

TaqPath™ COVID-19 Combo Kit

Multiplex real-time RT-PCR test intended for the presumptive qualitative detection of nucleic acid from SARS-CoV-2

Catalog Numbers A47813 and A47814

Pub. No. MAN0019181 Rev. A.0



WARNING! Read the Safety Data Sheets (SDSs) and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves. Safety Data Sheets (SDSs) are available from [thermofisher.com/support](https://www.thermofisher.com/support).

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IVD

For Emergency Use Authorization Only | Rx Only

Intended Use

TaqPath™ COVID-19 Combo Kit contains the assays and controls for a real-time reverse transcription polymerase chain reaction (RT-PCR) test intended for the presumptive qualitative detection of nucleic acid from SARS-CoV-2 in nasopharyngeal swab, nasopharyngeal aspirate, and bronchoalveolar lavage (BAL) specimens from individuals meeting CDC COVID-19 clinical criteria (e.g., clinical signs and symptoms associated with SARS-CoV-2 infection) in conjunction with CDC COVID-19 epidemiological criteria (e.g., history of residence in or travel to a geographic region with active SARS-CoV-2 transmission at the time of travel, or other epidemiologic criteria for which SARS-CoV-2 testing may be indicated).

Testing is limited to laboratories certified under the Clinical Laboratory Improvement Amendments of 1988 (CLIA), 42 U.S.C. §263a, to perform high complexity tests, or by similarly qualified non-U.S. laboratories.

Results are for the identification of SARS-CoV-2 RNA. The SARS-CoV-2 RNA is generally detectable in nasopharyngeal swab, nasopharyngeal aspirate, and bronchoalveolar lavage (BAL) specimens 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 viruses. The agent detected may not be the definite cause of disease. Laboratories within the United States and its territories are required to report all positive results to the appropriate public health authorities.

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

Testing with the TaqPath™ COVID-19 Combo Kit is intended for use by trained clinical laboratory personnel specifically instructed and trained in the techniques of real-time PCR and *in vitro* diagnostic procedures. The TaqPath™ COVID-19 Combo Kit is only for use under the Food and Drug Administration's Emergency Use Authorization.

Product description

The TaqPath™ RT-PCR COVID-19 Kit, packaged as part of the TaqPath™ COVID-19 Combo Kit, includes the assays and controls for a multiplex real-time RT-PCR test for the presumptive qualitative detection of RNA from SARS-CoV-2 in nasopharyngeal swab, nasopharyngeal aspirate, and bronchoalveolar lavage (BAL) specimens from patients who meet CDC COVID-19 clinical criteria in conjunction with CDC COVID-19 epidemiological criteria. TaqPath™ COVID-19 Combo Kit includes the following components:

- TaqPath™ RT-PCR COVID-19 Kit
 - COVID-19 Real Time PCR Assay Multiplex—Multiplexed assays that contain three primer/probe sets specific to different SARS-CoV-2 genomic regions and primers/probes for bacteriophage MS2
 - MS2 Phage Control—Internal process control for nucleic acid extraction
- TaqPath™ COVID-19 Control—RNA control that contains targets specific to the SARS-CoV-2 genomic regions targeted by the assays

Contents and storage

Table 1 TaqPath™ COVID-19 Combo Kit (Cat. No. A47813)

Component	Description	Amount	Storage
TaqPath™ RT-PCR COVID-19 Kit, 100 reactions (Cat. No. A47815) ^[1]	COVID-19 Real Time PCR Assay Multiplex (Gene Orf-1ab, N Protein, S Protein, MS2)	150 µL	–30°C to –10°C
	MS2 Phage Control	2 × 500 µL	–30°C to –10°C
TaqPath™ COVID-19 Control Kit (Cat. No. A47816) ^[1]	TaqPath™ COVID-19 Control (1 × 10 ⁴ copies/µL)	2 × 10 µL	≤ –70°C
	TaqPath™ COVID-19 Control Dilution Buffer	2 × 250 µL	–30°C to –10°C

^[1] This kit can be ordered as a stand-alone kit.

