Date: August 17, 2023

Cepheid
℅ Yen Nguyen
Senior Manager, Regulatory Affairs
Cepheid
904 Caribbean Drive
Sunnyvale, California 94089

Re: K231481
Trade/Device Name: Xpert Xpress CoV-2/Flu/RSV *plus*
Regulation Number: 21 CFR 866.3981
Regulation Name: 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
Regulatory Class: Class II
Product Code: QOF, OOI
Dated: May 22, 2023
Received: May 23, 2023

Dear Yen Nguyen:

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. Although this letter refers to your product as a device, please be aware that some cleared products may instead be combination products. The 510(k) Premarket Notification Database located at https://www.accessdata.fda.gov/scripts/cdrh/cfdocs/cfpmn/pmn.cfm identifies combination product submissions. 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 Part 801 and Part 809); medical device reporting (reporting of medical device-related adverse events) (21 CFR 803) for devices or postmarketing safety reporting (21 CFR 4, Subpart B) for combination products (see https://www.fda.gov/combination-products/guidance-regulatory-information/postmarketing-safety-reporting-combination-products); good manufacturing practice requirements as set forth in the quality systems (QS) regulation (21 CFR Part 820) for devices or current good manufacturing practices (21 CFR 4, Subpart A) for combination products; and, if applicable, the electronic product radiation control provisions (Sections 531-542 of the Act); 21 CFR 1000-1050.

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 https://www.fda.gov/medical-devices/medical-device-safety/medical-device-reporting-mdr-how-report-medical-device-problems.

For comprehensive regulatory information about medical devices and radiation-emitting products, including information about labeling regulations, please see Device Advice (https://www.fda.gov/medical-devices/device-advice-comprehensive-regulatory-assistance) and CDRH Learn (https://www.fda.gov/training-and-continuing-education/cdrh-learn). Additionally, you may contact the Division of Industry and Consumer Education (DICE) to ask a question about a specific regulatory topic. See the DICE website (https://www.fda.gov/medical-devices/device-advice-comprehensive-regulatory-assistance/contact-us-division-industry-and-consumer-education-dice) for more information or contact DICE by email (DICE@fda.hhs.gov) or phone (1-800-638-2041 or 301-796-7100).

Sincerely,

Joseph Briggs -S

Joseph Briggs, Ph.D.
Deputy Branch Chief
Division of Microbiology Devices
OHT7: Office of In Vitro Diagnostics
Office of Product Evaluation and Quality
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: 06/30/2023
See PRA Statement below.

510(k) Number *(if known)*
K231481

Device Name
Xpert Xpress CoV-2/Flu/RSV *plus*

Indications for Use *(Describe)*

The Xpert Xpress CoV-2/Flu/RSV *plus* test, performed on the GeneXpert Dx and GeneXpert Infinity Systems, 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 disease.

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.

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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510(k) Summary
for
Xpert® Xpress CoV-2/Flu/RSV *plus*

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5. 510(k) Summary

As required by 21 CFR Section 807.92(c).

Submitted by:	Cepheid 904 Caribbean Drive Sunnyvale, CA 94089 Phone number: (669) 246-2271
Contact:	Yen H. Nguyen, Ph.D.
Date of Preparation:	May 22, 2023

Device:

Trade name:	Xpert® Xpress CoV-2/Flu/RSV *plus*
Common name:	Xpert Xpress CoV-2/Flu/RSV *plus*
Type of Test:	Qualitative real-time reverse transcription polymerase chain reaction (RT-PCR) and detection test
Regulation number:	21 CFR 866.3981
Classification name:	Multi-Target Respiratory Specimen Nucleic Acid Test Including SARS-CoV-2 and Other Microbial Agents
Primary Product code:	QOF
Secondary Product code:	OOI
Classification Advisory Panel:	Microbiology (83)
Prescription Use:	Yes
Predicate Device Assay:	BioFire Respiratory Panel 2.1 (DEN200031)

5.1. Device Description

The Xpert Xpress CoV-2/Flu/RSV *plus* test is an automated *in vitro* diagnostic test for the simultaneous qualitative detection and differentiation of SARS-CoV-2, Flu A, Flu B, and RSV viral RNA in nasopharyngeal swab (NPS) and anterior nasal swab (NS) specimens collected from individuals showing signs and symptoms of respiratory viral infection.

The Xpert Xpress CoV-2/Flu/RSV *plus* test is performed on GeneXpert Instrument Systems (GeneXpert Dx, GeneXpert Infinity-48s and GeneXpert Infinity-80 systems), which consist of an instrument, computer, and preloaded software for running tests and viewing the results. The GeneXpert Instrument Systems automate and integrate sample preparation, nucleic acid extraction and amplification, and detection of the target sequences in simple or complex samples using real-time reverse transcription (RT)-polymerase chain reaction (PCR) and PCR assays. Depending on the instrument, the GeneXpert Instrument Systems can have from 1 and up to 80 randomly accessible modules, each capable of performing separate sample preparation and real-time RT-PCR and 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 RT-PCR and PCR as well as detection. The systems require 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. Because the cartridges are self-contained, cross-contamination between samples is minimized.

The Xpert Xpress CoV-2/Flu/RSV *plus* test includes reagents for the detection of SARS-CoV-2, Flu A, Flu B and RSV viral RNA from NPS and NS specimens. The primers and probes in the Xpert Xpress CoV-2/Flu/RSV *plus* test are designed to amplify and detect unique sequences in the genes that encode the following proteins: SARS-CoV-2 nucleocapsid (N), SARS-CoV-2 envelope (E), SARS-CoV-2 RNA-dependent RNA polymerase (RdRP), influenza A matrix (M), influenza A basic polymerase (PB2), influenza A acidic protein (PA), influenza B matrix (M), influenza B non-structural protein (NS), and the RSV A and RSV B nucleocapsid. 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 functional. 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.

The Xpert Xpress CoV-2/Flu/RSV *plus* test is designed for use with NPS or NS specimens collected with nylon flocked swabs and placed into viral transport medium (VTM), Universal Transport Medium (UTM), or eNAT®. The ancillary specimen collection kits, swabs and transport media validated for use with the Xpert Xpress CoV-2/Flu/RSV *plus* test included:

- **Nasopharyngeal Sample Collection Kit for Viruses**
    - Copan UTM® 3C057N (Flexible Minitip Flocked Swab with UTM® Medium without Beads)
    - Copan eNAT® Molecular Collection and Preservation Medium P/N 6U074S01 (Flexible Minitip Flocked Swab with eNAT® Medium)
    - Becton Dickinson Universal Viral Transport Kit P/N 220531 (Flexible Minitip Flocked Swab with UVT Medium)

- **Nasal Sample Collection Kit for Viruses**
    - Copan UTM® 3C064N (Regular Flocked Swab with UTM® Medium without Beads)
    - Copan eNAT® Molecular Collection and Preservation Medium P/N 6U073S01 (Regular Flocked Swab with eNAT® Medium)

- **Alternatively, swabs and transport media can be obtained separately:**
    - Nylon flocked swab (Copan P/N 502CS01, 503CS01)
    - Viral transport medium, 3 mL (Copan P/N 330C, 3C047N, BD Universal Transport Medium, Remel M4RT or Remel M5)

These ancillary reagents allow NPS and NS specimens from patients to be collected, preserved and transported to laboratory prior to analysis with the Xpert Xpress CoV-2/Flu/RSV *plus* test.

5.2. Device Intended Use

The Xpert® Xpress CoV-2/Flu/RSV *plus* test, performed on the GeneXpert® Dx and GeneXpert® Infinity Systems, 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 disease.

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.

5.3. Substantial Equivalence

Table 5-1 shows the similarities and differences between Xpert Xpress CoV-2/Flu/RSV *plus* and the predicate device, BioFire Respiratory Panel 2.1 (DEN20031).

Table 5-1: Comparison of Similarities and Differences Between Xpert Xpress CoV-2/Flu/RSV *plus* and the Predicate Device

Attribute	Investigational Device	Predicate Device – DEN200031
	Cepheid **Xpert® Xpress CoV-2/Flu/RSV *plus***	**BioFire Diagnostics, LLC** **BioFire Respiratory Panel 2.1**
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/ Classification Name	Same	QOF Multi-target respiratory specimen nucleic acid test including SARS-CoV-2 and other microbial agents
Device Class	Same	II (Special Controls)
Technology/ Detection	Real-time reverse transcription polymerase chain reaction (RT-qPCR)	Nested multiplex RT-PCR followed by high resolution melting analysis to confirm identity of amplified product
Intended Use	The Xpert® Xpress CoV-2/Flu/RSV *plus* test, performed on the GeneXpert® Dx and GeneXpert® Infinity Systems, 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	The BioFire Respiratory Panel 2.1 (RP2.1) is a PCR-based multiplexed nucleic acid test intended for use with the BioFire® FilmArray® 2.0 or BioFire® FilmArray® Torch Systems for the simultaneous qualitative detection and identification of multiple respiratory viral and bacterial nucleic acids in nasopharyngeal swabs (NPS) obtained from individuals suspected of respiratory tract infections, including COVID-19. Nucleic acids from the respiratory viral and bacterial organisms identified by this test are generally detectable in NPS specimens during the acute phase of infection. The detection and identification of specific viral

Attribute	Investigational Device Cepheid Xpert® Xpress CoV-2/Flu/RSV *plus*	Predicate Device – DEN200031 BioFire Diagnostics, LLC BioFire Respiratory Panel 2.1
	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 disease. 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.	and bacterial nucleic acids from individuals exhibiting signs and/or symptoms of respiratory infection is indicative of the presence of the identified microorganism and aids in the diagnosis of respiratory infection if used in conjunction with other clinical and epidemiological information. The results of this test should not be used as the sole basis for diagnosis, treatment, or other patient management decisions. The following organism types and subtypes are identified using the BioFire RP2.1: • Adenovirus • Coronavirus 229E • Coronavirus HKU1 • Coronavirus NL63 • Coronavirus OC43 • Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) • Human Metapneumovirus • Human Rhinovirus/Enterovirus • Influenza A, including subtypes • H1, H3 and H1-2009 • Influenza B • Parainfluenza Virus 1 • Parainfluenza Virus 2 • Parainfluenza Virus 3 • Parainfluenza Virus 4 • Respiratory Syncytial Virus • *Bordetella parapertussis* • *Bordetella pertussis* • *Chlamydia pneumoniae* • *Mycoplasma pneumoniae* Negative results in the setting of a respiratory illness may be due to infection with pathogens that are not detected by this test, or lower respiratory tract infection that may not be detected by an NPS specimen. Positive results do not rule out co-infection with other organisms. The agent(s) detected by the BioFire RP2.1 may not be the definite cause of disease. Additional laboratory testing (e.g., bacterial and viral culture, immunofluorescence, and radiography) may be necessary when evaluating a patient with possible respiratory tract infection.

