definite cause of disease.</p> <p>Negative results do not preclude SARS-CoV-2, influenza A, influenza B and/or RSV infection. The results of this test should not be used as the sole basis for diagnosis, treatment, or other patient management decisions.</p>	<p>reverse transcriptase polymerase chain reaction (RT-PCR) test intended for use in the simultaneous <i>in vitro</i> qualitative detection and differentiation of severe acute respiratory syndrome coronavirus (SARS-CoV-2), influenza A, influenza B, and/or respiratory syncytial virus (RSV) viral RNA in nasopharyngeal swab and anterior nasal swab specimens collected from individuals with signs and symptoms of respiratory tract infection. Clinical signs and symptoms of respiratory tract infection due to SARS-CoV-2, influenza A, influenza B, and RSV can be similar.</p> <p>The Xpert Xpress CoV-2/Flu/RSV <i>plus</i> is intended for use in the differential detection of SARS-CoV-2, influenza A, influenza B and/or RSV RNA and aids in the diagnosis of COVID-19, influenza and/or RSV infections if used in conjunction with other clinical and epidemiological information, and laboratory findings. SARS-CoV-2, influenza A, influenza B, and RSV viral RNA are generally detectable in nasopharyngeal swab and anterior nasal swab specimens during the acute phase of infection.</p> <p>Positive results are indicative of the presence of the identified virus, but do not rule out bacterial infection or co-infection with other pathogens not detected by the test. The agent(s) detected by the Xpert Xpress CoV-2/Flu/RSV <i>plus</i> test may not be the definite cause of disease.</p> <p>Negative results do not preclude SARS-CoV-2, influenza A, influenza B and/or RSV infection. The results of this test should not be used as the sole basis for diagnosis, treatment, or other patient management decisions.</p>
Assay Targets	Same	SARS-CoV-2, Influenza A, Influenza B, RSV viral RNA
Specimen Type	Same	<ul style="list-style-type: none"> Nasopharyngeal swab (NPS) Anterior nasal swab (NS)
Transport Media	Same	<ul style="list-style-type: none"> Universal Transport Medium (UTM) / Viral Transport Medium (VTM) eNAT
Test Format	Same	Single Use
Automation	Same	Automated Nucleic Acid Extraction, Detection and Results Interpretation

Combinatorial Assay Definition Files (ADFs)	Same (+ video to guide user through ordering and running the test)	<ul style="list-style-type: none"> • Xpress SARS-CoV-2 Flu RSV <i>plus</i> • Xpress SARS-CoV-2 Flu <i>plus</i> • Xpress SARS-CoV-2 <i>plus</i> • Xpress Flu <i>plus</i> • Xpress Flu RSV <i>plus</i>
Assay Results	Same	Qualitative
Non-Determinate Results Text	NO RESULTS REPEAT TEST INSTRUMENT ERROR	INVALID ERROR NO RESULT
Internal Control	Same	Sample Processing Control (SPC) Probe Check Control (PCC)
Instrument Systems	Cepheid GeneXpert Xpress System	Cepheid GeneXpert Instrument Systems (Dx and Infinity)
GeneXpert Software	GeneXpert Xpress software	GeneXpert Dx software GeneXpert Infinity software
Time to Result	Same	≤ 36 min for sample preparation and RT-PCR

VI Standards/Guidance Documents Referenced:

Consensus Standards

FDA FR Recognition Number	Document Number	Title	Application of Standards
5-125	ISO 14971:2019-12	Medical Devices – Application of Risk Management to Medical Devices	Declaration of Conformity
7-152	CLSI EP12-A2	User Protocol for Evaluation of Qualitative Test Performance; Approved Guideline – Second Edition.	General Use
7-233	CLSI EP17-A2	Evaluation of Detection Capability for Clinical Laboratory Measurement Procedures; Approved Guideline – Second Edition.	General Use
7-235	CLSI EP25-A	Evaluation of Stability of <i>In Vitro</i> Diagnostic Reagents; Approved Guideline.	General Use
7-251	CLSI EP05-A3	Evaluation of Precision of Quantitative Measurement Procedures; Approved Guideline – Third Edition	General Use
7-260	CLSI MM03-A3	Molecular Diagnostic Methods for Infectious Disease; Approved Guideline – Third Edition.	General Use
7-275	CLSI EP07-A3	Interference Testing in Clinical Chemistry; Approved Guideline – Third Edition.	General Use
7-289	CLSI MM17	Validation and Verification of Multiplex Nucleic Acid Assays – Second Edition	General Use
7-300	CLSI MM13-A2	Collection, Transport, Preparation, and Storage of Specimens for Molecular Methods – Second Edition	General Use

19-42	IEC 61326-1 Edition 3.0 2020-10	Electrical equipment for measurement, control and laboratory use - EMC requirements - Part 1: General requirements	General Use
19-43	IEC 61326-2-6 Edition 3.0 2020-10	Electrical equipment for measurement, control and laboratory use - EMC requirements - Part 2-6: Particular requirements - <i>In vitro</i> diagnostic (IVD) medical equipment	General Use
13-79	IEC 62304 Edition 1.1 2015-06	Medical device software - Software life cycle processes	Declaration of Conformity
19-36	IEC 60601-1-2 Edition 4.1 2020-09	Medical electrical equipment - Part 1-2: General requirements for basic safety and essential performance - Collateral Standard: Electromagnetic disturbances - Requirements and tests	General Use

FDA Special Controls

The Xpert Xpress CoV-2/Flu/RSV *plus* test was developed in accordance with the special controls for 21 CFR 866.3981 as detailed in the reclassification order DEN200031.

Guidance Documents

Document
Guidance for Industry and Staff – Recommendations for Clinical Laboratory Improvement Amendments of 1988 (CLIA) Waiver Applications for Manufacturers of <i>In Vitro</i> Diagnostic Devices, issued on February 26, 2020.
Guidance for Industry and FDA Staff – Recommendations for Dual 510(k) and CLIA Waiver by Application Studies, issued February 26, 2020.
Guidance for Industry and FDA Staff – Appropriate Use of Voluntary Consensus Standards in Premarket Submissions for Medical Devices, issued September 14, 2018
Guidance for Sponsors, Institutional Review Boards, Clinical Investigators and FDA Staff – Guidance on Informed Consent for <i>In Vitro</i> Diagnostic Device Studies Using Leftover Human Specimens that are Not Individually Identifiable, issued April 25, 2006.
Guidance for Industry and FDA Staff – Design Considerations and Premarket Submission Recommendations for Interoperable Medical Devices, issued September 6, 2017.
Guidance for Industry and FDA Staff – Content of Premarket Submissions for Device Software Functions, issued June 14, 2023.
Guidance for Industry and FDA Staff – Electromagnetic Compatibility (EMC) of Medical Devices, issued on June 6, 2022.
Guidance for Industry and FDA Staff – General Principles of Software Validation, issued January 11, 2002.
Guidance for Industry – Cybersecurity for Networked Medical Devices Containing Off-the-Shelf (OTS) Software, issued January 14, 2005.
Guidance for Industry and FDA Staff – Cybersecurity in Medical Devices: Quality System Considerations and Content of Premarket Submissions, issued September 27, 2023.
Guidance for Industry and FDA Staff – Electronic Submission Template for Medical Device 510(k) Submission, issued October 2, 2023.
Guidance for Test Developers and FDA Staff – Policy for Evaluating Impact of Viral Mutations on COVID-19 Tests (Revised), issued January 12, 2023.

