October 13, 2023

Cepheid
Jennifer Motto
Principal Regulatory Affairs Specialist
904 Caribbean Drive
Sunnyvale, California 94089

Re: K230440
Trade/Device Name: Xpert Xpress CoV-2 *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: QQX, OOI
Dated: September 1, 2023
Received: September 12, 2023

Dear Jennifer Motto:

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 (the 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 available 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.

Additional information about changes that may require a new premarket notification are provided in the FDA guidance documents entitled "Deciding When to Submit a 510(k) for a Change to an Existing Device"

(https://www.fda.gov/media/99812/download) and "Deciding When to Submit a 510(k) for a Software Change to an Existing Device" (https://www.fda.gov/media/99785/download).

Your device is also subject to, among other requirements, the Quality System (QS) regulation (21 CFR Part 820), which includes, but is not limited to, 21 CFR 820.30, Design controls; 21 CFR 820.90, Nonconforming product; and 21 CFR 820.100, Corrective and preventive action. Please note that regardless of whether a change requires premarket review, the QS regulation requires device manufacturers to review and approve changes to device design and production (21 CFR 820.30 and 21 CFR 820.70) and document changes and approvals in the device master record (21 CFR 820.181).

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 Part 803) for devices or postmarketing safety reporting (21 CFR Part 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 Part 4, Subpart A) for combination products; and, if applicable, the electronic product radiation control provisions (Sections 531-542 of the Act); 21 CFR Parts 1000-1050.

Also, please note the regulation entitled, "Misbranding by reference to premarket notification" (21 CFR 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,

Himani Bisht -S

Himani Bisht, Ph.D.
Assistant Director
Viral Respiratory and HPV Branch
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: 07/31/2026
See PRA Statement below.

510(k) Number *(if known)*
K230440

Device Name
Xpert Xpress CoV-2 plus

Indications for Use *(Describe)*
The Xpert Xpress CoV-2 plus test, performed on the GeneXpert Dx and GeneXpert Infinity Systems, is a rapid real-time RT-PCR test intended for the qualitative detection of SARS-CoV-2 RNA in nasopharyngeal and anterior nasal swab specimens collected from individuals with signs and symptoms of respiratory tract infection.

The Xpert Xpress CoV-2 plus test is intended for use as an aid in the diagnosis of COVID-19 if used in conjunction with other clinical, epidemiologic, and laboratory findings. Positive results are indicative of the presence of SARS-CoV-2 RNA. Positive results do not rule out bacterial infection or co-infection with other pathogens.

Negative results do not preclude SARS-CoV-2 infection. The results of this test should not be used as the sole basis for diagnosis and 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.

5. 510(k) Summary

As required by 21 CFR Section 807.92(c).

Submitted by:	Cepheid 904 Caribbean Drive Sunnyvale, CA 90489 Phone number: (408) 541-4191
Contact:	Jennifer Motto, M.S., RAC
Date of Preparation:	February 17, 2023

Device:

Trade name:	Xpert® Xpress CoV-2 ***plus***
Common name:	Xpert Xpress CoV-2 *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, Respiratory Specimen Nucleic Acid SARS-CoV-2 Test,
Primary product code:	QQX
Secondary product code	OOI
Classification Advisory Panel	Microbiology (83)
Prescription Use	Yes
Predicate Device Assay:	BioFire COVID-19 Test 2 (K211079)

5.1. Device Description

The Xpert Xpress CoV-2 *plus* test is a rapid, automated *in vitro* diagnostic test for the qualitative detection of viral RNA from SARS-CoV-2 in nasopharyngeal swab (NPS) and anterior nasal swab (NS) specimens obtained from individuals with signs and symptoms of respiratory tract infection.

The Xpert Xpress CoV-2 *plus* test is performed on the Cepheid 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 cartridges during the testing process is minimized.

The Xpert Xpress CoV-2 *plus* test includes reagents for the detection of viral RNA from SARS-CoV-2 in NPS and NS specimens. The primers and probes in the Xpert Xpress CoV-2 *plus* test are designed to amplify and detect sequences in the genes that encode the following SARS-CoV-2 proteins: nucleocapsid (N2), envelope (E), and RNA-dependent RNA polymerase (RdRP). 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 *plus* test is designed for use with NPS or NS specimen collected with nylon flocked swabs and placed into a viral transport medium (VTM), Universal Transport Medium (UTM) or eNAT®. Examples of the ancillary specimen collection kits that are compatible for use with the Xpert Xpress CoV-2 *plus* test include:

- **Nasopharyngeal Sample Collection Kit for Viruses**
  - Copan UTM® 3C057N (Flexible Minitip Flocked Swab with UTM® Medium w/o Beads)
  - Becton Dickinson Universal Viral Transport Kit P/N 220531 (Flexible Minitip Flocked Swab with UVT Medium)
  - Copan eNAT® Molecular Collection and Preservation Medium P/N 6U074S01 (Flexible Minitip Flocked Swab with eNAT® Medium)

- **Nasal Sample Collection Kit for Viruses**
  - Copan UTM® 3C064N (Regular Flocked Swab with UTM® Medium w/o 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 3C047N, BD Universal Transport Medium, Remel M4RT, Remel M5) or equivalent

These sample collection kits for viruses allow NPS and NS specimens from patients to be collected, preserved and transported to laboratory prior to analysis with the Xpert Xpress CoV-2 *plus* test.

5.2. Device Intended Use

The Xpert Xpress CoV-2 *plus* test, performed on the GeneXpert Dx and GeneXpert Infinity Systems, is a rapid real-time RT-PCR test intended for the qualitative detection of SARS-CoV-2 RNA in nasopharyngeal and anterior nasal swab specimens collected from individuals with signs and symptoms of respiratory tract infection.

The Xpert Xpress CoV-2 *plus* test is intended for use as an aid in the diagnosis of COVID-19 if used in conjunction with other clinical, epidemiologic, and laboratory findings. Positive results are indicative of the presence of SARS-CoV-2 RNA. Positive results do not rule out bacterial infection or co-infection with other pathogens.

Negative results do not preclude SARS-CoV-2 infection. The results of this test should not be used as the sole basis for diagnosis and patient management decisions.

5.3. Substantial Equivalence

Table 5-1 shows the similarities and differences between Xpert Xpress CoV-2 *plus* and the predicate device, BioFire COVID-19 Test 2 [K211079].

