



January 8, 2025

Life Technologies Corporation
Stacey Moltchanoff
Regulatory Affairs Manager
6055 Sunol Boulevard
Pleasanton, California 94566

Re: K241806

Trade/Device Name: Applied Biosystems TaqPath COVID-19, Flu A, Flu B, RSV Select Panel

Regulation Number: 21 CFR 866.3981

Regulation Name: Device To Detect And Identify Nucleic Acid Targets In Respiratory Specimens
From Microbial Agents That Cause The Sars-Cov-2 Respiratory Infection And Other
Microbial Agents When In A Multi-Target Test

Regulatory Class: Class II

Product Code: QOF

Dated: June 21, 2024

Received: June 21, 2024

Dear Stacey Moltchanoff:

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.

All medical devices, including Class I and unclassified devices and combination product device constituent parts are required to be in compliance with the final Unique Device Identification System rule ("UDI Rule"). The UDI Rule requires, among other things, that a device bear a unique device identifier (UDI) on its label and package (21 CFR 801.20(a)) unless an exception or alternative applies (21 CFR 801.20(b)) and that the dates on the device label be formatted in accordance with 21 CFR 801.18. The UDI Rule (21 CFR 830.300(a) and 830.320(b)) also requires that certain information be submitted to the Global Unique Device Identification Database (GUDID) (21 CFR Part 830 Subpart E). For additional information on these requirements, please see the UDI System webpage at <https://www.fda.gov/medical-devices/device-advice-comprehensive-regulatory-assistance/unique-device-identification-system-udi-system>.

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,

Anna M. Mielech -
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Anna Mielech, PhD.
Deputy Branch Chief (Acting)
Division of Microbiology Devices
OHT7: Office of In Vitro Diagnostics
Office of Product Evaluation and Quality
Center for Devices and Radiological Health

Enclosure

Indications for Use

510(k) Number (if known)

K241806

Device Name

Applied Biosystems TaqPath COVID-19, Flu A, Flu B, RSV Select Panel

Indications for Use (Describe)

The Applied Biosystems TaqPath COVID-19, Flu A, Flu B, RSV Select Panel is a multiplex, real-time reverse transcription polymerase chain reaction (RT-PCR) in vitro diagnostic test for the qualitative detection and differentiation of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), influenza A virus, influenza B virus, and respiratory syncytial virus (RSV). Nucleic acids are isolated and purified from nasopharyngeal (NP) swab and anterior nasal (AN) swab specimens obtained from individuals exhibiting signs and symptoms of a respiratory tract infection. Clinical signs and symptoms of respiratory viral infection due to SARS-CoV-2, influenza, and RSV can be similar. This test is intended to aid in the differential diagnosis of SARS-CoV-2, influenza A, influenza B, and RSV A/B (undifferentiated) infections in humans and is not intended to detect influenza C virus infections.

Nucleic acids from the viral organisms identified by this test are generally detectable in NP and AN swab specimens during the acute phase of infection. The detection and identification of specific viral nucleic acids from individuals exhibiting signs and symptoms of respiratory tract infection are indicative of the presence of the identified virus and aids in diagnosis if used in conjunction with other clinical and epidemiological information, and laboratory findings.

The results of this test should not be used as the sole basis for diagnosis, treatment, or other patient management decisions. Positive results do not rule out coinfection with other organisms. The organism(s) detected by the Applied Biosystems TaqPath COVID-19, Flu A, Flu B, RSV Select Panel may not be the definite cause of disease. Negative results do not preclude SARS-CoV-2, influenza A virus, influenza B virus, or RSV infections.

The Applied Biosystems TaqPath COVID-19, Flu A, Flu B, RSV Select Panel is intended for use by qualified and trained 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, Pleasanton, CA 94566, USA
Phone: (1) 760-603-7200
Fax: N/A
Contact Person: Stacey Moltchanoff
Contact email: stacey.moltchanoff@thermofisher.com
Contact Phone Number: 1 (707) 494-1756
Date: June 21, 2024
Proprietary Name: Applied Biosystems™ TaqPath™ COVID-19, Flu A, Flu B, RSV Select Panel
Measurand: The assay detects and identifies RNA from: SARS-CoV-2, influenza A virus, influenza B virus, and respiratory syncytial virus (RSV)
Regulation: 866.3981
Classification: Class II
Product Code: QOF
Panel: MI - Microbiology
Predicate Device: BioFire® Respiratory Panel 2.1 (DEN200031)

The Applied Biosystems™ TaqPath™ COVID-19, Flu A, Flu B, RSV Select Panel is a multiplex, real-time reverse transcription polymerase chain reaction (RT-PCR) test. The severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), influenza A, influenza B, respiratory syncytial virus (RSV) A/B and RNase P primer and probe sets are designed to detect viral RNA in nasopharyngeal (NP) and anterior nasal (AN) swab specimens from individuals exhibiting signs and symptoms of a respiratory tract infection.

Each TaqPath™ COVID-19, Flu A, Flu B, RSV Select Panel includes the following components:

- TaqPath™ COVID-19, Flu A, Flu B, RSV Select Assay—Multiplex assays that contain primer and probe sets specific to the following targets:
 - Three SARS-CoV-2 targets (Orf1a, Orf1b, and N genes)
 - Two influenza A virus targets (PB1 & M genes)
 - Two influenza B virus targets (M & NS genes)
 - Three RSV targets (NP, M, and L protein genes)
 - RNase P (internal human sample collection control)

- TaqPath™ COVID-19, Flu A, Flu B, RSV Select Positive Control—Inactivated viral control that contains SARS-CoV-2, influenza A, influenza B, and RSV.
- TaqPath™ COVID-19, Flu A, Flu B, RSV Select Negative Control—MS2 packaged RNA control that contains targets specific to RNase P genomic regions targeted by the assay.
- TaqPath™ 1-Step Select Master Mix (No ROX)—Ready-to-use PCR mix, including reverse transcriptase, polymerase, dNTPs, salts, and buffer.
- Package Insert —Provides the instructions and the link to download the instructions for use and other assets (including the ADF)
- An Assay Definition File (ADF) applicable to the instrument used in the workflow (available via download).

In addition to the SARS-CoV-2, influenza A, influenza B and RSV viral assay targets, the assay portion of the panel includes RNase P, which serves as an endogenous internal process control to monitor extraction and amplification of each clinical sample. The TaqPath™ COVID-19, Flu A, Flu B, RSV Select Panel also contains external process positive and negative controls. The positive control (PC) component included is an inactivated viral control that contains SARS-CoV-2, influenza A, influenza B, and RSV viruses. The PC monitors extraction and real-time RT-PCR by demonstrating that each of the four viruses can be detected when present and that RNase P is not detected when absent. The negative control (NC) component included is an MS2 packaged RNA control that contains targets specific to RNase P genomic regions targeted by the assay. The NC also monitors extraction and real-time RT-PCR by demonstrating RNase P can be detected when present and that the four viruses are not detected when absent. The TaqPath™ 1-Step Select Master Mix (No ROX) included as a component of the kit is a ready-to-use PCR mix which contains a deoxyribonucleotide triphosphate mix (dNTPs), enzymes, and other components to permit reverse transcription and amplification of the assay targets. The TaqPath™ 1-Step Select Master Mix (No ROX) also contains ribonuclease (RNase) inhibitors as well as deoxyuridine triphosphate (dUTP) and uracil N-glycosylase (known as UNG or UDG).

The TaqPath™ COVID-19, Flu A, Flu B, RSV Select Panel is provided in two overall kit configurations: either 200 reactions or 1,000 reactions. The materials provided within each TaqPath™ COVID-19, Flu A, Flu B, RSV Select Panel kit are shown in **Table 1** (200 reactions, Cat. No. A56123) and **Table 2** (1,000 reactions, Cat. No. A56124).

Table 1: TaqPath™ COVID-19, Flu A, Flu B, RSV Select Panel Components (200 reactions, Cat. No. A56123)

Component	SKU (box SKU)	Quantity	Volume per tube or bottle
TaqPath™ COVID 19, Flu A, Flu B, RSV Select Assay	A56440	2 tubes	150 µL
TaqPath™ COVID 19, Flu A, Flu B, RSV Select Positive Control	963612	6 tubes	250 µL
TaqPath™ COVID 19, Flu A, Flu B, RSV Select Negative Control	963609	6 tubes	250 µL
TaqPath™ 1-Step Select Master Mix (No ROX)	A56518	2 tubes	1 mL

Table 2: TaqPath™ COVID-19, Flu A, Flu B, RSV Select Panel Components (1,000 reactions, Cat. No. A56124)

Component	SKU (box SKU)	Quantity	Volume per tube or bottle
TaqPath™ COVID 19, Flu A, Flu B, RSV Select Assay	A56441	10 tubes	150 µL
TaqPath™ COVID 19, Flu A, Flu B, RSV Select Positive Control	963613	12 tubes	250 µL
TaqPath™ COVID 19, Flu A, Flu B, RSV Select Negative Control	963608	12 tubes	250 µL
TaqPath™ 1-Step Select Master Mix (No ROX)	A56519	10 tubes	1 mL

Materials required but not provided with the TaqPath™ COVID-19, Flu A, Flu B, RSV Select Panel:

- Applied Biosystems™ QuantStudio™ 5 Dx Real-Time PCR Instrument
- MagMAX™ Dx Viral/Pathogen NA Isolation Kit
- KingFisher™ Apex Dx Purification System
- Additional reagents and materials listed in the instructions for use

A high-level overview of the TaqPath™ COVID-19, Flu A, Flu B, RSV Select Panel workflow is shown in **Figure 1** below.

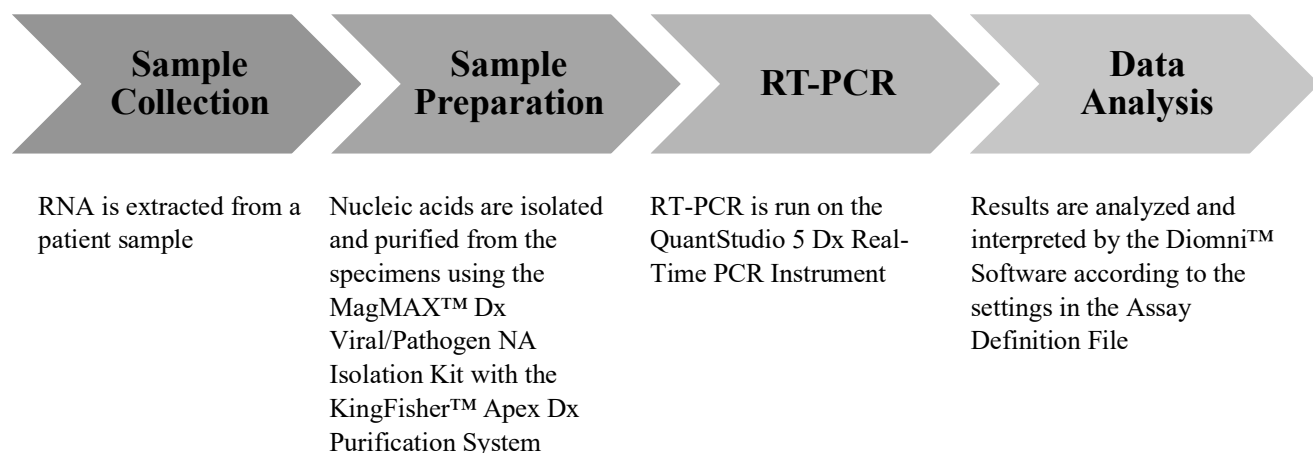


Figure 1: TaqPath™ COVID-19, Flu A, Flu B, RSV Select Panel Workflow

The TaqPath™ COVID-19, Flu A, Flu B, RSV Select Panel workflow begins with nucleic acid extraction from nasopharyngeal and anterior nasal swab specimens that arrive at the testing site stored in viral transport media (VTM) or universal transport medium (UTM). Nucleic acids are isolated and purified from the specimens using the MagMAX™ Dx Viral/Pathogen NA Isolation Kit with the KingFisher™ Apex Dx Purification System.

The purified nucleic acid is reverse transcribed into cDNA and amplified using the TaqPath™ COVID-19, Flu A, Flu B, RSV Select Panel and an Applied Biosystems™ QuantStudio™ 5 Dx Real Time PCR Instrument. The Applied Biosystems™ Diomni™ Software, which is used for results interpretation, is supplied with the PCR instrument.

Once the reaction plate is loaded onto the real-time PCR instrument, RNA is reverse transcribed into cDNA using the same gene-specific primers used for PCR. After heat inactivation of the reverse transcriptase enzyme, thermal cycling for PCR amplification commences.

During the anneal/extension phase of a PCR cycle, primers and probes specific to the target genes bind to corresponding DNA sequences in the indicated targets. 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 cycle, additional reporter dye molecules are cleaved

from their respective probes, increasing the overall fluorescence intensity. Fluorescence intensity is monitored at each PCR cycle by the real-time PCR instrument.

The Diomni™ Software implements the thermal cycling program, secondary analysis and clinical interpretation of the results using assay-specific parameters from the Assay Definition File (ADF) that is installed in the software.

Following PCR, results are analyzed and interpreted by the Diomni™ Software according to the settings in the ADF. A detailed explanation of the calling rules, plate validity and Cq cutoff values for assay targets is provided in the instructions for use. A minimum of one negative control and one positive control must be present for each run for Diomni™ Software to call the run valid. Furthermore, all control wells must pass for the real-time RT-PCR plate to be considered valid.

Control well interpretive rules are show in **Table 3** below.

Table 3: Control Well Interpretive Rules

Negative Control (NC)					Well Call	Status
C19	Flu A	Flu B	RSV AB	RNase P		
NEG	NEG	NEG	NEG	POS	Negative	Passed
All other scenarios					Invalid	Failed

Positive Control (PC)					Well Call	Status
C19	Flu A	Flu B	RSV AB	RNase P		
POS	POS	POS	POS	NEG	Positive	Passed
All other scenarios					Invalid	Failed

Results interpretation for patient samples is described in **Table 4** below. A Positive call for RNase P is only required for samples that are Negative for all four viral targets; samples with no amplification are called Invalid.

