OPTI SARS-CoV-2 RT-PCR Test

English Version

Used for real-time PCR identification of SARS-CoV-2 RNA extracted from upper respiratory specimens (anterior nasal, mid-turbinate, nasopharyngeal, or oropharyngeal swabs, and nasopharyngeal wash/aspirate or nasal aspirate) and bronchoalveolar lavage.

IVD CE Rx

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

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OPTIMedical

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OPTI SARS-CoV-2 RT-PCR Test

Intended Use

The OPTI SARS-CoV-2 RT-PCR Test is a real-time fluorescent reverse transcription polymerase chain reaction test for the qualitative detection of nucleic acid from SARS-CoV-2 in upper respiratory specimens (anterior nasal, mid-turbinate, nasopharyngeal, or oropharyngeal swabs, and nasopharyngeal wash/aspirate or nasal aspirate) and bronchoalveolar lavage from patients suspected of COVID-19 by their healthcare provider (HCP) as well as upper respiratory specimens (anterior nasal, mid-turbinate, nasopharyngeal, or oropharyngeal swabs) collected from any individual, including from individuals without symptoms or other reasons to suspect COVID-19 infection. 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.

This test is also for the qualitative detection of nucleic acid from SARS-CoV-2 in pooled samples containing up to five individual upper respiratory swab specimens (anterior nasal, mid-turbinate, nasopharyngeal or oropharyngeal swabs) that are collected by an HCP using individual vials containing transport media from individuals suspected of COVID-19 by their HCP. Negative results from pooled testing should not be treated as definitive. If patient's clinical signs and symptoms are inconsistent with a negative result or results are necessary for patient management, then the patient should be considered for individual testing. Specimens included in pools with a positive or invalid result must be tested individually prior to reporting a result. Specimens with low viral loads may not be detected in sample pools due to the decreased sensitivity of pooled testing.

Results are for the identification of SARS-CoV-2 RNA. The SARS-CoV-2 RNA is generally detectable in respiratory samples during the acute phase of infection. Positive results are indicative of the presence of SARS-CoV-2 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 test results to the appropriate public health authorities.

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

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

The OPTI SARS-CoV-2 RT-PCR Test is only for use under the Food and Drug Administration's Emergency Use Authorization.

Product Description

The OPTI SARS-CoV-2 RT-PCR Test is a real-time reverse transcription polymerase chain reaction (rRT-PCR) test that uses the N1 and N2 primer and probe sequences which are described by the CDC design[1]. The OPTI SARS-CoV-2 RNA Mix (SARS-CoV-2 Mix) includes primers and probes for the detection of SARS-

CoV-2 RNA when amplified with the OPTI RNA Master Mix (RNA MMx). SARS-CoV-2 RNA targets (N1 and N2) are both detected in the FAM channel. The internal control for the test is RNase P (RP), which is detected in the HEX channel. The internal control for the test is based on the detection of a conserved nucleic acid sequence present in human samples. This host target is referred to as the internal sample control (ISC). Detection of endogenous nucleic acid in the test sample controls for sample addition, extraction, and amplification. Primers and probe for detection of the internal sample control are included in the SARS-CoV-2 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 RT-PCR Test utilizes the OPTI Positive Control (PC) and OPTI PCR Grade Water (Negative Control). The OPTI Positive Control (PC) contains SARS-CoV-2 and ISC synthetic material and works as a positive control for the reaction. OPTI PCR Grade Water is used as the PCR negative control, as well as to reconstitute the dried SARS-CoV-2 Mix and the PC.

1. "Coronavirus Disease 2019 (COVID-19) Real-Time rRT-PCR Panel Primers and Probes." Centers for Disease Control and Prevention, Centers for Disease Control and Prevention, 6 Mar. 2020, www.cdc.gov/coronavirus/2019-ncov/lab/rt-pcr-panel-primer-probes.html.

Materials and Storage

Identification/ General Information	Cap color	Quantity		Storage		Freeze/ Thaw cycles
		100 tests 99-57003	500 tests 99-57004	At receipt	After reconstitution	
OPTI SARS-CoV-2 Mix (SARS-CoV-2 Mix), dried 61-56616-00	Red	1 x 1.0 mL	5 x 1.0 mL	–25 to 8°C	–25 to –15°C	≤6
Contains N1, N2 and ISC primers and probes. Reconstitute to 1 mL in PCR Grade Water. Store the SARS-CoV-2 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	Black	1 x 1.0 mL	5 x 1.0 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 Positive Control, dried (PC) 44-56617-00	Blue	1 x 200 µL	1 x 200 µL	–25 to 8°C	–25 to –15°C	≤6
The PC contains the targets for SARS-CoV-2 (N1 target region) and the internal control (RNase P). 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 61-56619-00	Clear	2 x 1.0 mL	7 x 1.0 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 Mix and PC. It is also used as the negative control for PCR. 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 PCR plate Optical plate cover	7500 instrument (4351106) and 7500software v2.0.6 QS5 instrument (A28138) and QuantStudio Design and Analysis Desktop software (v1.5.1) plate: 4346906 cover: 4311971
Agilent	
Agilent Mx3005P™ 96 well PCR plate Optical cap strips	3005P instrument (401449) and MxPro qPCR software v4.10 plate: 401334 caps: 401425
IDEXX Laboratories	
Bio Molecular Systems Mic qPCR Tubes and caps	Instrument (98-0012758-00) and micPCR software v2.8.10 tubes + caps: 98-0012759-01
Roche	
Roche LightCycler® 480 96 well PCR plate + cover	Instrument (05015278001) and LightCycler 480 SW v1.5.1 plate + cover: 04 729 692 081
Extraction Equipment and Consumables	**Source and part number**
RealPCR DNA/RNA Magnetic Bead Kit NucleoMag VET Magnetic Extraction Kit OPTI RNA/DNA Magnetic Bead Kit PurePrep Pathogens Extraction Kit	IDEXX 99-56102 (384 samples) / 99-56106 (96 samples) Macherey Nagel 744200.4 OPTI Medical Systems 99-58015 MolGen BV OE00290096 (n=96) and OE00290960 (n=960)
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
Thermo Scientific™ KingFisher™ Duo Prime 96 deep well plate 12-tip elution strip for deep well plate 12-tip comb for deep well plate	Duo instrument (5400110) and software v1.02.27. RT18 Deep well plate: 95040460 Elution strip: 97003520 Tip comb: 97003500
Manual Magnetic Extraction (OPTI) Magnetic separator for 96 well plates Plate shaker/heater Multichannel pipette	Manual Extraction (OPTI RNA/DNA Magnetic Bead Kit) MLS MLS MLS
Extraction control containing human specimen (HSC) material	See Quality Controls section

Rapid Lysis Reagent	Source and part number
OPTI Rapid Lysis Buffer	OPTI 99-57011
Equipment and Lab Consumables	**Source and part number**
Molecular grade water (used as extraction negative control)	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, Fisher Scientific, Eppendorf

Warnings and Precautions

General

- The assay is for in vitro diagnostic (IVD) use under the FDA Emergency Use Authorization Only.
- For prescription use only.
- This product has not been FDA cleared or approved, but has been authorized for emergency use by FDA under an Emergency Use Authorization (EUA) for use by authorized laboratories.
- This product has been authorized only for the detection of nucleic acid from SARS-CoV-2, 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 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-biosafetyguidelines.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 test results to the appropriate public health authorities.