Table 5-1. Comparison of Similarities and Differences Between Xpert Xpress CoV-2 *plus* and the Predicate Device

Attribute	New Device Cepheid Xpert® Xpress CoV-2 *plus*	Predicate Device – K211079 BioFire Defense, LLC BioFire COVID-19 Test 2
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	QQX Respiratory Specimen Nucleic Acid SARS-CoV-2 Test
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 *plus* test, performed on the GeneXpert Dx and GeneXpert Infinity Systems, is a rapid real-time RT-PCR test intended for the qualitative detection of SARS-CoV-2 RNA in nasopharyngeal and anterior nasal swab specimens collected from individuals with signs and symptoms of respiratory tract infection. The Xpert Xpress CoV-2 *plus* test is intended for use as an aid in the diagnosis of COVID-19 if used in conjunction with other clinical, epidemiologic, and laboratory findings. Positive results are indicative of the presence of SARS-CoV-2 RNA. Positive results do not rule out bacterial infection or co-infection with other pathogens. Negative results do not preclude SARS-CoV-2 infection. The results of this test should not be used as the sole basis for diagnosis and patient management decisions.	The BioFire COVID-19 Test 2 is a qualitative nested multiplexed RT-PCR in vitro diagnostic test intended for use with the BioFire FilmArray 2.0 and BioFire FilmArray Torch Systems. The BioFire COVID-19 Test 2 detects nucleic acids from severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) in nasopharyngeal swabs (NPS) from symptomatic individuals suspected of COVID-19 by their healthcare provider. Results are for the identification of SARS-CoV-2 RNA. The SARS-CoV-2 RNA is generally detectable in NPS specimens during the acute phase of infection. Positive results are indicative of the presence of SARS-CoV-2 RNA; clinical correlation with patient history and other diagnostic information is necessary to determine patient infection status. Positive results do not rule out co-infection with other pathogens. Results are meant to be used in conjunction with other clinical, epidemiologic, and laboratory data, in accordance with the guidelines provided by the relevant public health authorities. The BioFire COVID-19 Test 2 is intended for use by trained medical and laboratory professionals in a

Attribute	New Device Cepheid Xpert® Xpress CoV-2 *plus*	Predicate Device – K211079 BioFire Defense, LLC BioFire COVID-19 Test 2
		laboratory setting or under the supervision of a trained laboratory professional.
Assay Targets	SARS-CoV-2 (E, N2, RdRP)	SARS-CoV-2 (Multiple Targets)
Specimen Type	• Nasopharyngeal swab (NPS) • Anterior nasal swab (NS)	Nasopharyngeal swab (NPS)
Transport Media	• Universal Transport Medium (UTM) / Viral Transport Medium (VTM) • eNAT	• Transport Medium • 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	~30 minutes	~ 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/Limit of Detection (LoD) – Clinical NPS Matrix

The analytical sensitivity of the Xpert Xpress CoV-2 *plus* test was first estimated by testing limiting dilutions of a NATtrol inactivated SARS-CoV-2 virus in pooled negative clinical NPS-UTM/VTM matrix, using two reagent lots and following the guidance in Clinical and Laboratory Standards Institute (CLSI) document EP17-A2. LoD was estimated by considering each target gene (E, N2, and RdRP) in addition to the overall positivity rate for the Xpert Xpress CoV-2 *plus* test. The estimated LoD value as determined by Probit regression analysis was based on the weakest target gene (N2) and verified using two lots of Xpert Xpress CoV-2 *plus* reagents in replicates of 20 per lot for two clinical NPS matrices (UTM/VTM and eNAT). The concentration levels with observed hit rates greater than or equal to 95% in the LoD determination study were 403, 200 and 70 copies/mL for the N2 target, RdRP target and E target, respectively. The claimed LoD is 403 copies/mL (**Tables 5-2 and 5-3**).

Table 5-2. Xpert Xpress CoV-2 *plus* Limit of Detection – Verification of NATtrol SARS-CoV-2 LoD in NPS-UTM/VTM Matrix

Virus Strain	Reagent Lot	LoD (copies/mL)	Positive Results out of # Valid Replicates	% Positive	Mean E Ct	Mean N2 Ct	Mean RdRP Ct
NATtrol™ SARS-CoV-2	Reagent Lot 1	403	20/20	100%	34.3	38.3	36.6
	Reagent Lot 2	403	20/20	100%	34.1	37.7	36.5

Table 5-3. Xpert Xpress CoV-2 *plus* Limit of Detection – Verification of NATtrol SARS-CoV-2 LoD in NPS-eNAT Matrix

Virus Strain	Reagent Lot	LoD (copies/mL)	Positives out of # Valid Replicates	% Positive	Mean E Ct	Mean N2 Ct	Mean RdRP Ct
NATtrol™ SARS-CoV-2	Reagent Lot 1	403	20/20[a]	100%	33.4	36.8	35.5
	Reagent Lot 2	403	20/20	100%	33.5	36.8	35.5

a. One of 20 replicates tested reported INVALID. The run was successfully repeated to obtain 20 valid replicates.

Analytical Sensitivity/Limit of Detection (LoD) – Clinical NS-UTM/VTM Matrix

The analytical sensitivity of the Xpert Xpress CoV-2 *plus* test was first estimated by testing limiting dilutions of a NATtrol inactivated SARS-CoV-2 virus in pooled negative clinical NS-UTM/VTM matrix, using two reagent lots and following the guidance in Clinical and Laboratory Standards Institute (CLSI) document EP17-A2. LoD was estimated by considering each target gene (E, N2, and RdRP) in addition to the overall positivity rate for the Xpert Xpress CoV-2 *plus* test. The LoD point estimates and 95% upper and lower confidence intervals (CI) for individual SARS-CoV-2 targets (E, N2 and RdRP) in clinical

NS-UTM/VTM matrix were determined using Probit regression analysis and the point estimated LoD values were 97, 462 and 157 copies/mL for the E target, N2 target and RdRP target, respectively. The estimated LoD value of the weakest target gene (N2) was verified using two lots of Xpert Xpress CoV-2 *plus* reagents in replicates of 20 per lot for clinical NS-UTM/VTM matrix. The claimed LoD is 462 copies/mL (**Table 5-4**).

Table 5-4. Xpert Xpress CoV-2 *plus* Limit of Detection – Verification of NATtrol SARS-CoV-2 LoD in NS-UTM/VTM Matrix

Virus Strain	Reagent Lot	LoD (copies/mL)	Positive Results out of # of Valid Replicates	% Positive	Mean E Ct	Mean N2 Ct	Mean RdRP Ct
NATtrol™ SARS-CoV-2	Reagent Lot 1	462	20/20	100%	34.4	38.9	36.8
	Reagent Lot 2	462	20/20	100%	34.1	37.6	36.1

Analytical Sensitivity (Limit of Detection) – WHO First International Standard SARS-CoV-2 RNA in clinical NS-UTM/VTM Matrix

The analytical sensitivity of the Xpert Xpress CoV-2 *plus* test was estimated by testing limiting dilutions of WHO First International Standard for SARS-CoV-2 RNA in pooled negative clinical NS-UTM/VTM matrix, using one reagent lot. The study was performed in a random and blinded fashion.

The lowest concentration level of the First WHO International Standard for SARS-CoV-2 RNA that generated ≥95% positive results (19/20 positive results) for the weakest target gene (N2) by the Xpert Xpress CoV-2 *plus* test was used to confirm be the analytical sensitivity or Limit of Detection (LoD). The verified LoD for the First WHO International Standard for SARS-CoV-2 RNA tested in NS-UTM/VTM matrix with the Xpert Xpress CoV-2 *plus* test was 1000 IU/mL. The positivity rates for the overall SARS-CoV-2 results and the analyte targets, including the mean Ct values for E, N2 and RdRP at the verified LoD are presented in **Table 5-5**.