Table 2 TaqPath™ COVID-19 Combo Kit, 1,000 reactions (Cat. No. A47814)

Component	Description	Amount	Storage
TaqPath™ RT-PCR COVID-19 Kit, 1,000 reactions (Cat. No. A47817) ^[1]	COVID-19 Real Time PCR Assay Multiplex (Gene Orf-1ab, N Protein, S Protein, MS2)	1,500 µL	–30°C to –10°C
	MS2 Phage Control	20 × 500 µL	–30°C to –10°C
TaqPath™ COVID-19 Control Kit (Cat. No. A47816) ^[1]	TaqPath™ COVID-19 Control (1 × 10 ⁴ copies/µL)	2 × 10 µL per kit; 5 kits included	≤ –70°C
	TaqPath™ COVID-19 Control Dilution Buffer	2 × 250 µL per kit; 5 kits included	–30°C to –10°C

^[1] This kit can be ordered as a stand-alone kit.

Required materials not supplied

Unless otherwise indicated, all materials are available through thermofisher.com. "MLS" indicates that the material is available from fisherscientific.com or another major laboratory supplier.

Item	Source
Real-time PCR instrument and equipment	
Applied Biosystems™ 7500 Fast Dx Real-Time PCR Instrument (used with SDS Software v1.4.1)	4406984 (with laptop computer) 4406985 (with tower computer)
Laboratory freezers <ul style="list-style-type: none"> • -30°C to -10°C • ≤ -70°C 	MLS
Centrifuge, with a rotor for microplates	MLS
Microcentrifuge	MLS
Laboratory mixer, Vortex or equivalent	MLS
Single and multichannel adjustable pipettors (1.00 µL to 1,000.0 µL)	MLS
Cold block 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™ Deepwell 96 Plate	95040450
KingFisher™ 96 KF microplate	97002540
KingFisher™ 96 tip comb for DW magnets	97002534
Kits and reagents	
MagMAX™ Viral/Pathogen Nucleic Acid Isolation Kit	A42352 (100 preparations) Also available for 1,000 preparations
TaqPath™ 1-Step Multiplex Master Mix (No ROX™)	A28521, A28522, A28523
100% ethanol, ACS reagent grade or equivalent	MLS
Nuclease-free Water (not DEPC-Treated)	MLS
Tubes, plates, and other consumables	
ABY™ Dye Spectral Calibration Plate for Multiplex qPCR, Fast 96-well	A24734
JUN™ Dye Spectral Calibration Plate for Mutlplex qPCR, Fast 96-well	A24735
MicroAmp™ Fast Optical 96-Well Reaction Plate with Barcode, 0.1 mL	4346906, 4366932
MicroAmp™ Clear Adhesive Film	4306311
MicroAmp™ Optical Adhesive Film	4311971 and 4360954
MicroAmp™ Adhesive Film Applicator	4333183
Nonstick, RNase-free microcentrifuge tubes (1.5 mL and 2.0 mL)	thermofisher.com/plastics
Sterilize aerosol barrier (filtered) pipette tips	thermofisher.com/pipettetips
Data analysis software	
Applied Biosystems™ COVID-19 Interpretive Software v1.0 ^[1]	InstrumentServices@thermofisher.com or 1 800 955 6288 (Select option 3, then option 1)

^[1] For software installation instructions, see *COVID-19 Interpretive Software Installation Quick Reference* (Pub. No. MAN0019184).

Warnings and precautions

The TaqPath™ RT-PCR COVID-19 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 under a laminar airflow hood or biological safety cabinet.

- The assay is for *in vitro* diagnostic use under the FDA Emergency Use Authorization Only.
- Specimens 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.
- Do not use the kit after the indicated expiry date.
- Dispose of waste in compliance with the local, state, and federal regulations.
- Safety Data Sheets are available upon request.