- Only samples collected in non-denaturing/non-inactivating transport media such as the Viral Transport Media, Universal Transport Media, or normal saline can be tested using the OPTI Rapid Lysis Buffer specimen processing method.
- Do not use the OPTI Rapid Lysis Buffer to process specimens in media containing lithium or guanidine salt.
- Do not use the OPTI Rapid Lysis Buffer to process specimens that appear bloody or that contain particulate matter. Such specimens should be extracted using an extraction method listed in this document.
- Contamination may occur if carryover of samples is not adequately controlled during sample pool preparation, handling, and processing.
- Testing of pooled specimens may impact the detection capability of the OPTI SARS-CoV-2 RT-PCR Test and impact sensitivity.

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 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 Mix and Positive Control 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 SARS-CoV-2 Mix and the Positive Control are reconstituted, 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 and Thermo Scientific™ KingFisher™ Duo Prime)

- RealPCR DNA/RNA Magnetic Bead Kit (IDEXX, Part#99-56102/ 99-56106) for use on Thermo Scientific™ KingFisher™ Flex
  - Sample input volume: 200 uL; elution volume 100 uL
- NucleoMag VET Magnetic Extraction Kit (Macherey Nagel, Part #744200.4) for use on Thermo Scientific™ KingFisher™ Duo Prime
  - Sample input volume: 200 uL; elution volume 100 uL
- OPTI DNA/RNA Magnetic Bead Kit (OPTI Medical Systems 99-58015) for use on Thermo Scientific™ KingFisher™ Flex and KingFisher™ Duo Prime instruments or Manual protocol
  - Sample input volume: 200 uL; elution volume 100 uL
- PurePrep Pathogens Extraction Kit (MolGen BV, Part # OE00290096, 96 samples and OE00290960, 960 samples) for use on Thermo Scientific™ KingFisher™ Duo Prime instrument
  - Sample input volume: 200 uL; elution volume 100 uL

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

Rapid Lysis Preparation

The OPTI Rapid Lysis Buffer Kit (OPTI Medical Systems 99-57011) may be used for sample processing. Do not use rapid lysates that appear bloody or contain particulate matter. Do not use rapid lysates for pooling.

Rapid lysates can be stored refrigerated (2-8°C) and ambient (18-26°C) temperatures for up to 4 days.

Sample Pooling for Extraction

The OPTI SARS-CoV-2 RT-PCR Test has been validated on nucleic acid extracts for the use of 5-specimen pool testing. The decision to pool samples should be based on the positivity rate at each location. For further information about pooling, please see Appendix A: Specimen Pooling Implementation and Monitoring.

Prepare pools, by adding equal volumes of each specimen to achieve a final 200uL volume that will be used for extraction. For example, use 40uL from each specimen for a 5-specimen pool.

Mix pools and proceed with the nucleic acid extraction protocol according to the instructions for use.

Quality Controls

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

- PCR 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 Positive Control): A positive template control is needed to confirm the PCR plate is valid. Synthetic nucleic acid for the N1 target region is used at 20 copies per µL. The positive control should be included on each PCR run and should test positive for both the SARS CoV-2 target 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. 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 RT-PCR Test are listed below:

- Extraction Negative Control: A "no sample" (negative) control is needed to confirm that the extraction process is valid. Molecular grade water should be included as a sample for each extraction run. The purpose of an Extraction Negative Control is to monitor for cross-contamination of samples with amplifiable materials from SARS-CoV-2 and/or human sourced material (RNase P) within the extraction run. The Extraction Negative Control should always test negative for the SARS CoV-2 target.
- Extraction Sample 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 target 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.

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 Mix and 10 µL RNA MMx for each reaction.
- When preparing the PCR Mix, first pipette SARS-CoV-2 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.
- Pipette the PCR Mix slowly into the PCR plate.

Load the PCR plate within 20 minutes or store at 2 to 8°C for up to 4 hrs. The PCR Mix can be stored at –25 to –15°C for up to 2 weeks. Protect from light.

2 Pipette 20 µL of the PCR Mix into the required wells of the multiwell plate.

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

4 Include 5 µL each of the Positive Control, PCR Negative Control, Extraction Negative Control and Extraction Sample Control (5 µL) for each test run.

5 Cover the plate and briefly spin the plate, if necessary, to settle contents and remove air bubbles.

6 Load the plate into the PCR instrument. Set up thermal cycler with Cycling Program below. Start the run.

Settings for Reporter and Quencher

Target	Reporter	Quencher
SARS-CoV-2	FAM™	BHQ® (none)
Internal Control (RNase P)	HEX™ (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.

7 Examination and Interpretation of Quality Control Results

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 and internal control targets on the plate. To obtain appropriate Ct values, analysis for both the SARS CoV-2 target and internal control 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 curve and above background signal. This is best done while viewing all amplification curves, for each respective target for a given run, on a logarithmic scale. 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.

Control	SARS-CoV-2 FAM Ct Value	SARS-CoV-2 FAM Result	Internal Control HEX Ct Value	Internal Control HEX Ct Result
Positive Control	<40	Positive	<36	Positive
PCR Negative Control	No Signal	Negative	No signal*	Negative
Extraction Negative Control	No Signal	Negative	No signal*	Negative
Extraction Sample Control	No Signal	Negative	<36	Positive

*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. HEX 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.

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 and internal control target for each sample.

8 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	SARS-CoV-2 FAM Ct Value	Internal Control HEX Ct Value	Other Characteristics
SARS-CoV-2 RNA POSITIVE	≤40	Any Ct value	A characteristic amplification curve in comparison to the PCR negative control. An internal control amplification curve in the HEX (VIC) channel is expected. A strong positive SARS CoV-2 sample may result in a negative internal control result.
SARS-CoV-2 RNA NEGATIVE	> 40	≤36	Amplification curve in the HEX (VIC) internal control channel
Invalid Sample**	No Ct value	>36	Absence of an amplification curve in the FAM and HEX (VIC) channels indicates an invalid result for 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.

