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

English Version

Used for real-time PCR identification and differentiation of SARS-CoV-2, Influenza A and Influenza B RNA extracted from upper respiratory specimens (such as nasal, nasopharyngeal, oropharyngeal swabs, and nasopharyngeal wash/aspirate or nasal aspirate).

IVD CE ℞

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

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06-57014-00

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OPTIMedical

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Intended Use

The OPTI SARS-CoV-2 / Influenza A/B RT-PCR Test is a multiplexed real-time fluorescent reverse transcription polymerase chain reaction test for the qualitative detection and differentiation of RNA from SARS-CoV-2, Influenza A and Influenza B in upper respiratory specimens (such as nasal, nasopharyngeal, oropharyngeal swabs, and nasopharyngeal wash/aspirate or nasal aspirate) from patients.

Results are for the simultaneous detection and differentiation of SARS-CoV-2, Influenza A and Influenza B RNA which are generally detectable in upper respiratory samples during the acute phase of infection. The test is not intended to detect Influenza C RNA. Positive results are indicative of active infection; 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. Laboratories within the United States and its territories are required to report all positive results to the appropriate public health authorities.

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

The OPTI SARS-CoV-2 / Influenza A/B RT-PCR Test is intended to be used by qualified and trained clinical laboratory personnel specifically instructed and trained in the techniques of real-time nucleic acid amplification and *in vitro* diagnostic procedures.

Product Description

The 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 Cy 5 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 the PCR thermal cycler instrument. The OPTI SARS-CoV-2 / Influenza A/B RT-PCR Test is only compatible with the ABI instruments listed in "Materials Required but Not Provided" section.

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
		500 tests 99-57014	At receipt	After reconstitution	
OPTI SARS-CoV-2/Flu Mix (SARS-CoV-2/Flu Mix), dried	Red	5 x 1.0 mL	–25 to 8°C	–25 to –15°C	≤6
REF 61-56625-00 Contains primers and probes for SARS-CoV-2 (N1 and N4), Influenza A, Influenza B and internal control. Reconstitute to 1 mL 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)	Black	5 x 1.0 mL	–25 to –15°C (Long-term)	N/A	≤6
REF 61-56618-00 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)	Blue	1 x 200 µL	–25 to 8°C	–25 to –15°C	≤6
REF 44-56627-00 The PC contains the targets for SARS-CoV-2 (N1 target region), Influenza A, Influenza B and the internal control. Reconstitute to 200 µL in PCR Grade Water. The expiration date on the vial is valid for either the dry or reconstituted form.					
OPTI PCR Grade Water	Clear	7 x 1.0 mL	–25 to 8°C		N/A
REF 61-56619-00 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 (for use with upper respiratory specimens)	
Applied Biosystems® 7500 FAST Applied Biosystems® QuantStudio 5 96 well PCR plate Optical plate cover	7500 instrument (4351106) and 7500 software v2.0.6 QS5 instrument (A28138) and QuantStudio Design and Analysis Desktop software (v1.5.1) plate: 4346906 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

MLS = Major Laboratory Supplier, such as vwr.com or fisherscientific.com

Warnings and Precautions

General

- The assay is for *in vitro* diagnostic (IVD) use under the FDA Emergency Use Authorization Only.
- For prescription use only.
- The OPTI SARS-CoV-2/Influenza A/B RT-PCR Test has been authorized only for the detection of nucleic acid from SARS-CoV-2, Influenza A and Influenza B, not for any other viruses or pathogens
- Handle all specimens as of 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.
- Laboratories within the United States and its territories are required to report all positive results to the appropriate public health authorities.

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 FDA recommended swabs and transport media. Refer to Interim Guidelines for Collecting, Handling, and Testing Clinical Specimens from Patients Under Investigation (PUIs) for 2019 Novel Coronavirus (2019-nCoV): 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 viral 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.

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. 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

Magnetic Bead Extraction kits
For automated use on Thermo Scientific™ KingFisher™ Flex

OPTI DNA/RNA Magnetic Bead Kit (OPTI Medical System, #99-58015)
for use on Thermo Scientific™ KingFisher™ Flex

Sample input volume: 200 µL; elution volume 100 µL

Store the purified RNA at 2–8°C for up to 8 hours. Store at –25 to –15°C for long term storage.

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, Influenza A, and Influenza B targets and the 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 acid for all three targets and the internal control are used at 20 copies per µL. The positive control should be included on each PCR run and should test positive for the SARS-CoV-2, Influenza A, and Influenza B targets and the internal control. 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. The internal control is expected to test positive for each sample tested.

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 nucleic acids during the extraction process and should test negative for the SARS-CoV-2, Influenza A and Influenza B targets, and positive for the 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.

