OPTI SARS CoV 2/ Influenza A/B RT PCR Test Version 1

English Version

Used for real-time PCR identification and differentiation of SARS-CoV-2, Influenza A and/or Influenza B RNA extracted from nasopharyngeal swabs.

99-57014	OPTI SARS-CoV-2 / Influenza A/B RT-PCR Test Version 1 500/Box
99-57016	OPTI SARS-CoV-2 / Influenza A/B RT-PCR Test Version 1 5000/Box

IVD CE Rx

For *in vitro* diagnostic use
For use under Emergency Use Authorization (EUA) only
For Prescription Use only

REF 99-57014, 99-57016

Version 06-57014-01

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Intended Use

The OPTI SARS-CoV-2/Influenza A/B RT-PCR Test is a multiplex real-time reverse transcription polymerase chain reaction test intended for the simultaneous qualitative detection and differentiation of SARS-CoV-2, Influenza A, and/or Influenza B virus RNA in nasopharyngeal 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 requirements to perform high complexity tests.

The OPTI SARS-CoV-2/Influenza A/B RT-PCR Test is intended for use in the simultaneous detection and differentiation of SARS-CoV-2, Influenza A, and Influenza B nucleic acid in nasopharyngeal swab specimens, and is not intended to detect Influenza C virus. RNA from SARS-CoV-2, Influenza A, and/or Influenza B is generally detectable in nasopharyngeal swab specimens during the acute phase of infection. Positive results are indicative of the presence of SARS-CoV-2, influenza A, and/or influenza B RNA; clinical correlation with patient history and other diagnostic information is necessary to determine patient infection status. Positive results 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 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 patient management decisions. Negative results must be combined with clinical observations, patient history, and/or epidemiological information.

The OPTI SARS-CoV-2/Influenza A/B RT-PCR Test is intended to be used by qualified laboratory personnel specifically instructed and trained in the techniques of real-time PCR and in vitro diagnostic procedures. The OPTI SARS-CoV-2/Influenza A/B RT-PCR Test is only for use under the Food and Drug Administration's Emergency Use Authorization.

Product Description

The OPTI SARS-CoV-2/Influenza A/B RT-PCR Test is a real-time reverse transcription polymerase chain reaction (rRT-PCR) test. OPTI SARS-CoV-2/Flu RNA Mix (SARS-CoV-2/Flu Mix) includes primers and probes for the detection of SARS-CoV-2, Influenza A and Influenza B RNA when amplified with the OPTI RNA Master Mix (RNA MMx). Influenza A is detected in the FAM channel; Influenza B is detected in the NED channel and SARS-CoV-2 RNA targets (N1 and N4) are detected in the Cy5 channel. The internal control is based on the detection of a conserved nucleic acid sequence present in human samples and is detected in the VIC™ channel. Detection of endogenous nucleic acid in the test sample, controls for sample addition, extraction, and amplification. Primers and probe for detection of the internal control are included in the SARS-CoV-2/Flu Mix.

During the real-time reverse transcription polymerase chain reaction, viral RNA is reverse transcribed

into cDNA and subsequently amplified in a real-time PCR cycling protocol. During the process, the probe anneals to a specific target sequence located between the forward and reverse primers. 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 exponentially. Fluorescence intensity is monitored at each PCR cycle by one of the PCR thermal cycler instruments listed in Section "Materials Required but Not Provided".

In addition, the OPTI SARS-CoV-2/Influenza A/B RT-PCR Test utilizes the OPTI SARS-CoV-2/Flu PC (Positive Control) and OPTI PCR Grade Water (Negative Control). The OPTI SARS-CoV-2/Flu PC contains SARS-CoV-2 (N1), Influenza A, Influenza B and internal control synthetic material and works as a positive control for each target in the reaction. OPTI PCR Grade Water is used as the RT-PCR negative control, as well as to reconstitute the dried SARS-CoV-2/Flu Mix and the PC.

Materials and Storage

Identification/ General Information	Cap color	Quantity	Storage		Freeze/Thaw cycles
			At receipt	After reconstitution	
OPTI SARS-CoV-2/Flu Mix (SARS-CoV-2/Flu Mix), dried **61-56625-00 (500 reactions)** **61-56635-00 (5,000 reactions)**	Red	5 x 1.0 mL 5 x 10 mL	–25 to 8°C	–25 to –15°C	≤6
Contains primers and probes for SARS-CoV-2 (N1 and N4), Influenza A, Influenza B and internal control. Reconstitute to 1 mL (in 500 reaction kit) or 10 mL (in 5,000 reaction kit) in PCR Grade Water. Store the SARS-CoV-2/Flu Mix in the dark. The expiration date on the vial is valid for either the dry or reconstituted form.					
OPTI RNA Master Mix (RNA MMx) **61-56618-00 (500 reactions)** **61-56638-00 (5,000 reactions)**	Black	5 x 1.0 mL 5 x 10 mL	–25 to –15°C (Long-term)	N/A	≤6
Concentrated master mix that includes reverse transcriptase and hot-start polymerase. The RNA MMx is more viscous than most master mixes– see the Test Procedure section for handling recommendations. A reference dye (ROX) has been added for normalizing volume inaccuracies. Protect the RNA MMx from light.					
OPTI SARS-CoV-2/Flu PC, dried (PC) 44-56627-01	Blue	1 x 1.0 mL	–25 to 8°C	–25 to –15°C	≤6
The PC contains the targets for SARS-CoV-2 (N1 target region), Influenza A, Influenza B and the internal control. Reconstitute to 1 mL in PCR Grade Water. The expiration date on the vial is valid for either the dry or reconstituted form.					
OPTI PCR Grade Water **61-56619-00 (500 reactions)** **61-56639-00 (5,000 reactions)**	Clear	7 x 1.0 mL 3 x 25 mL	–25 to 8°C		N/A
PCR Grade Water has been qualified for reverse transcription-PCR (RT-PCR) use. It is used for the reconstitution of the SARS-CoV-2/Flu Mix and PC. It is also used as the PCR negative control for each test run.Do not transport PCR Grade Water vials between PCR work areas. Separate vials of water are needed for each area to avoid contamination risk.					

Note: See table at the end of the insert for a description of symbols used on the insert and labels.

Materials Required but Not Provided

Real-Time PCR Instrument and consumables	Source and part number
Thermo Scientific	
Applied Biosystems® 7500 FAST Applied Biosystems® QuantStudio 5 (96-well)	7500 instrument (4351106) and 7500 software v2.0.6 QS5 instrument (A28138) and QuantStudio Design and Analysis Desktop software (v1.5.1 and v2.5.1)
96 well PCR plate 384 well PCR plate Optical plate cover	plate: 4346906 plate: 4309849 cover: 4311971
Extraction Equipment and Consumables	**Source and part number**
OPTI DNA/RNA Magnetic Bead Kit	OPTI Medical Systems 99-58015
Thermo Scientific	
Thermo Scientific™ KingFisher™ Flex 96 deep well plate 96 well elution plate 96 tip comb for deep well magnet	Flex instrument (5400630) and software v1.0.1.0 Deep well plate: 95040460 Elution plate: 97002540 Tip Comb: 97002534
Extraction control containing human specimen (HSC) material	See Quality Controls section
96-well cold plate	MLS
Micro-centrifuge for 2 mL microtubes capable of 1500–3000 x g	MLS
Vortex mixer	MLS
1.5 mL microcentrifuge tubes (DNase/ RNase free)	MLS
Pipettes and multi-channel pipettes (5–1000 µL); dedicated pipettes for preparation of PCR Mix	MLS
Nuclease-free, aerosol resistant pipette tips	MLS
Personal protective equipment consistent with current guidelines for handling infectious samples	MLS
Optional: Centrifuge with rotor and adapters for multi-well plates	MLS
PCR plate cooler	MLS
External Positive Control	**Source and part number**
Option 1 (Extraction required) NATtrol™ Flu/RSV/SARS-CoV-2 Positive Control	Zeptometrix (Catalogue# NATFRC-6C)
Option 2 (No extraction required) Twist synthetic influenza H1N1 RNA (2009) RNA Control *and* Twist synthetic influenza B RNA Control *and*	Twist Bioscience (Catalogue# 103001) Twist Bioscience (Catalogue#103003)

Twist synthetic SARS-CoV-2 RNA Control Option 3 (No extraction required) Genomic RNA from influenza A/Indiana/10/2011 *and* Genomic RNA from influenza B/Wisconsin/1/2010 *and* Genomic RNA from SARS-CoV-2, Isolate USA-WA/2020	Twist Bioscience (Catalogue#102024) International Reagent Resources (Catalogue# FR986) International Reagent Resources (Catalogue# FR1044) BEI resources (Catalogue# NR-52347)

MLS = Major Laboratory Supplier, such as VWR, Fisher Scientific, Eppendorf.

Warnings and Precautions

General

- For *in vitro* diagnostic (IVD) use.
- For prescription use only.
- For use under Emergency Use Authorization (EUA) 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 and differentiation of nucleic acid from SARS-CoV-2, influenza A, and/or 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.
- Handle all specimens as infectious using safe laboratory procedures. Refer to Interim Laboratory Biosafety Guidelines for Handling and Processing Specimens Associated with SARS-CoV-2: https://www.cdc.gov/coronavirus/2019-ncov/lab/lab-biosafety-guidelines.html
- Use personal protective equipment (PPE) consistent with current guidelines for the handling of potentially infectious samples.
- Do not eat, drink, smoke, apply cosmetics or handle contact lenses in areas where reagents and human specimens are handled.
- Modifications to assay reagents, assay protocol, or instrumentation are not permitted, and are in violation of the product Emergency Use Authorization.
- Dispose of waste in compliance with the local, state, and federal regulations.

PCR

- Reagents must be stored and handled as specified in these instructions for use. Do not use reagents past expiration date.
- The entire procedure must be performed under nuclease-free conditions.
- Wear powder-free gloves when working with the reagents and nucleic acids.
- Always use pipette tips with aerosol barriers. Tips that are used must be sterile and free from DNases and RNases.
- Keep reagents and PCR Mix tubes capped or covered as much as possible.
- To avoid cross-contamination, use nuclease-free, aerosol-resistant pipette tips for all pipetting, and physically separate the workplaces for nucleic acid extraction/handling, PCR setup and PCR.
- Work surfaces, pipettes, and centrifuges should be cleaned and decontaminated with cleaning products such as 10% bleach, "DNAZap™" or "RNase AWAY®" to minimize risk of nucleic acid contamination. Residual bleach should be removed using 70% ethanol.
- The internal control for the test detects human nucleic acid; it is important to avoid environmental sources of human nucleic acid contamination.

Specimen Collection

- The sample collection device is not a part of the test kit. The OPTI SARS-CoV-2/Influenza A/B RT-PCR Test is compatible with Universal Transport Medium (UTM) and CDC recommended swabs (https://www.cdc.gov/coronavirus/2019-nCoV/guidelines-clinical-specimens.html).
- Follow specimen collection manufacturer instructions for proper collection methods.
- Swab specimens should be collected using only swabs with a synthetic tip, such as nylon or Dacron® and an aluminum or plastic shaft. Calcium alginate swabs should not be used and cotton swabs with wooden shafts are not recommended. Place swabs immediately into sterile tubes containing 2–3 mL of universal transport medium.

Transporting Specimens

- Specimens must be packaged, shipped, and transported according to the current edition of the International Air Transport Association (IATA) Dangerous Goods Regulation. Follow shipping regulations for UN 3373 Biological Substance, Category B when sending potential 2019-nCoV specimens.
- Store specimens at 2–8°C and ship on ice packs.

Storing Specimens

- Specimens can be stored at 2–8°C for up to 72 hours after collection.
- If a delay in extraction is expected, store specimens at –70°C or lower temperature, per CDC guidelines.

Reconstitution of Dried Components

Reconstitute the SARS-CoV-2/Flu Mix and SARS-CoV-2/Flu PC by pipetting PCR Grade Water to the volume indicated on the component label. Allow to sit at 18 to 26°C for at least 10 minutes; mix and microcentrifuge briefly prior to use. Once the components are reconstituted, the target mix can be kept at 2–8°C for up to 8 days. For the SARS-CoV-2/Flu PC and long-term storage of the SARS-CoV-2/Flu Mix, aliquot as appropriate and store the solutions frozen at -25°C to -15°C with the expiration date stated in the kit. When handling frozen components, thaw at 18 to 26°C for approximately 15 to 30 minutes, mix gently and then microcentrifuge briefly (~1,500 – 3,000 × g).

Extraction

Nucleic acids are extracted using the OPTI DNA/RNA Magnetic Bead Extraction kit (OPTI Medical System, #99-58015) on the Thermo Scientific™ KingFisher™ Flex Magnetic Partide Processor with 96 Deep-Well Head and plates. Refer to the online manual at https://www.optimedical.com/files/art-06-58015-02-opti-dna-rna-mag-bead.pdf for detailed instructions on preparing samples, wash plates, and elution plate.

On the KingFisher™ Flex Magnetic Particle Processor, download and install the run protocol (OPTI-FLEX-01) from https://www.optimedical.com/files/opti-flex-01.bdz. Download instructions can be found in the *BindIt Software user Manual* (https://assets.thermofisher.com/TFS-Assets/LSG/manuals/BindIt_4_KingFisherInstrumentsUserManual.pdf).

Store the purified RNA at <–15°C if testing is not performed immediately after RNA Extraction.

Quality Controls

Control(s) that are provided with the OPTI SARS-CoV-2/Influenza A/B RT-PCR Test are listed below:

- Negative Control (OPTI PCR Grade Water): A "no template" (negative) control is needed to confirm the PCR plate is valid. PCR Grade water is used and should be included for each PCR run. The negative control should test negative for the SARS-CoV-2 target and internal control. The no template control is not included during extraction.
- Positive Control (OPTI SARS-CoV-2/Flu PC): A positive template control is needed to confirm the PCR plate is valid. Synthetic nucleic acids for the N1 target region of SARS-CoV-2, and target regions for influenza A, influenza B, and RNase P (ISC) are used. The positive control should be included on each PCR run and should test positive for all assay targets and internal control channels. The positive control is not included during extraction.
- The internal control for the test is a human endogenous nucleic acid sequence (RNase P) and controls for sample addition, extraction and PCR.

Control(s) that are required but not provided with the OPTI SARS-CoV-2/Influenza A/B RT-PCR Test are listed below:

- Extraction control: An extraction control containing human specimen control (HSC) material should be extracted and tested with each set of patient samples. The extraction control is used to demonstrate

successful recovery of RNA during the extraction process and should test negative for the SARS-CoV-2, Influenza A and Influenza B targets, and positive for the RNase P internal control. Laboratories may use confirmed negative human specimen material (e.g., a negative respiratory specimen). This material should be prepared in enough volume to be used across multiple runs. Material should be tested prior to use as the extraction control to ensure it generates the expected results.

- External positive control: A viral RNA control should be tested with each set of patient samples to demonstrate successful reverse transcription during PCR. The external positive control should test positive for SARS-CoV-2, Influenza A, and Influenza B.

  External Control option 1: Extract RNA from the NATtrol™ Flu/RSV/SARS-CoV-2 Positive Control using the OPTI DNA/RNA Magnetic Bead Kit (input volume = 500 µL, elution volume = 100 µL).

  External Control option 2: Use Twist synthetic control RNA for Influenza A, Influenza B, and SARS-CoV-2 (no extraction needed).

  External Control option 3: Use genomic RNA for Influenza A, Influenza B, and SARS-CoV-2 (no extraction needed).

  Materials should be tested prior to use as the external positive control to ensure it generates the expected results. Store RNA in small aliquots at –70°C.

Test Procedure (96-well block)

1 Preparation of the PCR Mix.

- Mix the thawed RNA MMx by inversion or gentle vortex.
- The RNA MMx is a viscous solution; always pipette it slowly.
- To prepare the PCR Mix, add 10 µL OPTI SARS-CoV-2/Flu Mix and 10 µL RNA MMx for each reaction.
- When preparing the PCR Mix, first pipette OPTI SARS-CoV-2/Flu Mix into the tube and then add the RNA MMx. Pipette up and down a few times to rinse the MMx pipette tip.
- Gently vortex the solution to ensure the components are mixed well.
- Keep the PCR Mix on ice until it is pipetted onto the PCR plate.

Important: *Plate set-up must be completed, and plate loaded into the instrument within 30 minutes of PCR Mix preparation. The PCR Mix must be kept cold at all times. Protect from light.*

2 Use a chilled block to keep the PCR plate cool during set-up.

3 Carefully pipette 20 µL of the PCR Mix into the required wells of the PCR plate.

4 Add 5 µL of sample RNA to each well. The final reaction volume is 25 µL.

5 Include the Positive Control from the kit (5 µL), PCR negative control (5 µL PCR Grade Water), Extraction control (5 µL), and extracted Positive Control RNA (5 µL), or synthetic or genomic RNA (5 µL), for each test run.