Table 4: Patient Samples Interpretive Rules

Target					Call	Result
C19	Flu A	Flu B	RSV AB	RNase P		
POS	POS	POS	POS	POS or NEG	Positive	SARS-CoV-2, Flu A, Flu B, RSVAB Detected
POS	POS	POS	NEG	POS or NEG	Positive	SARS-CoV-2, Flu A, Flu B Detected
POS	POS	NEG	POS	POS or NEG	Positive	SARS-CoV-2, Flu A, RSVAB Detected
POS	POS	NEG	NEG	POS or NEG	Positive	SARS-CoV-2, Flu A Detected
POS	NEG	POS	POS	POS or NEG	Positive	SARS-CoV-2, Flu B, RSVAB Detected
POS	NEG	POS	NEG	POS or NEG	Positive	SARS-CoV-2, Flu B Detected
POS	NEG	NEG	POS	POS or NEG	Positive	SARS-CoV-2, RSVAB Detected
POS	NEG	NEG	NEG	POS or NEG	Positive	SARS-CoV-2 Detected
NEG	POS	POS	POS	POS or NEG	Positive	Flu A, Flu B, RSVAB Detected
NEG	POS	POS	NEG	POS or NEG	Positive	Flu A, Flu B Detected
NEG	POS	NEG	POS	POS or NEG	Positive	Flu A, RSVAB Detected
NEG	POS	NEG	NEG	POS or NEG	Positive	Flu A Detected
NEG	NEG	POS	POS	POS or NEG	Positive	Flu B, RSVAB Detected
NEG	NEG	POS	NEG	POS or NEG	Positive	Flu B Detected
NEG	NEG	NEG	POS	POS or NEG	Positive	RSVAB Detected
NEG	NEG	NEG	NEG	POS	Negative	SARS-CoV-2, Flu A, Flu B, RSVAB Not Detected
NEG	NEG	NEG	NEG	NEG	Invalid	RETEST ^[1]

[1] Retesting must be performed by re-extracting the original sample and repeating the real-time PCR. If the repeat result remains invalid, collection of a new specimen should be considered.

Intended Use

The Applied Biosystems™ TaqPath™ COVID-19, Flu A, Flu B, RSV Select Panel is a multiplex, real-time reverse transcription polymerase chain reaction (RT-PCR) in vitro diagnostic test for the qualitative detection and differentiation of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), influenza A virus, influenza B virus, and respiratory syncytial virus (RSV). Nucleic acids are isolated and purified from nasopharyngeal (NP) swab and anterior nasal (AN) swab specimens obtained from individuals exhibiting signs and symptoms of a respiratory tract infection. Clinical signs and symptoms of respiratory viral infection due to SARS-CoV-2,

influenza, and RSV can be similar. This test is intended to aid in the differential diagnosis of SARS-CoV-2, influenza A, influenza B, and RSV A/B (undifferentiated) infections in humans and is not intended to detect influenza C virus infections.

Nucleic acids from the viral organisms identified by this test are generally detectable in NP and AN swab specimens during the acute phase of infection. The detection and identification of specific viral nucleic acids from individuals exhibiting signs and symptoms of respiratory tract infection are indicative of the presence of the identified virus and aids in diagnosis if used in conjunction with other clinical and epidemiological information, and laboratory findings.

The results of this test should not be used as the sole basis for diagnosis, treatment, or other patient management decisions. Positive results do not rule out coinfection with other organisms. The organism(s) detected by the Applied Biosystems™ TaqPath™ COVID-19, Flu A, Flu B, RSV Select Panel may not be the definite cause of disease. Negative results do not preclude SARS-CoV-2, influenza A virus, influenza B virus, or RSV infections.

The Applied Biosystems™ TaqPath™ COVID-19, Flu A, Flu B, RSV Select Panel is intended for use by qualified and trained clinical laboratory personnel specifically instructed and trained in the techniques of real-time PCR and in vitro diagnostic procedures.

Predicate Device Comparison

The similarities and differences between TaqPath™ COVID-19, Flu A, Flu B, RSV Select Panel and the predicate device, BioFire® Respiratory Panel 2.1 (DEN20031) are described in **Table 5** and **Table 6** below.

Table 5: Predicate Device Similarities

Device & Predicate Device	New Device	DEN200031
Device Trade Name	TaqPath™ COVID-19, Flu A, Flu B, RSV Select Panel	BioFire® Respiratory Panel 2.1 (RP2.1)
General Device Characteristic Similarities		
Organisms Detected	SARS-CoV-2, Influenza A, Influenza B, Respiratory Syncytial Virus	SARS-CoV-2, Influenza A, Influenza B, Respiratory Syncytial Virus
Analyte	RNA	RNA
Technological Principles	real-time reverse transcription PCR	reverse transcription PCR
Specimen Types	nasopharyngeal swabs	nasopharyngeal swabs
Test Interpretation	Automated test interpretation	Automated test interpretation
Transport Media	Universal Transport Medium (UTM) / Viral Transport Medium (VTM)	Universal Transport Medium (UTM) / Viral Transport Medium (VTM)

Table 6: Predicate Device Differences

General Device Characteristic Differences	New Device	DEN200031
Device Trade Name	TaqPath™ COVID-19, Flu A, Flu B, RSV Select Panel	BioFire® Respiratory Panel 2.1 (RP2.1)
Intended Use/ Indications For Use	The Applied Biosystems™ TaqPath™ COVID-19, Flu A, Flu B, RSV Select Panel is a multiplex, real-time reverse transcription polymerase chain reaction (RT-PCR) <i>in vitro</i> diagnostic test for the qualitative detection and differentiation of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), influenza A virus, influenza B virus, and respiratory syncytial virus (RSV). Nucleic acids are isolated and purified from nasopharyngeal (NP) swab and anterior nasal (AN) swab specimens obtained from individuals exhibiting signs and symptoms of a respiratory tract infection. Clinical signs and symptoms of respiratory viral infection due to SARS-CoV-2, influenza, and RSV can be similar. This test is intended to aid in the differential diagnosis of SARS-CoV-2, influenza A, influenza B, and RSV A/B (undifferentiated) infections in humans and is not intended to detect influenza C virus infections. Nucleic acids from the viral organisms identified by this test are generally detectable in NP and AN swab specimens during the acute phase of infection. The detection and identification of specific viral nucleic acids from individuals exhibiting signs and symptoms of respiratory tract infection are indicative of the presence of the identified virus and aids in diagnosis if	The BioFire® Respiratory Panel 2.1 (RP2.1) is a PCR-based multiplexed nucleic acid test intended for use with the BioFire FilmArray® 2.0 or BioFire FilmArray® Torch systems for the simultaneous qualitative detection and identification of multiple respiratory viral and bacterial nucleic acids in nasopharyngeal swabs (NPS) obtained from individuals suspected of respiratory tract infections, including COVID-19. The following organism types and subtypes are identified using the BioFire® RP2.1: • Adenovirus, • Coronavirus 229E, • Coronavirus HKU1, • Coronavirus NL63, • Coronavirus OC43, • Severe Acute Respiratory Syndrome Coronavirus (SARS-CoV-2), • Human Metapneumovirus, • Human Rhinovirus/Enterovirus, • Influenza A, including subtypes H1, H1-2009, and H3, • Influenza B,

General Device Characteristic Differences	New Device	DEN200031
	used in conjunction with other clinical and epidemiological information, and laboratory findings. The results of this test should not be used as the sole basis for diagnosis, treatment, or other patient management decisions. Positive results do not rule out coinfection with other organisms. The organism(s) detected by the Applied Biosystems™ TaqPath™ COVID-19, Flu A, Flu B, RSV Select Panel may not be the definite cause of disease. Negative results do not preclude SARS-CoV-2, influenza A virus, influenza B virus, or RSV infections. The Applied Biosystems™ TaqPath™ COVID-19, Flu A, Flu B, RSV Select Panel is intended for use by qualified and trained clinical laboratory personnel specifically instructed and trained in the techniques of real-time PCR and <i>in vitro</i> diagnostic procedures.	• Parainfluenza Virus 1, • Parainfluenza Virus 2, • Parainfluenza Virus 3, • Parainfluenza Virus 4, • Respiratory Syncytial Virus, • <i>Bordetella parapertussis</i> (IS1001), • <i>Bordetella pertussis</i> (ptxP), • <i>Chlamydia pneumoniae</i>, and • <i>Mycoplasma pneumoniae</i> Nucleic acids from the respiratory viral and bacterial organisms identified by this test are generally detectable in NPS specimens during the acute phase of infection. The detection and identification of specific viral and bacterial nucleic acids from individuals exhibiting signs and/or symptoms of respiratory infection is indicative of the presence of the identified microorganism and aids in the diagnosis of respiratory infection if used in conjunction with other clinical and epidemiological information. The results of this test should not be used as the sole basis for diagnosis, treatment, or other patient management decisions. Negative results in the setting of a respiratory illness may be due to infection with pathogens that are not detected by this test, or lower respiratory tract infection that may not be detected by an NPS

General Device Characteristic Differences	New Device	DEN200031
		specimen. Positive results do not rule out coinfection with other organisms. The agent(s) detected by the BioFire® RP2.1 may not be the definite cause of disease. Additional laboratory testing (e.g. bacterial and viral culture, immunofluorescence, and radiography) may be necessary when evaluating a patient with possible respiratory tract infection.
Specimen Types	Anterior Nasal Swabs and Nasopharyngeal swabs	Nasopharyngeal swabs only
Instrumentation	Sample Prep/Nucleic Acid Purification: • MagMAX™ Dx Viral/Pathogen NA Isolation Kit (magnetic bead-based sample prep chemistry) • KingFisher™ Apex Dx Purification System (sample prep instrumentation) Real-time PCR instrument: • QuantStudio 5 Dx	Sample Prep/Nucleic Acid Purification: • Magnetic bead-based chemistry is contained within the BioFire® RP2.1 pouch Instruments: • FilmArray® 2.0 • FilmArray® Torch
Controls	One negative control and one positive control are run for each plate (external process controls). The assay detects endogenous RNase P present in the specimen as an internal positive/process control.	Two internal controls are included in each reagent pouch for quality control of sample processing and both PCR stages and melt analysis.

Analytical/Clinical Performance

Note that the terms “Cq” and “Ct” are used interchangeably throughout the documentation and refer to equivalent values. Quantification cycle (Cq) is an algorithm-agnostic term across different methods for determining the fractional cycle used for PCR reaction quantification, whereas “Ct”, or threshold cycle, refers specifically to the baseline-threshold algorithm term used by the software for determining this same value.

Analytical Performance Summaries

The analytical performance testing that was conducted to support substantial equivalence is summarized below.

Limit of Detection Determination

The preliminary LoD was determined by probit analysis at a hit rate of 0.95. LoD was determined for two (2) strains of influenza A (Flu A) H1N1, two (2) strains of Flu A H3N2, one (1) strain of influenza B (Flu B) of Victoria lineage, two (2) strains of Flu B of Yamagata lineage, one (1) strain of SARS-CoV-2, one (1) strain of RSV A and one (1) strain of RSV B. Each virus was spiked into pooled, negative nasopharyngeal (NP) swab specimens in VTM/UTM, extracted on the KingFisher™ Apex Purification System, then real-time RT-PCR was performed on the QuantStudio™ 5 Dx (QS5 Dx) PCR instrument across three (3) days, with two (2) reagent lots. LoDs for all viruses except the Flu B Victoria lineage strain were also determined in anterior nasal (AN) swab specimens in VTM/UTM. The NP and AN swab specimens in VTM/UTM were obtained as de-identified clinical remnants which were confirmed negative and pooled. In this study, the preliminary LoD was determined by probit analysis separately for each virus in each specimen type with each reagent lot, and the highest (i.e., worst-case) LoD value across reagent lots was selected as the preliminary LoD for that virus in that specimen type. In parallel, the Limit of Blank (LoB) was assigned as zero and confirmed as such in this LoD Determination Study.

Probit analysis was used to determine the preliminary LoD at a hit rate of 0.95 for each virus strain tested. The preliminary LoDs for each virus strain and specimen type across reagent lots is

provided in **Table 7**. The concentrations below are provided in both GCE/mL (as determined by in-house ddPCR) and in TCID₅₀/mL (as reported by the virus supplier in a Certificate of Analysis).

Table 7: Preliminary LoD as determined by probit analysis for each specimen type across reagent lots on the QS5Dx.

Virus	Strain	LoD Concentration (GCE/mL)		LoD Concentration (TCID ₅₀ /mL)	
		NP	AN	NP	AN
SARS-CoV-2	USA-WA1/2020	82	82	0.121	0.120
Flu A H1N1 strain #1	Michigan/45/15	166	305	0.022	0.041
Flu A H1N1 strain #2	California/07/09	88	183	0.008	0.017
Flu A H3N2 strain #1	South Australia/55/14	203	335	0.025	0.041
Flu A H3N2 strain #2	Texas/50/12	142	232	0.042	0.068
Flu B Victoria strain	Malaysia/2506/2004	4287	N/A	0.092	N/A
Flu B Yamagata strain #1	Victoria/504/00	547	552	0.047	0.047
Flu B Yamagata strain #2	Florida/04/06	232	227	0.024	0.024
RSV A	2013 Isolate Culture Fluid	982	753	0.008	0.006
RSV B	CH93(18)-18	664	614	0.020	0.019

Limit of Detection Confirmation

The preliminary LoDs determined by probit analysis were confirmed in the LoD Confirmation Study. The previously determined preliminary LoDs were confirmed for the same two (2) strains of Flu A H1N1, two (2) strains of Flu A H3N2, one (1) strain of Flu B of Victoria lineage, two (2) strains of Flu B of Yamagata lineage, one (1) strain of SARS-CoV-2, one (1) strain of RSV A and one (1) strain of RSV B. Each virus was spiked into pooled, negative NP (all viruses) and AN (all except Flu B Victoria lineage) swab specimens in VTM/UTM and extracted on the KingFisher™ Apex Purification System, then real-time RT-PCR performed on the QS5 Dx instrument across three (3) days with one (1) reagent lot. The preliminary LoD was confirmed as the lowest concentration (i.e., most dilute) that results in at least 95% positive detection ($\geq 95\%$ positive) when tested with at least 24 replicates. Viral concentrations are reported in both GCE/mL and TCID₅₀/mL.

The LoD was confirmed for each virus strain and specimen type as the lowest concentration (i.e., most dilute) that resulted in at least 95% positive detection ($\geq 95\%$ positive) and is presented in both GCE/mL and TCID₅₀/mL. The overall LoD concentrations (i.e., worst case) across matrices (NP and AN) are provided in **Table 8** below.

