Life Technologies Corporation
Ian Mcgill
Regulatory Affairs Manager
6055 Sunol Boulevard
Pleasanton, California 94566

Re: K233453
Trade/Device Name: Applied Biosystems TaqPath COVID-19 Diagnostic PCR Kit
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
Dated: October 20, 2023
Received: October 20, 2023

Dear Ian Mcgill:

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,

FDA

Stefanie Akselrod -S
2024.07.10 13:37:01 -04'00'

For

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)*
K233453

Device Name
Applied BioSystems™ TaqPath™ COVID-19 Diagnostic PCR Kit

Indications for Use *(Describe)*
The TaqPath™ COVID-19 Diagnostic PCR Kit is a real-time reverse transcription polymerase chain reaction (RT-PCR) test intended for the qualitative detection of nucleic acid from SARS-CoV-2 in nasopharyngeal and anterior nasal specimens from individuals with signs and symptoms of respiratory tract infection.

The TaqPath™ COVID-19 Diagnostic PCR Kit is intended for use as an aid in the diagnosis of COVID-19 if used in conjunction with other clinical observations, epidemiological information and laboratory findings. The SARS-CoV-2 RNA is generally detectable in upper respiratory (anterior nasal and nasopharyngeal swabs) specimens during the acute phase of infection. 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. The agent detected may not be the definite cause of disease.

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

The TaqPath™ COVID-19 Diagnostic PCR Kit is intended for use by qualified clinical laboratory personnel specifically instructed and trained in the techniques of real-time PCR and in vitro diagnostic procedures.

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

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

CONTINUE ON A SEPARATE PAGE IF NEEDED.

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510(k) Summary

Submitter:	Life Technologies Corporation (a legal entity of Thermo Fisher Scientific)
Address:	6055 Sunol Blvd Building C, Pleasanton, CA 94566
Phone:	(1)760-603-7200
Fax:	N/A
Contact Person:	Ian McGill
Contact email:	Ian.mcgill@thermofisher.com
Contact Phone Number:	1 (480) 536-4400

Date:	July 08, 2024
Proprietary Name:	Applied BioSystems™ TaqPath™ COVID-19 Diagnostic PCR Kit
Measurand:	SARS-CoV-2
Regulation:	866.3981
Classification:	Class II
Product Code:	QQX
Panel:	MI - Microbiology
Predicate Device:	BioFire COVID-19 Test 2 (K211079)

Life Technologies Corporation is a legal entity of Thermo Fisher Scientific. The Applied BioSystems™ TaqPath™ COVID-19 Diagnostic PCR Kit (TaqPath™ COVID-19 Diagnostic PCR Kit) includes the assays and controls for a multiplex real-time RT-PCR test for the qualitative detection of RNA from SARS-CoV-2 in nasopharyngeal and anterior nasal specimens from individuals with signs and symptoms of respiratory tract infection.

Each kit includes the following components:

- Multiplexed assays that contain three primer/probe sets specific to different SARS-CoV-2 genomic regions and primers/probes for bacteriophage MS2
- MS2 Phage Control as an internal process control for nucleic acid extraction
- TaqPath™ COVID-19 Diagnostic PCR Control as a positive RNA control that contains targets specific to the SARS-CoV-2 genomic regions targeted by the assays.

Assay workflow

Extract RNA from a patient sample

Set up the 7500 Fast Dx Real-Time PCR Instrument
OR
Set up the QuantStudio™ 5 Dx Real-Time PCR Instrument

Prepare and run the RT-PCR reaction

Import the data and review the results in COVID-19 Interpretive Software IVD Edition

The workflow begins with nucleic acid extraction from upper respiratory specimens (nasopharyngeal and anterior nasal swabs) that arrive in the testing site in transport media. Nucleic acids are isolated and purified from the specimens using the MagMAX™ Viral/Pathogen II Nucleic Acid Isolation Kit. Nucleic acid isolation is performed via an automated process using the KingFisher™ Flex Purification System. The nucleic acid is reverse transcribed into cDNA and amplified using the TaqPath™ COVID-19 Diagnostic PCR Kit and one of the following real-time PCR instruments:

- Applied Biosystems™ 7500 Fast Dx Real-Time PCR Instrument
- Applied Biosystems™ QuantStudio™ 5 Dx Real-Time PCR Instrument

In the process, the probes anneal to three (3) specific SARS-CoV-2 target sequences located between three (3) unique forward and reverse primers for the following genes:

- ORF1ab
- N gene
- S gene

During the extension phase of the PCR cycle, the 5’ nuclease activity of Taq polymerase degrades the probe, causing the reporter dye to separate from the quencher dye, generating a fluorescent signal. With each PCR cycle, additional reporter dye molecules are cleaved from their respective

probes, increasing the fluorescent intensity, which is measured at each cycle by the real-time PCR instrument.

Following RT-PCR, the data from the instrument's data collection software are imported into COVID-19 Interpretive Software IVD Edition for analysis and interpretation. After data import, the software analyzes the run data, performs quality check (QC) analysis, and calculates the interpretive results for each sample and control. The imported data and interpretive results for each run are saved as a batch in the software. Results can be exported as CSV files and reports can be generated in PDF format.

Validation of the results is performed automatically by the COVID-19 Interpretive Software based on performance of the positive and negative controls. The following results are automatically generated using the calling rules, plate validity and the C_T cutoff values for assay targets:

Table 1: Patient Samples

ORF1ab	N gene	S gene	MS2	Status	Result	Action
NEG	NEG	NEG	NEG	INVALID	NA	RETEST Repeat test by re-extracting the original sample and repeating the RT-PCR. If the repeat result remains invalid, consider collecting a new specimen.
NEG	NEG	NEG	POS	VALID	SARS-CoV-2 Not Detected	REPORT Report the results to the healthcare provider.

ORF1ab	N gene	S gene	MS2	Status	Result	Action
Only one SARS-CoV-2 target = POS			POS or NEG	VALID	SARS-CoV-2 Inconclusive	RETEST/REPORT 1. Repeat the test by re-extracting the original sample and repeating the RT-PCR. IMPORTANT! Samples with a result of SARS-CoV-2 Inconclusive shall be retested one time. 2. After retesting one time, report the results to the healthcare provider. 3. If the repeat result remains inconclusive, the healthcare provider should conduct additional confirmation testing with a new specimen, if clinically indicated.
Two or more SARS-CoV-2 targets = POS			POS or NEG	VALID	Positive SARS-CoV-2	REPORT Report the results to the healthcare provider.

A minimum of one negative control and one positive control must be present for each run. Additional negative control wells shall be run for each extraction that is represented on a real-time RT-PCR plate. All control wells must pass for the real-time RT-PCR plate to be considered valid. Recommended actions of retest or report are also provided depending on the results generated.

Table 2: Materials provided in each TaqPath™ COVID-19 Diagnostic PCR Kit, 1000 reactions:

Component	Quantity	Amount
TaqPath™ COVID-19 Diagnostic PCR Assay Kit	1 Tube Multiplex Assay	1,500 µL
	10 Tubes MS2 Phage	10 × 1,000 µL
TaqPath™ COVID-19 Diagnostic PCR Control	5 Boxes, 2 Tubes per Box	2 × 10 µL per box; 5 boxes per kit
TaqPath™ COVID-19 Diagnostic PCR Control Dilution Buffer	5 Boxes, 2 Tubes per Box	2 × 250 µL per box; 5 boxes per kit

Materials required but not provided:

- MagMAX™ Viral/Pathogen II Nucleic Acid Isolation Kit
- KingFisher™ Flex Purification System

- TaqPath™ 1-Step Multiplex Master Mix (No ROX™)
- Applied Biosystems™ 7500 Fast Dx Real-Time PCR Instrument
- Applied Biosystems™ QuantStudio™ 5 Dx Real-Time PCR Instrument
- Additional reagents and materials required are listed in the instructions for use document.

