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

I Background Information:

A 510(k) Number

K230440

B Applicant

Cepheid

C Proprietary and Established Names

Xpert Xpress CoV-2 *plus*

D Regulatory Information

Product Code(s)	Classification	Regulation Section	Panel
QQX	Class II	21 CFR 866.3981 - Device To Detect And Identify Nucleic Acid Targets In Respiratory Specimens From Microbial Agents That Cause The SARS-Cov-2 Respiratory Infection And Other Microbial Agents When In A Multi-Target Test	MI - Microbiology
OOI	Class II	21 CFR 862.2570 - Instrumentation for clinical multiplex test systems	CH - Clinical Chemistry

II Submission/Device Overview:

A Purpose for Submission:

To obtain a substantial equivalence determination for the Xpert Xpress CoV-2 *plus* test performed on the Cepheid GeneXpert Instrument Systems (GeneXpert Dx, GeneXpert Infinity-48s and GeneXpert Infinity-80 systems).

B Measurand:
Nucleocapsid (N2), envelope (E), and RNA-dependent RNA polymerase (RdRP) genes of SARS-CoV-2.

C Type of Test:
The Xpert Xpress CoV-2 *plus* test is a real-time reverse transcription polymerase chain reaction (RT-PCR) based *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.

III Intended Use/Indications for Use:

A Intended Use(s):
See Indications for Use below.

B Indication(s) for Use:
The Xpert Xpress CoV-2 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. .

C Special Conditions for Use Statement(s):
Rx - For Prescription Use Only

For in vitro diagnostic use Only

D Special Instrument Requirements:

The Xpert Xpress CoV-2 *plus* test is performed on the Cepheid GeneXpert Instrument Systems (GeneXpert Dx, GeneXpert Infinity-48s and GeneXpert Infinity-80s systems).

IV Device/System Characteristics:

A 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.

B Principle of Operation:

The Xpert Xpress CoV-2 *plus* test utilizes automated reverse transcription cDNA synthesis from RNA and real-time polymerase chain reaction (PCR) for gene specific sequence amplification. Detection of the amplified DNA is by fluorogenic target-specific probe hybridization followed by 5'-nuclease cleavage of the probe to release the fluorophore (TaqMan).

The technology utilizes lyophilized reagents in the form of freeze-dried beads, which provide all the components (enzymes, primers, probes, salts, buffers and divalent metal ion) to support PCR when reconstituted.

C Instrument Description Information:

1. Instrument Name:

Cepheid GeneXpert Instrument Systems [GeneXpert Dx (versions 4.7b or higher), GeneXpert Infinity-48s and GeneXpert Infinity-80s systems (versions 6.4b or higher)].

2. Specimen Identification:

Anterior nasal (NS) or nasopharyngeal (NPS) swab specimen identification is automated.

3. Specimen Sampling and Handling:
The NPS or NS specimen is collected and placed into a transport tube containing 3 mL of Viral Transport Medium (VTM)/ Universal Transport Medium (UTM) or 2 mL of eNAT. The specimen is briefly mixed by rapidly inverting the collection tube 5 times. Using the supplied transfer pipette, the specimen is transferred to the sample chamber of the Xpert Xpress CoV-2 *plus* cartridge. The GeneXpert cartridge is loaded onto the GeneXpert instrument, which performs hands-off, automated specimen processing, and real-time RT-PCR for detection of viral RNA.

4. Calibration:
GeneXpert instruments are calibrated at the factory. Routine calibration of the GeneXpert Instrument systems may be performed by Cepheid Field Service Engineers during annual maintenance.

5. Quality Control:
Internal Controls: Each cartridge includes a Sample Processing Control (SPC) and Probe Check Control (PCC).
   - Sample Processing Control (SPC) – The SPC is an encapsidated RNA pseudovirus that ensures adequate lysis of target virus, monitors the presence of PCR inhibitors, and verifies the use of proper PCR conditions. The SPC passes if it meets the validated acceptance criteria. The SPC result should be PASS in a sample that is negative for all three SARS-CoV-2 target analytes and can be NEGATIVE or POSITIVE in a sample containing detectable levels of one or more of the SARS-CoV-2 target analytes.
   - Probe Check Control (PCC) – Before the start of the PCR reaction, the GeneXpert System measures the fluorescence signal from the probes to monitor bead rehydration, reaction tube filling, probe integrity, and dye stability. The PCC passes if it meets the validated acceptance criteria.