Samples and controls

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

Include the following controls:

Control	Used to monitor	Assays
Positive Control (TaqPath™ COVID-19 Control Kit)	RT-PCR reaction setup and reagent integrity	All three SARS-CoV-2 assays
MS2 Phage Control	RNA extraction	MS2 assay
Negative Control	Cross-contamination during RNA extraction and reaction setup	All three SARS-CoV-2 assays
		MS2 assay

Workflow

Extract RNA from patient sample using the MagMAX™ Viral/Pathogen Nucleic Acid Isolation Kit



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



Analyze data using the Applied Biosystems™ COVID-19 Interpretive Software



Review results interpretation for patient samples

The workflow begins with nucleic acid extraction from nasopharyngeal swab, nasopharyngeal aspirate, or bronchoalveolar lavage (BAL) specimens which arrive in the testing site in Universal Viral Transport Media (VTM). Nucleic acids are isolated and purified from the specimens using the KingFisher™ Flex Purification System (KingFisher) and the MagMAX™ Viral/Pathogen Nucleic Acid Isolation Kit.

Instructions for extracting RNA using the MagMAX™ Viral/Pathogen Nucleic Acid Isolation Kit are found in the *MagMAX™ Viral/Pathogen Nucleic Acid Isolation Kit (automated extraction) User Guide* (Pub. No. MAN0018073).

The purified nucleic acid is reverse transcribed into cDNA and amplified using the TaqPath™ RT-PCR COVID-19 Kit and the Applied Biosystems™ 7500 Fast Dx Real-Time PCR instrument. In the process, the probes anneal to three (3) specific target sequences located between three (3) unique forward and reverse primers for the following genes:

- ORF1ab
- N Protein
- S Protein

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 Applied Biosystems™ 7500 Fast Dx Real-Time PCR instrument.

The data are analyzed, then interpreted by the Applied Biosystems™ COVID-19 Interpretive Software.

Extract RNA with the MagMAX™ Viral/Pathogen Nucleic Acid Isolation Kit

Before you begin

- Determine the number of required reactions based on the number patient samples to be processed, plus one Negative Control per plate.
- Prepare fresh 80% Ethanol using 100% absolute Ethanol and Nuclease-free Water (not DEPC-Treated), sufficient for 1.5 mL per reaction, plus 10% overage.
- Label each KingFisher™ Deepwell 96 Plate (5):

Label	Number of plates
Sample plate	1
Wash 1	1
Wash 2	1
Wash 3	1
Elution plate	1

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

Label	Number of plates
Tip comb	1

- Mark the Negative Control well on the plate.

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 proper program (**MVP_Flex**) has been downloaded from the product page 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, 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™ Deepwell 96 Plate	Wash Buffer	1,000 µL
Wash 2 Plate	3		80% Ethanol	1,000 µL
Wash 3 Plate	4		80% Ethanol	500 µL
Elution Plate	5		Elution Solution	50 µL
Tip Comb	6	Place a KingFisher™ 96 tip comb for DW magnets in a KingFisher™ 96 KF microplate		

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 homogenous.
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 sample plate

1. Add 10 µL of Proteinase K to each well in the KingFisher™ Deepwell 96 Plate labeled "Sample Plate".
2. Add samples and controls to the appropriate wells:

Component	Instructions
Sample	Add 400 µL of sample to a well.
Negative Control	Add 400 µL of Nuclease-free Water (not DEPC-Treated) to the Negative Control well.

3. Invert the Binding Bead Mix five 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 mixture containing the Binding Beads is viscous. Therefore, 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 volume added.

4. Add 10 µL of MS2 Phage Control to each sample well and to the Negative Control well.

Process the samples

1. Select the program **MVP_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 (~25 minutes after start), immediately remove the Elution Plate from the instrument, then cover the plate.

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

Store the Elution Plate on ice for immediate use in real-time RT-PCR or seal the plate and store at -20°C for up to 1 month, or at ≤ 70°C for long-term storage.

Perform RT-PCR

Guidelines for RT-PCR with the TaqPath™ RT-PCR COVID-19 Kit

IMPORTANT!

- To prevent contamination, prepare reagents in a PCR workstation or equivalent amplicon-free area. Do not use the same pipette for controls and samples, and always use aerosol barrier pipette tips.
- Maintain an RNase-free environment.
- Protect assays from light.
- Keep samples and components on ice during use.
- Prepare the run plate on ice.
- Run the plate within two hours of preparation.
- Include one Positive Control and one Negative Control on each plate.