Table 5-5. LoD Concentration, Positivity Rate and Mean Ct Values for SARS-CoV-2 for the First WHO International Standard for SARS-CoV-2 RNA in Clinical NS-UTM/VTM Matrix

Virus	LoD Concentration	SARS-CoV-2 Positive Results / Test Replicates	E Target		N2 Target		RdRP Target	
			Positive Results / Test Replicates	Mean Ct	Positive Results / Test Replicates	Mean Ct	Positive Results / Test Replicates	Mean Ct
1st WHO International Standard for SARS-CoV-2 RNA Virus	1000 IU/mL	20/20	20/20	34.3	19/20	39.5	20/20	36.5

Analytical Inclusivity (Reactivity)

SARS-CoV-2 *in silico* Analyses

The inclusivity of Xpert Xpress CoV-2 *plus* primers was evaluated on June 30th, 2022 using *in silico* analysis of the assay amplicons in relation to 11,650,640 SARS-CoV-2 sequences available in the GISAID gene database for three targets, E, N2 and RdRP. The 11,650,640 SARS-CoV-2 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 focuses on the combined, non-ambiguous sequences from the variants of interest and variants of concern as of June 30th, 2022. These constituted 10,469,612 sequences for the E target, 10,587,381 sequences for the N2 target and 10,333,656 sequences for the RdRP target. **Table 5-6** summarizes the effective predicted inclusivity for E, N2 and RdRP amplicons for the variants of interests and concern.

Table 5-6. Predicted Inclusivity for E, N2 and RdRP Amplicons for SARS-CoV-2 Variants of Interests and Concern

SARS-CoV-2 Target Amplicon	Exact Match	1 Mismatch [a]	2 or More Mismatches	Predicted Inclusivity
E	10,420,248 of 10,469,612 total (99.5%)	48,562 (0.5%)	802 (0.01%)	100%
N2	10,386,068 of 10,587,381 total (98.1%)	196,336 (1.9%)	4,977 (0.05%)	99.95%
RdRP	10,247,146 of 10,333,656 total (99.2%)	85,373 (0.8%)	1,137 (0.01%)	100%

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

The *in silico* inclusivity of the Xpert Xpress CoV-2 *plus* probe oligonucleotides for E, N2 and RdRP were also assessed against the top 20 most frequent matches in the GISAID EpiCoV sequence database as of June 15th, 2022, which constituted 10,310, 839 for the E target, 10,428,014 for the N2 target and 10,178,602 for the RdRP target. For each of the probe oligonucleotides used in the Xpert Xpress CoV-2 *plus* test, **Table 5-7** summarizes the number sequences as well as the corresponding percentage of sequences from this dataset with exact match, 1 mismatch/insertion, and 2 or more mismatches/insertions in the alignment.

Table 5-7. Predicted Inclusivity for E, N2 and RdRP Probes for SARS-CoV-2 Variants of Interests and Concern

SARS-CoV-2 Target Probe	Exact Match	1 Mismatch/Insertion [a]	2 or More Mismatches/ Insertions	Predicted Inclusivity
E	10,300,688 of 10,310,839 total (99.9%)	9,853 (0.1%)	22 (0.0002%)	100%
N2	10,351,581 of 10,428,014 total (99.3%)	72,957 (0.7%)	0 (0%)	100%
RdRP	0	10,140,254 of 10,178602 total (99.6%)	37,492 (0.4%)	99.6%

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

The *in silico* inclusivity of the Xpert Xpress CoV-2 *plus* primer/probe sequences for E, N2 and RdRP was further assessed against SAR-CoV-2 sequences submitted to the GISAID EpiCoV database up to 180 days prior to and including July 31, 2023 for SARS-CoV-2 isolates currently circulating in the United States. There were no instances where a specific primer/probe sequence was observed with greater than 0.5% frequency of mismatch during this 180-day period. The analysis predicted that the Xpert Xpress CoV-2 *plus* test will detect all currently circulating variants/lineages of SARS-CoV-2.

Moreover, based on the built-in redundancy of the Xpert Xpress CoV-2 *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 sequences would be missed by the Xpert Xpress CoV-2 *plus* test.

SARS-CoV-2 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 *plus* test was evaluated by bench testing against multiple strains of SARS-CoV-2 at levels near the analytical LoD (~3x LoD). A total of 61 strains comprised of 23 intact SARS-CoV-2 viral particles (18 inactivated viral cultures and 5 BSL-3 live viral cultures), and 38 SARS-CoV-2 *in vitro* RNA transcripts representing variant strains were tested in this study with the Xpert Xpress CoV-2 *plus* test. Three replicates were tested for each strain. All SARS-CoV-2 strains tested positive in all three replicates. Results are shown in **Table 5-8**.

Table 5-8. Analytical Reactivity (Inclusivity) of the Xpert Xpress CoV-2 *plus* Test

Count No.	SARS-CoV-2 Strain	Tested Concentration	SARS-CoV-2 Test Results	Number of Positive Results Obtained out of the Total Number of Valid Replicates		
				E	N2	RdRP
1	2019-nCoV/Italy-INMI1 [a]	5 $TCID_{50}$/mL	POS	3/3	3/3	3/3
2	England/204820464/2020 [a]	0.5 $TCID_{50}$/mL	POS [e]	3/3	3/3	3/3
3	Hong Kong/VM20001061/2020 [a]	0.25 $TCID_{50}$/mL	POS	3/3	3/3	3/3
4	South Africa/KRISP-K005325/2020 [a]	0.25 $TCID_{50}$/mL	POS	3/3	3/3	3/3
5	USA/CA_CDC_5574/2020 [a]	0.25 $TCID_{50}$/mL	POS	3/3	3/3	3/3
6	USA/MD-HP01542/2021 [a]	1.3e3 genome equivalents/mL	POS	3/3	3/3	3/3
7	USA/GA-EHC-2811C/2021 [a]	1.3e3 genome equivalents/mL	POS	3/3	3/3	3/3
8	NY-Wadsworth-103677-01/2020 [a]	0.15 $TCID_{50}$/mL	POS	3/3	3/3	3/3
9	NY-Wadsworth-21006055-01/2021 [a]	0.04 $TCID_{50}$/mL	POS	3/3	3/3	3/3
10	NY-Wadsworth-21025952-01/2021 [a] (Isolate 1)	0.625 $TCID_{50}$/mL	POS	3/3	3/3	3/3
11	NY-Wadsworth-21018781-01/2021 [a] (Isolate 2)	0.625 $TCID_{50}$/mL	POS	3/3	3/3	3/3