External Controls:
External controls should be used in accordance with local, state, and federal accrediting organizations as applicable.

V Substantial Equivalence Information:

A Predicate Device Name(s):
BioFire COVID-19 Test 2

B Predicate 510(k) Number(s):
K211079

C Comparison with Predicate(s):

Device & Predicate Device(s):	K230440	K211079
Device Trade Name	Xpert Xpress CoV-2 *plus*	BioFire COVID-19 Test 2
Product Code	Same	QQX

		Respiratory Specimen Nucleic Acid SARS-CoV-2 Test
Regulation Number/Name	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
Device Class	Same	II (Special Controls)
General Device Characteristic Similarities		
Intended Use/Indications For 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 SARSCoV-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 laboratory setting or under the supervision of a trained laboratory professional.
Analyte Target	Same	SARS-CoV-2
Control	Same	Internal Controls
Test Format	Same	Single Use
Result Interpretation	Same	Automated
Test Result	Same	Qualitative
Automation	Same	Automated Nucleic Acid Extraction, Detection and Results Interpretation
Intended Environment for Use	Same	Professional use
Prescription Use Only	Same	Yes
General Device Characteristic Differences		
Technology	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.
SARS-CoV-2 Target Type	E, N2, RdRP	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
Instrumentation	Cepheid GeneXpert Instrument Systems	FilmArray 2.0 or FilmArray Torch systems
Internal Control	• Sample Processing Control (SPC) • Probe Check Control (PCC)	• Sample Processing Control • PCR and Melt Analysis Control
Time to Result	~ 30 minutes	~ 45 minutes

VI Standards/Guidance Documents Referenced:

- Class II Special Controls as per 21 CFR 866.3981
- Guidance for Industry and FDA Staff: General Principles of Software Validation, issued January 11, 2002.

- Guidance for the Content of Premarket Submissions for Software Contained in Medical Devices, May 11, 2005.
- Guidance for Industry and FDA Staff: Statistical Guidance on Reporting Results from Studies Evaluating Diagnostic Tests, issued on March 13, 2007.
- Clinical and Laboratory Standards Institute (CLSI) documents: EP05-A3 (Evaluation of Precision of Quantitative Measurement Procedures, 3rd Edition).
- Clinical and Laboratory Standards Institute (CLSI) documents: EP07-A3 (Interference Testing in Clinical Chemistry, 3rd Edition).
- Clinical and Laboratory Standards Institute (CLSI) documents: EP12-A2 (User Protocol For Evaluation Of Qualitative Test Performance; Approved Guideline, 2nd Edition).
- Clinical and Laboratory Standards Institute (CLSI) documents: EP17-A2 (Evaluation of Detection Capability for Clinical Laboratory Measurement Procedures, 2nd Edition).

VII Performance Characteristics (if/when applicable):

A Analytical Performance:

1. Reproducibility and Within-Laboratory Precision Study:

a) Reproducibility Study

The reproducibility of the Xpert Xpress CoV-2 *plus* test was established at three sites (two external and one internal) using a three-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 prepared by spiking the NATtrol inactivated SARS-CoV- 2 virus strain USA-WA1/2020 (ZeptoMetrix). Testing was conducted over six days with the GeneXpert Dx and Infinity systems, using three lots of Xpert Xpress CoV-2 *plus* cartridges at three participating sites each with two 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 external controls, consisting of one positive and one negative control, were tested prior to testing panel samples at the site on each day of the study.

The percent agreement of the qualitative results for SARS-CoV-2 detection for each panel member analyzed by each of the six operators and each site is shown in **Table 1**. 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 1: Summary of the Reproducibility Study Results - % Agreement

Panel Member	Site 1			Site 2			Site 3			% Total Agreement and 95% CI
	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 performed. 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 2**.