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 SARS-CoV-2/Flu Mix and 10 µL RNA MMx for each reaction.
- When preparing the PCR Mix, first pipette 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 RNA 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 (5 µL), PCR negative control (5 µL PCR Grade Water) 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	Reporter	Quencher
Influenza A	FAM™	BHQ® (none)
Influenza B	NED™	BHQ® (none)
SARS-CoV-2	Cy5	BHQ® (none)
Internal Control	VIC™	BHQ® (none)
Passive Reference	ROX™	N/A

Cycling Program (used for all instruments)

Step	Temperature	Time	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 Analyze data

All test controls should 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 in order for each PCR run to be considered valid. If the plate controls are not valid, the patient results for that target cannot be interpreted, and the test must be repeated.

Control	SARS-CoV-2/ Flu PC	PCR Negative Control	Extraction Control
Influenza A FAM Ct/result	<40 Positive	No Signal Negative	No Signal Negative
Influenza B NED Ct/result			
SARS-CoV-2 Cy5 Ct/result			
Internal Control VIC™ Ct/result	<40 Positive	>36* Negative	<36 Positive

*The negative control is expected to test negative for the SARS-CoV-2, Influenza A, Influenza B and Internal Control targets. Trace environmental human contamination can result in late VIC Ct values >36. This is because the internal control target detects human nucleic acid and trace amounts can be present in the laboratory environment.

Sample Validity: The validity for each sample is determined by the internal control result for the respective sample. 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 should 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	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 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	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

- All users, analysts, and any person reporting diagnostic results should be trained to perform this procedure by a competent instructor and be able to demonstrate their ability to perform the test and interpret the results independently prior to performing the assay.
- The OPTI SARS-CoV-2 / Influenza A/B RT-PCR Test can be used with the specimens listed in the Intended Use statement. Other specimen types should not be tested with this assay. Negative results do not preclude infection with SARS-CoV-2, Influenza A or Influenza B viruses and should not be the sole basis of a patient management decision.
- Laboratories are required to report all positive 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, processing systems, and PCR instruments not listed under Materials Required but not Provided have not been validated.
- Do not use any reagent past the expiration date or with possible improper temperature control, as this may affect performance of the assay.
- A false negative result may occur if a specimen is improperly collected, transported, handled or stored. 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.

- 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.
- In the case of Influenza A and B viruses, children tend to shed virus more abundantly and for longer periods of time than adults. Therefore, testing specimens from adults for the presence of RNA from Influenza viruses will have lower sensitivity than testing specimens from children.
- 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 agent 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 A or SARS-CoV-2 virus have not been determined. Collection of multiple specimens from the same patient may be necessary to detect the viruses.

Assay Performance

11 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 the synthetic RNA for SARS-CoV-2, Influenza A and Influenza B sourced from Twist Bioscience, San Francisco, CA (REF 103001, REF 103003, and REF 102024, respectively) 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 5™ PCR instrument (software v1.5.1). To confirm the LoD, 20 replicates of each sample matrix spiked with SARS-CoV-2, Influenza A and Influenza B RNA 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™ 5 PCR instrument (software v1.5.1). The LoD was confirmed to be 3.1 copies/μL for SARS-CoV-2 (19/20), 1.68 copies/μL for Influenza A (20/20) and 3.1 copies/μL for Influenza B (19/20). Results are shown in Tables 1 and 2 below.

Table 1: LoD Initial Determination

SARS-CoV-2 RNA copies/μL	Mean Ct	Hit rate	% Detection
15.23	32.07	3/3	100
5.08	33.84	3/3	100
1.69	37.27	3/3	100
0.56	37.98	2/3	67
Influenza A RNA copies/μL	**Mean Ct**	**Hit rate**	**% Detection**
15.23	33.21	3/3	100
5.08	35.90	3/3	100
1.69	35.79	2/3	67
0.56	38.22	2/3	67

Influenza B RNA copies/μL	Mean Ct	Hit rate	% Detection
15.23	33.94	3/3	100
5.08	36.20	3/3	100
1.69	36.43	3/3	100
0.56	No Ct	0/3	0

Table 2: LoD Confirmation – Applied Biosystems® QuantStudio™ 5 PCR Instrument

PCR Target	RNA copies/μL	Mean Ct	Detection Rate	LoD copies/μL
SARS-CoV-2	3.1	35.88	19/20	3.1
	1.69	36.63	18/20	
Influenza A	1.69	35.16	20/20	1.69
	0.56	36.84	8/20	
Influenza B	3.1	36.49	19/20	3.1
	1.69	36.86	17/20	