VII Performance Characteristics (if/when applicable):

A. Analytical Performance:

The studies conducted to support the analytical performance of the Xpert Xpress CoV-2/Flu/RSV *plus* test, performed on the GeneXpert Instrument Systems, were previously reviewed and described in K231481. In this submission, analytical studies data generated on the GeneXpert Instrument Systems were reanalyzed using the latest 6.4a GeneXpert Xpress software to assess the analytical performance on the GeneXpert Xpress System.

The results from the analysis are described in sections below.

1. Precision/Reproducibility

a. Within-laboratory Precision

Within-laboratory precision was previously evaluated as described in K231481. A total of 687 test results (654 samples and 33 controls) were processed with the GeneXpert Xpress software version 6.4a. In all cases, the runs gave the same results as described in K231481.

The reanalysis of the data from the within-laboratory precision study demonstrates equivalent performance of the Xpert Xpress CoV-2/Flu/RSV *plus* test on the GeneXpert Xpress System.

b. Reproducibility

A blinded, multi-site reproducibility study was conducted to assess the total variability of the Xpert Xpress CoV-2/Flu/RSV *plus* test on the GeneXpert Xpress System across operators, study sites, testing days, runs and instruments. The reproducibility study design incorporated two contrived reproducibility panels consisting of five panel members each as indicated in **Table 1**.

Table 1. Reproducibility Panels

Reproducibility Panel 1 (Low Positive)		
Panel Member	Target	Level
1	Negative - 1	Negative
2	SARS-CoV-2	~1.5x LoD
3	Flu A	~1.5x LoD
4	Flu B	~1.5x LoD
5	RSV	~1.5x LoD
Reproducibility Panel 2 (Moderate Positive)		
Panel Member	Target	Level
1	Negative - 2	Negative
2	SARS-CoV-2	~3x LoD
3	Flu A	~3x LoD
4	Flu B	~3x LoD
5	RSV	~3x LoD

Panel members were contrived using inactivated NATtrol SARS-CoV-2 (ZeptoMetrix, Buffalo, NY, catalog number NATSARS(COV2)-ERC), and cultured viruses influenza A/Idaho/7/2018, influenza B/Wisconsin/10/2016, and RSV B/Wash/18537/62 in simulated nasal/NP swab matrix. The “Negative” sample does not contain any target microorganism or target RNA. The study was conducted at three (3) external CLIA-Waived sites with three (3) operators at each site, one (1) lot of Xpert Xpress CoV-2/Flu/RSV *plus* cartridges over five (5) independent days of testing per operator with one (1) run per operator per day, with a run consisting of two (2) replicates. Study results are summarized in **Table 2**.

Table 2. Summary of Reproducibility Results by Site and Operator

Panel	Panel Member	Site 1				Site 2				Site 3				Agreement (%)
		OP1	OP2	OP3	Site	OP1	OP2	OP3	Site	OP1	OP2	OP3	Site	
Panel One	Negative	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100% (90/90; 95.9-100)
		10/10	10/10	10/10	30/30	10/10	10/10	10/10	30/30	10/10	10/10	10/10	30/30	
	SARS-CoV-2 ~1.5x LoD	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100% (90/90; 95.9-100)
		10/10	10/10	10/10	30/30	10/10	10/10	10/10	30/30	10/10	10/10	10/10	30/30	
	Flu A ~1.5x LoD	100%	100%	100%	100%	90%	90%	100%	93.3%	100%	100%	100%	100%	97.8% (88/90; 92.3-99.4)
		10/10	10/10	10/10	30/30	9/10	9/10	10/10	28/30	10/10	10/10	10/10	30/30	
	Flu B ~1.5x LoD	100%	90%	100%	96.7%	100%	100%	90%	96/7%	100%	100%	100%	100%	97.8% (88/90; 92.3-99.4)
		10/10	9/10	10/10	29/30	10/10	10/10	9/10	29/30	10/10	10/10	10/10	30/30	
	RSV ~1.5x LoD	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100% (90/90; 95.9-100)
		10/10	10/10	10/10	30/30	10/10	10/10	10/10	30/30	10/10	10/10	10/10	30/30	
Panel Two	Negative	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100% (90/90; 95.9-100)
		10/10	10/10	10/10	30/30	10/10	10/10	10/10	30/30	10/10	10/10	10/10	30/30	
	SARS-CoV-2 ~3x LoD	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100% (89/89; 95.9-100)
		10/10	10/10	10/10	30/30	10/10	10/10	10/10	30/30	9/9 ^a	10/10	10/10	29/29	
	Flu A ~3x LoD	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100% (90/90; 95.9-100)
		10/10	10/10	10/10	30/30	10/10	10/10	10/10	30/30	10/10	10/10	10/10	30/30	
	Flu B ~3x LoD	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100% (90/90; 95.9-100)
		10/10	10/10	10/10	30/30	10/10	10/10	10/10	30/30	10/10	10/10	10/10	30/30	
	RSV ~3x LoD	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100% (90/90; 95.9-100)
		10/10	10/10	10/10	30/30	10/10	10/10	10/10	30/30	10/10	10/10	10/10	30/30	

OP = Operator

^a Operator initially had 10 samples. One sample produced a non-determinate result twice upon testing.

The Xpert Xpress CoV-2/Flu/RSV *plus* test demonstrated 100% agreement with expected results for all panel members except for the Flu A low positive (97.8%) and Flu B low positive (97.8%). The results of the study demonstrate acceptable assay reproducibility for the Xpert Xpress CoV-2/Flu/RSV *plus* test on the GeneXpert Xpress System.

2. Analytical Specificity/Interference:

Analytical Reactivity (Inclusivity)

Wet-Testing

Inclusivity was previously evaluated as described in K231481. Seven hundred twenty-six (726) tests from the analytical reactivity (inclusivity) study for K231481 were reanalyzed

using the latest 6.4a GeneXpert Xpress software. There were 709 valid results and 17 non-determinate results. All 709 valid tests gave the exact same test results after reanalysis. While the number of non-determinant results was unchanged, two (2) “ERROR” messages were changed to “INSTRUMENT ERROR”. Three (3) additional “ERROR” messages were changed to “NO RESULT – REPEAT TEST” and one (1) “INVALID” was changed to “NO RESULT – REPEAT TEST.” The remaining eleven (11) “INVALID” results were unchanged with Xpress 6.4a software.

The reanalysis of the inclusivity study data from K231481 demonstrates equivalent performance of the Xpert Xpress Cov-2/Flu/RSV *plus* test on the GeneXpert Xpress System.

SARS-CoV-2 *In silico* Analysis

In silico SARS-CoV-2 analysis was previously reviewed and described in K231481.

Analytical Specificity (Cross-Reactivity)

Cross-Reactivity Wet-Testing

Analytical specificity (cross-reactivity) was previously evaluated as described in K231481. Thirty-six (36) tests from the analytical specificity study were reanalyzed using the latest 6.4a GeneXpert Xpress software. There were 36 valid results and no non-determinate results in K231481. All 36 valid tests gave the exact same test results after reanalysis.