Table 2: 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

b) Within-Laboratory Precision Study

The within-laboratory precision of the Xpert Xpress CoV-2 *plus* test was evaluated at a single site using a three-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 swab matrix without target microorganism or target RNA. The positive samples were contrived samples in a simulated NS/NPS matrix prepared by spiking the NATtrol inactivated SARS-CoV- 2 virus strain USA-WA1/2020 (ZeptoMetrix).

Testing was conducted over 20 days, using one lot of Xpert Xpress CoV-2 *plus* cartridges at a single site and with one operator to yield a total of eighty 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 agreement of the qualitative results for SARS-CoV-2 detection for each panel member, including the overall percent agreement for each sample (% total agreement) and the two-sided Wilson Score confidence intervals (CI), are presented in **Table 3**. The mean, standard deviation (SD), and coefficient of variation (CV) between-days, between-runs, and within-run for each panel member are presented in **Table 4.**

Table 3: Summary of the With-in Laboratory 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%)

Table 4: Within-Laboratory Precision – Nested ANOVA by Coefficient of Variation

Sample	Analyte of Ct Values	N	Mean Ct	Between Days		Between Runs		Within Run		Total	
				SD	CV	SD	CV	SD	CV	SD	CV
Negative	SPC	80	30.34	0.26	0.8	0.00	0.0	1.03	3.4	1.06	3.5
SARS-CoV-2 ~1.5 x LoD	E	80	34.12	0.00	0.0	0.21	0.6	0.5	1.5	0.55	1.6
	N2	80	37.66	0.00	0.0	0.11	0.3	0.57	1.5	0.58	1.5
	RdRP	80	36.77	0.00	0.0	0.00	0.0	0.66	1.8	0.66	1.8
SARS-CoV-2 ~3 x LoD	E	80	33.37	0.00	0.0	0.00	0.0	1.03	3.1	1.03	3.1
	N2	80	36.89	0.17	0.4	0.00	0.0	1.02	2.8	1.03	2.8
	RdRP	80	35.99	0.27	0.7	0.37	1.0	0.95	2.6	1.05	2.9

*CV = SD/Mean Ct * 100

2. Linearity:

Not applicable; this is a qualitative assay.

3. Analytical Reactivity:

a) *In silico* Inclusivity Analysis

i) 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** summarizes the effective predicted inclusivity for E, N2 and RdRP amplicons for the variants of interests and concern.

Table 5: 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 6** 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 6: 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.

ii) Additional evaluation of inclusivity of Xpert Xpress CoV-2 *plus* primers was conducted on July 31, 2023 using *in silico* analysis of the assay amplicons in relation to SARS-CoV-2 sequences available in the GISAID gene database for three targets, E, N2 and RdRP. U.S.-specific sequences were then partitioned into the four time periods of 14, 30, 90 and 180 days (**Tables 7-10**) prior to the cutoff date of July 31, 2023 and were stratified by number and frequency of sequences with exact or mismatched sequences. The *in silico* inclusivity statistics for each time period are presented in **Tables 7-10**. In each of these tables, the results for the two N2 probes were combined into a single row which represents the best match of either probe.

Table 7: Inclusivity Statistic - Sequences Collected Within 14 Days Prior to July 31, 2023

SARS-CoV-2 Target		*In Silico* Inclusivity Statistics				
		Exact Match	**1 Mismatch**	**2 Mismatches**	**3 Mismatches**	**3+ Mismatches**
E	Forward	97.7% (173/177)	2.3% (4/177)	0.0% (0/177)	0.0% (0/177)	0.0% (0/177)
	Probe	100.0% (177/177)	0.0% (0/177)	0.0% (0/177)	0.0% (0/177)	0.0% (0/177)
	Reverse	100.0% (177/177)	0.0% (0/177)	0.0% (0/177)	0.0% (0/177)	0.0% (0/177)
		Exact Match	**1 Mismatch**	**2 Mismatches**	**3 Mismatches**	**3+ Mismatches**
N2	Forward	97.7% (172/176)	2.3% (4/176)	0.0% (0/176)	0.0% (0/176)	0.0% (0/176)
	Probe	100% (176/176)	0.0% (0/176)	0.0% (0/176)	0.0% (0/176)	0.0% (0/176)
	Reverse	99.4% (175/176)	0.6% (1/176)	0.0% (0/176)	0.0% (0/176)	0.0% (0/176)
		Exact Match	**1 Mismatch**	**2 Mismatches**	**3 Mismatches**	**3+ Mismatches**
RdRP	Forward	99.4% (172/173)	0.6% (1/173)	0.0% (0/173)	0.0% (0/173)	0.0% (0/173)
	Reverse	100.0% (173/173)	0.0% (0/173)	0.0% (0/173)	0.0% (0/173)	0.0% (0/173)
	Probe	**1 Insertion***	**1 Insertion + 1 Mismatch**	**1 Insertion + 2 Mismatches**	**1 Insertion + 3 Mismatches**	**1 Insertion + > 3 Mismatches**
		99.4% (172/173)	0.6% (1/173)	0.0% (0/173)	0.0% (0/173)	0.0% (0/173)