The use of the TaqPath™ COVID-19 Diagnostic PCR Kit assay as an *In vitro* diagnostic is limited to laboratories that are certified under the Clinical Laboratory Improvement Amendments of 1988 (CLIA), 42 U.S.C. § 263a, that meet requirements to perform high complexity tests.

Intended Use

The TaqPath™ COVID-19 Diagnostic PCR Kit is a real-time reverse transcription polymerase chain reaction (RT-PCR) test intended for the qualitative detection of nucleic acid from SARS-CoV-2 in nasopharyngeal and anterior nasal specimens from individuals with signs and symptoms of respiratory tract infection.

The TaqPath™ COVID-19 Diagnostic PCR Kit is intended for use as an aid in the diagnosis of COVID-19 if used in conjunction with other clinical observations, epidemiological information and laboratory findings. The SARS-CoV-2 RNA is generally detectable in upper respiratory (anterior nasal and nasopharyngeal swabs) specimens during the acute phase of infection. 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. The agent detected may not be the definite cause of disease.

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

The TaqPath™ COVID-19 Diagnostic PCR Kit is intended for use by qualified clinical laboratory personnel specifically instructed and trained in the techniques of real-time PCR and *in vitro* diagnostic procedures.

Table 3: Comparison of the TaqPath™ COVID-19 Diagnostic PCR Kit and the predicate device

Device & Predicate Device	New Device	K211079
Device Trade Name	TaqPath™ COVID-19 Diagnostic PCR Kit	BioFire COVID-19 Test 2
General Device Characteristic Similarities		
Organisms Detected	SARS-CoV-2	SARS-CoV-2
Analyte	RNA	RNA
Technological Principles	RT-PCR	RT-PCR
Test Interpretation	Automated test interpretation	Automated test interpretation
General Device Characteristic Differences	**New Device**	**K211079**
Intended Use/ Indications For Use	The TaqPath™ COVID-19 Diagnostic PCR Kit is a real-time reverse transcription polymerase chain reaction (RT-PCR) test intended for the qualitative detection of nucleic acid from SARS-CoV-2 in nasopharyngeal and anterior nasal specimens from individuals with signs and symptoms of respiratory tract infection. The TaqPath™ COVID-19 Diagnostic PCR Kit is intended for use as an aid in the diagnosis of COVID-19 if used in conjunction with other clinical observations, epidemiological information and laboratory findings. The SARS-CoV-2 RNA is generally detectable in upper respiratory (anterior nasal and nasopharyngeal swabs) specimens during the acute phase of infection. 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. The agent detected	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 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

	may not be the definite cause of disease. Negative results do not preclude SARS-CoV-2 infection and should not be used as the sole basis for patient management decisions. The TaqPath™ COVID-19 Diagnostic PCR Kit is intended for use by qualified clinical laboratory personnel specifically instructed and trained in the techniques of real-time PCR and in vitro diagnostic procedures.	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.
Specimen Types	Anterior Nasal Swabs and Nasopharyngeal swabs	Nasopharyngeal swabs only
Instrumentation	7500 Fast Dx, QuantStudio™ 5 Dx	FilmArray 2.0 or FilmArray Torch
Controls	One negative control and one positive control are run for each assay.	Two internal controls are included in each reagent pouch for quality control of sample processing and both PCR stages and melt analysis
Target Genes	ORF1ab, S gene, N Gene	ORF1ab, S gene, N Gene, ORF8

Performance Characteristics:

a) Limit of Detection

The limit of detection (LoD) for the TaqPath™ COVID-19 Diagnostic PCR Kit was determined using inactivated SARS-CoV-2 virus (USA-WA1/2020) in pooled negative NP (Nasopharyngeal swab) specimen. Samples were randomized and blinded prior to testing by the operator. A preliminary LoD was determined by testing serial dilutions of NP spiked with inactivated virus. Samples were tested on two (2) PCR instruments: QuantStudio™ 5 Dx (QS5 Dx) and 7500 Fast Dx.

The preliminary LoD was confirmed by testing twenty (20) replicates of contrived positive samples formulated at three concentration levels based around the preliminary LoD in Phase I: 3x (150 GCE/mL), 1x (50 GCE/mL), 0.33x (17 GCE/mL). Confirmatory testing results determined the LoD for both QS5 Dx and 7500 Fast Dx to be 50 GCE/mL as the lowest concentration tested

for each instrument that resulted in ≥ 95% positivity (19/20 replicates). The results of Phase II testing are summarized in **Table 4** below.

Table 4: LoD Phase II Summary

Viral Target	Concentration (GCE/mL)	QuantStudio™ 5 Dx	7500 Fast Dx
SARS-CoV-2 virus (USA-WA1/2020)	150	100% (20/20)	100% (20/20)
	50	100% (20/20)	100% (20/20)
	17	85% (17/20)	70% (14/20)
	0	0% (0/20)	0% (0/20)

b) Reproducibility and Within-Laboratory Precision

The reproducibility of the TaqPath™ COVID-19 Diagnostic PCR kit was assessed separately for each PCR instrument (7500 Fast Dx and QS5 Dx). Testing was performed at three sites (two external and one internal) using samples prepared at three (3) concentrations; negative (0x LoD), a low positive (1.5x LoD), and a moderate positive (5x LoD). Positive samples were spiked with inactivated SARS-CoV-2 virus in pooled negative NP specimen matrix. All samples were blinded and randomized before testing.

Testing included three (3) sites x two (2) operators x three (3) replicates x three (3) lots of TaqPath™ COVID-19 Diagnostic PCR Kits x five (5) non-consecutive days, for a total of 270 replicates per sample at each test level (0x LoD, 1.5x LoD, 5x LoD) on each PCR instrument model (7500 Fast Dx and QS5 Dx) to account for potential sources of variation.

The TaqPath™ COVID-19 Diagnostic PCR kit met acceptance criteria and generated 100% PPA for 5x LoD, 100% PPA for 1.5x LoD and 100% NPA for 0x LoD for each lot tested across all three sites. Qualitative study results are summarized below in **Table 5** and **Table 6** below. Overall reproducibility results for each instrument are summarized in **Table 7** and **Table 8.** Precision and reproducibility results for each instrument by site are summarized in **Table 9** and **Table 10**. The

number of positive target detections (n/N), mean Ct, standard deviation (SD) and coefficient of variation (CV) are shown by instrument and site for all assay targets.