Count No.	SARS-CoV-2 Strain	Tested Concentration	SARS-CoV-2 Test Results	Number of Positive Results Obtained out of the Total Number of Valid Replicates		
				E	N2	RdRP
12	NY-Wadsworth-21033899-01/2021 [a]	1.25 $TCID_{50}$/mL	POS	3/3	3/3	3/3
13	NY-Wadsworth-33126-01/2020 [a]	0.15 $TCID_{50}$/mL	POS	3/3	3/3	3/3
14	USA/CA-Stanford-15_S02/2021[a]	0.04 $TCID_{50}$/mL	POS	3/3	3/3	3/3
15	USA/PHC658/2021[a]	25 $TCID_{50}$/mL	POS	3/3	3/3	3/3
16	Japan/TY7-503/2021[a]	0.75 $TCID_{50}$/mL	POS	3/3	3/3	3/3
17	hCoV-19/USA/MD-HP30386/2022 [a]	27.5 copies/mL	POS	3/3	3/3	3/3
18	hCoV-19/USA/COR-22-063113/2022 [a]	19 copies/mL	POS	3/3	3/3	3/3
19	Australia/VIC01/2020 [b]	1.2e3 copies/mL	POS	3/3	3/3	3/3
20	Belgium/ULG/10004/2020 [b]	1.2e3 copies/mL	POS	3/3	3/3	3/3
21	England/205041766/2020 [b]	1.2e3 copies/mL	POS	3/3	3/3	3/3
22	England/MILK-9E05B3/2020 [b]	1.2e3 copies/mL	POS	3/3	3/3	3/3
23	England/SHEF-C05B2/2020 [b]	1.2e3 copies/mL	POS	3/3	3/3	3/3
24	France/HF2393/2020 [b]	1.2e3 copies/mL	POS	3/3	3/3	3/3
25	Hong Kong/HKU-211129-001/2021 [b]	1.2e3 copies/mL	POS	3/3	3/3	3/3
26	Iceland/5/2020 [b]	1.2e3 copies/mL	POS	3/3	3/3	3/3
27	India/CT-ILSGS00361/2021 [b]	1.2e3 copies/mL	POS	3/3	3/3	3/3
28	India/MH-NCCS-P1162000182735/2021 [b]	1.2e3 copies/mL	POS	3/3	3/3	3/3
29	India/MH-SEQ-221_S66_R1_001/2021 [b]	1.2e3 copies/mL	POS	3/3	3/3	3/3
30	Japan/Hu_DP_Kng_19-020/2020 [b]	1.2e3 copies/mL	POS	3/3	3/3	3/3
31	Japan/IC-0564/2021 [b]	1.2e3 copies/mL	POS	3/3	3/3	3/3
32	Portugal/PT9543/2021[b]	1.2e3 copies/mL	POS	3/3	3/3	3/3
33	South Africa/KRISP-EC-K005299/2020 [b]	1.2e3 copies/mL	POS	3/3	3/3	3/3
34	Taiwan/NTU02/2020 [b]	1.2e3 copies/mL	POS	3/3	3/3	3/3
35	USA/CA9/2020 [b]	1.2e3 copies/mL	POS	3/3	3/3	3/3
36	USA/CA-PC101P/2020 [b]	1.2e3 copies/mL	POS	3/3	3/3	3/3
37	USA/CA-CZB-12943/2020 [b]	1.2e3 copies/mL	POS	3/3	3/3	3/3
38	USA/MN2-MDH2/2020 [b]	1.2e3 copies/mL	POS	3/3	3/3	3/3
39	USA/NY-MSHSPSP-PV24650/2020 [b]	1.2e3 copies/mL	POS	3/3	3/3	3/3
40	USA/TX1/2020 [b]	1.2e3 copies/mL	POS	3/3	3/3	3/3
41	USA/WA2/2020 [b]	1.2e3 copies/mL	POS	3/3	3/3	3/3
42	USA/WA-CDC-UW21061750277/2021 [b]	1.2e3 copies/mL	POS	3/3	3/3	3/3
43	Wuhan-Hu-1 [b]	1.2e3 copies/mL	POS	3/3	3/3	3/3

Count No.	SARS-CoV-2 Strain	Tested Concentration	SARS-CoV-2 Test Results	Number of Positive Results Obtained out of the Total Number of Valid Replicates		
				E	N2	RdRP
44	Germany/BavPat1/2020[c]	1.2e3 genome copies/mL	POS	3/3	3/3	3/3
45	Singapore/2/2020[c]	300 genome equivalents/mL	POS	3/3	3/3	3/3
46	USA/AZ1/2020[c]	300 genome equivalents/mL	POS	3/3	3/3	3/3
47	USA/CA1/2020[c]	1.2e3 genome equivalents/mL	POS	3/3	3/3	3/3
48	USA/CA2/2020[c]	1.2e3 genome equivalents/mL	POS	3/3	3/3	3/3
49	USA/CA3/2020[c]	400 genome equivalents/mL	POS	3/3	3/3	3/3
50	USA/CA4/2020[c]	1.2e3 genome equivalents/mL	POS	3/3	3/3	3/3
51	USA/IL1/2020[c]	150 genome equivalents/mL	POS	3/3	3/3	3/3
52	USA/New York-PV08001/2020[c]	1.2e3 genome copies/mL	POS	3/3	3/3	3/3
53	USA/New York-PV08410/2020[c]	300 genome equivalents/mL	POS	3/3	3/3	3/3
54	USA/New York-PV08449/2020[c]	300 genome equivalents/mL	POS	3/3	3/3	3/3
55	USA/New York-PV09158/2020[c]	300 genome equivalents/mL	POS	3/3	3/3	3/3
56	USA/WI1/2020[c]	1.2e3 genome equivalents/mL	POS	3/3	3/3	3/3
57	hCoV-19/USA/MD-HP38861/2022[d]	0.29 $TCID_{50}$/mL	POS	3/3	3/3	3/3
58	hCoV-19/USA/MD-HP38960/2022[d]	0.043 $TCID_{50}$/mL	POS	3/3	3/3	3/3
59	hCoV-19/USA/CO-CDPHE-2102544747/2021[d]	0.028 $TCID_{50}$/mL	POS	3/3	3/3	3/3
60	hCoV-19/USA/MD-HP38288/2022[d]	0.24 $TCID_{50}$/mL	POS	3/3	3/3	3/3
61	hCoV-19/USA/MD-HP40900/2022[d]	0.72 $TCID_{50}$/mL	POS	3/3	3/3	3/3

a. Inactivated viral culture fluid (intact viral particles)
b. Synthetic, *in vitro* RNA transcript, TWIST controls
c. Genomic RNA
d. BSL-3 live viral culture fluid (intact viral particles)
e. One of 3 replicates reported ERROR. The run was successfully repeated to obtain 3 valid replicates.

Analytical Exclusivity (Specificity)

In Silico Analyses

The analytical specificity/cross-reactivity of the Xpert Xpress CoV-2 *plus* plan included evaluation of the SARS-CoV-2 test primer and probes with potentially cross-reactive microorganisms by *in silico* analysis. The analysis was conducted by mapping the primers and probes of Xpert Xpress CoV-2 *plus* individually to the microorganism sequences downloaded from the NCBI database. E primers and probes are not specific for SARS-COV-2 and will detect Human and Bat SARS-coronavirus. No potential unintended cross reactivity with other organisms listed in **Table 5-9** is expected based on the *in silico* analysis.

Table 5-9. 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	Adenovirus (e.g., C1 Ad. 71)
Human coronavirus OC43	Cytomegalovirus
Human coronavirus HKU1	Enterovirus (e.g., EV68)
Human coronavirus NL63	Epstein-Barr virus
SARS-coronavirus	Human Metapneumovirus (hMPV)
MERS-coronavirus	Influenza A & B
Bat coronavirus	Measles
	Mumps
	Parainfluenza virus 1-4
	Parechovirus
	Respiratory syncytial virus
	Rhinovirus
	Bacillus anthracis (Anthrax)
	Bordetella pertussis
	Bordetella parapertussis
	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

Microorganisms from the Same Genetic Family	High Priority Organisms
	Pneumocystis jirovecii (PJP)
	Pseudomonas aeruginosa
	Staphylococcus aureus
	Staphylococcus epidermidis
	Staphylococcus salivarius
	Streptococcus pneumoniae
	Streptococcus pyogenes
	Aspergillus sp
	Candida albicans

Wet-Testing

In addition to the *in silico* analysis of the SARS-CoV-2 primers and probes for cross-reactivity, the analytical specificity of the Xpert Xpress CoV-2 *plus* test was evaluated by bench-testing a panel of 62 microorganisms comprising (4) human coronaviruses, (1) MERS-coronavirus, (1) SARS-coronavirus, (20) other respiratory viruses, (30) respiratory bacteria, (2) yeast strains, (3) fungal strain, and 1 human nasal wash fluid representing a diverse microbial flora in the human respiratory tract.

