

TaqPath™ COVID-19, FluA, FluB Combo Kit

INSTRUCTIONS FOR USE

Multiplex real-time RT-PCR test for the detection and differentiation of SARS-CoV-2, influenza A, and influenza B RNA

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Revision D.0

IVD

For In Vitro Diagnostic Use. For Emergency Use Authorization Only | Rx
Only

ThermoFisher
S C I E N T I F I C



Revision history: MAN0019582 D.0 (English)

Revision	Date	Description
D.0	20 March 2023	<ul style="list-style-type: none"> • The statement for EUA was updated (“Warnings and precautions” on page 11). • The amount of MS2 Phage Control was increased to 11 (“Contents and storage” on page 9). • <i>In-silico</i> information was updated (“Reactivity (Inclusivity)” on page 53). • Compatible firmware and software versions were updated in the following sections: <ul style="list-style-type: none"> – “Product description” on page 8 – “Required materials not supplied” on page 9 – “Transfer the template (EDT) file for the QuantStudio™ 5 Real-Time PCR Instrument” on page 29 – “Software and instrument compatibility” on page 37 – “Obtain the software” on page 38 – “Install the assay panel” on page 39
C.0	9 March 2022	<ul style="list-style-type: none"> • Sample storage and transport statement updated in “Sample collection, transport, and storage” on page 15. • Clinical performance statement was updated in “Assay limitations” on page 13. • Specific instrument reference to QuantStudio™ 7 Flex Real-Time PCR Instrument added to “Prepare the RT-PCR reactions (384-well plates)” on page 23. • References to MicroAmp™ Optical Film Compression Pad added to “Required materials not supplied” on page 9. • Compression pad information added and spelling of HIPAA corrected in “Set up and run the QuantStudio™ 5 Real-Time PCR Instrument” on page 30. • Spelling of HIPAA corrected in “Set up and run the 7500 Fast Dx Real-Time PCR Instrument” on page 27 and “Set up and run the QuantStudio™ 7 Flex Real-Time PCR Instrument (384-well block)” on page 34. • A prospective clinical evaluation study added to Chapter 9, “Performance characteristics”. • Section for preparing RT-PCR reactions (384-well plates) added to “Prepare the RT-PCR reactions (384-well plates)” on page 23. • In-use stability information added to (“In-use stability” on page 21). • Section for performing RT-PCR using the Applied Biosystems™ QuantStudio™ 7 Flex Real-Time PCR Instrument (384-well block) added to (Chapter 6, “Perform RT-PCR using the Applied Biosystems™ QuantStudio™ 7 Flex Real-Time PCR Instrument (384-well block)”). • Software and instrument compatibility information added to (“Software and instrument compatibility” on page 37). • Assay panel information added to the following sections: <ul style="list-style-type: none"> – “Software and instrument compatibility” on page 37 – “Obtain the software” on page 38 – “Install the assay panel” on page 39 • The “Product description” on page 8 was updated to include the following information: <ul style="list-style-type: none"> – QuantStudio™ 7 Flex Real-Time PCR Instrument – Added a note that indicates the upgrade to Pathogen Interpretive Software is not required for the 7500 Fast Dx Real-Time PCR Instrument • The <i>in silico</i> analysis for SARS-CoV-2 (“Reactivity (Inclusivity)” on page 53) was updated. • Reference to the version of Pathogen Interpretive Software for the QuantStudio™ 5 Real-Time PCR Instrument was removed in (“Transfer the template (EDT) file for the QuantStudio™ 5 Real-Time PCR Instrument” on page 29). • Reference to the version of Pathogen Interpretive Software for the 7500 Fast Dx Real-Time PCR Instrument was removed in (“Transfer the template (SDT) file for the 7500 Fast Dx Real-Time PCR Instrument” on page 26).

Revision	Date	Description
C.0 (continued)	9 March 2022	<ul style="list-style-type: none"> Reference to the version of Pathogen Interpretive Software in the note regarding the Pathogen Interpretive Software being pre-installed on the 7500 Fast Dx Real-Time PCR Instrument and the QuantStudio™ 5 Real-Time PCR Instrument was removed in the following sections: <ul style="list-style-type: none"> “Obtain the software” on page 38 “Install the assay panel” on page 39
B.0	30 July 2021	<ul style="list-style-type: none"> Specified the versions for the Pathogen Interpretive Software (v1.1) and the SAE Administrator Console Dx (v1.0). Pathogen Interpretive Software is updated to v1.1. Updated the information about storing patient samples (“Samples and controls” on page 14). Specified that the Binding Bead Mix in the RNA extraction procedures is not compatible with bleach. Specified the steps to be performed on ice when preparing RT-PCR (“Prepare the RT-PCR reactions (96-well plates)” on page 21). Added information about HIPPA (Health Insurance Portability and Accountability Act) to the following sections: <ul style="list-style-type: none"> “Warnings and precautions” on page 11 “Samples and controls” on page 14 “Set up and run the 7500 Fast Dx Real-Time PCR Instrument” on page 27 “Set up and run the QuantStudio™ 5 Real-Time PCR Instrument” on page 30 Added information about the assay panel that is pre-installed in the Pathogen Interpretive Software Added a step to specify loading the plate into the instrument for the QuantStudio™ 5 Real-Time PCR Instrument. Updated the information in “Warnings and precautions” on page 11 and Chapter 8, “Conditions of authorization for labs”. Corrected the lineage of influenza B virus strain B/Wisconsin/1/2010 from Victoria lineage to Yamagata lineage in “Limit of detection (LoD)” on page 43. Corrected the name of the additional software information to Software Release Notes (“Customer and technical support” on page 73). Updated the instructions to obtain the software (“Obtain the software” on page 38). Added Appendix B, “EUO label for RUO instrument”. Added the <i>MagMAX™ Viral/Pathogen II Nucleic Acid Isolation Kit Instructions For Use</i> and the <i>SAE Administrator Console Dx User Guide</i> to the list of related documentation.
A.0	11 February 2021	New document for the TaqPath™ COVID-19, FluA, FluB Combo Kit.

The customer is responsible for compliance with regulatory requirements that pertain to their procedures and uses of the instrument.

The information in this guide is subject to change without notice.

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Intended Use

The TaqPath™ COVID-19, FluA, FluB Combo Kit is a multiplex real-time RT-PCR test intended for the simultaneous qualitative detection and differentiation of RNA from the SARS-CoV-2, influenza A, and/or influenza B viruses in nasopharyngeal swab and anterior nasal swab specimens collected from individuals suspected of respiratory viral infection consistent with COVID-19 by their healthcare provider. Clinical signs and symptoms of respiratory viral infection due to SARS-CoV-2 and influenza can be similar. Testing is limited to laboratories certified under the Clinical Laboratory Improvement Amendments of 1988 (CLIA), 42 U.S.C. §263a, that meet the requirements to perform high-complexity tests.

The TaqPath™ COVID-19, FluA, FluB Combo Kit is intended for use in simultaneous detection and differentiation of SARS-CoV-2, influenza A, and/or influenza B nucleic acid in clinical specimens and is not intended to detect influenza C virus. The SARS-CoV-2, influenza A, and influenza B RNA is generally detectable in upper respiratory samples during the acute phase of infection.

Positive results are indicative of active infection but do not rule out bacterial infection or co-infection with other pathogens not detected by the test. Clinical correlation with patient history and other diagnostic information is necessary to determine patient infection status. The agent detected may not be the definite cause of disease.

Laboratories within the United States and its territories are required to report all SARS-CoV-2 results to the appropriate public health authorities.

Negative results do not preclude SARS-CoV-2, influenza A, and/or influenza B infection and should not be used as the sole basis for treatment or other patient management decisions. Negative results must be combined with clinical observations, patient history, and/or epidemiological information.

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

The TaqPath™ COVID-19, FluA, FluB Combo Kit is only for use under the Food and Drug Administration's Emergency Use Authorization.

Product description

The TaqPath™ COVID-19, FluA, FluB Combo Kit includes the assays and controls for a multiplex real-time RT-PCR test for the qualitative detection and differentiation of RNA from SARS-CoV-2, influenza A, and influenza B viruses in nasopharyngeal swabs and anterior nasal swabs from individuals suspected of COVID-19 by their healthcare provider.

The kit includes the following components:

Note: The information that follows refers to systems using Pathogen Interpretive Software v1.1. The upgrade to Pathogen Interpretive Software v1.1 for the 7500 Fast Real-Time PCR Instrument is not required. For information on compatible analysis software and assay panel versions for these instruments, see “Software and instrument compatibility” on page 37.

- TaqPath™ RT-PCR COVID-19, FluA, FluB Kit
 - TaqPath™ COVID-19, FluA, FluB RT-PCR Assay Multiplex—Multiplexed assays that contain primer and probe sets specific to the following targets:
 - Two SARS-CoV-2 targets
 - One influenza A target
 - One influenza B target
 - Bacteriophage MS2
 - MS2 Phage Control—Internal process control for nucleic acid extraction
- TaqPath™ COVID-19, FluA, FluB Control—RNA control that contains targets specific to the SARS-CoV-2, influenza A, and influenza B genomic regions targeted by the assays

Results are analyzed using the following software:

- Applied Biosystems™ Pathogen Interpretive Software v1.1
- SAE Administrator Console Dx v1.0 (for security and audit functions)
- The appropriate assay panel for your instrument:
 - C19-FluAB_7500-FAST_1.1.0
 - C19-FluAB_QS5-9602_1.1.0
 - C19-FluAB_QS7F-384_1.0.0

Note: The assay panels for the 7500 Fast Real-Time PCR Instrument and the QuantStudio™ 5 Real-Time PCR Instrument are pre-installed in the Pathogen Interpretive Software.

Contents and storage

Table 1 TaqPath™ COVID-19, FluA, FluB Combo Kit, 1,000 reactions (Cat. No. A49868)

Component	Contents	Amount	Storage
TaqPath™ RT-PCR COVID-19, FluA, FluB Kit, 1,000 reactions	TaqPath™ COVID-19, FluA, FluB RT-PCR Assay Multiplex	1,500 µL	-30°C to -10°C
	MS2 Phage Control	11 × 1,000 µL	-30°C to -10°C
TaqPath™ COVID-19, FluA, FluB Control		10 × 10 µL	≤ -70°C
TaqPath™ Control Dilution Buffer		10 × 250 µL	-30°C to -10°C

Required materials not supplied

Unless otherwise indicated, all materials are available through [thermofisher.com](https://www.thermofisher.com). "MLS" indicates that the material is available from [fisherscientific.com](https://www.fisherscientific.com) or another major laboratory supplier.

Catalog numbers that appear as links open the web pages for those products.

Item	Source
Real-time PCR instrument (for firmware and software compatibility for each instrument, see “Software and instrument compatibility” on page 37)	
Applied Biosystems™ 7500 Fast Dx Real-Time PCR Instrument	4406984 (with laptop computer) 4406985 (with tower computer)
Applied Biosystems™ QuantStudio™ 5 Real-Time PCR Instrument, 96-well, 0.2-mL block	A28569 (with laptop computer) A28574 (with desktop computer) A28139 (instrument only)
Applied Biosystems™ QuantStudio™ 7 Flex Real-Time PCR Instrument, 384-well block	4485695 (with laptop computer) 4485701 (with desktop computer)
Software	
Pathogen Interpretive Software v1.1	See “Obtain the software” on page 38
SAE Administrator Console Dx v1.0	
Equipment	
Laboratory freezers <ul style="list-style-type: none"> -30°C to -10°C ≤ -70°C 	MLS
Centrifuge, with a rotor that accommodates standard and deepwell microplates	MLS

(continued)

Item	Source
Microcentrifuge	MLS
Laboratory mixer, vortex or equivalent	MLS
Single and multichannel adjustable pipettors (1.00 µL to 1,000.0 µL)	MLS
Cold block (96-well or 384-well) or ice	MLS
Automated nucleic acid extraction system and materials	
KingFisher™ Flex Magnetic Particle Processor with 96 Deep-Well Head	5400630
KingFisher™ Flex 96 Deep-Well Heating Block	24075430
KingFisher™ 96 Deep-Well Plate	95040450 , A48305 , A48424 , 95040455
96-well plate for the tip comb, one of the following: <ul style="list-style-type: none"> KingFisher™ 96 KF microplate Tip Comb Presenting Plate for KF 96 Nunc™ MicroWell™ 96-Well Microplate, Flat Bottom Nunc™ MicroWell™ 96-Well Microplate, barcoded ABgene™ 96-Well Polypropylene Storage Microplate ABgene™ 96-Well 1.2-mL Polypropylene Deepwell Storage Plate Nunc™ F96 MicroWell™ Black Polystyrene Plate Nunc™ F96 MicroWell™ White Polystyrene Plate KingFisher™ 96 Deep-Well Plate 	<ul style="list-style-type: none"> 97002540 267600 167008 269787 AB0796 AB1127 137101 136101 95040450, A48305, A48424, 95040455
KingFisher™ 96 tip comb for DW magnets	97002534 , A48438 , A48414
Kits and reagents	
MagMAX™ Viral/Pathogen II Nucleic Acid Isolation Kit (up to 2,000 preparations, when 200 µL of sample is used)	A48383
TaqPath™ 1-Step Multiplex Master Mix (No ROX™)	A28521 , A28522 , A28523
Fisher BioReagents™ Ethanol, Absolute, Molecular Biology Grade ^[1] , or equivalent	BP2818100 , BP2818500 , BP28184
Nuclease-free Water (not DEPC-Treated)	MLS
Calibration plates (7500 Real-Time PCR Instrument series)	
ABY™ Dye Spectral Calibration Plate for Multiplex qPCR, Fast 96-well (0.1-mL)	A24734
JUN™ Dye Spectral Calibration Plate for Multiplex qPCR, Fast 96-well (0.1-mL)	A24735

(continued)

Item	Source
Calibration plates (QuantStudio™ 7 Flex Real-Time PCR Instrument)	
ABY™ Dye Spectral Calibration Plate for Multiplex qPCR, 384-well	A24736
JUN™ Dye Spectral Calibration Plate for Multiplex qPCR, 384-well	A24733
Tubes, plates, and other consumables	
MicroAmp™ Fast Optical 96-Well Reaction Plate with Barcode, 0.1 mL	4346906 , 4366932
MicroAmp™ Fast Optical 96-Well Reaction Plate, 0.1 mL	4346907
MicroAmp™ Optical 96-Well Reaction Plate with Barcode, 0.2 mL	4306737 , 4326659
MicroAmp™ Optical 96-Well Reaction Plate, 0.2 mL	N8010560 , 4316813
MicroAmp™ Optical 384-Well Reaction Plate with Barcode	4309849 , 4326270 , 4343814
MicroAmp™ Optical 384-Well Reaction Plate	4343370
MicroAmp™ Clear Adhesive Film	4306311
MicroAmp™ Optical Adhesive Film	4311971 , 4360954
MicroAmp™ Adhesive Film Applicator	4333183
MicroAmp™ Optical Film Compression Pad ^[2]	4312639
Nonstick, RNase-free microcentrifuge tubes (1.5 mL and 2.0 mL)	thermofisher.com/plastics
Sterile aerosol barrier (filtered) pipette tips	thermofisher.com/pipettetips

^[1] Available at fisherscientific.com.

^[2] Required for QuantStudio™ 5 Real-Time PCR Instrument (96-well, 0.2-mL block) and a MicroAmp™ Optical 96-Well Reaction Plate (with or without barcode).