The reanalysis of the analytical specificity (cross-reactivity) study data demonstrates equivalent performance of the Xpert Xpress Cov-2/Flu/RSV *plus* test on the GeneXpert Xpress System.

Interference Testing

Microbial Interference

Microbial interference was previously evaluated as described in K231481. Four hundred forty-two (442) tests were reanalyzed using the latest 6.4a GeneXpert Xpress software. There were 440 valid results and two non-determinate results. While the number of non-determinate results was unchanged, the two (2) “NO RESULT” messages were changed to “NO RESULT – REPEAT TEST”.

The reanalysis of the analytical microbial interference study data demonstrates equivalent performance of the Xpert Xpress CoV-2/Flu/RSV *plus* test on the GeneXpert Xpress System.

Competitive Interference

Competitive interference was previously evaluated as described in K231481. Eighty-four (84) tests from these competitive interference studies were reanalyzed using the latest 6.4a GeneXpert Xpress software. There were 84 valid results and zero (0) non-determinate results. All 84 valid tests gave the exact same test results after the reanalysis.

The reanalysis of the data from the competitive interference studies demonstrates equivalent performance of the Xpert Xpress CoV-2/Flu/RSV *plus* test on the GeneXpert Xpress System.

Potentially Interfering Substances

The impact of potentially interfering substance on the Xpert Xpress CoV-2/Flu/RSV *plus* test was previously evaluated as described in K231481. Two thousand five hundred seventy-one (2571) tests from the potentially interfering substances study were reanalyzed using the latest 6.4a GeneXpert Xpress software. There were 2533 valid results and 38 non-determinate results. All 2533 valid tests gave the exact same test results after the reanalysis. The number of non-determinate results (38) was unchanged, including 13 "NO RESULT", 20 "ERROR", and five "INVALID".

The reanalysis of the data from the interfering substances study demonstrates equivalent performance of the Xpert Xpress CoV-2/Flu/RSV *plus* test on the GeneXpert Xpress System.

3. Assay Reportable Range:

Not Applicable; this is a qualitative assay.

4. Traceability, Stability, Expected Values (Controls, Calibrators, or Methods):

a. Controls

External Control Evaluation

One hundred twenty-one (121) tests from the external control evaluation study in K231481 were reanalyzed. There were 120 valid results and 1 non-determinate result. All 120 valid tests gave the exact same test results after the reanalysis while the number of non-determinate results was unchanged. One "ERROR" message changed to "NO RESULT - REPEAT TEST".

The reanalysis of the data from the external control validation study from K231481 demonstrates equivalent performance of the Xpert Xpress CoV-2/Flu/RSV *plus* test on the GeneXpert Xpress System.

b. Specimen Stability

NPS in UTM/VTM Matrix

Two hundred seventy-seven (277) tests from the NPS Specimen Stability in UTM/VTM Matrix Study in K231481 were reanalyzed using the latest 6.4a GeneXpert Xpress software. There were 276 valid results and one non-determinate result. All 276 valid tests gave the exact same test results after the reanalysis while the number of non-determinate results was unchanged. One "NO RESULT" message was changed to "NO RESULT-REPEAT TEST".

This reanalysis supports specimen storage conditions in clinical NPS UTM/VTM matrix at 2-8°C temperatures for up to seven (7) days and at room temperature (15-30°C) for up to 48 hours until testing is performed on the GeneXpert Xpress Systems.

NPS in eNAT Matrix

Two hundred seventy-eight (278) tests from the NPS Specimen Stability in eNAT Matrix

Study in K231481 were reanalyzed using the latest 6.4a GeneXpert Xpress software. There were 276 valid results and two non-determinate results. All 276 valid tests gave the exact same test results after the reanalysis, the number of non-determinate results was unchanged, and the two "ERROR" messages remained unchanged, after the reanalysis. This reanalysis supports specimen storage conditions in clinical NPS eNAT matrix at 2-8°C temperatures for up to six (6) days and at room temperature (15-30°C) for up to 48 hours until testing is performed on the GeneXpert Xpress Systems.

Freeze-Thaw Study at -80°C

Three hundred seventy-seven (377) tests from the fresh versus frozen samples study conducted at -80°C in K231481 were reanalyzed using the latest 6.4a GeneXpert Xpress software. There were 360 valid results and 17 non-determinate results. All 360 valid tests gave the exact same test results after the reanalysis, the number of non-determinate results was unchanged, and seven "ERROR" messages and ten "INVALID" messages remained unchanged after the reanalysis.

This reanalysis supports storage and freeze-thaw stability claims for NPS and NS samples collected in VTM and eNAT that can undergo up to one (1) freeze/thaw cycle when stored at -80°C.

Freeze-Thaw Study at -20°C

Three hundred sixty-eight (368) tests from the fresh versus frozen samples study at -20°C in K231481 were reanalyzed using the latest 6.4a GeneXpert Xpress software. There were 360 valid results and 8 non-determinate results. All 360 valid tests gave the exact same test results after the reanalysis.

The number of non-determinate GeneXpert results was unchanged, and four "NO RESULT" and four "ERROR" messages remained unchanged after the reanalysis.

This reanalysis supports storage and freeze-thaw stability claims for NPS and NS samples collected in VTM and eNAT that can undergo up to one (1) freeze/thaw cycle when stored at -20°C.

c. Prepared Cartridge Hold Time

Two hundred fifty-six (256) tests from the cartridge hold time study in K231481 were reanalyzed using the latest 6.4a GeneXpert Xpress software. There were 256 valid results and 0 non-determinate results. All 256 valid tests gave the exact same test results after the reanalysis.

The reanalysis of the data from the cartridge hold time study from K231481 demonstrates equivalent performance of the Xpert Xpress CoV-2/Flu/RSV *plus* test on the GeneXpert Xpress System and support a cartridge hold time of 4.5 hours.

5. Detection Limit:

Analytical Sensitivity/ Limit of Detection (LoD)

LoD in Clinical NPS in UTM/VTM Matrix

The LoD in clinical NPS in UTM/VTM was previously established as described in K231481 in two steps. First, 2927 tests from the LoD estimation in clinical NPS in UTM/VTM matrix were reanalyzed using the latest 6.4a GeneXpert Xpress software. There were 2900 valid results and 27 non-determinate results. All 2900 valid tests gave the exact same test results after the reanalysis. The number of non-determinate results was unchanged after the reanalysis. Four (4) “NO RESULT” messages were changed to “NO RESULT – REPEAT TEST”, 2 “ERROR” messages were changed to “INSTRUMENT ERROR”, and 1 “ERROR” message was changed to “NO RESULT-REPEAT TEST”. Twelve (12) “NO RESULT” and 8 “ERROR” messages were unchanged.

Then, 586 tests from the LoD verification in NPS in UTM/VTM matrix were reanalyzed using the latest 6.4a GeneXpert Xpress software. There were 580 valid results and 6 non-determinate results. All 586 valid tests gave the exact same test results after the reanalysis. The number of non-determinate results was unchanged, and one "NO RESULT" message and five "ERROR" messages remained unchanged after the reanalysis.

The reanalysis of the data from the LoD in clinical NPS in UTM/VTM matrix studies from K231481 demonstrates equivalent performance of the Xpert Xpress CoV-2/Flu/RSV *plus* test on the GeneXpert Xpress System.