Table 5: Positive Percent Agreement (PPA) Across All Sites

Instrument Type	LoD	Site	Positive SARS-CoV-2		PPA Across all Sites (%, Low CI %, High CI %)
			N	Percent	
7500 Fast Dx System	1.5x	1	90/90	100%	100% (98.6%, 100%)
		2	90/90	100%	
		3	90/90	100%	
	5x	1	90/90	100%	100% (98.6%, 100%)
		2	90/90	100%	
		3	90/90	100%	
QuantStudio™ 5 Dx System	1.5x	1	90/90	100%	100% (98.6%, 100%)
		2	90/90	100%	
		3	90/90	100%	
	5x	1	90/90	100%	100% (98.6%, 100%)
		2	90/90	100%	
		3	90/90	100%	

Table 6: Negative Percent Agreement (NPA) Across All Sites

Instrument Type	LoD	Site	SARS-CoV-2 Not Detected		NPA Across all Sites (%, Low CI %, High CI %)
			N	Percent	
7500 Fast Dx System	0x	1	90/90	100%	100% (98.6%, 100%)
		2	90/90	100%	
		3	90/90	100%	
QuantStudio™ 5 Dx System	0x	1	90/90	100%	100% (98.6%, 100%)
		2	90/90	100%	
		3	90/90	100%	

Table 7: Overall Reproducibility - 7500 Fast Dx Real-Time PCR Instrument

Panel member	Target	Detected (n/N)	mean Ct	Between-lots		Between-sites		Between-days		Between-operators/runs		Repeatability (Within-Run)		Reproducibility	
				SD	%CV	SD	%CV	SD	%CV	SD	%CV	SD	%CV	SD	%CV
1.5x LoD	ORF1ab	270/270	31.82	0.02	0.07	0.56	1.76	0.14	0.43	0.15	0.48	0.55	1.72	0.81	2.54
	N gene	270/270	32.27	0.02	0.06	0.43	1.34	0.00	0.00	0.19	0.60	0.58	1.81	0.75	2.33
	S gene	264/270	32.27	0.07	0.20	0.05	0.15	0.00	0.00	0.26	0.82	0.80	2.47	0.84	2.61
	MS2	270/270	24.48	0.14	0.58	0.26	1.06	0.28	1.14	0.23	0.92	0.18	0.73	0.50	2.04
5x LoD	ORF1ab	270/270	30.14	0.06	0.21	0.62	2.07	0.06	0.21	0.14	0.45	0.39	1.31	0.75	2.50
	N gene	270/270	30.57	0.01	0.04	0.48	1.58	0.16	0.53	0.00	0.00	0.34	1.12	0.61	2.01
	S gene	270/270	30.41	0.08	0.25	0.26	0.87	0.08	0.28	0.05	0.17	0.36	1.19	0.47	1.53
	MS2	270/270	24.46	0.13	0.52	0.33	1.33	0.33	1.33	0.27	1.09	0.22	0.92	0.59	2.42
0x LoD	MS2	270/270	24.50	0.11	0.46	0.18	0.75	0.27	1.09	0.30	1.21	0.17	0.69	0.48	1.97

Table 8: Overall Reproducibility - QuantStudio™ 5 Dx Real-Time PCR Instrument

Panel member	Target	Detected (n/N)	mean Ct	Between-lots		Between-sites		Between-days		Between-operators/runs		Repeatability (Within-Run)		Reproducibility	
				SD	%CV	SD	%CV	SD	%CV	SD	%CV	SD	%CV	SD	%CV
1.5x LoD	ORF1ab	270/270	32.56	0.11	0.35	0.10	0.29	0.00	0.00	0.17	0.53	0.51	1.56	0.56	1.71
	N gene	269/270	33.07	0.00	0.00	0.15	0.44	0.23	0.70	0.10	0.30	0.61	1.84	0.67	2.04
	S gene	264/270	33.35	0.09	0.26	0.10	0.31	0.00	0.00	0.37	1.12	0.77	2.31	0.87	2.60
	MS2	270/270	25.55	0.05	0.19	0.12	0.46	0.30	1.17	0.40	1.58	0.17	0.65	0.54	2.13
5x LoD	ORF1ab	270/270	30.94	0.11	0.34	0.00	0.00	0.00	0.00	0.13	0.43	0.30	0.99	0.35	1.13
	N gene	270/270	31.34	0.10	0.31	0.05	0.15	0.07	0.22	0.14	0.44	0.35	1.10	0.39	1.25
	S gene	270/270	31.35	0.11	0.34	0.11	0.35	0.00	0.00	0.19	0.59	0.27	0.87	0.36	1.16
	MS2	270/270	25.54	0.06	0.22	0.17	0.67	0.32	1.25	0.32	1.24	0.23	0.90	0.54	2.10

Panel member	Target	Detected (n/N)	mean Ct	Between-lots		Between-sites		Between-days		Between-operators/runs		Repeatability (Within-Run)		Reproducibility	
				SD	%CV	SD	%CV	SD	%CV	SD	%CV	SD	%CV	SD	%CV
0x LoD	MS2	270/270	25.52	0.00	0.00	0.25	0.97	0.31	1.23	0.29	1.13	0.14	0.56	0.51	2.01

Table 9: Summary of Within-lab Precision Results by Site -7500 Fast Dx Real-Time PCR Instrument

Site	Panel member	Target	Detected (n/N)	Mean Ct	Between-lots		Between-days		Between-operators/runs		Repeatability (Within-run)		Within-lab Precision	
					SD	%CV	SD	%CV	SD	%CV	SD	%CV	SD	%CV
Site 1	1.5x LoD	ORF1ab	90/90	32.19	0.00	0.00	0.14	0.42	0.14	0.44	0.54	1.69	0.58	1.80
		N gene	90/90	32.56	0.00	0.00	0.00	0.00	0.19	0.58	0.49	1.50	0.52	1.61
		S gene	89/90	32.38	0.00	0.00	0.14	0.43	0.00	0.00	0.57	1.76	0.59	1.81
		MS2	90/90	24.64	0.42	1.70	0.22	0.89	0.12	0.50	0.18	0.75	0.52	2.12
	5x LoD	ORF1ab	90/90	30.39	0.06	0.18	0.12	0.41	0.00	0.00	0.38	1.24	0.40	1.32
		N gene	90/90	30.90	0.15	0.48	0.11	0.35	0.00	0.00	0.34	1.11	0.39	1.26
		S gene	90/90	30.65	0.17	0.55	0.08	0.27	0.00	0.00	0.34	1.10	0.38	1.26
		MS2	90/90	24.55	0.48	1.94	0.08	0.32	0.18	0.74	0.20	0.83	0.55	2.26
	0x LoD	MS2	90/90	24.48	0.51	2.08	0.11	0.46	0.19	0.78	0.21	0.87	0.60	2.43
Site 2	1.5x LoD	ORF1ab	90/90	31.17	0.00	0.00	0.19	0.60	0.06	0.18	0.53	1.71	0.57	1.82
		N gene	90/90	31.76	0.00	0.00	0.00	0.00	0.22	0.69	0.58	1.83	0.62	1.95
		S gene	86/90	32.18	0.00	0.00	0.00	0.00	0.00	0.00	0.74	2.30	0.74	2.30
		MS2	90/90	24.17	0.10	0.40	0.26	1.06	0.22	0.93	0.14	0.59	0.38	1.58
	5x LoD	ORF1ab	90/90	29.42	0.05	0.16	0.12	0.41	0.18	0.60	0.44	1.48	0.49	1.66
		N gene	90/90	30.02	0.08	0.26	0.11	0.36	0.00	0.00	0.36	1.21	0.39	1.29
		S gene	90/90	30.12	0.04	0.12	0.10	0.34	0.00	0.00	0.36	1.21	0.38	1.26
		MS2	90/90	24.09	0.15	0.61	0.17	0.69	0.27	1.12	0.27	1.12	0.44	1.83
	0x LoD	MS2	90/90	24.30	0.23	0.94	0.00	0.00	0.32	1.34	0.13	0.52	0.42	1.71
Site 3	1.5x LoD	ORF1ab	90/90	32.11	0.00	0.00	0.12	0.39	0.22	0.67	0.56	1.75	0.62	1.92
		N gene	90/90	32.47	0.18	0.57	0.00	0.00	0.13	0.39	0.67	2.07	0.71	2.18
		S gene	89/90	32.27	0.00	0.00	0.00	0.00	0.54	1.66	1.01	3.13	1.14	3.55
		MS2	90/90	24.64	0.17	0.71	0.12	0.47	0.30	1.20	0.20	0.83	0.42	1.69