* RdRp probe design lacks dC at position 14 from the 5'-end of the oligonucleotide.

As shown in **Table 7**, there are two instances where a specific oligonucleotide was observed to have a mismatch frequency greater than 1% in the 14-day period. The total number of sequences collected in the 14-day period is small, which can be a source of bias and may not be representative of the test performance. Consistent with this account, there were no instances where a specific primer/probe sequence was observed with greater than 1% frequency of

mismatch in any of the longer time stratifications (30 days, 90 days and 180 days), which would warrant a risk assessment for adverse impact to the test performance. 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.

Table 8: Inclusivity Statistic - Sequences Collected Within 30 Days Prior to July 31, 2023

SARS-CoV-2 Target		*In Silico* Inclusivity Statistics				
		Exact Match	**1 Mismatch**	**2 Mismatches**	**3 Mismatches**	**3+ Mismatches**
E	Forward	99.2% (2505/2526)	0.8% (21/2526)	0.0% (0/2526)	0.0% (0/2526)	0.0% (0/2526)
	Probe	99.8% (2522/2526)	0.2% (4/2526)	0.0% (0/2526)	0.0% (0/2526)	0.0% (0/2526)
	Reverse	100.0% (2525/2526)	0.0% (1/2526)	0.0% (0/2526)	0.0% (0/2526)	0.0% (0/2526)
		Exact Match	**1 Mismatch**	**2 Mismatches**	**3 Mismatches**	**3+ Mismatches**
N2	Forward	99.2% (2509/2528)	0.8% (19/2528)	0.0% (0/2528)	0.0% (0/2528)	0.0% (0/2528)
	Probe	99.8% (2522/2528)	0.2% (6/2528)	0.0% (0/2528)	0.0% (0/2528)	0.0% (0/2528)
	Reverse	99.3% (2510/2528)	0.7% (18/2528)	0.0% (0/2528)	0.0% (0/2528)	0.0% (0/2528)
		Exact Match	**1 Mismatch**	**2 Mismatches**	**3 Mismatches**	**3+ Mismatches**
RdRp	Forward	99.6% (2488/2499)	0.4% (11/2499)	0.0% (0/2499)	0.0% (0/2499)	0.0% (0/2499)
	Reverse	99.9% (2497/2499)	0.1% (2/2499)	0.0% (0/2499)	0.0% (0/2499)	0.0% (0/2499)
	Probe	**1 Insertion***	**1 Insertion + 1 Mismatch**	**1 Insertion + 2 Mismatches**	**1 Insertion + 3 Mismatches**	**1 Insertion + > 3 Mismatches**
		99.5% (2486/2499)	0.5% (13/2499)	0.0% (0/2499)	0.0% (0/2499)	0.0% (0/2499)

* RdRp probe design lacks dC at position 14 from the 5'-end of the oligonucleotide.