Warnings and precautions

The TaqPath™ COVID-19, FluA, FluB Combo Kit workflow should be performed by qualified and trained staff to avoid the risk of erroneous results. Use separate areas for the preparation of patient samples and controls to prevent false positive results. Samples and reagents must be handled in a biological safety cabinet.

- For *in vitro* diagnostic use.
- For use under Emergency Use Authorization (EUA) only.
- For Prescription Use Only.
- This product has not been FDA cleared or approved but has been authorized for emergency use by FDA under an EUA for use by authorized laboratories.
- This product has been authorized only for the detection of nucleic acid from SARS-CoV-2, influenza A, and influenza B, not for any other viruses or pathogens.

- The emergency use of this product is only authorized for the duration of the declaration that circumstances exist justifying the authorization of emergency use of *in vitro* diagnostics for detection and/or diagnosis of COVID-19 under Section 564(b)(1) of the Federal Food, Drug and Cosmetic Act, 21 U.S.C. § 360bbb-3(b)(1), unless the declaration is terminated or authorization is revoked sooner.
- Samples and controls should always be treated as if infectious and/or biohazardous in accordance with safe laboratory procedures.
- Follow necessary precautions when handling specimens. Use personal protective equipment (PPE) consistent with current guidelines for the handling of potentially infectious samples.
- Always use pipette tips with aerosol barriers. Tips that are used must be sterile and free from DNases and RNases.
- Do not eat, drink, smoke, or apply cosmetic products in the work areas.
- Modifications to assay reagents, assay protocol, or instrumentation are not permitted, and are in violation of the product Emergency Use Authorization.
- Each compression pad may be used up to 20 times before discarding. Do not use more than 20 times.
- Reagents must be stored and handled as specified in Table 1 on page 9.
- Do not use the kit after the indicated expiry date.
- Dispose of waste in compliance with local, state, and federal regulations.
- Safety Data Sheets are available upon request.
- Laboratories within the United States and its territories are required to report all SARS-CoV-2 results to the appropriate public health authorities.
- Positive results are indicative of the presence of SARS-CoV-2 RNA, influenza A, and/or influenza B.
- Laboratories shall encrypt, pseudonymize, and anonymize personal data where possible following the requirements of HIPAA (Health Insurance Portability and Accountability Act).

Assay limitations

- The TaqPath™ COVID-19, FluA, FluB Combo Kit performance was established using nasopharyngeal swabs. Anterior nasal swabs are considered an acceptable specimen type for use with the TaqPath™ COVID-19, FluA, FluB Combo Kit, but performance with this specimen type has not been established. Refer to FDA's *FAQs on Diagnostic Testing for SARS-CoV-2* for additional information. Specimen types other than nasopharyngeal and anterior nasal swabs should not be tested with this assay.
- Influenza A and influenza B positive specimens were validated by testing archived specimens only. No influenza A or influenza B positive anterior nasal swabs or nasopharyngeal swab specimens were procured during the prospective clinical study. If an influenza A or influenza B result is inconsistent with clinical presentation and/or other clinical and epidemiological information, FDA-cleared influenza NAATs are available for confirmation if clinically indicated.
- The performance of this test was established based on the evaluation of a limited number of clinical specimens. Clinical performance has not been established with all circulating variants but is anticipated to be reflective of the prevalent variants in circulation at the time and location of the clinical evaluation. Performance at the time of testing may vary depending on the variants circulating, including newly emerging strains of SARS-CoV-2 and their prevalence, which change over time.
- Samples must be collected, transported, and stored using appropriate procedures and conditions. Improper collection, transport, or storage of specimens may hinder the ability of the assay to detect the target sequences.
- Extraction and amplification of nucleic acid from clinical samples must be performed according to the specified methods listed in this procedure. Other extraction approaches and processing systems have not been evaluated.
- FluMist™ was not tested as a potential interferent. It is anticipated that a live virus vaccine such as FluMist™ may produce a positive result for influenza A.
- Afrin™ nasal spray causes inhibition at 10% v/v. False negative results may occur if samples contain Afrin™ nasal spray.
- False-negative results may arise from:
 - Improper sample collection
 - Degradation of the SARS-CoV-2 RNA, influenza A RNA, or influenza B RNA during shipping/storage
 - Specimen collection after SARS-CoV-2 RNA, influenza A RNA, or influenza B RNA can no longer be found in the specimen matrix
 - Using unauthorized extraction or assay reagents
 - The presence of RNA extraction inhibitors or RT-PCR inhibitors
 - Mutation in the SARS-CoV-2 virus, the influenza A virus, or the influenza B virus
 - Improper vortexing and centrifuging when preparing RT-PCR reactions
 - Failure to follow instructions for use

- False-positive results may arise from:
 - Cross contamination during specimen handling or preparation
 - Cross contamination between patient samples
 - Specimen mix-up
 - RNA contamination during product handling
 - Improper sealing of the PCR plate
- The impacts of vaccines, antiviral therapeutics, antibiotics, chemotherapeutic or immunosuppressant drugs have not been evaluated. The TaqPath™ COVID-19, FluA, FluB Combo Kit cannot rule out diseases caused by other bacterial or viral pathogens.
- Negative results do not preclude infection with SARS-CoV-2 virus, influenza A virus, or influenza B virus, and should not be the sole basis of a patient management decision.

General laboratory recommendations

- Implement standard operating procedures in your laboratory to prevent contamination, such as the following:
 - Frequent glove changes
 - Frequent decontamination of surfaces, equipment, and pipettes with 10% bleach or decontamination solution, followed by 70% ethanol
 - Use of ultraviolet light during biosafety cabinet decontamination (when available)
- To prevent degradation, keep eluted sample RNA, master mixes, assays, and controls on ice or in cold blocks while in use.
- Limit freeze-thaw cycles.
- Aliquot reagents to prevent stock contamination and reduce the number of freeze-thaw cycles.
- After each run, review the amplification curves in the interpretive software for signs of inadequate vortexing or centrifugation. Contact your Applications Support team for additional information or training on data QC in your instrument software.

Samples and controls

Note: Handle all samples and controls as if they are capable of transmitting infectious agents.

Patient samples must be collected according to appropriate laboratory guidelines. Positive and negative test controls must be included to accurately interpret patient test results.

IMPORTANT! It is the responsibility of laboratories to encrypt, pseudonymize, and anonymize personal data where possible following the requirements of HIPAA (Health Insurance Portability and Accountability Act).

Include the following controls:

Control	Used to monitor	Assays
Positive Control (TaqPath™ COVID-19, FluA, FluB Control)	RT-PCR reaction setup and reagent integrity	Two SARS-CoV-2 assays, one influenza A assay, and one influenza B assay
MS2 Phage Control	RNA extraction	MS2 assay
Negative Control	Cross-contamination during RNA extraction and reaction setup	Two SARS-CoV-2 assays, one influenza A assay, and one influenza B assay
		MS2 assay

Sample collection, transport, and storage

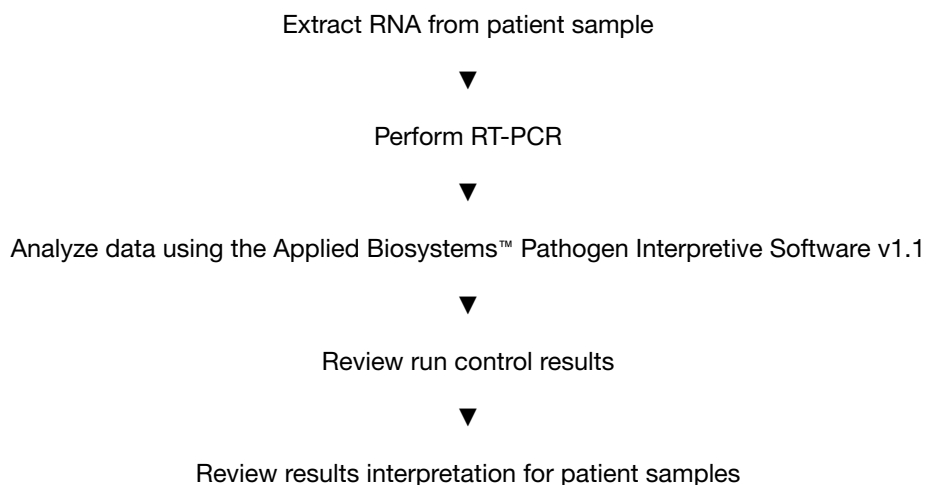
Note: Handle all samples and controls as if they are capable of transmitting infectious agents.

- Refer to the *Interim Guidelines for Collecting, Handling, and Testing Clinical Specimens from Persons for Coronavirus Disease 2019 (COVID-19)*, published by the Centers for Disease Control and Prevention, for specimen collection guidelines.
- Specimens must be packaged, shipped, and transported according to the current edition of the *International Air Transport Association (IATA) Dangerous Goods Regulations* ([iata.org/en/programs/cargo/dgr](https://www.iata.org/en/programs/cargo/dgr)).

Transport specimen stability for patient samples collected in Viral Transport Media (VTM) has been demonstrated at 15–30°C for up to 72 hours.

Store patient samples collected in VTM at 2–8°C for up to 72 hours.

Workflow



The workflow begins with nucleic acid extraction from nasopharyngeal or anterior nasal swabs that arrive at the testing site in transport media. Nucleic acids are isolated and purified from the specimens using the MagMAX™ Viral/Pathogen II Nucleic Acid Isolation Kit. Nucleic acid isolation is performed via an automated process using the KingFisher™ Flex Purification System (KingFisher). For more information about using the kit, see “Related documentation” on page 73.

The purified nucleic acid is reverse transcribed into cDNA and amplified using the TaqPath™ COVID-19, FluA, FluB Combo Kit and one of the following real-time PCR instruments:

- Applied Biosystems™ 7500 Fast Dx Real-Time PCR Instrument
- Applied Biosystems™ QuantStudio™ 5 Real-Time PCR Instrument, 96-well, 0.2-mL block
- Applied Biosystems™ QuantStudio™ 7 Flex Real-Time PCR Instrument, 384-well block

In the process, the probes anneal to the following target sequences located between unique forward and reverse primers:

- SARS-CoV-2 (N Gene)
- SARS-Cov-2 (S Gene)
- Influenza A
- Influenza B

Note: The targets for SARS-CoV-2 N Gene and the SARS-CoV-2 S Gene are in a single optical channel. They cannot be differentiated.

During the extension phase of the PCR cycle, the 5' nuclease activity of Taq polymerase degrades the probe, causing the reporter dye to separate from the quencher dye, generating a fluorescent signal. With each cycle, additional reporter dye molecules are cleaved from their respective probes, increasing the fluorescence intensity. Fluorescence intensity is monitored at each PCR cycle by the real-time PCR instrument.

The data are analyzed, then interpreted by the Applied Biosystems™ Pathogen Interpretive Software v1.1.

2

Extract RNA

RNA extraction is performed using the KingFisher™ Flex Magnetic Particle Processor with 96 Deep-Well Head and the MagMAX™ Viral/Pathogen II Nucleic Acid Isolation Kit with a sample input volume of 400 µL.

Before you begin

IMPORTANT! The Binding Bead Mix is not compatible with bleach. For more information, see the SDS.

Note: During the wash steps, the Wash Solution may develop inert white or brown particulates that float in solution. This is not a cause for concern and does not negatively affect performance.

- Determine the number of required reactions based on the number of patient samples to be processed, plus one Negative Control per plate.
- Prepare fresh 80% Ethanol using Ethanol, Absolute, Molecular Biology Grade and Nuclease-free Water (not DEPC-Treated) for the required number of reactions, sufficient for 1 mL per reaction, plus 10% overage.
- Label the short side of each KingFisher™ 96 Deep-Well Plate (4):

Label	Number of plates
Sample plate	1
Wash 1	1
Wash 2	1
Elution plate	1

- Label the short side of the KingFisher™ 96 KF microplate (1):

Label	Number of plates
Tip comb	1

Note: The following items can be used to hold the tip comb instead of the KingFisher™ 96 KF microplate:

- Tip Comb Presenting Plate for KF 96
- Nunc™ MicroWell™ 96-Well Microplate, Flat Bottom
- Nunc™ MicroWell™ 96-Well Microplate, barcoded
- ABgene™ 96-Well Polypropylene Storage Microplate
- ABgene™ 96-Well 1.2-mL Polypropylene Deepwell Storage Plate

- Nunc™ F96 MicroWell™ Black Polystyrene Plate
 - Nunc™ F96 MicroWell™ White Polystyrene Plate
 - KingFisher™ 96 Deep-Well Plate
-
- Mark the Negative Control well on the plate.

Extract RNA

Set up the instrument

1. Ensure that the KingFisher™ Flex Magnetic Particle Processor with 96 Deep-Well Head is set up with the KingFisher™ Flex 96 Deep-Well Heating Block.

IMPORTANT! Failure to use the proper magnetic head and heat block results in lower yields and potential harm to the instrument.

2. Ensure that the **MVP_2Wash_400_Flex** program has been downloaded from the MagMAX™ Viral/Pathogen II Nucleic Acid Isolation Kit product page at www.thermofisher.com and loaded onto the instrument.

Prepare the processing plates

Prepare the processing plates according to the following table. Cover the plates with a temporary seal (such as MicroAmp™ Clear Adhesive Film), then store at room temperature for up to 1 hour while you set up the sample plate.

Plate ID	Plate position	Plate type	Reagent	Volume per well
Wash 1 Plate	2	KingFisher™ 96 Deep-Well Plate	Wash Solution	1,000 µL
Wash 2 Plate	3		80% Ethanol	1,000 µL
Elution Plate	4		Elution Solution	50 µL
Tip Comb Plate	5	Place a KingFisher™ 96 tip comb for DW magnets in a KingFisher™ 96 KF microplate		

Note: The following items can be used to hold the tip comb instead of the KingFisher™ 96 KF microplate:

- Tip Comb Presenting Plate for KF 96
- Nunc™ MicroWell™ 96-Well Microplate, Flat Bottom
- Nunc™ MicroWell™ 96-Well Microplate, barcoded
- ABgene™ 96-Well Polypropylene Storage Microplate
- ABgene™ 96-Well 1.2-mL Polypropylene Deepwell Storage Plate
- Nunc™ F96 MicroWell™ Black Polystyrene Plate

- Nunc™ F96 MicroWell™ White Polystyrene Plate
- KingFisher™ 96 Deep-Well Plate

Prepare Binding Bead Mix

Prepare the required amount of Binding Bead Mix on each day of use.

1. Vortex the Total Nucleic Acid Magnetic Beads to ensure that the bead mixture is homogeneous.
2. For the number of required reactions, prepare the Binding Bead Mix according to the following table:

Component	Volume per well ^[1]
Binding Solution	530 µL
Total Nucleic Acid Magnetic Beads	20 µL
Total volume per well	550 µL

^[1] Include 10% overage when making the Binding Bead Mix for use with multiple reactions.

3. Mix well by inversion, then store at room temperature.

Prepare a Proteinase K and MS2 Phage Control Mix

Prepare the required amount of the Proteinase K and MS2 Phage Control Mix on each day of use. Keep on ice.

1. Thaw the vial of MS2 Phage Control.
2. For the number of required reactions, prepare the Proteinase K and MS2 Phage Control Mix according to the following table:

Component	Volume per well ^[1]	Volume per 96-well plate
Proteinase K	10 µL	1,056 µL
MS2 Phage Control	10 µL	1,056 µL
Total volume per well	20 µL	2,112 µL

^[1] Include 10% overage when preparing the Proteinase K and MS2 Phage Control Mix for use with multiple reactions.

3. Mix well by inversion, then store on ice.

Prepare sample plate

1. Invert the Binding Bead Mix 5 times gently to mix, then add 550 µL to each sample well and the Negative Control well in the Sample Plate.