Site	Panel member	Target	Detected (n/N)	Mean Ct	Between-lots		Between-days		Between-operators/runs		Repeatability (Within-run)		Within-lab Precision	
					SD	%CV	SD	%CV	SD	%CV	SD	%CV	SD	%CV
	5x LoD	ORF1ab	90/90	30.60	0.11	0.38	0.00	0.00	0.23	0.74	0.36	1.19	0.44	1.45
		N gene	90/90	30.80	0.14	0.46	0.15	0.49	0.00	0.00	0.32	1.03	0.38	1.23
		S gene	90/90	30.44	0.10	0.32	0.00	0.00	0.15	0.49	0.38	1.26	0.42	1.39
		MS2	90/90	24.75	0.23	0.91	0.32	1.30	0.33	1.33	0.19	0.77	0.55	2.21
	0x LoD	MS2	90/90	24.71	0.10	0.42	0.15	0.61	0.35	1.42	0.16	0.64	0.43	1.72

Table 10: Summary of Within-lab Precision Results by Site - QuantStudio™ 5 Dx Real-Time PCR Instrument

Site	Panel member	Target	Detected (n/N)	Mean Ct	Between-lots		Between-days		Between-operators/runs		Repeatability (Within-run)		Within-lab Precision	
					SD	%CV	SD	%CV	SD	%CV	SD	%CV	SD	%CV
Site 1	1.5x LoD	ORF1ab	90/90	32.50	0.14	0.42	0.00	0.00	0.20	0.62	0.51	1.58	0.57	1.75
		N gene	90/90	33.11	0.00	0.00	0.27	0.82	0.00	0.00	0.56	1.69	0.62	1.88
		S gene	90/90	33.20	0.11	0.33	0.23	0.71	0.00	0.00	0.53	1.59	0.59	1.77
		MS2	90/90	25.36	0.41	1.62	0.19	0.75	0.18	0.71	0.14	0.55	0.51	2.00
	5x LoD	ORF1ab	90/90	30.93	0.10	0.33	0.00	0.00	0.07	0.22	0.30	0.97	0.33	1.05
		N gene	90/90	31.41	0.12	0.38	0.11	0.34	0.00	0.00	0.35	1.10	0.38	1.21
		S gene	90/90	31.47	0.15	0.47	0.00	0.00	0.09	0.29	0.27	0.87	0.32	1.03
		MS2	90/90	25.31	0.45	1.77	0.09	0.37	0.16	0.63	0.14	0.56	0.51	2.00
	0x LoD	MS2	90/90	25.21	0.46	1.81	0.10	0.39	0.22	0.89	0.12	0.47	0.53	2.11
Site 2	1.5x LoD	ORF1ab	90/90	32.69	0.06	0.17	0.00	0.00	0.32	0.98	0.42	1.27	0.53	1.61
		N gene	89/90	33.22	0.00	0.00	0.22	0.67	0.06	0.19	0.61	1.84	0.65	1.97
		S gene	90/90	33.43	0.26	0.77	0.00	0.00	0.34	1.02	0.85	2.55	0.95	2.85
		MS2	90/90	25.63	0.06	0.22	0.18	0.70	0.36	1.40	0.12	0.46	0.42	1.65
	5x LoD	ORF1ab	90/90	30.98	0.09	0.28	0.00	0.00	0.00	0.00	0.31	1.01	0.32	1.05
		N gene	90/90	31.33	0.07	0.23	0.00	0.00	0.00	0.00	0.36	1.14	0.37	1.17
		S gene	90/90	31.24	0.10	0.31	0.00	0.00	0.17	0.56	0.27	0.86	0.34	1.07
		MS2	90/90	25.58	0.13	0.52	0.05	0.21	0.27	1.05	0.28	1.08	0.41	1.61

Site	Panel member	Target	Detected (n/N)	Mean Ct	Between-lots		Between-days		Between-operators/runs		Repeatability (Within-run)		Within-lab Precision	
					SD	%CV	SD	%CV	SD	%CV	SD	%CV	SD	%CV
	0x LoD	MS2	90/90	25.70	0.17	0.68	0.06	0.25	0.27	1.06	0.13	0.51	0.35	1.38
Site 3	1.5x LoD	ORF1ab	90/90	32.48	0.16	0.49	0.21	0.65	0.00	0.00	0.58	1.78	0.64	1.96
		N gene	90/90	32.89	0.12	0.36	0.24	0.72	0.27	0.83	0.65	1.97	0.75	2.28
		S gene	84/90	33.44	0.00	0.00	0.00	0.00	0.61	1.82	0.89	2.65	1.08	3.22
		MS2	90/90	25.65	0.25	0.98	0.15	0.58	0.58	2.27	0.22	0.86	0.69	2.68
	5x LoD	ORF1ab	90/90	30.91	0.16	0.52	0.03	0.09	0.23	0.74	0.30	0.97	0.41	1.33
		N gene	90/90	31.28	0.21	0.68	0.00	0.00	0.25	0.79	0.33	1.06	0.47	1.49
		S gene	90/90	31.34	0.07	0.22	0.00	0.00	0.26	0.82	0.28	0.88	0.38	1.22
		MS2	90/90	25.72	0.35	1.37	0.25	0.96	0.45	1.74	0.25	0.96	0.67	2.60
	0x LoD	MS2	90/90	25.66	0.29	1.11	0.22	0.86	0.36	1.39	0.17	0.67	0.54	2.09

c) In Use Stability

The In-use stability of the TaqPath™ COVID-19 Diagnostic PCR Kit reagents under expected use conditions was evaluated.

In-use stability of the TaqPath™ COVID-19 Diagnostic PCR Control was evaluated at undiluted (1 x 10^4) and working concentrations (25 copies/ µL) under refrigeration at 25 and 50 hours. Additionally, the working stability of the TaqPath™ COVID-19 Diagnostic PCR Assay Multiplex reagents under freeze thaw conditions was also evaluated. Freeze thaw stability of the TaqPath™ COVID-19 Diagnostic PCR Control, TaqPath™ COVID-19 Diagnostic PCR Control Dilution Buffer, and MS2 Phage Control were not evaluated as multiple tubes are provided in with the TaqPath™ COVID-19 Diagnostic PCR Kit and freeze-thaw cycles are not recommended. Likewise, the refrigerated stability of the TaqPath™ COVID-19 Diagnostic PCR Assay Multiplex was not evaluated as storage under refrigeration is not recommended.

The study data supports the in-use stability under the conditions listed in **Table 11.**

Table 11: TaqPath™ COVID-19 Diagnostic PCR Kit In-use Stability

Component Tested	Storage Holding Condition	In-Use Stability claim
TaqPath™ COVID-19 Diagnostic PCR Assay Multiplex (Freeze-Thaw)	–30°C to –10°C	Stable up to 10 Freeze-thaw cycles
TaqPath™ COVID-19 Diagnostic PCR Control Stock – Not Diluted (Refrigerated)	2°C to 8°C	Stable up to 48 hours
TaqPath™ COVID-19 Diagnostic PCR Control Working Stock – Diluted (Refrigerated)	2°C to 8°C	Stable up to 48 hours

d) Transport Simulation

The purpose of the transport simulation study was to demonstrate that the shipping configurations developed for the TaqPath™ COVID-19 Diagnostic PCR Kit reagents provide adequate thermal

and physical protection as packages are transported to customer sites. Three (3) lots of TaqPath™ COVID-19 Diagnostic PCR Kit, components of the kit were exposed to thermal challenges and vibration in their final shipping configuration followed by functional testing. Simulated transport challenges were performed according to the American Society for Testing and Materials (ASTM) D4169, International Safe Transit Association (ISTA) 7E requirements by a third-party testing organization followed by functional testing at Thermo Fisher Scientific.