The LoD concentrations and mean Ct values at the LoDs for clinical NPS in UTM/VTM specimens tested using two reagent lots are shown in **Table 3**.

Table 3. LoD Concentrations and Mean Ct Values for Clinical NPS in UTM/VTM Matrix

Virus	Strains	Xpert Xpress CoV-2/Flu/RSV <i>plus</i>		
		Reagent	LoD	Mean Ct
SARS-CoV-2	USA-WA1/2020 (NATrol)	Lot 00402 (MMLV/CAT)	138 copies/mL	39.8
		Lot 00502 (MMLV/PHX)	111 copies/mL	40.0
	1 st WHO International Standard	Lot 02271 (MMLV/CAT)	94 IU/mL	40.6
		Lot 02601 (MMLV/PHX)	94 IU/mL	39.7
Influenza A H1N1	Influenza A/ Idaho/07/2018	Lot 00402 (MMLV/CAT)	0.007 TCID ₅₀ /mL	35.7
		Lot 00502 (MMLV/PHX)	0.007 TCID ₅₀ /mL	35.6
	Influenza A/ California/07/2009	Lot 02005 (MMLV/CAT)	0.0022 TCID ₅₀ /mL	36.6
		Lot 02601 (MMLV/PHX)	0.0022 TCID ₅₀ /mL	36.7
Influenza A H3N2	Influenza A/ Hong Kong/45/2019	Lot 00402 (MMLV/CAT)	0.34 FFU/mL	36.7
		Lot 00502 (MMLV/PHX)	0.44 FFU/mL	35.9
	Influenza A/ Victoria/361/2011	Lot 02005 (MMLV/CAT)	0.05 TCID ₅₀ /mL	35.6
		Lot 02601 (MMLV/PHX)	0.05 TCID ₅₀ /mL	36.2
Influenza B	Influenza B/ Washington/2/2019	Lot 00402 (MMLV/CAT)	12.9 CEID ₅₀ /mL	36.6
		Lot 00502 (MMLV/PHX)	12.9 CEID ₅₀ /mL	36.7
	Influenza B/ Wisconsin/10/2016	Lot 00402 (MMLV/CAT)	2.4 TCID ₅₀ /mL	36.6
		Lot 00502 (MMLV/PHX)	2.4 TCID ₅₀ /mL	36.2
RSV A	RSV-A/2/Australia/61	Lot 00402 (MMLV/CAT)	0.33 TCID ₅₀ /mL	35.9
		Lot 00502 (MMLV/PHX)	0.33 TCID ₅₀ /mL	35.9
	RSV-A/Long/MD/56	Lot 02005 (MMLV/CAT)	0.17 TCID ₅₀ /mL	36.2
		Lot 02601 (MMLV/PHX)	0.17 TCID ₅₀ /mL	36.6

Virus	Strains	Xpert Xpress CoV-2/Flu/RSV <i>plus</i>		
		Reagent	LoD	Mean Ct
RSV B	RSV-B/9320/MA/77	Lot 00402 (MMLV/CAT)	0.37 TCID ₅₀ /mL	35.9
		Lot 00502 (MMLV/PHX)	0.37 TCID ₅₀ /mL	35.8
	RSV-B/Wash/18537/62	Lot 02005 (MMLV/CAT)	0.2 TCID ₅₀ /mL	36.2
		Lot 02601 (MMLV/PHX)	0.2 TCID ₅₀ /mL	36.8

LoD in Clinical ANS in UTM/VTM Matrix

The LoD in clinical ANS in UTM/VTM was previously established as described in K231481 in two steps. First, 3064 tests from the LoD estimation in clinical ANS in UTM/VTM matrix were reanalyzed using the latest 6.4a GeneXpert Xpress software. There were 3040 valid results and 24 non-determinate results. All 3040 valid tests gave the exact same test results after the reanalysis. The number of non-determinate results was unchanged, and ten "NO RESULT" messages and 14 "ERROR" messages remained unchanged, after the reanalysis.

Then, 765 tests from the LoD verification in clinical ANS in UTM/VTM matrix in K231481 were reanalyzed using the latest 6.4a GeneXpert Xpress software. There were 760 valid results and 5 non-determinate results. All 760 valid tests gave the exact same test results after the reanalysis. The number of non-determinate results was unchanged, and five "ERROR" messages remained unchanged, after the analysis.

The reanalysis of the data from the LoD in clinical ANS in UTM/VTM matrix studies from K231481 demonstrates equivalent performance of the Xpert Xpress CoV-2/Flu/RSV *plus* test on the GeneXpert Xpress System.

The LoD concentrations and mean Ct values at the LoDs for clinical ANS in UTM/VTM specimens tested using two reagent lots are shown in **Table 4**.

Table 4. LoD Concentrations and Mean Ct Values for Clinical NS UTM/VTM Matrix

Virus	Strains	Xpert Xpress CoV-2/Flu/RSV <i>plus</i>		
		Reagent	LoD	Mean Ct
SARS-CoV-2	USA-WA1/2020 (NATtrol)	Lot 02005 (MMLV/CAT)	50 copies/mL	40
		Lot 02601 (MMLV/PHX)	64 copies/mL	39.8
	1 st WHO International Standard	Lot 02271 (MMLV/CAT)	99 IU/mL	40.4
		Lot 02601 (MMLV/PHX)	143 IU/mL	40.1
Influenza A H1N1	Influenza A/Idaho/07/2018	Lot 02005 (MMLV/CAT)	0.0058 TCID ₅₀ /mL	36.6
		Lot 02601 (MMLV/PHX)	0.012 TCID ₅₀ /mL	36.2
Influenza A H1N1	Influenza A/California/7/2009	Lot 02005 (MMLV/CAT)	0.0028 TCID ₅₀ /mL	36.5
		Lot 02601 (MMLV/PHX)	0.0028 TCID ₅₀ /mL	36.7
Influenza A H3N2	Influenza A/Hong Kong/45/2019	Lot 02005 (MMLV/CAT)	0.38 FFU ₅₀ /mL	36.4
		Lot 02601 (MMLV/PHX)	0.49 FFU ₅₀ /mL	36.0
Influenza A H3N2	Influenza A/Victoria/361/2011	Lot 02005 (MMLV/CAT)	0.065 TCID ₅₀ /mL	35.9
		Lot 02601 (MMLV/PHX)	0.065 TCID ₅₀ /mL	36.9

Virus	Strains	Xpert Xpress CoV-2/Flu/RSV <i>plus</i>		
		Reagent	LoD	Mean Ct
Influenza B	Influenza B/Washington/2/2019	Lot 02005 (MMLV/CAT)	18.9 CEID ₅₀ /mL	35.9
		Lot 02601 (MMLV/PHX)	26.3 CEID ₅₀ /mL	35.6
Influenza B	Influenza B/Wisconsin/10/2016	Lot 02005 (MMLV/CAT)	2.41 TCID ₅₀ /mL	36.0
		Lot 02601 (MMLV/PHX)	2.41 TCID ₅₀ /mL	36.6
RSV A	RSV-A/2/Australia/61	Lot 02005 (MMLV/CAT)	0.28 TCID ₅₀ /mL	36.1
		Lot 02601 (MMLV/PHX)	0.28 TCID ₅₀ /mL	36.3
RSV A	RSV-A/Long/MD/56	Lot 02005 (MMLV/CAT)	0.22 TCID ₅₀ /mL	36.4
		Lot 02601 (MMLV/PHX)	0.22 TCID ₅₀ /mL	36.6
RSV B	RSV-B/9320/MA/77	Lot 02005 (MMLV/CAT)	0.27 TCID ₅₀ /mL	36.5
		Lot 02601 (MMLV/PHX)	0.27 TCID ₅₀ /mL	36.9
RSV B	RSV-B/Wash/18537/62	Lot 02005 (MMLV/CAT)	0.24 TCID ₅₀ /mL	36.7
		Lot 02601 (MMLV/PHX)	0.4 TCID ₅₀ /mL	36.1

6. Assay Cut-Off:

Assay cut-off was previously reviewed and described in K231481.