6 Seal the plate and briefly spin the plate, if necessary, to settle contents and remove air bubbles.

7 Load the plate into the PCR instrument. Set up the Cycling Program below. Start the run.

Settings for Reporter and Quencher

Target	Dye	Reporter	Quencher
Influenza A	FAM™	FAM™	BHQ® (none)
Influenza B	NED™	NED™	BHQ® (none)
SARS-CoV-2	Cy5	Cy5	BHQ® (none)
Internal Control (RNase P)	VIC™	VIC™	BHQ® (none)
Passive Reference	ROX™	ROX™	N/A

Cycling Program (used for all instruments)

Step	Temperature	Time	No. of Cycles
Reverse transcription (RT)	50°C	15 min	1
Denaturation	95°C	1 min.	1
Amplification**	95°C 60°C	15 sec. 30 sec.	45

**Ensure the instrument is set to record fluorescence following the 60°C amplification step.

Test Procedure (384-well block)

1 Preparation of the PCR Mix.

- Mix the thawed RNA MMx by inversion or gentle vortex.
- The RNA MMx is a viscous solution; always pipette it slowly.
- To prepare the PCR Mix, add 7.5 µL OPTI SARS-CoV-2/Flu Mix and 7.5 µL RNA MMx for each reaction.
- When preparing the PCR Mix, first pipette OPTI SARS-CoV-2/Flu Mix into the tube and then add the RNA MMx. Pipette up and down a few times to rinse the MMx pipette tip.
- Gently vortex the solution to ensure the components are mixed well.
- Keep the PCR Mix on ice until it is pipetted onto the PCR plate.

Important: *Plate set-up must be completed, and plate loaded into the instrument within 30 minutes of PCR Mix preparation. The PCR Mix must be kept cold at all times. Protect from light.*

2 Use a chilled block to keep the PCR plate cool during set-up.

3 Carefully pipette 15 µL of the PCR Mix into the required wells of the PCR plate.

4 Add 5 µL of sample RNA to each well. The final reaction volume is 20 µL.

5 Include the Positive Control (5 µL), PCR negative control (5 µL PCR Grade Water), Extract from external positive control or synthetic RNA (5 µL) and Extraction Control (5 µL) for each test run.

6 Seal the plate and briefly spin the plate, if necessary, to settle contents and remove air bubbles.

7 Load the plate into the PCR instrument. Set up the Cycling Program below. Start the run.

Settings for Reporter and Quencher

Target	Dye	Reporter	Quencher
Influenza A	FAM™	FAM™	BHQ® (none)
Influenza B	NED™	NED™	BHQ® (none)
SARS-CoV-2	Cy5	Cy5	BHQ® (none)
Internal Control (RNase P)	VIC™	VIC™	BHQ® (none)
Passive Reference	ROX™	ROX™	N/A

Cycling Program (used for all instruments)

Step	Temperature	Time	No. of Cycles
Reverse transcription (RT)	50°C	15 min.	1
Denaturation	95°C	1 min.	1
Amplification**	95°C 60°C	15 sec. 30 sec	45

**Ensure the instrument is set to record fluorescence following the 60°C amplification step.

8 Data analysis

All test controls must be examined prior to interpretation of patient results. If the controls are not valid, the patient results cannot be interpreted.

Using the PCR instrument software, assign a unique identifier for the SARS-CoV-2, Influenza A, Influenza B and internal control targets on the plate. To obtain appropriate Ct values, analysis for each target should be performed by manually setting the threshold. Each target threshold should be set separately. The threshold should be adjusted to the inflection point for the exponential phase of the positive control curve and above background signal of the negative control.
Confirm threshold placement by viewing the curves for each target. It is important to follow the same procedure run to run when setting the manual threshold.

Refer to specific instrument's user manual for guidance on how to analyze data.

Plate Validity Criteria

The following control results must be obtained for each PCR run in order for the run to be deemed valid. If the plate controls are not valid, the patient results cannot be interpreted, are not valid, and the plate must be repeated. If a positive/negative control fails, retesting the same eluates is recommended first to determine if there were some process-related issues in the initial run. If the extraction control fails, the extraction must be repeated from the original samples.

Control	SARS-CoV-2/Flu Positive Control	PCR Negative Control	Extraction Control	External Positive Control
Influenza A FAM Ct/result	<40 Positive	No Signal Negative	No Signal Negative	<40 Positive
Influenza B NED Ct/result				
SARS-CoV-2 Cy5 Ct/result				
Internal Control VIC™ Ct/result	<40 Positive	No signal Negative	<36 Positive	n/a

Sample Validity: The table below details the results interpretation of the SARS-CoV-2, Influenza A, Influenza B and internal control target for each sample.

9 Examination and Interpretation of Patient Specimen Results

Assessment of clinical specimen test results must be performed after the positive and negative controls have been examined and determined to be valid and acceptable. If the controls are not valid, the patient results cannot be interpreted.

Sample Result	Target Channel	Target Ct Value	Internal Control VIC™ Ct Value	Other Characteristics
Influenza A RNA POSITIVE	FAM	≤40	Any Ct value	A characteristic amplification curve in comparison to the PCR negative control. An internal control amplification curve in the VIC channel expected. A strong positive SARS-CoV-2, Influenza A or Influenza B sample may result in a negative internal contro result.
Influenza B RNA POSITIVE	NED			
SARS-CoV-2 RNA POSITIVE	Cy5			
Influenza A RNA NEGATIVE	FAM	>40	≤36	Amplification curve in the VIC internal control channel
Influenza B RNA NEGATIVE	NED			
SARS-CoV-2 RNA NEGATIVE	Cy5			
Invalid Sample**	Any Target	>40	>36	Absence of an amplification curve in the VIC channel indicates an invalid result for any negative targets in the sample.

**An invalid sample can be an indication of failed sample addition, extraction and/or PCR. It is recommended that the RNA be diluted five-fold into PCR grade water and retested; include the undiluted RNA as a sample. If the test is still not valid, a new extraction is recommended.

Limitations

10 Limitations

- Performance of the OPTI SARS-CoV-2/Influenza A/B RT-PCR Test has only been established in nasopharyngeal swabs. Other specimen types have not been evaluated and should not be tested with this assay.
- The OPTI SARS-CoV-2/Influenza A/B RT-PCR Test is for qualitative detection only and not for quantitative detection of SARS-CoV-2, Influenza and Influenza B.
- The OPTI SARS-CoV-2/Influenza A/B RT-PCR Test can detect SARS-CoV-2 but cannot identify any specific variant strain present in the circulation at any place and time.
- If the virus mutates in the test target region, SARS-CoV-2, Influenza A or Influenza B RNA may not be detected or may be detected less predictably. Inhibitors or other types of interference may produce a false negative result.
- A high concentration of the SARS-CoV-2 analyte may inhibit the detection of Influenza A and B.
- Negative results do not preclude infection with SARS-CoV-2, influenza A, and/or influenza B virus and should not be the sole basis of a patient management decision.
- Clinical performance has not been established in 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.
- Laboratories are required to report all SARS-CoV-2 results to the appropriate public health authorities.
- Samples must be collected, transported, and stored using appropriate procedures and conditions. Improper collection, transport, or storage of specimens may affect the test performance.
- 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 validated.
- A false negative result may occur if a specimen is improperly collected, transported. False negative results may also occur if amplification inhibitors are present in the specimen or if inadequate numbers of organisms are present in the specimen.
- False-positive results may arise from cross contamination during specimen handling, preparation, nucleic acid extraction, PCR assay set-up or product handling.
- The performance of the test has not been established in individuals who received nasal administered Influenza vaccine. Individuals who received nasal administered Influenza A vaccine may have positive Influenza A test results for up to three days after vaccination. https://www.cdc.gov/mmwr/preview/mmwrhtml/rr57e717a1.htm
- Detection of viral RNA may not indicate the presence of infectious virus or that Influenza or SARS-CoV-2 viruses are the causative agents for clinical symptoms.
- The performance of this test has not been established for screening of blood or blood products for the presence of Influenza A, Influenza B or SARS-CoV-2.
- This test cannot rule out diseases caused by other bacterial or viral pathogens.
- Optimum specimen types and timing for peak viral levels during infections caused by a novel Influenza or SARS-CoV-2 virus have not been determined. Collection of multiple specimens from the same patient may be necessary to detect the viruses.

CONDITIONS OF AUTHORIZATION FOR THE LABORATORY

11 Conditions

The OPTI SARS-CoV-2/Influenza A/B RT-PCR Test 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/in-vitro-diagnostics-euas-molecular-diagnostic-tests-sars-cov-2

To assist clinical laboratories using the OPTI SARS-CoV-2/Influenza A/B RT-PCR Test the relevant Conditions of Authorization are listed verbatim below, and are required to be met by laboratories performing the EUA test.

a) Authorized laboratories using your product[1] must include with test result reports all authorized Fact Sheets. Under exigent circumstances, other appropriate methods for disseminating these Fact Sheets may be used, which may include mass media.

b) Authorized laboratories using your product must use your product as outlined in the authorized labeling. Deviation 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 are not permitted.

c) Authorized laboratories that receive your product must notify the relevant public health authorities of their intent to run your product prior to initiating testing.

d) Authorized laboratories using your product must have a process in place for reporting test results to healthcare providers and relevant public health authorities, as appropriate.

e) Authorized laboratories must collect information on the performance of your product and report to DMD/OHT7-OIR/OPEQ/CDRH (via email: CDRH_EUA-Reporting@fda.hhs.gov) and OPTI Medical Inc (via email: covid19@optimedical.com) any suspected occurrence of false positive or false negative results and significant deviations from the established performance characteristics of your product of which they become aware.

f) All laboratory personnel using your product must be appropriately trained in PCR techniques and use appropriate laboratory and personal protective equipment when handling this kit and use this product in accordance with the authorized labeling.

g) OPTI Medical Systems, Inc., authorized distributors, and authorized laboratories using your product must ensure that any records associated with this EUA are maintained until otherwise notified by FDA. Such records will be made available to FDA for inspection upon request.

1 "Your product" refers to the OPTI SARS-CoV-2/Influenza A/B RT-PCR Test. 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".

Assay Performance

12 Limit of Detection (LoD)

Limit of detection (LoD) is defined as the lowest concentration of SARS-CoV-2, Influenza A and Influenza B at which greater than or equal to 95% of all replicates test positive. LoD for the OPTI SARS-CoV-2/Influenza A/B RT-PCR Test was determined using serial dilutions of inactivated SARS-CoV-2 virus from BEI, Manassas, VA (NR-52286), live influenza A/California/08/2009 pdm09/H1N1 (VR-1895), live influenza A/Hong Kong/4801/2014/H3N2 (VR-1990), live influenza B/Florida/78/2015 Victoria (VR-1930), and live influenza B/Florida/4/2006 Yamagata (VR-1804) from American Type Culture Collection, Manassas, VA, prepared in nasopharyngeal (NP) swab sample pools.

The initial LoD was determined with 3-fold serial dilutions tested in triplicate. Each replicate was extracted using the OPTI DNA/RNA Magnetic Bead Kit on Thermo Scientific™ KingFisher™ Flex following the standard protocol. Extracted RNA was tested on the Applied Biosystems® QuantStudio and 7500 FAST PCR instrument. To confirm the LoD, 20 replicates of each sample matrix spiked with SARS-CoV-2, Influenza A and Influenza B viruses were extracted with the OPTI DNA/RNA Magnetic Bead Kit on the Thermo Scientific™ KingFisher™ Flex. Extracted RNA was tested on the Applied Biosystems® QuantStudio™ and 7500 FAST PCR instrument. The LoDs were confirmed to be:

SARS-CoV-2 virus

1.09 GCE/µL (20/20) on Applied Biosystems QuantStudio 5 PCR System (96-well)

1.09 GCE/µL (20/20) on Applied Biosystems QuantStudio 5 PCR System (384-well)

0.36 GCE/µL (20/20) on 7500 FAST PCR System

Influenza A/H3N2

0.14 CEID50/µL (19/20) on Applied Biosystems QuantStudio 5 PCR System (96-well)

0.14 CEID50/µL (20/20) on Applied Biosystems QuantStudio 5 PCR System (384-well)

0.14 CEID50/µL (19/20) on 7500 FAST PCR System

Influenza A/H1N1

0.14 CEID50/µL (20/20) on Applied Biosystems QuantStudio 5 PCR System (96-well)

0.05 CEID50/µL (20/20) on 7500 FAST PCR System

1 "Your product" refers to the OPTI SARS-CoV-2/Influenza A/B RT-PCR Test. 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".

Influenza B/Victoria

0.14 CEID50/µL (20/20) on Applied Biosystems QuantStudio 5 PCR System (96-well)
0.14 CEID50/µL (20/20) on Applied Biosystems QuantStudio 5 PCR System (384-well)
0.14 CEID50/µL (20/20) on 7500 FAST PCR System

Influenza B/Yamagata

0.14 CEID50/µL on (20/20) Applied Biosystems QuantStudio 5 PCR System (96-well)
0.05 CEID50/µL on (20/20) 7500 FAST PCR System

Results are shown in Tables 1 and 2 below.

Table 1: LoD Initial Determination

SARS-CoV-2, 96-well QuantStudio 5 PCR System

SARS-CoV-2 GCE/µL	Mean Ct	Hit rate	% Detection
3.28	33.88	3/3	100
1.09	35.14	3/3	100
0.36	35.88	3/3	100
0.12	37.04	2/3	67

SARS-CoV-2, 384-well QuantStudio 5 PCR System

SARS-CoV-2 GCE/µL	Mean Ct	Hit rate	% Detection
3.28	34.17	3/3	100
1.09	35.42	3/3	100
0.36	36.65	1/3	33
0.12	No Ct	0/3	0

Influenza A/H3N2, 96-well QuantStudio 5 PCR System

A/H3N2 CEID50/µL	Mean Ct	Hit rate	% Detection
1.23	33.19	3/3	100
0.41	33.66	3/3	100
0.14	36.06	3/3	100
0.05	36.04	1/3	33

Influenza A/H1N1, 96-well QuantStudio 5 PCR System

A/H1N1 CEID50/µL	Mean Ct	Hit rate	% Detection
0.41	32.83	3/3	100
0.14	33.31	3/3	100
0.05	35.53	3/3	100
0.02	35.90	1/3	33

Influenza A/H3N2, 384-well QuantStudio 5 PCR System

A/H3N2 CEID50/µL	Mean Ct	Hit rate	% Detection
0.41	34.05	3/3	100
0.14	35.87	3/3	100
0.05	36.69	2/3	67
0.02	37.79	2/3	67

Influenza B/Victoria, 96-well QuantStudio 5 PCR System

B/Victoria CEID50/µL	Mean Ct	Hit rate	% Detection
1.23	32.32	3/3	100
0.41	34.66	3/3	100
0.14	36.91	3/3	100
0.05	37.03	1/3	33

Influenza B/Yamagata, 96-well QuantStudio 5 PCR System

B/Yamagata CEID50/µL	Mean Ct	Hit rate	% Detection
0.41	33.60	3/3	100
0.14	35.45	3/3	100
0.05	37.10	3/3	100
0.02	36.36	2/3	67

Influenza B/Victoria, 384-well QuantStudio 5 PCR System

B/Victoria CEID50/µL	Mean Ct	Hit rate	% Detection
0.41	33.60	3/3	100
0.14	35.29	3/3	100
0.05	34.48	2/3	67
0.02	No Ct	0/3	0

Table 2: LoD Confirmation

SARS-CoV-2, 96-well QuantStudio 5 PCR System

PCR Instrument	SARS-CoV-2 GCE/µL	Mean Ct	Detection Rate	LoD copies/µL
QuantStudio 5	1.09	34.01	20/20	1.09
	0.36	35.81	17/20	
ABI 7500 FAST	0.36	35.21	20/20	0.36
	0.12	35.35	11/20	

SARS-CoV-2, 384-well QuantStudio 5 PCR System

PCR Instrument	SARS-CoV-2 GCE/µL	Mean Ct	Detection Rate	LoD copies/µL
QuantStudio 5	1.09	36.23	20/20	1.09
	0.36	36.85	13/20	

Influenza A/H3N2, 96-well QuantStudio 5 PCR System

PCR Instrument	A/H3N2 CEID50/µL	Mean Ct	Detection Rate	LoD copies/µL
QuantStudio 5	0.14	35.54	19/20	0.14
	0.05	36.10	14/20	
ABI 7500 FAST	0.14	33.80	19/20	0.14
	0.05	34.81	15/20	

Influenza A/H1N1, 96-well QuantStudio 5 PCR System

PCR Instrument	A/H1N1 CEID50/µL	Mean Ct	Detection Rate	LoD copies/µL
QuantStudio 5	0.14	33.78	20/20	0.14
	0.05	35.88	18/20	
ABI 7500 FAST	0.05	35.01	20/20	0.05
	0.02	33.67	15/20	

Influenza A/H3N2, 384-well QuantStudio 5 PCR System

PCR Instrument	A/H3N2 CEID50/µL	Mean Ct	Detection Rate	LoD copies/µL
QuantStudio 5	0.14	35.32	20/20	0.14
	0.05	36.43	12/20	

Influenza B/Victoria, 96-well QuantStudio 5 PCR System

PCR Instrument	B/Victoria CEID50/µL	Mean Ct	Detection Rate	LoD copies/µL
QuantStudio 5	0.14	33.82	20/20	0.14
	0.05	35.54	16/20	
ABI 7500 FAST	0.14	34.01	20/20	0.14
	0.05	35.98	13/20	

Influenza B/Yamagata, 96-well QuantStudio 5 PCR System

PCR Instrument	B/Yamagata CEID50/µL	Mean Ct	Detection Rate	LoD copies/µL
QuantStudio 5	0.14	34.33	20/20	0.14
	0.05	35.77	17/20	
ABI 7500 FAST	0.05	34.78	20/20	0.05
	0.02	35.63	15/20	

Influenza B/Victoria, 384-well QuantStudio 5 PCR System

PCR Instrument	B/Victoria CEID50/µL	Mean Ct	Detection Rate	LoD copies/µL
QuantStudio 5	0.14	35.07	20/20	0.14
	0.05	35.45	17/20	

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2 Inclusivity (analytical reactivity)

In silico analysis

a) SARS-CoV-2

The OPTI SARS-CoV-2/Influenza A/B RT-PCR Test uses the primer and probe sequences that are described by the CDC. A dual target design is chosen to increase and maintain inclusivity in the event of any point mutation in one of the target regions. CDC has given the right of reference for leveraging all data including wet-testing.