Table 8: Overall LoD Values for the TaqPath™ COVID-19, Flu A, Flu B, RSV Select Panel

Virus	Strain	LoD Concentration (GCE/mL)	LoD Concentration (TCID₅₀/mL)
SARS-CoV-2	USA-WA1/2020	107	0.158
Flu A H1N1 strain #1	Michigan/45/15	340	0.045
Flu A H1N1 strain #2	California/07/09	282	0.026
Flu A H3N2 strain #1	South Australia/55/14	281	0.034
Flu A H3N2 strain #2	Texas/50/12	268	0.079
Flu B Victoria strain	Malaysia/2506/2004	3500	0.075
Flu B Yamagata strain #1	Victoria/504/00	1405	0.120
Flu B Yamagata strain #2	Florida/04/06	464	0.048
RSV A	2013 Isolate Culture Fluid	1614	0.013
RSV B	CH93(18)-18	1478	0.046

Limit of Detection with WHO Standard

The study was performed using two-phase approach to determine the TaqPath™ COVID-19, Flu A, Flu B, RSV Select Panel LoD using the WHO Standard on the QS5Dx PCR instrument. Contrived specimens were formulated with the First WHO International Standard for SARS-CoV-2 RNA spiked into pooled, negative anterior nasal (AN) swab specimens in VTM/UTM at various concentrations. Once formulated, contrived specimens were extracted and detected using the TaqPath™ COVID-19, Flu A, Flu B, RSV Select Panel in Phase I (range finding). The results of Phase I were used to establish the preliminary LoD (which was the lowest concentration to generate 100% positivity when tested in triplicate). In Phase II, the preliminary LoD was confirmed as the lowest concentration that results in at least 95% positive detection ($\geq 95\%$ positive) when tested with 20 replicates. Phase II testing was performed in a blinded format.

The results of Phase I testing produced a preliminary LoD of 5.012×10^1 IU/mL, which was the lowest (most dilute) concentration that yielded 100% positivity. Results are summarized in **Table 9** below.

Table 9: Summary of Phase I Results with WHO Standard

Concentration	Positive / Total Replicates (% Positive)
5.012×10^5 IU/mL	3 / 3 (100%)
5.012×10^4 IU/mL	3 / 3 (100%)
5.012×10^3 IU/mL	3 / 3 (100%)
5.012×10^2 IU/mL	3 / 3 (100%)
5.012×10^1 IU/mL	3 / 3 (100%)
5.012×10^0 IU/mL	1 / 3 (33.3%)
5.012×10^{-1} IU/mL	0 / 3 (0%)
5.012×10^{-2} IU/mL	0 / 3 (0%)
5.012×10^{-3} IU/mL	0 / 3 (0%)
Negative	0 / 3 (0%)

In Phase II, the LoD was confirmed at 150.36 IU/mL as the lowest concentration that resulted in $\geq 95\%$ positive detection as summarized in **Table 10** below.

Table 10: Summary of Phase II Results with WHO Standard

Concentration	Positive / Total Replicates (% Positive)
451.08 IU/mL (9x Preliminary LoD)	20 / 20 (100%)
150.36 IU/mL (3x Preliminary LoD)	20 / 20 (100%)
50.12 IU/mL (Preliminary LoD)	18 / 20 (90%)
16.71 IU/mL (0.33x Preliminary LoD)	12 / 20 (60%)
Negative	0 / 3 (0%)

In silico Reactivity (Inclusivity)

In silico analysis was conducted for RSV A/B, Flu A, Flu B, COVID-19 assay primers and probes using BLAST against known strains/isolates in the GISAID and GenBank (NCBI) databases to determine reactivity (inclusivity) of the TaqPath™ COVID-19, Flu A, Flu B, RSV Select Panel. The study included a summary of mismatch and melting temperature analysis. The acceptance criteria for this study was that less than 1% (SARS-CoV-2) and less than 5% (Flu A, Flu B, and RSV) of publicly available sequences have mutations that map to all gene targets and also reduce the melting temperature below annealing temperature.

A strain must have 100% homology or a predicted melting temperature (T_m) above the PCR annealing temperature (T_a) to at least one of the individual assays targeting that organism to be considered a positive for inclusivity. Greater than 99.99% of SARS-CoV-2 had $T_m > T_a$ for at least one of the three SARS-CoV-2 assays. Likewise, 99.9% of Flu B strains and 99.49% of RSV strains had at least one assay with $T_m > T_a$. Whereas 10.43% of the Flu A strains had mismatches that cause the predicted T_m to fall below the T_a for both the MP and PB1 gene assays, the most frequent mismatch in the MP gene was wet tested and found to not impact detection. After accounting for the number of strains with the mismatch in the MP gene, 95.27% of Flu A strains can be considered reactive with the TaqPath™ COVID-19, Flu A, Flu B, RSV Select Panel.

Reactivity (Inclusivity)

Reactivity (inclusivity) was determined for eleven (11) strains of Flu A (H1N1), twelve (12) strains of Flu A (H3N2), five (5) strains of influenza B (two (2) of Victoria lineage and three (3) of Yamagata lineage), six (6) strains of RSV (three (3) each of RSV A and B), five (5) strains of SARS-CoV-2 and five (5) avian influenza strains (one (1) each of H5N1, H5N2, H7N2, H7N7 and H7N9). Each virus was introduced at near-LoD levels (approximately 3x LoD) into negative, pooled NP swab specimens in VTM/UTM, then extracted and detected with the TaqPath™ COVID-19, Flu A, Flu B, RSV Select Panel. Sample extraction was performed using the KingFisher™ Apex Purification System, and real-time RT-PCR was performed with the QS5Dx using one (1) lot of TaqPath™ COVID-19, Flu A, Flu B, RSV Select Panel reagents. Each sample was formulated with live virus, inactivated virus or genomic RNA depending on availability, and tested in triplicate (N=3). Virus strains that did not produce 100% detection at the initial test concentration were further tested at progressively higher concentrations until 100% detection was achieved.

Each virus was tested and observed to be reactive (unless otherwise noted) with the TaqPath™ COVID-19, Flu A, Flu B, RSV Select Panel at approximately 3x LoD. As multiple strains per species were used for LoD determination for Flu A, Flu B and RSV, there was not a single LoD value for these viruses. For Flu A viruses, in which the LoD was determined for two strains each for subtypes H1N1 and H3N2, the worst-case LoD for the same subtype was used as the LoD. For

avian flu, the worst case for Flu A across both subtypes was used as the LoD. For Flu B and RSV, the subtype- or lineage-specific LoD was used. Results are shown in **Table 11** below.

Table 11: Reactivity (Inclusivity) Study Results

Strain	Pos Replicates / Total (% Positivity)	Concentration	
		GCE/mL	TCID ₅₀ /mL
Influenza A H1N1 (Georgia/M5081/2012)	3 / 3 (100%)	1020 GCE/mL	42.83 TCID ₅₀ /mL
Influenza A H1N1 (New Caledonia/20/99)	3 / 3 (100%)	1020 GCE/mL	0.44 TCID ₅₀ /mL
Influenza A H1N1 (NY/03/09) ^{[1],[2]}	3 / 3 (100%) ^[1]	4080 GCE/mL ^[1]	6.88 TCID ₅₀ /mL ^[1]
Influenza A H1N1 (Singapore/63/04)	3 / 3 (100%)	1020 GCE/mL	0.05 TCID ₅₀ /mL
Influenza A H1N1 (Brisbane/02/2018) ^[2]	3 / 3 (100%)	1020 GCE/mL	0.11 TCID ₅₀ /mL
Influenza A H1N1 (Solomon Islands/3/2006)	3 / 3 (100%)	1020 GCE/mL	1.67 TCID ₅₀ /mL
Influenza A H1N1 (Puerto Rico/08/1934)	3 / 3 (100%)	1020 GCE/mL	0.11 TCID ₅₀ /mL
Influenza A H1N1 (Mexico/4108/09) ^[2]	3 / 3 (100%)	1020 GCE/mL	7.75 TCID ₅₀ /mL
Influenza A H1N1 (Taiwan/42/06)	3 / 3 (100%)	1020 GCE/mL	0.99 TCID ₅₀ /mL
Influenza A H1N1 (Brisbane/59/07)	3 / 3 (100%)	1020 GCE/mL	0.07 TCID ₅₀ /mL
Influenza A H1N1 (Victoria/2570/19) ^[2]	3 / 3 (100%)	1020 GCE/mL	0.03 TCID ₅₀ /mL
Influenza A H3N2 (Wisconsin/67/2005)	3 / 3 (100%)	1278 GCE/mL	0.20 TCID ₅₀ /mL
Influenza A H3N2 (Switzerland/9715293/13)	3 / 3 (100%)	1278 GCE/mL	0.007 TCID ₅₀ /mL
Influenza A H3N2 (Kansas/14/2017)	3 / 3 (100%)	1278 GCE/mL	0.69 TCID ₅₀ /mL
Influenza A H3N2 (Singapore/INFIMH-16-0019/16)	3 / 3 (100%)	1278 GCE/mL	0.57 TCID ₅₀ /mL
Influenza A H3N2 (Perth/16/09)	3 / 3 (100%)	1278 GCE/mL	0.07 TCID ₅₀ /mL
Influenza A H3N2 (Victoria/361/2011)	3 / 3 (100%)	1278 GCE/mL	0.02 TCID ₅₀ /mL
Influenza A H3N2 (Hong Kong/8/68)	3 / 3 (100%)	1278 GCE/mL	0.12 TCID ₅₀ /mL
Influenza A H3N2 (Brisbane/10/07)	3 / 3 (100%)	1278 GCE/mL	0.20 TCID ₅₀ /mL
Influenza A H3N2 (California/7/04)	3 / 3 (100%)	1278 GCE/mL	0.72 TCID ₅₀ /mL
Influenza A H3N2 (Hong Kong/4801/14)	3 / 3 (100%)	1278 GCE/mL	2.32 TCID ₅₀ /mL
Influenza A H3N2 (Hong Kong/2671/19)	3 / 3 (100%)	1280 GCE/mL	1.07 TCID ₅₀ /mL
Influenza A H3N2 (Macha/O1237/2021)	3 / 3 (100%)	1280 GCE/mL	2.62 TCID ₅₀ /mL
Influenza B Victoria (Colorado/06/2017)	3 / 3 (100%)	4215 GCE/mL ^[4]	0.08 TCID ₅₀ /mL
Influenza B Victoria (Victoria/2/87)	3 / 3 (100%)	4215 GCE/mL ^[4]	1.74 TCID ₅₀ /mL
Influenza B Yamagata (Massachusetts/02/2012)	3 / 3 (100%)	1392 GCE/mL	0.02 TCID ₅₀ /mL
Influenza B Yamagata (Phuket/3073/13)	3 / 3 (100%)	1392 GCE/mL	0.004 TCID ₅₀ /mL
Influenza B Yamagata (Wisconsin/01/2010)	3 / 3 (100%)	1392 GCE/mL	0.06 TCID ₅₀ /mL
RSV A (Long)	3 / 3 (100%)	4842 GCE/mL	4.56 TCID ₅₀ /mL
RSV A (2014 Isolate 342)	3 / 3 (100%)	4842 GCE/mL	0.26 TCID ₅₀ /mL
RSV A (2006 isolate)	3 / 3 (100%)	4842 GCE/mL	0.05 TCID ₅₀ /mL
RSV B (9320) ^[5]	3 / 3 (100%)	4434 GCE/mL	Unknown ^[3]

Strain	Pos Replicates / Total (% Positivity)	Concentration	
		GCE/mL	TCID ₅₀ /mL
RSV B (3/2015 Isolate #2)	3 / 3 (100%)	4434 GCE/mL	2.07 TCID ₅₀ /mL
RSV B (12/2014 Isolate #1)	3 / 3 (100%)	4434 GCE/mL	0.56 TCID ₅₀ /mL
SARS-CoV-2 (Alpha Variant (B.1.1.7) England/204820464/2020) ^[5]	3 / 3 (100%)	321 GCE/mL	0.26 TCID ₅₀ /mL
SARS-CoV-2 (Beta (B.1.351) South Africa/KRISP- K005325/2020) ^[5]	3 / 3 (100%)	321 GCE/mL	0.09 TCID ₅₀ /mL
SARS-CoV-2 (Gamma (P1) Japan/TY7-503/2021) ^[5]	3 / 3 (100%)	321 GCE/mL	0.03 TCID ₅₀ /mL
SARS-CoV-2 (Delta (B.1.617.2) USA/PHC658/2021) ^[5]	3 / 3 (100%)	321 GCE/mL	0.02 TCID ₅₀ /mL
SARS-CoV-2 (Omicron (BA.2.3) USA/MD-HP24556/2022) ^[5]	3 / 3 (100%)	321 GCE/mL	0.02 TCID ₅₀ /mL
Avian influenza H5N1 (India/NIV/2006) ^[5]	3 / 3 (100%)	1278 GCE/mL	Unknown ^[3]
Avian influenza H5N2 (pheasant/New Jersey/1355/1998) ^[5]	3 / 3 (100%)	1278 GCE/mL	Unknown ^[3]
Avian influenza H7N2 (turkey/Virginia/4529/2002) ^[5]	3 / 3 (100%)	1278 GCE/mL	Unknown ^[3]
Avian influenza H7N7 (mallard/Netherlands/12/2000) ^[5]	3 / 3 (100%)	1278 GCE/mL	Unknown ^[3]
Avian influenza H7N9 (Anhui/1/2013) ^[5]	3 / 3 (100%)	1278 GCE/mL	Unknown ^[3]

[1] Reactive with the TaqPath™ COVID-19, Flu A, Flu B, RSV Select Panel at 12x LoD.

[2] H1N1pdm09 strain.

[3] TCID₅₀/mL concentrations unknown (not reported by supplier).

[4] 1.2x LoD relative to LoD of 3500 GCE/mL determined for Flu B Victoria lineage strain Malaysia/2506/2004.

[5] Viral genomic RNA or inactivated virus

Within-Laboratory Precision - Testing Extracted RNA

The Within-Laboratory Precision Study was performed using a panel of extracted RNA from nine (9) specimens contrived by spiking quantified virus from one (1) representative viral strain each of SARS-CoV-2, Flu A, Flu B, and RSV into a negative pool of nasopharyngeal swab (NP) specimen in VTM/UTM at concentrations relative to the LoD confirmed for that strain. RNA from each sample was extracted, pooled, aliquoted, and frozen pending use in the study. Use of extracted RNA for this study provides a separate precision value for the real-time PCR portion of the workflow alone and independent of sample extraction. Repeatability and within-lab precision values that include the sample extraction portion of the workflow were calculated from the Site-to-site Reproducibility study. NP was used to evaluate within-lab precision as the representative and more challenging sample type.