9 Examination and Interpretation of Pooled Patient Specimens

Only if a test run is valid can the results for a patient sample pool be interpreted and reported. For a patient sample pool, a sample is considered positive when SARS-CoV-2 has a Ct ≤42. Internal Control is considered positive when the Ct ≤36.

SARS-CoV-2 FAM Ct Value	Internal Control HEX Ct Value	Pool Result	Action
≤42	Any Ct value	SARS-CoV-2 Detected in Pool	Individually test each sample from the pool
> 42	≤36	SARS-CoV-2 Not Detected in Pool	Report results to sender
No Ct value	> 36	Invalid	Individually test each sample from the pool

Positive - Specimens with a positive sample pool result must be tested individually prior to reporting a result. Specimens with low viral loads may not be detected in sample pools due to the decreased sensitivity of pooled testing.

Negative – Negative results from pooled sample testing should not be treated as definitive. If the patient's clinical signs and symptoms are inconsistent with a negative result and if results are necessary for patient management, then the patient should be considered for individual testing. The utilization of sample pooling should be indicated for any specimens with reported negative results.

Invalid - Specimens with an invalid pool result must be tested individually prior to reporting a result. However, in instances of an invalid run, repeat testing of pooled specimens may be appropriate depending on laboratory workflow and required result reporting time.

Conditions of Authorization

The OPTI SARS-CoV-2 RT-PCR Test's Letter of Authorization, User Manual, and Labeling are available on FDA website: https://www.fda.gov/medical-devices/coronavirus-disease-2019-covid-19-emergency-use-authorizations-medical-devices/vitro-diagnostics-euas.

To assist clinical laboratories using the OPTI SARS-CoV-2 RT-PCR Test, the relevant Conditions of Authorization are listed below.

a) Authorized laboratories[1] using the OPTI SARS-CoV-2 RT-PCR Test 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 the OPTI SARS-CoV-2 RT-PCR Test must use your product as outlined in the authorized labeling. Deviation from the authorized procedures, such as the authorized instruments, authorized extraction methods, authorized clinical specimen types, authorized control materials, authorized other ancillary reagents and authorized materials required to use your product are not permitted.

c) Authorized laboratories that receive the OPTI SARS-CoV-2 RT-PCR Test must notify the relevant public health authorities of their intent to run your test prior to initiating testing.

d) Authorized laboratories using the OPTI SARS-CoV-2 RT-PCR Test must have a process in place for reporting test results to healthcare providers and relevant public health authorities.

e) Authorized laboratories must collect information on the performance of the OPTI SARS-CoV-2 RT-PCR Test 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) if they become aware of any suspected occurrence of false positive or false negative results and significant deviations from the established performance characteristics of the OPTI SARS-CoV-2 RT-PCR Test.

f) All laboratory personnel using the OPTI SARS-CoV-2 RT-PCR Test must be appropriately trained in RT-PCR techniques and use appropriate laboratory and personal protective equipment when handling this kit and use your test 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.

h) Authorized laboratories using specimen pooling strategies when testing patient specimens with the authorized test must include with test result reports for specific patients whose specimen(s) were the subject of pooling, a notice that pooling was used during testing and that "Patient specimens with low viral loads may not be detected in sample pools due to the decreased sensitivity of pooled testing."

i) Authorized laboratories implementing pooling strategies for testing patient specimens must use the "Specimen Pooling Implementation and Monitoring Guidelines" provided in the authorized labeling to evaluate the appropriateness of continuing to use such strategies based on the recommendations in the protocol.

j) Authorized laboratories must keep records of specimen pooling strategies implemented including type of strategy, date implemented, and quantities tested, and test result data generated as part of the Protocol for Monitoring of Specimen Pooling Strategies. For the first 12 months from the date of their creation, such records must be made available to FDA within 48 business hours for inspection upon request, and must be made available within a reasonable time after 12 months from the date of their creation.

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

Limitations

- The use of this assay as an In vitro diagnostic under the FDA Emergency Use Authorization (EUA) is limited to laboratories certified under the Clinical Laboratory Improvement Amendments of 1988 (CLIA), 42 U.S.C. § 263a, and meet requirements to perform high complexity tests.
- The OPTI SARS-CoV-2 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 virus and should not be the sole basis of a patient management decision.
- Laboratories are required to report all test 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.
- A false negative result may occur if a specimen is improperly collected, transported or handled. 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.
- 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.
- The Rapid Lysis protocol only works on clinical specimens collected in non-denaturing transport media and normal saline.
- If the virus mutates in the test target region, SARS-CoV-2 RNA may not be detected or may be detected less predictably. Inhibitors or other types of interference may produce a false negative result.
- False-positive results may arise from cross contamination during specimen handling, preparation, nucleic acid extraction, PCR assay set-up or product handling.
- This test cannot rule out diseases caused by other bacterial or viral pathogens.
- The impacts of vaccines, antiviral therapeutics, antibiotics, chemotherapeutic, immunosuppressant drugs or cold medications have not been evaluated.
- Nasopharyngeal wash/aspirate and nasal aspirates specimen types should not be pooled.
- Sample pooling has only been validated using nasopharyngeal swab specimens.
- Samples should only be pooled when testing demand exceeds laboratory capacity and/or when testing reagents are in short supply.
- The 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.

Assay Performance

Limit of Detection (LoD) with nucleic acid extracts

Limit of detection (LoD) is defined as the lowest concentration of SARS-CoV-2 RNA at which greater than or equal to 95% of all replicates test positive. LoD for the OPTI SARS-CoV-2 RT-PCR Test was determined using serial dilutions of synthetic SARS-CoV-2 RNA (Twist Bioscience, San Francisco, CA, part # MT007544.1) prepared in nasopharyngeal (NP) swab (upper respiratory sample type) sample pools. Samples were collected prior to 2020 and were considered negative for SARS-CoV-2.

The initial LoD was determined with 3-fold serial dilutions tested in triplicate. Each replicate was extracted using the RealPCR DNA/RNA Magnetic Bead Kit on Thermo Scientific™ KingFisher™ Flex following the standard protocol. Extracted RNA was tested on the Applied Biosystems® 7500 PCR instrument (software v2.0.6). To confirm the LoD, 20 replicates of each sample matrix spiked with SARS-CoV-2 RNA were extracted with the RealPCR DNA/RNA Magnetic Bead Kit on the Thermo Scientific™ KingFisher™ Flex and the NucleoMag VET Magnetic Extraction Kit on the Thermo Scientific™ KingFisher™ Duo Prime. Extracted RNA was tested on the Applied Biosystems® 7500 FAST PCR instrument (software v2.0.6). The LoD was confirmed to be 0.9 copies/ µL in NP swab samples (19/20) with both extraction methods. Results are shown in Tables 1 and 2 below.