7. Accuracy (Instrument):

Not Applicable.

8. Carry-Over:

Eighty-five (85) tests from the carry-over contamination study in K231481 were reanalyzed using the latest 6.4a GeneXpert Xpress software. There were 82 valid results and three non-determinate results. All 82 valid tests gave the exact same test results after the reanalysis. While the number of non-determinate results was unchanged, one "NO RESULT" message was changed to "NO RESULT - REPEAT TEST", and two "ERROR" messages were changed to "NO RESULT - REPEAT TEST".

The reanalysis of the data from the carry-over contamination study from K231481 demonstrates equivalent performance of the Xpert Xpress CoV-2/Flu/RSV *plus* test on the GeneXpert Xpress System.

9. Matrix Comparison:

These studies were conducted to establish equivalent performance of Xpert Xpress CoV-2/Flu/RSV *plus* between

- (a) clinical nasopharyngeal swab (NPS), clinical anterior nasal swab (ANS) and simulated matrices in Universal Transport Medium (UTM)/Viral Transport Medium (VTM),
- (b) clinical NPS in UTM/VTM and eNAT.

Clinical NPS UTM/VTM, Clinical NS UTM/VTM and Simulated NPS/NS UTM/VTM Matrices

Nine hundred thirty-eight (938) tests from the matrix equivalency study with clinical NPS, clinical NS, and simulated NPS/NS matrices, in K231481, were reanalyzed using the latest 6.4a GeneXpert Xpress software. There were 930 valid results and eight non-determinate results. All 930 valid tests gave the exact same test results after the reanalysis. The number of non-determinate results was unchanged, and five "NO RESULT" and three "ERROR" messages were unchanged, after the reanalysis.

The reanalysis of the data from the matrix equivalency study with clinical NPS, clinical NS, and simulated NPS/NS matrices from K231481 demonstrates equivalent performance of the Xpert Xpress CoV-2/Flu/RSV *plus* test on the GeneXpert Xpress System when testing natural NPS or ANS collected in UTM/VTM matrix and simulated NPS/ANS in UTM/VTM matrices.

Clinical NPS UTM/VTM and Clinical NPS eNAT Matrices

Six hundred twenty-five (625) tests from the matrix equivalency study with clinical NPS UTM/VTM and clinical NPS eNAT in K231481 were reanalyzed using the latest 6.4a GeneXpert Xpress software. There were 620 valid results and five non-determinate results. All 620 valid tests gave the exact same test results after the reanalysis. The number of non-determinate results was unchanged between the two sets of data, and two "NO RESULT" and 2 "ERROR" and one "INVALID" message remained unchanged after the reanalysis.

The reanalysis of the data from the matrix equivalency study with clinical NPS UTM/VTM and clinical NPS eNAT from K231481 demonstrates equivalent performance of the Xpert Xpress CoV-2/Flu/RSV *plus* test on the GeneXpert Xpress System when testing NPS collected in UTM/VTM and NPS collected in eNAT.

10. Method Comparison with Predicate Device:

Not Applicable.

B. Clinical Studies:

Prospective Clinical Study:

The performance of the Xpert Xpress CoV-2/Flu/RSV *plus* on the GeneXpert Xpress System was established in a multi-site prospective clinical study that included 23 geographically diverse sites in the United States (US) in specimens collected from individuals showing signs and symptoms of respiratory infection. Of the 23 sites, 22 performed GeneXpert Xpress System testing and specimen collection, and one site performed comparator testing and discrepant investigation.

Prospectively collected, fresh (Category I) and frozen (Category II) nasopharyngeal swab (NPS) and anterior nasal swab (ANS) specimens in UTM/VTM, from individuals with signs and symptoms of respiratory viral infection, were enrolled from January 24, 2022 to July 19, 2022 in

the US in an all-comers fashion. In addition, frozen NPS and ANS specimens in UTM/VTM that were prospectively collected from individuals with signs and symptoms of respiratory viral infection during the 2016-2017 influenza season in the US in an all-comers fashion (Category II specimens) were also enrolled in the prospective clinical study to assess performance for the influenza A, influenza B, and RSV analytes.

Subject/Specimen Enrollment

Specimens tested for the SARS-CoV-2 Analyte

There were 3807 prospective subjects/specimens enrolled for evaluating the performance of the Xpert Xpress CoV-2/Flu/RSV *plus* on the GeneXpert Xpress System in testing for the SARS-CoV-2 analyte. Six participants withdrew after enrollment without providing specimens resulting in a total of 3801 subjects/specimens (1888 NPS and 1913 ANS). Seventy-five (75) specimens were excluded (39 NPS and 36 ANS) as a result of not meeting the inclusion/exclusion criteria or as a result of an issue with informed consent documentation resulting in 3726 eligible specimens (1849 NPS and 1877 ANS). A further 392 specimens (194 NPS and 198 ANS) were excluded due to protocol deviations leaving 3334 specimens (1655 NPS and 1679 ANS). Unresolved repeat non-determinate (ND) for GeneXpert Xpress System testing resulted in the exclusion of 18 additional samples (3 NPS and 15 ANS) from performance assessment, leaving 3316 specimens (1652 NPS and 1664 ANS). Finally, 169 specimens (87 NPS and 82 ANS) were excluded from performance assessment due to non-evaluable/invalid comparator test results, leaving 3147 prospective specimens (1565 NPS and 1582 ANS, Category I and II) eligible for performance evaluation.

Specimens tested for the influenza A, influenza B, and RSV Analytes

There were 5166 prospective subjects/specimens enrolled for evaluating the performance of the Xpert Xpress CoV-2/Flu/RSV *plus* on the GeneXpert Xpress System in testing for the Flu A/ Flu B/ RSV analytes. Six participants withdrew after enrollment without providing specimens resulting in a total of 5160 subjects/specimens. Seventy-five (75) specimens were excluded (39 NPS and 36 ANS) as a result of not meeting the inclusion/exclusion criteria or as a result of an issue with informed consent documentation resulting in 5085 eligible specimens (2571 NPS and 2514 ANS). An additional 524 specimens (271 NPS and 253 ANS) were excluded due to protocol deviations leaving 4561 specimens (2300 NPS and 2261 ANS) eligible for performance assessment. **Table 5** describes demographic data for the prospective specimens available for performance assessments. Of the 4561 specimens remaining, a further 26 samples (7 NPS and 19 ANS) were excluded from performance analysis due to unresolved ND (repeat ND or missing repeat testing results) for GeneXpert Xpress System testing. This left 4535 specimens for performance analysis (2293 NPS and 2242 ANS). Finally, 225 additional specimens (118 NPS and 107 ANS) were excluded from performance analysis due to non-evaluable/invalid comparator test results. This resulted in 4310 specimens (2175 NPS and 2135 ANS) eligible for performance evaluation.