Table 9: Inclusivity Statistic - Sequences Collected Within 90 Days Prior to July 31, 2023

SARS-CoV-2 Target		*In Silico* Inclusivity Statistics				
		Exact Match	**1 Mismatch**	**2 Mismatches**	**3 Mismatches**	**3+ Mismatches**
E	Forward	99.3% (25156/25321)	0.7% (165/25321)	0.0% (0/25321)	0.0% (0/25321)	0.0% (0/25321)
	Probe	99.9% (25292/25321)	0.1% (29/25321)	0.0% (0/25321)	0.0% (0/25321)	0.0% (0/25321)
	Reverse	99.9% (25304/25321)	0.1% (16/25321)	0.0% (1/25321)	0.0% (0/25321)	0.0% (0/25321)
		Exact Match	**1 Mismatch**	**2 Mismatches**	**3 Mismatches**	**3+ Mismatches**
N2	Forward	99.2% (25272/25483)	0.8% (211/25483)	0.0% (0/25483)	0.0% (0/25483)	0.0% (0/25483)
	Probe	99.6% (25389/25483)	0.4% (93/25483)	0.0% (1/25483)	0.0% (0/25483)	0.0% (0/25483)
	Reverse	99.5% (25353/25483)	0.5% (130/25483)	0.0% (0/25483)	0.0% (0/25483)	0.0% (0/25483)
		Exact Match	**1 Mismatch**	**2 Mismatches**	**3 Mismatches**	**3+ Mismatches**
RdRp	Forward	99.7% (25216/25302)	0.3% (86/25302)	0.0% (0/25302)	0.0% (0/25302)	0.0% (0/25302)
	Reverse	99.8% (25242/25302)	0.2% (60/25302)	0.0% (0/25302)	0.0% (0/25302)	0.0% (0/25302)
	Probe	**1 Insertion***	**1 Insertion + 1 Mismatch**	**1 Insertion + 2 Mismatches**	**1 Insertion + 3 Mismatches**	**1 Insertion + > 3 Mismatches**
		99.6% (25192/25302)	0.4% (110/25302)	0.0% (0/25302)	0.0% (0/25302)	0.0% (0/25302)

* RdRp probe design lacks dC at position 14 from the 5'-end of the oligonucleotide.

Table 10: Inclusivity Statistic - Sequences Collected Within 180 Days Prior to July 31, 2023

SARS-CoV-2 Target		*In Silico* Inclusivity Statistics				
		Exact Match	**1 Mismatch**	**2 Mismatches**	**3 Mismatches**	**3+ Mismatches**
E	Forward	99.5% (147492/148212)	0.5% (717/148212)	0.0% (3/148212)	0.0% (0/148212)	0.0% (0/148212)
	Probe	99.8% (147963/148212)	0.2% (249/148212)	0.0% (0/148212)	0.0% (0/148212)	0.0% (0/148212)
	Reverse	99.9% (148106/148212)	0.1% (105/148212)	0.0% (1/148212)	0.0% (0/148212)	0.0% (0/148212)
		Exact Match	**1 Mismatch**	**2 Mismatches**	**3 Mismatches**	**3+ Mismatches**
N2	Forward	99.1% (149625/150916)	0.9% (1287/150916)	0.0% (4/150916)	0.0% (0/150916)	0.0% (0/150916)
	Probe	99.8% (150597/150916)	0.2% (315/150916)	0.0% (4/150916)	0.0% (0/150916)	0.0% (0/150916)
	Reverse	99.6% (150373/150916)	0.4% (543/150916)	0.0% (0/150916)	0.0% (0/150916)	0.0% (0/150916)
		Exact Match	**1 Mismatch**	**2 Mismatches**	**3 Mismatches**	**3+ Mismatches**
RdRp	Forward	99.8% (146465/146703)	0.2% (238/146703)	0.0% (0/146703)	0.0% (0/146703)	0.0% (0/146703)
	Reverse	99.9% (146489/146703)	0.1% (214/146703)	0.0% (0/146703)	0.0% (0/146703)	0.0% (0/146703)
	Probe	**1 Insertion***	**1 Insertion + 1 Mismatch**	**1 Insertion + 2 Mismatches**	**1 Insertion + 3 Mismatches**	**1 Insertion + > 3 Mismatches**
		99.7% (146204/146703)	0.3% (499/146703)	0.0% (0/146703)	0.0% (0/146703)	0.0% (0/146703)

* RdRp probe design lacks dC at position 14 from the 5'-end of the oligonucleotide.

b) Inclusivity Wet-Testing

In addition to the *in silico* analysis of the SARS-CoV-2 primers and probes, the inclusivity of the Xpert Xpress CoV-2 *plus* test was evaluated by wet testing against multiple strains of SARS-CoV-2 at ~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 11**.