Attribute	Investigational Device **Cepheid** **Xpert® Xpress CoV-2/Flu/RSV *plus***	Predicate Device – DEN200031 **BioFire Diagnostics, LLC** **BioFire Respiratory Panel 2.1**
Assay Targets	SARS-CoV-2, Influenza A, Influenza B, RSV viral RNA	• Adenovirus • Coronavirus 229E • Coronavirus HKU1 • Coronavirus NL63 • Coronavirus OC43 • Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) • Human Metapneumovirus • Human Rhinovirus/Enterovirus • Influenza A, including subtypes • H1, H3 and H1-2009 • Influenza B • Parainfluenza Virus 1 • Parainfluenza Virus 2 • Parainfluenza Virus 3 • Parainfluenza Virus 4 • Respiratory Syncytial Virus • *Bordetella parapertussis* • *Bordetella pertussis* • *Chlamydia pneumoniae* • *Mycoplasma pneumoniae*
Specimen Type	• Nasopharyngeal swab (NPS) • Anterior nasal swab (NS)	• Nasopharyngeal swab (NPS)
Transport Media	• Universal Transport Medium (UTM) / Viral Transport Medium (VTM) • eNAT	• Universal Transport Medium (UTM)/ Viral Transport Medium (VTM) • Saline
Test Format	Same	Single Use
Automation	Same	Automated Nucleic Acid Extraction, Detection and Results Interpretation
Assay Results	Same	Qualitative
Internal Control	Sample Processing Control (SPC) Probe Check Control (PCC)	Sample Processing Control PCR and Melt Analysis Control
Instrument Systems	Cepheid GeneXpert® Instrument Systems	BioFire® FilmArray® 2.0 or BioFire® FilmArray® Torch Systems
Time to Result	~ 36 min for sample preparation and RT-PCR	~ 45 minutes

The following performance data (analytical and clinical) were provided in support of the substantial equivalence determination.

5.4. Performance Studies

5.4.1. Analytical Performance

Analytical Sensitivity – Clinical Nasopharyngeal Swab (NPS) Matrix

The analytical sensitivity of the Xpert Xpress CoV-2/Flu/RSV *plus* test was first estimated by using 2 reagent lots and testing limiting dilutions of viruses (NATtrol SARS-CoV-2, 1st World Health Organization (WHO) International Standard for SARS-CoV-2, Flu A H1, Flu A H3 , Flu B Victoria linage, Flu B Yamagata lineage, RSV A and RSV B) in pooled negative clinical NPS-UTM/VTM matrix, following the guidance in Clinical and Laboratory Standards Institute (CLSI) document EP17-A2. The LoD is defined as the lowest concentration for each strain at which 95% (19/20) of replicates yield a positive result. The estimated LoD values as determined by Probit regression analysis were verified using 2 lots of Xpert Xpress CoV-2/Flu/RSV *plus* reagents, by testing 20 replicates per virus/lot combination. The highest (least sensitive) LoD value for the two lots was reported as the final, verified LoD. The verified LoD values for the viruses tested are summarized in **Table 5-2**.

Table 5-2. Xpert Xpress CoV-2/Flu/RSV *plus* Limit of Detection in Clinical NPS-UTM/VTM Matrix

Virus/Strain	LoD Concentration
USA-WA1/2020 (NATtrol)	138 copies/mL
1st WHO International Standard	94 IU/mL
Flu A/Idaho/07/2018	0.007 $TCID_{50}$/mL
Flu A/California/07/2009	0.0022 $TCID_{50}$/mL
Flu A/Hong Kong/45/2019	0.44 FFU/mL
Flu A/Victoria/361/2011	0.05 $TCID_{50}$/mL
Flu B/Washington/2/2019	12.9 $CEID_{50}$/mL
Flu B/Wisconsin/10/2016	2.4 $TCID_{50}$/mL
RSV A/2/Australia/61	0.33 $TCID_{50}$/mL
RSV A/Long/MD/56	0.17 $TCID_{50}$/mL
RSV B/9320/MA/77	0.37 $TCID_{50}$/mL
RSV B/Wash/18537/62	0.2 $TCID_{50}$/mL

Analytical Sensitivity – Clinical Anterior Nasal Swab (NS) Matrix

The analytical sensitivity of the Xpert Xpress CoV-2/Flu/RSV *plus* test in clinical anterior nasal swab (NS) matrix was first estimated by using 2 lots and testing limiting dilutions of viruses (NATtrol SARS-CoV-2, 1st World Health Organization (WHO) International Standard for SARS-CoV-2, Flu A H1, Flu A H3, Flu B Victoria linage, Flu B Yamagata lineage, RSV A and RSV B) in pooled negative clinical NS UTM/VTM matrix, following the guidance in Clinical and Laboratory Standards Institute (CLSI) document EP17-A2. The estimated LoD values as determined by Probit regression analysis were verified using 2 lots of Xpert Xpress CoV-2/Flu/RSV *plus* reagents, by testing 20 replicates per virus/lot

combination. The highest (least sensitive) LoD value for the two lots was reported as the final, verified LoD. The verified LoD values for the viruses tested are summarized in **Table 5-3.**

Table 5-3. Xpert Xpress CoV-2/Flu/RSV *plus* Limit of Detection in Clinical NS-UTM/VTM Matrix

Virus/Strain	LoD Concentration
USA-WA1/2020 (NATtrol)	64 copies/mL
1st WHO International Standard	143 IU/mL
Flu A/Idaho/07/2018	0.012 $TCID_{50}$/mL
Flu A/California/07/2009	0.0028 $TCID_{50}$/mL
Flu A/Hong Kong/45/2019	0.49 FFU/mL
Flu A/Victoria/361/2011	0.065 $TCID_{50}$/mL
Flu B/Washington/2/2019	26.3 $CEID_{50}$/mL
Flu B/Wisconsin/10/2016	2.41 $TCID_{50}$/mL
RSV A/2/Australia/61	0.28 $TCID_{50}$/mL
RSV A/Long/MD/56	0.22 $TCID_{50}$/mL
RSV B/9320/MA/77	0.27 $TCID_{50}$/mL
RSV B/Wash/18537/62	0.4 $TCID_{50}$/mL

Analytical Reactivity (Inclusivity)

SARS-CoV-2 in silico Analyses

The inclusivity of Xpert Xpress CoV-2/Flu/RSV *plus* was evaluated using *in silico* analysis of the assay amplicons in relation to SARS-CoV-2 sequences available in the GISAID gene database as of June 15, 2022. The sequences were separated into the lineages of interest based on the Pango Lineage assigned to each genome by GISAID, and those with ambiguous nucleotides were removed. Thus, the following inclusivity analyses focus on the combined, non-ambiguous sequences from the variants of interest and variants of concern as of June 15, 2022. These constituted 10,310,839 sequences for the E target, 10,428,014 sequences for the N2 target, and 10,178,602 sequences for the RdRP target. **Table 5-4** summarizes the effective predicted inclusivity for E, N2 and RdRP amplicons for the variants of interests and concern.

Table 5-4. Predicted Inclusivity for E, N2 and RdRP Amplicons for SARS-CoV-2 Variants of Interests and Concern

Amplicon	Exact Match	1 Mismatch	2 or More Mismatches	% Total <2 Mismatches
CEP-COV-E-PLUS	10,262,080 of 10,310,839 (99.5%)	47,959 (0.5%)	800 (0.01%)	100%
CEP-COV-N2	10,228,739 of 10,428,014 (98.1%)	194,319 (1.9%)	4,956 (0.05%)	99.9%
CEP-COV-RDRP	10,092,873 of 10,178,602 (99.2%)	84,595 (0.8%)	1,134 (0.01%)	100%

a. Single-nucleotide mismatches are predicted to not impact the performance of the test.

Based on the built-in redundancy of the Xpert Xpress CoV-2/Flu/RSV *plus* test's SARS-CoV-2 amplification system (i.e., 3 independent targets, only 1 of 3 must be detected to assign a positive result), it is not anticipated that any of the evaluated SARS-CoV-2 sequences would be missed by the Xpert Xpress CoV-2/Flu/RSV *plus* test.

SARS-CoV-2, Flu A, Flu B, and RSV Inclusivity Wet-Testing

In addition to the *in silico* analysis of the SARS-CoV-2 primers and probes for inclusivity, the inclusivity of the Xpert Xpress CoV-2/Flu/RSV *plus* test was evaluated by bench testing against multiple strains of SARS-CoV-2, 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 concentrations of ~3x LoD in simulated matrix. A total of 102 respiratory viral strains comprised of 18 SARS-CoV-2 strains, 69 influenza viruses (48 influenza A and 21 influenza B) and 15 RSV strains were evaluated for analytical reactivity (inclusivity) with the Xpert Xpress CoV-2/Flu/RSV *plus* test. Three replicates were tested for each strain. All SARS-CoV-2, Flu and RSV strains tested positive in all 3 replicates. Results are shown in **Table 5-5**.

Table 5-5. Analytical Reactivity (Inclusivity) of the Xpert Xpress CoV-2/Flu/RSV *plus* Test

Virus	Strain	Target Conc.	Result			
			SARS-CoV-2	Flu A	Flu B	RSV
SARS-CoV-2	NATtrol SARS-CoV-2 USA-WA1/2020	412 copies/mL	POS	NEG	NEG	NEG
	SARS-CoV-2/HongKong/VM20001061/2020	0.03 $TCID_{50}$/mL	POS[a]	NEG	NEG	NEG
	SARS-CoV-2/Italy-INMI1	1 $TCID_{50}$/mL	POS	NEG	NEG	NEG
	SARS-CoV-2/Africa/KRISPK005325/2020 (Beta)	0.025 $TCID_{50}$/mL	POS	NEG	NEG	NEG
	SARS-CoV-2/England/204820464/2020	0.05 $TCID_{50}$/mL	POS	NEG	NEG	NEG
	SARS-CoV-2 (NY-Wadsworth-21033899-01/2021) P1_2021 (Gamma)	0.01 $TCID_{50}$/mL	POS	NEG	NEG	NEG
	SARS-CoV-2 (NY-Wadsworth-21006055-01/2021) P2_2021 (Zeta)	0.03 $TCID_{50}$/mL	POS	NEG	NEG	NEG
	SARS-CoV-2 (NYWadsworth-21025952-01/2021) B.1.526_2021 (Iota)	0.1 $TCID_{50}$/mL	POS	NEG	NEG	NEG
	SARS-CoV-2 (NY-Wadsworth-103677-01/2020) B.1_2020	0.003 $TCID_{50}$/mL	POS	NEG	NEG	NEG
	SARS-CoV-2 (NY-Wadsworth-33126-01/2020) B.1.595_2020	0.0015 $TCID_{50}$/mL	POS	NEG	NEG	NEG
	SARS-CoV-2 (USA/CA-Stanford-15_S02/2021) B.1.617.1 (Kappa)	1.7 $TCID_{50}$/mL	POS	NEG	NEG	NEG
	SARS-CoV-2 (USA/PHC658/2021) B.1.617.2 (Delta)	0.01 $TCID_{50}$/mL	POS	NEG	NEG	NEG
	SARS-CoV-2 (USA/MDHP01542/2021) B.1.351 (Beta)	100 (genome equivalents/mL)	POS	NEG	NEG	NEG
	SARS-CoV-2 (USA/GA-EHC-2811C/20221) B.1.1.529 (Omicron)	100 (genome equivalents/mL)	POS	NEG	NEG	NEG