This study demonstrated that primary and distribution packaging integrity including that of labels has been maintained, temperature ranges for materials distributed are maintained within allowable limits and all reagents perform as expected according to requirements and specifications upon exposure to the simulated distribution environment conditions.

e) Interfering Substances

The potential impact of endogenous and exogenous interferents found in respiratory specimens on the performance of the TaqPath™ COVID-19 Diagnostic PCR Kit were evaluated. A total of 27 interfering substances were spiked in contrived negative matrix prepared using negative pooled nasopharyngeal swab samples (0x LoD) and contrived positive samples using inactivated SARS-CoV-2 virus at a concentration of 3x LoD, to evaluate potential false positive interference and false negative interference respectively.

All interfering substances are considered non-interfering at the tested concentrations as shown below.

Results are summarized in **Table 12** below.

Table 12: Interfering substances

Substance	Concentration	PPA	NPA
Leukocytes (human)	1% (v/v)	100% (3/3)	100% (3/3)
Mucin: bovine submaxillary gland, type I-S	0.1 mg/mL	100% (3/3)	100% (3/3)
Blood (human)	1% (v/v)	100% (3/3)	100% (3/3)

Substance	Concentration	PPA	NPA
Throat lozenges: Benzocaine (7.5 mg), Dextromethorphan HBr (5 mg)	1% (w/v)	100% (3/3)	100% (3/3)
Throat lozenges: Menthol (5.4 mg)	2.2 mg/mL	100% (3/3)	100% (3/3)
Nasal sprays or drops: Phenylephrine	10% (v/v)	100% (3/3)	100% (3/3)
Nasal sprays or drops: Oxymetazoline (Afrin – Allergy Sinus)	10% (v/v)	100% (3/3)	100% (3/3)
Nasal sprays or drops: Oxymetazoline (Afrin – No drip, extra moisturizing)[1]	10% (v/v)	100% (6/6)	83% (5/6)
	5% (v/v)	100% (3/3)	100% (3/3)
Nasal sprays or drops: Sodium chloride with preservatives	10% (v/v)	100% (3/3)	100% (3/3)
Bronchodilator: Albuterol	0.83 mg/mL	100% (3/3)	100% (3/3)
Nasal corticosteroids: Beclomethasone	2 mg/mL	100% (3/3)	100% (3/3)
Nasal corticosteroids: Dexamethasone	1.5 mg/mL	100% (3/3)	100% (3/3)
Nasal corticosteroids: Flunisolide	2 mg/mL	100% (3/3)	100% (3/3)
Nasal corticosteroids: Budesonide	1% (v/v)	100% (3/3)	100% (3/3)
Nasal corticosteroids: Mometasone	1 mg/mL	100% (3/3)	100% (3/3)
Nasal corticosteroid: Triamcinolone[1]	10% (v/v)	83% (5/6)	17% (1/6)
	5% (v/v)	100% (3/3)	100% (3/3)
Nasal corticosteroid: Fluticasone	5 ug/mL	100% (3/3)	100% (3/3)
Nasal gel: Luffa opperculata, sulfur	1% (v/v)	100% (3/3)	100% (3/3)
Zinc Acetate	7.5 mg/mL	100% (3/3)	100% (3/3)
Anti-viral drug: Remdesivir	6.7 µg/mL	100% (3/3)	100% (3/3)
Anti-viral drug: Zanamivir	5.5 mg/mL	100% (3/3)	100% (3/3)
Anti-viral drug: Oseltamivir	33 ug/mL	100% (3/3)	100% (3/3)
Antibiotic, nasal ointment: Mupirocin	5 mg/mL	100% (3/3)	100% (3/3)
Antibacterial, systemic: Tobramycin	4 µg/mL	100% (3/3)	100% (3/3)
Homeopathic allergy medicine: Galphimia glauca, Histaminum hydrochloricum	10% (w/v)	100% (3/3)	100% (3/3)
Tobacco product	1% w/v	100% (3/3)	100% (3/3)
Analgesic (e.g., ibuprofen)	21.9 mg/dL	100% (3/3)	100% (3/3)

[1] Interference was observed at concentrations >5% (v/v).

f) Cross-Reactivity and Microbial Interference

These studies evaluated the potential for cross-reactivity and microbial interference for the TaqPath™ COVID-19 Diagnostic PCR Kit assay with microorganisms found in respiratory samples.

In the cross-reactivity study, a total of 46 potentially cross-reactive organisms were spiked into pooled negative NP specimen matrix at a high, clinically relevant concentration (10^6 CFU/mL or higher for bacteria/fungi and 10^5 PFU/mL or $TCID_{50}$/mL or higher for viruses). In the microbial interference study, the same 46 organisms were spiked into contrived positive NP specimen matrix formulated at a concentration of 3x LoD with inactivated SARS-CoV-2 virus. Additionally, testing was also performed with nasal wash solution contrived with 3x LoD (150 GCE/mL) with SARS-CoV-2 virus. Organisms were obtained from vendors at the highest commercially available concentration. Organisms that did not meet the target testing concentration or did not come in equivalent quantitative units (i.e., copies/mL or Ct values) were tested at the highest concentration possible. Three (3) replicates of all microorganisms were tested using one lot of TaqPath™ COVID-19 Diagnostic PCR Kit on one (1) QuantStudio™ 5 Dx Real-Time PCR instrument for each segment of the study. The microorganisms and concentrations tested and a summary of the cross-reactivity and microbial interference results is provided in **Table 13** below. For all organisms tested, no cross-reactivity or microbial interference was observed.

Table 13: Microorganisms and Concentrations Tested

Type	Organism	Test concentration	Units
Virus	Human coronavirus 229E[1]	2.82×10^4	$TCID_{50}$/mL
Virus	Human coronavirus OC43	1.00×10^5	$TCID_{50}$/mL
Virus	Human coronavirus NL63[1]	2.34×10^4	$TCID_{50}$/mL
Virus	MERS-CoV	29.4	C_t
Virus	Coronavirus-SARS	28.8	C_t
Virus	Adenovirus Type 1	1.00×10^5	$TCID_{50}$/mL
Virus	Mastadenovirus B Type 7	1.00×10^5	$TCID_{50}$/mL
Virus	Human Metapneumovirus (hMPV)	1.00×10^5	$TCID_{50}$/mL