Table 11: 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
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

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
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
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

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
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.

4. Analytical Specificity/Interference:

a) *In Silico* Analysis

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 the 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 12** is expected based on the *in silico* analysis.

Table 12: 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

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

b) Cross-reactivity Wet-Testing

In addition to *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 strains, 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 sample with 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 produces a false positive result was identified.

The results of the cross-reactivity study demonstrated that the primer/probe sets included in the Xpert Xpress CoV-2 *plus* do 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 SARS-CoV-2 POSITIVE test result. The cross-reactivity for the E gene target was expected 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 13.**

Table 13: Cross-reactivity testing 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				

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
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				
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.

c) 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 spiked at 1.15x-3x LoD into simulated NPS/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 14**. SARS-CoV-2 virus and each potential microbial interference strain combination was tested in replicates of eight. All eight 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 14: Microbial Interference Study Results

Commensal Strain	Concentration Tested	Number of Correct Test Results/ Number Tested
Adenovirus Type 1C[a]	$1x10^5$ TCID50/mL	8/8
Adenovirus Type 2C[b]	$1x10^5$ TCID50/mL	8/8[c]
Adenovirus Type 3B[b]	$1x10^5$ TCID50/mL	8/8
Human Coronavirus-OC43[a]	$1x10^5$ copies/mL	8/8
Human Coronavirus-229E[b]	$1x10^5$ TCID50/mL	8/8
Human Coronavirus-NL63[b]	$1x10^5$ TCID50/mL	8/8
Human Coronavirus-HKU1[b]	$1x10^5$ copies/mL	8/8
Metapneumovirus 5, Type B1[a]	$1x10^5$ TCID50/mL	8/8
Parainfluenza Type 1[a]	$1x10^5$ TCID50/mL	8/8
Parainfluenza Type 2[a]	$1x10^5$ TCID50/mL	8/8
Parainfluenza Type 3[a]	$1x10^5$ TCID50/mL	8/8
Rhinovirus Type 1A[a]	$1x10^5$ PFU/mL	8/8
Influenza A H1N1[b]	$1x10^5$ CEID50/mL	8/8
Influenza B[b]	$1x10^5$ TCID50/mL	8/8[d]
Respiratory Syncytial Virus A[b]	$1x10^5$ TCID50/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.

d) Endogenous/Exogenous Interference Substances

An interfering substances study was performed to evaluate if substances naturally present or artificially introduced into the nasal cavity or nasopharynx interfere with the Xpert Xpress CoV-2 *plus* test performance. Positive and negative samples were prepared in simulated NPS/NS matrix. Each interfering substance was tested at the concentration listed below (**Table 15)** in the absence of SARS-CoV-2 and when SARS-CoV-2 is present at 3x LoD. All specimens were tested in eight replicates on the Xpert Xpress CoV-2 *plus* test according to the test procedure. For substances that resulted in an INVALID test result, the concentration of the substance was reduced by half and re-tested.

Expected results were obtained for all interfering substances tested, except for Mucin (Type I-S) (2.5 mg/mL) and Fluticasone Propionate Nasal Spray (5 µg/mL). **Table 15** below presents the concentration at which no interference was seen for each substance tested.

Table 15: Interfering Substances Study Results

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**	**-**
SARS-CoV-2 Positive (No substance) Control	**100% (v/v)**	**-**	**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	-
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 and no interference was observed.

5. Assay Reportable Range:

Not applicable; this is a qualitative assay.

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

a) Assay Controls

Please refer to Instrument Description Information (Section C.5) above for assay controls.

b) Specimen Stability Studies

Room Temperature (15 to 30°C) and Refrigerated (2 to 8°C) Stability Studies
A study was conducted to evaluate the stability of NPS specimens stored in UTM/VTM, followed by testing with the Xpert Xpress CoV-2 *plus* test. Clinical NPS specimens collected in UTM/VTM and eNAT, and confirmed SARS-CoV-2 negative were pooled for this study. The positive sample consisted of the NATtrol inactivated SARS-CoV-2 virus strain USA-