Virus	Strain	Target Conc.	Result			
			SARS-CoV-2	Flu A	Flu B	RSV
	SARS-CoV-2 RNA, USA/WA2/2020 (C09)[b]	100 copies/mL	POS	NEG	NEG	NEG
	SARS-CoV-2 RNA,England/205041766/2020 (C14) (alpha)[b]	100 copies/mL	POS	NEG	NEG	NEG
	SARS-CoV-2 RNA, England/MILK-9E05B3/2020 (C15) (alpha)[b]	200 copies/mL	POS	NEG	NEG	NEG
	SARS-CoV-2 RNA /Japan (Brazil)/IC-0564/2021 (C17) (gamma)[b]	100 copies/mL	POS	NEG	NEG	NEG
Flu A H1N1 (pre-2009)	A/swine/Iowa/15/30	10 $TCID_{50}$/mL	NEG	POS	NEG	NEG
	A/WS/33	0.6 $CEID_{50}$/mL	NEG	POS	NEG	NEG
	A/PR/8/34	1.25 $CEID_{50}$/mL	NEG	POS	NEG	NEG
	A/Mal/302/54	0.156 $CEID_{50}$/mL	NEG	POS	NEG	NEG
	A/Denver/1/57	1.5 $CEID_{50}$/mL	NEG	POS	NEG	NEG
	A/New Jersey/8/76	5 $CEID_{50}$/mL	NEG	POS	NEG	NEG
	A/New Caledonia/20/1999	0.10 $TCID_{50}$/mL	NEG	POS	NEG	NEG
	A/New York/55/2004	9 $TCID_{50}$/mL	NEG	POS	NEG	NEG
	A/Solomon Island/3/2006	0.0159$TCID_{50}$/mL	NEG	POS	NEG	NEG
	A/Taiwan/42/06	0.002 $TCID_{50}$/mL	NEG	POS	NEG	NEG
	A/Brisbane/59/2007	0.008 $TCID_{50}$/mL	NEG	POS	NEG	NEG
	A/Swine/NY/02/2009	3.2 $TCID_{50}$/mL	NEG	POS	NEG	NEG
Flu A H1N1 (pdm 2009)	A/Colorado/14/2012	0.04 $TCID_{50}$/mL	NEG	POS	NEG	NEG
	A/Michigan/45/2015	15 $CEID_{50}$/mL	NEG	POS	NEG	NEG
	A/Iowa/53/2015	6 $CEID_{50}$/mL	NEG	POS	NEG	NEG
	A/Michigan/272/2017	0.07 $TCID_{50}$/mL	NEG	POS	NEG	NEG
	A/Idaho/07/2018	0.0159$TCID_{50}$/mL	NEG	POS	NEG	NEG
	A/Wisconsin/505/2018	0.08 $TCID_{50}$/mL	NEG	POS	NEG	NEG
	A/Hawaii/66/2019	100 $CEID_{50}$/mL	NEG	POS	NEG	NEG
	A/Indiana/02/2020	NA[c]	NEG	POS	NEG	NEG
Flu A H3N2	A/Aichi/2/68	2 $CEID_{50}$/mL	NEG	POS	NEG	NEG
	A/Hong Kong/8/68	0.25 $CEID_{50}$/mL	NEG	POS	NEG	NEG
	A/Port Chalmers/1/73	8 $CEID_{50}$/mL	NEG	POS	NEG	NEG
	A/Hawaii/15/2001	33 $CEID_{50}$/mL	NEG	POS	NEG	NEG
	A/Wisconsin/67/05c	0.22 $TCID_{50}$/mL	NEG	POS	NEG	NEG
	A/Brisbane/10/2007	0.003 $TCID_{50}$/mL	NEG	POS	NEG	NEG
	A/Minnesota/11/2010	2.4 $CEID_{50}$/mL	NEG	POS	NEG	NEG
	A/Indiana/08/2011	0.02 $TCID_{50}$/mL	NEG	POS	NEG	NEG
	A/Texas/50/2012	0.008 $TCID_{50}$/mL	NEG	POS	NEG	NEG
	A/Alaska/232/2015	2 $CEID_{50}$/mL	NEG	POS	NEG	NEG
	A/Singapore/INFIMH-16-0019/2016	2.5 $CEID_{50}$/mL	NEG	POS	NEG	NEG
	A/Texas/71/2017	1 FFU/mL	NEG	POS	NEG	NEG
	A/Kansas/14/2017	0.15 FFU/mL	NEG	POS	NEG	NEG
	A/Wisconsin/04/2018[d]	0.15 FFU/mL	NEG	POS	NEG	NEG
	A/Arizona/45/2018	2 FFU/mL	NEG	POS	NEG	NEG
	A/Hong Kong/45/2019	0.8 FFU/mL	NEG	POS	NEG	NEG
Avian Flu A[e]	A/Mallard/NY/6750/78 (H2N2)	< 1 pg/uL	NEG	POS	NEG	NEG
	A/duck/Hunan/795/2002 (H5N1)	< 1 pg/uL	NEG	POS	NEG	NEG
	A/Vietnam/1194/2004 (H5N1)	< 1 pg/uL	NEG	POS	NEG	NEG
	A/Anhui/01/2005 (H5N1)	< 1 pg/uL	NEG	POS	NEG	NEG

Virus	Strain	Target Conc.	Result			
			SARS-CoV-2	Flu A	Flu B	RSV
	A/Japanese white eye/Hong Kong/1038/2006 (H5N1)	< 1 pg/uL	NEG	POS	NEG	NEG
	A/mallard/WI/34/75 (H5N2)	< 1 pg/uL	NEG	POS	NEG	NEG
	A/turkey/Massachusetts/3740/ 1965 (H6N2)	0.1 fg/uL	NEG	POS	NEG	NEG
	A/duck/LTC-10-82743 (H7N2)	5 fg/uL	NEG	POS	NEG	NEG
	A/chicken/New Jersey/15086/3 (H7N3)	4 fg/uL	NEG	POS	NEG	NEG
	A/Anhui/1/2013 (H7N9)	0.612 ng/uL	NEG	POS	NEG	NEG
	A/Shanghai/1/2013 (H7N9)	NA[f]	NEG	POS	NEG	NEG
	A/chicken/New Jersey/12220/1997 (H9N2)	0.05 pg/uL	NEG	POS	NEG	NEG
Flu B	B/Lee/40	0.08 PFU/mL	NEG	NEG	POS	NEG
	B/Allen/45	0.25 $CEID_{50}$/mL	NEG	NEG	POS	NEG
	B/GL/1739/54	0.50 $CEID_{50}$/mL	NEG	NEG	POS	NEG
	B/Maryland/1/59	0.2 $CEID_{50}$/mL	NEG	NEG	POS	NEG
	B/Taiwan/2/62	0.7 $CEID_{50}$/mL	NEG	NEG	POS	NEG
	B/Hong Kong/5/72	1 $CEID_{50}$/mL	NEG	NEG	POS	NEG
Flu B (Victoria Lineage)	B/Panama/45/90	0.125 $TCID_{50}$/mL	NEG	NEG	POS	NEG
	B/Malaysia/2506/04	0.001 $TCID_{50}$/mL	NEG	NEG	POS	NEG
	B/Florida/02/06	0.004 $TCID_{50}$/mL	NEG	NEG	POS	NEG
	B/Brisbane/60/2008	0.005 $TCID_{50}$/mL	NEG	NEG	POS	NEG
	B/Maryland/15/2016	0.06 $TCID_{50}$/mL	NEG	NEG	POS	NEG
	B/Colorado/6/2017	0.01 $TCID_{50}$/mL	NEG	NEG	POS	NEG
	B/Hawaii/01/2018	1 $TCID_{50}$/mL	NEG	NEG	POS	NEG
	B/Missouri/12/2018 (NA D197E)	1.2 $TCID_{50}$/mL	NEG	NEG	POS	NEG
	B/Washington/02/2019	60 $TCID_{50}$/mL	NEG	NEG	POS	NEG
Flu B (Yamagata Lineage)	B/Florida/07/2004	0.03 $TCID_{50}$/mL	NEG	NEG	POS	NEG
	B/Florida/04/06	0.03 $TCID_{50}$/mL	NEG	NEG	POS	NEG
	B/Wisconsin/01/2010	0.025 $CEID_{50}$/mL	NEG	NEG	POS	NEG
	B/Wisconsin/10/2016	2 $TCID_{50}$/mL	NEG	NEG	POS	NEG
	B/Indiana/17/2017	0.5 $TCID_{50}$/mL	NEG	NEG	POS	NEG
	B/Oklahoma/10/2018	1 $TCID_{50}$/mL	NEG	NEG	POS	NEG
RSV A	RSV-A/NY	0.386 $TCID_{50}$/mL	NEG	NEG	NEG	POS
	RSV-A/WI-629.8.2/2007	0.50 $TCID_{50}$/mL	NEG	NEG	NEG	POS
	RSV-A/WI/629-11-1_2008	0.50 $TCID_{50}$/mL	NEG	NEG	NEG	POS
	RSV-A, Strain: 4/2015 Isolate #1	0.03 $TCID_{50}$/mL	NEG	NEG	NEG	POS
	RSV-A (2014, Isolate 342)	0.38 IU/mL	NEG	NEG	NEG	POS
	RSV-A (A2 cpts-248 mutant)	1600 copies/mL	NEG	NEG	NEG	POS
	RSV-A (2000/3-4)	0.0015$TCID_{50}$/mL	NEG	NEG	NEG	POS
	RSV-A (2001/3-12)	0.28 $TCID_{50}$/mL	NEG	NEG	NEG	POS
	RSV-A (1997/12-35)	0.5 $TCID_{50}$/mL	NEG	NEG	NEG	POS
	RSV-A (*Homo sapiens*/ARG/177/2006)	0.089 $TCID_{50}$/mL	NEG	NEG	NEG	POS
	RSV-A (1998/3-2)	0.0089$TCID_{50}$/mL	NEG	NEG	NEG	POS
RSV B	RSV-B/WV14617/85	0.04 $TCID_{50}$/mL	NEG	NEG	NEG	POS
	RSV-B-CH93(18)-18-01	0.004 $TCID_{50}$/mL	NEG	NEG	NEG	POS
	RSV-B (12/2014, Isolate #1)	0.008 $TCID_{50}$/mL	NEG	NEG	NEG	POS

Virus	Strain	Target Conc.	Result			
			SARS-CoV-2	Flu A	Flu B	RSV
	RSV-B (cp23 Clone 1A2)	4200 copies/mL	NEG	NEG	NEG	POS

a. One of three replicates was Invalid. The run was successfully repeated to obtain three valid replicates.
b. *In vitro* transcripts from Twist Biosciences.
c. Influenza A/Indiana/02/2020 virus was without titer and the stock was diluted 48,000-fold in simulated matrix for testing.
d. One of three replicates yielded an ERROR result. The run was successfully repeated to obtain three valid replicates.
e. Purified viral RNA in TE and diluted in simulated matrix was tested due to biosafety regulations.
f. Inactivated avian influenza A (H7N9) viral RNA without viral titer was diluted 100,000-fold in simulated matrix for testing due to biosafety regulations.