Note: Remix the Binding Bead Mix by inversion frequently during pipetting to ensure even distribution of beads to all samples or wells. The Binding Bead Mix is viscous, so pipet slowly to ensure that the correct amount is added. DO NOT reuse pipette tips to add Binding Bead Mix to the samples, as the high viscosity will cause variations in the volumes added.

2. Add 400 μ L of sample to each sample well.
3. Add 400 μ L of Nuclease-free Water (not DEPC-Treated) to the Negative Control well.
4. Add 20 μ L of the Proteinase K and MS2 Phage Control Mix to each well in the KingFisher™ 96 Deep-Well Plate labeled "Sample Plate", including the Negative Control well.

Process the samples

1. Select the **MVP_2Wash_400_Flex** on the KingFisher™ Flex Magnetic Particle Processor with 96 Deep-Well Head.
2. Start the run, then load the prepared plates into position when prompted by the instrument.
3. After the run is complete (~24 minutes after start), immediately remove the Elution Plate from the instrument, then cover the plate with MicroAmp™ Clear Adhesive Film.

IMPORTANT! To prevent evaporation, seal the plate containing the eluate immediately.

The samples are eluted in 50 μ L of Elution Solution (see "Prepare the processing plates" on page 18).

Note: Significant bead carry over may adversely impact RT-PCR performance.

Place the Elution Plate on ice for immediate use in real-time RT-PCR.

3

Prepare RT-PCR reactions

Guidelines for RT-PCR

IMPORTANT!

- Prepare the run plate on ice and keep it on ice until it is loaded into the real-time PCR instrument.
 - Run the plate immediately after preparation. Failure to do so could result in degraded RNA samples.
 - To prevent contamination, prepare reagents in a PCR workstation or equivalent amplicon-free area. Do not use the same pipette for controls and RNA samples, and always use aerosol barrier pipette tips.
 - Maintain an RNase-free environment.
 - Protect assays from light.
 - Keep RNA samples and components on ice during use.
 - For each RT-PCR plate, include the following controls:
 - One Positive Control
 - One Negative Control from each extraction run.
-

In-use stability

The TaqPath™ COVID-19, FluA, FluB RT-PCR Assay Multiplex is stable for up to 12 freeze-thaw cycles at –30°C to –10°C when used with the TaqPath™ 1-Step Multiplex Master Mix (No ROX).

The TaqPath™ COVID-19, FluA, FluB Control is stable for 72 hours at 2–8°C after it has been diluted with the TaqPath™ Control Dilution Buffer as specified in “Prepare the RT-PCR reactions (96-well plates)” on page 21 and “Prepare the RT-PCR reactions (384-well plates)” on page 23.

Prepare the RT-PCR reactions (96-well plates)

1. If frozen, thaw the reagents on ice.
2. Gently vortex the reagents, then centrifuge briefly to collect liquid at the bottom of the tube.
3. Dilute TaqPath™ COVID-19, FluA, FluB Control to a working stock on ice:
 - a. Pipet 98.0 µL of TaqPath™ Control Dilution Buffer into a microcentrifuge tube, then add 2.0 µL of TaqPath™ COVID-19, FluA, FluB Control. Mix well, then centrifuge briefly.
 - b. Pipet 98.0 µL of TaqPath™ Control Dilution Buffer into a second microcentrifuge tube, then add 2.0 µL of the dilution created in substep 3a. Mix well, then centrifuge briefly.

Note: The TaqPath™ COVID-19, FluA, FluB Control does not contain the MS2 template.

4. Prepare the Reaction Mix on ice:
 - a. For each run, combine the following components sufficient for the number of RNA samples to be tested plus one Positive Control and one Negative Control.

All volumes include 10% overage for pipette error.

Component	Volume per RNA Sample or Control	Volume for n RNA Samples plus 2 Controls	Volume for 94 RNA Samples plus 2 Controls
TaqPath™ 1-Step Multiplex Master Mix (No ROX™) (4X)	6.25 μ L	$6.875 \times (n + 2)$ μ L	660 μ L
TaqPath™ COVID-19, FluA, FluB RT-PCR Assay Multiplex	1.25 μ L	$1.375 \times (n + 2)$ μ L	132 μ L
Total Reaction Mix volume	7.5 μL	—	792 μL

5. Set up the reaction plate on ice:
 - a. Pipet 7.5 μ L of the Reaction Mix prepared in step 4 into each well of a MicroAmp™ Fast Optical 96-Well Reaction Plate with Barcode, 0.1 mL or a MicroAmp™ Optical 96-Well Reaction Plate with Barcode, 0.2 mL.
Plates without a barcode can be used (see “Required materials not supplied” on page 9).

Note: Use the 0.1-mL plates for the 7500 Fast Dx Real-Time PCR Instrument. Use the 0.2-mL plates for the QuantStudio™ 5 Real-Time PCR Instrument (0.2-mL block).

 - b. Gently vortex the sealed plate containing the purified sample RNA and Negative Control from the RNA extraction procedure, then centrifuge briefly to collect liquid at the bottom of the plate.
 - c. Unseal the plate containing the purified sample RNA and Negative Control from the RNA extraction procedure. Add either sample RNA, Negative Control, or Positive Control to each well of the reaction plate according to Table 2 on page 23.
 - d. Seal the plate thoroughly with MicroAmp™ Optical Adhesive Film.

IMPORTANT!

- Use ONLY MicroAmp™ Optical Adhesive Film (Cat. No. [4311971](#), [4360954](#)).
 - Do NOT use MicroAmp™ Clear Adhesive Film (Cat. No. [4306311](#)) or any other film or sealing methods.
 - Do NOT heat seal the plates.
 - When applying the MicroAmp™ Optical Adhesive Film, ensure that pressure is applied across the entire plate and that there is a tight seal across every individual well. Failure to do so runs the risk of an improperly sealed well, leading to potential well-to-well contamination during vortexing and evaporation during PCR.
-

- e. Vortex the plate at the highest setting speed for 10–30 seconds with medium pressure. Move the plate around to ensure equal contact on the vortex mixer platform.

IMPORTANT! Vortex for 10–30 seconds to ensure proper mixing. Failure to do so might result in false classification of samples.

- f. Centrifuge the reaction plate for 1–2 minutes at $\geq 650 \times g$ (≥ 650 RCF) to remove bubbles and to collect the liquid at the bottom of the reaction plate.

IMPORTANT! Failure to do so can produce inaccurate results.

Table 2 Reaction plate

Component	Volume per reaction		
	RNA Sample reaction	Positive Control reaction	Negative Control reaction
Reaction Mix	7.5 μ L	7.5 μ L	7.5 μ L
Purified sample RNA (from RNA extraction)	17.5 μ L	—	—
Positive Control (diluted TaqPath™ COVID-19, FluA, FluB Control from step 3)	—	2.0 μ L	—
Purified Negative Control (from RNA extraction)	—	—	17.5 μ L
Nuclease-free water	—	15.5 μ L	—
Total volume	25.0 μL	25.0 μL	25.0 μL

Prepare the RT-PCR reactions (384-well plates)

Use this procedure for the QuantStudio™ 7 Flex Real-Time PCR Instrument with 384-well RT-PCR plates.

1. If frozen, thaw the reagents on ice.
2. Gently vortex the reagents, then centrifuge briefly to collect liquid at the bottom of the tube.
3. Dilute TaqPath™ COVID-19, FluA, FluB Control to a working stock on ice:
 - a. Pipet 98.0 μ L of TaqPath™ Control Dilution Buffer into a microcentrifuge tube, then add 2.0 μ L of TaqPath™ COVID-19, FluA, FluB Control. Mix well, then centrifuge briefly.
 - b. Pipet 98.0 μ L of TaqPath™ Control Dilution Buffer into a second microcentrifuge tube, then add 2.0 μ L of the dilution created in substep 3a. Mix well, then centrifuge briefly.

Note: The TaqPath™ COVID-19, FluA, FluB Control does not contain the MS2 template.

4. Prepare the Reaction Mix on ice:

- a. For each run, combine the following components sufficient for the number of RNA samples to be tested plus one Positive Control and one Negative Control.

All volumes include 10% overage for pipette error.

Component	Volume per RNA Sample or Control	Volume for n RNA Samples plus y Negative Controls plus 1 Positive Control	Volume for 379 RNA Samples plus 4 Negative Controls plus 1 Positive Control
TaqPath™ 1-Step Multiplex Master Mix (No ROX™) (4X)	5.0 µL	$5.5 \times (n + y + 1)$ µL	2,112.0 µL
TaqPath™ COVID-19, FluA, FluB RT-PCR Assay Multiplex	1.0 µL	$1.1 \times (n + y + 1)$ µL	422.4 µL
Total Reaction Mix volume	6.0 µL	—	2,534.4 µL

5. Set up the reaction plate on ice:

- a. Pipet 6.0 µL of the Reaction Mix prepared in step 4 into each well of a MicroAmp™ Optical 384-Well Reaction Plate with Barcode.
Plates without a barcode can be used (see “Required materials not supplied” on page 9).
- b. Gently vortex the sealed plate containing the purified sample RNA and Negative Control from the RNA extraction procedure, then centrifuge briefly to collect liquid at the bottom of the plate.
- c. Unseal the plate containing the purified sample RNA and Negative Control from the RNA extraction procedure. Add either sample RNA, Negative Control, or Positive Control to each well of the reaction plate according to Table 3.
- d. Seal the plate thoroughly with MicroAmp™ Optical Adhesive Film.

IMPORTANT!

- Use ONLY MicroAmp™ Optical Adhesive Film (Cat. No. [4311971](#), [4360954](#)).
- Do NOT use MicroAmp™ Clear Adhesive Film (Cat. No. [4306311](#)) or any other film or sealing methods.
- Do NOT heat seal the plates.
- When applying the MicroAmp™ Optical Adhesive Film, ensure that pressure is applied across the entire plate and that there is a tight seal across every individual well. Failure to do so runs the risk of an improperly sealed well, leading to potential well-to-well contamination during vortexing and evaporation during PCR.

- e. Vortex the plate at the highest setting speed for 10–30 seconds with medium pressure. Move the plate around to ensure equal contact on the vortex mixer platform.

IMPORTANT! Vortex for 10–30 seconds to ensure proper mixing. Failure to do so might result in false classification of samples.

- f. Centrifuge the reaction plate for 1–2 minutes at $\geq 650 \times g$ (≥ 650 RCF) to remove bubbles and to collect the liquid at the bottom of the reaction plate.

IMPORTANT! Failure to do so can produce inaccurate results.

Table 3 Reaction plate volumes

Component	Volume per reaction		
	RNA Sample reaction	Positive Control reaction	Negative Control reaction
Reaction Mix	6.0 μ L	6.0 μ L	6.0 μ L
Purified sample RNA (from RNA extraction)	14.0 μ L	—	—
Positive Control (diluted TaqPath™ COVID-19, FluA, FluB Control from step 3)	—	2.0 μ L	—
Purified Negative Control (from RNA extraction)	—	—	14.0 μ L
Nuclease-free water	—	12.0 μ L	—
Total volume	20.0 μL	20.0 μL	20.0 μL



Perform RT-PCR using the Applied Biosystems™ 7500 Fast Dx Real-Time PCR Instrument

- Dye calibration for the 7500 Real-Time PCR Instrument series 26
- Transfer the template (SDT) file for the 7500 Fast Dx Real-Time PCR Instrument 26
- Set up and run the 7500 Fast Dx Real-Time PCR Instrument 27

Dye calibration for the 7500 Real-Time PCR Instrument series

A maintained instrument will be calibrated for many dyes. In addition to those dyes, the instrument operator must calibrate the instrument for ABY™ dye and JUN™ dye that are used with this kit. For all other assays, refer to the standard calibration process.

Transfer the template (SDT) file for the 7500 Fast Dx Real-Time PCR Instrument

The template (SDT) file contains the settings for the instrument run.

It is installed on the computer with the Applied Biosystems™ Pathogen Interpretive Software.

The template file must be transferred via a USB drive or other method to the computer on which SDS Software v1.4.1 is installed.

IMPORTANT! Be careful to select the appropriate template file for your instrument. Failure to do so can cause errors in the analysis.

1. In the Pathogen Interpretive Software, click **Help ▶ Run Templates**.
2. Click **Download** to access the template file for your version of the data collection software.
3. In the **Save** dialog box, select the location to save the template file, then save the file.

4. Ensure that the correct template file was downloaded and saved.

TaqPath COVID-19 FluA FluB template 7500fast Dx sds1_4_1 v1_0.sdt

5. Transfer the SDT file to the computer with SDS Software v1.4.1, using a USB drive or other method.

Set up and run the 7500 Fast Dx Real-Time PCR Instrument

For more information about the instrument, see the documents listed in “Related documentation” on page 73.

1. Using SDS Software v1.4.1, open the SDT file that you transferred in “Transfer the template (SDT) file for the 7500 Fast Dx Real-Time PCR Instrument” on page 26.

IMPORTANT! Be careful to select the appropriate template file for your instrument and software version. Failure to do so can cause errors in the analysis.

2. Confirm the run settings in the template and adjust as necessary.

- **Assay: Standard Curve (Absolute Quantitation)**
- **Run mode: Standard 7500**
- **Passive reference: None**
- **Sample volume: 25 µL**

IMPORTANT! The passive reference must be set to **None**.

3. Confirm that the reporter dye and the detector pairs are correct in the **Detector Manager** in the **Tools** menu.

Reporter dye	Detector
FAM	FluA
VIC	C19 ^[1]
ABY	FluB
JUN	MS2

^[1] SARS-CoV-2 N gene and SARS-CoV-2 S gene

IMPORTANT! The detectors are case-sensitive.

4. Confirm that the targets above are assigned to each well in the plate layout.

5. Confirm the labeling of the control wells.

- The template has one positive control (PC) and one negative control (NC) assigned to wells for reference.
- The positive control must be named **PC**. If additional characters are included, it must be named **PC<>**, where <> is defined by the user, for example **PC1**.
- The negative control must be named **NC**. If additional characters are included, it must be named **NC<>**, where <> is defined by the user, for example **NC1**.
- Move the control well assignments by copying the existing control wells and pasting them according to their location on the physical plate.

IMPORTANT! The positive and negative controls must be named as described.

6. For wells with a positive control, confirm that **Task** is set to **Standard**.

7. For wells with a negative control, confirm that **Task** is set to **NTC**.

8. Edit the plate layout to assign a unique sample name to each well in the physical plate.

For wells with a patient sample, confirm that **Task** is set to **Unknown** for all detectors.

Note: Wells that do not have a sample name will not be analyzed by the software.

IMPORTANT! Encrypt, pseudonymize, or anonymize personal data where possible following the requirements of HIPAA (Health Insurance Portability and Accountability Act).

9. Confirm the thermal protocol.

Step	Temperature	Time	Number of cycles
UNG incubation	25°C	2 minutes	1
Reverse transcription	53°C	10 minutes	1
Preincubation	85°C	10 minutes	1
Activation	95°C	2 minutes	1
Denaturation	95°C	3 seconds	46
Anneal / extension	60°C	30 seconds	

10. Click **Save As**, enter a file name, then click **Save**.

11. Reopen the file to connect the computer to the instrument, load the plate, then start the run on the real-time PCR instrument.