WA1/2020 (ZeptoMetrix Inc., USA) spiked at 2.75x LoD (1108 copies/mL) into the pooled negative clinical NPS in UTM/VTM and into the pooled clinical NPS in eNAT. The same negative clinical matrix pool was used to provide the negative sample. Aliquots of each positive and negative sample were stored at 15°C and 30°C to cover the extremes of the recommended room temperature storage temperature range, and at 2°C and 8°C to cover the extremes of the recommended refrigerated storage temperature range. All four storage temperatures were monitored continuously by automated temperature monitoring and access control system. Eight (8) replicates of the positive sample and 4 replicates of the negative sample were tested at T=0, 4 hours, 8 hours, 24 hours, 48 hours and 50 hours after storage at 15°C and 30°C. Eight (8) replicates of the positive sample and 4 replicates of the negative sample were tested at T=0, 1 day, 2 days, 4 days, 6 days, 7 days, and 8 days after storage at 2°C and 8°C.

External positive and negative controls, run daily during the study, reported the expected Xpert Xpress CoV-2 *plus* test results. Aliquots of the negative and positive specimens stored at 15°C, 30°C, 2°C and 8°C, and tested at their respective timepoints gave expected results.

The study data supports the following specimen stability claims:

- NPS and NS specimens can be stored in UTM/VTM or in eNAT matrix at refrigerated (2°C - 8°C) temperatures for 7 days and at room temperature (15°C - 30°C) for up to 48 hours until testing is performed on the GeneXpert Instrument Systems.

Freeze/Thaw Testing for Samples Frozen at -20°C or -80°C in VTM or eNAT
A study was performed to determine equivalency of frozen SARS-CoV-2 samples compared to fresh samples when tested with the Xpert Xpress CoV-2 *plus* test. Positive samples used for the fresh and frozen specimen equivalency study were prepared by spiking the NATtrol SARS-CoV-2 (USA-WA1/2020) strain into pooled negative clinical NPS-UTM/VTM and NPS-eNAT at concentrations of < 1x LoD, 1-3x LoD and 5x LoD. A negative sample consisting of negative clinical NPS-UTM/VTM and NPS-eNAT matrices were also included in the study. The positive and negative samples were tested fresh, and each sample was subsequently allowed to undergo two freeze-thaw cycles consisting of freezing at -80°C for at least 24 hours and thawing at room temperature prior to testing. Thawed samples were kept on ice prior to testing.

The study data supports the following specimen stability claims:
Frozen clinical NPS-UTM/VTM, clinical NS-UTM/VTM and clinical NPS-eNAT specimens positive for SARS-CoV- 2 can undergo one cycle of freeze-thaw before testing with the Xpert Xpress CoV-2 plus test.

7. Analytical Sensitivity/Limit of Detection (LoD):
The LoD of the Xpert Xpress CoV-2 *plus* test was established using NATtrol inactivated SARS-CoV-2 virus strain USA-WA1/2020 (ZeptoMetrix Inc., USA) diluted into pooled negative clinical NPS matrix and negative clinical NS matrix. Clinical NPS specimens were collected in VTM/UTM and in eNAT, while clinical NS specimens were collected in VTM/UTM only. The LoD is defined as the lowest concentration for SARS-CoV-2 strain at which 95% (19/20) of replicates yield a positive result.

The virus strain was initially estimated in a range finding study at >10 concentrations in replicates of 20 per concentration of virus. Range finding was performed with two cartridge lots. Probit analysis was performed on the range finding results to estimate the LoDs, and the estimated LoDs were subsequently confirmed using two lots of Xpert Xpress CoV-2 *plus* cartridges. For the confirmatory study, testing was also performed in replicates of 20 per cartridge lot. The highest (least sensitive) LoD value for the two lots evaluated in the confirmatory study was reported as the final LoD for the Xpert Xpress CoV-2 *plus* test. The claimed LoD for SARS-CoV-2 is 403 copies/ mL in Clinical NPS-UTM/VTM (**Table 16).** An equivalent LoD (403 copies/ mL) was also established for SARS-CoV-2 in Clinical NPS-eNAT. The claimed LoD is 462 copies/mL in Clinical NS-UTM/VTM (**Table 17)**.