The study was performed by two (2) operators each completing two (2) real-time PCR runs per day (4 runs total per day) at one (1) internal test site on 12 testing days spread over a ≥ 20 -day

study duration. All testing was completed using one (1) real-time PCR reagent lot and on one (1) QuantStudio™ 5 Dx (QS5 Dx) real-time PCR instrument. Each panel member was tested with two (2) replicates in every run with at least one (1) Negative Control (NC) and one (1) Positive Control (PC) included in each reaction plate as run controls.

The positivity rate for the negative sample was 0% (100% NPA) as expected. The overall PPA for all targets at approximately 1x LoD and 3x LoD on the QS5Dx was 100.00%. The observed precision and repeatability %CV at 1x and 3x LoD were less than 2 %CV for all targets. The results of the Cq Variability Analysis are shown in **Table 12**.

Table 12: Summary of Within-Laboratory Precision (Testing Extracted RNA) – Cq Variability Analysis Results

Panel Member	N	Mean Cq	Random Effect	Variance	95% Lower	95% Upper	SD	CV (%)
Negative	96/96	25.16	Operator	0.00	-0.01	0.01	0.05	0.18
			Day	0.01	-0.01	0.02	0.08	0.31
			Run	0.03	0.01	0.05	0.17	0.68
			Repeatability	0.01	0.01	0.02	0.10	0.40
			Within-Lab Precision	0.05	0.03	0.08	0.22	0.87
SARS-CoV-2 1x LoD	96/96	35.52	Operator	0.00	-0.03	0.04	0.06	0.18
			Day	0.04	-0.02	0.11	0.21	0.59
			Run	-0.03	-0.11	0.05	0.00	0.00
			Repeatability	0.27	0.19	0.42	0.52	1.47
			Within-Lab Precision	0.32	0.22	0.50	0.56	1.59
SARS-CoV-2 3x LoD	96/96	34.11	Operator	0.00	-0.01	0.01	0.04	0.13
			Day	-0.03	-0.06	-0.01	0.00	0.00
			Run	0.03	-0.02	0.09	0.18	0.54
			Repeatability	0.10	0.07	0.15	0.31	0.92
			Within-Lab Precision	0.14	0.10	0.21	0.37	1.08
Flu A 1x LoD	96/96	33.05	Operator	0.00	0.00	0.00	0.00	0.00
			Day	0.01	-0.01	0.03	0.10	0.30
			Run	0.00	-0.02	0.03	0.07	0.20
			Repeatability	0.06	0.04	0.09	0.24	0.73
			Within-Lab Precision	0.07	0.05	0.10	0.27	0.82

Panel Member	N	Mean Cq	Random Effect	Variance	95% Lower	95% Upper	SD	CV (%)
Flu A 3x LoD	96/96	32.48	Operator	0.00	-0.01	0.01	0.04	0.13
			Day	0.00	-0.01	0.01	0.05	0.17
			Run	0.00	-0.02	0.01	0.00	0.00
			Repeatability	0.05	0.03	0.07	0.21	0.66
			Within-Lab Precision	0.05	0.03	0.08	0.22	0.69
Flu B 1x LoD	96/96	34.03	Operator	0.00	-0.01	0.01	0.04	0.10
			Day	-0.01	-0.03	0.00	0.00	0.00
			Run	0.02	-0.01	0.05	0.13	0.38
			Repeatability	0.06	0.04	0.10	0.25	0.73
			Within-Lab Precision	0.08	0.06	0.12	0.28	0.83
Flu B 3x LoD	96/96	32.35	Operator	0.00	0.00	0.00	0.00	0.00
			Day	0.01	0.00	0.02	0.09	0.27
			Run	0.01	-0.01	0.02	0.08	0.23
			Repeatability	0.02	0.02	0.04	0.15	0.47
			Within-Lab Precision	0.04	0.03	0.05	0.19	0.59
RSV 1x LoD	96/96	34.28	Operator	0.00	-0.01	0.00	0.00	0.00
			Day	-0.01	-0.05	0.03	0.00	0.00
			Run	0.02	-0.05	0.09	0.15	0.45
			Repeatability	0.17	0.12	0.27	0.42	1.22
			Within-Lab Precision	0.20	0.14	0.29	0.44	1.30
RSV 3x LoD	96/96	32.30	Operator	-0.01	-0.01	0.00	0.00	0.00
			Day	0.06	0.00	0.12	0.25	0.78
			Run	0.03	-0.01	0.07	0.17	0.52
			Repeatability	0.08	0.05	0.12	0.28	0.87
			Within-Lab Precision	0.17	0.12	0.26	0.41	1.28

Site-to-Site Reproducibility

The Site-to-Site Reproducibility study was performed using a panel of nine (9) specimens contrived by spiking quantified virus into negative pool of NP swab specimens in VTM/UTM (NP specimen pool). One (1) representative viral strain each of SARS-CoV-2, Flu, Flu B, and RSV was selected to be spiked into negative NP specimen pool at concentrations relative to the LoD determined for each analyte strain.

Reproducibility of the TaqPath™ COVID-19, Flu A, Flu B, RSV Select Panel was assessed for the QuantStudio™ 5 Dx (QS5 Dx) real-time PCR instrument. Testing was performed at three (3) test sites across five (5) non-consecutive testing days with two (2) instruments per site. Two of the testing sites were external to Thermo Fisher Scientific and one site was internal. Each panel member was tested with each of the three (3) real-time PCR reagent lots in triplicate, for a total of nine (9) replicates per panel member in every run. At least one (1) Negative Control (NC) and one (1) Positive Control (PC) were included on each plate as run controls.

Two operators per site tested the samples following the full TaqPath™ COVID-19, Flu A, Flu B, RSV Select Panel workflow, with each site using a different lot of nucleic acid extraction kit (three (3) lots total) and a single KingFisher™ Apex Purification System at each site for performing the automated RNA extractions. Each operator performed one (1) run on the PCR instrument per day.

The NPA for the negative sample was 99.63%. The overall Site-to-Site reproducibility PPA for all targets at 1x LoD ranged from 92.59% to 98.89% and at 3x LoD, the reproducibility PPA was 100.00% for all targets (**Table 13**). The observed reproducibility, within-lab precision and repeatability % CV at approximately 1x and 3x LoD was less than 5% CV for all targets. Results are shown in **Table 14** below.

Table 13: Reproducibility Study – Qualitative Results

Analyte	Test Level	Agreement/Expected (% Agreement)			Overall Agreement (95% Score CI)
		Site 1	Site 2	Site 3	
N/A	Negative	90/90 (100%)	90/90 (100%)	89/90 (98.89%) ^[1]	99.63% (97.93%, 99.93%)
SARS-CoV-2	~1x LoD	85/90 (94.44%)	82/90 (91.11%)	83/90 (92.22%)	92.59% (88.84%, 95.15%)
	~3x LoD	90/90 (100%)	90/90 (100%)	90/90 (100%)	100% (98.60%, 100%)
Flu A	~1x LoD	88/90 (97.78%)	90/90 (100%)	89/90 (98.89%)	98.89% (96.78%, 99.62%)
	~3x LoD	90/90 (100%)	90/90 (100%)	90/90 (100%)	100% (98.60%, 100%)
Flu B	~1x LoD	86/90 (95.56%)	89/90 (98.89%)	81/90 (90.00%)	94.81% (91.49%, 96.89%)
	~3x LoD	90/90 (100%)	90/90 (100%)	90/90 (100%)	100% (98.60%, 100%)
RSV	~1x LoD	87/90 (96.67%)	87/90 (96.67%)	90/90 (100%)	97.78% (95.24%, 98.98%)
	~3x LoD	90/90 (100%)	90/90 (100%)	90/90 (100%)	100% (98.60%, 100%)

^[1] One replicate was called positive for Flu A.

Table 14: Summary of Site-to-site Reproducibility - Ct Variability Analysis Results

Panel Member	N	Mean Cq	Random Effect	Variance	95% Lower	95% Upper	SD	CV (%)
Negative RNase P	269/270	25.35	Site	0.08	-0.08	0.24	0.28	1.11
			Lot	0.00	-0.01	0.01	0.06	0.24
			Day	-0.11	-0.17	-0.06	0.00	0.00
			Instrument/Operator	0.25	0.15	0.36	0.50	1.99
			Repeatability	0.01	0.01	0.01	0.10	0.39
			Within-lab Precision	0.27			0.52	2.04
			Reproducibility	0.35	0.21	0.65	0.59	2.32
SARS-CoV-2 1x LoD	250/270	36.09	Site	0.08	-0.09	0.26	0.29	0.79
			Lot	0.01	-0.03	0.06	0.12	0.33
			Day	-0.05	-0.13	0.03	0.00	0.00
			Instrument/Operator	0.16	0.02	0.29	0.39	1.09
			Repeatability	0.43	0.35	0.54	0.66	1.82
			Within-lab Precision	0.60			0.77	2.14
			Reproducibility	0.68	0.50	0.98	0.82	2.29
SARS-CoV-2 3x LoD	270/270	34.50	Site	0.07	-0.09	0.23	0.26	0.76
			Lot	0.01	-0.03	0.04	0.08	0.23
			Day	0.07	-0.01	0.14	0.26	0.75
			Instrument/Operator	0.05	-0.02	0.13	0.23	0.67
			Repeatability	0.37	0.31	0.46	0.61	1.77
			Within-lab Precision	0.50			0.71	2.05
			Reproducibility	0.57	0.42	0.80	0.75	2.18
Flu A 1x LoD	267/270	34.75	Site	0.01	-0.03	0.05	0.10	0.28
			Lot	-0.01	-0.02	-0.01	0.00	0.00
			Day	-0.06	-0.18	0.06	0.00	0.00
			Instrument/Operator	0.26	0.06	0.46	0.51	1.46
			Repeatability	0.61	0.50	0.76	0.78	2.25

Panel Member	N	Mean Cq	Random Effect	Variance	95% Lower	95% Upper	SD	CV (%)
			Within-lab Precision	0.87			0.93	2.69
			Reproducibility	0.88	0.70	1.14	0.94	2.70
Flu A 3x LoD	270/270	33.05	Site	0.00	-0.01	0.02	0.05	0.16
			Lot	0.00	-0.01	0.00	0.00	0.00
			Day	-0.08	-0.16	0.00	0.00	0.00
			Instrument/Operator	0.25	0.10	0.40	0.50	1.51
			Repeatability	0.30	0.24	0.37	0.54	1.65
			Within-lab Precision	0.54			0.74	2.23
			Reproducibility	0.55	0.42	0.74	0.74	2.24
Flu B 1x LoD	256/270	35.01	Site	0.11	-0.14	0.36	0.33	0.96
			Lot	-0.01	-0.02	0.00	0.00	0.00
			Day	0.05	-0.06	0.16	0.22	0.63
			Instrument/Operator	0.04	-0.11	0.18	0.20	0.56
			Repeatability	0.77	0.63	0.97	0.88	2.51
			Within-lab Precision	0.86			0.93	2.65
			Reproducibility	0.97	0.74	1.34	0.98	2.81
Flu B 3x LoD	270/270	33.50	Site	0.22	-0.23	0.67	0.47	1.40
			Lot	-0.01	-0.01	0.00	0.00	0.00
			Day	-0.06	-0.17	0.04	0.00	0.00
			Instrument/Operator	0.25	0.08	0.42	0.50	1.50
			Repeatability	0.48	0.40	0.60	0.70	2.08
			Within-lab Precision	0.74			0.86	2.56
			Reproducibility	0.96	0.61	1.71	0.98	2.92
RSV 1x LoD	264/270	35.43	Site	0.09	-0.14	0.32	0.30	0.86
			Lot	-0.02	-0.03	0.00	0.00	0.00
			Day	-0.02	-0.24	0.20	0.00	0.00
			Instrument/Operator	0.24	-0.09	0.57	0.49	1.37
			Repeatability	1.54	1.26	1.92	1.24	3.50

Panel Member	N	Mean Cq	Random Effect	Variance	95% Lower	95% Upper	SD	CV (%)
RSV 3x LoD	270/270	33.81	Within-lab Precision	1.77			1.33	3.76
			Reproducibility	1.86	1.49	2.40	1.37	3.85
			Site	0.00	-0.02	0.03	0.04	0.11
			Lot	0.02	-0.04	0.08	0.14	0.42
			Day	-0.26	-0.45	-0.07	0.00	0.00
			Instrument/Operator	0.43	0.06	0.79	0.65	1.93
			Repeatability	1.28	1.05	1.59	1.13	3.34
			Within-lab Precision	1.72			1.31	3.88
			Reproducibility	1.72	1.38	2.20	1.31	3.88

Interfering Substances

Interference was assessed by testing contrived positive samples consisting of one strain each of SARS-CoV-2, Flu A, Flu B and RSV at 3x their respective limits of detection (LoDs) as well as negative samples in the presence or absence of the twenty-six (26) interfering substances, two (2) solvents, one (1) no interferent control, and five (5) viral transport media (VTM). Additionally, potential interference due to extraction reagent carryover was assessed with four (4) carryover conditions and one (1) no-carryover control. For all potential interferents other than VTM, contrived samples were prepared in pooled negative NP specimens. VTM testing used pooled negative AN swab specimens that had been collected in the VTM being tested. Each substance was tested in triplicate (N=3) for each positive and negative sample through the full workflow with one (1) lot of reagents on the QuantStudio™ 5 Dx (QS5 Dx) real-time PCR instrument.

Each of the conditions tested produced the expected results (i.e., each of the Positive samples was “Positive” for its relevant target, and each of the Negative samples was “Negative”). Detection of both Flu A and Flu B in all samples tested with FluMist® (0.05%, 0.01% and 0.005%) was expected since it is a live-attenuated virus vaccine against influenza A and influenza B viruses. Importantly, the presence of FluMist® did not result in false positive (FP) or false negative (FN) results for the other targets at any of the three concentrations tested. None of the other exogenous or endogenous substances, transport media or extraction kit reagents tested interfered with the TaqPath™ COVID-19, Flu A, Flu B, RSV Select Panel. Results showing positivity and mean Cq values by sample are shown in **Table 15** below. Concentrations (Conc.) listed are per volume of sample before extraction for all conditions except Extraction Reagent Carryover. Extraction Reagent Carryover substances were added directly to the post-extraction eluate at the indicated concentrations per volume of eluate.