12. After the instrument run is complete, open the SDS file in SDS Software v1.4.1. Analyze, then save the file.



Perform RT-PCR using the Applied Biosystems™ QuantStudio™ 5 Real-Time PCR Instrument

- Dye calibration for the QuantStudio™ 5 Real-Time PCR Instrument 29
- Transfer the template (EDT) file for the QuantStudio™ 5 Real-Time PCR Instrument 29
- Set up and run the QuantStudio™ 5 Real-Time PCR Instrument 30

Dye calibration for the QuantStudio™ 5 Real-Time PCR Instrument

A maintained instrument will be calibrated for all dyes that are used with this kit. Ensure that the calibrations for FAM™ dye, VIC™ dye, ABY™ dye, and JUN™ dye are current. If calibration is required, refer to the standard calibration process in the instrument user guide.

Transfer the template (EDT) file for the QuantStudio™ 5 Real-Time PCR Instrument

The template (EDT) file contains the settings for the instrument run.

It is installed on the computer with the Applied Biosystems™ Pathogen Interpretive Software.

The template must be transferred via a USB drive or other method to the computer on which QuantStudio™ Design and Analysis Desktop Software is installed.

IMPORTANT! Be careful to select the appropriate template file for your instrument. Failure to do so can cause errors in the analysis.

1. In the Pathogen Interpretive Software, click **Help ▶ Run Templates**.
2. Click **Download** to access the template file for your version of the data collection software.
3. In the **Save** dialog box, select the location to save the template file, then save the file.

4. Ensure that the correct template file was downloaded and saved.

TaqPath COVID-19 FluA FluB template QS5 0_2ml da1_5_1 v1_0.edt

5. Transfer the EDT file to the computer with QuantStudio™ Design and Analysis Desktop Software, using a USB drive or other method.

Set up and run the QuantStudio™ 5 Real-Time PCR Instrument

For more information about the instrument, see the documents listed in “Related documentation” on page 73.

1. In the QuantStudio™ Design and Analysis Desktop Software, in the **New Experiment** box, select **Create New Experiment ▶ Template**.
2. Browse to, then open the EDT file that you transferred in “Transfer the template (EDT) file for the QuantStudio™ 5 Real-Time PCR Instrument” on page 29.

IMPORTANT! Be careful to select the appropriate template file for your instrument. Failure to do so can cause errors in the analysis.

3. In the **Properties** tab, enter or confirm the following.

- **Name:** Enter a unique name
- **Instrument type:** QuantStudio™ 5 System
- **Block type:** 96-Well 0.2-mL Block
- **Experiment type:** Standard Curve
- **Chemistry:** TaqMan™ Reagents
- **Run Mode:** Standard

4. In the **Method** tab, confirm that the **Volume** is 25 µL, then confirm the thermal protocol.

Step	Temperature	Time	Number of cycles
UNG incubation	25°C	2 minutes	1
Reverse transcription	53°C	10 minutes	1
Preincubation	85°C	10 minutes	1
Activation	95°C	2 minutes	1
Denaturation	95°C	3 seconds	46
Anneal / extension	60°C	30 seconds	

5. In the **Plate** tab, click **Quick Setup**.
6. In the **Plate Attributes** pane, confirm that the **Passive Reference** is set to **None**.
7. In the **Plate** tab, click **Advanced Setup**.

8. In the **Targets** table, confirm that the reporter dye and the target pairs are correct.

Reporter dye	Detector	Quencher
FAM	FluA	None
VIC	C19 ^[1]	None
ABY	FluB	None
JUN	MS2	None

^[1] SARS-CoV-2 N gene and SARS-CoV-2 S gene

IMPORTANT! The detectors are case-sensitive.

9. Confirm that the targets above are assigned to each well in the plate layout.
10. In the plate layout pane, confirm the labeling of the control wells.
- The template has one positive control (PC) and one negative control (NC) assigned to wells for reference.
 - The positive control must be named **PC**. If additional characters are included, it must be named **PC<>**, where <> is defined by the user, for example **PC1**.
 - The negative control must be named **NC**. If additional characters are included, it must be named **NC<>**, where <> is defined by the user, for example **NC1**.
 - Move the control well assignments by copying the existing control wells and pasting them according to their location on the physical plate.

IMPORTANT! The positive and negative controls must be named as described.

11. For all targets in the positive control well, confirm that **Task** is set to **S (Standard)**.
12. For all targets in the negative control well, confirm that **Task** is set to **N (Negative Control)**.
13. In the **Samples** table, click **Add** to define the sample names. Create a unique sample name for each well in the physical plate.
14. To assign a sample to a well, select the well in the plate layout, then select the sample from the **Samples** table.

For all targets in the patient sample wells, confirm that **Task** is set to **U (Unknown)**.

Note: Wells that do not have a sample name will not be analyzed by the software.

IMPORTANT! Encrypt, pseudonymize, or anonymize personal data where possible following the requirements of HIPAA (Health Insurance Portability and Accountability Act).

15. Load the plate into the instrument.

16. Place a MicroAmp™ Optical Film Compression Pad gray side down on the surface of the RT-PCR reaction plate, to ensure a proper seal between the thermal cycler and the adhesive film.

IMPORTANT!

- Be careful to place the compression pad with the brown side up and the gray side down, centered on top of the plate.
 - Ensure the compression pad is free from wrinkles and signs of deterioration prior to use.
-

17. In the **Run** tab, click **Start Run**, then select your instrument from the drop-down list.
18. Enter a file name in the dialog box that prompts you to save the run file, then save the file.
19. At the end of the run, remove the pad from the plate and store the pad inside the pack.

IMPORTANT!

- If the compression pad becomes stuck inside the thermal cycler, call service to clean the heated cover.
 - Between each use, place the pad back in the pouch so that it does not dry out.
 - Each compression pad may be used up to 20 times before discarding. Do not use more than 20 times.
 - Do NOT use the pad with other instruments, unless expressly instructed to do so in the user documentation.
-



Perform RT-PCR using the Applied Biosystems™ QuantStudio™ 7 Flex Real-Time PCR Instrument (384-well block)

Dye calibration for the QuantStudio™ 7 Flex Real-Time PCR Instrument

A maintained instrument will be calibrated for many dyes. In addition to those dyes, the instrument operator must calibrate the instrument for ABY™ dye and JUN™ dye that are used with this kit. For all other assays, refer to the standard calibration process.

Transfer the template (EDT) file for the QuantStudio™ 7 Flex Real-Time PCR Instrument (384-well block)

The template (EDT) file contains the settings for the instrument run.

It is in the same compressed folder as the Pathogen Interpretive Software v1.1. The folder is C19-FluAB_EUA_QS7_384_AssayPanel_Template

The template must be transferred via a USB drive or other method to the computer on which the QuantStudio™ Real-Time PCR Software v1.3 is installed.

IMPORTANT! Be careful to select the appropriate template file for your instrument. Failure to do so can cause errors in the analysis.

1. After extracting the files from the compressed folder, select the following EDT file:
`TaqPath COVID-19 FluA FluB template QS7 384 1_3 v1_0.edt`
2. Transfer the EDT file to the computer with QuantStudio™ Real-Time PCR Software v1.3, using a USB drive or other method.

Set up and run the QuantStudio™ 7 Flex Real-Time PCR Instrument (384-well block)

For more information about the instrument, see the documents listed in “Related documentation” on page 73.

1. In the QuantStudio™ Real-Time PCR Software v1.3 home screen, click **Template**.
2. Browse to, then open the EDT file that you transferred in “Transfer the template (EDT) file for the QuantStudio™ 7 Flex Real-Time PCR Instrument (384-well block)” on page 33.

IMPORTANT! Be careful to select the appropriate template file for your instrument, block type, and assay type. Failure to do so can cause errors in the analysis.

3. In the **Experiment Properties** tab, enter or confirm the following.
 - **Experiment Name:** Enter a unique name
 - **Instrument type:** **QuantStudio™ 7 Flex System**
 - **Block:** **384-well**
 - **Type of Experiment:** **Standard Curve**
 - **Reagents:** **TaqMan™ Reagents**
 - **Properties:** **Standard**
4. In the **Define** tab, in the **Targets** pane, confirm that the targets, reporter dyes, and quenchers are listed correctly.

Reporter dye	Target	Quencher
FAM	FluA	None
VIC	C19 ^[1]	None
ABY	FluB	None
JUN	MS2	None

^[1] SARS-CoV-2 N gene and SARS-CoV-2 S gene

IMPORTANT! Target names are case-sensitive and must be named as described.

5. In the **Define** tab, in the **Samples** pane, define a sample name for each sample. Create a unique sample name for each well in the physical plate.

Note: Wells that do not have a sample name will not be analyzed by the software.

IMPORTANT! Encrypt, pseudonymize, or anonymize personal data where possible following the requirements of HIPAA (Health Insurance Portability and Accountability Act)

6. In the **Define** screen, confirm that the **Passive Reference** is set to **None**.

7. In the **Assign** screen, confirm that targets are assigned to each well in the **Plate Layout** tab. To assign a target to a well, select the well in the plate layout, then select the targets from the **Targets** table.
8. In the **Assign** screen, in the **Plate Layout** tab, confirm the labeling of the control wells.
 - The template has one positive control (PC) and one negative control (NC) assigned to wells for reference.
 - The positive control must be named **PC**. If additional characters are included, it must be named **PC<>**, where <> is defined by the user, for example **PC1**.
 - The negative control must be named **NC**. If additional characters are included, it must be named **NC<>**, where <> is defined by the user, for example **NC1**.
 - Move the control well assignments by copying the existing control wells and pasting them according to their location on the physical plate.

IMPORTANT! The positive and negative controls must be named as described.

9. In the **Assign** screen, confirm the **Task** assignments.
 - For wells with a Positive Control (**PC**), confirm that the **Task** is set to **S (Standard)** for all of the targets.
 - For wells with a Negative Control (**NC**), confirm that the **Task** is set to **N (Negative Control)** for all of the targets.
 - For the wells with a patient sample, confirm that the **Task** is set to **U (Unknown)** for all of the targets.
10. In the **Assign** screen, assign a sample name to each well to match the physical plate. To assign a sample to a well, select the well in the plate layout, then select the sample from the **Samples** table.

Note: Wells that do not have a sample name will not be analyzed by the software.

11. In the **Run Method** tab, confirm that the **Reaction Volume Per Well** is 20 µL, then confirm the thermal protocol.

Step	Temperature	Time	Number of cycles
UNG incubation	25°C	2 minutes	1
Reverse transcription	53°C	10 minutes	1
Preincubation	85°C	10 minutes	1
Activation	95°C	2 minutes	1
Denaturation	95°C	3 seconds	46
Anneal / extension	60°C	30 seconds	

12. In the **Run Method** screen, select **Optical Filters**, then select the 8 PCR filters shown in Table 4 on page 36. To enable **Optical Filters**, navigate to **Tools ▶ Preferences**, then in the **Defaults** tab, select **Show optical filters for run method**.

Note: Five filters are pre-selected by default.

Table 4 PCR Filters

		Emission Filter					
		m1(520±15)	m2(558±11)	m3(586±10)	m4(623±14)	m5(682±14)	m6(711±12)
Excitation Filter	x1(470±15)	✓	✓				
	x2(520±10)		✓	✓			
	x3(550±11)			✓	✓		
	x4(580±10)				✓		
	x5(640±10)					✓	
	x6(662±10)						

13. Load the prepared and sealed RT-PCR reaction plate into the real-time PCR instrument.
14. In the **Run** screen, click **Start Run**, then select your instrument from the drop-down list.
15. Enter a file name in the dialog box that prompts you to save the run file, then save the file.



Analysis and results

Software and instrument compatibility

Software	Description
Pathogen Interpretive Software	Data analysis and results interpretation software
SAE Administrator Console Dx v1.0	Security and audit tool
C19-FluAB_7500-FAST_1.0.0 <i>or</i> C19-FluAB_7500-FAST_1.1.0	Assay panel for the 7500 Fast Dx Real-Time PCR Instrument ^[1]
C19-FluAB_QS5-9602_1.1.0	Assay panel for QuantStudio™ 5 Real-Time PCR Instrument (96-well 0.2-mL block) ^[1]
C19-FluAB_QS7F-384_1.0.0	Assay panel for QuantStudio™ 7 Flex Real-Time PCR Instrument (384-well block)

^[1] Pre-installed in the Pathogen Interpretive Software.

For information on how to obtain the software, see “Obtain the software” on page 38.

The following table lists the assay panel that is compatible with the Pathogen Interpretive Software, your instrument, and its associated analysis software.

To obtain the correct analysis software or firmware version for your real-time PCR instrument, go to [thermofisher.com/qpcrsoftware](https://www.thermofisher.com/qpcrsoftware), then select your instrument in the **Real-Time PCR** section. If you have questions or problems finding the correct version, contact technical support (see Appendix D, “Documentation and support”).

Instrument	Analysis software associated with the instrument	Pathogen Interpretive Software	Assay panel code and version
7500 Fast Dx Real-Time PCR Instrument	SDS Software v1.4.1	v1.0	C19-FluAB_7500-FAST_1.0.0
		v1.1	C19-FluAB_7500-FAST_1.1.0
QuantStudio™ 5 Real-Time PCR Instrument with instrument firmware v1.3.3 (96-well 0.2-mL block)	QuantStudio™ Design and Analysis Desktop Software v1.5.1 or v1.5.2	v1.1	C19-FluAB_QS5-9602_1.1.0
QuantStudio™ 5 Real-Time PCR Instrument with instrument firmware v1.5.1 (96-well 0.2-mL block)	QuantStudio™ Design and Analysis Desktop Software v1.5.2	v1.1	C19-FluAB_QS5-9602_1.1.0
QuantStudio™ 7 Flex Real-Time PCR Instrument with instrument firmware v1.0.4 (384-well block)	QuantStudio™ Real-Time PCR Software v1.3	v1.1	C19-FluAB_QS7F-384_1.0.0

Obtain the software

To perform data analysis and results interpretation, you must use the following software:

- Applied Biosystems™ Pathogen Interpretive Software v1.1 with an installed assay panel
- SAE Administrator Console Dx v1.0

Note: The assay panel for the 7500 Fast Dx Real-Time PCR Instrument and QuantStudio™ 5 Real-Time PCR Instrument are pre-installed in the Pathogen Interpretive Software.

For instructions to obtain the software, see *TaqPath™ COVID-19, FluA, FluB Combo Kit Product Information Sheet* (Pub. No. 100097258). The product information sheet is included with the TaqPath™ COVID-19, FluA, FluB Combo Kit.

- The appropriate assay panel for your instrument:
 - C19-FluAB_7500-FAST_1.1.0
 - C19-FluAB_QS5-9602_1.1.0
 - C19-FluAB_QS7F-384_1.0.0

Install the assay panel

An assay panel contains the analysis settings that are used for data analysis in the interpretive software.

User-installed assay panels are located in the same compressed folder as the software and is instrument-specific. Select the folder for your instrument.

Only one matching assay panel is required to analyze a data file.

Note: Your instrument assay panel may be pre-installed in the Pathogen Interpretive Software, see “Software and instrument compatibility” on page 37 to confirm.

1. Extract the files from the compressed folder.

Instrument	Folder name
QuantStudio™ 7 Flex Real-Time PCR Instrument	C19-FluAB_EUA_QS7_384_AssayPanel_Template

2. In the Pathogen Interpretive Software v1.1, in the **Assay Panels** library, click **Actions ▶ Install**.

3. Navigate to, then open the assay panel for your instrument:

Instrument	Assay panel name and version
QuantStudio™ 7 Flex Real-Time PCR Instrument	C19-FluAB_QS7F-384_1.0.0

4. Click **Install**.

Note: If the assay panel is already installed, you will be prompted to confirm the assay panel upgrade.