Analytical Specificity (Exclusivity)

In silico Analyses

An *in silico* analysis for possible cross-reactions with all the organisms listed in **Table 5-6** was conducted by mapping the SARS-CoV-2 oligonucleotides and amplicons in the Xpert Xpress CoV-2/Flu/RSV *plus* test individually to the sequences downloaded from the GISAID database. E gene primers and probes are not specific for SARS-CoV-2 and will detect Human and Bat SARS-coronavirus.

In silico exclusivity analysis using Flu A, Flu B and RSV B primer and probe oligonucleotides against the GenBank database (which encompasses essentially all species) did not identify matches, with at least 80% similarity to each oligonucleotide, for any non-target organism expected to be found in a human respiratory tract sample. RSV A primer and probe oligonucleotides exhibited >80% homology with two Pangolin RSV A isolates. Therefore, the RSV A primer and probe may cross-react with Pangolin RSV A if the strain is circulating in a human population and present in a sample tested with the Xpert Xpress CoV-2/Flu/RSV *plus* test. While there was some homology ≥80% to human genomic DNA, the matches were to different chromosomal regions, and there were no cases where a forward and reverse primer for a specific target matched to the same human genomic DNA fragment.

In silico exclusivity analysis using the five Flu amplicons (Flu A MP, Flu A PB2, Flu A PA, Flu B MP and Flu B NS) against the GenBank database produced no significant matches to non-influenza-related sequences. Similarly, no matches to RSV isolates from other species or to genomic sequences from non-RSV species were observed with the RSV B amplicon. While no matches of the RSV A amplicon to genomic sequences from non-RSV species of ≥80% homology were observed, the RSV A amplicon shared a 95% identity with two Pangolin RSV A isolates.

No cross reactivity with non-SARS-CoV-2, non-influenza and non-RSV viruses listed in **Table 5-6** is expected based on the *in silico* analysis.

Table 5-6. Microorganisms Analyzed in the *in silico* Analysis for the SARS-CoV-2 Target

Microorganisms from the Same Genetic Family	High Priority Organisms
Human coronavirus 229E	**Viruses**
Human coronavirus OC43	Adenovirus (e.g., C1 Ad. 71)
Human coronavirus HKU1	Cytomegalovirus
Human coronavirus NL63	Enterovirus (e.g., EV68)
SARS-coronavirus	Epstein-Barr virus
MERS-coronavirus	Human Metapneumovirus (hMPV)
Bat coronavirus	Influenza A & B
	Measles
	Mumps
	Parainfluenza virus 1-4
	Parechovirus
	Respiratory syncytial virus
	Rhinovirus
	Bacteria
	Bacillus anthracis
	Bordetella pertussis
	Bordetella parapertussis
	Candida albicans
	Chlamydia pneumoniae
	Chlamydia psittaci
	Corynebacterium diphtheriae
	Coxiella burnetii (Q-Fever)
	Escherichia coli
	Fusobacterium necrophorum
	Haemophilus influenzae
	Lactobacillus sp.
	Legionella non-*pneumophila*
	Legionella pneumophila
	Leptospira
	Moraxella catarrhalis
	Mycobacterium tuberculosis
	Mycoplasma genitalium
	Mycoplasma pneumoniae
	Neisseria elongata
	Neisseria meningitidis
	Pneumocystis jirovecii (PJP)
	Pseudomonas aeruginosa
	Staphylococcus aureus
	Staphylococcus epidermidis
	Staphylococcus salivarius
	Streptococcus pneumoniae
	Streptococcus pyogenes

Microorganisms from the Same Genetic Family	High Priority Organisms
	Fungi
	Aspergillus sp

Wet Testing

In addition to the *in silico* analysis of the SARS-CoV-2, influenza A, influenza B, and RSV oligonucleotides and amplicons for cross-reactivity, the analytical specificity of the Xpert Xpress CoV-2/Flu/RSV *plus* test was evaluated by bench testing a panel of 48 microorganisms, comprising 4 human coronaviruses, 1 MERS coronavirus and 43 common respiratory pathogens or those potentially encountered in the nasopharynx. The panel was tested in different pools of microorganisms in simulated matrix; if a pool produced a positive result, then each member of the pool would have been tested individually. Three replicates of each pool were tested. A sample was considered negative if all three replicates were negative. The bacterial and yeast strains were tested at concentrations of $\geq 1 \times 10^6$ CFU/mL except for *Chlamydia pneumoniae* which was tested at 1.2×10^6 IFU/mL and *Lactobacillus reuteri* which was tested at 5×10^7 copies/mL of genomic DNA. Viruses were tested at concentrations of $\geq 1 \times 10^5$ $TCID_{50}$/mL. The analytical specificity was 100%. Results are shown in **Table 5-7**.

Table 5-7. Respiratory Microorganisms and Human Coronavirus Tested, Concentrations and Xpert Xpress CoV-2/Flu/RSV *plus* Test Results

Count	Strain	Tested Concentration	SARS-CoV-2	Flu A	Flu B	RSV
0	Negative Control	NA	NEG	NEG	NEG	NEG
00	Positive Control (NATFRC-6C)	NA	POS	POS	POS	POS
1	Human coronavirus NL63	1.17e5 $TCID_{50}$/mL	NEG	NEG	NEG	NEG
2	MERS-coronavirus	1.17e5 $TCID_{50}$/mL	NEG	NEG	NEG	NEG
3	Human coronavirus 229E	1.21e5 $TCID_{50}$/mL	NEG	NEG	NEG	NEG
4	Human coronavirus OC43	1.02e5 $TCID_{50}$/mL	NEG	NEG	NEG	NEG
5	Human coronavirus HKU1[a]	1.23e6 copies/mL	NEG	NEG	NEG	NEG
6	Adenovirus Type 1	4.07e5 $TCID_{50}$/mL	NEG	NEG	NEG	NEG
7	Adenovirus Type 7	1.15e5 $TCID_{50}$/mL	NEG	NEG	NEG	NEG
8	Cytomegalovirus	1.0e5 $TCID_{50}$/mL	NEG	NEG	NEG	NEG
9	Echovirus	1.14e5 $TCID_{50}$/mL	NEG	NEG	NEG	NEG
10	Enterovirus	2.80e5 $TCID_{50}$/mL	NEG	NEG	NEG	NEG
11	Epstein Barr Virus	5.60e6 $TCID_{50}$/mL	NEG	NEG	NEG	NEG
12	HSV	1.97e5 $TCID_{50}$/mL	NEG	NEG	NEG	NEG
13	Human metapneumovirus	4.07e5 $TCID_{50}$/mL	NEG	NEG	NEG	NEG
14	Human parainfluenza Type 1	1.0e5 $TCID_{50}$/mL	NEG	NEG	NEG	NEG
15	Human parainfluenza Type 2	1.2e5 $TCID_{50}$/mL	NEG	NEG	NEG	NEG
16	Human parainfluenza Type 3	1.2e5 $TCID_{50}$/mL	NEG	NEG	NEG	NEG
17	Human parainfluenza Type 4	1.19e6 $TCID_{50}$/mL	NEG	NEG	NEG	NEG
18	Measles	1.2e5 $TCID_{50}$/mL	NEG	NEG	NEG	NEG
19	Mumps virus	1.2e5 $TCID_{50}$/mL	NEG	NEG	NEG	NEG
20	Rhinovirus Type 1A	1.0e5 $TCID_{50}$/mL	NEG	NEG	NEG	NEG
21	*Acinetobacter baumannii*	1.30e7 CFU/mL	NEG	NEG	NEG	NEG
22	*Bordetella pertussis*	6.40e7 CFU/mL	NEG	NEG	NEG	NEG
23	*Burkholderia cepacia*	1.90e8 CFU/mL	NEG	NEG	NEG	NEG
24	*Candida albicans*	6.30e6 CFU/mL	NEG	NEG	NEG	NEG

Count	Strain	Tested Concentration	SARS-CoV-2	Flu A	Flu B	RSV
25	*Candida parapsilosis*	1.45e6 CFU/mL	NEG	NEG	NEG	NEG
26	*Citrobacter freundii*	1.73e8 CFU/mL	NEG	NEG	NEG	NEG
27	*Corynebacterium sp.*	1.27e7 CFU/mL	NEG	NEG	NEG	NEG
28	*Enterococcus faecalis*	5.87e7CFU/mL	NEG	NEG	NEG	NEG
29	*Escherichia coli*	1.55e8 CFU/mL	NEG	NEG	NEG	NEG
30	*Hemophilus influenzae*	6.62e6 CFU/mL	NEG	NEG	NEG	NEG
31	*Lactobacillus reuteri* [b]	5.0e7 copies/mL	NEG	NEG	NEG	NEG
32	*Legionella pneumophila*	1.42e8 CFU/mL	NEG	NEG	NEG	NEG
33	*Moraxella catarrhalis*	2.46e6 CFU/mL	NEG	NEG	NEG	NEG
34	*Mycoplasma pneumoniae*	2.7e6 CFU/mL	NEG	NEG	NEG	NEG
35	*Neisseria meningitides*	4.2e6 CFU/mL	NEG	NEG	NEG	NEG
36	*Neisseria mucosa*	1.0e8 CFU/mL	NEG	NEG	NEG	NEG
37	*Propionibacterium acnes*	8.25e7 CFU/mL	NEG	NEG	NEG	NEG
38	*Pseudomonas aeruginosa*	1.05e7 CFU/mL	NEG	NEG	NEG	NEG
39	*Staphylococcus haemolyticus*	2.66e6 CFU/mL	NEG	NEG	NEG	NEG
40	*Staphylococcus aureus*	5.87e7 CFU/mL	NEG	NEG	NEG	NEG
41	*Staphylococcus epidermidis*	2.47e7 CFU/mL	NEG	NEG	NEG	NEG
42	*Streptococcus agalactiae*	1.75e7 CFU/mL	NEG	NEG	NEG	NEG
43	*Streptococcus pneumoniae*	2.26e7 CFU/mL	NEG	NEG	NEG	NEG
44	*Streptococcus pyogenes*	9.0e6 CFU/mL	NEG	NEG	NEG	NEG
45	*Streptococcus salivarius*	4.19e6 CFU/mL	NEG	NEG	NEG	NEG
46	*Streptococcus sanguinis*	8.67e6 CFU/mL	NEG	NEG	NEG	NEG
47	*Chlamydia pneumoniae*	1.20e6 CFU/mL	NEG	NEG	NEG	NEG
48	*Mycobacterium tuberculosis* (avirulent)	1.20e6 CFU/mL	NEG	NEG	NEG	NEG

NA – Not Applicable
[a]Live virus was not available. Synthetic RNA was used.
[b]Live organism was not available. Genomic DNA was used.