Table 5. Demographic Data for Prospectively Collected Category I and II Specimens

Prospectively Collected Clinical Specimens	NPS (N=2300)	ANS (N=2261)	Overall (N=4561)^a
Gender			
Female	1296 (56.3%)	1374 (60.8%)	2670 (58.5%)
Male	1004 (43.7%)	887 (39.2%)	1891 (41.5%)
Age Group (Years)			
≤5	273 (11.9%)	402 (17.8%)	675 (14.8%)
6-21	600 (26.1%)	550 (24.3%)	1150 (25.2%)
22-59	1129 (49.1%)	1015 (44.9%)	2144 (47.0%)
≥60	298 (13.0%)	294 (13.0%)	592 (13.0%)
Specimen Testing			
Fresh	1654 (71.9%)	1679 (74.3%)	3333 (73.1%)
Frozen	646 (28.1%)	582 (25.7%)	1228 (26.9%)
Race			
Prospectively Collected in 2022			
American Indian or Alaska Native	1 (0.0%)	3 (0.1%)	4 (0.1%)
Asian	53 (2.3%)	58 (2.6%)	111 (2.4%)
Asian, White	6 (0.3%)	2 (0.1%)	8 (0.2%)
Black or African American	403 (17.5%)	389 (17.2%)	792 (17.4%)
Black or African American, White	6 (0.3%)	7 (0.3%)	13 (0.3%)
Native Hawaiian or Other Pacific Islander	1 (0.0%)	1 (0.0%)	2 (0.0%)
White	1042 (45.3%)	1035 (45.8%)	2077 (45.5%)
Other Mixed	2 (0.1%)	4 (0.2%)	6 (0.1%)
Missing, Declined to Answer or unknown	141 (6.1%)	180 (8.0%)	321 (7.0%)
Prospectively Collected Pre-Pandemic			
Not Available	645 (28.0%)	582 (25.7%)	1227 (26.9%)
Ethnicity			
Prospectively Collected in 2022			
Hispanic	136 (5.9%)	146 (6.5%)	282 (6.2%)
Non-Hispanic	1411 (61.3%)	1413 (62.5%)	2824 (61.9%)
Missing, Declined to Answer or Unknown	108 (4.7%)	120 (5.3%)	228 (5.0%)
Prospectively Collected Pre-Pandemic			
Not Available	645 (28.0%)	582 (25.7%)	1227 (26.9%)
COVID-19 Vaccination Status			
Prospectively Collected in 2022			
Vaccinated	1293 (56.2%)	1184 (52.4%)	2477 (54.3%)
Not Vaccinated	328 (14.3%)	468 (20.7%)	796 (17.5%)
Unknown	34 (1.5%)	27 (1.2%)	61 (1.3%)
Prospectively Collected Pre-Pandemic			
Not Available (Not Vaccinated)	645 (28.0%)	582 (25.7%)	1227 (26.9%)

^a The 4561 specimens comprise 3333 fresh (Category I) specimens and 1228 frozen (Category II) specimens.

The Xpert Xpress CoV-2/Flu/RSV *plus* test on the GeneXpert Xpress System was evaluated for SARS-CoV-2 performance by comparing to a U.S. FDA-cleared molecular respiratory panel that includes SARS-CoV-2. For SARS-CoV-2 performance evaluations, only the prospective specimens collected in 2022 were tested with the SARS-CoV-2 comparator, as specimens collected prior to the COVID-19 pandemic (*i.e.*, 2016-2017) were assumed to be SARS-CoV-2 negative.

The Xpert Xpress CoV-2/Flu/RSV *plus* test on the GeneXpert Xpress System was evaluated for Flu A, Flu B, and RSV performance by comparing to a U.S. FDA-cleared molecular Flu A/B/RSV assay. For Flu A, Flu B and RSV performance assessment, prospective specimens collected in both 2022 and 2016-2017 were evaluated.

Positive Percent Agreement (PPA) was calculated as $100\% \times (TP / (TP + FN))$. True positive (TP) indicates that both the Xpert Xpress CoV-2/Flu/RSV *plus* and the comparator method had a positive result for the specific analyte, and false negative (FN) indicates that the Xpert Xpress CoV-2/Flu/RSV *plus* was negative while the comparator result was positive. Negative Percent Agreement (NPA) was calculated as $100\% \times (TN / (TN + FP))$. True negative (TN) indicates that both the Xpert Xpress CoV-2/Flu/RSV *plus* and the comparator method had negative results, and false positive (FP) indicates that the Xpert Xpress CoV-2/Flu/RSV *plus* was positive while the comparator result was negative. Specimens that obtained discordant SARS-CoV-2 results underwent additional testing with a U.S. FDA EUA SARS-CoV-2 molecular test, while specimens that obtained discordant Flu A, Flu B, or RSV results underwent additional testing with a U.S. FDA-cleared molecular respiratory panel.

Prospective Clinical Performance

Nasopharyngeal Swab Specimens

Of the 1565 prospective NPS specimens that were analyzed for SARS-CoV-2 performance, 99.9% (1564/1565) were tested fresh (Category I), while 0.06% (1/1565) were tested frozen (Category II) with the Xpert Xpress CoV-2/Flu/RSV *plus*. Of the 2175 prospective NPS specimens that were analyzed for Flu A, Flu B, and RSV performance, 72.7% (1582/2175) were tested fresh (Category I), while 27.3% (593/2175) were tested frozen (Category II) with the Xpert Xpress CoV-2/Flu/RSV *plus*.

A summary of the Xpert Xpress CoV-2/Flu/RSV *plus* test prospective clinical study performance is provided in **Table 6** for NPS specimens (Category I and II).

Table 6. Xpert Xpress CoV-2/Flu/RSV *plus* Prospective Clinical Performance - NPS Specimens (Category I/Fresh & Category II/Frozen Specimens)

Analyte	Specimen Category	Number of Specimens	True Positives	False Negatives	False Positive	True Negative	PPA (%)	95% CI	NPA (%)	95% CI
SARS-CoV-2	Fresh	1564	294	4 ^a	19 ^b	1247	98.7	96.6-99.5	98.5	97.7-99.0
	Frozen	1	1	0	0	0	100	20.7-100	-	-
	Overall	1565	295	4	19	1247	98.7	96.6-99.5	98.5	97.7-99.0