SARS-CoV-2 inclusivity was assessed by comparing the OPTI SARS-CoV-2 RT-PCR test primer and probe design to recently circulating SARS-CoV-2 virus sequences. High coverage SARS-CoV-2 genomic sequences from September 1st, 2021 and December 20th, 2021 for VOC alpha, beta, gamma, and omicron were downloaded from the GISAID database. Due to the large number of delta variant submissions, 105,346 delta sequences within the same period were pulled without biases. The N gene region was aligned to the test design using the MAFFT version 7 software (RIMD, Osaka, Japan). The analysis showed that over 99% of all VOC SARS-CoV-2 variants have a perfect match to both or at least one of the two N-gene targets and therefore will be amplified and detected by the OPTI SARS-CoV-2/Influenza A/B RT-PCR test (see Tables below). In this analysis, it is noted that 98.7% of the omicron variant population has a conserved single nucleotide mutation (C -> T) in the N1 probe. However, this mutation is unlikely to affect the amplification of the target because it is located at the 5′ end. A preliminary study using a synthetic N1 template containing the prevalent omicron mutation also showed that the mutation has no impact on the amplification of the target.

Probe sequence: ACCCCGCATTACGTTTGGTGGACC

Omicron variant: ACTCCGCATTACGTTTGGTGGACC

Alpha variant sequence alignment summary

ALIGNMENT	COUNT	PERCENTAGE
N1,N4 Perfect Match	724	93.54%
N1 match (mismatch in N4)	16	2.07%
N4 match (mismatch in N1)	33	4.26%
Mismatches in N1 and N4	1	0.13%
TOTAL	**774**	**100.00%**

Beta variant sequence alignment summary

ALIGNMENT	COUNT	PERCENTAGE
N1,N4 Perfect Match	85	64.39%
N1 match (mismatch in N4)	14	10.61%
N4 match (mismatch in N1)	31	23.48%
Mismatches in N1 and N4	0	0.00%
TOTAL	**132**	**100.00%**

Gamma variant sequence alignment summary

ALIGNMENT	COUNT	PERCENTAGE
N1,N4 Perfect Match	906	89.88%
N1 match (mismatch in N4)	76	7.54%
N4 match (mismatch in N1)	25	2.48%
Mismatches in N1 and N4	1	0.10%
TOTAL	**1008**	**100.00%**

Delta variant sequence alignment summary

ALIGNMENT	COUNT	PERCENTAGE
N1,N4 Perfect Match	82713	78.52%
N1 match (mismatch in N4)	10423	9.89%
N4 match (mismatch in N1)	11753	11.16%
Mismatches in N1 and N4	457	0.43%
TOTAL	**105346**	**100.00%**

Omicron variant sequence alignment summary

ALIGNMENT	COUNT	PERCENTAGE
N1,N4 Perfect Match	60	0.48%
N1 match (mismatch in N4)	3	0.02%
N4 match (mismatch in N1)	12351	98.96%
Mismatches in N1 and N4	67	0.54%
TOTAL	**12481**	**100.00%**

Wet testing data showed that the OPTI SARS-CoV-2/Influenza A/B RT-PCR Test detected the BA.2 variant sequence with the same efficiency when compared to the N1 and N4 synthetic DNA targets.

b) Influenza A

The OPTI SARS-CoV-2/Influenza A/B RT-PCR Test uses identical Influenza A primer sequences described in the CDC test design and an exact reverse complement of the CDC Influenza A probe sequence[1]. However, to assess the *in silico* inclusivity of the OPTI Influenza A Test, a Master Sequence Alignment (MSA) was generated from the GISAID Influenza A database[3] sequences for H1N1 and H3N2 and compared for identity to the test primers and probes. Only full-length H1N1 and H3N2, high coverage sequences from human hosts from North America, Europe and Asia were included, resulting in over 75,000 sequences in the design region. 98.7% of sequenced strains match the Influenza A forward primer, 94.6% match the Influenza A probe and 98.7% match the Influenza A reverse primer. Based on this analysis the OPT SARS-CoV-2/Influenza A/B RT-PCR Test is predicted to detect all currently circulating strains of influenza A.

c) Influenza B

The OPTI SARS-CoV-2/Influenza A/B RT-PCR Test uses identical Influenza B primer sequences described in the CDC test design and uses an Influenza B probe that contains a single degenerate base not included in the CDC Influenza B probe[2]. However, to assess the *in silico* inclusivity of the OPTI Influenza B Test, an MSA was generated from the GISAID Influenza B database[3] sequences and compared for identity to the test primers and probes. Only full-length Victoria and Yamagata lineages with high coverage sequences from human hosts from North America, Europe and Asia were included, resulting in over 19,000 sequences in the design region. 95.9% of sequenced strains match the Influenza B forward primer, 98.5% match the Influenza B probe and 99.1% match the Influenza B reverse primer. The addition of the degenerate nucleotide base to the probe adds an additional 555 (2.9%) sequences to match the design. Based on this analysis the OPT SARS-CoV-2/Influenza A/B RT-PCR Test is predicted to detect all currently circulating strains of influenza B.

Wet testing

The inclusivity of OPTI SARS-CoV-2/Influenza A/B for the detection of influenza A was evaluated by testing five isolates of A/H1N1 and five isolates of B/H3N2 from within the past five years, and for the detection of influenza B by testing five isolates of influenza B/Victoria and three isolates of B/Yamagata lineages from within the past five years. The lowest target analyte concentration at which all four tested replicates tested positive are reported in Table 3. Additionally, two influenza B isolates from St. Jude Children's Research Hospital also tested positive correctly using the OPTI SARS-CoV-2/Influenza A/B Tests.

Table 3: Wet Testing of Influenza A and B inclusivity.

Influenza organism	Lowest detectable concentration
Influenza A Virus, A/Hawaii/66/2019 (H1N1)pdm09	3.70E+03 CEID50/mL
Influenza A Virus, A/Idaho/07/2018 (H1N1)pdm09	1.60E-01 TCID50/mL
Influenza A Virus, A/Indiana/02/2020 (H1N1)pdm09	4.85E+02 CEID50/mL
Influenza A Virus, A/Michigan/272/2017 (H1N1)pdm09	4.80E+00 TCID50/mL
Influenza A Virus, A/Wisconsin/588/2019 (H1N1)pdm09	1.63E+01 FFU/mL
Influenza A Virus, A/Arizona/45/2018 (H3N2)	3.38E+01 FFU/mL
Influenza A Virus, A/Hong Kong/2671/2019 (H3N2)	3.61E+03 CEID50/mL
Influenza A Virus, A/Kansas/14/2017 (H3N2)	3.96E+01 FFU/mL
Influenza A Virus, A/Wisconsin/04/2018 (H3N2)	1.40E+02 CEID50/mL
Influenza A Virus, A/Texas/71/2017 (H3N2)	1.40E+02 TCID50/mL
Influenza B Virus, B/Hong Kong/286/2017 (Victoria Lineage)	1.35E+00 TCID50/mL
Influenza B Virus, B/Colorado/6/2017 (Victoria Lineage)	7.00E-01 TCID50/mL
Influenza B Virus, B/Hawaii/01/2018 (NA D197N) (Victoria Lineage)	3.26E+02 TCID50/mL
Influenza B Virus, B/Washington/02/2019 (Victoria Lineage)	1.50E+03 CEID50/mL
Influenza B Virus, B/Missouri/12/2018 (NA D197E) (Victoria Lineage)	2.80E+01 TCID50/mL
Influenza B Virus, B/Wisconsin/10/2016 (NA I221V) (Yamagata lineage)	1.60E+02 TCID50/mL
Influenza B Virus, B/Indiana/17/2017 (NA I221T) (Yamagata Lineage)	5.00E+01 TCID50/mL
Influenza B Virus, B/Oklahoma/10/2018 (NA D197N) (Yamagata Lineage)	3.80E+01 TCID50/mL
B/Memphis/47/2016	*not determined*
B/Memphis/FMT001-A1/2015	*not determined*

13 Analytical Specificity

a) *In silico* Cross-reactivity: SARS-CoV-2

The OPTI SARS-CoV-2/Influenza A/B RT-PCR Test uses identical N1, N4 primer and probe sequences as described for the CDC design. To assess the *in silico* exclusivity of the OPTI SARS-CoV-2/Influenza A/B RT-PCR Test, an MSA was generated from several high priority pathogens from the same genetic family as SARS-CoV-2 as well as other high-profile pathogens likely in the same biological niche as SARS-CoV-2. This alignment was then compared for identity to the test primers and probes. The N1 and N4 design regions were aligned with SARS coronavirus (NC_004718), MERS coronavirus (NC_019843), and human coronaviruses NL63 (NC_005831), OC43 (KX344031), 229E (NC_002645), and HKU1 (NC_006577). No single primer or probe sequence contains greater than 80% identity to the design region other than NC_004718 (SARS coronavirus Tor2) which contains 91.7% identity with the N1 probe. Based on the mismatches in the overall design region, it is highly unlikely the N1 primers will amplify the target region of NC_004718.

A BLAST analysis was performed using the N1 and N4 amplicon sequences, lenient parameters and excluding SARS-CoV-2 and unidentified viral sequences. No significant similarity to any sequences in the NCBI database were returned.

Similarly, a directed BLAST search was performed against the genome sequences from other upper respiratory tract microorganisms listed in Table 4. Again, no significant similarities were returned.

b) *In silico* Cross-reactivity: Influenza A

A BLAST analysis was performed using the Influenza A amplicon, lenient parameters and excluding Influenza B and unidentified viral sequences. No significant similarity to any sequences in the NCBI database were returned. Similarly, a directed BLAST search was performed against the genome sequences from other upper respiratory tract microorganisms listed in Table 4. Again, no significant similarities were returned.

c) *In silico* Cross-reactivity: Influenza B

A BLAST analysis was performed using the Influenza B amplicon, lenient parameters and excluding Influenza B and unidentified viral sequences. No significant similarity to any sequences in the NCBI database were returned. Similarly, a directed BLAST search was performed against the genome sequences from other upper respiratory tract microorganisms listed in Table 4. Again, no significant similarities were returned.

Table 4: List of organisms analyzed *in silico*.

Organism	Strain	Accession or WGS number
Bordetella bronchiseptica	NCTC10543	NZ_LR134326
Bordetella pertussis	18323	HE965805
Candida albicans	TIMM 1768	GCA_003454735
Chlamydia pneumoniae	CWL029	AE001363
Chlamydia trachomatis	D/UW-3/CX	NC_000117
Corynebacterium diphtheriae	NCTC11397	NZ_LN831026
Escherichia coli	K-12	NC_000913
Haemophilus influenzae	NCTC8143	LN831035
Klebsiella pneumoniae	HS11286	NC_016845
Lactobacillus plantarum	SK151	NZ_CP030105
Legionella pneumophila	Phil.1	CP015928
Moraxella catarrhalis	BBH18	NC_014147
Mycobacterium tuberculosis	HN-506	AP018036
Mycoplasma pneumoniae	FH	CP010546
Neisseria gonorrhoeae	35/02	NZ_CP012028
Neisseria meningitidis	MC58	NC_003112
Neisseria mucosa	FDAARGOS_758	NZ_CP053939
Pneumocystis jirovecii (PJP)	RU7	GCA_001477535
Proteus mirabilis	HI4320	NC_010554
Proteus vulgaris	NCTC13145	NZ_LR590468
Pseudomonas aeruginosa	PAO1	AE004091

Staphylococcus aureus	NCTC 8325	NC_007795
Staphylococcus epidermidis	ATCC 12228	NC_004461
Streptococcus pneumoniae	NCTC7465	LN831051
Streptococcus pyogenes	NGAS638	NZ_CP010450
Streptococcus salivarius	NCTC8618	NZ_LR134274
Adenovirus	A	NC_001460
Coxsackievirus	B5	JX843811
Echovirus	NGR_2014	MH745407
Enterovirus	EV68	KT266905
Epstein Barr Virus	YCCEL1	AP015016
Human coronavirus	OC43	KX344031
Human coronavirus	HKU1	MH940245
Human coronavirus	NL63	MK334047
Human rhinovirus A	p311	KX398052
Human rhinovirus B	R93	KF958309
Human rhinovirus C	25	EF582386
Influenza virus A	A/California/VRDL/179/2009	CY092759
Influenza virus B	B/Iowa/03/2002	CY019567
MERS-coronavirus	011/LOM/C20	MK357909
Metapneumovirus	00-1	NC_039199
Parainfluenza virus 1	NM001	KX639498
Parainfluenza virus 2	VIROAF10	KM190939
Parainfluenza virus 3	CFI1849	KJ672618
Parainfluenza virus 4	SC3019	KY986647
Respiratory Syncytial virus	B/WI/629-Q0190/10	JN032120
SARS-coronavirus	MA-15	DQ497008

d) Cross-reactivity and Microbial Interference Study (Wet testing)

The OPTI SARS-CoV-2/Influenza A/B RT-PCR Test was evaluated for cross-reactivity with a panel of bacteria, viruses and yeast that represents common respiratory pathogens, and a pool of 30 negative human nasopharyngeal specimens that represents a microbial flora seen in human respiratory specimens. Live bacteria and yeast at the listed concentrations were spiked with inactivated SARS-CoV-2 virus, live influenza A/H3N2, or B/Victoria viruses at ≤3xLoD. Nucleic acids were extracted using the OPTI DNA/RNA Magnetic Bead Kit with the Thermo Scientific KingFisher Flex Purification System and test on an Applied Biosystems QuantStudio 5 Real-Time PCR instrument. No cross-reactivity was observed (Table 5). Detection of the target analytes was not affected by the presence of the other microbial species tested (Table 6).