Prepare the RT-PCR reactions

1. If frozen, thaw the purified nucleic acid samples and reagents on ice.
 2. Gently vortex the samples and reagents, then centrifuge briefly to collect liquid at the bottom of the tube or sample plate.
 3. **Prepare the Positive Control**—Dilute TaqPath™ COVID-19 Control (1×10^4 copies/ μL) to a working stock of 25 copies/ μL .
 - a. Pipet 98 μL of TaqPath™ COVID-19 Control Dilution Buffer into a microcentrifuge tube, then add 2 μL of TaqPath™ COVID-19 Control. Mix well, then centrifuge briefly.
 - b. Pipet 87.5 μL of TaqPath™ COVID-19 Control Dilution Buffer into a second microcentrifuge tube, then add 12.5 μL of the dilution created in substep 3a. Mix well, then centrifuge briefly.
- Note:** The TaqPath™ COVID-19 Control does not contain the MS2 template.
4. **Prepare the Reaction Mix**—For each run, combine the following components sufficient for the number of tests, plus one Positive Control and one Negative Control.

All volumes include 10% overage for pipette error.

Component	Volume per Sample or Control	Volume for n Samples plus 2 Controls	Volume for 94 Samples plus 2 Controls
TaqPath™ 1-Step Multiplex Master Mix (No ROX™) (4X)	6.25 μL	$6.875 \times (n + 2)$ μL	660 μL
COVID-19 Real Time PCR Assay Multiplex	1.25 μL	$1.375 \times (n + 2)$ μL	132 μL
Nuclease-free Water	12.50 μL	$13.75 \times (n + 2)$ μL	1320 μL
Total Reaction Mix volume	20.0 μL	—	2112 μL

5. **Set up the reaction plate**—Pipette 20.0 μL 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, then combine with the Sample or the Control according to the following table.

Component	Volume per reaction		
	Sample reaction	Purified Positive Control (TaqPath™ COVID-19 Control)	Negative Control reaction
Reaction Mix	20.0 μL	20.0 μL	20.0 μL
Purified Sample nucleic acid	5.0 μL	—	—
Positive Control (TaqPath™ COVID-19 Control) ^[1]	—	2.0 μL	—
Nuclease-free Water	—	3.0 μL	—
Purified Negative Control	—	—	5.0 μL
Total volume	25.0 μL	25.0 μL	25.0 μL

^[1] From step 3.

Set up and run the Applied Biosystems™ 7500 Fast Dx Real-Time PCR instrument

See the *Applied Biosystems™ 7500 Fast Dx Real-Time PCR Instrument Reference Guide* (Pub. No. 4406991) for detailed instructions.

The instrument must be calibrated for ABY™ dye and JUN™ dye.

1. From the laptop or tower computer, open the SDT file.

The SDT file is at the following location: <installation directory>\Applied Biosystems\COVID-19 Interpretive Software Client\docs\User Documents.

The SDT file contains the settings for the run.

2. Confirm the run settings.

- 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. Using the **Detector Manager** in the **Tools** menu create the following detectors with the quencher set as none. The detector name must be an exact match with the names shown in the table below, including upper and lower case letters.

Reporter dye	Detector
FAM	ORF1ab
VIC	N gene
ABY	S gene
JUN	MS2

4. Set up the plate layout by assigning a unique sample name to each well.

5. Assign a **Task** to each well.

- **Unknown**—for patient samples
- **Standard**—for Positive Control
- **NTC**—for Negative Control

IMPORTANT! Ensure that **Standard** is used for the Positive Control and that **NTC** is used for the Negative Control.

6. Confirm the thermal protocol.

Step	Temperature	Time	Number of cycles
UNG incubation	25°C	2 minutes	1
Reverse transcription	53°C	10 minutes	1
Activation	95°C	2 minutes	1
Denaturation	95°C	3 seconds	40
Anneal / extension	60°C	30 seconds	

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

8. In the **Reason for Change Entry** dialog box, enter a description, then click **OK**.

9. Reopen the file, load the plate, then start the run on the Applied Biosystems™ 7500 Fast Dx Real-Time PCR instrument.

Analyze the data

(Required) To obtain the Applied Biosystems™ COVID-19 Interpretive Software, contact InstrumentServices@thermofisher.com or 1 800 955 6288 (Select option 3, then option 1).