Flu A	Fresh	1582	79	0	8 ^c	1495	100	95.4-100	99.5	99.0-99.7
	Frozen	593	130	2 ^d	21 ^e	440	98.5	94.6-99.6	95.4	93.1-97.0
	Overall	2175	209	2	29	1935	99.1	96.6-99.7	98.5	97.9-99.0
Flu B	Fresh	1582	0	0	0	1582	-	-	100	99.8-100
	Frozen	593	57	2 ^f	2 ^g	532	96.6	88.5-99.1	99.6	98.6-99.9
	Overall	2175	57	2	2	2114	96.6	88.5-99.1	99.9	99.7-100
RSV	Fresh	1582	6	0	0	1576	100	61.0-100	100	99.8-100
	Frozen	593	84	2 ^h	1 ⁱ	506	97.7	91.9-99.4	99.8	98.9-100
	Overall	2175	90	2	1	2082	97.8	92.4-99.4	100	99.7-100

a. Discrepant test results based on U.S. FDA EUA SARS-CoV-2 molecular test: 1/4 SARS-CoV-2 positive; 3/4 SARS-CoV-2 negative

b. Discrepant test results based on U.S. FDA EUA SARS-CoV-2 molecular test: 6/19 SARS-CoV-2 positive; 13/19 SARS-CoV-2 negative

c. Discrepant test results based on U.S. FDA-cleared molecular respiratory panel: 6/8 Flu A positive; 2/8 Flu A negative

d. Discrepant test results based on U.S. FDA-cleared molecular respiratory panel: 2/2 tests not performed due to the specimens being stored for a longer duration than allowed per the package insert.

e. Discrepant test results based on U.S. FDA-cleared molecular respiratory panel: 21/21 tests not performed due to the specimens being stored for a longer duration than allowed per the package insert.

f. Discrepant test results based on U.S. FDA-cleared molecular respiratory panel: 2/2 tests not performed due to the specimens being stored for a longer duration than allowed per the package insert.

g. Discrepant test results based on U.S. FDA-cleared molecular respiratory panel: 2/2 tests not performed due to the specimens being stored for a longer duration than allowed per the package insert.

h. Discrepant test results based on U.S. FDA-cleared molecular respiratory panel: 2/2 tests not performed due to the specimens being stored for a longer duration than allowed per the package insert.

i. Discrepant test results based on U.S. FDA-cleared molecular respiratory panel: 1/1 test not performed due to the specimens being stored for a longer duration than allowed per the package insert.

Anterior Nasal Swab Specimens

Of the 1582 ANS specimens analyzed for SARS-CoV-2, 100% (1582/1582) were tested fresh (Category I) with the Xpert Xpress CoV-2/Flu/RSV *plus*. Of the 2135 ANS specimens that were analyzed for Flu A, FluB, and RSV, 75.1% (1603/2135) were tested fresh (Category I), while 24.9% (532/2135) were tested frozen (Category II) with the Xpert Xpress CoV-2/Flu/RSV *plus*.

A summary of the Xpert Xpress CoV-2/Flu/RSV *plus* test prospective clinical study performance is provided in **Table 7** for ANS specimens (Category I and II).

Table 7. Xpert Xpress CoV-2/Flu/RSV *plus* Prospective Clinical Performance - ANS Specimens (Category I/Fresh & II/Frozen Specimens)

Analyte	Specimen Category	Number of Specimens	True Positives	False Negatives	False Positive	True Negative	PPA (%)	95% CI	NPA (%)	95% CI
SARS-CoV-2	Fresh	1582	302	5 ^a	9 ^b	1266	98.4	96.2-99.3	99.3	98.7-99.6
	Frozen	0	0	0	0	0	-	-	-	-
	Overall	1582	302	5	9	1266	98.4	96.2-99.3	99.3	98.7-99.6
Flu A	Fresh	1603	107	4 ^c	3 ^d	1489	96.4	91.1-98.6	99.8	99.4-99.9
	Frozen	532	99	1 ^e	19 ^f	413	99.0	94.6-99.8	95.6	93.2-97.2
	Overall	2135	206	5	22	1902	97.6	94.6-99.0	98.9	98.3-99.2
Flu B	Fresh	1603	0	0	0	1603	-	-	100	99.8-100
	Frozen	532	34	0	3	495	100	89.9-100	99.4	98.2-99.8
	Overall	2135	34	0	3 ^g	2098	100	89.9-100	99.9	99.6-100

RSV	Fresh	1603	6	1 ^h	0	1596	85.7	48.7-97.4	100	99.8-100
	Frozen	532	91	2 ⁱ	2 ^j	437	97.8	92.5-99.4	99.5	98.4-99.9
	Overall	2135	97	3	2	2033	97.0	91.6-99.0	99.9	99.6-100

a. Discrepant test results based on U.S. FDA EUA SARS-CoV-2 molecular test: 1/5 SARS-CoV-2 positive; 4/5 SARS-CoV-2 negative.

b. Discrepant test results based on U.S. FDA EUA SARS-CoV-2 molecular test: 2/9 SARS-CoV-2 positive; 4/9 SARS-CoV-2 negative; 2/9 invalid results; 1/9 discrepant testing was inadvertently not performed.

c. Discrepant test results based on U.S. FDA-cleared molecular respiratory panel: 2/4 Flu A positive; 2/4 Flu A negative.

d. Discrepant test results based on U.S. FDA-cleared molecular respiratory panel: 2/3 Flu A positive; 1/3 Flu A negative.

e. Discrepant test results based on U.S. FDA-cleared molecular respiratory panel: 1/1 tests not performed due to the specimens being stored for a longer duration than allowed per the package insert.

f. Discrepant test results based on U.S. FDA-cleared molecular respiratory panel: 19/19 tests not performed due to the specimens being stored for a longer duration than allowed per the package insert.

g. Discrepant test results based on U.S. FDA-cleared molecular respiratory panel: 3/3 tests not performed due to the specimens being stored for a longer duration than allowed per the package insert.

h. Discrepant test results based on U.S. FDA-cleared molecular respiratory panel: 1/1 RSV positive.

i. Discrepant test results based on U.S. FDA-cleared molecular respiratory panel: 2/2 test not performed due to the specimens being stored for a longer duration than allowed per the package insert.

j. Discrepant test results based on U.S. FDA-cleared molecular respiratory panel: 2/2 test not performed due to the specimens being stored for a longer duration than allowed per the package insert.

Non-determinate rates

The Xpert Xpress CoV-2/Flu/RSV *plus* test on the GeneXpert Xpress System non-determinate (ND) rate for prospective specimens (Category I and II) collected in 2022 and 2016-2017, combined, was initially 2.7% (123/4561) and decreased to 0.6% (26/4561) upon retest.

For NPS specimens, the initial ND result was 2.5% (58/2300) and decreased to 0.3% (7/2300) upon retest. With ANS specimens, the initial ND was 2.9% (65/2261) and, upon retest, was decreased to 0.8% (19/2261).

For fresh vs frozen, the initial ND rate for fresh specimens was 2.7% (90/3333) and decreased to 0.5% (18/3333) with retesting. For frozen specimens, the initial ND rate was 2.7% (33/1228) and it decreased to 0.7% (8/1228) with retesting.

Multi-target Detection

The number of specimens with positive results for one or more target analyte by the Xpert Xpress CoV-2/Flu/RSV *plus* and/or comparator assay(s) is presented in **Table 8** and **Table 9**, where bolded values indicate concordant results. As shown in **Table 8**, a total of 3028 prospective specimens collected in 2022 yielded valid results for all four targets for both the Xpert Xpress CoV-2/Flu/RSV *plus* and comparator assays. Note that influenza B is not presented in **Table 8**, as no influenza B positives were obtained during the 2022 study. The rate of co-infection for Xpert Xpress CoV-2/Flu/RSV *plus* was 0.1% (1/797) and the rate of co-infection by the comparators was 0.3% (2/774). In total, 2 co-infections were detected by either the Xpert Xpress CoV-2/Flu/RSV *plus* test or the comparator assays for specimens collected in 2022. One of these co-infections was discordant between the Xpert Xpress CoV-2/Flu/RSV *plus* and the comparator assays (see **Table 8**).