Microbial Interference

Microbial interference of the Xpert Xpress CoV-2/Flu/RSV *plus* test caused by the presence of bacterial or viral strains that might be encountered in human upper respiratory tract specimens, was evaluated by testing a panel of 10 potentially interfering microorganisms, consisting of 7 viral strains and 3 bacterial strains. Contrived samples consisted of SARS-CoV-2, Flu A, Flu B, RSV A, or RSV B viruses seeded at 3x the Limit of Detection (LoD) into simulated nasopharyngeal swab (NPS)/nasal swab (NS) matrix in the presence of Adenovirus Type 1C, Human Coronavirus OC43, Rhinovirus Type 1A, Human metapneumovirus, Human parainfluenza Types 1, 2, and 3 (each seeded at $1x10^5$ $TCID_{50}$/mL), *Hemophilus influenzae* (seeded at $1x10^6$ CFU/mL), *Staphylococcus aureus* or *Staphylococcus epidermidis* (each seeded at $1x10^7$ CFU/mL).

Eight (8) replicates of positive samples were tested for each target virus (SARS-CoV-2, Flu A, Flu B, RSV A, or RSV B) and each potential microbial interference strain combination. For each target, all 8 of 8 replicate samples were correctly identified using the Xpert Xpress CoV-2/Flu/RSV *plus* test. No microbial interference by the viral or bacterial strains was reported.

Competitive Interference

Competitive interference of the Xpert Xpress CoV-2/Flu/RSV *plus* caused by co-infections were evaluated by testing contrived samples of individual SARS-CoV-2, Flu A, Flu B or RSV strains at 3x LoD in the presence of different target strains at a higher concentration in a simulated background matrix. The concentration at 3x LoD was 414 copies/mL for SARS-CoV-2 (inactivated USA-WA1/2020); 0.021 $TCID_{50}$/mL for Flu A/Idaho/072018, 38.7 $CEID_{50}$/mL for Flu B/Washington/2/2019; 0.99 $TCID_{50}$/mL for RSV A/2/Australia/61), and 1.11 $TCID_{50}$/mL for RSV B/9320/MA/77. The competitive strains were evaluated at $\geq 10^5$ RNA copies/mL, as determined by droplet digital PCR (ddPCR).

Replicates of 3 were tested for each target strain and each competitive strain combination. The virus at high concentration shows no competitive inhibitory effects if 3 of 3 replicates for the target strain report positive results. If the results reported less than 3 of 3 positive replicates, the concentration of the competing virus was reduced by 10-fold increments until no interference was observed. The results for competitive interference study are presented in **Tables 5-8** through **5-12** for high concentration of Flu A, Flu B, RSV A, RSV B and SARS-CoV-2, respectively.

Table 5-8. Summary of Competitive Interference Study with Flu A at High Concentration

Test Viruses at 3X LoD	Interferent Virus	Correct Calls (n/3)			
		at 1.7e8 RNA copies/mL	at 1.7e7 RNA copies/mL	at 1.7e6 RNA copies/mL	at 1.7e5 RNA copies/mL
Flu B	Flu A	0/3	0/3	2/3	3/3
RSV A		0/3	0/3	3/3	Not tested
RSV B		3/3	Not tested	Not tested	Not tested
SARS-CoV-2		3/3	Not tested	Not tested	Not tested

Table 5-9. Summary of Competitive Interference Study with Flu B at High Concentration

Test Viruses at 3X LoD	Interferent Virus	Correct Calls (n/3) at 1.4e5 RNA copies/mL
Flu A	Flu B	3/3
RSV A		3/3
RSV B		3/3
SARS-CoV-2		3/3

Table 5-10. Summary of Competitive Interference Study with RSV A at High Concentration

Test Viruses at 3X LoD	Interferent Virus	Correct Calls (n/3) at 4.6e6 RNA copies/mL
Flu A	RSV A	3/3
Flu B		3/3
SARS-CoV-2		3/3

Table 5-11. Summary of Competitive Interference Study with RSV B at High Concentration

Test Viruses at 3X LoD	Interferent Virus	Correct Calls (n/3) at 1.9e5 RNA copies/mL
Flu A	RSV B	3/3
Flu B		3/3
SARS-CoV-2		3/3

Table 5-12. Summary of Competitive Interference Study with SARS-CoV-2 at High Concentration

Test Viruses at 3X LoD	Interferent Virus	Correct Calls (n/3)	
		at 1e6 RNA copies/mL	at 1e5 RNA copies/mL
Flu A	SARS-CoV-2	3/3	Not tested
Flu B		1/3	3/3
RSV A		3/3	Not tested
RSV B		3/3	Not tested

The study showed that Flu A/Idaho/07/2018 at concentrations above 1.7e5 RNA copies/mL inhibited detection of Flu B at 3x LoD, and at concentrations above 1.7e6 RNA copies/mL inhibited detection of RSV A at 3x LoD (**Table 5-8**). In addition, SARS-CoV-2 at concentrations above 1e5 RNA copies/mL inhibited detection of Flu B at 3x LoD (**Table 5-12**). No other competitive interference was observed for the potential co-infections evaluated in the study at the concentrations tested.

Potentially Interfering Substances

Substances that are normally found in or may be introduced into clinical NPS or NS matrix that could potentially interfere with accurate detection of SARS-CoV-2, Flu A, Flu B and RSV were evaluated with direct testing on the Xpert Xpress CoV-2/Flu/RSV *plus*.

Potentially interfering substances in the nasal passage and 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. Positive and negative samples were prepared in simulated nasopharyngeal swab (NPS)/ nasal swab (NS) matrix. Negative samples (N = 8) were tested in the presence of each substance to determine the effect on the performance of the sample processing control (SPC). Positive samples (N = 8) were tested per substance with viruses spiked at 3x the LoD determined for each strain. Positive samples tested with the Xpert Xpress CoV-2/Flu/RSV *plus* included one SARS-CoV-2, two influenza A H1N1, two influenza A H3N2, two influenza B and two RSV (RSV A and RSV B) strains. The substances, with active ingredients and test concentrations, that were evaluated are listed in **Table 5-13**.

Table 5-13. Potentially Interfering Substances Tested

Substance ID	Substance/Class	Substance/Active Ingredient	Concentrations Tested
No substance	Control	Simulated NPS/NS Matrix	100% (v/v)
Albuterol Sulfate	Beta-adrenergic bronchodilator	Albuterol Sulfate (5mg/mL)	0.83 mg/mL (equivalent to 1 dose per day)
Afrin	Nasal Spray	Oxymetazoline, 0.05%	15% (v/v)
BD Universal Transport Medium	Transport Media	N/A	100% (v/v)
Blood	Blood	Blood (Human)	2% (v/v)
Copan Swab M	Transport Media	N/A	100% (v/v)
FluMist Quadrivalent	Vaccine	Live attenuated influenza viruses	6.7e-4% (v/v)
			6.7e-6% (v/v)
			6.7e-7% (v/v)
Fluticasone Propionate Nasal Spray	Nasal corticosteroid	Fluticasone Propionate	5 µg/mL
Human peripheral blood mononuclear cells	Human cells	PBMC	1 x 10^6 cells/mL
			0.5 x 10^6 cells/mL
			0.25 x 10^6 cells/mL
Ibuprofen	Nonsteroidal anti-inflammatory drug	Ibuprofen 200 mg/tablet	5% w/v
Menthol	Throat lozenges, oral anesthetic and analgesic	Benzocaine, Menthol	1.7 mg/mL
Mucin	Mucin	Purified Mucin protein (Bovine or porcine submaxillary gland)	0.1 (w/v)
Mupirocin	Antibiotic, nasal ointment	Mupirocin (20 mg/g = 2%)	10 mg/mL
PHNY	Nasal Drops	Phenylephrine, 1%	15% (v/v)
Remel M4RT	Transport Media	N/A	100% (v/v)
Remel M5	Transport Media	N/A	100% (v/v)
Saline	Saline Nasal Spray	Sodium Chloride (0.65%)	15% (v/v)
Snuff	Tobacco product	Nicotine	1% (w/v)
			0.5% (w/v)
			0.25% (w/v)
			0.1% (w/v)
Tamiflu	Anti-viral drugs	Zanamivir	7.5 mg/mL
Tobramycin	Antibacterial, systemic	Tobramycin	4 µg/mL
Zicam	Nasal Gel	Luffa opperculata, Galphimia glauca, Histaminum hydrochloricum Sulfur (0.05%)	15% (w/v)
			7.5% (w/v)
Zinc	Zinc supplement	Zinc Gluconate	0.1 µg/mL

The results from the study (**Table 5-14**) show that for most cases, 8 out of 8 replicates reported positive results for each combination of virus and substance tested and no interference was observed. In the presence of FluMist at 6.7e-4% (v/v), interfering effects were observed when testing SARS-CoV-2, RSV A and RSV B strains. Inhibitory effects were not observed when testing these viruses in the presence of FluMist at 6.7e-6% (v/v) except for RSV A/Long/MD/56. For RSV A/Long/MD/56, the inhibitory effect was not observed when FluMist concentration was further reduced to 6.7e-7% (v/v). In the presence of human PBMC at 1 x 10^6 cells/mL, interfering effects were observed when testing Flu B/Washington /2/2019. Inhibitory effects were not observed when the PBMC concentration was reduced to 2.5 x 10^5 cells/mL. In the presence of snuff at 1% (w/v), interfering effects were observed when testing Flu A /California/07/2009 and Flu B/Washington/2/2019. Inhibitory effects were not observed when testing the viruses at a snuff concentration of 0.1% (w/v). In the presence of Zicam at 15% (w/v), interfering effects were observed when testing Flu A, Flu B and RSV A strains. Inhibitory effects were not observed when testing the viruses in the presence of Zicam at 7.5% (w/v).