The intact viruses were tested at concentrations of $\geq 10^5$ $TCID_{50}$/mL, $\geq 10^5$ $CEID_{50}$/mL, and $\geq 10^5$ copies/mL. Bacteria and yeast were tested at $\geq 10^6$ CFU/mL. The bacteria *Chlamydia pneumoniae* and *Mycoplasma pneumoniae* were tested at concentrations of $\geq 10^6$ IFU/mL and $\geq 10^6$ CCU/mL, respectively. The live strains of *Coxiella burnetii, Lactobacillus reuteri,* and *Neisseria meningitides* were not available, therefore genomic DNA at $\geq 10^6$ genome equivalent copies/mL was used. The *in vitro* transcribed (IVT) RNA sample was tested at $\geq 10^6$ genome equivalent/mL for human Coronavirus HKU1, and the genomic RNA sample was tested at $\geq 10^6$ genome equivalent/mL for SARS-coronavirus Urbani.

The microorganisms being evaluated for cross-reactivity were tested in groups or individually and spiked into negative simulated NPS/NS background matrix for testing. If a grouped microorganisms produced a SARS-CoV-2 positive result, then each member of the group was tested separately. If the individual non-target organism yielded a positive result, retesting was performed at lower concentrations until a concentration that no longer produce a false positive result was identified.

The results of the analytical specificity/exclusivity study demonstrate that the primer/probe sets included in the Xpert Xpress CoV-2 *plus* does not cross-react with the nucleic acids from non-intended respiratory microorganisms and phylogenetically related human coronavirus species. The one exception was the SARS-coronavirus, Urbani which yielded the expected test result of SARS-CoV-2 POSITIVE. We expected cross-reactivity for the E gene target with the SARS-coronavirus Urbani strain which has the highly conserved E gene target shared among coronaviruses in the B lineage *Betacoronavirus*. Results are shown in **Table 5-10**.

Table 5-10. Analytical Specificity (Exclusivity) of the Xpert Xpress CoV-2 *plus* Test

Respiratory Microorganisms	Test Group	Tested Concentration	Final SARS-CoV-2 Result Call-Out	Number of Positive Results Obtained from Total Number of Replicates Tested		
				E	N2	RdRP
Human coronavirus, 229E	G1	1.1e5 $TCID_{50}$/mL	NEG	0/3	0/3	0/3
Human coronavirus, OC43		1.1e5 $TCID_{50}$/mL				
MERS-coronavirus		1.1e5 $TCID_{50}$/mL				
Human coronavirus, NL63	G2	1.1e5 $TCID_{50}$/mL	NEG	0/3	0/3	0/3
Human coronavirus, HKU1[a]	G3	1.1e6 genome cp/mL	NEG	0/3	0/3	0/3
SARS-coronavirus, Urbani [a]	G4	1.1e6 genome cp/mL	POS	3/3	0/3	0/3
Influenza A H1N1 (pdm2009), Michigan/272/2017	G5	1.1e5 $TCID_{50}$/mL	NEG	0/3	0/3	0/3
Influenza B (Victoria Lineage), Hawaii/01/2018 (NA D197N)		1.1e5 $TCID_{50}$/mL				
RSV-A, Strain: 4/2015 Isolate #1		1.1e5 TCID50/mL				
Adenovirus Type 1	G6	1.1e5 $TCID_{50}$/mL	NEG	0/3	0/3	0/3
Adenovirus Type 7A		1.1e5 $TCID_{50}$/mL				
Cytomegalovirus		1.1e5 $TCID_{50}$/mL				
Echovirus	G7	1.1e5 $TCID_{50}$/mL	NEG	0/3	0/3	0/3
Enterovirus, D68 strain US/KY/14-18953		1.1e5 $TCID_{50}$/mL				
Epstein Barr Virus (Human Herpes Virus 4 [Hhv-4])		1.1e5 cp/mL				
Herpes Simplex Virus (HSV) type 1		1.1e5 $TCID_{50}$/mL				
Human metapneumovirus (hMPV-5, type B1)		1.1e5 $TCID_{50}$/mL				
Measles		1.1e5 $TCID_{50}$/mL				
Mumps virus		1.1e5 $TCID_{50}$/mL				
Human parainfluenza Type 1	G8	1.1e5 $TCID_{50}$/mL	NEG	0/3	0/3	0/3
Human parainfluenza Type 2		1.1e5 $TCID_{50}$/mL				
Human parainfluenza Type 3		1.1e5 $TCID_{50}$/mL				
Human parainfluenza Type 4		1.1e5 $TCID_{50}$/mL				
Rhinovirus, Type 1A [b]		4.5e4 $TCID_{50}$/mL				
Acinetobacter baumannii	G9	1.1e6 CFU/mL	NEG	0/3	0/3	0/3
Burkholderia cepacia		1.1e6 CFU/mL				
Candida albicans		1.1e6 CFU/mL				
Candida parapsilosis		1.1e6 CFU/mL				
Bordetella pertussis		1.1e6 CFU/mL				
Chlamydia pneumoniae		1.1e6 IFU/mL				
Citrobacter freundii		1.1e6 CFU/mL				
Corynebacterium xerosis	G10	1.1e6 CFU/mL	NEG	0/3	0/3	0/3
Escherichia coli		1.1e6 CFU/mL				
Enterococcus faecalis		1.1e6 CFU/mL				
Hemophilus influenzae		1.1e6 CFU/mL				
Legionella spp [d]		1.1e6 CFU/mL				
Moraxella catarrhalis		1.1e6 CFU/mL				
Mycobacterium tuberculosis (avirulent)	G11	1.1e6 CFU/mL	NEG	0/3	0/3	0/3
Mycoplasma pneumoniae		1.1e6 CCU/mL				
Neisseria mucosa		1.1e6 CFU/mL				
Propionibacterium acnes (= Cutibacterium acnes) Z144		1.1e6 CFU/mL				
Pseudomonas aeruginosa, Z139		1.1e6 CFU/mL				
Staphylococcus aureus		1.1e6 CFU/mL				
Staphylococcus epidermidis	G12	1.1e6 CFU/mL	NEG	0/3	0/3	0/3
Staphyloccus haemolyticus		1.1e6 CFU/mL				

Respiratory Microorganisms	Test Group	Tested Concentration	Final SARS-CoV-2 Result Call-Out	Number of Positive Results Obtained from Total Number of Replicates Tested		
				E	N2	RdRP
Streptococcus agalactiae		1.1e6 CFU/mL				
Streptococcus pneumoniae		1.1e6 CFU/mL				
Streptococcus pyogenes		1.1e6 CFU/mL				
Streptococcus salivarius		1.1e6 CFU/mL				
Streptococcus sanguinis		1.1e6 CFU/mL				
Pneumocystis jirovecii (PJP)		1.1e6 CFU/mL				
Lactobacillus reuteri, F275 [c]	G13	1.1e6 genome cp/mL	NEG	0/3	0/3	0/3
Neisseria meningitides [c]		1.1e6 genome cp/mL				
Pooled human nasal wash	G14	N/A	NEG	0/3	0/3	0/3
Influenza C (Taylor/1233/1947)	G15	1.1e5 $CEID_{50}$/mL	NEG	0/3	0/3	0/3
Rhinovirus, Type 1A[b]	G16	1.1e5 $TCID_{50}$/mL	NEG	0/3	0/3	0/3
Aspergillus flavus	G17	1.35e7 CFU/mL	NEG	0/3	0/3	0/3
Aspergillus fumigatus	G18	3.67e6 CFU/mL	NEG	0/3	0/3	0/3
Coxiella burnetii [c]	G19	2.5e6 genome cp/mL	NEG	0/3	0/3	0/3
Leptospira broomii	G20	2.9e6 CFU/mL	NEG	0/3	0/3	0/3
Parechovirus type 1	G21	3.39e5 TCID50/mL	NEG	0/3	0/3	0/3
Fusobacterium necrophorum	G22	6.52e6 CFU/mL	NEG	0/3	0/3	0/3
Mycoplasma genitalium [c]	G23	3.6e6 genome cp/mL	NEG	0/3	0/3	0/3