Table 16: Results for Verification of SARS-CoV-2 LoD in Clinical NPS-UTM/VTM

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 17: Results for Verification of SARS-CoV-2 LoD in Clinical NS-UTM/VTM

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

The analytical sensitivity of the Xpert Xpress CoV-2 *plus* test was also estimated by testing WHO First International Standard for SARS-CoV-2 RNA in pooled negative clinical NS-UTM/VTM matrix. The study was performed in a random and blinded fashion. The 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 (**Table 18)**.

Table 18: 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

8. Assay Cut-Off:

The Xpert Xpress CoV-2 *plus* test detects defined cycle threshold (Ct) ranges for the SARS-CoV-2 targets and SPC. The Ct cut-offs are hard coded values and are included as automatic calculations in the assay definition file (ADF) of the Xpert Xpress CoV-2 *plus* test.

9. Carry-Over Contamination Study:

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 study utilized two GeneXpert instrument modules; both modules were cleaned prior to starting the study. 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 $5x10^4$ 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.

B Comparison Studies:

1. Method Comparison with Predicate Device:

Not applicable. Please refer to the Clinical Studies Section of this document.

2. Matrix Comparison:

The following studies were performed to establish equivalent performance of Xpert Xpress CoV-2 *plus* between (a) clinical nasopharyngeal swab (NPS), clinical anterior nasal swab (NS) and simulated NPS/NS matrices in Universal Transport Medium (UTM)/Viral Transport Medium (VTM), and (b) clinical NPS in UTM/VTM and eNAT.

Aliquots of clinical NPS-UTM/VTM, clinical NS-UTM/VTM, and simulated NPS/NS matrices were screened with the Xpert Xpress CoV-2 *plus* test and negative clinical samples were pooled. NATtrol inactivated SARS-CoV-2 virus strain USA-WA1/2020 (ZeptoMetrix Inc., USA) was spiked into the negative matrices at 0.05x, 1.5x, 5x, and 10x LoD. Aliquots of the negative and SARS-CoV-2 positive matrices were tested in replicates 10 (except for 30 replicates in case of 1.5x LoD). Expected results were obtained for all replicates tested.

A similar study as described above, except the simulated NPS/NS matrix, was used to establish equivalent performance between clinical NPS-UTM/VTM and clinical NPS-eNAT**.** Expected results were obtained for all replicates tested.

Clinical Studies:

The clinical performance of the Xpert Xpress CoV-2 *plus* test was evaluated in a multi-site, observational and method comparison study in 2022 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.

To be enrolled in the study, patients had to be presenting at the study centers showing signs and symptoms of respiratory infection. One nasal (NS) or nasopharyngeal (NPS) swab collected in a randomized and blinded fashion from each patient was tested using the Xpert Xpress CoV-2 *plus* test side by side with an FDA-cleared molecular respiratory panel that includes SARS-CoV-2. The demographics of study participants are presented below (**Table 19**). Of the 4047 specimens collected from individuals showing signs and symptoms of respiratory infection, a total of 3075 specimens (869 NPS and 1871 NS) passed all study criteria and were used for performance calculations. The Positive Percent Agreement (PPA) and Negative Percent Agreement of Xpert Xpress CoV-2 *plus* test with the FDA cleared molecular comparator test are presented in **Table 20** below. 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.

Table 19: Demographic Information 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%)

Table 20: Performance of Xpert Xpress CoV-2 *plus* in Symptomatic Individuals (Nasal and Nasopharyngeal Swabs Combined)

	Comparator Result		
Xpert Xpress CoV-2 *plus*	**Positive**	**Negative**	**Total**
Positive	574	55	629
Negative	11	3110	3121
Total	585	3165	3750
PPA (95% CI)	98.1% (96.7% - 98.9%)		
NPA (95% CI)	98.3% (97.7% - 98.7%)		

C Clinical Cut-Off:

Not applicable.

D Expected Values/Reference Range:

Expected values as determined by Xpert Xpress CoV-2 *plus* are presented for nasopharyngeal swab (NPS) and anterior nasal swab (NS) specimen types (**Table 21**).

Table 21: Positivity Rate by Specimen Type

Specimen Type	
NPS	**NS**
17% (320/1879)	16.5% (309/1871)

VIII Proposed Labeling:

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

IX Conclusion:

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