Table 5: SARS-CoV-2, Influenza A and B cross-reactivity test results

Microorganism	Test Concentration	SARS-CoV-2 Result	Flu A Result	Flu B Result
Bordetella pertussis	2.37E+07 CFU/mL	-	-	-
Candida albicans	1.20E+06 CFU/mL	-	-	-
Chlamydia pneumoniae	5.36E+06 CFU/mL	-	-	-
Corynebacterium spp.	1.77E+07 CFU/mL	-	-	-
Escherichia coli (respiratory)	1.92E+07 CFU/mL	-	-	-
Haemophilus influenzae	1.77E+07 CFU/mL	-	-	-
Lactobacillus sp.	1.65E+07 CFU/mL	-	-	-
Legionella pneumophila	1.0E+07 CFU/mL	-	-	-
Moraxella Catarrhalis	1.74E+07 CFU/mL	-	-	-

Mycoplasma pneumoniae	1.36E+06 CFU/mL	-	-	-
Mycoplasma tuberculosis	152.6 µg/mL	-	-	-
Neisseria Meningitidis	2.10E+07 CFU/mL	-	-	-
Neisseria sp.	2.04E+07 CFU/mL	-	-	-
Pneumocystis Jirovecii (PJP)	Not Available	-	-	-
Pseudomonas aeruginosa	1.80E+07 CFU/mL	-	-	-
Staphylococcus aureus	2.16E+07 CFU/mL	-	-	-
Staphylococcus epidermis	1.95E+07 CFU/mL	-	-	-
Streptococcus pneumoniae	1.62E+07 CFU/mL	-	-	-
Streptococcus pyogenes	2.34E+07 CFU/mL	-	-	-
Streptococcus salivarius	2.31E+07 CFU/mL	-	-	-
Enterovirus D68	1.00E+05 PFU/mL	-	-	-
Epstein Barr Virus	Not Available	-	-	-
Human Adenovirus, Type 1	1.00E+05 PFU/mL	-	-	-
Human Adenovirus, Type 7	3 µg/mL	-	-	-
Human coronavirus 229E	2.67E+05	-	-	-
Human coronavirus HKU1	3.67E+05	-	-	-
Human coronavirus NL63	2.67E+03	-	-	-
Human coronavirus OC43	1.00E+05 PFU/mL	-	-	-
Human cytomegalovirus	1.00E+05 PFU/mL	-	-	-
Human metapneumovirus	2.8 ng/100µL	-	-	-
Human parainfluenza 1	1.67E+05 PFU/mL	-	-	-
Human parainfluenza 2	1.00E+05 PFU/mL	-	-	-
Human parainfluenza 3	1.00E+05 PFU/mL	-	-	-
Human parainfluenza 4a	1.00E+05 PFU/mL	-	-	-
Human Rhinovirus 14	1.00E+05 PFU/mL	-	-	-
MERS-coronavirus	1.48E+05 TCID50/mL	-	-	-
Respiratory syncytial virus	1.03E+05 PFU/mL	-	-	-
SARS-coronavirus	1.67E+05 PFU/mL	-	-	-
Influenza A (A/H3N2)	9.60E+05 CEID/mL	-	n/a	-
Influenza B (B/Yamagata)	1.10E+08 CEID/mL	-	-	n/a
30 pooled negative human NP	n/a	-	-	-

Table 6: Interfering microorganisms study results

Micro-organism	Test Concentration	SARS-CoV-2, Influenza A & B at ≤3xLoD		
		SARS-CoV-2 Result	Flu A Result	Flu B Result
Bordetella pertussis	2.37E+07 CFU/mL	+	+	+
Candida albicans	1.20E+06 CFU/mL	+	+	+
Chlamydia pneumoniae	5.36E+06 CFU/mL	+	+	+
Corynebacterium sp	1.92E+07 CFU/mL	+	+	+
Escherichia coli (respiratory)	2.13E+07 CFU/mL	+	+	+
Haemophilus influenzae	1.77E+07 CFU/mL	+	+	+
Lactobacillus sp.	1.65E+07 CFU/mL	+	+	+
Legionella pneumophila	1.34E+06 CFU/mL	+	+	+
Moraxella Catarrhalis	1.98E+07 CFU/mL	+	+	+
Mycoplasma pneumoniae	1.36E+06 CFU/mL	+	+	+

Mycoplasma tuberculosis	152.6 µg/mL	+	+	+
Neisseria Meningitidis	2.10E+07 CFU/mL	+	+	+
Neisseria sp.	2.04E+07 CFU/mL	+	+	+
Pneumocystis Jirovecii (PJP)	Not available	+	+	+
Pseudomonas aeruginosa	1.80E+07 CFU/mL	+	+	+
Staphylococcus aureus	2.16E+07 CFU/mL	+	+	+
Staphylococcus epidermidis	1.95E+07 CFU/mL	+	+	+
Streptococcus pneumoniae	1.62E+07 CFU/mL	+	+	+
Streptococcus pyogenes	2.34E+07 CFU/mL	+	+	+
Streptococcus salivarius	2.31E+07 CFU/mL	+	+	+
Enterovirus D68	1.00E+05 PFU/mL	+	+	+
Epstein Barr Virus	not available	+	+	+
Human Adenovirus, Type 1	1.00E+05 PFU/mL	+	+	+
Human Adenovirus, Type 7	2.67E+05 TCID50/mL	+	+	+
Human coronavirus 229E	2.67E+05 TCID50/mL	+	+	+
Human coronavirus HKU1	3.67E+05 copies/µL	+	+	+
Human coronavirus NL63	2.67E+03 TCID50/mL	+	+	+
Human coronavirus OC43	1.00E+05 PFU/mL	+	+	+
Human cytomegalovirus	1.00E+05 PFU/mL	+	+	+
Human metapneumovirus	2.8 ng/100 µL	+	+	+
Human parainfluenza 1	1.67E+05 PFU/mL	+	+	+
Human parainfluenza 2	1.00E+05 PFU/mL	+	+	+
Human parainfluenza 3	1.00E+05 PFU/mL	+	+	+
Human parainfluenza 4a	1.00E+05 PFU/mL	+	+	+
Human Rhinovirus 14	1.00E+05 PFU/mL	+	+	+
MERS-coronavirus	1.48E+05 TCID50/mL	+	+	+
Respiratory syncytial virus	1.03E+05 PFU/mL	+	+	+
SARS-coronavirus	1.67E+05 PFU/mL	+	+	+
Influenza A (A/H3N2)	9.60E+05 CEID/mL	+	+	+
Influenza B (B/Yamagata)	1.10E+08 CEID/mL	+	+	+
30 pooled negative human NP	n/a	+	+	+

14. Interfering Substances Study

Potentially interfering substances in the nasal passage and nasopharynx may include, but are not limited to, blood, mucus or nasal secretions, medications for the relief of nasal congestion or dryness, irritation, or asthma and allergy symptoms, aswell as antibiotics and antiviral treatment. Negative nasopharyngeal samples containing spiked viruses (3x LoD) were tested in the presence of each substance to determine the effect on the detection of the targets in the OPTI SARS- CoV-2/Influenza A/B RT-PCR Test. The interferents and their concentrations evaluated are listed in Table 7. None of the substances caused interference with the assay performance at the concentrations tested in this study. All positive replicates were correctly detected by the OPTI SARS-CoV-2/Influenza A/B RT-PCR Test.

Table 7: Interfering substances study results

Interferent	Concentration
Endogenous Substances	
Whole Blood	1% v/v
Mucin	2.5 mg/ml
Spray and Gel	
Zicam Nasal gel	10% v/v
NeoSynephrine Spray	20% v/v
Normal saline Spray	20% v/v
Otrivin Nasal Spray	20% v/v
Zicam Nasal Spray	20% v/v
Nasal Corticosteroid	
Beclomethasone	2 mg/ml
Budesonide	1 mg/ml
Dexamethasone	3 mg/ml
Flunisolide	5 mg/ml
Fluticasone	0.25 mg/ml
Mometasone	1 mg/ml
Triamcinolone	1.5 mg/ml
Antiviral Agents and Antibiotics	
Chloroseptic Max	20% w/v
Lozenges	10 mg/ml
Mupirocin	4.3 mg/mL
Peridex chlorhexidine	20% v/v
Tobryamycin	2 mg/ml
Zanamivir (Relenza)	5 mg/ml
Others	
Oral Zinc	0.67 mg/ml

15. Co-infection Study

Analytical sensitivity of the OPTI SARS-CoV-2/Influenza A/B RT-PCR Test in the context of a co-infection scenario was evaluated using negative nasopharyngeal samples spiked with inactivated SARS-CoV-2, live influenza A/H3N2, and live B/Victoria viruses. Two of three targets were spiked at a starting concentration of 3x LoD in the presence of a third target a high concentration. Triplicate samples were extracted and tested. The test data shows that a high concentration of influenza A did not affect the detection of influenza B and SARS-CoV-2 at 3x LoD. Similarly, a high concentration of influenza B did not affect the detection of influenza A and SARS-CoV-2 at 3x LoD. However, a high concentration of SARS-CoV-2 in a clinical sample adversely affected the detection of the influenza A and B viruses at 3x LoD, leading to false negative results for these analytes.

Table 8: Co-infection study results with high concentrations of SARS-CoV-2

		SARS-CoV-2/Influenza A/B version 1 Test			
		Ct 1	Ct 2	Ct 3	Mean
Single infection	Flu A	34.66	32.49	34.58	33.91
	Flu B	34.02	34.88	33.36	34.09
Co-infection 1	High SARS-CoV-2 (clinical sample 1)	17.28	17.37	17.28	17.31
	Low Flu A	37.08	37.27	36.40	36.92
	Low Flu B	39.72	39.43	**41.02**	39.58
Co-infection 2	High SARS-CoV-2 (clinical sample 2)	23.07	22.82	22.92	22.94
	Low Flu A	**41.93**	**40.53**	37.68	n/a
	Low Flu B	**44.92**	**43.73**	**41.09**	n/a
Co-infection 3	High SARS-CoV-2 (clinical sample 3)	24.93	25.12	25.25	25.10
	Low Flu A	**40.59**	**40.21**	**40.42**	n/a
	Low Flu B	**No Ct**	**40.59**	**No Ct**	n/a

* Ct values indicated in **bold** are either beyond threshold limit (Ct ≤ 40) or not detected. n/a: not available

16. Precision/Reproducibility and Cross-contamination Studies

The precision and reproducibility of the OPTI SARS-CoV-2/Influenza A/B RT-PCR Test were evaluated using ten replicates of nasopharyngeal samples containing the three target analytes at three concentrations (1x, 4x, and 40x LoD). The study showed concordant data across three different lots of reagents. The potential for carryover and cross-contamination was assessed with samples containing high concentration of contrived viral samples adjacent to negative samples in an alternating pattern.

17. Clinical Evaluation

The performance of the OPTI SARS-CoV-2/Influenza A/B RT-PCR Test was evaluated using archived clinical nasopharyngeal (NP) swab samples in viral transport medium. Results for SARS-CoV-2 detection were compared to results from a highly sensitive, FDA-authorized molecular assay. Results for influenza A and B detection were compared to results from a highly sensitive, FDA-cleared molecular assay. Nucleic acid was extracted with the OPTI DNA/RNA Magnetic Bead Extraction Kit, and PCR was performed using the Applied Biosystems® QuantStudio™ 5 PCR instrument (software v1.5.1). Table 9 summarizes the results, including the positive and negative percent agreement with 95% confidence limits.

Table 9: OPTI SARS-CoV-2 Influenza A/B RT-PCR Test Performance Results

Clinical Evaluation – QuantStudio 5 (96-well format)

Target	Number of specimens	TP	FP	TN	FN	PPA (95% CI)	NPA (95% CI)
SARS-CoV-2	164	41	0	123	0	100% (91.43–100%)	100% (96.97–100%)
Flu A	143	50	0	93	0	100% (92.87–100%)	100% (96.03–100%)
Flu B	143	59	0	84	0	100% (93.9–100.0%)	99.42% (95.63–100%)

Clinical Evaluation – QuantStudio 5 (384-well format)

Target	Number of specimens	TP	FP	TN	FN	PPA (95% CI)	NPA (95% CI)
SARS-CoV-2	73	38	0	35	0	100% (90.82–100%)	100% (90.11–100%)
Flu A	65	29	0	35	1	96.7% (83.33–100%)	100% (90.11–100%)
Flu B	80	44	0	35	1	97.8% (88.43–99.61%)	99.42% (90.11–100%)

TP: True Positive, FP: False Positive, TN: True Negative, FN: False Negative, CI: Confidence Level

Emergency Use Only Labelling

18. Additional Label

An Emergency Use Only (EUO) label is required for instruments authorized for use under Emergency use Authorization (EUA).

ThermoFisher Scientific has provided a blanket Right of Reference to the Master File held by FDA which certifies that the Applied Biosystem 7500 FAST and QuantStudio 5 PCR instruments are manufactured under quality systems that are compliant with the applicable parts of 21 CFR 820 and ISO 13485:2016. Therefore, the testing laboratories are not required to perform any qualification studies prior to use of the instrument for testing patient specimens and reporting

test results of the qualification studies.

Please print and place the following label on the front panel on each instrument. If the instruments include labeling indicating "For Research Use Only", please cover with the below "Emergency Use Only" labeling. Retain this label throughout the EUA use of the OPTI SARS-CoV-2/Influenza A/B RT-PCR Test.

Emergency Use Only
This instrument is authorized for use with OPTI Medical Systems assays that have received Emergency Use Authorization (EUA)

References

1 Real-time RT-PCR Primers and Probes for COVID-19. (n.d.). Retrieved October 28, 2020, from https://www.cdc.gov/coronavirus/2019-ncov/lab/rt-pcr-panel-primer-probes.html

2 Research Use Only CDC Influenza SARS-CoV-2 (Flu SC2) Multiplex Assay Real-Time RT-PCR Primers and Probes. (n.d.). Retrieved October 28, 2020, from https://www.cdc.gov/coronavirus/2019-ncov/lab/multiplex-primer-probes.html

3 Asdfsadf(n.d.). Retrieved October 28, 2020, from https://platform.gisaid.org/epi3/cfrontend

For technical assistance on the OPTI SARS-CoV-2/Influenza A/B RT-PCR Test:
IDEXX USA Tel: +1 800 548 9997 or +1 207 556 4895
IDEXX Europe Tel: +800 727 43399
Contact your IDEXX area manager or distributor or visit our website.

Made in France

Symbol Descriptions

LOT	Batch Code (Lot)
SN	Serial Number
REF	Catalog Number
EC	Authorized Representative in the European Community
	Use by date
	Date of manufacture
	Manufacturer
	Temperature limitation
	Consult instructions for use
	Major change in the user instructions
IVD	*In vitro* diagnostic
CE	CE marking - European conformity
Rx	Prescription use only

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EC REP

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OPTIMedical

OPTI SARS CoV 2/ Influenza A/B RT PCR Test Version 2

English Version

Used for real-time PCR identification and differentiation of SARS-CoV-2, Influenza A and/or Influenza B RNA extracted from nasopharyngeal swabs.

99-57015 OPTI SARS-CoV-2 / Influenza A/B RT-PCR Test Version 2 500/box
99-57017 OPTI SARS-CoV-2 / Influenza A/B RT-PCR Test Version 2 5000/box

IVD CE Rx

For *in vitro* diagnostic use
For use under Emergency Use Authorization (EUA) Only
For Prescription Use only

REF 99-57015
99-57017

Version
06-57015-00

Approval Date: XX-XXX-2021

DRAFT 12-APR 2022

OPTIMedical

Table of Content

OPTI SARS CoV 2/ Influenza A/B RT PCR Test Version 2

Intended Use

The OPTI SARS-CoV-2/Influenza A/B RT-PCR Test is a multiplex real-time reverse transcription polymerase chain reaction test intended for the simultaneous qualitative detection and differentiation of SARS-CoV-2, Influenza A, and/or Influenza B virus RNA in nasopharyngeal 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 requirements to perform high complexity tests.

The OPTI SARS-CoV-2/Influenza A/B RT-PCR Test is intended for use in the simultaneous detection and differentiation of SARS-CoV-2, Influenza A, and Influenza B nucleic acid in nasopharyngeal swab clinical specimens, and is not intended to detect Influenza C virus. RNA from SARS-CoV-2, Influenza A, and/or Influenza B is generally detectable in nasopharyngeal swab specimens during the acute phase of infection. Positive results are indicative of the presence of SARS-CoV-2, influenza A, and/or influenza B RNA; clinical correlation with patient history and other diagnostic information is necessary to determine patient infection status. Positive results 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 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 patient management decisions. Negative results must be combined with clinical observations, patient history, and/or epidemiological information.

The OPTI SARS-CoV-2/Influenza A/B RT-PCR Test is intended to be used by qualified laboratory personnel specifically instructed and trained in the techniques of real-time PCR and *in vitro* diagnostic procedures. The OPTI SARS-CoV-2/Influenza A/B RT-PCR Test is only for use under the Food and Drug Administration's Emergency Use Authorization.

Product Description

The OPTI SARS-CoV-2/Influenza A/B RT-PCR Test is a real-time reverse transcription polymerase chain reaction (rRT-PCR) test. OPTI SARS-CoV-2/Flu RNA Mix (SARS-CoV-2/Flu Mix) includes primers and probes for the detection of SARS-CoV-2, Influenza A and Influenza B RNA when amplified with the OPTI RNA Master Mix (RNA MMx). Influenza A is detected in the FAM channel; Influenza B is detected in the CAL Fluor Red 610 channel and SARS-CoV-2 RNA targets (N1 and N4) are detected in the Cy5 channel. The internal control is based on the detection of a conserved nucleic acid sequence present in human samples and is detected in the CAL Fluor Orange 560 channel. Detection of endogenous nucleic acid in the test sample, controls for sample addition, extraction, and amplification. Primers and probe for detection of the internal control are included in the SARS-CoV-2/Flu Mix.