Additional studies showed comparable LoD results (within 3X of the original confirmed LoD) when using the OPTI DNA/RNA Magnetic Bead Kit (OPTI Medical Systems 99-58015) and PurePrep Pathogens Extraction Kit (MolGen BV OE00290096) on the Thermo Scientific™ KingFisher™ Duo Prime with extracted RNA tested on the Applied Biosystems® 7500 FAST PCR instrument. The Manual protocol for the OPTI DNA/RNA Magnetic Bead Kit was also shown to have comparable LoD results (within 3X of the original confirmed LoD) with the RealPCR DNA/RNA Magnetic Bead Kit, NucleoMag VET Magnetic Extraction Kit and OPTI DNA/RNA Magnetic Bead Kit on the Thermo Scientific™ KingFisher™ Duo Prime or Flex with extracted RNA tested on the Applied Biosystems® 7500 FAST PCR instrument.

Table 1: LoD Initial Determination with nucleic acid extracts

NP Swab			
RNA copies/µL	**Mean Ct (N1/N2)**	**Detection Rate**	**% Detection**
6.7	NT	NT	NT
2.2	36.9	3/3	100%
0.7	38.9	2/3	67%
0.2	40.0	1/3	33%

Table 2. LoD Confirmation with nucleic acid extracts - Applied Biosystems® 7500 FAST PCR Instrument

	NP Swab			
	RNA copies/µL	Mean Ct (N1/N2)	Detection Rate	LoD copies/µL
RealPCR DNA/RNA Magnetic Bead 100uL elution (KingFisher Flex)	**0.9**	**37.6**	**19/20**	**0.9**
	0.3	38.5	12/20	
Macherey Nagel NucleoMag VET 100uL elution (KingFisher DUO Prime)	**0.9**	**37.3**	**19/20**	**0.9**
	0.3	38.3	10/20	

Alternate Instrument Testing

An additional study was conducted to determine the LoD for the OPTI SARS-CoV-2 RT-PCR Test using additional PCR instruments. Applied Biosystems QuantStudio 5, Agilent Mx3005P, Roche LightCycler® 480, and Bio Molecular Systems Mic PCR instruments were included in this study. See section "Materials Required but Not Provided" for software versions.

The LoD with was evaluated by testing 20 replicates of pooled nasopharyngeal (NP) swab matrix spiked with SARS-CoV-2 synthetic RNA sourced from Twist Bioscience (part # MT007544.1). Samples were spiked at the LoD concentration which had been previously confirmed for the Applied Biosystems® 7500 FAST PCR instrument. Twenty replicates at the LoD were tested on each instrument. The lowest concentration at which 95% of the replicates were detected was considered the LoD for the instrument. The LoD was 0.9 copies/ul in NP swabs on each of the instruments tested. These results are shown in table 3, below.

Table 3. PCR Instrument LoD Determination with nucleic acid extracts

	NP Swab			
PCR Instrument	RNA copies/µL	Mean Ct (N1/N2)	Detection Rate	LoD copies/µL
ABI 7500 FAST	0.9	37.6	19/20	0.9
	0.3	38.5	12/20	
ABI QuantStudio 5	0.9	36.5	20/20	0.9
	0.3	37.9	14/20	
Agilent MX3005P	0.9	33.7	19/20	0.9
	0.3	35.2	15/20	
Roche LC480	0.9	36.2	20/20	0.9
	0.3	36.4	13/20	
Bio Molecular Systems MIC	0.9	33.7	20/20	0.9
	0.3	35.0	17/20	

Inclusivity (analytical reactivity)

As of February 26, 2021, complete and high coverage SARS-CoV-2 genomic sequences were downloaded from the GISAID database. The region containing the N gene was selected and submitted for alignment using the MAFFT version 7 software (RIMD, Osaka, Japan). Sequences corresponding to the N1 and N2 target regions were compared to the OPTI SARS-CoV-2 RT-PCR Test primer and probe design.

A summary of the alignments for the sequences corresponding to the N1 and N2 target regions compared to the OPTI SARS-CoV-2 RT-PCR Test primer and probe design is provided in the tables below.

B.1.1.7 sequence alignment summary (UK Variant)

ALIGNMENT	COUNT	PERCENTAGE
N1,N2 Perfect Match	84287	97.4%
N1 match (mismatch in N2)	846	1.0%
N2 match (mismatch in N1)	1353	1.6%
Mismatches in N1 and N2	21	0.0%
Ambiguous sequences in N1 and N2	0	0.0%
TOTAL	**86507**	**100%**

P1 Lineage sequence alignment summary (Brazilian Variant)

ALIGNMENT	COUNT	PERCENTAGE
N1,N2 Perfect Match	240	99.2%
N1 match (mismatch in N2)	2	0.8%
N2 match (mismatch in N1)	0	0.0%
Mismatches in N1 and N2	0	0.0%
Ambiguous sequences in N1 and N2	0	0.0%
TOTAL	**242**	**100%**

B.1.351 sequence alignment summary (South African Variant)

ALIGNMENT	COUNT	PERCENTAGE
N1,N2 Perfect Match	1249	90.5%
N1 match (mismatch in N2)	12	0.9%
N2 match (mismatch in N1)	119	8.6%
Mismatches in N1 and N2	0	0.0%
Ambiguous sequences in N1 and N2	0	0.0%
TOTAL	**1380**	**100%**

B.1.429 + B.1.427 sequence alignment summary (California Variant)

ALIGNMENT	COUNT	PERCENTAGE
N1,N2 Perfect Match	3641	97.0%
N1 match (mismatch in N2)	59	1.6%
N2 match (mismatch in N1)	49	1.3%
Mismatches in N1 and N2	3	0.1%
Ambiguous sequences in N1 and N2	0	0.0%
TOTAL	**3752**	**100%**

EpiCoV sequence alignment summary

ALIGNMENT	COUNT	PERCENTAGE
N1,N2 Perfect Match	430269	91.263%
N1 match (mismatch in N2)	14681	3.114%
N2 match (mismatch in N1)	25177	5.340%
Mismatches in N1 and N2	1329	0.282%
Ambiguous sequences in N1 and N2	5	0.001%
TOTAL	**471461**	**100%**

The *in silico* inclusivity analysis showed over 99% of sequences 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. Collectively, all SARS-CoV-2 sequences analyzed and categorized with mismatches in N1 and N2 represent no more than 0.282% of the total SARS-CoV-2 sequence population.

Specificity (Cross-Reactivity)

To access the *in silico* exclusivity of the OPTI SARS-CoV-2 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 N2 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 contained greater than 80% identity to the design region.
For the organisms listed in Table 4 below, there was insufficient identity to align any of the additional organisms listed. No single organisms contained greater than 80% identity to the design region.