a. Microorganism in the form of genomic RNA were tested in Tris-EDTA+ ($[NH_4]_2SO_4$) buffer using an assay definition file (ADF) without sample preparation.
b. Rhinovirus, Type 1A was initially tested at 4.5 e4 $TCID_{50}$/mL in test group G8 using virus lot 325725. It was re-tested individually (G16) at a higher concentration of 1.1e5 $TCID_{50}$/mL using virus lot 326527.
c. Microorganisms in the form of genomic DNA were tested in simulated NPS/NS background matrix using the ADF with full sample preparation.
d. *Legionella pneumophila* was tested in this study.

Microbial Interference

A microbial interference study was performed to assess the inhibitory effects of commensal microorganisms potentially encountered in upper respiratory tract specimens on the performance of the Xpert Xpress CoV-2 *plus* test. A panel of 18 commensal microorganisms, consisting of 15 viral strains and 3 bacterial strains was tested. Contrived samples consisted of SARS-CoV-2 virus seeded at 1.15x-3x LoD into simulated nasopharyngeal swab (NPS)/ anterior nasal swab (NS) matrix in the presence of fifteen (15) commensal virus strains and three (3) commensal bacterial strains (*Haemophilus influenzae, Staphylococcus aureus, Staphylococcus epidermidis*) spiked at their respective concentrations listed in **Table 5-11**.

Replicates of 8 positive samples were tested with SARS-CoV-2 virus and each potential microbial interference strain combination. All 8 of 8 positive replicate samples were correctly identified as SARS-CoV-2 POSITIVE using the Xpert Xpress CoV-2 *plus* test. No interference by the listed above commensal viral or bacterial strains was reported at the concentrations tested.

Table 5-11: Microbial Interference Study Results

Commensal Strain	Concentration Tested	Number of Correct Test Results/ Number Tested
Adenovirus Type 1C [a]	$1x10^5$ $TCID_{50}$/mL	8/8
Adenovirus Type 2C [b]	$1x10^5$ $TCID_{50}$/mL	8/8 [c]
Adenovirus Type 3B[b]	$1x10^5$ $TCID_{50}$/mL	8/8
Human Coronavirus-OC43 [a]	$1x10^5$ copies/mL	8/8
Human Coronavirus-229E [b]	$1x10^5$ $TCID_{50}$/mL	8/8
Human Coronavirus-NL63 [b]	$1x10^5$ $TCID_{50}$/mL	8/8
Human Coronavirus-HKU1 [b]	$1x10^5$ copies/mL	8/8
Metapneumovirus 5, Type B1 [a]	$1x10^5$ $TCID_{50}$/mL	8/8
Parainfluenza Type 1 [a]	$1x10^5$ $TCID_{50}$/mL	8/8
Parainfluenza Type 2 [a]	$1x10^5$ $TCID_{50}$/mL	8/8
Parainfluenza Type 3 [a]	$1x10^5$ $TCID_{50}$/mL	8/8
Rhinovirus Type 1A [a]	$1x10^5$ PFU/mL	8/8
Influenza A H1N1 [b]	$1x10^5$ $CEID_{50}$/mL	8/8
Influenza B [b]	$1x10^5$ $TCID_{50}$/mL	8/8 [d]
Respiratory Syncytial Virus A [b]	$1x10^5$ $TCID_{50}$/mL	8/8
Haemophilus influenzae [a]	$1x10^7$ CFU/mL	8/8
Staphylococcus aureus [a]	$1x10^7$ CFU/mL	8/8
Staphylococcus epidermidis [a]	$1x10^7$ CFU/mL	8/8

a. These commensal strains were tested with SARS-CoV-2 at a concentration of 3x LoD.
b. These commensal strains were tested with SARS-CoV-2 at a concentration of 1.15x LoD.
c. Two of 8 replicates reported as ERROR. The runs were successfully repeated to obtain 8 valid replicates.
d. One of 8 replicates reported as ERROR. The run was successfully repeated to obtain 8 valid replicates.

Potentially Interfering Substances

Substances that could be present in the nasopharynx (or introduced during specimen collection and handling) and potentially interfere with accurate detection of SARS-CoV-2 were evaluated with direct testing on the Xpert Xpress CoV-2 *plus* test. 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)/ anterior 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 SARS-CoV-2 virus spiked at 3x LoD. The controls were samples with and without SARS-CoV-2 virus spiked at 3x LoD into simulated NPS/ NS matrix containing no potentially interfering substance.

The 23 potentially substances, with active ingredients, that were evaluated are listed in **Table 5-12**. For substances that resulted in an INVALID test result, the concentration of the substance was reduced by half and re-tested. Interfering effects were observed for fluticasone propionate and mucin type I-S.

Table 5-12. Potentially Interfering Substances Tested

Substance ID	Substance/Class	Substance/Active Ingredient	Concentrations Tested
No substance	Control	Simulated NPS/NS Matrix	100% (v/v)
Afrin	Nasal Spray	Oxymetazoline (0.05%)	15% (v/v)
Albuterol Sulfate	Beta-adrenergic bronchodilator	Albuterol Sulfate (5mg/mL)	0.83 mg/mL (equivalent to 1 dose per day)
BD Universal Transport Medium	Transport Media	BD Universal Transport Medium	100% (v/v)
Blood	Blood	Blood (Human)	2% (v/v)
Copan Swab M	Transport Media	Copan Swab M	100% (v/v)
FluMist	FluMist®	Live intranasal influenza virus vaccine	6.7% (v/v)
Fluticasone Propionate Nasal Spray	Nasal corticosteroid	Fluticasone Propionate	5 µg/mL, 2.5 µg/mL
Ibuprofen	Analgesic (nonsteroidal anti-inflammatory drugs (NSAID))	Ibuprofen	21.9 mg/dL
Leukocytes	Leukocytes	Leukocytes (Human)	1 x 10^6 cells/mL
Menthol	Throat lozenges, oral anesthetic, and analgesic	Benzocaine, Menthol	1.7 mg/mL
Mucin type II	Mucin	Purified Mucin protein (Porcine submaxillary gland, type II)	0.1% (w/v)
Mucin type I-S	Mucin	Purified Mucin protein (Bovine submaxillary gland, type I-S)	2.5 mg/mL, 1.25 mg/mL
Mupirocin	Antibiotic, nasal ointment	Mupirocin (2%)	10 mg/mL
Human peripheral blood mononuclear cells (PBMC)	Human peripheral blood mononuclear cells (PBMC)	Human peripheral blood mononuclear cells (PBMC)	1x10^3 cells/µL
PHNY	Nasal Drops	Phenylephrine (1%)	15% (v/v)
Remel M4RT	Transport Media	Remel M4RT	100% (v/v)
Remel M5	Transport Media	Remel M5	100% (v/v)
Saline	Saline Nasal Spray	Sodium Chloride (0.65%)	15% (v/v)
Snuff	Tobacco	Nicotine	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)
Zinc	Zinc supplement	Zinc Gluconate	0.1 µg/mL

The results for the negative and positive samples are presented in **Table 5-13**.