For detailed instructions about using the software, click the **Help** menu to access the *COVID-19 Interpretive Software Help*.

1. In the COVID-19 Interpretive Software **Home** screen, click the **Import Samples** button.
2. Select the SDS files to import, then click **Open**.
After import, the software analyzes the run data, performs Quality Check (QC) analysis, and calculates the interpretive results for each sample and control.
3. In the **Batches** pane of the **Home** screen, select a batch to view the status and result for each sample in the **Samples** list.
4. To generate a batch export file (CSV or XLSX), select the checkbox for the batch, then click the **Export Batch** button at the top of the **Home** screen. Click **Open folder location** in the dialog box, then navigate to the exported file.
5. To generate a batch report file (PDF), select the checkbox for the batch, then click the **Report Batch** button at the top of the **Home** screen. Click **Open folder location** in the dialog box, then navigate to the report.

Results interpretation for patient samples

Results interpretation is performed by the Applied Biosystems™ COVID-19 Interpretive Software.

Table 3 Result interpretation for patient samples

ORF1ab	N gene	S gene	MS2	Status	Result	Action
NEG	NEG	NEG	NEG	Invalid	NA	Repeat test. If the repeat result remains invalid, consider collecting a new specimen.
NEG	NEG	NEG	POS	Valid	SARS-CoV-2 Not Detected	Report results to healthcare provider. Consider testing for other viruses
Only one SARS-CoV-2 target = POS			POS or NEG	Valid	SARS-CoV-2 Inconclusive ^[1]	Repeat test. If the repeat result remains inconclusive, contact CDC immediately for instructions for transfer of the specimen to CDC for additional testing and guidance.
Two or more SARS-CoV-2 targets = POS			POS or NEG	Valid	Presumptive Positive SARS-CoV-2	Report results to healthcare provider and CDC. Contact CDC immediately for instructions for transfer of the specimen to CDC for additional testing and guidance.

^[1] Samples with a result of SARS-CoV-2 Inconclusive shall be retested one time.

Assay limitations

- The use of this assay as an *In vitro* diagnostic under the FDA Emergency Use Authorization (EUA) is limited to laboratories that are certified under the Clinical Laboratory Improvement Amendments of 1988 (CLIA), 42 U.S.C. § 263a, to perform high complexity tests.
- The TaqPath™ RT-PCR COVID-19 Kit performance was established using nasopharyngeal swab, nasopharyngeal aspirate, and bronchoalveolar lavage samples only. Other specimen types have not been evaluated and should not be tested with this assay.
- 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 the specified methods listed in this procedure. Other extraction approaches and processing systems have not been evaluated.
- False-negative results may arise from:
 - Improper sample collection
 - Degradation of the viral RNA during shipping/storage
 - Specimen collection after nucleic acid can no longer be found in the specimen matrix
 - Using unauthorized extraction or assay reagents
 - The presence of RT-PCR inhibitors
 - Mutation in the SARS-CoV-2 virus

- 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
- The impacts of vaccines, antiviral therapeutics, antibiotics, chemotherapeutic or immunosuppressant drugs have not been evaluated. The TaqPath™ RT-PCR COVID-19 Kit cannot rule out diseases caused by other bacterial or viral pathogens.
- Negative results do not preclude infection with SARS-CoV-2 virus, and should not be the sole basis of a patient management decision.
- A patient matched serum specimen is required for serological follow up testing of all positive RT-PCR results, per the CDC testing algorithm.
- Laboratories are required to report all positive results to the appropriate public health authorities.

Conditions of authorization for labs

The TaqPath™ RT-PCR COVID-19 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/MedicalDevices/Safety/EmergencySituations/ucm161496.htm>.

However, to assist clinical laboratories using the TaqPath™ RT-PCR COVID-19 Kit, the relevant Conditions of Authorization are listed below.