It can reasonably be concluded that the N1 and N2 primers and probes will not amplify and detect any of the virus, bacterial or yeast sequences analyzed.

Table 4: List of organisms analyzed *in silico*

Organism	Strain	Accession or WGS number
Human Adenovirus	A	NC_001460
Human Metapneumovirus (hMPV)	00-1	NC_039199
Parainfluenza virus 1	NM001	KX639498
Parainfluenza virus 2	VIROAF10	KM190939
Parainfluenza virus 3	CFI1849/2012	KJ672618
Parainfluenza virus 4	SC3019/2015	KY986647
Influenza A	8/1934(H1N1)	NC_002016 to NC_002023
Influenza B	2/2012 BX-51C	MT056021 to MT056028
Enterovirus	D68	MN389735
Respiratory syncytial virus	B/WI/629-Q0190/10	JN032120
Human Rhinovirus	14	NC_001490
Chlamydia pneumoniae	CWL029	AE001363
Haemophilus influenzae	NCTC8143	LN831035
Legionella pneumophila	Phil. 1	CP015928
Mycobacterium tuberculosis	HN-506	AP018036
Streptococcus pneumoniae	NCTC7465	LN831051
Streptococcus pyogenes	NCTC8198	LN831034
Bordetella pertussis	18323	HE965805
Mycoplasma pneumoniae	FH	CP010546
Pneumocystis jirovecii	E2178	NJFV01000001 to NJFV01000219
Candida albicans	SC5314	CP017623 to CP017630
Pseudomonas aeruginosa	PAO1	AE004091
Staphylococcus epidermis	ATCC 12228	NC_004461
Staphylococcus (Streptococcus) salivarius	NCTC8618	NZ_LR134274

Clinical Evaluation

A clinical evaluation study was conducted using real NP swab specimens from patients suspected of COVID-19 by their health care provider that were collected from a CLIA laboratory in the United States. A total of 34 positive (including 7 low positives or 20.6%) and 32 negative NP swab samples were tested with the OPTI SARS-CoV-2 RT PCR Test and compared to results obtained with an FDA EUA authorized RT-PCR test. Samples were extracted with either the OPTI DNA/RNA Magnetic Bead Kit (automated protocol) or RealPCR DNA/RNA Magnetic Bead Kit and RT-PCR was performed using the Applied Biosystems® 7500 FAST PCR instrument. Table 5 and Table 6 summarizes the results including the positive and negative percent agreement with 95% confidence limits.

Table 5. Clinical Samples- OPTI DNA/RNA Magnetic Bead Kit

		FDA EUA RT-PCR Test		
		Positive Patient Specimen	Negative Patient Specimen	**Total**
OPTI SARS-CoV-2 RT PCR Test	Positive Patient Specimen	**34**	**0**	**34**
	Negative Patient Specimen	**0**	**32**	**32**
	Total	**34**	**32**	

The positive and negative percent agreements between the OPTI SARS-CoV-2 RT PCR Test and FDA EUA test with the OPTI DNA/RNA Magnetic Bead Kit is:

PPA = 100% * 34/34 = 100%. (95% C.I. = 89.85% - 100%)

NPA = 100% * 32/32 = 100%. (95% C.I. = 89.28% - 100%)

Table 6. Clinical samples - RealPCR DNA/RNA Magnetic Bead Kit

		FDA EUA RT-PCR Test		
		Positive Patient Specimen	Negative Patient Specimen	**Total**
OPTI SARS-CoV-2 RT PCR Test	Positive Patient Specimen	**34**	**0**	**34**
	Negative Patient Specimen	**0**	**32**	**32**
	Total	**34**	**32**	

The positive and negative percent agreements between the OPTI SARS-CoV-2 RT PCR Test and FDA EUA test with the RealPCR DNA/RNA Magnetic Bead Kit is:

PPA = 100% * 34/34 = 100%. (95% C.I. = 89.85% - 100%)

NPA = 100% * 32/32 = 100%. (95% C.I. = 89.28% - 100%)

FDA SARS-CoV-2 Reference Panel Testing

The evaluation of sensitivity and MERS-CoV cross-reactivity was performed using reference material (T1), blinded samples and a standard protocol provided by the FDA. The study included a range finding study and a confirmatory study for LoD. Blinded sample testing was used to establish specificity and to confirm the LoD. The extraction method and instrument used were the OPTI DNA/RNA Magnetic Bead Kit (automated protocol) and the Applied Biosystems® 7500 FAST PCR instrument. The results are summarized in Table 7.

Table 7: Summary of LoD Confirmation Result using the FDA SARS-CoV-2 Reference Panel

Reference Materials Provided by FDA	Specimen Type	Product LoD	Cross-Reactivity
SARS-CoV-2	NP swab	1800 NDU/mL	N/A
MERS-CoV		N/A	ND

NDU/mL = RNA NAAT detectable units/mL

N/A: Not applicable

ND: Not detected

Clinical performance with pooling of 5 specimens

The clinical performance of the OPTI SARS-CoV-2 RT PCR Test was evaluated with a pool size of 5 specimens. Testing included positive and negative nasopharyngeal (NP) specimen pools. Each positive specimen pool consisted of one positive specimen with the remaining specimens being negative, whereas the negative specimen pools consisted only of negative specimens. 20 positive and 20 negative specimen pools were evaluated. The positive specimens used in the study covered the detectable range of the assay and included 5 (25%) low positive specimens (defined as within 3 Ct of the assay LoD). Both the pooled and individual specimens were evaluated with the OPTI SARS-CoV-2 RT PCR Test. The Positive Percent Agreement (PPA) and Negative Percent Agreement (NPA) were calculated in relation to the expected (individual) result for each evaluated pool size, as shown in Table 8.

Table 8**:** Results of pooled vs. individual samples including positive percent agreement and negative percent agreement

			Pooled samples			
		n	Positive	Negative	Invalid	% Agreement
Individual samples	Positive	20	20	0	0	100% (20/20) 95% CI: 80.6% - 100%
	Negative	20	0	20	0	100% (20/20) 95% CI: 80.6% - 100%

***In silico* sensitivity of customer data set**

An *in silico* analysis was performed to assess the loss of sensitivity (Ct value shift) caused by pooling five samples into a single extraction. The Ct value shift was calculated using data generated from twenty positive samples that were processed both individually and as a pool of five after combining with four negative samples. The positive percent agreement was calculated for three geographically diverse US sites using the Ct value shift. The positive percent agreement was > 85% for each of the three sites.