Type	Organism	Test concentration	Units
Virus	Parainfluenza virus 1	1.00×10^5	$TCID_{50}$/mL
Virus	Parainfluenza virus 2	1.00×10^5	$TCID_{50}$/mL
Virus	Parainfluenza virus 3	1.00×10^5	$TCID_{50}$/mL
Virus	Parainfluenza virus 4	1.00×10^5	$TCID_{50}$/mL
Virus	Influenza A[1]	8.34×10^4	$TCID_{50}$/mL
Virus	Influenza B[1]	8.34×10^4	$TCID_{50}$/mL
Virus	Respiratory syncytial virus A	1.00×10^5	$TCID_{50}$/mL
Virus	Respiratory syncytial virus B	1.00×10^5	$TCID_{50}$/mL
Virus	Rhinovirus[1]	2.82×10^4	$TCID_{50}$/mL
Virus	Measles	1.00×10^5	$TCID_{50}$/mL
Virus	Mumps	1.00×10^5	$TCID_{50}$/mL
Virus	Cytomegalovirus[1]	3.20×10^4	$TCID_{50}$/mL
Virus	Epstein-Barr virus	1.57×10^7 (cross-reactivity) 5.88×10^6 (microbial interference)	copies/mL
Virus	Human Immunodeficiency Virus type 1[1]	1.00×10^4	IU/mL
Virus	Human coronovirus (HKU1)[1] [2]	9.99×10^4 copies/mL	copies/mL
Virus	Enterovirus (EV-D68)[1] [2]	9.88×10^4 copies/mL	copies/mL
Bacteria	*Chlamydia pneumoniae*	1.00×10^6	IFU/mL
Bacteria	*Haemophilus influenzae*	1.00×10^6	CFU/mL
Bacteria	*Legionella pneumophila*	1.00×10^6	CFU/mL
Bacteria	*Mycobacterium tuberculosis*	5.40×10^7 (cross-reactivity) 8.60×10^7 (microbial interference)	GCE/mL
Bacteria	*Streptococcus pneumoniae*	1.00×10^6	CFU/mL
Bacteria	*Fusobacterium necrophorum*	1.00×10^6	CFU/mL
Bacteria	*Pseudomonas aeruginosa*	1.00×10^6	CFU/mL
Bacteria	*Staphylococcus epidermis, MRSE*	1.00×10^6	CFU/mL
Bacteria	*Streptococcus salivarius*	1.00×10^6	CFU/mL
Bacteria	*Corynebacterium diphtheriae*	1.00×10^6	CFU/mL
Bacteria	*Escherichia coli*	1.00×10^6	CFU/mL

Type	Organism	Test concentration	Units
Bacteria	*Lactobacillus acidophilus*	1.00×10^6	CFU/mL
Bacteria	*Moraxella catarrhalis*	1.00×10^6	CFU/mL
Bacteria	*Neisseria meningitidis*	1.00×10^6	CFU/mL
Bacteria	*Neisseria elongata*	1.00×10^6	CFU/mL
Bacteria	*Staphylococcus aureus, MRSA*	1.00×10^6	CFU/mL
Bacteria	*Streptococcus pyogenes*	1.00×10^6	CFU/mL
Bacteria	*Bordetella pertussis*	1.00×10^6	CFU/mL
Bacteria	*Bordetella parapertussis*	1.00×10^6	CFU/mL
Bacteria	*Mycoplasma pneumoniae*	1.00×10^6	CCU/mL
Bacteria	*Mycoplasma genitalium*[1]	4.00×10^5	Bacteria/mL
Fungi	*Candida albicans*	1.00×10^6	CFU/mL
Fungi	*Aspergillus flavus*	1.00×10^6	CFU/mL
Fungi	*Pneumocystis carinii*	1.00×10^6	Cells/mL
Biofluid	Pooled human nasal wash[3]	Neat	N/A

[1] A lower concentration was tested due to inability to obtain stock material with high titer.

[2] Microbial interference was not evaluated

[3] Used to represent diverse microbial flora in the human respiratory tract.

g) *In silico* Cross-Reactivity

In silico analysis was performed to demonstrate that the TaqPath™ COVID-19 Diagnostic PCR kit probes and primers do not cross-react with common microorganisms found in respiratory clinical specimen.

Genome sequences of respiratory pathogens including 31,011 virus isolates, 9,996 bacterial isolates and 14 fungal isolates from GenBank were analyzed using BLAST to compare against the TaqPath™ COVID-19 Diagnostic PCR kit assay forward primers, reverse primers, and probes. Complete genomes for all isolates were available and downloaded except for *Candida albicans* and *Pneumocystis jirovecii* which did not possess any complete genomes on NCBI (National Center for Biotechnology Information). Instead, the RefSeq reference sequence was tested. Rhinovirus Type 1A did not have any complete genomes or a RefSeq, so all 18 available partial

genomes downloaded from NCBI were tested. Atypical genomes were excluded. Atypical genomes are genomes with one or more problems that have been identified by NCBI relating to quality, unusual size, or other flaws in the genome assembly.

A total of 101 sequences aligned with exactly one individual component (primer or probe) of the TaqPath™ COVID-19 Diagnostic PCR Kit (COVID-19 Kit) with greater than or equal to 80% homology. None of the isolates matched to two or more individual components of any TaqPath™ COVID-19 Diagnostic PCR Kit assay. An amplicon requires all three individual assay components to be generated and detected by qPCR, therefore, the isolates evaluated are predicated to be negative for cross-reactivity.

h) Carry-Over Cross-Contamination

The carry-over cross-contamination rate of the TaqPath™ COVID-19 Diagnostic PCR Kit was evaluated in a study using contrived SARS-CoV-2 samples with high viral titers (formulated at approximately 1×10^5 PFU/mL in negative NP matrix) plated in an alternating checkerboard pattern with negative samples. This study tested a total of ten (10) extraction runs across two (2) KingFisher™ Flex Purification Instruments, two (2) operators, and one (1) of each real time PCR instruments - 7500 Fast Dx Real-Time PCR Instrument and QuantStudio™ 5 Dx Real-Time PCR Instrument. Each extraction run tested forty-seven (47) replicates of contrived positive samples and forty-seven (47) replicates of negative NP matrix samples. Out of a total of four hundred and seventy (470) negative samples tested, a total of four (4) false positive SARS-CoV-2 calls (2 false positive calls on 7500 Fast Dx Real-Time PCR Instrument and 2 false positive calls on QuantStudio™ 5 Dx Real-Time PCR Instrument) were observed. The carry-over cross-contamination rate of the TaqPath™ COVID-19 Diagnostic PCR Kit was determined to be 0.85% (4/470).

i) Analytical Reactivity (Inclusivity)

The inclusivity of the TaqPath™ Diagnostic PCR Kit for detection of SARS-CoV-2 was confirmed by testing fifteen variants of SARS-CoV-2. Positive samples were contrived by spiking inactivated SARS-CoV-2 virus into negative pooled NP specimen at a concentration of 3x LoD. Each SARS-

CoV-2 strain below was tested in triplicate along with negative a sample (0x LoD) in a blinded and randomized fashion. All fifteen (15) strains of SARS-CoV-2 tested met the acceptance criteria and generated 100% analytical inclusivity. This study demonstrated that the TaqPath™ COVID-19 Diagnostic PCR Kit has an analytical inclusivity of 100% for the common SARS-CoV-2 variants tested. Results are summarized in **Table 14**.