12 Alternate Instrument Testing

An additional study was conducted to determine the LoD for the OPTI SARS-CoV-2/Influenza A/B RT-PCR Test using Applied Biosystems® 7500 Fast PCR instrument (Software v2.0.6). The LoD was evaluated by testing 20 replicates of pooled nasopharyngeal (NP) swab matrix spiked with synthetic RNA for SARS-CoV-2, Influenza A, and Influenza B sourced from Twist Bioscience ((REF 103001, REF 103003, and REF 102024, respectively) at the LoD concentration which had been previously confirmed for the Applied Biosystems® QuantStudio™ 5 PCR instrument. Twenty replicates at the LoD were tested on the instrument. The lowest concentration at which 95% of the replicates were detected was considered the LoD for the instrument. The LoD was 3.1 copies/μL for SARS-CoV-2 (19/20), 1.68 copies/μL for Influenza A (20/20) and 3.1 copies/μL for Influenza B (19/20). Results are shown in Table 3, below.

Table 3: Applied Biosystems® 7500 Fast PCR instrument LoD Determination

PCR Target	RNA copies/μL	Mean Ct	Detection Rate	LoD copies/μL
SARS-CoV-2	3.1	34.78	19/20	3.1
	1.69	37.35	14/20	
Influenza A	1.69	33.58	20/20	1.69
	0.56	42.40	4/20	
Influenza B	3.1	33.92	19/20	3.1
	1.69	42.17	4/20	

13 Inclusivity (analytical reactivity)

a) SARS-CoV-2
The OPTI SARS-CoV-2/Influenza A/B RT-PCR Test uses identical N1 and N4 primer and probe sequences described in the CDC test designs[1,2]. However, to assess the *in silico* inclusivity of the OPTI SARS-CoV-2/Influenza A/B RT-PCR Test, a multiple sequence alignment (MSA) was generated from the GISAID CoV database[3] sequences submitted between the dates of December 23, 2019 and October 23, 2020 and compared for identity to the test primers and probes. Only full-length, high coverage sequences were used, resulting in over 115,000 sequences in the design regions. 99.7% of sequenced strains match the N1 forward primer, 98.1% match the N1 probe and 99.6% match the N1 reverse primer. Likewise, 98.2% of sequences strains match the N4 forward primer, 99.5% match the N4 probe and 98.1% match the N4 reverse primer. In total, the combined N1 and N4 designs match 115,292 strains of 115,332 strains with known sequence in either design region (99.95% match, data not shown).

A total of 40 sequences were identified with one or more mismatches within the same viral genome sequence, in both the N1 and N4 design regions. None of the any given oligo region has more than a single mismatch for all 40 sequences. All mismatches found in the N1 primers and 35 mismatches found in the N4 primers are located outside the critical 3' clamp (i.e., the terminal five nucleotides). Thus, it can be reasonably predicted that these 40 sequences would be amplified and detected with minimal to no loss in sensitivity by the N1 test design. Likewise, the respective 35 sequences would also be amplified and detected with minimal to no loss in sensitivity by the N4 test design. Five sequences contain mismatches to an N4 primer that occur within the 3' clamp. For these five sequences, it can be predicted the N4 design would fail to properly amplify the target region and would instead only be amplified and detected by the N1 test design.

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, an 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.

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.

14 Specificity (Cross-Reactivity)

a) *In silico*: 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*: 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*: 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

Organism	Strain	Accession or WGS number
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) Microbial Interference Study and Cross-reactivity
The OPTI SARS-CoV-2/Influenza A/B RT-PCR Test was evaluated for cross-reactivity with a panel of bacteria and yeast. Nucleic acids from high titer preparations (typically $>10^8$ CFU/mL or 10 ng/μL except for fastidious organisms such as Chlamydia pneumoniae and Mycobacterium pneumoniae) of 17 bacteria and 1 yeast representing respiratory pathogens or flora commonly present in human respiratory specimens were extracted using the OPTI DNA/RNA Magnetic Bead Kit and tested. No cross-reactivity was observed (Table 5). Detection of Influenza A, Influenza B, and SARS-CoV-2 RNA at ~5x LoD was not affected by the presence of the microbial nucleic acid extracts (Table 6). Potential cross-reactivity of a broader list of bacterial pathogens are highly unlikely as shown in the *in silico* analysis above.