Table 15: Summary of Interfering Substances Testing Results for the TaqPath™ COVID-19, Flu A, Flu B, RSV Select Panel

Substance Description	Conc.	NEG	Flu A		Flu B		SARS-CoV-2		RSV	
		% Positive (nPositive/Total)	% Positive (nPositive/Total)	Mean Cq	% Positive (nPositive/Total)	Mean Cq	% Positive (nPositive/Total)	Mean Cq	% Positive (nPositive/Total)	Mean Cq
No interferent control (NP)	N/A	0% (0/9)	100% (6/6)	33.20	100% (9/9)	33.32	100% (6/6)	34.20	100% (6/6)	33.42
Mucin	5 mg/mL	0% (0/3)	100% (3/3)	33.94	100% (3/3)	33.23	100% (3/3)	35.20	100% (3/3)	33.23
Whole blood	5% (v/v)	0% (0/3)	100% (3/3)	32.76	100% (3/3)	33.08	100% (3/3)	34.09	100% (3/3)	32.78
Jurkat cell gDNA	7 µg/mL	0% (0/3)	100% (3/3)	33.65	100% (3/3)	33.09	100% (3/3)	34.02	100% (3/3)	33.15
Nasal decongestant – Phenylephrine (Equate™)	10% (v/v)	0% (0/3)	100% (3/3)	32.23	100% (3/3)	33.30	100% (3/3)	34.72	100% (3/3)	33.60
Nasal decongestant – Oxymetazoline (Afrin®)	10% (v/v)	0% (0/3)	100% (3/3)	30.50	100% (3/3)	31.20	100% (3/3)	34.42	100% (3/3)	32.83
Saline nasal spray (Equate™)	10% (v/v)	0% (0/3)	100% (3/3)	32.69	100% (3/3)	33.04	100% (3/3)	34.04	100% (3/3)	33.29
Bronchodilator (Ventolin® Evohaler®)	0.83 mg/mL	0% (0/3)	100% (3/3)	33.67	100% (3/3)	33.35	100% (3/3)	34.26	100% (3/3)	33.51
Nasal corticosteroids – Dexamethasone	1.5 mg/mL	0% (0/3)	100% (3/3)	33.60	100% (3/3)	32.87	100% (3/3)	34.44	100% (3/3)	33.48
Nasal corticosteroids – Flunisolide	2 mg/mL	0% (0/3)	100% (3/3)	34.16	100% (3/3)	32.93	100% (3/3)	34.78	100% (3/3)	31.27
Nasal corticosteroids – Budesonide (Apotex®)	1% (v/v)	0% (0/3)	100% (3/3)	33.71	100% (3/3)	33.00	100% (3/3)	34.10	100% (3/3)	32.73
Nasal corticosteroids – Fluticasone (Equate™)	1% (v/v)	0% (0/3)	100% (3/3)	33.17	100% (3/3)	33.57	100% (3/3)	33.74	100% (3/3)	33.11
Nasal gel (NeilMed® Nasogel®)	1% (w/v)	0% (0/3)	100% (3/3)	33.38	100% (3/3)	33.27	100% (3/3)	34.60	100% (3/3)	33.29
Homeopathic allergy relief medicine (Zicam®)	1% (w/v)	0% (0/3)	100% (3/3)	33.36	100% (3/3)	33.84	100% (3/3)	34.74	100% (3/3)	33.12

Substance Description	Conc.	NEG	Flu A		Flu B		SARS-CoV-2		RSV	
		% Positive (nPositive/ Total)	% Positive (nPositive/ Total)	Mean Cq	% Positive (nPositive/ Total)	Mean Cq	% Positive (nPositive/ Total)	Mean Cq	% Positive (nPositive/ Total)	Mean Cq
Throat lozenges (Chloraseptic® Max)	2.2 mg/mL methanol; 3.3 mg/mL benzocaine	0% (0/3)	100% (3/3)	33.71	100% (3/3)	33.46	100% (3/3)	34.33	100% (3/3)	33.25
Zinc lozenges (Life Extension®)	7.5 mg/mL	0% (0/3)	100% (3/3)	33.69	100% (3/3)	33.17	100% (3/3)	34.06	100% (3/3)	33.20
Antiviral – Zanamivir	5.5 mg/mL	0% (0/6)	100% (4/4)	33.46	100% (6/6)	32.95	100% (3/3)	34.33	100% (3/3)	33.39
Antiviral - Oseltamivir phosphate	33 µg/mL	0% (0/3)	100% (3/3)	33.25	100% (3/3)	31.93	100% (3/3)	34.52	100% (3/3)	33.38
Antiviral – Remdesivir	6.7 µg/mL	0% (0/3)	100% (3/3)	33.45	100% (3/3)	33.48	100% (3/3)	33.88	100% (3/3)	34.03
Topical antibiotic (Pseudomonic Acid)	3.3 mg/mL	0% (0/3)	100% (3/3)	32.09	100% (3/3)	33.06	100% (3/3)	34.56	100% (3/3)	32.43
Systemic antibiotic (Tobramycin)	4 µg/mL	0% (0/3)	100% (3/3)	33.33	100% (3/3)	33.24	100% (3/3)	34.40	100% (3/3)	32.92
Flu vaccine: FluMist®	0.05% (v/v)	100% (3/3)^[1]	100% (3/3)	21.59	100% (3/3)	20.85	100% (3/3)	33.19	100% (3/3)	32.82
	0.01% (v/v)	100% (3/3)^[1]	100% (3/3)	23.89	100% (3/3)	23.36	100% (3/3)	32.85	100% (3/3)	32.99
	0.005% (v/v)	100% (3/3)^[1]	100% (3/3)	24.86	100% (3/3)	24.24	100% (3/3)	33.37	100% (3/3)	32.88
Analgesic (Ibuprofen)	220 µg/mL	0% (0/3)	100% (3/3)	33.30	100% (3/3)	32.69	100% (3/3)	34.00	100% (3/3)	33.48
Tobacco (Camel Snus®)	1% (w/v)	0% (0/3)	100% (3/3)	32.93	100% (3/3)	32.99	100% (3/3)	33.85	100% (3/3)	32.82
Solvent DMSO	20% (v/v)	0% (0/6)	100% (3/3)	33.53	100% (6/6)	32.74	100% (3/3)	34.69	100% (3/3)	31.27
Solvent Ethanol	20% (v/v)	0% (0/3)	100% (3/3)	32.34	100% (3/3)	32.79	100% (3/3)	34.01	100% (3/3)	33.60
VTM Remel® M4RT	Neat ^[2]	0% (0/3)	100% (3/3)	33.35	100% (3/3)	33.37	100% (3/3)	34.73	100% (3/3)	32.10

Substance Description	Conc.	NEG	Flu A		Flu B		SARS-CoV-2		RSV	
		% Positive (nPositive/ Total)	% Positive (nPositive/ Total)	Mean Cq	% Positive (nPositive/ Total)	Mean Cq	% Positive (nPositive/ Total)	Mean Cq	% Positive (nPositive/ Total)	Mean Cq
VTM Remel® M5	Neat ^[2]	0% (0/3)	100% (3/3)	33.00	100% (3/3)	33.19	100% (3/3)	34.76	100% (3/3)	33.06
VTM Remel® M6	Neat ^[2]	0% (0/3)	100% (3/3)	33.31	100% (3/3)	33.05	100% (3/3)	35.42	100% (3/3)	33.15
VTM Copan® UTM-RT	Neat ^[2]	0% (0/3)	100% (3/3)	32.88	100% (3/3)	31.92	100% (3/3)	34.57	100% (3/3)	32.76
VTM BD® UVT	Neat ^[2]	0% (0/3)	100% (3/3)	31.77	100% (3/3)	33.40	100% (3/3)	34.47	100% (3/3)	32.96
No Extraction Reagent Carryover Control	N/A	0% (0/3)	100% (3/3)	33.43	100% (3/3)	32.97	100% (3/3)	33.94	100% (3/3)	33.26
Extraction Reagent Carryover - Sample Plate	1% (v/v) ^[3]	0% (0/3)	100% (3/3)	33.47	100% (3/3)	33.06	100% (3/3)	34.59	100% (3/3)	33.52
Extraction Reagent Carryover - Wash 1	1% (v/v) ^[3]	0% (0/3)	100% (3/3)	33.67	100% (3/3)	33.44	100% (3/3)	34.25	100% (3/3)	33.33
Extraction Reagent Carryover - Wash 2	6% (v/v) ^[3]	0% (0/3)	100% (3/3)	33.90	100% (3/3)	33.13	100% (3/3)	34.02	100% (3/3)	33.54
	3% (v/v) ^[3]	0% (0/3)	100% (3/3)	33.32	100% (3/3)	32.79	100% (3/3)	34.41	100% (3/3)	33.33

^[1] As expected, NEG samples containing FluMist® were positive for Flu A and Flu B and negative for SARS-CoV-2 and RSV

^[2] Neat = undiluted; AN samples originally collected in indicated VTM

^[3] Interferent added to eluates (post-extraction), and concentrations are per volume of eluate.

Competitive Interference

Competitive interference testing was performed using contrived samples containing pairs of Flu A, Flu B, SARS-CoV-2 or RSV with one virus at a low concentration (3x LoD) and the other virus at a high concentration (1.0×10^5 TCID₅₀/mL) spiked into pooled negative NP specimens. In parallel, viruses were tested at both levels individually to serve as controls and demonstrate within-panel specificity of each of the target channels. Sample extraction was performed using the KingFisher™ Apex Purification System and real-time RT-PCR was performed with the QS5Dx real-time PCR instrument with one (1) lot of TaqPath™ COVID-19, Flu A, Flu B, RSV Select Panel reagents. Note that different virus strains were used to prepare the high-concentration virus samples because the quantified virus stocks used for the 3x LoD level were either insufficiently concentrated to achieve the target of 10^5 TCID₅₀/mL or had limited stock.

The results of each competitive interference condition tested satisfied the acceptance criteria. Three positive test results among the three total replicates (N=3) (100% positivity) for each viral target for both low-concentration virus and the high-concentration virus were produced. There were no off-target false-positive results in any target channel observed for the within-panel specificity testing component of the study (i.e., single targets present at 10^5 TCID₅₀/mL). The negative, pooled NP specimens (alone, with no virus added) were tested in the workflow as a negative control condition and yielded negative test results, as expected. The results of the study are summarized in **Table 16**.

Table 16: Summary of Competitive Interference Testing Results for the TaqPath™ COVID-19, Flu A, Flu B, RSV Select Panel

Viral Test Condition Combination	Low Target (Group 1)		High Target (Group 2)	
	Virus (Concentration)	nPositive/ Total (%) Positive)	Virus (Concentration)	nPositive/ Total (%) Positive)
SARS-CoV-2 (low), Flu A (high)	SARS-CoV-2 (3x LoD)	3 / 3 (100%)	Flu A (10^5 TCID ₅₀ /mL)	3 / 3 (100%)
SARS-CoV-2 (low), Flu B (high)	SARS-CoV-2 (3x LoD)	3 / 3 (100%)	Flu B (10^5 TCID ₅₀ /mL)	3 / 3 (100%)
SARS-CoV-2 (low), RSV (high)	SARS-CoV-2 (3x LoD)	3 / 3 (100%)	RSV (10^5 TCID ₅₀ /mL)	3 / 3 (100%)

Viral Test Condition Combination	Low Target (Group 1)		High Target (Group 2)	
	Virus (Concentration)	nPositive/ Total (%) Positive)	Virus (Concentration)	nPositive/ Total (%) Positive)
Flu A (low), SARS-CoV-2 (high)	Flu A (3x LoD)	3 / 3 (100%)	SARS-CoV-2 (10 ⁵ TCID ₅₀ /mL)	3 / 3 (100%)
Flu A (low), Flu B (high)	Flu A (3x LoD)	3 / 3 (100%)	Flu B (10 ⁵ TCID ₅₀ /mL)	3 / 3 (100%)
Flu A (low), RSV (high)	Flu A (3x LoD)	3 / 3 (100%)	RSV (10 ⁵ TCID ₅₀ /mL)	3 / 3 (100%)
Flu B (low), SARS-CoV-2 (high)	Flu B (3x LoD)	3 / 3 (100%)	SARS-CoV-2 (10 ⁵ TCID ₅₀ /mL)	3 / 3 (100%)
Flu B (low), Flu A (high)	Flu B (3x LoD)	3 / 3 (100%)	Flu A (10 ⁵ TCID ₅₀ /mL)	3 / 3 (100%)
Flu B (low), RSV (high)	Flu B (3x LoD)	3 / 3 (100%)	RSV (10 ⁵ TCID ₅₀ /mL)	3 / 3 (100%)
RSV (low), SARS-CoV-2 (high)	RSV (3x LoD)	3 / 3 (100%)	SARS-CoV-2 (10 ⁵ TCID ₅₀ /mL)	3 / 3 (100%)
RSV (low), Flu A (high)	RSV (3x LoD)	3 / 3 (100%)	Flu A (10 ⁵ TCID ₅₀ /mL)	3 / 3 (100%)
RSV (low), Flu B (high)	RSV (3x LoD)	3 / 3 (100%)	Flu B (10 ⁵ TCID ₅₀ /mL)	3 / 3 (100%)
SARS-CoV-2 (low)	SARS-CoV-2 (3x LoD)	3 / 3 (100%)	N/A	N/A
SARS-CoV-2 (high)	N/A	N/A	SARS-CoV-2 (10 ⁵ TCID ₅₀ /mL)	3 / 3 (100%)
Flu A (low)	Flu A (3x LoD)	3 / 3 (100%)	N/A	N/A
Flu A (high)	N/A	N/A	Flu A (10 ⁵ TCID ₅₀ /mL)	3 / 3 (100%)
Flu B (low)	Flu B (3x LoD)	3 / 3 (100%)	N/A	N/A
Flu B (high)	N/A	N/A	Flu B (10 ⁵ TCID ₅₀ /mL)	3 / 3 (100%)
RSV (low)	RSV (3x LoD)	3 / 3 (100%)	N/A	N/A
RSV (high)	N/A	N/A	RSV (10 ⁵ TCID ₅₀ /mL)	3 / 3 (100%)
Negative (NP alone)	N/A	N/A	N/A	N/A

Cross-Reactivity & Microbial Interference

Contrived samples were prepared by spiking one strain each of 49 microorganisms into negative pooled NP samples to determine whether false positive (FP) results would be produced for any of the targets of the test. In parallel, the same microorganisms were tested in the presence of one representative viral strain for each of SARS-CoV-2, Flu A, Flu B, and RSV at 3x LoD to determine whether false negative (FN) results would be produced for these targets. Potentially cross-reactive microbes were either tested individually or in groups (multi-spikes). Each sample was tested in triplicate (N=3) through the full workflow with one (1) lot of reagents on one (1) representative PCR instrument model. Microbes with stock concentrations too low to achieve the target concentration were tested at 20% (v/v) in negative pooled NP samples. In addition to contrived samples, results from samples tested during the Clinical Validation study that had been called positive for coronavirus HKU1 (CoV-HKU1) by the comparator test were assessed for evidence of cross-reactivity with SARS-CoV-2, Flu A, Flu B or RSV.