The assay panel is added to the **Assay Panels** library.

For more information about assay panels, including uninstalling assay panels, see the documentation for the interpretive software.

Analyze the data

For detailed instructions about using the software, click the **Help** menu in the Pathogen Interpretive Software v1.1.

1. Using a USB drive or other method, transfer the SDS or EDS files from the computer with the data collection software to the computer with the Pathogen Interpretive Software v1.1.
2. In the software screen, select one of the following options:

Option	Description
In the Data Gallery , click Actions ▶ Open File .	The data file will open in the current window.
In the Data Gallery , click Actions ▶ Open File in New Window .	The data file will open in a new window.

3. Navigate to, then open the data file.
 - The data file opens and the results are displayed in the **Presence Absence** tab.
 - The data file is added to the **Data Gallery**.

Note: If the data file has already been added to the **Data Gallery**, click the file to open the file in the current window or hover over the file, then click **⋮ (Actions) ▶ Open in new window** to open the file in a new window.

Interpretation of the results

Interpretation of the results is performed by the Applied Biosystems™ Pathogen Interpretive Software v1.1. For information about the C_t values that are used by the software to interpret results, see Appendix A, “Ct cutoff values for assay targets”.

Quality control and validity of results

A minimum of one Negative Control and one Positive Control must be present for each run. Additional Negative Control wells must be run for each extraction that is represented on a real-time RT-PCR plate. All control wells must pass for the real-time RT-PCR plate to be considered valid (Table 5).

Validation of results is performed automatically by the Applied Biosystems™ Pathogen Interpretive Software v1.1 based on performance of the Positive and Negative Controls.

Table 5 Control wells

Negative Control (NC)				Positive Control (PC)				Overall Control Call
C19	FluA	FluB	MS2	C19	FluA	FluB	MS2	
NEG	NEG	NEG	POS	POS	POS	POS	NEG	Pass
All other scenarios								Fail

Table 6 Results interpretation for viral targets in patient samples

Sample ^[1]				Call	Assessment
C19	FluA	FluB	MS2		
POS	POS	POS	POS or NEG	Presence	REPORT - SARS-CoV-2, Flu A, Flu B Detected
POS	POS	NEG	POS or NEG	Presence	REPORT - SARS-CoV-2, Flu A Detected
POS	NEG	POS	POS or NEG	Presence	REPORT - SARS-CoV-2, Flu B Detected
POS	NEG	NEG	POS or NEG	Presence	REPORT - SARS-CoV-2 Detected
NEG	NEG	NEG	POS	Absence	REPORT - SARS-CoV-2, Flu A, Flu B Not Detected
NEG	NEG	NEG	NEG	Invalid	RETEST ^[2]
NEG	POS	POS	POS or NEG	Presence	REPORT - Flu A, Flu B Detected
NEG	POS	NEG	POS or NEG	Presence	REPORT - Flu A Detected
NEG	NEG	POS	POS or NEG	Presence	REPORT - Flu B Detected

^[1] Controls must pass for viral targets to be interpreted (Table 5).

^[2] Retesting must be performed by re-extracting the original sample and repeating the RT-PCR. If the repeat result remains invalid, consider collecting a new specimen.



Conditions of authorization for labs

The TaqPath™ COVID-19, FluA, FluB Combo Kit Letter of Authorization, along with the authorized Fact Sheet for Healthcare Providers, the authorized Fact Sheet for Patients, and authorized labeling are available on the FDA website: <https://www.fda.gov/medical-devices/coronavirus-disease-2019-covid-19-emergency-use-authorizations-medical-devices/vitro-diagnostics-euas>.

To assist clinical laboratories using the TaqPath™ COVID-19, FluA, FluB Combo Kit, the relevant Conditions of Authorization are listed below.

- Authorized laboratories^[1] using the TaqPath™ COVID-19, FluA, FluB Combo Kit must include with test result reports of the TaqPath™ COVID-19, FluA, FluB Combo Kit all authorized Fact Sheets. Under exigent circumstances, other appropriate methods for disseminating these Fact Sheets may be used, which may include mass media.
- Authorized laboratories using the TaqPath™ COVID-19, FluA, FluB Combo Kit must perform the TaqPath™ COVID-19, FluA, FluB Combo Kit as outlined in the *TaqPath™ COVID-19, FluA, FluB Combo Kit Instructions for Use*. Deviations from the authorized procedures, including the authorized instruments, authorized extraction methods, authorized clinical specimen types, authorized control materials, authorized other ancillary reagents, and authorized materials required to perform the TaqPath™ COVID-19, FluA, FluB Combo Kit are not permitted.
- Authorized laboratories that receive the TaqPath™ COVID-19, FluA, FluB Combo Kit must notify the relevant public health authorities of their intent to run the test prior to initiating testing.
- Authorized laboratories using the TaqPath™ COVID-19, FluA, FluB Combo Kit will have a process in place for reporting test results to healthcare providers and relevant public health authorities, as appropriate.
- Authorized laboratories must collect information on the performance of the test and report to DMD/OHT7-OIR/OPEQ/CDRH (via email: CDRH-EUA-Reporting@fda.hhs.gov) and Thermo Fisher Scientific (techservices@thermofisher.com; 1 800 955 6288) any suspected occurrence of false positive or false negative results and significant deviations from the established performance characteristics of the test of which they become aware.
- All laboratory personnel using this test must be appropriately trained in RT-PCR techniques and use appropriate laboratory and personal protective equipment when handling this kit, and use the test in accordance with the authorized labeling.
- Thermo Fisher Scientific, its authorized distributor(s), and authorized laboratories using the TaqPath™ COVID-19, FluA, FluB Combo Kit must ensure that any records associated with this EUA are maintained until otherwise notified by FDA. Such records must be made available to FDA for inspection upon request.

^[1] The letter of authorization refers to, “Laboratories certified under the Clinical Laboratory Improvement Amendments of 1988 (CLIA), 42 U.S.C. §263a, that meet requirements to perform high complexity tests” as “authorized laboratories.”



Performance characteristics

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Analytical performance of the TaqPath™ COVID-19, FluA, FluB Combo Kit was evaluated by determining limit of detection (LoD), characterizing the impact of competitive interference and endogenous interfering substances, and assessing reactivity and cross-reactivity. In addition, a Clinical Evaluation study was performed for the TaqPath™ COVID-19, FluA, FluB Combo Kit. All studies are described in the following sections.

Limit of detection (LoD)

The LoD study established the lowest SARS-CoV-2, influenza A, and influenza B viral concentrations (Genomic Copy Equivalents or GCE, or TCID₅₀/mL, as indicated) that can be detected by the TaqPath™ COVID-19, FluA, FluB Combo Kit at least 95% of the time.

Negative nasopharyngeal swab (NP) specimens were pooled and spiked with the indicated strains of SARS-CoV-2 virus, influenza A virus, or influenza B virus, at several concentrations and processed through the TaqPath™ COVID-19, FluA, FluB Combo Kit workflow. A three-phase approach was used to determine the LoD for each virus. In phases I and II, the preliminary LoD was established and confirmed in phase III by testing 20 replicates.

Table 7 LoD determination in NP specimens spiked with gamma-irradiated SARS-CoV-2 isolate USA-WA1/2020 (7500 Fast Dx Real-Time PCR Instrument)

Concentration	Replicate	C _t value		Interpretation	% Positive
		C19 (SARS-CoV-2)	MS2		
100 GCE/mL	1	32.43	22.94	Positive	100%
	2	32.54	22.75	Positive	
	3	32.76	22.71	Positive	
	4	32.63	22.64	Positive	

Table 7 LoD determination in NP specimens spiked with gamma-irradiated SARS-CoV-2 isolate USA-WA1/2020 (7500 Fast Dx Real-Time PCR Instrument) (continued)

Concentration	Replicate	C _t value		Interpretation	% Positive
		C19 (SARS-CoV-2)	MS2		
100 GCE/mL	5	32.86	22.78	Positive	100%
	6	32.68	22.71	Positive	
	7	32.95	22.58	Positive	
	8	32.89	22.46	Positive	
	9	32.52	22.53	Positive	
	10	32.99	22.56	Positive	
	11	32.90	22.98	Positive	
	12	32.85	22.84	Positive	
	13	33.09	22.71	Positive	
	14	32.66	22.77	Positive	
	15	32.63	22.64	Positive	
	16	32.42	22.62	Positive	
	17	32.50	22.89	Positive	
	18	32.81	22.87	Positive	
19	32.98	22.74	Positive		
20	33.04	22.46	Positive		

Table 8 LoD determination in NP specimens spiked with gamma-irradiated SARS-CoV-2 isolate USA-WA1/2020 (QuantStudio™ 5 Real-Time PCR Instrument, 96-well, 0.2-mL block)

Concentration	Replicate	C _t value		Interpretation	% Positive
		C19 (SARS-CoV-2)	MS2		
100 GCE/mL	1	34.22	24.66	Positive	100%
	2	34.17	24.57	Positive	
	3	34.07	24.51	Positive	
	4	33.91	24.48	Positive	
	5	34.81	24.37	Positive	
	6	34.13	24.63	Positive	
	7	34.23	24.42	Positive	

Table 8 LoD determination in NP specimens spiked with gamma-irradiated SARS-CoV-2 isolate USA-WA1/2020 (QuantStudio 5 Real-Time PCR Instrument, 96-well, 0.2-mL block) (continued)

Concentration	Replicate	C _t value		Interpretation	% Positive
		C19 (SARS-CoV-2)	MS2		
100 GCE/mL	8	34.95	24.43	Positive	100%
	9	34.11	23.99	Positive	
	10	34.28	24.29	Positive	
	11	34.66	24.93	Positive	
	12	34.62	24.82	Positive	
	13	34.79	24.47	Positive	
	14	34.57	24.47	Positive	
	15	34.34	24.39	Positive	
	16	34.60	24.44	Positive	
	17	34.14	24.50	Positive	
	18	34.13	24.62	Positive	
	19	34.14	24.40	Positive	
20	34.65	24.48	Positive		

Table 9 LoD determination in NP specimens spiked with live influenza A virus strain A/Perth/16/2009 (H3N2) (7500 Fast Dx Real-Time PCR Instrument)

Concentration	Replicate	C _t value		Interpretation	% Positive
		Flu A	MS2		
200 GCE/mL	1	35.58	22.62	Positive	100%
	2	36.63	22.91	Positive	
	3	36.60	22.87	Positive	
	4	36.54	23.01	Positive	
	5	36.13	22.87	Positive	
	6	35.61	22.84	Positive	
	7	35.86	22.84	Positive	
	8	36.93	22.70	Positive	
	9	41.95	22.81	Positive	
	10	36.57	22.90	Positive	

Table 9 LoD determination in NP specimens spiked with live influenza A virus strain A/Perth/16/2009 (H3N2) (7500 Fast Dx Real-Time PCR Instrument) (continued)

Concentration	Replicate	C _t value		Interpretation	% Positive
		Flu A	MS2		
200 GCE/mL	11	35.68	23.26	Positive	100%
	12	39.28	22.70	Positive	
	13	36.57	22.80	Positive	
	14	34.24	22.58	Positive	
	15	38.07	22.67	Positive	
	16	41.25	22.78	Positive	
	17	38.62	22.72	Positive	
	18	36.80	22.72	Positive	
	19	37.19	22.82	Positive	
	20	37.12	22.58	Positive	

Table 10 LoD determination in NP specimens spiked with live influenza A virus strain A/Perth/16/2009 (H3N2) (QuantStudio™ 5 Real-Time PCR Instrument, 96-well, 0.2-mL block)

Concentration	Replicate	C _t value		Interpretation	% Positive
		Flu A	MS2		
200 GCE/mL	1	41.91	25.50	Positive	95%
	2	39.25	25.88	Positive	
	3	37.68	25.07	Positive	
	4	37.27	25.23	Positive	
	5	36.66	25.21	Positive	
	6	40.03	25.37	Positive	
	7	38.58	24.57	Positive	
	8	38.19	24.69	Positive	
	9	43.06	24.71	Positive	
	10	44.91	24.76	Positive	
	11	Undetermined	25.80	Negative	
	12	44.87	25.40	Positive	
	13	37.03	25.19	Positive	

Table 10 LoD determination in NP specimens spiked with live influenza A virus strain A/Perth/16/2009 (H3N2) (QuantStudio 5 Real-Time PCR Instrument, 96-well, 0.2-mL block) (continued)

Concentration	Replicate	C _t value		Interpretation	% Positive
		Flu A	MS2		
200 GCE/mL	14	35.83	25.10	Positive	95%
	15	37.44	24.83	Positive	
	16	35.78	24.92	Positive	
	17	37.06	25.33	Positive	
	18	37.81	24.89	Positive	
	19	43.77	24.41	Positive	
	20	38.58	24.17	Positive	

Table 11 LoD determination in NP specimens spiked with live influenza A virus strain A/Brisbane/59/2007 (H1N1) (7500 Fast Dx Real-Time PCR Instrument)

Concentration	Replicate	C _t value		Interpretation	% Positive
		Flu A	MS2		
500 GCE/mL	1	35.72	22.99	Positive	100%
	2	32.98	23.18	Positive	
	3	34.81	22.86	Positive	
	4	36.51	22.61	Positive	
	5	37.40	22.35	Positive	
	6	33.14	22.35	Positive	
	7	36.03	22.56	Positive	
	8	36.06	22.77	Positive	
	9	36.37	22.73	Positive	
	10	39.29	22.67	Positive	
	11	40.24	23.51	Positive	
	12	34.71	22.92	Positive	
	13	36.02	22.81	Positive	
	14	35.90	23.10	Positive	
	15	36.94	22.58	Positive	
	16	35.42	22.76	Positive	

Table 11 LoD determination in NP specimens spiked with live influenza A virus strain A/Brisbane/59/2007 (H1N1) (7500 Fast Dx Real-Time PCR Instrument) (continued)

Concentration	Replicate	C _t value		Interpretation	% Positive
		Flu A	MS2		
500 GCE/mL	17	35.32	22.55	Positive	100%
	18	35.18	22.64	Positive	
	19	38.19	23.11	Positive	
	20	37.60	22.49	Positive	

Table 12 LoD determination in NP specimens spiked with live influenza A virus strain A/Brisbane/59/2007 (H1N1) (QuantStudio™ 5 Real-Time PCR Instrument, 96-well, 0.2-mL block)

Concentration	Replicate	C _t value		Interpretation	% Positive
		Flu A	MS2		
500 GCE/mL	1	42.74	25.32	Positive	95%
	2	33.12	25.27	Positive	
	3	37.67	25.62	Positive	
	4	36.02	24.92	Positive	
	5	37.51	25.34	Positive	
	6	34.53	25.02	Positive	
	7	38.67	24.96	Positive	
	8	39.48	24.90	Positive	
	9	39.31	25.41	Positive	
	10	Undetermined	24.99	Negative	
	11	40.79	25.90	Positive	
	12	36.36	24.57	Positive	
	13	38.23	25.23	Positive	
	14	39.40	24.94	Positive	
	15	37.79	25.28	Positive	
	16	39.48	24.72	Positive	
	17	38.41	25.30	Positive	
	18	36.77	24.84	Positive	

Table 12 LoD determination in NP specimens spiked with live influenza A virus strain A/Brisbane/59/2007 (H1N1) (QuantStudio 5 Real-Time PCR Instrument, 96-well, 0.2-mL block) (continued)

Concentration	Replicate	C _t value		Interpretation	% Positive
		Flu A	MS2		
500 GCE/mL	19	44.31	24.76	Positive	95%
	20	38.61	25.03	Positive	