Table 5: SARS-CoV-1, Influenza A and B cross-reactivity test results

Microorganism	Test Concentration	SARS-CoV-2 Result	Flu A Result	Flu B Result
Bordetella pertussis	22.9 ng/μL	-	-	-
Candida albicans	$1.08x10^8$ CFU/mL	-	-	-
Chlamydia pneumoniae	$5 x 10^3$ CFU/mL	-	-	-
Corynebacterium spp	3×10^8 CFU/mL	-	-	-
Escherichia coli (respiratory)	3×10^8 CFU/mL	-	-	-
Haemophilus influenzae	$1x10^8$ CFU/mL	-	-	-
Klebsiella pneumoniae	$3x10^8$ CFU/mL	-	-	-
Legionella pneumophila	$2.24x10^9$ CFU/mL	-	-	-
Mycobacterium pneumoniae	$4.2x10^6$ CFU/mL	-	-	-
Mycobacterium tuberculosis	34 ng/μL	-	-	-
Neisseria spp	3×10^8 CFU/mL	-	-	-
Proteus vulgaris	$3x10^8$ CFU/mL	-	-	-
Pseudomonas aeruginosa	3×10^8 CFU/mL	-	-	-
Staphylococcus aureus	3×10^8 CFU/mL	-	-	-
Staphylococcus epidermidis	12.2 ng/μL	-	-	-
Streptococcus pneumoniae	11.0 ng/μL	-	-	-
Streptococcus pyogenes	3×10^8 CFU/mL	-	-	-
Streptococcus salivarius	$1x10^8$ CFU/mL	-	-	-

Table 6: Interfering microorganisms study results

Micro-organism	Test Concentration	SARS-CoV-2, Influenza A & B at ~5x LoD		
		SARS-CoV-2 Result	Flu A Result	Flu B Result
Bordetella pertussis	22.9 ng/μL	+	+	+
Candida albicans	1.08×10^8 CFU/mL	+	+	+
Chlamydia pneumoniae	5×10^3 CFU/mL	+	+	+
Corynebacterium spp	3×10^8 CFU/mL	+	+	+
Escherichia coli (respiratory)	3×10^8 CFU/mL	+	+	+
Klebsiella pneumoniae	3×10^8 CFU/mL	+	+	+
Mycobacterium pneumoniae	4.2×10^6 CFU/mL	+	+	+
Neisseria spp	3×10^8 CFU/mL	+	+	+
Proteus vulgaris	3×10^8 CFU/mL	+	+	+
Pseudomonas aeruginosa	3×10^8 CFU/mL	+	+	+
Staphylococcus aureus	3×10^8 CFU/mL	+	+	+
Staphylococcus epidermidis	12.2 ng/μL	+	+	+
Streptococcus pneumoniae	11.0 ng/μL	+	+	+
Streptococcus pyogenes	3×10^8 CFU/mL	+	+	+

15. Interfering Substances Study

Potentially interfering substances in the nasal passage and nasopharynx may include, but not limited to, blood, mucus or nasal secretions, medications for the relief of nasal congestion or dryness, irritation, or asthma and allergy symptoms, as well as antibiotics and antiviral treatment. Negative nasopharyngeal samples containing spiked synthetic RNA (~5x 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 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

Potential Interferent	**Test Concentration**
Beclomethasone	0.068 mg/mL
Betadine Sore Throat Spray	20% w/v
Budesonide	0.051 mg/mL
Chloroseptic Max	20% w/v
Dexamethasone	0.48 mg/mL
Flunisolide	0.04 mg/mL
Fluticasone	0.025 mg/mL
Mometasone	0.04 mg/mL
Mucin: bovine submaxillary gland, type I-S	0.5% w/v
Mupirocin	4.3 mg/mL
NeoSynephrine Spray	20% v/v
Normal saline	20% v/v
Otrivin Nasal Spray	20% v/v
Peridex chlorhexidine	20% w/v
Tobryamycin	1.44 mg/mL
Triamcinolone	0.04 mg/mL
Whole Blood	1% v/v
Zanamivir (Relenza)	0.284 mg/mL
Zicam	20% v/v

16. 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 synthetic RNA where two of three targets were spiked at <5x LoD. Triplicate samples were tested. All controls performed as expected and low-level spiked RNA were detectable in all simulated co-infection experiments. Test data are presented in Table 8.

Table 8: Co-infection study results

Simulated Co-infection Scenario	Synthetic RNA Spike at ~5x LoD		
	SARS-CoV-2 Result	Flu A Result	Flu B Result
High Flu A, Low Flu B and Low SARS-CoV-2	Pos		Pos
High Flu B, Low Flu A and Low SARS-CoV-2	Pos	Pos	
High SARS-CoV-2, Low Flu A and Low B		Pos	Pos

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. A total of 224 NP swab specimens with previously known analyte results were tested. RNA 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

Target	Number of specimens	TP	FP	TN	FN	PPA (95% CI)	NPA (95% CI)
SARS-CoV-2	224	30	0	194	0	100% (86.2–100%)	100% (97.6–100%)
Flu A	224	44	0	180	0	100% (90.3–100%)	100% (97.4–100%)
Flu B	224	46	1	177	0	100% (90.38–100.0%)	99.42% (96.5–100%)

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

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

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

OPTIMedical