Cross-reactivity was evaluated by assessing the occurrence of false positive results in negative pooled NP samples spiked with the microbes tested; pooled human nasal wash was tested alone. None of the potentially cross-reactive organisms tested produced FP results. Moreover, none of the microbes tested inhibited PCR or interfered with extraction or RNase P detection as evidenced by the RNase P signal in each sample. Within-panel cross-reactivity was assessed as part of the Competitive Interference Study. When each of the viruses was present at 10^5 TCID₅₀/mL, FPs were not observed for the other targets. All but one of the 39 CoV-HKU1-positive samples from the Clinical Performance study were called negative for all virus targets, with one sample producing a positive result of SARS-CoV-2 below the C_q cutoff for positivity. Results are shown in **Table 17** below.

Table 17: Cross-Reactivity & Microbial Interference Study Results for the TaqPath™ COVID-19, Flu A, Flu B, RSV Select Panel

Organism Type	Species	Test Concentration	Negative Samples	Contrived Positive Samples at 3x LoD			
			Number Negative / Total (% Negative)	Number Positive / Total (% Positive)			
				SARS-CoV-2	Flu A	Flu B	RSV
Bacteria	<i>Bordetella parapertussis</i>	1 x 10 ⁶ CFU/mL	3/3 (100%)	3/3 (100%)	3/3 (100%)	3/3 (100%)	3/3 (100%)
Bacteria	<i>Bordetella pertussis</i>	1 x 10 ⁶ CFU/mL	3/3 (100%)	3/3 (100%)	3/3 (100%)	3/3 (100%)	3/3 (100%)
Bacteria	<i>Chlamydophila pneumoniae</i>	1 x 10 ⁶ CFU/mL	3/3 (100%)	3/3 (100%)	3/3 (100%)	3/3 (100%)	3/3 (100%)
Bacteria	<i>Corynebacterium diphtheriae</i>	1 x 10 ⁶ CFU/mL	3/3 (100%)	3/3 (100%)	3/3 (100%)	3/3 (100%)	3/3 (100%)
Bacteria	<i>Escherichia coli</i>	1 x 10 ⁶ CFU/mL	3/3 (100%)	3/3 (100%)	3/3 (100%)	3/3 (100%)	3/3 (100%)
Bacteria	<i>Fusobacterium necrophorum</i>	1 x 10 ⁶ CFU/mL	3/3 (100%)	3/3 (100%)	3/3 (100%)	3/3 (100%)	3/3 (100%)
Bacteria	<i>Haemophilus influenzae type b</i>	1 x 10 ⁶ CFU/mL	3/3 (100%)	3/3 (100%)	3/3 (100%)	3/3 (100%)	3/3 (100%)
Bacteria	<i>Klebsiella pneumoniae</i>	1 x 10 ⁶ CFU/mL	3/3 (100%)	3/3 (100%)	3/3 (100%)	3/3 (100%)	3/3 (100%)
Bacteria	<i>Lactobacillus acidophilus</i>	1 x 10 ⁶ CFU/mL	3/3 (100%)	3/3 (100%)	3/3 (100%)	3/3 (100%)	3/3 (100%)
Bacteria	<i>Legionella pneumophila</i>	1 x 10 ⁶ CFU/mL	3/3 (100%)	3/3 (100%)	3/3 (100%)	3/3 (100%)	3/3 (100%)
Bacteria	<i>Moraxella catarrhalis</i>	1 x 10 ⁶ CFU/mL	3/3 (100%)	3/3 (100%)	3/3 (100%)	3/3 (100%)	3/3 (100%)
Bacteria	<i>Mycobacterium tuberculosis</i>	5.4 x 10 ⁷ copies/mL	3/3 (100%)	3/3 (100%)	3/3 (100%)	3/3 (100%)	3/3 (100%)
Bacteria	<i>Mycoplasma genitalium</i>	4 x 10 ⁵ cells/mL	3/3 (100%)	3/3 (100%)	3/3 (100%)	3/3 (100%)	3/3 (100%)
Bacteria	<i>Mycoplasma pneumoniae</i>	1 x 10 ⁶ CCU/mL	3/3 (100%)	3/3 (100%)	3/3 (100%)	3/3 (100%)	3/3 (100%)
Bacteria	<i>Neisseria elongata</i>	1 x 10 ⁶ CFU/mL	3/3 (100%)	3/3 (100%)	3/3 (100%)	3/3 (100%)	3/3 (100%)
Bacteria	<i>Neisseria meningitidis serogroup A</i>	1 x 10 ⁶ CFU/mL	3/3 (100%)	3/3 (100%)	3/3 (100%)	3/3 (100%)	3/3 (100%)
Bacteria	<i>Pseudomonas aeruginosa</i>	1 x 10 ⁶ CFU/mL	3/3 (100%)	3/3 (100%)	3/3 (100%)	3/3 (100%)	3/3 (100%)
Bacteria	<i>Staphylococcus aureus MRSA</i>	1 x 10 ⁶ CFU/mL	3/3 (100%)	3/3 (100%)	3/3 (100%)	3/3 (100%)	3/3 (100%)
Bacteria	<i>Staphylococcus epidermidis MRSE</i>	1 x 10 ⁶ CFU/mL	3/3 (100%)	3/3 (100%)	3/3 (100%)	3/3 (100%)	3/3 (100%)

Organism Type	Species	Test Concentration	Negative Samples	Contrived Positive Samples at 3x LoD			
			Number Negative / Total (% Negative)	Number Positive / Total (% Positive)			
				SARS-CoV-2	Flu A	Flu B	RSV
Bacteria	<i>Streptococcus pneumoniae</i>	1 x 10 ⁶ CFU/mL	3/3 (100%)	3/3 (100%)	3/3 (100%)	3/3 (100%)	3/3 (100%)
Bacteria	<i>Streptococcus pyogenes</i>	1 x 10 ⁶ CFU/mL	3/3 (100%)	3/3 (100%)	3/3 (100%)	3/3 (100%)	3/3 (100%)
Bacteria	<i>Streptococcus salivarius</i>	1 x 10 ⁶ CFU/mL	3/3 (100%)	3/3 (100%)	3/3 (100%)	3/3 (100%)	3/3 (100%)
Fungi	<i>Aspergillus flavus</i>	1 x 10 ⁶ CFU/mL	3/3 (100%)	3/3 (100%)	3/3 (100%)	3/3 (100%)	3/3 (100%)
Fungi	<i>Candida albicans</i>	1 x 10 ⁶ CFU/mL	3/3 (100%)	3/3 (100%)	3/3 (100%)	3/3 (100%)	3/3 (100%)
Fungi	<i>Pneumocystis carinii</i>	1 x 10 ⁶ cells/mL	3/3 (100%)	3/3 (100%)	3/3 (100%)	3/3 (100%)	3/3 (100%)
Virus	Adenovirus type 1, species C	1 x 10 ⁵ TCID ₅₀ /mL	3/3 (100%)	3/3 (100%)	3/3 (100%)	3/3 (100%)	3/3 (100%)
Virus	Mastadenovirus B type 7, Gomen	1 x 10 ⁵ TCID ₅₀ /mL	3/3 (100%)	3/3 (100%)	3/3 (100%)	3/3 (100%)	3/3 (100%)
Virus	Cytomegalovirus (HHV-5)	3.2 x 10 ⁴ TCID ₅₀ /mL	3/3 (100%)	3/3 (100%)	3/3 (100%)	3/3 (100%)	3/3 (100%)
Virus	Enterovirus type 68	1 x 10 ⁵ TCID ₅₀ /mL	3/3 (100%)	3/3 (100%)	3/3 (100%)	3/3 (100%)	3/3 (100%)
Virus	Epstein-Barr virus (HHV-4)	1.57 x 10 ⁷ copies/mL	3/3 (100%)	3/3 (100%)	3/3 (100%)	3/3 (100%)	3/3 (100%)
Virus	Hepatitis B virus	4.2 x 10 ⁷ IU/mL	3/3 (100%)	3/3 (100%)	3/3 (100%)	3/3 (100%)	3/3 (100%)
Virus	Hepatitis C virus	3.3 x 10 ⁷ IU/mL	3/3 (100%)	3/3 (100%)	3/3 (100%)	3/3 (100%)	3/3 (100%)
Virus	Herpes simplex virus 1 (HSV-1)	1 x 10 ⁵ TCID ₅₀ /mL	3/3 (100%)	3/3 (100%)	3/3 (100%)	3/3 (100%)	3/3 (100%)
Virus	Human coronavirus 229E	2.82 x 10 ⁴ TCID ₅₀ /mL	3/3 (100%)	3/3 (100%)	3/3 (100%)	3/3 (100%)	3/3 (100%)
Virus	Human coronavirus HKU1 ^[1]	2 x 10 ⁶ copies/mL	3/3 (100%)	3/3 (100%)	3/3 (100%)	3/3 (100%)	3/3 (100%)
Virus	Human coronavirus HKU1	39 positive clinical specimens	38/39 (97.4%) ^[2]	Not tested	Not tested	Not tested	Not tested
Virus	Human coronavirus NL63	2.34 x 10 ⁴ TCID ₅₀ /mL	3/3 (100%)	3/3 (100%)	3/3 (100%)	3/3 (100%)	3/3 (100%)
Virus	Human coronavirus OC43	1 x 10 ⁵ TCID ₅₀ /mL	3/3 (100%)	3/3 (100%)	3/3 (100%)	3/3 (100%)	3/3 (100%)

Organism Type	Species	Test Concentration	Negative Samples	Contrived Positive Samples at 3x LoD			
			Number Negative / Total (% Negative)	Number Positive / Total (% Positive)			
				SARS-CoV-2	Flu A	Flu B	RSV
Virus	Human immunodeficiency virus type IIIB	1 x 10 ⁴ IU/mL	3/3 (100%)	3/3 (100%)	3/3 (100%)	3/3 (100%)	3/3 (100%)
Virus	Human metapneumovirus	1 x 10 ⁵ TCID ₅₀ /mL	3/3 (100%)	3/3 (100%)	3/3 (100%)	3/3 (100%)	3/3 (100%)
Virus	Measles virus	1 x 10 ⁵ TCID ₅₀ /mL	3/3 (100%)	3/3 (100%)	3/3 (100%)	3/3 (100%)	3/3 (100%)
Virus	MERS coronavirus strain Florida/USA-2 Saudi Arabia 2014	Not provided ^[3]	3/3 (100%)	3/3 (100%)	3/3 (100%)	3/3 (100%)	3/3 (100%)
Virus	MERS coronavirus strain EMC/2012	1 x 10 ⁵ TCID ₅₀ /mL	3/3 (100%)	3/3 (100%)	3/3 (100%)	3/3 (100%)	3/3 (100%)
Virus	Mumps virus	1 x 10 ⁵ TCID ₅₀ /mL	3/3 (100%)	3/3 (100%)	3/3 (100%)	3/3 (100%)	3/3 (100%)
Virus	Parainfluenza virus type 1	1 x 10 ⁵ TCID ₅₀ /mL	3/3 (100%)	3/3 (100%)	3/3 (100%)	3/3 (100%)	3/3 (100%)
Virus	Parainfluenza virus type 2	1 x 10 ⁵ TCID ₅₀ /mL	3/3 (100%)	3/3 (100%)	3/3 (100%)	3/3 (100%)	3/3 (100%)
Virus	Parainfluenza virus type 3	1 x 10 ⁵ TCID ₅₀ /mL	3/3 (100%)	3/3 (100%)	3/3 (100%)	3/3 (100%)	3/3 (100%)
Virus	Parainfluenza virus type 4A	1 x 10 ⁵ TCID ₅₀ /mL	3/3 (100%)	3/3 (100%)	3/3 (100%)	3/3 (100%)	3/3 (100%)
Virus	Rhinovirus type 1A	2.82 x 10 ⁴ TCID ₅₀ /mL	3/3 (100%)	3/3 (100%)	3/3 (100%)	3/3 (100%)	3/3 (100%)
Virus	SARS coronavirus strain 2003-00592	Not provided ^[3]	3/3 (100%)	3/3 (100%)	3/3 (100%)	3/3 (100%)	3/3 (100%)
Virus	SARS coronavirus strain Urbani	1 x 10 ⁵ PFU/mL	3/3 (100%)	3/3 (100%)	3/3 (100%)	3/3 (100%)	3/3 (100%)
Virus	Varicella-zoster virus (HHV-3)	1.94 x 10 ⁸ copies/mL	3/3 (100%)	3/3 (100%)	3/3 (100%)	3/3 (100%)	3/3 (100%)
Biofluid	Pooled human nasal wash	Neat	3/3 (100%)	3/3 (100%)	3/3 (100%)	3/3 (100%)	3/3 (100%)

[1] Subgenomic synthetic control (ATCC VR-3262SD)

[2] One sample produced a positive for SARS-CoV-2.

[3] Concentration not provided by vendor.

In silico Cross-Reactivity

In silico analysis was conducted for the COVID-19, Flu A, Flu B, RSV and RNase P assay primers and probes using BLAST against known strains/isolates from the GenBank database, as of March

29, 2023. Cross-reactivity was evaluated *in silico* by determining whether any of the microbes had at least 80% homology with primers or probes and produced an amplicon of less than 10,000 bases.

No isolate out of the 1,155 tested matched to 3 or more individual components of any TaqPath™ COVID-19, Flu A, Flu B, RSV Select Panel assay. An amplicon will not be detected when less than 3 individual assay components bind to an isolate and an amplicon will not be produced unless two primers bind; therefore, all sequences were predicted to be negative for cross-reactivity.