During the real-time reverse transcription polymerase chain reaction, viral RNA is reverse transcribed into cDNA and subsequently amplified in a real-time PCR cycling protocol. During the process, the probe

anneals to a specific target sequence located between the forward and reverse primers. 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 exponentially. Fluorescence intensity is monitored at each PCR cycle by one of the PCR thermal cycler instruments listed in Section "Materials Required but Not Provided".

In addition, the OPTI SARS-CoV-2/Influenza A/B RT-PCR Test utilizes the OPTI SARS-CoV-2/Flu PC (Positive Control) and OPTI PCR Grade Water (Negative Control). The OPTI SARS-CoV-2 Flu PC contains SARS-CoV-2 (N1), Influenza A, Influenza B and internal control synthetic material and works as a positive control for each target in the reaction. OPTI PCR Grade Water is used as the RT-PCR negative control, as well as to reconstitute the dried SARS-CoV-2/Flu Mix and the PC.

Materials and Storage

Identification/ General Information	Cap color	Quantity	Storage		Freeze/Thaw cycles
			At receipt	After reconstitution	
OPTI SARS-CoV-2/Flu Mix (SARS-CoV-2/Flu Mix), dried 61-56626-00 (500 reactions) 61-56636-00 (5,000 reactions)	Red	5 x 1.0 mL 5 x 10 mL	–25 to 8°C	–25 to –15°C	≤6
Contains primers and probes for SARS-CoV-2 (N1 and N4), Influenza A, Influenza B and internal control. Reconstitute to 1 mL (in 500 reaction kit) or 10 mL (in 5,000 reaction kit) in PCR Grade Water. Store the SARS-CoV-2/Flu Mix in the dark. The expiration date on the vial is valid for either the dry or reconstituted form.					
OPTI RNA Master Mix (RNA MMx) 61-56630-00 (500 reactions) 61-56640-00 (5,000 reactions)	Black	5 x 1.0 mL 5 x 10 mL	–25 to –15°C (Long-term)	N/A	≤6
Concentrated master mix that includes reverse transcriptase and hot-start polymerase. The RNA MMx is more viscous than most master mixes– see the Test Procedure section for handling recommendations. Protect the RNA MMx from light.					
OPTI SARS-CoV-2/Flu PC, dried (PC) 44-56627-01	Blue	1 x 1.0 mL	–25 to 8°C	–25 to –15°C	≤6
The PC contains the targets for SARS-CoV-2 (N1 target region), Influenza A, Influenza B and the internal control. Reconstitute to 1 mL in PCR Grade Water. The expiration date on the vial is valid for either the dry or reconstituted form.					
OPTI PCR Grade Water 61-56619-00 (500 reactions) 61-56639-00 (5,000 reactions)	Clear	7 x 1.0 mL 3 x 25 mL	–25 to 8°C		N/A
PCR Grade Water has been qualified for reverse transcription-PCR (RT-PCR) use. It is used for the reconstitution of the SARS-CoV-2/Flu Mix and PC. It is also used as the PCR negative control for each test run. Do not transport PCR Grade Water vials between PCR work areas. Separate vials of water are needed for each area to avoid contamination risk.					

Note: See table at the end of the insert for a description of symbols used on the insert and labels.

Materials Required but Not Provided

Real-Time PCR Instrument and consumables	Source and part number
Roche Diagnostics	
LightCycler® 480* 96 well PCR plate and cover	instrument (05015278001) and software SW v1.5.1 Plate and cover: 040729692081
Bio-Rad	
Bio-Rad CFX96 Touch 96 well PCR plate Optical plate cover	instrument (1855196) and Maestro software 2.0 Plate: MSB1001 Cover: MSF1001
Bio Molecular Systems	
MIC qPCR instrument* Tubes and caps	instrument (98-0012758-00) and micPCR software v4.10 Tubes and Caps: 98-0012759-01
Extraction Equipmentand Consumables	**Source and part number**
OPTI DNA/RNA Magnetic Bead Kit	OPTI Medical Systems 99-58015
Thermo Scientific	
Thermo Scientific™ KingFisher™ Flex 96 deep well plate 96 well elution plate 96 tip comb for deep well magnet	Flex instrument (5400630) and software v1.0.1.0 Deep well plate: 95040460 Elution plate: 97002540 Tip Comb: 97002534
Extraction control containing human specimen (HSC) material	See Quality Controls section
96-well cold plate	MLS
Micro-centrifuge for 2 mL microtubes capable of 1500–3000 x g	MLS
Vortex mixer	MLS
1.5 mL microcentrifuge tubes (DNase/ RNase free)	MLS
Pipettes and multi-channel pipettes (5–1000 µL); dedicated pipettes for preparation of PCR Mix	MLS
Nuclease-free, aerosol resistant pipette tips	MLS
Personal protective equipment consistent with current guidelines for handling infectious samples	MLS
Optional: Centrifuge with rotor and adapters for multi-well plates	MLS
PCR plate cooler	MLS
External Positive Control	**Source and part number**
Option 1 (Extraction required): NATtrol™ Flu/RSV/SARS-CoV-2 Positive Control Option 2 (No extraction required):	Zeptometrix (Catalogue# NATFRC-6C)

Twist synthetic influenza H1N1 RNA (2009) RNA Control *and*	Twist Bioscience (Catalogue# 103001)
Twist synthetic influenza B RNA Control *and*	Twist Bioscience (Catalogue# 103003)
Twist synthetic SARS-CoV-2 RNA Control	Twist Bioscience (Catalogue# 102024)
Option 3 (No extraction required):	
Genomic RNA from influenza A/Indiana/10/2011 *and*	International Reagent Resources (Catalogue# FR986)
Genomic RNA from influenza B/Wisconsin/1/2010 *and*	International Reagent Resources (Catalogue# FR1044)
Genomic RNA from SARS-CoV-2, Isolate USA-WA/2020	BEI resources (Catalogue# NR-52347)

MLS = Major Laboratory Supplier, such as VWR, Fisher Scientific, Eppendorf.

*This RUO instrument requires qualification prior to testing samples with the OPTI SARS-CoV-2/ Influenza A/B RT-PCR Test Version 2. Refer to the Laboratory Procedure for Qualification of RUO Instruments Standard Operating Procedure for qualification procedures.

Warnings and Precautions

General

- For *in vitro* diagnostic (IVD) use.
- For prescription use only.
- For use under Emergency Use Authorization (EUA) 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 and differentiation of nucleic acid from SARS-CoV-2, influenza A, and/or 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.
- Handle all specimens as infectious using safe laboratory procedures. Refer to Interim Laboratory Biosafety Guidelines for Handling and Processing Specimens Associated with SARS-CoV-2: https://www.cdc.gov/coronavirus/2019-ncov/lab/lab-biosafety-guidelines.html
- Use personal protective equipment (PPE) consistent with current guidelines for the handling of potentially infectious samples.
- Do not eat, drink, smoke, apply cosmetics or handle contact lenses in areas where reagents and human specimens are handled.
- Modifications to assay reagents, assay protocol, or instrumentation are not permitted, and are in violation of the product Emergency Use Authorization.
- Dispose of waste in compliance with the local, state, and federal regulations.

PCR

- Reagents must be stored and handled as specified in these instructions for use. Do not use reagents past expiration date.
- The entire procedure must be performed under nuclease-free conditions.
- Wear powder-free gloves when working with the reagents and nucleic acids.
- Always use pipette tips with aerosol barriers. Tips that are used must be sterile and free from DNases and RNases.
- Keep reagents and PCR Mix tubes capped or covered as much as possible.
- To avoid cross-contamination, use nuclease-free, aerosol-resistant pipette tips for all pipetting, and physically separate the workplaces for nucleic acid extraction/handling, PCR setup and PCR.
- Work surfaces, pipettes, and centrifuges should be cleaned and decontaminated with cleaning products such as 10% bleach, "DNAZap™" or "RNase AWAY®" to minimize risk of nucleic acid contamination. Residual bleach should be removed using 70% ethanol.
- The internal control for the test detects human nucleic acid; it is important to avoid environmental sources of human nucleic acid contamination.

Specimen Collection

- The sample collection device is not a part of the test kit. The OPTI SARS-CoV-2/Influenza A/B RT-PCR Test is compatible with collection swabs and media stated in the FDA website (https://www.cdc.gov/coronavirus/2019-nCoV/guidelines-clinical-specimens.html).
- Follow specimen collection manufacturer instructions for proper collection methods.
- Swab specimens should be collected using only swabs with a synthetic tip, such as nylon or Dacron® and an aluminum or plastic shaft. Calcium alginate swabs should not be used and cotton swabs with wooden shafts are not recommended. Place swabs immediately into sterile tubes containing 2–3 mL of universal transport media.

Transporting Specimens

- Specimens must be packaged, shipped, and transported according to the current edition of the International Air Transport Association (IATA) Dangerous Goods Regulation. Follow shipping regulations for UN 3373 Biological Substance, Category B when sending potential 2019-nCoV specimens.
- Store specimens at 2–8°C and ship on ice packs.

Storing Specimens

- Specimens can be stored at 2–8°C for up to 72 hours after collection.
- If a delay in extraction is expected, store specimens at –70°C or lower temperature, per CDC guidelines.

Reconstitution of Dried Components

Reconstitute the SARS-CoV-2/Flu Mix and SARS-CoV-2/Flu PC by pipetting PCR Grade Water to the volume indicated on the component label. Allow to sit at 18 to 26°C for at least 10 minutes; mix and microcentrifuge briefly prior to use. Once the components are reconstituted, the target mix can be kept at 2–8°C for up to 8 days. For the SARS-CoV-2/Flu PC and long-term storage of the SARS-CoV-2/Flu Mix, aliquot as appropriate and store the solutions frozen at -25°C to -15°C with the expiration date stated in the kit. When handling frozen components, thaw at 18 to 26°C for approximately 15 to 30 minutes, mix gently and then microcentrifuge briefly (~1,500 – 3,000 × g).

Extraction

Nucleic acids are extracted using the OPTI DNA/RNA Magnetic Bead Extraction kit (OPTI Medical System, #99-58015) on the Thermo Scientific™ KingFisher™ Flex Magnetic Particle Processor with 96 Deep-Well Head and plates. Refer to the online manual at https://www.optimedical.com/files/art-06-58015-02-opti-dna-rna-mag-bead.pdf for detailed instructions on preparing samples, wash plates, and elution plate.

On the KingFisher™ Flex Magnetic Particle Processor, download and install the run protocol (OPTI-FLEX-01) from https://www.optimedical.com/files/opti-flex-01.bdz. Download instructions can be found in the *BindIt Software user Manual* (https://assets.thermofisher.com/TFS-Assets/LSG/manuals/BindIt_4_KingFisherInstrumentsUserManual.pdf).

Store the purified RNA at <–15°C if testing is not performed immediately after RNA extraction.

Quality Controls

Control(s) that are provided with the OPTI SARS-CoV-2/Influenza A/B RT-PCR Test are listed below:

- Negative Control (OPTI PCR Grade Water): A "no template" (negative) control is needed to confirm the PCR plate is valid. PCR Grade water is used and should be included for each PCR run. The negative control should test negative for the SARS-CoV-2 target and internal control. The no template control is not included during extraction.
- Positive Control (OPTI SARS-CoV-2/Flu PC): A positive template control is needed to confirm the PCR plate is valid. Synthetic nucleic acids for the N1 target region of SARS-CoV-2, and target regions for influenza A, influenza B, and RNase P (ISC) are used. The positive control should be included on each PCR run and should test positive for all assay targets and internal control channels. The positive control is not included during extraction.
- The internal control for the test is a human endogenous nucleic acid sequence (RNase P) and controls for sample addition, extraction and PCR.

Control(s) that are required but not provided with the OPTI SARS-CoV-2/Influenza A/B RT-PCR Test are listed below:

- Extraction control: An extraction control containing human specimen control (HSC) material should be extracted and tested with each set of patient samples. The extraction control is used to demonstrate successful recovery of RNA during the extraction process and should test negative for the SARS-CoV-2,

Influenza A and Influenza B targets, and positive for the RNase P internal control. Laboratories may use confirmed negative human specimen material (e.g. a negative respiratory specimen). This material should be prepared in enough volume to be used across multiple runs. Material should be tested prior to use as the extraction control to ensure it generates the expected results.

- External positive control: A viral RNA control should be tested with each set of patient samples to demonstrate successful reverse transcription during PCR. The external positive control should test positive for SARS-CoV-2, Influenza A, and Influenza B.

  External Control option 1: Extract RNA from the NATtrol™ Flu/RSV/SARS-CoV-2 Positive Control using the OPTI DNA/RNA Magnetic Bead Kit (input volume = 500 µL, elution volume = 100 µL).

  External Control option 2: Use Twist synthetic control RNA for Influenza A, Influenza B, and SARS-CoV-2 (no extraction needed).

  External Control option 3: Use genomic RNA for Influenza A, Influenza B, and SARS-CoV-2 (no extraction needed).

  Materials should be tested prior to use as the external positive control to ensure it generates the expected results. Store RNA in small aliquots at –70°C.

Test Procedure

1 Preparation of the PCR Mix.

- Mix the thawed RNA MMx by inversion or gentle vortex.
- The RNA MMx is a viscous solution; always pipette it slowly.
- To prepare the PCR Mix, add 10 µL OPTI SARS-CoV-2/Flu Mix and 10 µL RNA MMx for each reaction.
- When preparing the PCR Mix, first pipette OPTI SARS-CoV-2/Flu Mix into the tube and then add the RNA MMx. Pipette up and down a few times to rinse the MMx pipette tip.
- Gently vortex the solution to ensure the components are mixed well.
- Keep the PCR Mix on ice until it is pipetted onto the PCR plate.

Important: *Plate set-up must be completed, and plate loaded into the instrument within 30 minutes of PCR Mix preparation. The PCR Mix must be kept cold at all times. Protect from light.*

2 Use a chilled block to keep the PCR plate cool during set-up.

3 Carefully pipette 20 µL of the PCR Mix into the required wells of the PCR plate.

4 Add 5 µL of sample RNA to each well. The final reaction volume is 25 µL.

1 Include the Positive Control from the kit (5 µL), PCR negative control (5 µL PCR Grade Water), Extraction control (5 µL), and extracted Positive Control RNA (5 µL), or synthetic or genomic RNA (5 µL), for each test run.

2 Seal the plate and briefly spin the plate, if necessary, to settle contents and remove air bubbles.

3 Load the plate into the PCR instrument. Set up the Cycling Program below. Start the run.

Settings for Reporter and Quencher

Target	Dye	Reporter	Quencher
Influenza A	FAM™	FAM™	BHQ® (none)
Influenza B	CAL Fluor Red 610	ROX™	BHQ® (none)
SARS-CoV-2	Cy5	Cy5	BHQ® (none)
Internal Control (RNase P)	CAL Fluor Orange 560	VIC™	BHQ® (none)

Cycling Program (used for all instruments)

Step	Temperature	Time	No. of Cycles
Reverse transcription (RT)	50°C	15 min.	1
Denaturation	95°C	2 min.	1
Amplification**	95°C 60°C	15 sec. 30 sec	45

**Ensure the instrument is set to record fluorescence following the 60°C amplification step.

8 Data Analysis

All test controls must be examined prior to interpretation of patient results. If the controls are not valid, the patient results cannot be interpreted.

Using the PCR instrument software, assign a unique identifier for the SARS-CoV-2, Influenza A, Influenza B and internal control targets on the plate. To obtain appropriate Ct values, analysis for each target should be performed by manually setting the threshold. Each target threshold should be set separately. The threshold should be adjusted to the inflection point for the exponential phase of the positive control curve and above background signal of the negative control. Confirm threshold placement by viewing the curves for each target. It is important to follow the same procedure run to run when setting the manual threshold.

Refer to specific instrument's user manual for guidance on how to analyze data.

Plate Validity Criteria

The following control results must be obtained for each PCR run in order for the run to be deemed valid. If the plate controls are not valid, the patient results cannot be interpreted, are not valid, and the plate must be repeated. If a positive/negative control fails, retesting the same eluates is recommended first to determine if there were some process-related issues in the initial run. If the extraction control fails, the extraction must be repeated from the original samples.

Control	SARS-CoV-2/Flu Positive Control	PCR Negative Control	Extraction Control	External Positive Control
Influenza A FAM Ct/result	<40 Positive	No Signal Negative	No Signal Negative	<40 Positive
Influenza B CAL Fluor Red 610 Ct/result				
SARS-CoV-2 Cy5 Ct/result				
Internal Control CAL Fluor Orange 560 Ct/result	<40 Positive	No signal Negative	<36 Positive	n/a

Sample Validity: The table below details the results interpretation of the SARS-CoV-2, Influenza A, Influenza B and internal control target for each sample.