Table 14: Inclusivity Study Results

Variant	Isolate ID	Concentration (GCE/mL)	%PPA
N/A	USA-WA1/2020	150	100% (3/3)
N/A	Italy-INMI1	150	100% (3/3)
N/A	Hong Kong/VM20001061/2020	150	100% (3/3)
Alpha; B.1.1.7	England/204820464/2020	150	100% (3/3)
Beta; B.1.351	South Africa/KRISP-K005325/2020	150	100% (3/3)
Gamma; P.1	Japan/TY7-503/2021	150	100% (3/3)
Delta; B.1.617.2	USA/PHC658/2021	150	100% (3/3)
Lambda; C.37	Peru/UN-CDC-2-4069945/2021	150	100% (3/3)
Kappa; B.1.617.1	USA/CA-Stanford-15_S02/2021	150	100% (3/3)
Omicron; B.1.1.529	USA/MD-HP20874/2021	150	100% (3/3)
Iota; B.1.526	USA/NY-WADSWORTH-21025952-01/2021 Isolate 1	150	100% (3/3)
B.1	USA/NY-Wadsworth-103677-01/2020	150	100% (3/3)
B.1.595	USA/NY-Wadsworth-33126-01/2020	150	100% (3/3)
Zeta; P2	USA/NY-Wadsworth-21006055-01/2021	150	100% (3/3)
Omicron; BA 2.3	USA/MD-HP24556/2022	150	100% (3/3)

j) *In silico* Reactivity-Inclusivity

In silico analysis was performed to determine inclusivity (reactivity) of the TaqPath™ COVID-19 Diagnostic PCR Kit primer/probe sequences with all known strains/isolates of SARS-CoV-2 from GISAID and GenBank databases through April 2024. Sequences from major SARS-CoV-2 variants were evaluated and found to be reactive with the assay include but are not limited to Alpha, Beta, Delta, Epsilon, Eta, Gamma, Iota, Kappa, Lambda, Mu, Omicron, Theta, and

Zeta. Specific Omicron pango lineages evaluated and found to be reactive with the assay include but are not limited to BA.2.86, XBB.1.9.1, XBB.1.9.2, XBB.2.3, XBB.1.16, XBB.1.5, CH.1.1, JN.1, and KP.2. Mismatch and melting temperature analyses were performed and genomes with 100% identity and/or melting temperature (Tm) greater than the annealing temperature were considered reactive. Analysis indicates that for >99% of the sequences are reactive based on 100% homology or Tm greater than annealing temperature to at least two of the three assay gene targets. Evaluation of assay components that do not match 100% to target sequences indicates that most primer or probe mismatches are unlikely to affect assay function, thus the TaqPath™ COVID-19 Diagnostic PCR Kit is predicted to be highly inclusive for SARS-CoV-2.

k) Specimen Storage Stability

Specimen storage stability testing was performed to characterize the stability of SARS-CoV-2 in nasopharyngeal swabs (NP) after storage at ambient temperature (15-30°C), refrigerated (2°C to 8°C), frozen (-30°C to -10°C), and frozen at ≤-70°C. The stability of SARS-CoV-2 in nasopharyngeal (NP) swabs in viral transport media (VTM) was evaluated at several time points (T_0 – T_n) with contrived positive samples and negative samples (0x LoD) for each storage condition and tested with the TaqPath™ COVID-19 Diagnostic PCR Kit.

The study produced 100% PPA and 100% NPA for all timepoints tested for all storage conditions and established the specimen storage stability: up to 4 hours at ambient temperature (15°C to 30°C), up to 3 days (72 hours) refrigerated (2°C to 8°C), up to 30 days frozen (-30°C to -10°C) and up to 30 days frozen at ≤-70°C.

l) Fresh vs Frozen Equivalency Study

A fresh vs frozen study was performed to demonstrate equivalency between fresh and frozen SARS-CoV-2 nasopharyngeal (NP) samples in viral transport media (VTM). Contrived samples were prepared by spiking inactivated SARS-CoV-2 virus in negative pooled NP samples at a concentration of 0x LoD, 1.5x LoD and 5x LoD. Samples were tested at baseline and subsequently after 1x and 2x freeze-thaw cycles when stored at -10°C to -30°C, and after 1x, 2x, 3x and 4x freeze-thaw cycles when stored at ≤ -70°C. Ten replicates were performed for 0x and 5x LoD and 40 replicates were tested for 1.5x LoD. All test conditions produced expected results with 100% NPA at 0x LoD and 100% PPA at 1.5x LoD and 5x LoD. The results demonstrated that freeze-

thaw up to 2 cycles when stored at -10°C to -30°C and freeze-thaw up to 4 cycles when stored at ≤ -70°C does not impact the expected performance of the TaqPath™ COVID-19 Diagnostic PCR Kit.

m) LoD Determination with WHO Standard

An additional LoD Determination was performed to establish the analytical sensitivity of the TaqPath™ COVID-19 Diagnostic PCR Kit with the first WHO International Standard for SARS-CoV-2 RNA.

Phase I of testing determined a preliminary LoD using a 10-fold dilution series with three (3) replicate per concentration level. In Phase II, the LoD was confirmed with twenty (20) replicates at three concentration levels (3x, 1x and 0.33x) based around the preliminary LoD. The lowest concentration level with ≥ 95% positivity was designated as the confirmed LoD for each PCR instrument tested.

Phase II of testing confirmed the LoD for the 7500 Fast Dx instrument as 150 IU/mL and the LoD for the QuantStudio™ 5 Dx as 50 IU/mL. Results are summarized in **Table 15** below.

Table 15. LoD Phase II Summary

Viral Target	Concentration (IU/mL)	QuantStudio™ 5 Dx	7500 Fast Dx
SARS-CoV-2 virus (PN:20/146)	150	100% (20/20)	100% (20/20)
	50	95% (19/20)	90% (18/20)
	17	50% (10/20)	50% (10/20)

n) Clinical Validation Summary

The clinical performance of the TaqPath™ COVID-19 Diagnostic PCR Kit was established in multi-site prospective study in nasopharyngeal swabs (NP) and anterior nasal swabs (AN) collected in BD universal viral transport (UVT) medium from individuals with signs and symptoms of respiratory tract infection, following informed consent of study participants. All specimens were prospectively collected in all-comers fashion at 11 geographically diverse collection sites in the U.S.

Testing of the TaqPath™ COVID-19 Diagnostic PCR Kit was conducted on the Applied Biosystems™ 7500 Fast Dx and QuantStudio™ 5 Dx instruments following extraction of viral RNA using the KingFisher™ Flex purification system with MagMAX™ Viral/Pathogen Nucleic Acid Isolation Kit II reagents. Analysis/interpretation of the results was performed using the COVID-19 Interpretive Software Investigational Use Only (IUO) Edition V1.0. Testing was conducted at three qualified sites including one central lab that performed comparator testing.

A composite comparator approach was used to evaluate performance of the TaqPath™ COVID-19 Diagnostic PCR Kit. In this method, three highly sensitive SARS-CoV-2 molecular assays were selected as comparators and the clinical calls were compared between the TaqPath™ COVID-19 Diagnostic PCR Kit and the composite comparators. All samples were tested by two comparator assays and the third comparator assay was only used to test samples that showed discrepant clinical calls between the first two comparator methods, for a two-out-of-three result interpretation.

The TaqPath™ COVID-19 Diagnostic PCR Kit was evaluated with both Category I specimens (prospectively collected, tested fresh) and Category II specimens (prospectively collected, frozen, tested thawed). A total of one thousand seventy-six (1076) subjects from 11 geographically diverse collection sites were enrolled in this study between April 2023 and August 2023. After exclusions, a total of one thousand fifty-five (1055) nasopharyngeal swabs (NP) and one thousand fifty-two (1052) anterior nasal swabs (AN) were tested. Of the 1055 NP specimens, 1053 were considered evaluable NP specimens on both instruments after exclusions. Of the 1052 AN specimens, 3 were called inconclusive on 7500 Fast Dx and hence excluded from statistical analysis; none of the 1052 AN specimens were excluded from statistical analysis on QS5 Dx. This resulted in 1049 evaluable AN specimens on 7500 Fast Dx and 1052 evaluable specimens on QS5 Dx.