Specimen Stability

Specimen stability was assessed by examining the stability of contrived NP and AN swab specimens collected in VTM/UTM at the following storage conditions:

- Upper end of Room temperature (30°C) for up to 24 hours
- Upper end of Refrigerated temperature (8°C) for up to 72 hours
- Low frozen temperature ($\leq -70^{\circ}\text{C}$) for up to 30 days

Contrived samples were created by pooling individual negative clinical NP and AN swab specimens collected in VTM/UTM respectively, then aliquoting the pooled matrix and spiking with one representative strain for each of SARS-CoV-2, Flu A, Flu B and RSV at 3x LoD. Upon contriving, samples were aliquoted and stored at the various storage conditions prior to testing. At each timepoint, samples were removed from their storage condition and moved to $\leq -70^{\circ}\text{C}$ to prevent further aging. An isochronous design was employed where possible to reduce variability in testing. When ready to conduct testing, extraction was performed for all samples being tested together using the MagMAX™ Dx Viral/Pathogen Nucleic Acid Isolation Kit. Negative NP and AN samples were extracted in parallel. All samples were tested with the TaqPath™ COVID-19, Flu A, Flu B, RSV Select Panel on the Applied Biosystems™ QuantStudio™ 5 Dx. Specimen stability testing utilized one lot of extraction and PCR reagents for all storage conditions.

All targets for both NP and AN specimens produced 100% positivity at all the time points tested for the 8°C, 30°C and the $\leq -70^{\circ}\text{C}$ temperatures and therefore, support the claimed specimen stability in the Instruction for Use of 24 hours at room temperature (15–25°C), 72 hours refrigerated (2–8°C) and 30 days at $\leq -70^{\circ}\text{C}$.

Eluate Stability

The Eluate Stability Study evaluated the performance of refrigerated and frozen-and-thawed nucleic acid eluates with the TaqPath™ COVID-19, Flu A, Flu B, RSV Select Panel. NP and AN specimens contrived with SARS-CoV-2, Flu A, Flu B and RSV to 3x LoD were used with the MagMAX™ Dx Viral/Pathogen Nucleic Acid Isolation Kit to collect the eluates. Eluates were either tested fresh or stored at +8°C for 20 and 27 hours, then underwent two (2) freeze-and-thaw cycles (-70°C to +8°C) before testing with the TaqPath™ COVID-19, Flu A, Flu B, RSV Select Panel.

Eluate stability was assessed by the occurrence of FN results in contrived positive samples, each containing SARS-CoV-2, Flu A, Flu B or RSV at 3x LoD. 100% of contrived positive sample eluates produced the expected results (correct calls) at all three (3) time points, supporting the eluates storage claims for up to 24 hours at 2°C – 8°C, 5 days at ≤ -70°C and after one (1) freeze/thaw cycle, cumulatively.

Fresh vs Frozen Equivalency

The Fresh Versus Frozen Equivalency Study examined equivalency of fresh and frozen NP and AN swab specimens collected in VTM/UTM. The study served two purposes: first, to support the use of frozen samples during the Clinical Validation study and second, to evaluate freeze/thaw stability of specimens. Positive contrived samples were created by pooling individual negative clinical NP and AN swab specimens collected in VTM/UTM respectively, then aliquoting the pooled matrix and spiking with one representative strain for each of SARS-CoV-2, Flu A, Flu B and RSV at 2x LoD and 5x LoD. Negative samples from the same NP and AN specimen pools were also tested. Upon contriving, samples were aliquoted for immediate testing for the “fresh” study arm, and the remaining sample aliquots were stored at ≤ -70°C to undergo the freeze/thaw cycles prior to testing. Samples were frozen at ≤ -70°C for at least 2 hours (maximum 24 hours) or until fully frozen. After the thaw event (maximum 4 hours or until completely thawed), the aliquots were removed and stored for testing. Testing was conducted for samples that underwent two (2), three (3) and six (6) freeze/thaw (F/T) cycles.

Samples undergoing freeze/thaw were stored at $\leq -70^{\circ}\text{C}$ upon completion of their respective F/T cycles and tested together to reduce variability in testing. Extraction was performed using the MagMAX™ Dx Viral/Pathogen Nucleic Acid Isolation Kit. Negative NP and AN samples were also extracted in parallel. All samples were tested with the TaqPath™ COVID-19, Flu A, Flu B, RSV Select Panel on the Applied Biosystems™ QuantStudio™ 5 Dx. Fresh vs frozen equivalency testing utilized one lot of extraction and real-time PCR reagents for all conditions.

The results of this study support the use of frozen specimens in the clinical performance study and the claim in the TaqPath™ COVID-19, Flu A, Flu B, RSV Select Panel Instructions for Use that specimens collected in Copan® UTM Universal Transport Medium (UTM) or BD® Universal Viral Transport (UVT) Medium may undergo up to 2 freeze/thaw cycles. Do not exceed 1 freeze/thaw cycle for specimens collected in other transport media types.

In-Use and Hold Time Stability

The TaqPath™ COVID-19, Flu A, Flu B, RSV Select Panel has a recommended storage temperature of -30°C to -10°C and the kit components require thawing prior to use. The in-use stability of the reagents during typical in-use conditions were evaluated while executing the workflow, and the performance of the kit components was compared to the untreated baseline (i.e., time point T0 or freeze/thaw cycle FT0).

Assay Mix Tube and Master Mix were tested using contrived positive samples in NP matrix containing SARS-CoV-2, Flu A, Flu B or RSV at 3x LoD as well as negative samples consisting of NP matrix alone. Positive Control (PC) and Negative Control (NC) were used unstressed as batch controls for Master Mix and Assay Mix Tube testing. PC and NC were also stressed to determine their own in-use stability parameters. Testing utilized the isochronous design where possible in which reagents were returned to frozen storage at various time points and tested together at the end. A minimum of three time points or freeze/thaw cycles were tested per condition, including T0/FT0, and all testing extended beyond the claimed stability period. Samples were prepared in advance and extracted with the MagMAX™ Dx Viral/Pathogen Nucleic Acid Isolation Kit on the KingFisher™ Apex Purification System, and testing was performed on the QuantStudio™ 5 Dx. Data were analyzed qualitatively to determine if positive samples remained

positive, negative samples remained valid negative and PC and NC gave the required results after in-use testing.

Data from these studies support the storage conditions and freeze thaw cycles specified in the Instructions for Use for the TaqPath™ COVID-19, Flu A, Flu B, RSV Select Panel for the Assay Tube Mix, Master Mix and PC and NC.

The TaqPath™ COVID-19, Flu A, Flu B, RSV Select Panel components met all the acceptance criteria for all the test points in all the tested conditions and summarized as follows:

- The Assay Mix Tube and Master Mix demonstrated stability when held at 80 hours at 8°C, and thus the Assay Mix Tube and Master Mix are deemed stable when each are stored at 2°C to 8°C for up to 72 hours.
- The Positive Control and Negative Control demonstrated stability when held at 27 hours at 8°C prior to extraction, and therefore the Positive Control and Negative Control are deemed stable when each are stored at 2°C to 8°C for up to 24 hours.
- The Assay Tube Mix and Master Mix demonstrated stability to the fifth freeze/thaw cycle, and are deemed stable up to 4 freeze/thaw cycles (inclusive) from -30°C to -10°C to 2°C to 8°C.

Carryover Cross-Contamination

In the TaqPath™ COVID-19, Flu A, Flu B, RSV Select Panel workflow, PCR plate setup is performed manually and only the extraction step utilizes instrumentation that could result in carryover and/or cross-contamination. To this end, assessment of carryover and cross-contamination was performed beginning from sample extraction. A modified checkerboard layout was extracted in which 12 high-concentration samples containing inactivated SARS-CoV-2 viral material at 10^5 TCID₅₀/mL in pooled negative NP samples were interspersed with negative NP samples.

Six full plates were extracted and tested sequentially by two operators on two different KingFisher™ Apex Purification System instruments. After extraction, the eluates were tested

using the TaqPath™ COVID-19, Flu A, Flu B, RSV Select Panel. SARS-CoV-2 was used as a model for all targets because (a) SARS-CoV-2 has higher clinically relevant concentrations and (b) the SARS-CoV-2 LoD is lower (more sensitive) than the other target viruses. Of the 492 replicates run, 0 of the negative sample wells produced a positive call for SARS-CoV-2, providing a carryover/cross-contamination rate of 0%.

RNase P Internal Control Cutoff Confirmation

In the RNase P Internal Control Cutoff Confirmation study, viral target-negative “used” (i.e., collected from individual donors and containing RNase P) nasopharyngeal (NP) swab specimens and “unused” (i.e., unopened and not containing RNase P) NP swabs were tested according to the TaqPath™ COVID-19, Flu A, Flu B, RSV Select Panel Instructions for Use. Sample extraction was performed using the KingFisher™ Apex Purification System, and RT-PCR was performed on the QS5Dx with one (1) lot of TaqPath™ COVID-19, Flu A, Flu B, RSV Select Panel reagents. The RNase P IC cutoff was determined to be $C_q = 33.0$ in a development study and was confirmed in the execution of this study.

The IC cutoff was confirmed at $C_q = 33.0$ while testing individually collected NP swabs on the QS5Dx instrument. The results of the experiment demonstrated an overall positive percent agreement (PPA) of 98.07% (95% CI of 97.31-98.63%) and negative percent agreement (NPA) of 100% (95% CI of 97.49-100%) for RNase P detection in NP swabs. For AN swabs, PPA for RNase P detection was 92.30% (95% CI of 90.83-93.54%) and NPA was 100% (95% CI of 97.50-100%).

Due to the lower than expected PPA observed for AN specimens in the RNase P Cutoff confirmation study, an additional study was conducted utilizing an improved AN swab collection technique. All of the evaluated samples produced valid calls. Two (2) out of the one hundred thirty-eight (138) AN samples were positive for COVID-19, while the rest were negative for all viral targets. RNase P was detected in 100% of both negative and positive samples. The results are summarized in **Table 18**.

Table 18: Invalid Rate in AN Specimen Swab Collection Study Results

Sample Call	RNase P Call	Number of Samples	Percent Validity	RNase P (Cq)		
				Mean	Median	SD
Negative	Positive	136	100%	27.1	26.7	2.1
COVID-19 Positive	Positive	2	100%	26.9	26.9	1.9
All	Positive	138	100%	27.1	26.7	2.1

This study has demonstrated that the improved AN swab collection technique reduces the invalid rate for AN swabs when tested with the TaqPath COVID-19, Flu A, Flu B, RSV Select Panel. The TaqPath™ COVID-19, Flu A, Flu B, RSV Select Panel Instructions for Use include the improved AN swab collection instructions.

Matrix Equivalency

The matrix equivalency study evaluated the performance of NP and AN swab specimens (collected in VTM/UTM) with the TaqPath™ COVID-19, Flu A, Flu B, RSV Select Panel. Contrived samples prepared with one strain each of SARS-CoV-2, Flu A, Flu B and RSV at two concentrations (2x and 5x LoD) in NP and AN sample matrices were tested with one lot of the TaqPath™ COVID-19, Flu A, Flu B, RSV Select Panel on the QS5Dx real-time PCR instrument. The selected virus concentrations (2x and 5x relative to LoD) represent low positive and moderate positive specimens, respectively. Negative specimens were also tested. All samples produced 100% concordance with the expected results; thus, matrix equivalency between NP and AN swab specimens collected in VTM/UTM was demonstrated for the TaqPath™ COVID-19, Flu A, Flu B, RSV Select Panel.

Clinical Cut-Off

The clinical cut-off study was conducted in two phases. The first phase was a preliminary benchmarking investigation into the performance of the TaqPath™ COVID-19, Flu A, Flu B, RSV Select Panel against each two comparator devices to assess performance near the analytical limits of the TaqPath™ COVID-19, Flu A, Flu B, RSV Select Panel. The second testing phase, the

Receiver Operating Characteristic (ROC) justification study, was designed to further evaluate the TaqPath™ COVID-19, Flu A, Flu B, RSV Select Panel with clinical and contrived samples against each comparator device for the purpose of defining the clinical cut-off prior to design validation. The method used to determine the most appropriate clinical cutoff was the Receiver Operating Characteristic analysis according to CLSI EP24-A2.

The benchmarking and ROC justification studies established clinical cutoffs for the TaqPath™ COVID-19, Flu A, Flu B, RSV Select Panel by comparing to the predicate device.

Clinical Performance

The clinical performance testing that was conducted to support substantial equivalence is summarized below.

Clinical Performance (Prospective Study)

A clinical performance study was conducted using the comparator approach to evaluate the clinical performance of the TaqPath™ COVID-19, Flu A, Flu B, RSV Select Panel (investigational test) in nasopharyngeal (NP) swabs and anterior nasal (AN) swabs from individuals with signs and symptoms of respiratory tract infections including SARS-CoV-2, influenza A, influenza B, or RSV. The comparator device chosen in this study was an FDA cleared molecular test that detects and differentiates between SARS-CoV-2, influenza A virus, influenza B virus, and respiratory syncytial virus (RSV). NP and AN swab specimens were collected prospectively and during an enrichment phase from eligible subjects who provided consent or assent to participate in the study. Specimens collected in this study were tested by the TaqPath™ COVID-19, Flu A, Flu B, RSV Select Panel and the comparator device.

The study population was composed of participants who were experiencing signs and symptoms of respiratory viral infection. A total of one thousand nine hundred and nine (1,909) subjects from 14 different collection sites were enrolled in this study, of which 1,840 subjects were prospectively enrolled from all comers who met the inclusion criteria and 69 subjects were enrolled during an enrichment phase of the study. One nasopharyngeal swab and one anterior nasal swab in Universal

Viral Transport Medium was collected from each participant. Sample collection was completed between February 20, 2023 and February 29, 2024.

After applicable exclusions, a total of 1,620 NP and 1,541 AN swabs were included in final data analysis for the prospective cohort. Two (2) of the 1,541 AN specimens and four (4) of the 1,620 NP specimens observed inconclusive comparator results for Flu A, leaving 1,539 evaluable AN specimens and 1,616 NP specimens for Flu A. Specimens were tested either fresh as Category I samples or frozen as Category II samples. The performance comparison of the TaqPath™ COVID-19, Flu A, Flu B, RSV Select Panel and the comparator test results are presented in **Table 19**. Performance characteristics of the study included Positive Percent Agreement (PPA) and Negative Percent Agreement (NPA) for each clinical target using NP and AN swab specimen types on the QuantStudio™ 5 Dx (QS5 Dx) instrument. PPA and NPA are defined as follows:

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

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

Two-sided, Wilson Score confidence intervals (CI) around each estimate were also calculated. Samples that produced a discordant call between the TaqPath™ COVID-19, Flu A, Flu B, RSV Select Panel and the comparator test were further tested with another FDA cleared molecular assay. Results from discordant sample testing are presented as footnotes in each table.