Performance Characteristics using Rapid Lysis Sample Preparation Method

Limit of detection (LoD) is defined as the lowest concentration of inactivated SARS-CoV-2 virus at which greater than or equal to 95% of all replicate lysates test positive. Serial dilutions of inactivated SARS-CoV-2 (Isolate USA-WA1/2020) from BEI (Manassas, VA; Cat. No. NR-52286) were prepared in negative nasopharyngeal (NP) swab (upper respiratory sample type) sample pools. The initial LoD was determined with 3-fold serial dilutions tested in five replicates. Each replicate was processed according to the OPTI Rapid Lysis Buffer Kit product insert and tested with the OPTI SARS-CoV-2 RT-PCR Test on the Applied Biosystems®(ABI) QuantStudio 5 (QS5) PCR instrument (software v1.5.1). For comparison, each replicate was also processed using the validated OPTI DNA/RNA Magnetic Bead Kit on Thermo Scientific KingFisher Flex Magnetic Particle Processor with the OPTI SARS-CoV-2 RT-PCR Test on the Applied Biosystems® QuantStudio 5 PCR Instrument. The initial LoD was considered to be the lowest target level at which 5/5 replicates were positive. Furthermore, the LoD of the OPTI SARS-CoV-2 RT-PCR Test using the OPTI Rapid Lysis Buffer was considered comparable to the LoD of the OPTI SARS-CoV-2 RT-PCR Test using the validated OPTI DNA/RNA Magnetic Bead Kit when it was within approximately 3x the LoD established using the validated OPTI DNA/RNA Magnetic Bead Kit. Table 9 provides a summary of the results with both the OPTI Rapid Lysis Buffer Kit and the OPTI DNA/RNA Magnetic Bead Kit.

To confirm the LoD, 20 replicates of NP sample matrix spiked with inactivated SARS-CoV-2 virus were processed and tested on the Applied Biosystems® QuantStudio 5 PCR instrument (software v1.5.1) and the Roche® LightCycler (LC)480 PCR Instrument. The LoD was confirmed to be 1.09 copies/ µL in NP swab samples (19/20). Results are shown in Table 10 below.

Table 9. Limit of Detection comparison between specimens processed using the OPTI DNA/RNA Magnetic Bead Kit and the OPTI Rapid Lysis Buffer Kit

Platform	Parameter	OPTI SARS-CoV-2 RT-PCR Test					Observed LoD
OPTI DNA/RNA Magnetic Bead Kit	Viral copies/µL	9.83	3.28	1.09	0.36	0.12	0.36
	Detection Rate	5/5	5/5	5/5	5/5	2/5	
	Mean Ct value	32.14	33.67	35.17	36.68	37.25	
OPTI Rapid Lysis Buffer Kit (QS5)	Viral copies/µL	9.83	3.28	1.09	0.36	0.12	1.09
	Detection Rate	NT	5/5	5/5	4/5	1/5	
	Mean Ct value	NT	34.66	36.45	38.06	39.52	
OPTI Rapid Lysis Buffer Kit (LC480)	Viral copies/µL	9.83	3.28	1.09	0.36	0.12	1.09
	Detection Rate	NT	5/5	5/5	2/5	2/5	
	Mean Ct value	NT	36.17	36.88	37.05	38.22	

NT = Not Tested

Table 10. PCR instrument LoD determination with rapid lysates using the OPTI Rapid Lysis Buffer Kit

	NP Swab			
PCR Instrument	Viral copies/µL	Mean Ct (N1/N2)	Detection Rate	LoD copies/µL
ABI QuantStudio 5	1.09	36.42	19/20	1.09
	0.36	38.02	11/20	
Roche LC480	1.09	37.34	19/20	1.09
	0.36	38.15	7/20	

Clinical Evaluation

A clinical evaluation study was conducted using original NP swab specimens from patients suspected of COVID-19 by their health care provider that were collected from a CLIA laboratory in the United States. A total of 76 positive (including low positives) and 90 negative NP swab samples (positive and negative results as determined by an EUA molecular test) were processed using both the OPTI Rapid Lysis Buffer Kit and OPTI DNA/RNA Magnetic Bead Kit. Rapid lysates and extracts were tested in parallel with the OPTI SARS-CoV-2 RT-PCR Test on the Applied Biosystems® QuantStudio 5 PCR instrument. Table 11 summarizes the results including the positive and negative percent agreement with 95% confidence limits.

Table 11. Clinical Sample Comparison – OPTI DNA/RNA Magnetic Bead Kit and OPTI Rapid Lysis Buffer Kit

		OPTI Rapid Lysis Buffer Kit		
		Positive	Negative	**TOTAL**
OPTI DNA/RNA Magnetic Bead Kit	Positive	76	0	**76**
	Negative	0	90	**90**
	TOTAL	**76**	**90**	

Positive Percent Agreement = 100% (95% C.I. = 95.2-100%)
Negative Percent Agreement = 100% (95% C.I. = 95.9-100%)

Nine positive clinical samples were further diluted by 1,000 fold, processed in parallel using both the OPTI DNA/RNA Magnetic Bead Extraction Kit and the OPTI Rapid Lysis Buffer Kit, and retested. Results are shown in Table 12.

Table 12. Contrived low-positive samples – OPTI DNA/RNA Magnetic Bead Kit (extract) and OPTI Rapid Lysis Buffer Kit (rapid lysate)

Sample	**SARS-CoV-2 Ct value**	
	Extract	**Rapid Lysate**
1	33.94	35.04
2	32.70	35.92
3	34.48	37.34
4	34.93	38.07
5	34.21	38.40
6	34.67	37.45
7	33.30	38.13
8	33.80	38.02
9	34.82	38.22

Interfering substances

Medically and/or physiologically relevant interferants that can potentially be present in respiratory samples were assessed for their impact on the efficacy of the OPTI Rapid Lysis Buffer Kit specimen preparation method. Interferents were added to NP specimens containing inactivated SARS-CoV-2 at 3x LoD and processed using the Rapid Lysis method and tested. No interference was observed from the list of substances and at the concentrations examined (Table 13).

Table 13. Interfering substances study

Potential Interferent	Concentration
Control	–
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	0.5% w/v

Potential Interferent	Concentration
Mupirocin	4.3 mg/mL
NeoSynephrine Spray	20% v/v
Normal saline	20% v/v
Otrivin Nasal Spray	20% v/v
Tobryamycin	1.44 mg/mL
Triamcinolone	0.04 mg/mL
Whole Blood	1% v/v
Zanamivir (Relenza)	0.284 mg/mL
Zicam nasal spray	20% v/v

Appendix A: Specimen Pooling Implementation and Monitoring (Laboratory Responsibilities)

Sample Pooling Implementation (Laboratory Monitoring Part A)

After the sample pooling using the candidate test has been authorized by the FDA, before a test developer validated sample pooling strategy is implemented, a laboratory should determine the appropriate pool size based on percent positivity rate in the testing population and pooling testing efficiency (Table 1).