The results from the SARS-CoV-2 negative samples showed that in the presence of fluticasone propionate nasal spray at 5 µg/mL and mucin type I-S at 2.5 mg/mL, 7 of 8 replicates correctly reported **"SARS-CoV-2 NEGATIVE"** test results, and 1 of 8 replicates reported **"INVALID"** test result for each substance. When the concentrations of fluticasone

propionate nasal spray and mucin type I-S were reduced by half, 8/8 replicates provided valid test results and correctly reported **"SARS-CoV-2 NEGATIVE"** for each substance. No interference was observed at these lower concentrations.

The results from the SARS-CoV-2 positive samples showed that in the presence of fluticasone propionate nasal spray at 5 µg/mL, 7 of 8 replicates correctly reported **"SARS-CoV-2 POSITIVE"** test results and 1 of 8 replicate reported **"INVALID"** test result. When the concentration of the fluticasone propionate nasal spray was reduced to 2.5 µg/mL, 8/8 replicates provided valid test results and correctly reported **"SARS-CoV-2 POSITIVE"**. No interference was observed at this lower concentration.

Table 5-13. SARS-CoV-2 Negative Samples Tested in the Presence of Potentially Interfering Substances

Substance	Concentration Tested	Number SARS-CoV-2 Negative / Number of Valid Replicates Tested	Number SARS-CoV-2 Positive / Number of Valid Replicates Tested
SARS-CoV-2 Negative (No Substance) Control	**100% (v/v)**	**8/8**	**Not Applicable**
SARS-CoV-2 Positive (No Substance) Control	**100% (v/v)**	**Not Applicable**	**8/8**
Afrin	15% (v/v)	8/8	8/8
Albuterol Sulfate	0.83 mg/mL	8/8	8/8
BD Universal Transport Medium	100 (v/v)	8/8	8/8
Blood	2% (v/v)	8/8	8/8
Copan Swab M	100 (v/v)	8/8	8/8
FluMist®	6.7% (v/v)	8/8	8/8
Fluticasone Propionate Nasal Spray	5 µg/mL	7/8[a]	7/8[a]
	2.5 µg/mL	8/8	8/8
Ibuprofen	21.9 mg/dL	8/8	8/8
Leukocytes	1 x 10^6 cells/mL	8/8	8/8
Menthol	1.7 mg/mL	8/8	8/8
Mucin (Type II)	0.1% (w/v)	8/8	8/8
Mucin (Type I-S)	2.5 mg/mL	7/8[a]	8/8
	1.25 mg/mL	8/8	Not Applicable
Mupirocin	10 mg/mL	8/8	8/8
Human Peripheral Blood Mononuclear Cells (PBMC)	1 x 10^3 cells/µL	8/8	8/8
PHNY	15% (v/v)	8/8	8/8
Remel M4RT	100% (v/v)	8/8	8/8
Remel M5	100% (v/v)	8/8	8/8
Saline	15% (v/v)	8/8	8/8
Snuff	1% (w/v)	8/8	8/8
Tamiflu	7.5 mg/mL	8/8	8/8
Tobramycin	4 µg/mL	8/8	8/8
Zicam	15% (w/v)	8/8	8/8
Zinc	0.1 µg/mL	8/8	8/8

a. One of 8 replicates reported **INVALID** test result, indicating interference from the substance. The substance was subsequently tested with 8 replicates at half the initial concentration.

Carryover Contamination

A study was conducted to assess whether the single-use, self-contained Xpert Xpress CoV-2 *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 SARS-CoV-2 virus concentration (inactivated SARS-CoV-2 USA-WA1/2020 at 5e4 copies/mL) spiked into negative 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 and all 42 negative samples were correctly reported as SARS-CoV-2 NEGATIVE with the Xpert Xpress CoV-2 *plus* test. No specimen or amplicon carry-over contamination was observed in this study.

Reproducibility

The reproducibility of the Xpert Xpress CoV-2 *plus* test was established at 3 sites (2 external and 1 internal) using a 3-member panel including one negative sample, one low positive (~1.5x LoD) sample and one moderate positive (~3x LoD) sample. The negative sample consisted of simulated NS/NPS matrix without target microorganism or target RNA. The positive samples were contrived samples in a simulated matrix using inactivated NATtrol SARS-CoV-2 (ZeptoMetrix).

Testing was conducted over 6 days, using 3 lots of Xpert Xpress CoV-2 *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 percent agreement of the qualitative results for SARS-CoV-2 detection for each panel member analyzed by each of the 6 operators and each site is shown in **Table 5-14**. 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 5-14. Summary of the Reproducibility Results - % Agreement

Panel Member	Site 1			Site 2			Site 3			% Total Agreement and 95% CI by Panel Member
	Op1	Op2	Site	Op1	Op2	Site	Op1	Op2	Site	
Negative	100% (24/24)	95.8% (23/24)	97.9% (47/48)	100% (24/24)	100% (24/24)	100% (48/48)	100% (24/24)	100% (23/23) [a]	100% (47/47)	99.3% (142/143) [96.1% - 99.9%]
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%]
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%]

Abbreviation: Op, operator; Pos, positive; Mod, moderate; CI, confidence interval

a. One sample was non-determinate on both initial and retest and was excluded from the analyses.

The evaluation of reproducibility and within-laboratory precision of the underlying Ct values for E, N2 and RdRP obtained in the Xpert Xpress CoV-2 *plus* test was analyzed. The mean, 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 5-15**.

Table 5-15. Summary of Reproducibility Results – Nested ANOVA by Coefficient of Variation

Sample	Analyte	N	Mean Ct	Between Sites		Between Operators		Between Lots		Between Days		Between Runs		Within Run		Total	
				SD	CV (%)	SD	CV (%)	SD	CV (%)	SD	CV (%)	SD	CV (%)	SD	CV (%)	SD	CV (%)
Negative	**SPC**	143	31.1	0.29	0.9	0.16	0.5	0.42	1.3	0	0	0	0	1.47	4.7	1.56	5.0
SARS-CoV-2 Low Pos	**E**	144	34.0	0.06	0.2	0.19	0.6	0	0	0.14	0.4	0	0	0.55	1.6	0.60	1.8
	N2	144	37.5	0.12	0.3	0	0	0.2	0.5	0.11	0.3	0	0	0.68	1.8	0.73	1.9
	RdRP	144	36.3	0.07	0.2	0	0	0.25	0.7	0.06	0.2	0	0	0.61	1.7	0.66	1.8
SARS-CoV-2 Mod Pos	**E**	144	32.5	0.31	0.9	0.03	0.1	0	0	0.47	1.4	0	0	1.86	5.7	1.95	6.0
	N2	144	36.2	0.16	0.4	0	0	0.29	0.8	0.17	0.5	0	0	0.65	1.8	0.75	2.1
	RdRP	144	34.8	0.33	1.0	0	0	0	0	0.62	1.8	0	0	2.18	6.2	2.29	6.6

Single-Site Precision

The precision of the Xpert Xpress CoV-2 *plus* test was established at a single site using a 3-member panel including one negative sample, one low positive (~1.5x LoD) sample and one moderate positive (~3x LoD) sample. The negative sample consisted of simulated NS/NPS matrix without target microorganism or target RNA. The positive samples were contrived samples in a simulated NS/NPS matrix using inactivated NATtrol SARS-CoV-2 (ZeptoMetrix).