- Authorized laboratories will include with reports of the results of the TaqPath™ RT-PCR COVID-19 Kit the authorized Fact Sheet for Healthcare Providers and the authorized Fact Sheet for Patients. Under exigent circumstances, other appropriate methods for disseminating these Fact Sheets may be used, which may include mass media.
- Authorized laboratories will perform RT-PCR with the TaqPath™ RT-PCR COVID-19 Kit using sample collected via nasopharyngeal swab, nasopharyngeal aspirate, bronchoalveolar lavage, or other authorized specimen types.
- Authorized laboratories will perform RT-PCR with the TaqPath™ RT-PCR COVID-19 Kit using samples extracted using the KingFisher™ Flex Magnetic Particle Processor with 96 Deep-Well Head and MagMAX™ Viral/Pathogen Nucleic Acid Isolation Kit or with other authorized extraction methods.
- Authorized laboratories will perform RT-PCR with the TaqPath™ RT-PCR COVID-19 Kit on the Applied Biosystems™ 7500 Fast Dx Real-Time PCR instrument, or other authorized instruments.
- Authorized laboratories will have a process in place for reporting test results to healthcare providers and relevant public health authorities, as appropriate.
- Authorized laboratories will collect information on the performance of the test and report to DMD/OIR/CDRH (via email CDRH-EUA-Reporting@fda.hhs.gov) and Thermo Fisher Scientific any suspected occurrence of false positive or false negative results of which they become aware.
- All laboratory personnel using the test should 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, will ensure that any records associated with this EUA are maintained until notified by FDA. Such records will be made available to FDA for inspection upon request.

Performance characteristics

Analytical performance of the TaqPath™ RT-PCR COVID-19 Kit was evaluated by determining limit of detection (LoD), characterizing the impact of interfering substances and cross-reactivity, as described in the following sections.

Limit of detection (LoD)

The LoD study established the lowest SARS-CoV-2 viral concentration (Genomic Copy Equivalents or GCE) that can be detected by the TaqPath™ COVID-19 Combo Kit in a particular specimen type at least 95% of the time. Banked Nasopharyngeal swab (NP) and Bronchoalveolar lavage (BAL) samples, obtained from U.S. patients in the years 2015-2019, were pooled, respectively, and spiked with purified SARS-CoV-2 RNA at several concentrations and processed through the TaqPath™ COVID-19 Combo Kit workflow. A three-phase approach was used to determine the LoD for each specimen type.

Table 4 LoD results

Specimen type	Limit of Detection (GCE/mL)	Limit of Detection (GCE/reaction)
Bronchoalveolar lavage	250 GCE/mL	10 GCE/reaction
Nasopharyngeal swab	250 GCE/mL	10 GCE/reaction

Reactivity (Inclusivity)

The assays were mapped to 185 complete SARS-CoV-2 genomes of human host in GenBank and GISAID databases as of March 5, 2020. Primer and probes sequences for SARS-CoV-2 ORF1ab, S gene, and N gene assays had 100% homology to all SARS-CoV-2 isolates analyzed, with one exception. EPI_ISL_407084 showed a mismatch at position 7 from the 5' end of the reverse primer (23 nt length) corresponding to 95.6% homology. The mismatch is located at the 5' end of the primer and does not affect the test performance.

Cross-reactivity

In silico analysis of the following forty-two (42) organisms:

Table 5 Organisms used for *in silico* cross-reactivity analysis

Organism
Human coronavirus 229E
Human coronavirus OC43
Human coronavirus HKU1
Human coronavirus NL63
SARS-coronavirus
MERS-coronavirus
Adenovirus
Human Metapneumovirus (hMPV)
Parainfluenza 1
Parainfluenza 2
Parainfluenza 3
Parainfluenza 4
Influenza A
Influenza B
Influenza C
Enterovirus
Respiratory Syncytial Virus A
Respiratory Syncytial Virus B
Rhinovirus/Enterovirus
Parechovirus
<i>Candida albicans</i>
<i>Corynebacterium diphtheriae</i>
<i>Legionella</i> (non-pneumophila)
<i>Bacillus anthracis</i> (Anthrax)
<i>Moraxella catarrhalis</i>
<i>Neisseria elongata</i> and <i>N. meningitidis</i>
<i>Pseudomonas aeruginosa</i>
<i>Staphylococcus epidermidis</i>
<i>Streptococcus salivarius</i>
<i>Leptospira</i> sp.
<i>Chlamydophila pneumoniae</i>
<i>Chlamydophila psittaci</i>
<i>Coxiella burnetii</i> (Q-Fever)
<i>Staphylococcus aureus</i>
<i>Haemophilus influenzae</i>
<i>Legionella pneumophila</i>