Table 1. Efficiency of pooling based on the positivity of SARS-CoV-2 RNA in individual samples (as an example)

P, percent of positive subjects in the tested population	$n_{maxefficiency}$ (n corresponding to the maximal efficiency)	Efficiency of n-sample pooling corresponding to $n_{maxefficiency}$ (a maximum increase in the number of tested patients when Dorfman n- pooling strategy used)
5%	5	2.35
6%	5	2.15
7%	4	1.99
8%	4	1.87
9%	4	1.77
10%	4	1.68
11%	4	1.61
12%	4	1.54
13%	3	1.48
14%	3	1.43
15%	3	1.39
16%	3	1.35
17%	3	1.31
18%	3	1.28
19%	3	1.25
20%	3	1.22
21%	3	1.19
22%	3	1.16
23%	3	1.14
24%	3	1.12
25%	3	1.10

A.1 If Historical Data for Individual Specimens is Available

A.1.1 Positivity Rate of Individual Testing

- Estimate positivity rate ($P_{individual}$) in the laboratory based on individual sample testing. For this consider the 7-10 previous days and calculate the number of patients tested during those days. $P_{individual}$ is the number of positive results divided by the total number of tested patients during these 7-10 days.

A.1.2 Selection of test developer validated size of sample pools, n

- Use $P_{individual}$ and Table 1 to choose an appropriate validated pool size. Table 1 presents the pool size with the maximum efficiency for the validated pool sizes and positivity rates. If the positivity rate ($P_{individual}$) is in Table 1, choose n from Table 1 which corresponds to the maximum efficiency (F).
- If $P_{individual}$ in your laboratory does not correspond to the largest validated pool size in Table 1, the pool size with maximum efficiency for this positivity rate was not validated and you should choose the maximum n which was validated. For example, for the calculation of efficiency of 5-sample pooling, using formula $F=1/(1+1/5-(1-P)^5)$, when $P_{individual}$ is 1%, the efficiency F is 3.46 for n=5. It means that 1,000 tests can cover testing of 3,460 patients on average.
- If $P_{individual}$ is greater than 25%, then pooling patient samples is not efficient and should not be implemented.

A.2 If Historical Individual Data for Individual Specimens is Unavailable

If historical data from the previous 7-10 days is unavailable, the maximum pool size validated in the EUA and any smaller pool sizes can still be implemented, as the EUA test has been validated for the maximum pool size-specimen pooling. However, note that without $P_{individual}$, the laboratory may choose a pooling size that does not maximize pooling efficiency.

Sample Pooling Monitoring (Laboratory Monitoring Part B)

After implementing a n-sample pooling strategy, calculate the percent positivity rate (P_{pool}) based on n sample pooling strategy periodically using the data from pooled samples from the previous 7-10 days*.

B.1 If Historical Data for Individual Specimens is Available

If historical data for individual specimens is available, compare P_{pool} to $P_{individual}$ periodically. If P_{pool} is less than 85% of $P_{individual}$ ($P_{pool} < 0.85 \times P_{individual}$), it is recommended that:

- The n-samples pooling should be re-assessed by conducting a re-assessment study as described in "Laboratory Monitoring Part C" below.
- Alternatively, if the EUA test is a high sensitivity RT-PCR assay which uses a chemical lysis step followed by solid phase extraction of nucleic acid (e.g., silica bead extraction), and has established high sensitivity with an internationally recognized standard or FDA SARS-CoV-2 Reference Panel, size of pools may be increased taking into consideration Table 1, and the new n should not be more than the test developer validated maximum n in the EUA.
- If P_{pool} is greater than 25%, pooling of patient samples is not efficient and should be discontinued until the percent positivity rate decreases.

B.2 If Historical Data for Individual Specimens is Unavailable

- After implementing a n-sample pooling strategy, first calculate the positivity rate ($P_{pool\text{-}initial}$) based on n-sample pool size using the data from testing pooled samples from the first 7-10 days*.
  - If $P_{pool\text{-}initial}$ is greater than 25%, pooling of patient specimens is not efficient and should be discontinued until the percent positivity rate decreases.
  - If $P_{pool\text{-}initial}$ is less than or equal to 25%, pooling of patient specimens can be continued.
- Continue to monitor n-sample pooling strategy by calculating the positivity rate among patient samples during n-sample pooling ($P_{pools\text{-}x}$) for subsequent 7-10* day period based on n-sample pool testing. ($P_{pool\text{-}x}$) should be updated daily using a moving average.

Compare $P_{pool\text{-}initial}$ to $P_{pool\text{-}x}$ periodically. If $P_{pool\text{-}x}$ is less than 90% of $P_{pool\text{-}initial}$ ($P_{pool\text{-}x} < 0.90 \times P_{pool\text{-}initial}$), it is recommended that:

- The n-samples pooling should be re-assessed by conducting a re-assessment study as described in "Laboratory Monitoring Part C" below.
- Alternatively, if the EUA test is a high sensitivity RT-PCR assay which uses a chemical lysis step followed by solid phase extraction of nucleic acid (e.g., silica bead extraction), and has established high sensitivity with an internationally recognized standard or FDA SARS-CoV-2 Reference Panel, size of pools may be increased taking into consideration Table 1, and the new n should not be more than the test developer validated maximum n in the EUA.
- If P_{pool} is greater than 25%, pooling of patient samples is not efficient and should be discontinued until the percent positivity rate decreases.

* It is recommended that $P_{individual}$ be calculated from the previous 7-10 days, while P_{pool} and $P_{pool\text{-}x}$ are calculated from data collected during a 7-10 day time frame. However, when determining if 7-10 days is appropriate, take into consideration the laboratory testing volume and percent positivity, among other factors. Note that if the number of individual or pooled positive results collected during a given time frame is less than 10, $P_{individual}$, P_{pools}, and $P_{pool\text{-}x}$ may not be representative of the percent positivity in the testing population and the laboratory may want to consider extending the testing time period to increase the chance of capturing positives.

Sample Pooling Re-assessment (Laboratory Monitoring Part C)

Option 1: Stop n-sample pooling and return to individual testing

- Patient samples should be tested individually until 10 consecutive positive samples have been collected. The total number of samples, tested individually, depends on the positivity rate.
- Using these samples, 10 pools should be created and tested with 1 positive and (n-1) negative samples and the PPA between testing sample pools and individual samples should be calculated.