Testing was conducted over 20 days, using one lot of Xpert Xpress CoV-2 *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 5-16**.

Table 5-16. Summary of the Single-Site Precision Results – % Agreement

Panel Member	Agreement	% Total Agreement and 95% CI by Panel Member
Negative	80/80	100% (95.4%-100.0%)
SARS-CoV-2 Low Positive	80/80	100% (95.4%-100.0%)
SARS-CoV-2 Mod Positive	80/80	100% (95.4%-100.0%)

5.4.2. Clinical Performance

The clinical performance of the Xpert Xpress CoV-2 *plus* test was evaluated in a multi-site, observational and method comparison study that included 32 geographically diverse sites in the United States (US). Of the 32 sites, 5 sites participated in specimen collection only, 26 sites performed Xpert testing and specimen collection, and 1 site performed Xpert testing as well as comparator and discrepant testing.

The performance of the Xpert Xpress CoV-2 *plus* test was evaluated using prospectively collected fresh (98.6%) and frozen (1.4%) clinical nasopharyngeal swab (NPS) and anterior nasal swab (NS) specimens in viral transport medium or universal transport medium. These specimens were collected in 2022 from individuals with signs and symptoms of respiratory tract infection and tested using Xpert Xpress CoV-2 *plus* side by side with a U.S. FDA-cleared molecular respiratory panel that includes SARS-CoV-2, in a randomized and blinded fashion. A total of 4047 specimens (2029 NPS and 2018 NS) were evaluated from individuals with signs and symptoms of respiratory infection. Of these, 60 specimens yielded non-determinate results (INVALID, ERROR, and NO RESULT) with Xpert Xpress CoV-2 *plus*. Additionally, 237 specimens either yielded non-determinate result, or were not tested per comparator package insert. Therefore, 297 (60 + 237) specimens were excluded, and a total of 3750 (1879 NPS and 1871 NS) specimens that yielded valid results by both Xpert Xpress CoV-2 *plus* and the U.S. FDA-cleared molecular respiratory panel were included in

the clinical performance evaluation. Available demographic data collected from study participants are presented in **Table 5-17**.

Table 5-17. Demographic Data Summary of Participants Symptomatic of Respiratory Infection

	NPS (N=1879)	NS (N=1871)	Overall (N=3750)
Gender			
Female	1107 (58.9%)	1171 (62.6%)	2278 (60.7%)
Male	772 (41.1%)	700 (37.4%)	1472 (39.3%)
Age Group (Years)			
≤5	8 (0.4%)	64 (3.4%)	72 (1.9%)
6-21	389 (20.7%)	380 (20.3%)	769 (20.5%)
22-59	1228 (65.4%)	1167 (62.4%)	2395 (63.9%)
≥60	254 (13.5%)	260 (13.9%)	514 (13.7%)
Race			
American Indian or Alaska Native	4 (0.2%)	4 (0.2%)	8 (0.2%)
Asian	44 (2.3%)	44 (2.4%)	88 (2.3%)
Black or African American	525 (27.9%)	524 (28.0%)	1049 (28.0%)
White	1236 (65.8%)	1222 (65.3%)	2458 (65.5%)
Native Hawaiian or Other Pacific Islander	4 (0.2%)	0 (0%)	4 (0.1%)
Black or African American, White	7 (0.4%)	8 (0.4%)	15 (0.4%)
Other Mixed (each N≤5)	7 (0.4%)	2 (0.1%)	9 (0.2%)
Participant declined to answer, or unknown	52 (2.8%)	67 (3.6%)	119 (3.2%)
Ethnicity			
Hispanic	170 (9.0%)	165 (8.8%)	335 (8.9%)
Non-Hispanic	1681 (89.5%)	1668 (89.2%)	3349 (89.3%)
Participant declined to answer, or unknown	28 (1.5%)	38 (2.0%)	66 (1.8%)
Specimen Testing			
Fresh	1852 (98.6%)	1845 (98.6%)	3697 (98.6%)
Frozen	27 (1.4%)	26 (1.4%)	53 (1.4%)
Testing Environment			
Laboratory/NPT	996 (53.0%)	971 (51.9%)	1967 (52.5%)
CW	883 (47.0%)	900 (48.1%)	1783 (47.5%)
Vaccine Status			
Vaccinated	1336 (71.1%)	1344 (71.8%)	2680 (71.5%)
Not Vaccinated	523 (27.8%)	508 (27.2%)	1031 (27.5%)
Unknown	20 (1.1%)	19 (1.0%)	39 (1.0%)

Positive Percent Agreement (PPA) and Negative Percent Agreement (NPA) were determined by comparing the results of the Xpert Xpress CoV-2 *plus* test relative to the results of a U.S. FDA-cleared molecular respiratory panel for the SARS-CoV-2 target. Based on the specimens that yielded non-determinate results (INVALID, ERROR, and NO RESULT) with Xpert Xpress CoV-2 *plus*, the non-determinate rate was 1.6% (32/2029) with NPS and 1.4% (28/2018) with NS specimens.

A total of 3750 (1879 NPS and 1871 NS) specimens that yielded valid results by both Xpert Xpress CoV-2 *plus* and the U.S. FDA-cleared molecular respiratory panel were included in the clinical performance evaluation for SARS-CoV-2. The Xpert Xpress CoV-2 *plus* demonstrated an overall PPA and NPA of 98.1% and 98.3% for SARS-CoV-2, respectively (**Table 5-18**).

Table 5-18. Xpert Xpress CoV-2 *plus* Performance Results in Symptomatic Individuals

		Comparator Result								
		Overall			**NPS**			**NS**		
		Positive	Negative	Total	Positive	Negative	Total	Positive	Negative	Total
Xpert Xpress CoV-2 *plus*	Positive	574	55	629	292	28[b]	320	282	27[d]	309
	Negative	11	3110	3121	9[a]	1550	1559	2[c]	1560	1562
	Total	585	3165	3750	301	1578	1879	284	1587	1871
PPA		98.1% (95% CI: 96.7% - 98.9%)			97.0% (95% CI: 94.4% - 98.4%)			99.3% (95% CI: 97.5% - 99.8%)		
NPA		98.3% (95% CI: 97.7% - 98.7%)			98.2% (95% CI: 97.4% - 98.8%)			98.3% (95% CI: 97.5% - 98.8%)		

NPS: nasopharyngeal swab specimen; NS: anterior nasal swab specimen; PPA: Positive Percent Agreement; NPA: Negative Percent Agreement; CI: 95% two-sided Confidence Interval

[a] Discrepant test results based on a U.S. FDA EUA SARS-CoV-2 molecular test: 7/9 SARS-CoV-2 negative; 1/7 SARS-CoV-2 positive; 1/9 invalid.
[b] Discrepant test results based on a U.S. FDA EUA SARS-CoV-2 molecular test: 8/28 SARS-CoV-2 positive; 19/28 SARS-CoV-2 negative; 1/28 invalid.
[c] Discrepant test results based on a U.S. FDA EUA SARS-CoV-2 molecular test: 2/2 SARS-CoV-2 negative.
[d] Discrepant test results based on a U.S. FDA EUA SARS-CoV-2 molecular test: 8/27 SARS-CoV-2 positive; 18/27 SARS-CoV-2 negative; 1/27 invalid.

5.5. Conclusions

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