Of the 1055 Subjects included in the analysis, the mean age was 39.3 years (SD: 17.0) and the median age was 37 years (range: 2–87 years). The majority of the subjects were between 5 and 80 years (98.6%); 7 subjects (0.7%) were ≥81 years and 8 subjects (0.8%) were ≤ 5 years. Of the subjects enrolled, 62.8% were female, 56.7% self-identified as “not Hispanic or Latino” and 40.7% identified as “Hispanic or Latino”.

Specimens tested on investigational COVID-19 Test were either fresh (tested within 72 hours of collection when stored at 2°C to 8°C) or frozen as category II samples (stored ≤-70°C and tested within 30 days of collection). **Table 16** shows count and percentages of fresh frozen specimens and their respective clinical calls on QS5 Dx and 7500 Fast Dx.

Table 16: Distribution of Fresh and Frozen specimens used in the study and their corresponding clinical calls.

	COVID-19 test on QuantStudio™ 5 Dx				**COVID-19 test on 7500 Fast Dx**			
	Total Number of specimens	POS	NEG	Invalid/ Inconclusive	Total Number of specimens	POS	NEG	Invalid/ Inconclusive
NP (Fresh)	785 (74.4%)	54 (6.9%)	729 (92.9%)	2 (0.3%)	785 (74.4%)	54 (6.9%)	731 (93.1%)	0
NP (Frozen)	270 (25.6%)	47 (17.4%)	223 (82.6%)	0	270 (25.6%)	49 (18.1%)	219 (81.1%)	2 (0.7%)
AN (Fresh)	783 (74.4%)	51 (6.5%)	732 (93.5%)	0	783 (74.4%)	51 (6.5%)	732 (93.5%)	0
AN (Frozen)	269 (25.6%)	52 (19.3%)	217 (80.7%)	0	269 (25.6%)	54 (20.1%)	212 (78.8%)	3 (1.1%)

The comparator result is defined as the concordant results from two comparator assays (test A and test B). In case of discordance between the initial two comparator assays, the sample was tested by a third assay (test C) and the result of the third test determined the composite comparator result. Performance characteristics of the study included Positive Percent Agreement (PPA) and Negative Percent Agreement (NPA) for SARS-CoV-2 target using NP and AN specimen type on QS5Dx and 7500 Fast Dx instruments. PPA and NPA for each combination of specimen and instrument are defined as follows:

$$PPA\ (\%) = 100 \times \frac{Number\ of\ Positives\ by\ Both\ Candidate\ \ and\ \ Comparator\ Method}{Number\ of\ Positives\ by\ Comparator\ Method}$$

$$NPA\ (\%) = 100 \times \frac{Number\ of\ Negatives\ by\ Both\ Candidate\ Method\ and\ \ Comparator\ Method}{Number\ of\ Negatives\ by\ Comparator\ Method}$$

A two-sided 95% Confidence Interval (CI) was determined using Wilson's score method.

A summary of the TaqPath™ COVID-19 Diagnostic PCR Kit clinical performance by specimen and instrument type is include in **Table 17** – **Table 20**.

Table 17: Clinical Performance Estimates of the TaqPath™ COVID-19 Diagnostic PCR Kit on QuantStudio™ 5 Dx with NP Specimens.

QuantStudio™ 5 Dx	**Composite Comparator Result**		
COVID-19 test on QS5 Dx	Positive	Negative	Total
Positive	88	13	101
Negative	1	951	952
Total	89	964	1053
Agreement Calculations			95% CI (Wilson's score)
PPA	88/89	98.9%	93.9% to 99.8%
NPA	951/964	98.7%	97.7% to 99.2%

1055 NP samples were tested on QS5Dx, of which 2 were excluded from analysis because they were called inconclusive on QS5Dx

Table 18: Clinical Performance Estimates of the TaqPath™ COVID-19 Diagnostic PCR Kit on 7500 Fast Dx with NP Specimens.

7500 Fast Dx	**Composite Comparator Result**		
COVID-19 test on 7500 Fast Dx	Positive	Negative	Total
Positive	88	15	103
Negative	1	949	950
Total	89	964	1053
Agreement Calculations			95% CI (Wilson's score)
PPA	88/89	98.9%	93.9% to 99.8%
NPA	949/964	98.4%	97.4% to 99.1%

1055 NP samples were tested on 7500Fast Dx, of which 2 were excluded from analysis because 1 was invalid and 1 was inconclusive on 7500 Fast Dx

Table 19: Clinical Performance Estimates of the TaqPath™ COVID-19 Diagnostic PCR Kit on QuantStudio™ 5 Dx with AN specimens.

QuantStudio™ 5 Dx	Composite Comparator Result		
COVID-19 test on QS5 Dx	Positive	Negative	Total
Positive	84	19	103
Negative	1	948	949
Total	85	967	1052
Agreement Calculations			95% CI (Wilson's score)
PPA	84/85	98.8%	93.6% to 99.8%
NPA	948/967	98.0%	97.0% to 98.7%

Table 20: Clinical Performance Estimates of the TaqPath™ COVID-19 Diagnostic PCR Kit on 7500 Fast Dx with AN specimens.

7500 Fast Dx	Composite Comparator Result		
COVID-19 test on 7500 Fast Dx	Positive	Negative	Total
Positive	84	21	105
Negative	1	943	944
Total	85	964	1049
Agreement Calculations			95% CI (Wilson's score)
PPA	84/85	98.8%	93.6% to 99.8%
NPA	943/964	97.8%	96.7% to 98.6%

1052 AN samples were tested on 7500 Fast Dx, of which 3 were excluded from analysis because they were called inconclusive on 7500Fast Dx

Reference Ranges/Expected Values

Samples were collected from 11 geographically diverse collection sites across the U.S. and tested at three qualified testing sites. The stratification of positivity rates (as determined by the TaqPath™ COVID-19 Diagnostic PCR Kit) per collection site for the prospective samples is shown in **Table 21**.

Table 21: Prospective Specimens: Positivity Rates based on the TaqPath™ COVID-19 Diagnostic PCR Kit.

Collection Site	QuantStudio™ 5 Dx		7500 Fast Dx	
	NP	AN	NP	AN
D&H, Florida	28.2% (61/216)	29.0% (63/217)	28.2% (61/216)	30.1% (65/216)
KUR-B, New York	3.9% (3/76)	3.9% (3/77)	3.9% (3/77)	3.9% (3/77)
KUR-E, South Carolina	12.7% (13/102)	12.9% (13/101)	13.9% (14/101)	11.9% (12/101)
KUR-M, Texas	2.5% (2/81)	2.5% (2/81)	2.5% (2/81)	2.5% (2/81)
KUR_MB, California	7.7% (1/13)	7.7% (1/13)	7.7% (1/13)	7.7% (1/13)
KUR-P, South Carolina	5.8% (3/52)	5.8% (3/52)	5.8% (3/52)	5.8% (3/52)
KUR-R, California	0.0% (0/2)	0.0% (0/2)	0.0% (0/2)	0.0% (0/2)
WR-C, Tennessee	3.3% (2/60)	1.7% (1/59)	3.3% (2/60)	1.7% (1/58)
WR-L, Florida	6.7% (8/120)	5.0% (6/119)	6.7% (8/120)	5.1% (6/118)
WR-S, California	2.9% (7/240)	2.9% (7/240)	3.3% (8/240)	2.9% (7/240)
WR-V, Nevada	1.1% (1/91)	4.4% (4/91)	1.1% (1/91)	5.5% (5/91)
Overall	9.6% (101/1053)	9.8% (103/1052)	9.8% (103/1053)	10.0% (105/1049)

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