Organism
<i>Mycobacterium tuberculosis</i>
<i>Streptococcus pneumoniae</i>
<i>Streptococcus pyogenes</i>
<i>Bordetella pertussis</i>
<i>Mycoplasma pneumoniae</i>
<i>Pneumocystis jirovecii</i> (PJP)

Blast analysis showed $\geq 80\%$ homology for one assay component (forward primer, reverse primer, or probe) for select isolates. Despite $\geq 80\%$ homology of one assay component for select isolates, there is no anticipated amplification because hybridization of all three assay components are necessary to generate a signal. We also found instances where different assay components had $\geq 80\%$ homology to different isolates of the same species. For example, *Bacillus anthracis* strain AFS029987 had $\geq 80\%$ homology to the ORF1ab forward primer while strain MCCC 1A01412 had $\geq 80\%$ homology to the ORF1ab reverse primer. Since these are two different organisms, amplification will not occur. The *in silico* analysis indicates that significant amplification of non-target sequences will not occur that result in cross-reactivity or potentially interfere with detection of SARS-CoV-2.

Clinical evaluation

A clinical evaluation study was performed to evaluate the performance of the TaqPath™ RT-PCR COVID-19 Kit using nasopharyngeal swab (NP) and bronchoalveolar lavage (BAL) specimens.

A total of sixty (60) contrived positive specimens were tested:

- 30 contrived positive nasopharyngeal swab (NP) specimens
- 30 contrived positive bronchoalveolar lavage (BAL) specimens

Samples were contrived by spiking known concentrations of isolated SARS-CoV-2 RNA, relative to the product LoD, into matrices which were determined to be negative by the TaqPath™ RT-PCR COVID-19 Kit prior to spiking in the RNA.

In addition to the contrived positive specimens, sixty (60) negative specimens were tested:

- 30 negative nasopharyngeal swab (NP) specimens
- 30 negative samples bronchoalveolar lavage (BAL) specimens

Final clinical evaluation study outcome as shown below:

Table 6 BAL Clinical Evaluation Study

Final RNA Concentration in Sample	Interpretation
5X LoD	5/5 presumptive positive
3X LoD	5/5 presumptive positive
2X LoD	20/20 presumptive positive
Negative	30/30 not detected

Table 7 NP Clinical Evaluation Study

Final RNA Concentration in Sample	Interpretation
5X LoD	5/5 presumptive positive
3X LoD	5/5 presumptive positive
2X LoD	20/20 presumptive positive
Negative	30/30 not detected

Related documentation

Document	Publication Number
<i>Applied Biosystems™ 7500 Fast Dx Real-Time PCR Instrument Reference Guide</i>	4406991
<i>MagMAX™ Viral/Pathogen Nucleic Acid Isolation Kit (automated extraction) User Guide</i>	MAN0018073
<i>COVID-19 Interpretive Software Installation Quick Reference</i>	MAN0019184
<i>TaqPath™ COVID-19 Combo Kit Instructions for Use</i>	100092995

Customer and technical support

Visit thermofisher.com/support for the latest service and support information.

- Worldwide contact telephone numbers
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 - Product FAQs
 - Software, patches, and updates
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- Order and web support
- Product documentation
 - User guides, manuals, and protocols
 - Certificates of Analysis
 - Safety Data Sheets (SDSs; also known as MSDSs)

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

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

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Revision history: Pub. No. MAN0019181

Revision	Date	Description
A.0	12 March 2020	New document.

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