Table 5-14. Number of Correct Results for Xpert Xpress CoV-2/Flu/RSV *plus* Targets Tested in the Presence of Potentially Interfering Substances

Substance	Concentration Tested	Number of Correct Results/Number Tested for Each Virus and the No Virus Control											
		No Virus Control	**SARS-CoV-2 USA-WA-1**	**Flu A California/7/2009**	**Flu A Idaho/07/2018**	**Flu A Hong Kong/ 45/2019**	**Flu A/ Victoria/361/2011**	**Flu B Wisconsin/10/2016**	**Flu B Washington/02/2019**	**RSV A 2/Australia/61**	**RSV A Long/MD/56**	**RSV B 9320/MA/77**	**RSV B WA/18537/62**
Control Simulated NPS/NS Matrix (No substance)	100% (v/v)	32/32[a]	24/24	24/24	16/16	16/16	24/24[b]	24/24	32/32	32/32[b]	32/32	24/24	24/24
Albuterol Sulfate	0.83 mg/mL	16/16	8/8	8/8	8/8	8/8	8/8	8/8	8/8	8/8	8/8	8/8	8/8
Afrin	15% (v/v)	16/16	8/8	8/8[b]	8/8	8/8	8/8	8/8	8/9[c]	8/8	8/8	8/8	8/8
BD Universal Transport Medium	100% v/v	16/16	8/8	8/8	8/8	8/8	8/8[b]	8/8	8/8	8/8	8/8	8/8	8/8
Blood	2% (v/v)	16/16	8/8	8/8	8/8	8/8	8/8	8/8[a]	8/8	8/8[b]	8/8[b]	8/8	8/8
Copan Swab M	100% (v/v)	16/16	8/8	8/8	8/8	8/8	8/8	8/8	8/8	8/8	8/8	8/8	8/8
FluMist	6.7% (v/v)	8/8	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
	6.7e-4% (v/v)	N/A	**7/8**	N/A	N/A	N/A	N/A	N/A	N/A	**0/8**	**0/8**	**2/8**	**0/8**
	6.7e-6% (v/v)	N/A	8/8	N/A	N/A	N/A	N/A	N/A	N/A	8/8	**7/8**	8/8[b]	8/8
	6.7e-7% (v/v)	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	8/8[a]	N/A	N/A
Fluticasone Propionate Nasal Spray	5 µg/mL	16/16	8/8	8/8	8/8	8/8	8/8	8/8[a, b]	8/8	8/8[d]	8/8	8/8[a]	8/8
Human peripheral blood mononuclear cells	1e6 cells/mL	8/8	8/8	8/8[b]	8/8[b]	8/8	8/8	8/8	**6/8**	8/8[b]	8/8	8/8[b]	8/8[b]
	0.5e6 cells/mL	N/A	N/A	N/A	N/A	N/A	N/A	N/A	**7/8**	N/A	N/A	N/A	N/A
	0.25e6 cells/mL	N/A	N/A	N/A	N/A	N/A	N/A	N/A	8/8	N/A	N/A	N/A	N/A
Ibuprofen	5% (w/v)	8/8	8/8	8/8	8/8	8/8	8/8	8/8	8/8	8/8	8/8	8/8	8/8

Substance	Concentration Tested	Number of Correct Results/Number Tested for Each Virus and the No Virus Control											
		No Virus Control	**SARS-CoV-2 USA-WA-1**	**Flu A California/7/2009**	**Flu A Idaho/07/2018**	**Flu A Hong Kong/ 45/2019**	**Flu A/ Victoria/361/2011**	**Flu B Wisconsin/10/2016**	**Flu B Washington/02/2019**	**RSV A 2/Australia/61**	**RSV A Long/MD/56**	**RSV B 9320/MA/77**	**RSV B WA/18537/62**
Menthol	1.7 mg/mL	16/16[a]	8/8	8/8	8/8	8/8[a]	8/8	8/8[b]	8/8	8/8	8/8[b]	8/8	8/8
Mucin	0.1% (w/v)	16/16	8/8	8/8	8/8	8/8	8/8	8/8	8/8	8/8	8/8[a, b]	8/8	8/8
Mupirocin	10 mg/mL	16/16	8/8	8/8	8/8	8/8	8/8	8/8	8/8	8/8	8/8	8/8	8/8
PHNY	15% (v/v)	16/16	8/8	8/8	8/8	8/8	8/8	8/8	8/8	8/8	8/8	8/8	8/8
Remel M4RT	100% (v/v)	16/16[a]	8/8	8/8	8/8	8/8	8/8	8/8	8/8	8/8	8/8	8/8	8/8
Remel M5	100% (v/v)	16/16	8/8	8/8	8/8	8/8	8/8	8/8	8/8	8/8	8/8	8/8	8/8
Saline	15% (v/v)	16/16	8/8	8/8[a]	8/8	8/8	8/8	8/8	8/8	8/8	8/8	8/8	8/8[a]
Snuff	1% (w/v)	8/8	8/8	**6/8**	8/8	8/8[b]	8/8	8/8	**4/8**[b]	8/8	8/8	8/8	8/8[e]
	0.5% (w/v)	N/A	N/A	**7/8**	N/A	N/A	N/A	N/A	**3/8**	N/A	N/A	N/A	N/A
	0.25 % (w/v)	N/A	N/A	8/8	N/A	N/A	N/A	N/A	**7/8**	N/A	N/A	N/A	N/A
	0.1 % (w/v)	N/A	N/A	N/A	N/A	N/A	N/A	N/A	8/8	N/A	N/A	N/A	N/A
Tamiflu	7.5 mg/mL	16/16[a]	8/8	8/8	8/8	8/8	8/8	8/8	8/8	8/8	8/8	8/8	8/8
Tobramycin	4 µg/mL	16/16	8/8	8/8	8/8	8/8	8/8	8/8	8/8	8/8	8/8	8/8	8/8
Zicam	15% (w/v)	16/16	8/8	**7/8**	8/8	8/8	8/8	8/8[a]	**5/8**	**7/8**	8/8	8/8	8/8
	7.5% (w/v)	N/A	N/A	8/8	N/A	N/A	N/A	N/A	8/8	8/8	N/A	N/A	N/A
Zinc	0.1µg/mL	16/16	8/8	8/8	8/8	8/8	8/8	8/8	8/8	8/8	8/8	8/8	8/8

BOLD: False negative or INVALID results indicating interference from the substance

a. One replicate reported NO RESULT. The run was successfully repeated to obtain the required number of valid replicates.

b. One replicate reported ERROR. The run was successfully repeated to obtain the required number of valid replicates

c. One of 8 replicates reported a Flu B NEGATIVE result. The Flu B Probe check signals were reduced in this sample suggesting an issue with the EZR bead. The test was repeated and gave a Flu B positive result.

d. One of 8 replicates reported INVALID. The run was successfully repeated to obtain 8 valid replicates

e. Two of 8 replicates reported ERROR. The 2 runs were successfully repeated to obtain 8 valid replicates.

Carryover Contamination

A study was conducted to assess whether the single-use, self-contained Xpert Xpress CoV-2/Flu/RSV *plus* cartridge prevents specimen and amplicon carryover by testing a negative sample immediately after testing of a very high positive sample in the same GeneXpert module. The negative sample used in this study consisted of simulated NPS/NS matrix and the positive sample consisted of high Flu B and high SARS-CoV-2 virus concentrations (Flu B/Wisconsin/10/2016 at 1.0e6 $TCID_{50}$/mL and inactivated SARS-CoV-2 USA-WA1/2020 at 1e4 copies/mL) seeded into simulated NPS/NS matrix. The negative sample was tested in a GeneXpert module at the start of the study. Following the initial testing of the negative sample, the high positive sample was processed in the same GeneXpert module immediately followed by another negative sample. This was repeated 20 times in the same module, resulting in 20 positives and 21 negatives for the module. The study was repeated using a second GeneXpert module for a total of 40 positive and 42 negative samples. All 40 positive samples were correctly reported as **SARS-CoV-2 POSITIVE; Flu A NEGATIVE; Flu B POSITIVE; RSV NEGATIVE.** All 42 negative samples were correctly reported as **SARS-CoV-2 NEGATIVE**; Flu A NEGATIVE; **Flu B NEGATIVE; RSV NEGATIVE** with the Xpert Xpress CoV-2/Flu/RSV *plus* test. No specimen or amplicon carry-over contamination was observed in this study.

Single-Site Precision

The precision of the Xpert Xpress CoV-2/Flu/RSV *plus* test was established at a single site using a 9-member panel including one negative sample, 4 low positive (~1.5x LoD) samples and 4 moderate positive (~3x LoD) samples. The negative sample consisted of simulated matrix without target microorganism or target RNA. The positive samples were contrived using inactivated NATtrol SARS-CoV-2 (ZeptoMetrix, Buffalo, NY, catalog number NATSARS(COV2)-ST), and cultured viruses Influenza A/Idaho/07/2018, Influenza B/Wisconsin/10/2016, and RSV B/Wash/18537/62 in a simulated NPS/NS matrix.

Testing was conducted over 20 days, using 1 lot of Xpert Xpress CoV-2/Flu/RSV *plus* cartridges at a single site and with 1 operator to yield a total of 80 observations per panel member (1 Site x 1 Operator x 1 Lot x 20 Days x 2 Runs x 2 Replicates = 80 observations/panel member). The results from the study are summarized in **Table 5-15**.

Table 5-15. Summary of the Precision Results by Panel Member – % Agreement

Panel Member	Agreement	% Agreement (95% CI)
Negative	80/80	100% (95.4%-100%)
SARS-CoV-2 Low Positive (~1.5x LoD)	79/80	98.8% (93.3%-99.8%)
SARS-CoV-2 Moderate Positive (~3x LoD)	80/80	100% (95.4%-100%)
Flu A Low Positive (~1.5x LoD)	78/80	97.5% (91.3%-99.3%)
Flu A Moderate Positive (~3x LoD)	80/80	100% (95.4%-100%)
Flu B Low Positive (~1.5x LoD)	77/80	96.3% (89.5%-98.7%)
Flu B Moderate Positive (~3x LoD)	80/80	100% (95.4%-100%)
RSV Low Positive (~1.5x LoD)	78/80	97.5% (91.3%-99.3%)
RSV Moderate Positive (~3x LoD)	80/80	100% (95.4%-100%)

Reproducibility

The reproducibility of the Xpert Xpress CoV-2/Flu/RSV *plus* test was established at 3 sites (2 external and 1 internal) using a 9-member panel including 1 negative sample, 4 low positive (~1.5x LoD) and 4 moderate positive (~3x LoD) samples. The negative sample consisted of simulated matrix without target microorganism or target RNA. The positive samples were contrived using inactivated NATtrol SARS-CoV-2 (ZeptoMetrix), cultured viruses Influenza A/ Idaho/07/2018, Influenza B/Wisconsin/ 10/2016, and RSV B/Wash/18537/62 in a simulated NPS/NS matrix. Testing was conducted over 6 days, using 3 lots of Xpert Xpress CoV-2/Flu/RSV *plus* cartridges at 3 participating sites each with 2 operators to yield a total of 144 observations per panel member (3 Sites x 2 Operators x 3 Lots x 2 Days/Lot x 2 Runs x 2 Replicates = 144 observations/panel member). The results from the study are summarized in **Tables 5-16** and **5-17**.

The percent agreement of the correct results compared to the expected results analyzed by each of the 6 operators and each site is shown in **Table 5-17**. In addition, the overall percent agreement for each sample (% total agreement) and the two-sided Wilson Score confidence intervals (CI) are presented in the last column.