Individual testing	Study
Sample #1, Individual, negative	
Sample #2, Individual, negative	
Sample #3, Individual, positive	Convolute a pool with Sample#3 and (n-1) negative samples
Sample #4, Individual, negative	
……	

- Alternatively, if the laboratory is using an EUA RT-PCR test that can generate Ct values, the laboratory may be able to assess PPA (EUA Test $_{pool}$ vs. EUA Test $_{individual}$) in silico based on the individually tested sample results without performing any testing of pooled samples. In order to perform this in silico PPA assessment, the Instructions for Use (IFU) of this EUA test must include a reference table containing information regarding the in silico PPA (EUA Test $_{pool}$ vs. EUA Test $_{individual}$) analysis rules established by the test developer for each test target for all validated pool sizes. Refer to the "Clinical Sample Pooling Validation Study at Three Geographically Diverse US Sites" section of the template for more information regarding this reference table.

Option 2: Continue n-sample pooling

- Re-assessment study should start from time T0 and should consist of simultaneous testing individual sample testing in parallel with the normal workflow of pooled testing. However, since all non-negative sample pools require individual testing of all individual samples included in the pool as a part of the n-sample pooling and deconvoluting workflow, the re-assessment study essentially consists of testing individual samples from the negative n-sample pools.
- Re-assessment study may pause at time T1 when a minimum of 10 consecutive positive individual results are obtained, including both positive individual results generated from individual testing of samples from the non-negative sample pools following the n-sample pooling and deconvoluting workflow, and positive individual results obtained from individual testing of samples from the negative sample pools for the time period from T0 to T1 [T0, T1].

n-sample pools workflow	Study
Pool#1, negative	Deconvolute n individual samples
Pool#2, negative	Deconvolute n individual samples
Pool#3, negative	Deconvolute n individual samples
Pool#4, positive => n individual results	
……	

- Considering that number of positive individual sample results among negative pools is K, PPA between testing n-sample pools and assaying single specimens using the candidate test should be calculated as PPA (EUA Test $_{pool}$ vs. EUA Test $_{individual}$) = 100% x (10-K)/10. It is critical that all consecutive positive samples from time period [T0, T1] are included in the PPA calculations. With regard to calculating the PPA, all non-negative results testing pooled samples should be counted as in agreement with positive individually tested results.

Re-assessment Acceptance Criteria for Option 1 and Option 2

- If the PPA (EUA Test $_{pool}$ vs. EUA Test $_{individual}$) is ≥ 90% (9 out of 10 or 10 out of 10), then implementation of testing using n-sample pooling is acceptable.
- If the PPA between pooled-testing results and individual-testing results is less than 90%:
  - If PPA ≤70% (7 out of 10), reduce the pool size (consider a new n as n-1)
  - If PPA is 80% (8 out of 10), collect an additional 10 consecutive individually positive samples. Then, calculate the PPA from the combined data of 20 samples, between pooled testing results and individual testing results. If the PPA is ≥ 85%, then implementation of testing using n-sample pooling is acceptable. Or, to compensate for lost sensitivity, reduce the pool size (consider a new n as n-1) and continue with the re-assessment testing until PPA of pooled compared to individual testing is ≥ 90%.
- If PPA of at least 85% cannot be reached for any pool size evaluated in the re-assessment, cease pooling patient specimens.

If n-sample pooling is acceptable based on re-assessment, re-establish $P_{individual}$ in your laboratory by estimating the positivity rate from individual testing in the population from which the 10 (or 20) consecutive individual positive samples were collected. If the total number of samples (N*) that needed to be tested to obtain the 10 (or 20) consecutive positive samples is stopped at the 10^{th} (or 20^{th}) positive sample, then the positivity rate of 10/N* (or 20/N*) is overestimated. The positivity rate should be corrected by the following corresponding multiplier:

- Positivity rate for 10 samples is (10/N*) × (10/11)
- Positivity rate for 20 samples is (20/N*) × (20/21).

This updated new positivity rate should be used as $P_{individual}$ in the future laboratory monitoring (return to section B.1 of the "Laboratory Monitoring Part B").

Appendix B: Laboratory Procedure for Qualification of RUO Instruments

Testing laboratories should use this protocol to qualify RUO instrument(s) listed in the "Materials Required but Not Provided" section of the IFU for SARS-CoV-2 testing using the OPTI SARS-CoV-2 RT-PCR Test Kit.

Materials required:

Description	Included in the kit
OPTI PCR Grade Water	Yes
OPTI Positive Control, dried (PC)	Yes
A negative upper respiratory (UR) specimen (pool if necessary)	Not provided

Preparation of contrived positive specimens for RUO instrument qualification:

1. Reconstitute the dried OPTI Positive Control as described in the "Reconstitution of Dried Components" section of the IFU.
2. 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)	Reconstituted PC (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	SARS-CoV-2 FAM Ct value	SARS-CoV-2 FAM Result	Internal Control HEX Ct Value	Internal Control HEX Ct Result
Positive Control	<40	Positive	<36	Positive
PCR Negative Control	No signal	Negative	No signal*	Negative
Extraction Negative Control	No signal	Negative	No signal*	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. HEX 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.

Sample	SARS-CoV-2 FAM Ct value	SARS-CoV-2 FAM Result	Internal Control HEX Ct Value	Internal Control HEX Ct Result
Negative Specimen	No signal	Negative	<36	Positive
Contrived Positive Specimen 1	<40	Positive	<36	Positive
Contrived Positive Specimen 2	<40	Positive	<36	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

For Applied Biosystems® 7500 FAST, Applied Biosystems® QuantStudio 5, Agilent Mx3005P™, Bio Molecular Systems Mic qPCR and Roche LightCycler® 480.

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 RT-PCR Test.

* Refer to Appendix B: Laboratory Procedure for Qualification of RUO Instruments

Emergency Use Only

This instrument is authorized for use with the OPTI SARS-CoV-2 RT-PCR Test

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

OPTI SARS-CoV-2 RT-PCR Test

The latest version of the Instructions for Use (IFU) for the OPTI SARS-CoV-2 RT-PCR Test can be accessed as an electronic pdf from the OPTI Medical Systems website at https://www.optimedical.com/en/products-and-services/kits/opti-sars-cov-2-rt-pcr-test-kit/

A paper version of the IFU for the OPTI SARS-CoV-2 RT-PCR Test can also be requested at no additional cost.

For more information on the OPTI SARS-CoV-2 RT-PCR test kit, please contact your area manager or distributor, or visit www.optimedical.com

covid19@optimedical.com

IDEXX USA

+1 800 548 9997

IDEXX Europe

+800 727 43399

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 of nucleic acid from SARS-CoV-2, not for any other viruses or pathogens; and

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

REF XX-XXXXX

Version
XX-XXXXX-XX

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