9 Examination and Interpretation of Patient Specimen Results

Assessment of clinical specimen test results must be performed after the positive and negative controls have been examined and determined to be valid and acceptable. If the controls are not valid, the patient results cannot be interpreted.

Sample Result	Target Channel	Target Ct Value	Internal Control CAL Fluor Orange 560 Ct Value	Other Characteristics
Influenza A RNA POSITIVE	FAM	≤40	Any Ct value	A characteristic amplification curve in comparison to the PCR negative control. An internal control amplification curve in the CAL Fluor Orange 560 channel is expected. A strong positive SARS-CoV-2, Influenza A or Influenza B sample may result in a negative internal control result.
Influenza B RNA POSITIVE	CAL Fluor Red 610			
SARS-CoV-2 RNA POSITIVE	Cy5			
Influenza A RNA NEGATIVE	FAM	>40	≤36	Amplification curve in the CAL Fluor Orange 560 internal control channel
Influenza B RNA NEGATIVE	CAL Fluor Red 610			
SARS-CoV-2 RNA NEGATIVE	Cy5			
Invalid Sample**	Any Target	>40	>36	Absence of an amplification curve in the CAL Fluor Orange 560 channel indicates an invalid result for any negative targets in the sample.

**An invalid sample can be an indication of failed sample addition, extraction and/or PCR. It is recommended that the RNA be diluted five-fold into PCR grade water and retested; include the undiluted RNA as a sample. If the test is still not valid, a new extraction is recommended.

Limitations

10 Limitations

- Performance of the OPTI SARS-CoV-2/Influenza A/B RT-PCR Test has only been established in nasopharyngeal swabs. Other specimen types have not been evaluated and should not be tested with this assay.
- The OPTI SARS-CoV-2/Influenza A/B RT-PCR Test is for qualitative detection only and not for quantitative detection of SARS-CoV-2, Influenza and Influenza B.
- The OPTI SARS-CoV-2/Influenza A/B RT-PCR Test can detect SARS-CoV-2 but cannot identify any specific variant strain present in the circulation at any place and time.
- If the virus mutates in the test target region, SARS-CoV-2, Influenza A or Influenza B RNA may not be detected or may be detected less predictably. Inhibitors or other types of interference may produce a false negative result.
- A high concentration of the SARS-CoV-2 analyte may inhibit the detection of Influenza A and B.
- Negative results do not preclude infection with SARS-CoV-2, influenza A, and/or influenza B virus and should not be the sole basis of a patient management decision.
- Clinical performance has not been established in 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.
- Laboratories are required to report all SARS-CoV-2 results to the appropriate public health authorities.
- Samples must be collected, transported, and stored using appropriate procedures and conditions.

Improper collection, transport, or storage of specimens may affect the test performance.

- 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 validated.
- A false negative result may occur if a specimen is improperly collected, transported. False negative results may also occur if amplification inhibitors are present in the specimen or if inadequate numbers of organisms are present in the specimen.
- False-positive results may arise from cross contamination during specimen handling, preparation, nucleic acid extraction, PCR assay set-up or product handling.
- The performance of the test has not been established in individuals who received nasal administered Influenza vaccine. Individuals who received nasal administered Influenza A vaccine may have positive Influenza A test results for up to three days after vaccination. https://www.cdc.gov/mmwr/preview/mmwrhtml/rr57e717a1.htm
- Detection of viral RNA may not indicate the presence of infectious virus or that Influenza or SARS-CoV-2 viruses are the causative agents for clinical symptoms.
- The performance of this test has not been established for screening of blood or blood products for the presence of Influenza A, Influenza B or SARS-CoV-2.
- This test cannot rule out diseases caused by other bacterial or viral pathogens.
- Optimum specimen types and timing for peak viral levels during infections caused by a novel Influenza or SARS-CoV-2 virus have not been determined. Collection of multiple specimens from the same patient may be necessary to detect the viruses.

CONDITIONS OF AUTHORIZATION FOR THE LABORATORY

11 Conditions

The OPTI SARS-CoV-2/Influenza A/B RT-PCR Test 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/in-vitro-diagnostics-euas-molecular-diagnostic-tests-sars-cov-2

To assist clinical laboratories using the OPTI SARS-CoV-2/Influenza A/B RT-PCR Test the relevant Conditions of Authorization are listed verbatim below, and are required to be met by laboratories performing the EUA test.

a) Authorized laboratories using your product[1] must include with test result reports all authorized Fact Sheets. Under exigent circumstances, other appropriate methods for disseminating these Fact Sheets may be used, which may include mass media.

b) Authorized laboratories using your product must use your product as outlined in the authorized labeling. Deviation 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 are not permitted.

c) Authorized laboratories that receive your product must notify the relevant public health authorities of their intent to run your product prior to initiating testing.

d) Authorized laboratories using your product must have a process in place for reporting test results to healthcare providers and relevant public health authorities, as appropriate.

e) Authorized laboratories must collect information on the performance of your product and report to DMD/OHT7-OIR/OPEQ/CDRH (via email: CDRH_EUA-Reporting@fda.hhs.gov) and OPTI Medical Systems Inc. (via email: covid19@optimedical.com) any suspected occurrence of false positive or false negative results and significant deviations from the established performance characteristics of your product of which they become aware.

f) All laboratory personnel using your product must be appropriately trained in PCR techniques and use appropriate laboratory and personal protective equipment when handling this kit and use this product in accordance with the authorized labeling.

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1 "Your product" refers to the OPTI SARS-CoV-2/Influenza A/B RT-PCR Test. 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".

g) OPTI Medical Systems, Inc., authorized distributors, and authorized laboratories using your product must ensure that any records associated with this EUA are maintained until otherwise notified by FDA. Such records will be made available to FDA for inspection upon request.

Assay Performance

12 Limit of Detection (LoD)

Limit of detection (LoD) is defined as the lowest concentration of SARS-CoV-2, Influenza A and Influenza B at which greater than or equal to 95% of all replicates test positive. LoD for the OPTI SARS-CoV-2/Influenza A/B RT-PCR Test was determined using serial dilutions of inactivated SARS-CoV-2 virus from BEI, Manassas, VA (NR-52286), live influenza A/California/08/2009 pdm09/H1N1 (VR-1895), live influenza A/Hong Kong/4801/2014/H3N2 (VR-1990), live influenza B/Florida/78/2015 Victoria (VR-1930), and live influenza B/Florida/4/2006 Yamagata (VR-1804) from American Type Culture Collection, Manassas, VA, prepared in nasopharyngeal (NP) swab sample pools.

The initial LoD was determined with 3-fold serial dilutions tested in triplicate. Each replicate was extracted using the OPTI DNA/RNA Magnetic Bead Kit on Thermo Scientific™ KingFisher™ Flex following the standard protocol. Extracted RNA was tested on the Roche LightCycler 480, and Bio Molecular Systems MIC PCR instruments. To confirm the LoD, 20 replicates of each sample matrix spiked with SARS-CoV-2, Influenza A and Influenza B viruses were extracted with the OPTI DNA/RNA Magnetic Bead Kit on the Thermo Scientific™ KingFisher™ Flex. Extracted RNA was tested on Roche LightCycler 480, Bio-Rad CFX96 Touch, and Bio Molecular Systems MIC PCR instruments PCR instruments. The LoDs were confirmed to be:

SARS-CoV-2 virus	
Roche LightCycler 480	1.09 GCE/µL (20/20)
Bio-Rad CFX96 Touch	0.36 GCE/µL (19/20)
Bio Molecular Systems	0.36 GCE/µL (20/20)
Influenza A/H3N2	
Roche LightCycler 480	0.14 CEID50/µL (20/20)
Bio-Rad CFX96 Touch	0.14 CEID50/µL (20/20)
Bio Molecular Systems	0.14 CEID50/µL (20/20)
Influenza A/H1N1	
Roche LightCycler 480	0.05 CEID50/µL (19/20)
Bio-Rad CFX96 Touch	0.05 CEID50/µL (20/20)
Bio Molecular Systems	0.05 CEID50/µL (20/20)
Influenza B/Victoria	
Roche LightCycler 480	0.14 CEID50/µL (20/20)
Bio-Rad CFX96 Touch	0.14 CEID50/µL (20/20)
Bio Molecular Systems	0.14 CEID50/µL (20/20)
Influenza B/Yamagata	
Roche LightCycler 480	0.14 CEID50/µL (20/20)
Bio-Rad CFX96 Touch	0.05 CEID50/µL (20/20)
Bio Molecular Systems	0.05 CEID50/µL (20/20)

Results are shown in Tables 1 and 2 below.

Table 1: LoD Initial Determination

S-CoV-2, Roche LightCycler 480

SARS-CoV-2 GCE/µL	Mean Ct	Hit rate	% Detection
9.83	32.94	3/3	100
3.28	34.01	3/3	100
1.09	35.38	3/3	100
0.36	35.67	2/3	67

SARS-CoV-2, Bio Molecular Systems MIC qPCR

SARS-CoV-2 GCE/µL	Mean Ct	Hit rate	% Detection
3.28	33.32	3/3	100
1.09	34.44	3/3	100
0.36	35.64	3/3	100
0.12	35.99	2/3	67

Influenza A/H3N2, Roche LightCycler 480

A/H3N2 CEID50/µL	Mean Ct	Hit rate	% Detection
1.23	33.18	3/3	100
0.41	33.97	3/3	100
0.14	34.74	3/3	100
0.05	35.15	2/3	67

Influenza A/H3N2, Bio Molecular Systems MIC qPCR

A/H3N2 CEID50/µL	Mean Ct	Hit rate	% Detection
0.41	32.03	3/3	100
0.14	33.93	3/3	100
0.05	34.56	3/3	100
0.02	35.24	1/3	33

Influenza A/H1N1, Roche LightCycler 480

A/H1N1 CEID50/µL	Mean Ct	Hit rate	% Detection
0.41	33.45	3/3	100
0.14	34.05	3/3	100
0.05	35.47	3/3	100
0.02	35.36	2/3	67

Influenza A/H1N1, Bio Molecular Systems MIC qPCR

A/H1N1 CEID50/µL	Mean Ct	Hit rate	% Detection
0.41	30.82	3/3	100
0.14	32.22	3/3	100
0.05	34.26	3/3	100
0.02	35.08	2/3	67

Influenza B/Victoria, Roche LightCycler 480

B/Victoria CEID50/µL	Mean Ct	Hit rate	% Detection
0.41	33.82	3/3	100
0.14	34.55	3/3	100
0.05	35.28	3/3	100
0.02	No Ct	0/3	0

Influenza B/Victoria, Bio Molecular Systems MIC qPCR

B/Victoria CEID50/µL	Mean Ct	Hit rate	% Detection
1.23	30.96	3/3	100
0.41	32.41	3/3	100
0.14	34.35	3/3	100
0.05	34.99	2/3	67

Influenza B/Yamagata, Roche Light Cycler 480

B/Yamagata CEID50/µL	Mean Ct	Hit rate	% Detection
0.41	32.83	3/3	100
0.14	34.03	3/3	100
0.05	34.94	3/3	100
0.02	35.13	3/3	100

Influenza B/Yamagata, Bio Molecular Systems MIC qPCR

B/Yamagata CEID50/µL	Mean Ct	Hit rate	% Detection
1.23	30.03	3/3	100
0.41	31.48	3/3	100
0.14	32.09	3/3	100
0.05	35.02	1/3	33

Table 2: LoD Confirmation –

SARS-CoV-2

PCR Instrument	SARS-CoV-2 GCE/µL	Mean Ct	Detection Rate	LoD copies/µL
Roche LightCycler 480	1.09	34.54	20/20	1.09
	0.36	35.34	15/20	
Bio-Rad CFX96 Touch	0.36	35.22	19/20	0.36
	0.12	36.02	4/20	
Bio Molecular Systems MIC qPCR	0.36	32.97	20/20	0.36
	0.12	33.37	6/20	

Influenza A/H3N2

PCR Instrument	A/H3N2 CEID50/µL	Mean Ct	Detection Rate	LoD copies/µL
Roche LightCycler 480	0.14	34.42	20/20	0.14
	0.05	34.99	17/20	
Bio-Rad CFX96 Touch	0.14	32.78	19/20	0.14
	0.05	33.92	18/20	
Bio Molecular Systems MIC qPCR	0.14	33.09	20/20	0.14
	0.05	34.28	16/20	

Influenza A/H1N1

PCR Instrument	A/H1N1 CEID50/µL	Mean Ct	Detection Rate	LoD copies/µL
Roche LightCycler 480	0.05	34.89	19/20	0.05
	0.02	35.01	15/20	
Bio-Rad CFX96 Touch	0.05	33.42	20/20	0.05
	0.02	34.80	17/20	
Bio Molecular Systems MIC qPCR	0.05	34.46	20/20	0.05
	0.02	34.58	14/20	

Influenza B/Victoria

PCR Instrument	B/Victoria CEID50/µL	Mean Ct	Detection Rate	LoD copies/µL
Roche LightCycler 480	0.14	33.80	20/20	0.14
	0.05	34.77	10/20	
Bio-Rad CFX96 Touch	0.14	30.44	20/20	0.14
	0.05	31.02	18/20	
Bio Molecular Systems MIC qPCR	0.14	33.34	20/20	0.14
	0.05	34.08	16/20	

Influenza B/Yamagata

PCR Instrument	B/Yamagata CEID50/µL	Mean Ct	Detection Rate	LoD copies/µL
Roche LightCycler 480	0.14	34.43	20/20	0.14
	0.05	34.13	17/20	
Bio-Rad CFX96 Touch	0.05	30.73	20/20	0.05
	0.02	34.99	15/20	
Bio Molecular Systems MIC qPCR	0.05	33.45	20/20	0.05
	0.02	34.54	16/20	

12 Inclusivity (analytical reactivity)

a) SARS-CoV-2

The OPTI SARS-CoV-2/Influenza A/B RT-PCR Test uses the primer and probe sequences that are described by the CDC. A dual target design is chosen to increase and maintain inclusivity in the event of any point mutation in one of the target regions. CDC has given the right of reference for leveraging all data including wet-testing.

SARS-CoV-2 inclusivity was assessed by comparing the OPTI SARS-CoV-2 RT-PCR test primer and probe design to the recently circulating SARS-CoV-2 viruses. High coverage SARS-CoV-2 genomic sequences from September 1st, 2021 and December 20th, 2021 for VOC alpha, beta, gamma, and omicron were downloaded from the GISAID database. Due to the large number of delta variant submissions, 105,346 delta sequences within the same period were pulled without biases. The N gene region was aligned to the test design using the MAFFT version 7 software (RIMD, Osaka, Japan). The analysis showed that over 99% of all VOC SARS-CoV-2 variants have a perfect match to both or at least one of the two N-gene targets and therefore will be amplified and detected by the OPTI SARS-CoV-2 RT-PCR test (see Tables below). In this analysis, it is noted that 98.7% of the omicron variant population has a conserved single nucleotide mutation (C -> T) in the N1 probe. However, this mutation is unlikely to affect the amplification of the target because it is located at the 5' end. A preliminary study using a synthetic N1 template containing the prevalent omicron mutation also showed that the mutation has no impact on the amplification of the target.