Table 13 LoD determination in NP specimens spiked with live influenza B virus strain B/Florida/04/2006 (Yamagata lineage) (7500 Fast Dx Real-Time PCR Instrument)

Concentration	Replicate	C _t value		Interpretation	% Positive
		Flu B	MS2		
500 GCE/mL	1	36.78	22.97	Positive	95%
	2	36.96	23.06	Positive	
	3	37.40	22.89	Positive	
	4	Undetermined	23.14	Negative	
	5	36.56	23.02	Positive	
	6	37.65	23.58	Positive	
	7	36.26	22.66	Positive	
	8	36.40	22.67	Positive	
	9	35.35	22.68	Positive	
	10	37.97	23.33	Positive	
	11	37.64	23.28	Positive	
	12	36.04	23.06	Positive	
	13	36.71	23.19	Positive	
	14	37.18	23.20	Positive	
	15	36.49	22.76	Positive	
	16	36.66	22.68	Positive	
	17	37.64	22.82	Positive	
	18	37.09	22.96	Positive	
	19	35.70	22.54	Positive	
	20	36.66	22.98	Positive	

Table 14 LoD determination in NP specimens spiked with live influenza B virus strain B/Florida/04/2006 (Yamagata lineage) (QuantStudio™ 5 Real-Time PCR Instrument, 96-well, 0.2-mL block)

Concentration	Replicate	C _t value		Interpretation	% Positive
		Flu B	MS2		
500 GCE/mL	1	39.13	24.52	Positive	95%
	2	38.84	24.75	Positive	
	3	37.57	24.34	Positive	
	4	42.76	25.26	Positive	
	5	38.67	24.96	Positive	
	6	Undetermined	25.97	Negative	
	7	41.18	24.32	Positive	
	8	36.76	24.41	Positive	
	9	38.22	24.37	Positive	
	10	39.35	25.52	Positive	
	11	39.30	24.54	Positive	
	12	37.87	24.56	Positive	
	13	40.50	24.79	Positive	
	14	39.98	24.55	Positive	
	15	38.59	24.99	Positive	
	16	38.83	24.50	Positive	
	17	38.48	24.48	Positive	
	18	37.40	24.52	Positive	
	19	39.63	24.49	Positive	
	20	38.88	24.93	Positive	

Table 15 LoD determination in NP specimens spiked with live influenza B virus strain B/Wisconsin/1/2010 (Yamagata lineage) (7500 Fast Dx Real-Time PCR Instrument)

Concentration	Replicate	C _t value		Interpretation	% Positive
		Flu B	MS2		
1,000 GCE/mL	1	36.17	23.00	Positive	100%
	2	37.69	22.94	Positive	
	3	35.79	22.81	Positive	
	4	36.03	22.73	Positive	
	5	36.47	22.78	Positive	
	6	37.03	22.63	Positive	
	7	35.99	22.50	Positive	
	8	37.82	22.60	Positive	
	9	35.28	22.76	Positive	
	10	36.97	22.98	Positive	
	11	36.84	23.11	Positive	
	12	36.51	23.00	Positive	
	13	36.77	23.00	Positive	
	14	37.10	23.40	Positive	
	15	35.29	22.83	Positive	
	16	36.73	22.82	Positive	
	17	36.97	22.77	Positive	
	18	37.75	22.93	Positive	
	19	37.42	22.92	Positive	
	20	38.01	22.99	Positive	

Table 16 LoD determination in NP specimens spiked with live influenza B virus strain B/Wisconsin/1/2010 (Yamagata lineage) (QuantStudio™ 5 Real-Time PCR Instrument, 96-well, 0.2-mL block)

Concentration	Replicate	C _t value		Interpretation	% Positive
		Flu B	MS2		
1,000 GCE/mL	1	38.07	24.67	Positive	100%
	2	42.63	24.80	Positive	
	3	39.47	24.76	Positive	
	4	37.73	24.70	Positive	
	5	38.52	24.87	Positive	
	6	39.56	24.52	Positive	
	7	37.83	24.39	Positive	
	8	38.27	24.54	Positive	
	9	38.08	24.50	Positive	
	10	38.87	24.76	Positive	
	11	41.83	24.81	Positive	
	12	37.29	24.96	Positive	
	13	37.71	25.00	Positive	
	14	37.45	24.88	Positive	
	15	38.61	24.69	Positive	
	16	38.48	24.62	Positive	
	17	38.37	24.70	Positive	
	18	38.67	24.64	Positive	
	19	40.73	24.96	Positive	
	20	38.99	24.82	Positive	

Table 17 LoD results

Virus	Limit of Detection (7500 Fast Dx Real-Time PCR Instrument)		Limit of Detection (QuantStudio™ 5 Real-Time PCR Instrument, 96-well, 0.2-mL block)	
SARS-CoV-2 isolate USA-WA1/2020	100 GCE/mL	0.16 TCID ₅₀ /mL	100 GCE/mL	0.16 TCID ₅₀ /mL
Influenza A virus strain A/Perth/16/2009 (H3N2)	200 GCE/mL	2.49×10^{-3} TCID ₅₀ /mL	200 GCE/mL	2.49×10^{-3} TCID ₅₀ /mL
Influenza A virus strain A/Brisbane/59/2007 (H1N1)	500 GCE/mL	1.59×10^{-3} TCID ₅₀ /mL	500 GCE/mL	1.59×10^{-3} TCID ₅₀ /mL
Influenza B virus strain B/Florida/04/2006 (Yamagata lineage)	500 GCE/mL	5.88×10^{-2} TCID ₅₀ /mL	500 GCE/mL	5.88×10^{-2} TCID ₅₀ /mL
Influenza B virus strain B/Wisconsin/1/2010 (Yamagata lineage)	1,000 GCE/mL	5.60×10^{-3} TCID ₅₀ /mL	1,000 GCE/mL	5.60×10^{-3} TCID ₅₀ /mL

Reactivity (Inclusivity)

In silico analysis was performed from May 10 to June 10, 2022, containing 108,046 sequenced genomes to determine the current reactivity (inclusivity) of TaqPath™ COVID-19, FluA, FluB Combo Kit primer/probe sequences with all known strains/isolates of SARS-CoV-2, influenza A, and influenza B viruses. A positive was called when at least one assay shows a melting temperature higher than the annealing temperature. Based on the melting temperature analysis, a positive was called for 99.96% of SARS-CoV-2 strains. Evaluation of assay components that do not match 100% to target sequences indicates that most of the primer or probe mismatches are unlikely to impact assay function, thus the TaqPath™ COVID-19, FluA, FluB Combo Kit is expected to be highly inclusive for SARS-CoV-2 strain detection.

In silico analysis was performed in August 2020, using 32,460 full-length segment 7 sequences from human influenza A isolates from NCBI influenza Virus Sequence Database and 30,858 human influenza A segment 7 sequences (2009 to 2020) from the GISAID database. Influenza A assay primer/probes had 100% homology to 88% of known strains/isolates of influenza A analyzed as of August 2020.

Functional testing was also performed for the following 10 influenza A strains, starting at 3X LoD (1,500 GCE/mL for H1N1 strains and 600 GCE/mL for H3N2 strains, based on the results of the LoD study described in Table 17 on page 53). The lowest concentration tested that produced positive results for 3 of 3 replicates is indicated.

Table 18 Influenza A strains (7500 Fast Dx Real-Time PCR Instrument and QuantStudio™ 5 Real-Time PCR Instrument, 96-well 0.2-mL block)

Strain	Lowest concentration tested Positive for 3 of 3 replicates	
	GCE/mL	TCID ₅₀ /mL or CEID ₅₀ /mL
H1N1/Georgia/M5081/2012	1,500 GCE/mL	4.78 × 10 ¹ TCID ₅₀ /mL
H1N1/New Caledonia/20/99	1,500 GCE/mL	7.44 × 10 ⁻² TCID ₅₀ /mL
H1N1/Puerto Rico/08/1934	1,500 GCE/mL	Unknown
H1N1/Solomon Islands/3/2006	1,500 GCE/mL	5.40 × 10 ¹ CEID ₅₀ /mL
H1N1/California/04/2009	4,500 GCE/mL	4.20 × 10 ⁰ TCID ₅₀ /mL
H3N2/Wisconsin/15/2009	600 GCE/mL	Unknown
H3N2/Switzerland/9715293/2013	600 GCE/mL	≥1.15 × 10 ⁻⁴ CEID ₅₀ /mL ^[1]
H3N2/Wisconsin/67/2005	600 GCE/mL	3.23 × 10 ⁻² TCID ₅₀ /mL
H3N2/Aichi/2/68	600 GCE/mL	Unknown
H3N2/Hong Kong/8/68	600 GCE/mL	Unknown

^[1] Certificate of Analysis for this strain lists concentration as ≥5 × 10³ CEID₅₀/mL.

In silico analysis was performed in August 2020, using 8,660 full-length segment 7 sequences from human influenza B isolates from NCBI Influenza Virus Sequence Database and 12,577 human influenza B segment 7 sequences (2009 to 2020) from the GISAID database. Influenza B assay primer/probes had 100% homology to 41% of known strains/isolates of influenza B analyzed as of August 2020.

Functional testing was also performed for the following 5 influenza B strains, starting at 3X LoD (based on the LoD study described in Table 17 on page 53). The lowest concentration tested that produced positive results for 3 of 3 replicates is indicated.

Table 19 Influenza B strains (7500 Fast Dx Real-Time PCR Instrument and QuantStudio™ 5 Real-Time PCR Instrument, 96-well 0.2-mL block)

Strain	Lowest concentration tested Positive for 3 of 3 replicates	
	GCE/mL	TCID ₅₀ /mL
Unknown Lineage/Taiwan/2/62	1,500 GCE/mL	Unknown
Mixed Lineage/Malaysia/2506/2004	1,500 GCE/mL	1.09 × 10 ⁻² TCID ₅₀ /mL
Victoria/Colorado/06/2017	3,000 GCE/mL	1.91 × 10 ⁻² TCID ₅₀ /mL
Yamagata/Massachusetts/02/2012	1,500 GCE/mL	7.41 × 10 ⁻² TCID ₅₀ /mL
Yamagata/Brisbane/03/2007	1,500 GCE/mL	3.50 × 10 ⁻¹ TCID ₅₀ /mL

In silico analysis was performed from November 1st, 2021 to December 30th, 2021 containing 749,572 genomes. This study includes a summary of mismatch and melting temperature analysis. A positive is called when at least one assay shows a melting temperature higher than the annealing temperature.

Based on the melting temperature analysis, a positive is called for 99.9% of SARS-CoV-2 strains.

In silico analysis was performed on December 30th, 2021 using blastn and melting temperature analysis for reactivity of COVID-19, Flu A, Flu B Combo kit primers/probes with known human viral isolates.

Results for SARS-CoV-2 strain detection are shown below.

Table 20 Strains mapping with 100% identity to 0, 1, or 2 targets

Target(s)	GISAID (749,572 strains)	
	# Strains	% Strains
2	23,734	3.1
1	684,331	91.3
0	41,507	5.5

A positive detection result is called when at least one assay shows a melting temperature higher than the annealing temperature. Therefore, a positive is called for 745,002 of 746,490 (99.7%) of SARS-CoV-2 strains.

Table 21 Strains mapping with melting temperature > annealing temperature for 0, 1, or 2 targets

Target(s)	GISAID (746,490 strains ^[1])	
	# Strains	% Strains
2	25,715	3.4
1	719,287	96.4
0	1,448	0.19

^[1] For 3,154 strains the tm value could not be calculated, so these are not reported in the table.

Table 22 Summary of mismatches

SARS-CoV2_target	Sequence percentage with mismatch	Forward primer mismatches	Probe mismatches	Reverse primer mismatches
N_gene	91.23	1	0	0
N_gene	6.74	0	0	0
N_gene	1.16	1	0	1
S_gene	91.92	0	0	0
S_gene	3.3	0	deletion	0
S_gene	1.77	0	1	0
S_gene	1.65	0	0	1

Mutations of less than 1% are not reported.

The forward primer mutation in the N gene and deletion in the S gene are not linked. The S gene deletion reflects omicron which has a small representation in the dataset discussed here but overall is by now the major variant in the US. The forward primer mutation in the N gene is linked to Delta and is now less prevalent in the US population.

Competitive interference

Negative NP specimens were pooled and spiked with the indicated strains of SARS-CoV-2 (USA-WA1/2020), influenza A (A/Brisbane/59/07), or influenza B (B/Florida/04/06) virus in combinations where at least one virus was present at a low concentration and the other at a high concentration and were processed through the TaqPath™ COVID-19, FluA, FluB Combo Kit workflow.

Table 23 7500 Fast Dx Real-Time PCR Instrument

Combination	Target 1		Target 2		Target 3		Mean C _t (n=3) (Number detected/number tested)			
	Virus	Conc.	Virus	Conc.	Virus	Conc.	SARS-CoV-2 (VIC)	FluA (FAM)	FluB (ABY)	MS2 (JUN)
SARS-CoV-2 ^{lo} FluA ^{hi}	SARS-CoV-2	0.48 TCID ₅₀ /mL	Flu A	10 ⁵ TCID ₅₀ /mL	N/A	N/A	30.9 (3/3)	7.5 (3/3)	46.0 (0/3)	22.5 (3/3)
FluA ^{lo} SARS-CoV-2 ^{hi}	Flu A	4.78 × 10 ⁻³ TCID ₅₀ /mL	SARS-CoV-2	10 ⁵ TCID ₅₀ /mL	N/A	N/A	13.6 (3/3)	33.4 (3/3)	46.0 (0/3)	22.6 (3/3)
SARS-CoV-2 ^{lo} FluB ^{hi}	SARS-CoV-2	0.48 TCID ₅₀ /mL	Flu B	10 ⁵ TCID ₅₀ /mL	N/A	N/A	30.7 (3/3)	46.0 (0/3)	15.4 (3/3)	21.7 (3/3)
FluB ^{lo} SARS-CoV-2 ^{hi}	Flu B	0.18 TCID ₅₀ /mL	SARS-CoV-2	10 ⁵ TCID ₅₀ /mL	N/A	N/A	13.7 (3/3)	46.0 (0/3)	34.6 (3/3)	22.1 (3/3)
FluA ^{lo} FluB ^{hi}	Flu A	4.78 × 10 ⁻³ TCID ₅₀ /mL	Flu B	10 ⁵ TCID ₅₀ /mL	N/A	N/A	46.0 (0/3)	34.5 (3/3)	15.5 (3/3)	21.0 (3/3)
FluB ^{lo} FluA ^{hi}	Flu B	0.18 TCID ₅₀ /mL	Flu A	10 ⁵ TCID ₅₀ /mL	N/A	N/A	46.0 (0/3)	7.0 (3/3)	33.5 (3/3)	21.8 (3/3)
FluA ^{lo}	Flu A	4.78 × 10 ⁻³ TCID ₅₀ /mL	N/A	N/A	N/A	N/A	46.0 (0/3)	33.8 (3/3)	46.0 (0/3)	22.4 (3/3)
SARS-CoV-2 ^{lo}	SARS-CoV-2	0.48 TCID ₅₀ /mL	N/A	N/A	N/A	N/A	31.3 (3/3)	46.0 (0/3)	46.0 (0/3)	22.6 (3/3)
FluB ^{lo}	Flu B	0.18 TCID ₅₀ /mL	N/A	N/A	N/A	N/A	46.0 (0/3)	46.0 (0/3)	34.4 (3/3)	22.3 (3/3)
SARS-CoV-2 ^{lo} FluA ^{lo} FluB ^{lo}	SARS-CoV-2	0.48 TCID ₅₀ /mL	Flu A	4.78 × 10 ⁻³ TCID ₅₀ /mL	Flu B	0.18 TCID ₅₀ /mL	30.8 (3/3)	33.9 (3/3)	34.6 (3/3)	23.1 (3/3)