Of the 1,840 subjects enrolled in the prospective study, 1,647 had their AN swab specimens evaluated with the candidate device. One hundred seventy nine (179) of these specimens were invalid by the candidate device during testing, for an initial invalid rate of 10.9% (179/1,647). Upon retesting, the invalid rate for AN specimens decreased to 6.4% (105/1,647). Of the 1,840 subjects enrolled in the prospective study, 1,647 had their NP swab specimens evaluated with the candidate device. Forty five (45) of these specimens were invalid by the candidate device during testing, for an initial invalid rate of 2.7% (45/1,647). Upon retesting, the invalid rate for NP specimens decreased to 1.6% (27/1,647).

Table 19: Performance Estimates for the TaqPath™ COVID-19, Flu A, Flu B, RSV Select Panel (Prospective Cohort)

Target	Swab Type	Specimen Collection	Number of Specimens	True Positive	False Negative	True Negative	False Positive	PPA (%)	95% Two-Sided Confidence Interval	NPA (%)	95% Two-Sided Confidence Interval
SARS-CoV-2	Nasopharyngeal Swab	Fresh	1246	86	3	1114	43	96.6%	90.6% - 98.9%	96.28%	95.0% - 97.2%
		Frozen	374	56	2	307	9	96.6%	88.3% - 99.1%	97.15%	94.7% - 98.5%
		Overall	1620	142	5 ^a	1421	52 ^b	96.6%	92.3% - 98.5%	96.47%	95.4% - 97.3%
	Anterior Nasal Swab	Fresh	1192	80	3	1068	41	96.4%	89.9% - 98.8%	96.3%	95.0% - 97.3%
		Frozen	349	41	3	296	9	93.2%	81.8% - 97.7%	97.05%	94.5% - 98.4%
		Overall	1541	121	6 ^c	1364	50 ^d	95.3%	90.1% - 97.8%	96.46%	95.4% - 97.3%
Flu A	Nasopharyngeal Swab	Fresh	1242	43	1	1191	7	97.7%	88.2% - 99.6%	99.42%	98.8% - 99.7%
		Frozen	374	13	2	356	3	86.7%	62.1% - 96.3%	99.16%	97.6% - 99.7%
		Overall	1616	56	3 ^e	1547	10 ^f	94.9%	86.1% - 98.3%	99.36%	98.8% - 99.7%
	Anterior Nasal Swab	Fresh	1191	39	0	1140	12	100.0%	91.0% - 100.0%	98.96%	98.2% - 99.4%
		Frozen	348	6	1	339	2	85.7%	48.7% - 97.4%	99.41%	97.9% - 99.8%
		Overall	1539	45	1 ^g	1479	14 ^h	97.8%	88.7% - 99.6%	99.06%	98.4% - 99.4%
Flu B	Nasopharyngeal Swab	Fresh	1246	31	1	1213	1	96.9%	84.3% - 99.5%	99.92%	99.5% - 100.0%
		Frozen	374	18	0	355	1	100.0%	82.4% - 100.0%	99.72%	98.4% - 100.0%
		Overall	1620	49	1 ⁱ	1568	2 ^j	98.0%	89.5% - 99.7%	99.87%	99.5% - 100.0%
	Anterior Nasal Swab	Fresh	1192	30	0	1160	2	100.0%	88.7% - 100.0%	99.83%	99.4% - 100.0%
		Frozen	349	17	0	332	0	100.0%	81.6% - 100.0%	100.0%	98.9% - 100.0%
		Overall	1541	47	0	1492	2 ^k	100.0%	92.4% - 100.0%	99.87%	99.5% - 100.0%
RSV	Nasopharyngeal Swab	Fresh	1246	25	1	1216	4	96.2%	81.1% - 99.3%	99.67%	99.2% - 99.9%
		Frozen	374	5	1	368	0	83.3%	43.7% - 97.0%	100.0%	99.0% - 100.0%
		Overall	1620	30	2 ^l	1584	4 ^m	93.8%	79.9% - 98.3%	99.75%	99.4% - 99.9%
	Anterior Nasal Swab	Fresh	1192	24	0	1163	5	100.0%	86.2% - 100.0%	99.57%	99.0% - 99.8%
		Frozen	349	5	0	343	1	100.0%	56.6% - 100.0%	99.71%	98.4% - 100.0%
		Overall	1541	29	0	1506	6 ⁿ	100.0%	88.3% - 100.0%	99.6%	99.1% - 99.8%

^a Discordant test results based on resolver testing: 3/5 SARS-CoV-2 Positive and 2/5 SARS-CoV-2 Negative

^b Discordant test results based on resolver testing: 38/52 SARS-CoV-2 Positive and 14/52 SARS-CoV-2 Negative

- ^c Discordant test results based on resolver testing: 5/6 SARS-CoV-2 Positive and 1/6 SARS-CoV-2 Negative
- ^d Discordant test results based on resolver testing: 37/50 SARS-CoV-2 Positive and 13/50 SARS-CoV-2 Negative
- ^e Discordant test results based on resolver testing: 1/3 Flu A Positive and 2/3 Flu A Negative
- ^f Discordant test results based on resolver testing: 3/10 Flu A Positive and 7/10 Flu A Negative
- ^g Discordant test results based on resolver testing: 1/1 Flu A Negative
- ^h Discordant test results based on resolver testing: 4/14 Flu A Positive and 10/14 Flu A Negative.
- ⁱ Discordant test results based on resolver testing: 1/1 Flu B Negative
- ^j Discordant test results based on resolver testing: 1/2 Flu B Positive and 1/2 Flu B Negative
- ^k Discordant test results based on resolver testing: 2/2 Flu B Positive
- ^l Discordant test results based on resolver testing: 2/2 RSV Negative
- ^m Discordant test results based on resolver testing: 4/4 RSV Negative
- ⁿ Discordant test results based on resolver testing: 1/6 RSV Positive and 5/6 RSV Negative

Enrichment Study

After one thousand (1,000) subjects were enrolled in the prospective study and the desired minimum number of SARS-CoV-2 positive samples were collected, an enrichment phase of the study was initiated to supplement the prospective data for Flu A, Flu B and RSV. Three (3) of the fourteen (14) collection sites enrolled subjects for the enrichment phase. Subjects who were experiencing signs and symptoms of respiratory viral infection with a positive PCR test result for Flu A, Flu B and/or RSV within three (3) days prior to enrollment were included in the study.

A total of sixty-nine (69) subjects were enrolled during the enrichment phase of this study, one AN and one NP swab was collected from each of the 69 subjects. One AN swab was excluded from the performance analysis due to a collection site protocol deviation. Sixty-eight (68) AN and 69 NP swabs were included in the performance analysis for the enriched sample cohort. Sixty (60) specimens were tested fresh with the TaqPath COVID-19, Flu A, Flu B, RSV Select Panel and 9 NP swabs and 8 AN swabs specimens were tested after freezing. No invalid results were observed in the enrichment phase for an invalid rate of 0.0% for both AN and NP specimens. The performance comparison of the TaqPath™ COVID-19, Flu A, Flu B, RSV Select Panel and the comparator test results are presented in **Table 20**.

Table 20: Agreement of the TaqPath™ COVID-19, Flu A, Flu B, RSV Select Panel with the Comparator (Enriched Sample Cohort)

Target	Swab Type	Specimen Collection	Number of Specimens	True Positive	False Negative	True Negative	False Positive	PPA (%)	95% Two-Sided Confidence Interval	NPA (%)	95% Two-Sided Confidence Interval
Flu A	Nasopharyngeal Swab	Fresh	60	45	1 ^a	14	0	97.8%	88.7% - 99.6%	100.0%	78.5% - 100.0%
		Frozen	9	2	0	7	0	100.0%	34.2% - 100.0%	100.0%	64.6% - 100.0%
		Overall	69	47	1	21	0	97.9%	89.1% - 99.6%	100.0%	84.5% - 100.0%
	Anterior Nasal Swab	Fresh	60	45	0	14	1	100.0%	92.1% - 100.0%	93.3%	70.2% - 98.8%
		Frozen	8	1	0	7	0	100.0%	20.7% - 100.0%	100.0%	64.6% - 100.0%
		Overall	68	46	0	21	1 ^b	100.0%	92.3% - 100.0%	95.5%	78.2% - 99.2%
Flu B	Nasopharyngeal Swab	Fresh	60	8	1	50	1	88.9%	56.5% - 98.0%	98.0%	89.7% - 99.7%
		Frozen	9	4	0	5	0	100.0%	51.0% - 100.0%	100.0%	56.6% - 100.0%
		Overall	69	12	1 ^c	55	1 ^d	92.3%	66.7% - 98.6%	98.2%	90.6% - 99.7%
	Anterior Nasal Swab	Fresh	60	8	1	51	0	88.9%	56.5% - 98.0%	100.0%	93.0% - 100.0%
		Frozen	8	4	0	4	0	100.0%	51.0% - 100.0%	100.0%	51.0% - 100.0%
		Overall	68	12	1 ^e	55	0	92.3%	66.7% - 98.6%	100.0%	93.5% - 100.0%
RSV	Nasopharyngeal Swab	Fresh	60	7	0	52	1	100.0%	64.6% - 100.0%	98.1%	90.1% - 99.7%
		Frozen	9	3	0	4	2	100.0%	43.9% - 100.0%	66.7%	30.0% - 90.3%
		Overall	69	10	0	56	3 ^f	100.0%	72.3% - 100.0%	94.9%	86.1% - 98.3%
	Anterior Nasal Swab	Fresh	60	7	0	52	1	100.0%	64.6% - 100.0%	98.1%	90.1% - 99.7%
		Frozen	8	3	0	4	1	100.0%	43.9% - 100.0%	80.0%	37.6% - 96.4%
		Overall	68	10	0	56	2 ^g	100.0%	72.3% - 100.0%	96.6%	88.3% - 99.1%

^a Discordant test results based on resolver testing: 1/1 Flu A Negative

^b Discordant test results based on resolver testing: 1/1 Flu A Negative

^c Discordant test results based on resolver testing: 1/1 Flu B Positive

^d Discordant test results based on resolver testing: 1/1 Flu B Negative

^e Discordant test results based on resolver testing: 1/1 Flu B Positive

^f Discordant test results based on resolver testing: 1/3 RSV Positive and 2/3 RSV Negative

^g Discordant test results based on resolver testing: 1/2 RSV Positive and 1/2 RSV Negative

Reference Ranges/Expected Values

The prospective cohort of the TaqPath™ COVID-19, Flu A, Flu B, RSV Select Panel clinical performance study tested a total of one thousand six hundred twenty (1,620) nasopharyngeal swabs (NP) and one thousand five hundred forty one (1,541) anterior nasal swabs (AN) after exclusions. Samples were collected from 14 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, Flu A, Flu B, RSV Select Panel) per collection site for the prospective samples is shown in **Table 21**.

Table 21: TaqPath™ COVID-19, Flu A, Flu B, RSV Select Panel – Expected Values by Specimen Collection Site for NP and AN Swab Specimens

Collection Site	SARS-CoV-2		Flu A		Flu B		RSV	
	NP	AN	NP	AN	NP	AN	NP	AN
Site 1	14.2% (23/162)	13.5% (20/148)	0.0% (0/162)	0.0% (0/148)	0.0% (0/162)	0.0% (0/148)	0.0% (0/162)	0.0% (0/148)
Site 2	6.7% (9/135)	9.5% (12/126)	0.0% (0/135)	0.0% (0/126)	0.0% (0/135)	0.0% (0/126)	0.7% (1/135)	0.8% (1/126)
Site 3	13.8% (49/356)	10.9% (38/348)	2.2% (8/356)	1.7% (6/348)	0.8% (3/356)	0.9% (3/348)	2.0% (7/356)	2.0% (7/348)
Site 4	11.0% (9/82)	18.2% (14/77)	0.0% (0/82)	0.0% (0/77)	0.0% (0/82)	0.0% (0/77)	1.2% (1/82)	1.3% (1/77)
Site 5	9.0% (17/188)	3.9% (7/178)	0.0% (0/188)	0.0% (0/178)	0.0% (0/188)	0.0% (0/178)	0.0% (0/188)	0.0% (0/178)
Site 6	7.1% (13/182)	6.4% (11/171)	2.7% (5/182)	2.3% (4/171)	0.0% (0/182)	0.0% (0/171)	0.5% (1/182)	0.6% (1/171)
Site 7	21.7% (10/46)	15.6% (7/45)	10.9% (5/46)	6.7% (3/45)	15.2% (7/46)	15.6% (7/45)	2.2% (1/46)	2.2% (1/45)
Site 8	8.3% (1/12)	8.3% (1/12)	0.0% (0/12)	0.0% (0/12)	0.0% (0/12)	0.0% (0/12)	0.0% (0/12)	0.0% (0/12)
Site 9	0.0% (0/10)	0.0% (0/7)	0.0% (0/10)	0.0% (0/7)	0.0% (0/10)	0.0% (0/7)	0.0% (0/10)	0.0% (0/7)
Site 10	13.9% (28/202)	14.7% (29/197)	7.9% (16/202)	8.1% (16/197)	12.4% (25/202)	12.7% (25/197)	6.4% (13/202)	5.6% (11/197)
Site 11	8.0% (6/75)	6.6% (5/76)	28.0% (21/75)	30.3% (23/76)	12.0% (9/75)	11.8% (9/76)	9.3% (7/75)	11.8% (9/76)
Site 12	18.8% (15/80)	18.8% (15/80)	1.3% (1/80)	2.5% (2/80)	1.3% (1/80)	0.0% (0/80)	1.3% (1/80)	0.0% (0/80)
Site 13	22.7% (5/22)	16.7% (3/18)	9.1% (2/22)	11.1% (2/18)	9.1% (2/22)	11.1% (2/18)	4.5% (1/22)	5.6% (1/18)
Site 14	13.2% (9/68)	15.5% (9/58)	14.7% (10/68)	8.6% (5/58)	5.9% (4/68)	5.2% (3/58)	1.5% (1/68)	5.2% (3/58)
All	12.0% (194/1620)	11.1% (171/1541)	4.2% (68/1620)	4.0% (61/1541)	3.1% (51/1620)	3.2% (49/1541)	2.1% (34/1620)	2.3% (35/1541)

Conclusion

The results of the analytical and clinical performance studies summarized above demonstrated that the TaqPath™ COVID-19, Flu A, Flu B, RSV Select Panel is substantially equivalent to the predicate device.