Probe sequence: ACCCCGCATTACGTTTGGTGGACC

Omicron variant: ACTCCGCATTACGTTTGGTGGACC

Alpha variant sequence alignment summary

ALIGNMENT	COUNT	PERCENTAGE
N1,N4 Perfect Match	724	93.54%
N1 match (mismatch in N4)	16	2.07%
N4 match (mismatch in N1)	33	4.26%
Mismatches in N1 and N4	1	0.13%
TOTAL	**774**	**100.00%**

Beta variant sequence alignment summary

ALIGNMENT	COUNT	PERCENTAGE
N1,N4 Perfect Match	85	64.39%
N1 match (mismatch in N4)	14	10.61%
N4 match (mismatch in N1)	31	23.48%
Mismatches in N1 and N4	0	0.00%
TOTAL	**132**	**100.00%**

Gamma variant sequence alignment summary

ALIGNMENT	COUNT	PERCENTAGE
N1,N4 Perfect Match	906	89.88%
N1 match (mismatch in N4)	76	7.54%
N4 match (mismatch in N1)	25	2.48%
Mismatches in N1 and N4	1	0.10%
TOTAL	**1008**	**100.00%**

Delta variant sequence alignment summary

ALIGNMENT	COUNT	PERCENTAGE
N1,N4 Perfect Match	82713	78.52%
N1 match (mismatch in N4)	10423	9.89%
N4 match (mismatch in N1)	11753	11.16%
Mismatches in N1 and N4	457	0.43%
TOTAL	**105346**	**100.00%**

Omicron variant sequence alignment summary

ALIGNMENT	COUNT	PERCENTAGE
N1,N4 Perfect Match	60	0.48%
N1 match (mismatch in N4)	3	0.02%
N4 match (mismatch in N1)	12351	98.96%
Mismatches in N1 and N4	67	0.54%
TOTAL	**12481**	**100.00%**

Wet testing data showed that the OPTI SARS-CoV-2/Influenza A/B RT-PCR Test detected the BA.2 variant sequence with the same efficiency when compared to the N1 and N4 synthetic DNA targets.

b) Influenza A

The OPTI SARS-CoV-2/Influenza A/B RT-PCR Test uses identical Influenza A primer sequences described in the CDC test design and an exact reverse complement of the CDC Influenza A probe sequence[2]. However, to assess the *in silico* inclusivity of the OPTI Influenza A Test, a Master Sequence Alignment (MSA) was generated from the GISAID Influenza A database[3] sequences for H1N1 and H3N2 and compared for identity to the test primers and probes. Only full-length H1N1 and H3N2, high coverage sequences from human hosts from North America, Europe and Asia were included, resulting in over 75,000 sequences in the design region. 98.7% of sequenced strains match the Influenza A forward primer, 94.6% match the Influenza A probe and 98.7% match the Influenza A reverse primer. Based on this analysis the OPTI SARS-CoV-2/Influenza A/B RT-PCR Test is predicted to detect all currently circulating strains of influenza A.

c) Influenza B

The OPTI SARS-CoV-2/Influenza A/B RT-PCR Test uses identical Influenza B primer sequences described in the CDC test design and uses an Influenza B probe that contains a single degenerate base not included in the CDC Influenza B probe[3]. However, to assess the *in silico* inclusivity of the OPTI Influenza B Test, an MSA was generated from the GISAID Influenza B database[3] sequences and compared for identity to the test primers and probes. Only full-length Victoria and Yamagata lineages with high coverage sequences from human hosts from North America, Europe and Asia were included, resulting in over 19,000 sequences in the design region. 95.9% of sequenced strains match the Influenza B forward primer, 98.5% match the Influenza B probe and 99.1% match the Influenza Breverse primer. The addition of the degenerate nucleotide base to the probe adds an additional 555 (2.9%) sequences to match the design. Based on this analysis the OPTI SARS-CoV-2/Influenza A/B RT-PCR Test is predicted to detect all currently circulating strains of influenza A.

Wet testing

The inclusivity of OPTI SARS-CoV-2/Influenza A/B for the detection of influenza A was evaluated by testing five isolates of A/H1N1 and five isolates of B/H3N2 from within the past five years, and for the detection of influenza B by testing five isolates of influenza B/Victoria and three isolates of B/Yamagata lineages from within the past five years. The lowest target analyte concentration at which all four tested replicates were tested positive are reported in Table 3. Additionally, two influenza B isolates from St. Jude Children's Research Hospital were also tested positive correctly using the OPTI SARS-CoV-2/Influenza A/B Tests.

Table 3: Wet Testing of Influenza A and B inclusivity.

Influenza organism	Lowest detectable concentration
Influenza A Virus, A/Hawaii/66/2019 (H1N1)pdm09	3.70E+03 CEID50/mL
Influenza A Virus, A/Idaho/07/2018 (H1N1)pdm09	1.60E-01 TCID50/mL
Influenza A Virus, A/Indiana/02/2020 (H1N1)pdm09	4.85E+02 CEID50/mL
Influenza A Virus, A/Michigan/272/2017 (H1N1)pdm09	4.80E+00 TCID50/mL
Influenza A Virus, A/Wisconsin/588/2019 (H1N1)pdm09	1.63E+01 FFU/mL
Influenza A Virus, A/Arizona/45/2018 (H3N2)	3.38E+01 FFU/mL
Influenza A Virus, A/Hong Kong/2671/2019 (H3N2)	3.61E+03 CEID50/mL
Influenza A Virus, A/Kansas/14/2017 (H3N2)	3.96E+01 FFU/mL
Influenza A Virus, A/Wisconsin/04/2018 (H3N2)	1.40E+02 CEID50/mL
Influenza A Virus, A/Texas/71/2017 (H3N2)	1.40E+02 TCID50/mL
Influenza B Virus, B/Hong Kong/286/2017 (Victoria Lineage)	1.35E+00 TCID50/mL
Influenza B Virus, B/Colorado/6/2017 (Victoria Lineage)	7.00E-01 TCID50/mL
Influenza B Virus, B/Hawaii/01/2018 (NA D197N) (Victoria Lineage)	3.26E+02 TCID50/mL
Influenza B Virus, B/Washington/02/2019 (Victoria Lineage)	1.50E+03 CEID50/mL
Influenza B Virus, B/Missouri/12/2018 (NA D197E) (Victoria Lineage)	2.80E+01 TCID50/mL
Influenza B Virus, B/Wisconsin/10/2016 (NA I221V) (Yamagata lineage)	1.60E+02 TCID50/mL
Influenza B Virus, B/Indiana/17/2017 (NA I221T) (Yamagata Lineage)	5.00E+01 TCID50/mL
Influenza B Virus, B/Oklahoma/10/2018 (NA D197N) (Yamagata	3.80E+01 TCID50/mL
B/Memphis/47/2016	*not determined*
B/Memphis/FMT001-A1/2015	*not determined*

13 Analytical Specificity

a) *In silico* Cross-reactivity: SARS-CoV-2

The OPTI SARS-CoV-2/Influenza A/B RT-PCR Test uses identical N1, N4 primer and probe sequences as described for the CDC design. To assess the *in silico* exclusivity of the OPTI SARS-CoV-2/Influenza A/B RT-PCR Test, an MSA was generated from several high priority pathogens from the same genetic family as SARS-CoV-2 as well as other high-profile pathogens likely in the same biological niche as SARS-CoV-2. This alignment was then compared for identity to the test primers and probes. The N1 and N4 design regions were aligned with SARS coronavirus (NC_004718), MERS coronavirus (NC_019843), and human coronaviruses NL63 (NC_005831), OC43 (KX344031), 229E (NC_002645), and HKU1 (NC_006577). No single primer or probe sequence contains greater than 80% identity to the design region other than NC_004718 (SARS coronavirus Tor2) which contains 91.7% identity with the N1 probe. Based on the mismatches in the overall design region, it is highly unlikely the N1 primers will amplify the target region of NC_004718.

A BLAST analysis was performed using the N1 and N4 amplicon sequences, lenient parameters and excluding SARS-CoV-2 and unidentified viral sequences. No significant similarity to any sequences in the NCBI database were returned.

Similarly, a directed BLAST search was performed against the genome sequences from other upper respiratory tract microorganisms listed in Table 4. Again, no significant similarities were returned.

b) *In silico* Cross-reactivity: Influenza A

A BLAST analysis was performed using the Influenza A amplicon, lenient parameters and excluding Influenza B and unidentified viral sequences. No significant similarity to any sequences in the NCBI database were returned. Similarly, a directed BLAST search was performed against the genome sequences from other upper respiratory tract microorganisms listed in Table 4. Again, no significant similarities were returned.

c) *In silico* Cross-reactivity: Influenza B

A BLAST analysis was performed using the Influenza B amplicon, lenient parameters and excluding Influenza B and unidentified viral sequences. No significant similarity to any sequences in the NCBI database were returned. Similarly, a directed BLAST search was performed against the genome sequences from other upper respiratory tract microorganisms listed in Table 4. Again, no significant similarities were returned.

Table 4: List of organisms analyzed *in silico*.

Organism	Strain	Accession or WGS number
Bordetella bronchiseptica	NCTC10543	NZ_LR134326
Bordetella pertussis	18323	HE965805
Candida albicans	TIMM 1768	GCA_003454735
Chlamydia pneumoniae	CWL029	AE001363
Chlamydia trachomatis	D/UW-3/CX	NC_000117
Corynebacterium diphtheriae	NCTC11397	NZ_LN831026
Escherichia coli	K-12	NC_000913
Haemophilus influenzae	NCTC8143	LN831035
Klebsiella pneumoniae	HS11286	NC_016845

Table 4: List of organisms analyzed *in silico (cont'd)*

Organism	Strain	Accession or WGS number
Lactobacillus plantarum	SK151	NZ_CP030105
Legionella pneumophila	Phil.1	CP015928
Moraxella catarrhalis	BBH18	NC_014147
Mycobacterium tuberculosis	HN-506	AP018036
Mycoplasma pneumoniae	FH	CP010546
Neisseria gonorrhoeae	35/02	NZ_CP012028
Neisseria meningitidis	MC58	NC_003112
Neisseria mucosa	FDAARGOS_758	NZ_CP053939
Pneumocystis jirovecii (PJP)	RU7	GCA_001477535
Proteus mirabilis	HI4320	NC_010554
Proteus vulgaris	NCTC13145	NZ_LR590468
Pseudomonas aeruginosa	PAO1	AE004091
Staphylococcus aureus	NCTC 8325	NC_007795
Staphylococcus epidermidis	ATCC 12228	NC_004461
Streptococcus pneumoniae	NCTC7465	LN831051
Streptococcus pyogenes	NGAS638	NZ_CP010450
Streptococcus salivarius	NCTC8618	NZ_LR134274
Adenovirus	A	NC_001460
Coxsackievirus	B5	JX843811
Echovirus	NGR_2014	MH745407
Enterovirus	EV68	KT266905
Epstein Barr Virus	YCCEL1	AP015016
Human coronavirus	OC43	KX344031
Human coronavirus	HKU1	MH940245
Human coronavirus	NL63	MK334047
Human rhinovirus A	p311	KX398052
Human rhinovirus B	R93	KF958309
Human rhinovirus C	25	EF582386
Influenza virus A	A/California/VRDL/179/2009	CY092759
Influenza virus B	B/Iowa/03/2002	CY019567
MERS-coronavirus	011/LOM/C20	MK357909
Metapneumovirus	00-1	NC_039199
Parainfluenza virus 1	NM001	KX639498
Parainfluenza virus 2	VIROAF10	KM190939
Parainfluenza virus 3	CFI1849	KJ672618
Parainfluenza virus 4	SC3019	KY986647
Respiratory Syncytial virus	B/WI/629-Q0190/10	JN032120
SARS-coronavirus	MA-15	DQ497008

d) Cross-reactivity and Microbial Interference Study (Wet testing)

The OPTI SARS-CoV-2/Influenza A/B RT-PCR Test was evaluated for cross-reactivity with a panel of bacteria, viruses and yeast that represents common respiratory pathogens, and a pool of 30 negative human nasopharyngeal specimens that represents a microbial flora seen in human respiratory specimens. Live bacteria and yeast at the listed concentrations were spiked with inactivated SARS-CoV-2 virus, live influenza A/H3N2, or B/Victoria viruses at ≤3xLoD. Nucleic acids were extracted using the OPTI DNA/RNA Magnetic Bead Kit with the Thermo Scientific KingFisher Flex Purification System and test on Roche LC480 Real-Time PCR instrument. No cross-reactivity was observed (Table 5). Detection of the analytes was not affected by the presence of the other microbial species tested (Table 6).

Table 5: SARS-CoV-2, Influenza A and B cross-reactivity test results

Microorganism	Test Concentration	SARS-CoV-2 Result	Flu A Result	Flu B Result
Bordetella pertussis	2.37E+07 CFU/mL	-	-	-
Candida albicans	1.20E+06 CFU/mL	-	-	-
Chlamydia pneumoniae	5.36E+06 CFU/mL	-	-	-
Corynebacterium spp.	1.77E+07 CFU/mL	-	-	-
Escherichia coli (respiratory)	1.92E+07 CFU/mL	-	-	-
Haemophilus influenzae	1.77E+07 CFU/mL	-	-	-
Lactobacillus sp.	1.65E+07 CFU/mL	-	-	-
Legionella pneumophila	1.0E+07 CFU/mL	-	-	-
Moraxella Catarrhalis	1.74E+07 CFU/mL	-	-	-
Mycoplasma pneumoniae	1.36E+06 CFU/mL	-	-	-
Mycoplasma tuberculosis	152.6 µg/mL	-	-	-
Neisseria Meningitidis	2.10E+07 CFU/mL	-	-	-
Neisseria sp.	2.04E+07 CFU/mL	-	-	-
Pneumocystis Jirovecii (PJP)	Not Available	-	-	-
Pseudomonas aeruginosa	1.80E+07 CFU/mL	-	-	-
Staphylococcus aureus	2.16E+07 CFU/mL	-	-	-
Staphylococcus epidermis	1.95E+07 CFU/mL	-	-	-
Streptococcus pneumoniae	1.62E+07 CFU/mL	-	-	-
Streptococcus pyogenes	2.34E+07 CFU/mL	-	-	-
Streptococcus salivarius	2.31E+07 CFU/mL	-	-	-
Enterovirus D68	1.00E+05 PFU/mL	-	-	-
Epstein Barr Virus	Not Available	-	-	-
Human Adenovirus, Type 1	1.00E+05 PFU/mL	-	-	-
Human Adenovirus, Type 7	3 µg/mL	-	-	-
Human coronavirus 229E	2.67E+05 TCID50/mL	-	-	-
Human coronavirus HKU1	3.67E+05 copies/uL	-	-	-
Human coronavirus NL63	2.67E+03 TCID50/mL	-	-	-
Human coronavirus OC43	1.00E+05 PFU/mL	-	-	-
Human cytomegalovirus	1.00E+05 PFU/mL	-	-	-
Human metapneumovirus	2.8 ng/100µL	-	-	-
Human parainfluenza 1	1.67E+05 PFU/mL	-	-	-
Human parainfluenza 2	1.00E+05 PFU/mL	-	-	-
Human parainfluenza 3	1.00E+05 PFU/mL	-	-	-
Human parainfluenza 4a	1.00E+05 PFU/mL	-	-	-
Human Rhinovirus 14	1.00E+05 PFU/mL	-	-	-
MERS-coronavirus	1.48E+05 TCID50/mL	-	-	-
Respiratory syncytial virus	1.03E+05 PFU/mL	-	-	-
SARS-coronavirus	1.67E+05 PFU/mL	-	-	-
Influenza A (A/H3N2)	9.60E+05 CEID/mL	-	n/a	-
Influenza B (B/Yamagata)	1.10E+08 CEID/mL	-	-	n/a
30 pooled negative human NP	n/a	-	-	-

Table 6: Interfering microorganisms study results

Micro-organism	Test Concentration	SARS-CoV-2, Influenza A & B at ≤3xLoD		
		SARS-CoV-2 Result	Flu A Result	Flu B Result
Bordetella pertussis	2.37E+07 CFU/mL	+	+	+
Candida albicans	1.20E+06 CFU/mL	+	+	+
Chlamydia pneumoniae	5.36E+06 CFU/mL	+	+	+
Corynebacterium sp	1.92E+07 CFU/mL	+	+	+
Escherichia coli (respiratory)	2.13E+07 CFU/mL	+	+	+
Haemophilus influenzae	1.77E+07 CFU/mL	+	+	+
Lactobacillus sp.	1.65E+07 CFU/mL	+	+	+
Legionella pneumophila	1.34E+06 CFU/mL	+	+	+
Moraxella Catarrhalis	1.98E+07 CFU/mL	+	+	+
Mycoplasma pneumoniae	1.36E+06 CFU/mL	+	+	+
Mycoplasma tuberculosis	152.6 µg/mL	+	+	+
Neisseria Meningitidis	2.10E+07 CFU/mL	+	+	+
Neisseria sp.	2.04E+07 CFU/mL	+	+	+
Pneumocystis Jirovecii (PJP)	Not available	+	+	+
Pseudomonas aeruginosa	1.80E+07 CFU/mL	+	+	+
Staphylococcus aureus	2.16E+07 CFU/mL	+	+	+
Staphylococcus epidermidis	1.95E+07 CFU/mL	+	+	+
Streptococcus pneumoniae	1.62E+07 CFU/mL	+	+	+
Streptococcus pyogenes	2.34E+07 CFU/mL	+	+	+
Streptococcus salivarius	2.31E+07 CFU/mL	+	+	+
Enterovirus D68	1.00E+05 PFU/mL	+	+	+
Epstein Barr Virus	not available	+	+	+
Human Adenovirus, Type 1	1.00E+05 PFU/mL	+	+	+
Human Adenovirus, Type 7	2.67E+05 TCID50/mL	+	+	+
Human coronavirus 229E	2.67E+05 TCID50/mL	+	+	+
Human coronavirus HKU1	3.67E+05 copies/µL	+	+	+
Human coronavirus NL63	2.67E+03 TCID50/mL	+	+	+
Human coronavirus OC43	1.00E+05 PFU/mL	+	+	+
Human cytomegalovirus	1.00E+05 PFU/mL	+	+	+
Human metapneumovirus	2.8 ng/100 µL	+	+	+
Human parainfluenza 1	1.67E+05 PFU/mL	+	+	+
Human parainfluenza 2	1.00E+05 PFU/mL	+	+	+
Human parainfluenza 3	1.00E+05 PFU/mL	+	+	+
Human parainfluenza 4a	1.00E+05 PFU/mL	+	+	+
Human Rhinovirus 14	1.00E+05 PFU/mL	+	+	+
MERS-coronavirus	1.48E+05 TCID50/mL	+	+	+
Respiratory syncytial virus	1.03E+05 PFU/mL	+	+	+
SARS-coronavirus	1.67E+05 PFU/mL	+	+	+
Influenza A (A/H3N2)	9.60E+05 CEID/mL	+	+	+
Influenza B (B/Yamagata)	1.10E+08 CEID/mL	+	+	+
30 pooled negative human NP	n/a	+	+	+

14. Interfering Substances Study

Potentially interfering substances in the nasal passage and nasopharynx may include, but are not limited to, blood, mucus or nasal secretions, medications for the relief of nasal congestion or dryness, irritation, or asthma and allergy symptoms, aswell as antibiotics and antiviral treatment. Negative nasopharyngeal samples containing spiked viruses (3x LoD) were tested in the presence of each substance on Roche LC480 instrument to determine the effect on the detection of the targets in the OPTI SARS- CoV-2/Influenza A/B RT-PCR Test. The interferents and their concentrations evaluated are listed in Table 7. None of the substances caused interference of the assay performance at the concentrations tested in this study. All positive replicates were correctly detected by the OPTI SARS-CoV-2/Influenza A/B RT-PCR Test.