Table 24 QuantStudio™ 5 Real-Time PCR Instrument 96-well, 0.2-mL block

Combination	Target 1		Target 2		Target 3		Mean C _t (n=3) (Number detected/number tested)			
	Virus	Conc.	Virus	Conc.	Virus	Conc.	SARS-CoV-2 (VIC)	FluA (FAM)	FluB (ABY)	MS2 (JUN)
SARS-CoV-2 ^{lo} FluA ^{hi}	SARS-CoV-2	0.64 TCID ₅₀ /mL ^[1]	Flu A	10 ⁵ TCID ₅₀ /mL	N/A	N/A	32.1 (3/3)	8.1 (3/3)	46.0 (0/3)	23.7 (3/3)
FluA ^{lo} SARS-CoV-2 ^{hi}	Flu A	6.36 × 10 ⁻³ TCID ₅₀ /mL ^[1]	SARS-CoV-2	10 ⁵ TCID ₅₀ /mL	N/A	N/A	15.1 (3/3)	33.0 (3/3)	46.0 (0/3)	24.1 (3/3)
SARS-CoV-2 ^{lo} FluB ^{hi}	SARS-CoV-2	0.48 TCID ₅₀ /mL	Flu B	10 ⁵ TCID ₅₀ /mL	N/A	N/A	32.3 (3/3)	46.0 (0/3)	16.2 (3/3)	22.9 (3/3)
FluB ^{lo} SARS-CoV-2 ^{hi}	Flu B	0.18 TCID ₅₀ /mL	SARS-CoV-2	10 ⁵ TCID ₅₀ /mL	N/A	N/A	14.7 (3/3)	46.0 (0/3)	35.7 (3/3)	23.9 (3/3)
FluA ^{lo} FluB ^{hi}	Flu A	4.78 × 10 ⁻³ TCID ₅₀ /mL	Flu B	10 ⁵ TCID ₅₀ /mL	N/A	N/A	46.0 (0/3)	35.2 (3/3)	16.5 (3/3)	22.8 (3/3)
FluB ^{lo} FluA ^{hi}	Flu B	0.18 TCID ₅₀ /mL	Flu A	10 ⁵ TCID ₅₀ /mL	N/A	N/A	46.0 (0/3)	7.2 (3/3)	36.5 (3/3)	23.5 (3/3)
FluA ^{lo}	Flu A	6.36 × 10 ⁻³ TCID ₅₀ /mL ^[1]	N/A	N/A	N/A	N/A	46.0 (0/3)	33.5 (3/3)	46.0 (0/3)	24.1 (3/3)
SARS-CoV-2 ^{lo}	SARS-CoV-2	0.64 TCID ₅₀ /mL ^[1]	N/A	N/A	N/A	N/A	32.4 (3/3)	46.0 (0/3)	46.0 (0/3)	24.4 (3/3)
FluB ^{lo}	Flu B	0.18 TCID ₅₀ /mL	N/A	N/A	N/A	N/A	46.0 (0/3)	46.0 (0/3)	36.1 (3/3)	24.2 (3/3)
SARS-CoV-2 ^{lo} FluA ^{lo} FluB ^{lo}	SARS-CoV-2	0.48 TCID ₅₀ /mL	Flu A	4.78 × 10 ⁻³ TCID ₅₀ /mL	Flu B	0.18 TCID ₅₀ /mL	32.1 (3/3)	34.8 (3/3)	35.9 (3/3)	24.2 (3/3)

^[1] This virus combination produced 3/3 Positive at 3X LoD, but the shift in mean C_t versus 3X LoD alone was greater than 1.5. Therefore, the combination was repeated with the low-concentration virus at 4X LoD.

No competitive interference was detected at the 3X LoD level for the following viral combinations:

- SARS-CoV-2 (0.48 TCID₅₀/mL) and FluB (10⁵ TCID₅₀/mL)
- FluB (0.18 TCID₅₀/mL) and SARS-CoV-2 (10⁵ TCID₅₀/mL)
- FluA (4.78 × 10⁻³ TCID₅₀/mL) and FluB (10⁵ TCID₅₀/mL)
- FluB (0.18 TCID₅₀/mL) and FluA (10⁵ TCID₅₀/mL)
- SARS-CoV-2 (0.48 TCID₅₀/mL), FluA (4.78 × 10⁻³ TCID₅₀/mL), and FluB (0.18 TCID₅₀/mL)

No competitive interference was detected at the 3X LoD level for the following viral combinations (7500 Fast Dx Real-Time PCR Instrument only):

- SARS-CoV-2 (0.48 TCID₅₀/mL) and FluA (10⁵ TCID₅₀/mL)
- FluA (4.78 × 10⁻³ TCID₅₀/mL) and SARS-CoV-2 (10⁵ TCID₅₀/mL)

No competitive interference was detected at the 4X LoD level for the following viral combinations (QuantStudio™ 5 Real-Time PCR Instrument 96-well, 0.2-mL block only):

- SARS-CoV-2 (0.64 TCID₅₀/mL) and FluA (10⁵ TCID₅₀/mL)
- FluA (6.36 × 10⁻³ TCID₅₀/mL) and SARS-CoV-2 (10⁵ TCID₅₀/mL)

Cross-reactivity

Functional testing was performed using the following microorganisms at the indicated concentrations. No cross-reactivity with the microorganisms tested was observed for SARS-CoV-2, influenza A virus, influenza B virus, or the MS2 Phage Control with the TaqPath™ COVID-19, FluA, FluB Combo Kit at the concentrations tested.

Table 25 Cross-reactivity testing results

Organism	Equivalent concentration tested	Number detected / number tested
<i>Bacillus anthracis</i>	2.9 × 10 ⁸ GCE/mL	0/3
<i>Bordetella pertussis</i>	1.1 × 10 ⁶ CFU/mL	0/3
<i>Chlamydia psittaci</i>	1.5 × 10 ⁵ GCE/mL	0/3
<i>Chlamydophila pneumoniae</i>	1.1 × 10 ⁶ CFU/mL	0/3
<i>Corynebacterium diphtheriae</i>	1.1 × 10 ⁶ CFU/mL	0/3
<i>Coxiella burnetii</i>	2.6 × 10 ⁵ GCE/mL	0/3
<i>Haemophilus influenzae</i>	1.1 × 10 ⁶ CFU/mL	0/3
<i>Legionella non-pneumophila</i> (<i>Legionella longbeachae</i>)	1.1 × 10 ⁶ CFU/mL	0/3
<i>Legionella pneumophila</i>	1.1 × 10 ⁶ CFU/mL	0/3
<i>Leptospira interrogans</i>	1.7 × 10 ⁸ GCE/mL	0/3

Table 25 Cross-reactivity testing results (continued)

Organism	Equivalent concentration tested	Number detected / number tested
<i>Moraxella catarrhalis</i>	4.2×10^8 GCE/mL	0/3
<i>Mycobacterium tuberculosis</i>	7.9×10^6 GCE/mL	0/3
<i>Mycoplasma pneumoniae</i>	1.1×10^6 CFU/mL	0/3
<i>Neisseria elongata</i>	1.1×10^6 CFU/mL	0/3
<i>Neisseria meningitidis</i>	1.1×10^6 CFU/mL	0/3
<i>Pseudomonas aeruginosa</i>	1.1×10^6 CFU/mL	0/3
<i>Staphylococcus aureus</i>	1.1×10^6 CFU/mL	0/3
<i>Staphylococcus epidermidis</i>	1.1×10^6 CFU/mL	0/3
<i>Streptococcus pneumoniae</i>	3.8×10^8 GCE/mL	0/3
<i>Streptococcus pyogenes</i>	4.2×10^8 GCE/mL	0/3
<i>Streptococcus salivarius</i>	1.1×10^6 CFU/mL	0/3
Adenovirus	1.1×10^5 TCID ₅₀ /mL	0/3
Enterovirus	1.1×10^5 TCID ₅₀ /mL	0/3
Human coronavirus 229E	1.1×10^5 TCID ₅₀ /mL	0/3
Human coronavirus NL63	4.0×10^4 TCID ₅₀ /mL	0/3
Human coronavirus OC43	1.1×10^5 TCID ₅₀ /mL	0/3
Human metapneumovirus	1.1×10^5 TCID ₅₀ /mL	0/3
Influenza C virus	1.1×10^5 TCID ₅₀ /mL	0/3
MERS Coronavirus	Unknown ^[1]	0/3
Parainfluenza 1 virus	1.1×10^5 TCID ₅₀ /mL	0/3
Parainfluenza 2 virus	1.1×10^5 TCID ₅₀ /mL	0/3
Parainfluenza 3 virus	1.1×10^5 TCID ₅₀ /mL	0/3
Parainfluenza 4 virus	1.1×10^5 TCID ₅₀ /mL	0/3
Parechovirus	1.1×10^5 TCID ₅₀ /mL	0/3
Respiratory Syncytial Virus A	1.1×10^5 TCID ₅₀ /mL	0/3
Respiratory Syncytial Virus B	1.1×10^5 TCID ₅₀ /mL	0/3
Rhinovirus	1.1×10^5 TCID ₅₀ /mL	0/3
<i>Candida albicans</i>	1.1×10^6 CFU/mL	0/3

Table 25 Cross-reactivity testing results (continued)

Organism	Equivalent concentration tested	Number detected / number tested
<i>Pneumocystis carinii</i> (Substituted for <i>Pneumocystis jirovecii</i>)	1.1 × 10 ⁶ CFU/mL	0/3
Pooled human nasal wash	14% v/v	0/3

^[1] The concentration was not provided by the vendor. The concentration tested was unknown.

Additional testing was performed to assess the impact of most of the organisms listed above at the indicated concentrations on the detection of MS2 Phage Control.

Chlamydomyxa pneumoniae and *Coxiella burnetii* were not tested in the presence of the MS2 Phage Control.

None of the organisms that were tested had a significant impact on the MS2 Phage Control C_t except for *Streptococcus pneumoniae* gDNA when tested directly in PCR without extraction, which produced an approximately 4–5-fold C_t increase in the mean C_t. Dilution of *S. pneumoniae* gDNA by 1:10 eliminated the inhibition on both of the real-time PCR instruments.

Table 26 Cross-reactivity MS2 Phage Control testing results

Instrument	Equivalent concentration	Mean C _t for MS2 Phage Control (n=3)	Δ C _t
7500 Fast Dx Real-Time PCR Instrument	3.8 × 10 ⁸ GCE/mL	29.09	4.99
	3.8 × 10 ⁷ GCE/mL	23.88	-0.22
	3.8 × 10 ⁶ GCE/mL	23.94	-0.16
	3.8 × 10 ⁵ GCE/mL	24.07	-0.03
	0 GCE/mL	24.10	N/A
QuantStudio™ 5 Real-Time PCR Instrument	3.8 × 10 ⁸ GCE/mL	29.85	4.13
	3.8 × 10 ⁷ GCE/mL	25.57	-0.15
	3.8 × 10 ⁶ GCE/mL	25.69	-0.03
	3.8 × 10 ⁵ GCE/mL	25.91	0.19
	0 GCE/mL	25.72	N/A

Interfering substances

Pooled negative NP specimens were spiked with SARS-CoV-2 (USA-WA1/2020), influenza A (A/Brisbane/59/07), or influenza B (B/Florida/04/06) viruses at 3X LoD and run in triplicate for each of the 10 potentially interfering substances and one no-interferent control on the 7500 Fast Dx Real-Time PCR Instrument and the QuantStudio™ 5 Real-Time PCR Instrument 96-well 0.2-mL block.

Interference was not observed for mucin, blood, corticosteroid nasal spray, nasal gel, homeopathic allergy relief nasal spray, throat lozenges, Oseltamivir, antibiotic ointment, and systemic antibiotics at the concentrations tested. Afrin™ Original nasal spray showed interference at 10% v/v; the highest concentrations that did not produce interference for any viral target were 0.6% and 1.3% for the 7500 Fast Dx Real-Time PCR Instrument and the QuantStudio™ 5 Real-Time PCR Instrument, respectively.

Table 27 Interfering substances (7500 Fast Dx Real-Time PCR Instrument)

Interfering substance	Final concentration in sample	Agreement with expected results					
		SARS-CoV-2		Flu A		Flu B	
		Percent agreement	Number of positive / Number tested	Percent agreement	Number of positive / Number tested	Percent agreement	Number of positive / Number tested
None	N/A	100%	3/3	100%	3/3	100%	3/3
Mucin: bovine submaxillary gland, type I-S	0.1 mg/mL	100%	3/3	100%	3/3	100%	3/3
Blood (human)	1% v/v	100%	3/3	100%	3/3	100%	3/3
Nasal sprays or drops—Afrin™ Original	0.6% v/v	100% ^[1]	3/3	100% ^[1]	3/3	100% ^[2]	3/3
Nasal corticosteroids—Flonase™	5 µg/mL	100%	3/3	100%	3/3	100%	3/3
Nasal gel—NeilMed™ Nasogel™	1% w/v	100%	3/3	100%	3/3	100%	3/3
Homeopathic allergy relief medicine—NatraBio Allergy Relief	10% v/v	100%	3/3	100%	3/3	100%	3/3
Throat lozenges, oral anesthetic and analgesic—Chloraseptic™	1% w/v	100%	3/3	100%	3/3	100%	3/3

Table 27 Interfering substances (7500 Fast Dx Real-Time PCR Instrument) (continued)

Interfering substance	Final concentration in sample	Agreement with expected results					
		SARS-CoV-2		Flu A		Flu B	
		Percent agreement	Number of positive / Number tested	Percent agreement	Number of positive / Number tested	Percent agreement	Number of positive / Number tested
Oseltamivir phosphate	33 µg/mL	100%	3/3	100%	3/3	100%	3/3
Antibiotic, nasal ointment—Bactroban™	5 µg/mL	100%	3/3	100%	3/3	100%	3/3
Antibacterial, systemic—Tobramycin	0.6 mg/mL	100%	3/3	100%	3/3	100%	3/3

^[1] All replicates tested at 10%, 5%, and 2.5% were undetected.

^[2] All replicates tested at 10%, 5%, 2.5%, and 1.3% v/v were undetected.

Table 28 Interfering substances (QuantStudio™ 5 Real-Time PCR Instrument, 96-well, 0.2-mL block)

Interfering substance	Final concentration in sample	Agreement with expected results					
		SARS-CoV-2		Flu A		Flu B	
		Percent agreement	Number of positive / Number tested	Percent agreement	Number of positive / Number tested	Percent agreement	Number of positive / Number tested
None	N/A	100%	3/3	100%	3/3	100%	3/3
Mucin: bovine submaxillary gland, type I-S	0.1 mg/mL	100%	3/3	100%	3/3	100%	3/3
Blood (human)	1% v/v	100%	3/3	100%	3/3	100%	3/3
Nasal sprays or drops—Afrin™ Original	1.3% v/v	100% ^[1]	3/3	100% ^[1]	3/3	100% ^[1]	3/3
Nasal corticosteroids—Flonase™	5 µg/mL	100%	3/3	100%	3/3	100%	3/3
Nasal gel—NeilMed™ Nasogel™	1% w/v	100%	3/3	100%	3/3	100%	3/3

Table 28 Interfering substances (QuantStudio 5 Real-Time PCR Instrument, 96-well, 0.2-mL block) (continued)

Interfering substance	Final concentration in sample	Agreement with expected results					
		SARS-CoV-2		Flu A		Flu B	
		Percent agreement	Number of positive / Number tested	Percent agreement	Number of positive / Number tested	Percent agreement	Number of positive / Number tested
Homeopathic allergy relief medicine—NatraBio Allergy Relief	10% v/v	100%	3/3	100%	3/3	100%	3/3
Throat lozenges, oral anesthetic and analgesic—Chloraseptic™	1% w/v	100%	3/3	100%	3/3	100%	3/3
Oseltamivir phosphate	33 µg/mL	100%	3/3	100%	3/3	100%	3/3
Antibiotic, nasal ointment—Bactroban™	5 µg/mL	100%	3/3	100%	3/3	100%	3/3
Antibacterial, systemic—Tobramycin	0.6 mg/mL	100%	3/3	100%	3/3	100%	3/3

^[1] All replicates tested at 10%, 5%, and 2.5% were undetected.