Table 5-16. Summary of the Reproducibility Results – % Agreement

Sample	Site 1			Site 2			Site 3			% Total Agreement [95% CI]
	Op 1	Op 2	Site	Op 1	Op 2	Site	Op 1	Op 2	Site	
Negative	100% 24/24	100% 24/24	100% 48/48	100% 24/24	100% 24/24	100% 48/48	100% 24/24	100% 24/24	100% 48/48	100% (144/144) [97.4-100.0]
SARS-CoV-2 Low Pos	100% 24/24	100% 24/24	100% 48/48	100% 24/24	100% 24/24	100% 48/48	100% 24/24	100% 24/24	100% 48/48	100% (144/144) [97.4-100.0]
SARS-CoV-2 Mod Pos	100% 24/24	100% 24/24	100% 48/48	100% 24/24	100% 24/24	100% 48/48	100% 24/24	100% 24/24	100% 48/48	100% (144/144) [97.4-100.0]
Flu A Low Pos	100% 24/24	100% 24/24	100% 48/48	100% 24/24	100% 24/24	100% 48/48	100% 24/24	100% 24/24	100% 48/48	100% (144/144) [97.4-100.0]
Flu A Mod Pos	100% 24/24	100% 24/24	100% 48/48	100% 24/24	100% 24/24	100% 48/48	100% 24/24	100% 24/24	100% 48/48	100% (144/144) [97.4-100.0]
Flu B Low Pos	100% 24/24	100% 24/24	100% 48/48	95.8% 23/24	95.8% 23/24	95.8% 46/48	100% 24/24	100% 24/24	100% 48/48	98.6% (142/144) [95.1-99.6]
Flu B Mod Pos	100% 24/24	100% 24/24	100% 48/48	100% 24/24	100% 24/24	100% 48/48	100% 23/23	95.8% 23/24	97.9% 46/47	99.3% (142/143[a]) [96.1-99.9]
RSV Low Pos	100% 24/24	100% 24/24	100% 48/48	95.8% 23/24	100% 24/24	97.9% 47/48	100% 24/24	100% 24/24	100% 48/48	99.3% (143/144) [96.2-99.9]
RSV Mod Pos	100% 24/24	100% 24/24	100% 48/48	100% 24/24	100% 24/24	100% 48/48	100% 24/24	100% 24/24	100% 48/48	100% (144/144) [97.4-100.0]

a. One replicate was excluded because it was run on a day when an external positive control produced an incorrect result but was inadvertently not retested.

The evaluation of reproducibility and within-laboratory precision of the underlying analyte Ct values for the Xpert Xpress CoV-2/Flu/RSV *plus* test was analyzed using nested Analysis of Variance (ANOVA). The mean Ct, standard deviation (SD), and coefficient of variation (%CV) between-sites, between-operators, between-lots, between-days, between-runs and within-run for each panel member are presented in **Table 5-17**.

Table 5-17. Summary of Nested ANOVA by Coefficient of Variation

Sample	Analyte	N	Mean Ct	Variance Source													
				Site		OP		Lot		Day		Run		Within-Run		Total	
				SD	CV (%)	SD	CV (%)	SD	CV (%)	SD	CV (%)	SD	CV (%)	SD	CV (%)	SD	CV (%)
Negative	SPC	144	30.8	0.1	0.4	0.0	0.0	0.9	2.9	0.5	1.5	0.0	0.0	1.3	4.2	1.6	5.3
SARS-CoV-2 Low Pos	SARS-CoV-2	144	37.4	0.0	0.0	0.2	0.5	0.1	0.2	0.0	0.0	0.3	0.7	0.4	1.1	0.5	1.4
SARS-CoV-2 Mod Pos	SARS-CoV-2	144	36.2	0.0	0.1	0.1	0.3	0.0	0.0	0.1	0.3	0.2	0.4	0.4	1.0	0.4	1.2
Flu A Low Pos	Flu A1	144	35.7	0.2	0.6	0.0	0.0	0.1	0.2	0.0	0.0	0.0	0.0	0.6	1.6	0.6	1.7
	Flu A2	135[b]	37.9	0.3	0.8	0.0	0.0	0.2	0.5	0.0	0.0	0.4	1.1	0.9	2.5	1.1	2.9
Flu A Mod Pos[a]	Flu A1	144	34.7	0.0	0.0	0.1	0.2	0.0	0.0	0.1	0.3	0.0	0.0	0.4	1.2	0.4	1.3
	Flu A2	144	36.6	0.0	0.1	0.0	0.0	0.2	0.4	0.0	0.0	0.0	0.0	0.5	1.5	0.6	1.5
Flu B Low Pos	Flu B	144	36.3	0.3	0.8	0.0	0.1	0.0	0.0	0.1	0.2	0.3	0.7	0.7	2.1	0.8	2.3
Flu B Mod pos	Flu B	142[c]	35.1	0.0	0.0	0.1	0.4	0.1	0.3	0.3	0.8	0.0	0.0	0.7	2.0	0.8	2.2
RSV Low Pos	RSV	144	35.8	0.1	0.2	0.0	0.0	0.2	0.4	0.0	0.0	0.0	0.0	0.6	1.7	0.6	1.8
RSV Mod Pos	RSV	144	34.8	0.1	0.2	0.0	0.0	0.1	0.4	0.0	0.0	0.2	0.5	0.5	1.4	0.5	1.5

a. One replicate from Site 2 used the reagent lot 102 instead of the intended reagent lot 401. In the nested ANOVA analysis, this replicate was calculated under the intended lot 401 following the principle of "analyze as intended".
b. Nine replicates were excluded due to zero Flu A2 Ct values.
c. One replicate was excluded due to the zero Flu B Ct value; one replicate was excluded due to the incorrect external positive control result preceding the retest.

5.4.2. Clinical Performance

The clinical performance of the Xpert Xpress CoV-2/Flu/RSV *plus* test was evaluated in a multi-site, observational and method comparison study that included 33 geographically diverse sites in the United States (US) using specimens collected from individuals showing signs and symptoms of respiratory infection. Of the 33 sites, 5 sites participated in specimen collection only, 27 performed Xpert testing and specimen collection, and 1 site performed Xpert testing as well as comparator and discrepant testing.

Specimens tested included prospective clinical NPS and NS specimens collected in UTM/VTM. Prospectively collected fresh clinical specimens (Category I) tested in the study were from a larger US specimen collection protocol. Fresh (98.9%) and frozen (1.1%) specimens meeting the eligibility criteria were prospectively collected and tested in 2022. Due to low prevalence of Flu/RSV in 2022, archived prospectively collected frozen clinical specimens (Category II) collected during the 2016-2017 influenza season were used to supplement the sample size. These specimens represent contemporary Flu/RSV strains. Since these specimens were collected prior to the COVID-19 pandemic, they were expected to be negative for SARS-CoV-2 and therefore tested only for the Flu A, Flu B, and RSV targets. Available demographic data from the individuals from whom Category I and Category II specimens were collected are presented in **Table 5-18**.

Table 5-18. Demographic Summary for Category I and II Specimens

Prospectively Collected Fresh and Frozen Specimens from 2022 (Category I and Category II)	NP Swab (N=2672)	NS (N=2659)	Overall (N=5331)
Gender			
Female	1568 (58.7%)	1634 (61.5%)	3202 (60.1%)
Male	1104 (41.3%)	1025 (38.5%)	2129 (39.9%)
Age Group (Years)			
≤5	9 (0.3%)	183 (6.9%)	192 (3.6%)
6-21	623 (23.3%)	562 (21.1%)	1185 (22.2%)
22-59	1676 (62.7%)	1553 (58.4%)	3229 (60.6%)
≥60	364 (13.6%)	361 (13.6%)	725 (13.6%)
Race			
American Indian or Alaska Native	5 (0.2%)	6 (0.2%)	11 (0.2%)
Asian	73 (2.7%)	80 (3.0%)	153 (2.9%)
Asian, White	8 (0.3%)	1 (0.0%)	9 (0.2%)
Black or African American	734 (27.5%)	730 (27.5%)	1464 (27.5%)
Black or African American, White	10 (0.4%)	12 (0.5%)	22 (0.4%)
Native Hawaiian or Other Pacific Islander	6 (0.2%)	2 (0.1%)	8 (0.2%)
Other Mixed (N ≤ 3)	4 (0.1%)	5 (0.2%)	9 (0.2%)

Prospectively Collected Fresh and Frozen Specimens from 2022 (Category I and Category II)	NP Swab (N=2672)	NS (N=2659)	Overall (N=5331)
White	1685 (63.1%)	1641 (61.7%)	3326 (62.4%)
Participant Declined to Answer, or Unknown	147 (5.5%)	182 (6.8%)	329 (6.2%)
Ethnicity			
Hispanic	228 (8.5%)	213 (8.0%)	441 (8.3%)
Non-Hispanic	2333 (87.3%)	2323 (87.4%)	4656 (87.3%)
Participant Declined to Answer, or Unknown	111 (4.2%)	123 (4.6%)	234 (4.4%)
Specimen Testing			
Fresh	2641 (98.8%)	2631 (98.9%)	5272 (98.9%)
Frozen	31 (1.2%)	28 (1.1%)	59 (1.1%)
COVID-19 Vaccination Status			
Vaccinated	1969 (73.7%)	1865 (70.1%)	3834 (71.9%)
Not Vaccinated	665 (24.9%)	764 (28.7%)	1429 (26.8%)
Unknown	38 (1.4%)	30 (1.1%)	68 (1.3%)
Testing Environment			
CLIA Waiver	1603 (60.0%)	1619 (60.9%)	3222 (60.4%)
Laboratory/NPT	1069 (40.0%)	1040 (39.1%)	2109 (39.6%)
Prospectively Collected Frozen Specimens from 2016-2017 Influenza Season (Category II)	**NPS (N=422)**	**NS (N=368)**	**Overall (N=790)**
Gender			
Female	211 (50.0%)	223 (60.6%)	434 (54.9%)
Male	211 (50.0%)	145 (39.4%)	356 (45.1%)
Age Group (Years)			
≤5	164 (38.9%)	144 (39.1%)	308 (39.0%)
6-21	85 (20.1%)	72 (19.6%)	157 (19.9%)
22-59	134 (31.8%)	111 (30.2%)	245 (31.0%)
≥60	39 (9.2%)	41 (11.1%)	80 (10.1%)

Specimens were tested using Xpert Xpress CoV-2/Flu/RSV *plus* side-by-side with a U.S.FDA-cleared molecular respiratory panel that includes SARS-CoV-2 and a U.S. FDA-cleared molecular Flu A/B/RSV assay, in a randomized and blinded fashion.

Positive Percent Agreement (PPA) and Negative Percent Agreement (NPA), and non-determinate rate were determined by comparing the results of the Xpert Xpress CoV-2/Flu/RSV *plus* test relative to the results of a U.S. FDA-cleared molecular respiratory panel for the SARS-CoV-2 target, and a U.S.FDA-cleared molecular Flu A/B/RSV assay for the Flu A, Flu B, and RSV targets, respectively.

Discrepant results between Xpert Xpress CoV-2/Flu/RSV *plus* and the comparator for the SARS-CoV-2 target were investigated using a U.S. FDA EUA SARS-CoV-2 molecular test. Discrepant results between the Xpert Xpress CoV-2/Flu/RSV *plus* and the comparator for the Flu A/B/RSV targets were investigated using a U.S. FDA-cleared molecular respiratory panel.