Table 7: Interfering substances study results

Interferent	Concentration
Endogenous Substances	
Whole Blood	1% v/v
Mucin	2.5 mg/ml
Spray and Gel	
Zicam Nasal gel	10% v/v
NeoSynephrine Spray	20% v/v
Normal saline Spray	20% v/v
Otrivin Nasal Spray	20% v/v
Zicam Nasal Spray	20% v/v
Nasal Corticosteroid	
Beclomethasone	2 mg/ml
Budesonide	1 mg/ml
Dexamethasone	3 mg/ml
Flunisolide	5 mg/ml
Fluticasone	0.25 mg/ml
Mometasone	1 mg/ml
Triamcinolone	1.5 mg/ml
Antiviral Agents and Antibiotics	
Chloroseptic Max	20% w/v
Lozenges	2.5 mg/ml
Mupirocin	4.3 mg/mL
Peridex chlorhexidine	20% v/v
Tobryamycin	2 mg/ml
Zanamivir (Relenza)	5 mg/ml
Others	
Oral Zinc	0.67 mg/ml

15. Co-infection Study

Analytical sensitivity of the OPTI SARS-CoV-2/Influenza A/B RT-PCR Test in the context of a co-infection scenario was evaluated using negative nasopharyngeal samples spiked with inactivated SARS-CoV-2, live influenza A/H3N2, and live B/Victoria viruses. Two of three targets were spiked at a starting concentration of 3x LoD in the presence of a third target a high concentration. Triplicate samples were extracted and tested. The test data shows that a high concentration of influenza A did not affect the detection of influenza B and SARS-CoV-2 at 3x LoD. Similarly, a high concentration of influenza B did not affect the detection of influenza A and SARS-CoV-2 at 3x LoD. However, a high concentration of SARS-CoV-2 in a clinical sample adversely affected the detection of the influenza A and B viruses at 3xLoD, leading to false negative results for these analytes.

Table 8: Co-infection study results with high concentrations of SARS-CoV-2

		SARS-CoV-2/Influenza A/B version 2 Test			
		Ct 1	Ct 2	Ct 3	Mean
Single infection	Flu A	33.51	33.03	34.86	33.47
	Flu B	33.47	33.04	32.97	33.16
Co-infection 1	High SARS-CoV-2 (clinical sample 1)	19.35	18.28	18.24	18.62
	Low Flu A	**No Ct**	**No Ct**	**No Ct**	n/a
	Low Flu B	**No Ct**	**No Ct**	**No Ct**	n/a
Co-infection 2	High SARS-CoV-2 (clinical sample 2)	22.87	22.85	23.03	22.92
	Low Flu A	37.83	38.05	37.27	37.72
	Low Flu B	**No Ct**	**No Ct**	36.83	n/a
Co-infection 3	High SARS-CoV-2 (clinical sample 3)	25.55	25.09	24.82	25.15
	Low Flu A	37.83	38.05	37.27	37.72
	Low Flu B	**No Ct**	**No Ct**	**No Ct**	n/a

* Ct values indicated in **bold** are not detected. n/a: not available

16 Precision/Reproducibility and Cross-contamination Studies

The precision and reproducibility of the OPTI SARS-CoV-2/Influenza A/B RT-PCR Test were evaluated using ten replicates of nasopharyngeal samples containing the three target analytes at three concentrations (1x, 4x, and 40x LoD). The study showed concordant data across three different lots of reagents. The potential for carryover and cross-contamination was assessed with samples containing high concentration of contrived viral samples adjacent to negative samples in an alternating pattern.

17 Clinical Evaluation

The performance of the OPTI SARS-CoV-2/Influenza A/B RT-PCR Test was evaluated using archived clinical nasopharyngeal (NP) swab samples in viral transport medium. Results for SARS-CoV-2 detection were compared to results from a highly sensitive, FDA-authorized molecular assay. Results for influenza A and B detection were compared to results from a highly sensitive, FDA-cleared molecular assay. Nucleic acid was extracted with the OPTI DNA/RNA Magnetic Bead Extraction Kit, and PCR was performed using the Roche LC480 PCR instrument (software SW v1.5.1). Table 9 summarizes the results including the positive and negative percent agreement with 95% confidence limits.

Table 9: OPTI SARS-CoV-2 Influenza A/B RT-PCR Test Performance Results

Clinical Evaluation – LC480 (96-well format)

Target	Number of specimens	TP	FP	TN	FN	PPA (95% CI)	NPA (95% CI)
SARS-CoV-2	164	41	0	123	0	100% (91.43–100%)	100% (96.97–100%)
Flu A	143	50	0	93	0	100% (92.87–100%)	100% (96.03–100%)
Flu B	143	59	0	84	0	100% (93.9–100.0%)	99.42% (95.63–100%)

TP: True Positive, FP: False Positive, TN: True Negative, FN: False Negative, CI: Confidence Level

Emergency Use Only Labelling

18 Additional Label

An Emergency Use Only (EUO) label is required for instruments authorized for use under Emergency use Authorization (EUA).

For Bio Molecular Systems Mic qPCR and Roche LightCycler® 480 PCR instrument users, contact Opti Medical Systems Inc. (via email: covid19@optimedical.com) to obtain a RUO instrument verification protocol and complete the verification as required.

BioRad has provided a blanket Right of Reference to the Master File held by FDA which certifies that the BioRad CFX96 Touch PCR instrument is manufactured under quality systems that are compliant with the applicable parts of 21 CFR 820 and ISO 13485:2016. Therefore, the testing laboratories are not required to perform any qualification studies prior to use of the instrument for testing patient specimens and reporting test results of the qualification studies.

Please print and place the following label on the front panel on each instrument. If the instruments include labeling indicating "For Research Use Only", please cover with the below "Emergency Use Only" labeling. Retain this label throughout the EUA use of the OPTI SARS-CoV-2/Influenza A/B RT-PCR Test.

Emergency Use Only
This instrument is authorized for use with OPTI Medical Systems assays that have received Emergency Use Authorization (EUA)

References

1 Real-time RT-PCR Primers and Probes for COVID-19. (n.d.). Retrieved October 28, 2020, from https://www.cdc.gov/coronavirus/2019-ncov/lab/rt-pcr-panel-primer-probes.html

2 Research Use Only CDC Influenza SARS-CoV-2 (Flu SC2) Multiplex Assay Real-Time RT-PCR Primers and Probes. (n.d.). Retrieved October 28, 2020, from https://www.cdc.gov/coronavirus/2019-ncov/lab/multiplex-primer-probes.html

3 Asdfsadf(n.d.). Retrieved October 28, 2020, from https://platform.gisaid.org/epi3/cfrontend

For technical assistance on the OPTI SARS-CoV-2/Influenza A/B RT-PCR Test:
IDEXX USA Tel: +1 800 548 9997 or +1 207 556 4895
IDEXX Europe Tel: +800 727 43399
Contact your IDEXX area manager or distributor or visit our website.

Made in France

Symbol Descriptions

LOT	Batch Code (Lot)
SN	Serial Number
REF	Catalog Number
EC REP	Authorized Representative in the European Community
	Use by date
	Date of manufacture
	Manufacturer
	Temperature limitation
	Consult instructions for use
	Major change in the user instructions
IVD	*In vitro* diagnostic
CE	CE marking - European conformity

OPTI Medical Systems, Inc.
235 Hembree Park Drive
Roswell, Georgia 30076
USA

EC REP

MT Promedt Consulting GmbH
Altenhofstrasse 80
66386 St. Ingbert
Germany
Tel: +49 6894 581020
info@mt-procons.com

OPTIMedical

Appendix B: Laboratory Procedure for Qualification of RUO Instruments

Testing laboratories that use Bio Molecular Systems MIC qPCR or Roche® LightCycler 480 PCR systems should use this protocol to qualify their RUO instrument(s) for SARS-CoV-2/ Influenza A/B testing using the OPTI SARS-CoV-2/ Influenza A/B RT-PCR Test Kit.

Materials required:

Description	Included in the kit
OPTI PCR Grade Water	Yes
Genomic RNA from OPTI Medical	Available upon request
A negative upper respiratory (UR) specimen (pool if necessary)	Not provided

Preparation of contrived positive specimens for RUO instrument qualification:

Prepare contrived positive specimens for RUO instrument qualification as detailed below. Each contrived positive specimen will be extracted and tested in triplicate.

Description	Negative UR Specimen (µL)	Genomic RNA (µL)
Negative UR Specimen	1000	0
Contrived Positive Specimen 1	982	18
Contrived Positive Specimen 2	955	45

Set up extraction and assay:

1. For each extraction instrument, assign ten wells on an extraction plate. Load three wells each of the Negative UR Specimen, Contrived Positive Specimen 1, and Contrived Positive Specimen 2. Assign a separate well for a negative extraction control using molecular grade water only.
2. Use 200 µL of the prepared materials and extract nucleic acids according to the extraction kit instructions provided by the manufacturer. Refer to the "Extraction" section of the IFU.
3. Test each extract on the PCR instruments as described in the OPTI SARS-CoV-2 RT-PCR Test Instructions for Use. Include a positive and a negative control in the PCR run.

Analyze data

1. The following control results must be obtained for the PCR run to be deemed valid.

Control	Target	Ct value	Qualitative Result
PCR Positive Control	Flu A Positive Control	<40 (FAM)	Positive
	Flu B Positive Control	<40 (CAL Fluor red 610)	Positive
	SARS-CoV-2 Positive Control	<40 (Cy5)	Positive
	Internal Control	<36 (CAL Fluor Orange 560)	Positive
PCR Negative Control	Flu A Positive Control	No signal	Negative
	Flu B Positive Control	No signal	Negative
	SARS-CoV-2 Positive Control	No signal	Negative
	Internal Control	No signal* (CAL Fluor Orange 560)	Negative
Extraction Negative Control	Flu A Positive Control	No signal	Negative
	Flu B Positive Control	No signal	Negative
	SARS-CoV-2 Positive Control	No signal	Negative
	Internal Control	No signal* (CAL Fluor Orange 560)	Negative

*The negative controls are expected to test negative for both the SARS-CoV-2 and Internal Control targets. If the laboratory observes nucleic acid contamination (e.g. CAL Fluor Orange 560 Ct values >36), please review and evaluate your established laboratory procedures intended to prevent environmental sources of human nucleic acid contamination. The internal control target is human RNase P nucleic acid and trace amounts may be present in the laboratory environment.

2. The following results for the three replicates of Negative Specimen, Contrived Positive Specimen 1, and Contrived Specimen 2 must be obtained in order to qualify the extraction and PCR instruments for clinical testing.

Control	Target	Ct value	Qualitative Result
Negative Specimen	Flu A Positive Control	No signal (FAM)	Negative
	Flu B Positive Control	No signal (CAL Fluor red 610)	Negative
	SARS-CoV-2 Positive Control	No signal (Cy5)	Negative
	Internal Control	<36 (CAL Fluor Orange 560)	Positive
Contrived Positive Specimen 1	Flu A Positive Control	<40 (FAM)	Positive
	Flu B Positive Control	<40 (CAL Fluor red 610)	Positive
	SARS-CoV-2 Positive Control	<40 (Cy5)	Positive
	Internal Control	<36 (CAL Fluor Orange 560)	Positive
Contrived Positive Specimen 2	Flu A Positive Control	<40 (FAM)	Positive
	Flu B Positive Control	<40 (CAL Fluor red 610)	Positive
	SARS-CoV-2 Positive Control	<40 (Cy5)	Positive
	Internal Control	<36 (CAL Fluor Orange 560)	Positive

3. Any unexpected or invalid results would indicate that the instruments do not meet the established performance requirement. Review laboratory procedure to resolve and optimize performance if applicable.

Appendix C: Additional Label

Please print and place this label on the front panel of the instrument. If the instruments include labeling indicating "For Research Use Only", please cover with the below "Emergency Use Only" labeling. The instrument should retain this labeling throughout the EUA use of the OPTI SARS-CoV-2/ Influenza A/B RT-PCR Test.

Emergency Use Only
This instrument is authorized for use with OPTI Medical Systems assays that have received Emergency Use Authorization (EUA)

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

For technical assistance on the OPTI SARS-CoV-2 RT-PCR Test:
IDEXX USA Tel: +1 800 548 9997 or +1 207 556 4895
IDEXX Europe Tel: +800 727 43399
Contact your IDEXX area manager or distributor or visit our website.

Dye compounds in this product are sold under license from Biosearch Technologies, Inc. and protected by U.S. and world-wide patents either issued or in application.

Patent information: idexx.com/patents

Symbol Descriptions

LOT	Batch Code (Lot)
SN	Serial Number
REF	Catalog Number
EC REP	Authorized Representative in the European Community
	Use by date
	Date of manufacture
	Manufacturer
	Temperature limitation
	Consult instructions for use
	Major change in the user instructions
IVD	In vitro diagnostics

Manufactured in France for
OPTI Medical Systems, Inc.
235 Hembree Park Drive
Roswell, Georgia 30076, USA

EC - Representative
MT Promedt Consulting
Alenhofstrasse 80
66386 St. Ingbert Germany
+49 6894 581020
info@mtpromedt.com

IDEXX Laboratories, Inc.
One IDEXX Drive
Westbrook, Maine 04092, USA

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The latest version of the Instructions for Use (IFU) for the OPTI SARS-COV-2/Influenza A/B RT-PCR Test Version 1 can be accessed as an electronic pdf from the OPTI Medical Systems website at:

https://www.optimedical.com/files/06-57014-00-opti-sars-cov-2-influenza-a-b-rt-pcr-test-version1-fda.pdf

A paper version of the IFU for the OPTI SARS-COV-2/Influenza A/B RT-PCR Test Version 1 can also be requested at no additional cost by calling 1-800-548-9997 or emailing covid19@optimedical.com.

For more information on the OPTI SARS-COV-2/Influenza A/B RT-PCR Test Version 1:
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IVD CE Rx

For *in vitro* diagnostic use
For Emergency Use Authorization Only
For Prescription Use only

REF **99-57014 & 99-57016**

Version 06-57014-00

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The latest version of the Instructions for Use (IFU) for the OPTI SARS-COV-2/Influenza A/B RT-PCR Test Version 2 and Laboratory Procedure for Qualification of RUO Instruments Standard Operating Procedure can be accessed as an electronic pdf from the OPTI Medical Systems website at:

https://www.optimedical.com/files/06-57015-00-opti-sars-cov-2-influenza-a-b-rt-pcr-test-version2-fda.pdf
https://www.optimedical.com/files/06-57015-00 RUO protocol.pdf

A paper version of the IFU for the OPTI SARS-COV-2/Influenza A/B RT-PCR Test Version 2 and Laboratory can also be requested at no additional cost by calling 1-800-548-9997 or emailing covid19@optimedical.com.

For more information on the OPTI SARS-COV-2/Influenza A/B RT-PCR Test Version 2:
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covid19@optimedical.com
Contact your IDEXX area manager or distributor or visit our website: www.optimedical.com

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 and differentiation of nucleic acid from SARS-CoV-2, influenza A and/or 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.

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Version
06-57015-00

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