Clinical evaluation

A clinical evaluation study was carried out to evaluate the performance of the TaqPath™ COVID-19, FluA, FluB Combo Kit using archived nasopharyngeal specimens.

The following specimens were tested:

- 51 NP specimens positive for SARS-CoV-2
- 59 NP specimens negative for SARS-CoV-2
- 56 NP specimens positive for influenza A virus
- 104 NP specimens negative for influenza A virus
- 36 NP specimens positive for influenza B virus
- 124 NP specimens negative for influenza B virus

Samples were tested using the TaqPath™ COVID-19, FluA, FluB Combo Kit as well as the following comparator tests:

- Comparator for SARS-CoV-2: FDA EUA-Authorized Molecular SARS-CoV-2 Assay
- Comparator for influenza A/B virus: FDA-cleared Molecular Influenza A+B Assay

Samples were extracted using the MagMAX™ Viral/Pathogen II Nucleic Acid Isolation Kit for testing with TaqPath™ COVID-19, FluA, FluB Combo Kit or according to the Instructions for Use for the comparator assays.

Positive Percent Agreement (PPA) and Negative Percent Agreement (NPA) were calculated relative to the comparator test. Results are shown below.

Table 29 Clinical evaluation study for SARS-CoV-2 (7500 Fast Dx Real-Time PCR Instrument)

TaqPath™ COVID-19, FluA, FluB Combo Kit	Comparator assay		
	SARS-CoV-2 Positive	SARS-CoV-2 Negative	Total
SARS-CoV-2 Positive	49	0	49
SARS-CoV-2 Negative	2	59	61
Total	51	59	110

PPA: 96.1% (95% CI: 86.5% LCL - 99.5% UCL)

NPA: 100% (95% CI: 93.9% LCL - 100.0% UCL)

Table 30 Clinical evaluation study for SARS-CoV-2 (QuantStudio™ 5 Real-Time PCR Instrument, 96-well, 0.2-mL block)

TaqPath™ COVID-19, FluA, FluB Combo Kit	Comparator assay		
	SARS-CoV-2 Positive	SARS-CoV-2 Negative	Total
SARS-CoV-2 Positive	49	0	49
SARS-CoV-2 Negative	2	59	61
Total	51	59	110

PPA: 96.1% (95% CI: 86.5% LCL - 99.5% UCL)

NPA: 100% (95% CI: 93.9% LCL - 100.0% UCL)

Table 31 Clinical evaluation study for Flu A (7500 Fast Dx Real-Time PCR Instrument)

TaqPath™ COVID-19, FluA, FluB Combo Kit	Comparator assay		
	Flu A Positive	Flu A Negative	Total
Flu A Positive	54	1	55
Flu A Negative	2	103	105
Total	56	104	160

PPA: 96.4% (95% CI: 87.7% LCL - 99.6% UCL)

NPA: 99.0% (95% CI: 94.8% LCL - 100.0% UCL)

Table 32 Clinical evaluation study for Flu A (QuantStudio™ 5 Real-Time PCR Instrument, 96-well, 0.2-mL block)

TaqPath™ COVID-19, FluA, FluB Combo Kit	Comparator assay		
	Flu A Positive	Flu A Negative	Total
Flu A Positive	53	1	54
Flu A Negative	3	103	106
Total	56	104	160

PPA: 94.6% (95% CI: 85.1% LCL - 98.9% UCL)

NPA: 99.0% (95% CI: 94.8% LCL - 100.0% UCL)

Table 33 Clinical evaluation study for Flu B (7500 Fast Dx Real-Time PCR Instrument)

TaqPath™ COVID-19, FluA, FluB Combo Kit	Comparator assay		
	Flu B Positive	Flu B Negative	Total
Flu B Positive	33	4	37
Flu B Negative	3	120	123
Total	36	124	160

PPA: 91.7% (95% CI: 77.5% LCL - 98.2% UCL)

NPA: 96.8% (95% CI: 91.9% LCL - 99.1% UCL)

Table 34 Clinical evaluation study for Flu B (QuantStudio™ 5 Real-Time PCR Instrument, 96-well, 0.2-mL block)

TaqPath™ COVID-19, FluA, FluB Combo Kit	Comparator assay		
	Flu B Positive	Flu B Negative	Total
Flu B Positive	33	4	37
Flu B Negative	3	120	123
Total	36	124	160

PPA: 91.7% (95% CI: 77.5% LCL - 98.2% UCL)

NPA: 96.8% (95% CI: 91.9% LCL - 99.1% UCL)

Prospective clinical evaluation

A prospective clinical evaluation study was performed at three testing sites to evaluate the performance of the TaqPath™ COVID-19, FluA, FluB Combo Kit with nasopharyngeal (NP) swabs and anterior nasal swab (ANS) specimens.

384 participants with signs and symptoms of SARS-CoV-2, influenza A, or influenza B were enrolled in the study. Among the tested samples, two NP and two ANS samples were excluded due to incorrect processing, and one NP sample leaked during transit and was also excluded from testing. A total of 381 NP samples and 382 ANS were evaluated. Samples were tested using the TaqPath™ COVID-19, FluA, FluB Combo Kit and with an FDA cleared influenza A and influenza B molecular assay as a comparator method.

Samples were tested according to their respective Instructions for Use.

Testing with the TaqPath™ COVID-19, FluA, FluB Combo Kit was performed using the MagMAX™ Viral/Pathogen II Nucleic Acid Isolation Kit on the KingFisher Flex Purification System, and RT-qPCR was performed with the TaqPath™ 1-Step Multiplex Master Mix (No ROX™) on the Applied Biosystems™ 7500 Fast Dx Real-Time PCR Instrument.

No positive specimens for influenza A or influenza B were procured during the study. The Negative Percent Agreement (NPA) were calculated relative to the comparator test.

Table 35 Clinical evaluation study for influenza A with NP specimens (7500 Fast Dx Real-Time PCR Instrument)

TaqPath™ COVID-19, FluA, FluB Combo Kit	Comparator test		
	Influenza A Positive	Influenza A Negative	Total
Influenza A Positive	0	0	0
Influenza A Negative	0	381	381
Total	0	381	381

PPA: Not applicable, no positive result by the comparator method

NPA: 100.0% (95% CI: 99.0% LCL – 100.0% UCL)

Table 36 Clinical evaluation study for influenza B with NP specimens (7500 Fast Dx Real-Time PCR Instrument)

TaqPath™ COVID-19, FluA, FluB Combo Kit	Comparator test		
	Influenza B Positive	Influenza B Negative	Total
Influenza B Positive	0	0	0
Influenza B Negative	0	381	381
Total	0	381	381

PPA: Not applicable, no positive result by the comparator method

NPA: 100.0% (95% CI: 99.0% LCL – 100.0% UCL)

Table 37 Clinical evaluation study for influenza A with ANS specimens (7500 Fast Dx Real-Time PCR Instrument)

TaqPath™ COVID-19, FluA, FluB Combo Kit	Comparator test		
	Influenza A Positive	Influenza A Negative	Total
Influenza A Positive	0	0	0
Influenza A Negative	0	382	382
Total	0	382	382

PPA: Not applicable, no positive result by the comparator method

NPA: 100.0% (95% CI: 99.0% LCL – 100.0% UCL)

Table 38 Clinical evaluation study for influenza B with ANS specimens (7500 Fast Dx Real-Time PCR Instrument)

TaqPath™ COVID-19, FluA, FluB Combo Kit	Comparator test		
	Influenza B Positive	Influenza B Negative	Total
Influenza B Positive	0	0	0
Influenza B Negative	0	382	382
Total	0	382	382

PPA: Not applicable, no positive result by the comparator method

NPA: 100.0% (95% CI: 99.0% LCL – 100.0% UCL)



C_t cutoff values for assay targets

The Applied Biosystems™ Pathogen Interpretive Software v1.1 uses the following C_t cutoff values for assay targets during interpretation of the results.

Table 39 Assay C_t cutoff values

Sample or Control	Target	C _t cutoff
Positive Control	MS2	Valid C _t values are >37
	Flu A and Flu B targets	Valid C _t values are ≤45
	SARS-CoV-2 targets	Valid C _t values are ≤37
Negative Control	MS2	Valid C _t values are ≤32
	Flu A and Flu B targets	Valid C _t values are >45
	SARS-CoV-2 targets	Valid C _t values are >37
Clinical samples	MS2	Valid C _t values are ≤32 ^[1]
	SARS-CoV-2 targets	Positive C _t values are ≤37
	Flu A and Flu B targets	Positive C _t values are ≤45

^[1] If any of the viral targets is positive, the C_t for MS2 can be >32.



EUO label for RUO instrument

For the Applied Biosystems™ QuantStudio™ 5 Real-Time PCR Instrument and the Applied Biosystems™ QuantStudio™ 7 Flex Real-Time PCR Instrument, affix the Emergency Use Only (EUO) label on each instrument and retain this labeling throughout the Emergency Use Authorization (EUA) use of the Applied Biosystems™ QuantStudio™ 5 Real-Time PCR Instrument or the Applied Biosystems™ QuantStudio™ 7 Flex Real-Time PCR Instrument.

1. Print the following EUO label:



2. Visibly affix the EUO instrument verification label on your instrument. If the instrument includes labeling indicating “For Research Use Only”, cover with the EUO instrument verification label.



WARNING! GENERAL SAFETY. Using this product in a manner not specified in the user documentation may result in personal injury or damage to the instrument or device. Ensure that anyone using this product has received instructions in general safety practices for laboratories and the safety information provided in this document.

- Before using an instrument or device, read and understand the safety information provided in the user documentation provided by the manufacturer of the instrument or device.
- Before handling chemicals, read and understand all applicable Safety Data Sheets (SDSs) and use appropriate personal protective equipment (gloves, gowns, eye protection, and so on). To obtain SDSs, see the “Documentation and Support” section in this document.

Chemical safety



WARNING! GENERAL CHEMICAL HANDLING. To minimize hazards, ensure laboratory personnel read and practice the general safety guidelines for chemical usage, storage, and waste provided below. Consult the relevant SDS for specific precautions and instructions:

- Read and understand the Safety Data Sheets (SDSs) provided by the chemical manufacturer before you store, handle, or work with any chemicals or hazardous materials. To obtain SDSs, see the "Documentation and Support" section in this document.
- Minimize contact with chemicals. Wear appropriate personal protective equipment when handling chemicals (for example, safety glasses, gloves, or protective clothing).
- Minimize the inhalation of chemicals. Do not leave chemical containers open. Use only with sufficient ventilation (for example, fume hood).
- Check regularly for chemical leaks or spills. If a leak or spill occurs, follow the manufacturer cleanup procedures as recommended in the SDS.
- Handle chemical wastes in a fume hood.
- Ensure use of primary and secondary waste containers. (A primary waste container holds the immediate waste. A secondary container contains spills or leaks from the primary container. Both containers must be compatible with the waste material and meet federal, state, and local requirements for container storage.)
- After emptying a waste container, seal it with the cap provided.
- Characterize (by analysis if needed) the waste generated by the particular applications, reagents, and substrates used in your laboratory.
- Ensure that the waste is stored, transferred, transported, and disposed of according to all local, state/provincial, and/or national regulations.
- **IMPORTANT!** Radioactive or biohazardous materials may require special handling, and disposal limitations may apply.



Biological hazard safety



WARNING! Potential Biohazard. Depending on the samples used on this instrument, the surface may be considered a biohazard. Use appropriate decontamination methods when working with biohazards.



WARNING! BIOHAZARD. Biological samples such as tissues, body fluids, infectious agents, and blood of humans and other animals have the potential to transmit infectious diseases. Conduct all work in properly equipped facilities with the appropriate safety equipment (for example, physical containment devices). Safety equipment can also include items for personal protection, such as gloves, coats, gowns, shoe covers, boots, respirators, face shields, safety glasses, or goggles. Individuals should be trained according to applicable regulatory and company/ institution requirements before working with potentially biohazardous materials. Follow all applicable local, state/provincial, and/or national regulations. The following references provide general guidelines when handling biological samples in laboratory environment.

- U.S. Department of Health and Human Services, *Biosafety in Microbiological and Biomedical Laboratories (BMBL)*, 6th Edition, HHS Publication No. (CDC) 300859, Revised June 2020; found at:
<https://www.cdc.gov/labs/pdf/CDC-BiosafetyMicrobiologicalBiomedicalLaboratories-2020-P.pdf>
- Laboratory biosafety manual, fourth edition. Geneva: World Health Organization; 2020 (Laboratory biosafety manual, fourth edition and associated monographs); found at:
www.who.int/publications/i/item/9789240011311



Documentation and support

Related documentation

Document	Publication Number
<i>Applied Biosystems™ 7500 Fast Dx Real-Time PCR Instrument Reference Guide</i>	4406991
<i>QuantStudio™ 3 and 5 Real-Time PCR Systems Installation, Use, and Maintenance Guide</i>	MAN0010407
<i>QuantStudio™ 6 and 7 Flex Real-Time PCR Systems Maintenance and Administration Guide</i>	4489821
<i>QuantStudio™ 6 and 7 Flex Real-Time PCR Systems Quick Reference</i>	4489826
<i>QuantStudio™ Real-Time PCR Software Getting Started Guide</i>	4489822
<i>MagMAX™ Viral/Pathogen II Nucleic Acid Isolation Kit Instructions For Use</i>	MAN0019746
<i>MagMAX™ Viral/Pathogen Nucleic Acid Isolation Kit (automated extraction) User Guide</i>	MAN0018073
<i>Thermo Scientific™ KingFisher™ Flex User Manual</i>	N07669
<i>Pathogen Interpretive Software Installation Quick Reference</i>	MAN0019535
<i>SAE Administrator Console Dx User Guide</i>	MAN0019574

Customer and technical support

For additional documentation and information about this kit, visit:

<https://www.thermofisher.com/covid19flu>

For download instructions for the software, see “Obtain the software” on page 38.

Refer to the Software Release Notes provided with the software before contacting support for the software.

Visit: <https://www.thermofisher.com/contactus> for service and support information for this kit, including the following:

- Worldwide contact telephone numbers
- Product support information
- Order and web support



- Product documentation such as:
 - Certificates of Analysis
 - Safety Data Sheets (SDSs; also known as MSDSs)

Note: For SDSs for reagents and chemicals from other manufacturers, contact the manufacturer.

Limited product warranty

Life Technologies Corporation and/or its affiliate(s) warrant their products as set forth in the Life Technologies' General Terms and Conditions of Sale at www.thermofisher.com/us/en/home/global/terms-and-conditions.html. If you have any questions, please contact Life Technologies at www.thermofisher.com/support.