A total of 5051 specimens, including 2536 NPS and 2515 NS specimens that yielded valid results by both the Xpert Xpress CoV-2/Flu/RSV *plus* and the U.S. FDA-cleared molecular respiratory panel were included in the performance evaluation for SARS-CoV-2. A total of 5954 specimens, including 3011 NPS and 2943 NS (specimens that yielded valid results by both the Xpert Xpress CoV-2/Flu/RSV *plus* and the U.S. FDA-cleared molecular Flu A/B/RSV assay were included in the performance evaluation for Flu A, Flu B, and RSV targets.

For the NPS specimens (both fresh and frozen specimens, combined), Xpert Xpress CoV-2/Flu/RSV *plus* demonstrated a PPA and NPA of 97.1% and 98.2% for SARS-CoV-2, respectively; 99.0% and 99.1% for Flu A, respectively; 96.6% and 100.0% for Flu B, respectively; 98.6% and 100.0% for RSV, respectively (**Table 5-19**). The initial non-determinate rate for the Xpert Xpress CoV-2/Flu/RSV *plus* test was 2.4% (74/3094). On repeat testing, 66 specimens yielded valid results. The final non-determinate rate for the Xpert Xpress CoV-2/Flu/RSV *plus* test was 0.3% (8/3094).

Table 5-19. Xpert Xpress CoV-2/Flu/RSV *plus* Performance Results for NPS Specimens

Target	Specimen Collection	Numbers of Specimens	TP	FP	TN	FN	PPA (%)	95%CI	NPA (%)	95%CI
SARS-CoV-2	Fresh	2505	454	37[a]	2000	14[b]	97	95.0 - 98.2	98.2	97.5 - 98.7
	Frozen	31	8	0	23	0	100	67.6 - 100.0	100	85.7 - 100.0
	Overall	2536	462	37	2023	14	97.1	95.1 - 98.2	98.2	97.5 - 98.7
Flu A	Fresh	2562	98	11[c]	2451	2[d]	98	93.0 - 99.5	99.6	99.2 - 99.8
	Frozen	449	93	13[e]	343	0	100	96.0 - 100.0	96.3	93.9 - 97.9
	Overall	3011	191	24	2794	2	99.0	96.3 - 99.7	99.1	98.7 - 99.4
Flu B	Fresh	2562	0	0	2562	0	NA	NA	100	99.9 - 100.0
	Frozen	449	57	0	390	2[f]	96.6	88.5 - 99.1	100	99.0 - 100.0
	Overall	3011	57	0	2952	2	96.6	88.5 - 99.1	100	99.9 - 100.0
RSV	Fresh	2562	12	0	2550	0	100	75.8 - 100.0	100	99.8 - 100.0
	Frozen	449	59	0	389	1[g]	98.3	91.1 - 99.7	100	99.0 - 100.0
	Overall	3011	71	0	2939	1	98.6	92.5 - 99.8	100	99.9 - 100.0

TP: True Positive; FP: False Positive; TN: True Negative; FN: False Negative; CI: 95% two-sided Confidence Interval

a. Discrepant test results based on a U.S. FDA EUA SARS-CoV-2 molecular test: 15/37 SARS-CoV-2 positive; 22/37 SARS-CoV-2 negative
b. Discrepant test results based on a U.S. FDA EUA SARS-CoV-2 molecular test: 3/14 SARS-CoV-2 positive; 10/14 SARS-CoV-2 negative; 1/14 invalid result
c. Discrepant test results based on a U.S. FDA-cleared molecular respiratory panel: 8/11 Flu A positive; 3/11 Flu A negative
d. Discrepant test results based on a U.S. FDA-cleared molecular respiratory panel: 1/2 Flu A positive; 1/2 Flu A negative
e. Discrepant test results based on a U.S. FDA-cleared molecular respiratory panel: 13/13 tests not performed due to specimens being stored for a longer duration than recommended per the package insert
f. Discrepant test results based on a U.S. FDA-cleared molecular respiratory panel: 2/2 test not performed due to specimens being stored for a longer duration than recommended per the package insert
g. Discrepant test results based on a U.S. FDA-cleared molecular respiratory panel: 1/1 test not performed due to specimens being stored for a longer duration than recommended per the package insert

For the NS specimens (both fresh and frozen specimens, combined), Xpert Xpress CoV-2/Flu/RSV *plus* demonstrated a PPA and NPA of 98.2% and 98.8% for SARS CoV-2, respectively; 98.0% and 99.3% for Flu A, respectively; 100.0% and 99.9% for Flu B, respectively; 95.8% and 100.0% for RSV, respectively (**Table 5-20**). The initial non-determinate rate for the Xpert Xpress CoV-2/Flu/RSV *plus* test was 2.4% (74/3027). On repeat testing, 57 specimens gave valid results upon retest. The final non-determinate rate for the Xpert Xpress CoV-2/Flu/RSV *plus* test was 0.6% (17/3027).

Table 5-20. Xpert Xpress CoV-2/Flu/RSV *plus* Performance Results for NS Specimens

Target	Specimen Collection	Numbers of Specimens	TP	FP	TN	FN	PPA (%)	95%CI	NPA (%)	95%CI
SARS-CoV-2	Fresh	2489	442	23[a]	2017	7[b]	98.4	96.8 - 99.2	98.9	98.3 - 99.2
	Frozen	26	6	1[c]	18	1[d]	85.7	48.7 - 97.4	94.7	75.4 - 99.1
	Overall	2515	448	24	2035	8	98.2	96.6 - 99.1	98.8	98.3 - 99.2
Flu A	Fresh	2553	130	6[e]	2413	4[f]	97.0	92.6 - 98.8	99.8	99.5 - 99.9
	Frozen	390	66	12[g]	312	0	100	94.5 - 100.0	96.3	93.6 - 97.9
	Overall	2943	196	18	2725	4	98.0	95.0 - 99.2	99.3	99.0 - 99.6
Flu B	Fresh	2553	0	0	2553	0	NA	NA	100	99.8 - 100.0
	Frozen	390	34	3[h]	353	0	100	89.8 - 100.0	99.2	97.6 - 99.7
	Overall	2943	34	3	2906	0	100	89.8 - 100.0	99.9	99.7 - 100.0
RSV	Fresh	2553	14	0	2538	1[i]	93.3	70.2 - 98.8	100	99.8 - 100.0
	Frozen	390	55	0	333	2[j]	96.5	88.1 - 99.0	100	98.9 - 100.0
	Overall	2943	69	0	2871	3	95.8	88.5 - 98.6	100	99.9 - 100.0

TP: True Positive; FP: False Positive; TN: True Negative; FN: False Negative; CI: 95% two-sided Confidence Interval

a. Discrepant test results based on a U.S. FDA EUA SARS-CoV-2 molecular test: 6/23 SARS-CoV-2 positive; 14/23 SARS-CoV-2 negative; 2/23 invalid result; 1/23 discrepant testing was inadvertently not performed
b. Discrepant test results based on a U.S. FDA EUA SARS-CoV-2 molecular test: 2/7 SARS-CoV-2 positive; 5/7 SARS-CoV-2 negative
c. Discrepant test results based on a U.S. FDA EUA SARS-CoV-2 molecular test: 1/1 SARS-CoV-2 positive
d. Discrepant test results based on a U.S. FDA EUA SARS-CoV-2 molecular test: 1/1 SARS-CoV-2 negative
e. Discrepant test results based on a U.S. FDA-cleared molecular respiratory panel: 5/6 Flu A positive; 1/6 Flu A negative
f. Discrepant test results based on a U.S. FDA-cleared molecular respiratory panel: 2/4 Flu A positive; 2/4 Flu A negative
g. Discrepant test results based on a U.S. FDA-cleared molecular respiratory panel: 12/12 tests not performed due to specimens being stored for a longer duration than recommended per the package insert
h. Discrepant test results based on a U.S. FDA-cleared molecular respiratory panel: 3/3 tests not performed due to specimens being stored for a longer duration than recommended per the package insert
i. Discrepant test results based on a U.S. FDA-cleared molecular respiratory panel: 1/1 RSV positive
j. Discrepant test results based on a U.S. FDA-cleared molecular respiratory panel: 2/2 test not performed due to specimens being stored for a longer duration than recommended per the package insert

The number of specimens with positive results for more than one target as detected by Xpert Xpress CoV-2/Flu/RSV *plus* is presented in **Table 5-21** and **Table 5-22**, where bolded values indicate concordant results.

Table 5-21. Multi-Target Detection by Xpert Xpress CoV-2/Flu/RSV *plus* for Specimens Collected in 2022

Infection		Comparator Results						
		SARS-CoV-2 only	SARS-CoV-2 and Flu A	Flu A only	RSV only	Negative	Total	Co-Infection Rate (%)
Xpert Xpress CoV-2/Flu/RSV *plus*	SARS-CoV-2 only	**876**	1	0	0	57	934	0.3
	SARS-CoV-2 and Flu A	0	**2**	1	0	0	3	
	Flu A only	0	0	**220**	0	17	237	
	RSV only	0	0	0	**26**	0	26	
	Negative	22	0	5	1	**3693**	3721	
	Total	898	3	226	27	3767	4921	
	Co-Infection Rate (%)	0.3						

As presented in **Table 5-21**, a total of 4921 Category I specimens collected in 2022 yielded valid results for SARS-CoV-2, Flu A, and RSV targets for both the Xpert Xpress CoV-2/Flu/RSV *plus* test and the comparator test. The co-infection rate for Xpert Xpress CoV-2/Flu/RSV *plus* was 0.3% (3/1200) and the rate of co-infection by the comparator was 0.3% (3/1154).

Table 5-22. Flu A, Flu B, and RSV Multi-Target Detection by Xpert Xpress CoV-2/Flu/RSV *plus* for Specimens Collected in 2016-2017 and 2022

Infection		Comparator								
		Flu A only	Flu B Only	RSV Only	Flu A and Flu B	Flu A and RSV	Flu B and RSV	Negative	Total	Co-Infection Rate (%)
Xpert Xpress CoV-2/Flu/RSV *plus*	Flu A only	**381**	0	0	1	1	0	36	419	1.7
	Flu B Only	0	**85**	0	0	0	0	2	87	
	RSV Only	0	0	**135**	0	0	0	0	135	
	Flu A and Flu B	0	4	0	**1**	0	0	1	6	
	Flu A and RSV	0	0	1	0	**3**	0	0	4	
	Flu B and RSV	0	0	0	0	0	**1**	0	1	
	Negative	6	1	3	0	0	0	**5292**	5302	
	Total	387	90	139	2	4	1	5331	5954	
	Co-Infection Rate (%)	1.1								

As presented in **Table 5-22**, of the 5954 Category I and II specimens evaluated for Flu A, Flu B and RSV targets, the co-infection rate for Xpert Xpress CoV-2/Flu/RSV *plus* was 1.7% (11/652) and the rate of co-infection by the comparator was 1.1% (7/623).

5.5. Conclusions

The results of the analytical and clinical performance studies summarized above demonstrated that the Xpert Xpress CoV-2/Flu/RSV *plus* test is substantially equivalent to the predicate device.