Table 8. Summary of Multi-Target Detections for the Xpert Xpress CoV-2/Flu/RSV *plus* Test – NPS and ANS Prospective Specimens Collected in 2022

Infection		Comparator Results*						
		SARS-CoV-2 only	Flu A only	RSV only	SARS-CoV-2 & Flu A	Negative	Total	Co-Infection Rate (%)
Xpert Xpress CoV-2/Flu/RSV <i>plus</i>	SARS-CoV-2 only	568	0	0	1	25	594	0.1% (1/797)
	Flu A only	0	179	0	0	11	190	
	RSV only	0	0	12	0	0	12	
	SARS-CoV-2 and Flu A	0	0	0	1	0	1	
	Negative	9	3	1	0	2218	2231	
	Total	577	182	13	2	2254	3028	
	Co-Infection Rate (%) for the Comparator	0.3 % (2/774)						

*The comparator assay for SARS-CoV-2 was a U.S. FDA-cleared molecular respiratory panel (under 21 CFR 866.3981) that includes SARS-CoV-2. The comparator assay for Flu A, Flu B, and RSV was a U.S. FDA-cleared molecular Flu A/B/RSV assay.

As shown in **Table 9**, a total of 4310 prospective specimens, collected in 2022 and 2016-2017, yielded valid results for Flu A, Flu B, and RSV by both the Xpert Xpress CoV-2/Flu/RSV *plus* and comparator assay. The co-infection rate for Xpert Xpress CoV-2/Flu/RSV *plus* was 2.04% (15/737) and the co-infection rate by the comparator was 1.29% (9/698). In total, 17 co-infections were detected by either the Xpert Xpress CoV-2/Flu/RSV *plus* test or the comparator assay, of which ten were discordant (see **Table 9**).

Table 9 Co-Infection for Flu A, Flu B, and RSV in Prospectively Collected Specimens

Infection		Comparator Results*								
		Flu A Only	Flu B Only	RSV Only	Flu A & Flu B	Flu A & RSV	Flu B & RSV	Negative	Total	Co-Infection Rate (%)
Xpert Xpress CoV-2/Flu/RSV <i>plus</i>	Flu A Only	406	0	0	1	1	0	44	452	2.04% (15/737)
	Flu B Only	0	85	0	0	0	0	3	88	
	RSV Only	0	0	179	0	0	0	3	182	
	Flu A & Flu B	1	4	0	1	0	0	1	7	
	Flu A and RSV	0	0	2	0	5	0	0	7	
	Flu B & RSV	0	0	0	0	0	1	0	1	
	Negative	7	1	4	0	0	0	3561	3573	
	Total	414	90	185	2	6	1	3612	4310	
	Co-Infection Rate (%) for the Comparator	1.29% (9/698)								

*The comparator assay for Flu A, Flu B, and RSV was a U.S. FDA-cleared molecular Flu A/B/RSV assay.

Other Clinical Supportive Data:

Not Applicable.

C. Clinical Cut-Off:

Not Applicable.

D. Expected Values/Reference Range:

The 2022 prospective clinical study included a total of 3147 specimens (1565 NPS and 1582 ANS) with valid Xpert Xpress CoV-2/Flu/RSV *plus* test and comparator SARS-CoV-2 results. The 2022 and 2016-2017 prospective clinical studies included 3186 specimens (1583 NPS and 1603 ANS) and 1124 specimens (592 NPS and 532 ANS), respectively, with valid Flu A, Flu B, and RSV results. The number and percentage of cases positive for SARS-CoV-2, Flu A, Flu B, and RSV, as determined by the Xpert Xpress CoV-2/Flu/RSV *plus* test are shown in **Table 10**, stratified by collection period and age group, and **Table 11**, stratified by collection period and specimen type.

Table 10. Cepheid Xpert Xpress CoV-2/Flu/RSV *plus* - Expected Values Stratified by Collection Period and Age Group

Target	Expected Values for Prospectively Collected Specimens ^a from 2022 (Category I and II)				
	Overall	Age Group (years)			
		≤5	6-21	22-59	≥60
SARS-CoV-2	19.9% (625/3147)	12.5% (23/184)	14.1% (122/867)	22.9% (381/1661)	22.8% (99/435)
Flu A	6.2% (197/3186)	7.3% (13/179)	10.3% (91/880)	4.7% (80/1690)	3.0% (13/437)
Flu B	0.0% (0/3186)	0.0% (0/179)	0.0% (0/880)	0.0% (0/1690)	0.0% (0/437)
RSV	0.4% (12/3186)	0.6% (1/179)	0.7% (6/880)	0.3% (5/1690)	0.0% (0/437)
Target	Expected Values for Prospectively Collected Specimens ^b from 2016-2017 (Category II)				
	Overall	Age Group (years)			
		≤5	6-21	22-59	≥60
SARS-CoV-2	NA ^c	NA ^c	NA ^c	NA ^c	NA ^c
Flu A	23.9% (269/1124)	18.7% (82/438)	42.0% (87/207)	18.7% (67/358)	27.3% (33/121)
Flu B	8.5% (96/1124)	7.3% (32/438)	14.5% (30/207)	8.4% (30/358)	3.3% (4/121)
RSV	15.8% (178/1124)	33.1% (145/438)	0.5% (1/207)	3.4% (12/358)	16.5% (20/121)

a One prospectively collected specimen was frozen and retrospectively tested.

b Prospectively collected and stored thereafter at -70°C

c Not applicable - Specimens collected prior to the COVID-19 pandemic were expected to be negative for SARS-CoV-2 and were tested only for the Flu A, Flu B and RSV analyte.

Table 11. Cepheid Xpert Xpress CoV-2/Flu/RSV *plus* – Expected Values Stratified by Collection Period and Specimen Type

Analyte	Prospectively Collected Fresh and Frozen Specimens ^a from 2022 (Category I and II)			Prospectively Collected Frozen Specimens ^b from 2016-2017 Influenza Season (Category II)		
	Overall	NPS	ANS	Overall	NPS	NS
SARS-CoV-2	19.9% (625/3147)	20.1% (314/1565)	19.7% (311/1582)	NA ^c	NA ^c	NA ^c

Flu A	6.2% (197/3186)	5.5% (87/1583)	6.9% (110/1603)	23.9% (269/1124)	25.5% (151/592)	22.2% (118/532)
Flu B	0.0% (0/3186)	0.0% (0/1583)	0.0% (0/1603)	8.5% (96/1124)	10.0 (59/592)	7.0% (37/532)
RSV	0.4% (12/3186)	0.4% (6/1583)	0.4% (6/1603)	15.8% (178/1124)	14.4% (85/592)	17.5% (93/532)

^a One prospectively collected specimen was frozen and retrospectively tested.

^b Prospectively collected and stored thereafter at -70 °C.

^c Not Applicable – Specimens collected prior to the COVID-19 pandemic were expected to be negative for SARS-CoV-2 and were tested only for the Flu A, Flu B, and RSV analytes.

E Other Supportive Instrument Performance Characteristics Data:

Not Applicable.

VIII Proposed Labeling:

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

IX Conclusion:

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