OPTI SARS-CoV-2 RT-PCR Test

English Version

Used for real-time PCR identification of SARS-CoV-2 RNA extracted from upper and lower respiratory specimens (such as nasal, nasopharyngeal, oropharyngeal swabs, sputum, lower respiratory tract aspirates, bronchoalveolar lavage, and nasopharyngeal wash/aspirate or nasal aspirate).

IVD CE Rx

For *in vitro* diagnostic use only
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 the SARS-CoV-2 in upper and lower respiratory specimens (such as nasal, nasopharyngeal, oropharyngeal swabs, sputum, lower respiratory tract aspirates, bronchoalveolar lavage, and nasopharyngeal wash/aspirate or nasal aspirate) from patients suspected of COVID-19 by their health care provider.

Results are for the identification of SARS-CoV-2 RNA. The SARS-CoV-2 RNA is generally detectable in upper and lower respiratory samples during the acute phase of infection. 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 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 and trained clinical laboratory personnel specifically instructed and trained in the techniques of real-time nucleic acid amplification and *in vitro* diagnostic procedures.

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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 RT-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, 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	At receipt	After reconstitution	
OPTI SARS-CoV-2 Mix (SARS-CoV-2 Mix), dried	Red	1 x 1.0 mL	–25 to 8°C	–25 to –15°C	≤6
REF 61-56616-00 Contains N1 and N2 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)	Black	1 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 Positive Control, dried (PC)	Blue	1 x 200 µL	–25 to 8°C	–25 to –15°C	≤6
REF 44-56617-00 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	Clear	2 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 Mix and PC. It is also used as the PCR negative control for each test run. Do not transfer 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 7500 software 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 (for use with upper respiratory specimens)	
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	IDEXX 99-56102 (384 samples) / 99-56106 (96 samples) Macherey Nagel 744200.4 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
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
Extraction control containing human specimen (HSC) material	See Quality Controls section

Equipment and Lab Consumables	Source and part number
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.
- 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 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)

NucleoMag VET Magnetic Extraction Kit (Macherey Nagel, Part #744200.4)

OPTI RNA/DNA Magnetic Bead Kit (OPTI Medical System, #99-58015)

Other extraction or lysis methods may also be used once validated by the laboratory. Store the purified RNA at <–15°C if testing is not performed immediately after RNA extraction.

Quality Controls

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

- Negative Control (OPTI PCR Grade Water): A "no template" (negative) control is needed to confirm the PCR plate is valid. PCR Grade water is used and should be included for each PCR run. The negative control should test negative for the SARS CoV-2 target and internal control. The no template control is not included during extraction.
- Positive Control (OPTI 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 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 the Positive Control (5 μL), PCR negative control (5 μL PCR Grade Water) and Extraction 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 Set up thermal cycler with Cycling Program below.

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 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 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	≥36	Negative
Extraction Control	No Signal	Negative	<36	Positive

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	No Ct value	≤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.

Limitations

9 Limitations

- Performance of the OPTI SARS-CoV-2 RT-PCR Test has only been established in upper and lower respiratory specimens (such as nasal, nasopharyngeal, oropharyngeal swabs, sputum, lower respiratory tract aspirates, bronchoalveolar lavage, and nasopharyngeal wash/aspirate or nasal aspirate). Other specimen types have not been evaluated and 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 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 and processing systems have not been validated.
- 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.
- 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.

Assay Performance

10 Inclusivity (analytical reactivity)

To assess the *in silico* inclusivity of the OPTI SARS-CoV-2 RT-PCR Test, a multiple sequence alignment (MSA) was generated from the GISAID CoV database sequences submitted between the dates of December 23, 2019 and April 15, 2020 and compared for identity to the test primers and probes. Only full length, high coverage sequences were used, resulting in 6,267 sequences in the design regions. 6,260 sequences have a 100% match to the N1 forward primer, 6,218 have a 100% match to the N1 probe and 6,224 have a 100% to the N1 reverse primer. This represents 99.9%, 99.2% and 99.3% of N1 sequences analyzed. Likewise, 6,258 sequences have a 100% match to the N2 forward primer, 6,257 have a 100% match to the N2 probe and 6,261 have a 100% match to N2 reverse primer. This represents 99.9%, 99.8%, and 99.9% of N2 sequences analyzed. Of the remaining sequences with mismatches, only one sequence had a mismatch in more than one binding region of the N1 design (hCoV-19/USA/UN-UW-1407/2020|EPI_ISL_ 422983|2020-03-17). However, this strain maintains a perfect match to the N2 design and is therefore predicted to be amplified and detected by the combined target assay. No sequences were identified with a mismatch in both the N1 and N2 target regions. Therefore, the OPTI SARS-CoV-2 RT-PCR Test would be predicted to amplify and detect all analyzed sequences.

10.1 Exclusivity (Cross-Reactivity)

To assess 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 1 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 1: 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
Infuenza A	8/1934(H1N1)	NC_002016 to NC_002023
Infuenza 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	ATC 12228	NC_004461
Staphylococcus (Streptococcus) salivarius	NCTC8618	NZ_LR134274

10.2 Analytical Sensitivity- SARS-CoV-2 RNA

The analytical sensitivity was defined as the lowest concentration of analyte that could be reliably detected with 95% confidence. This was assessed by testing dilutions of RNA template representing the SARS-CoV-2 genomic region of interest. Seven dilutions were tested on the Applied Biosystem® 7500 Real-Time PCR instrument, in three sessions of 12 replicates each, resulting in 36 replicates for each concentration tested. The detection rate mean FAM (SARS CoV-2) Ct, Ct standard deviation (SD) and Ct coefficient of variation (%CV) were calculated for each dilution level.

Table 2 summarizes the analytical sensitivity test results. The data shows that the OPTI SARS CoV-2 RT-PCR Test detects 1 copy/μL of SARS-CoV-2 viral RNA with a confidence ≥95%. This concentration demonstrates the limit of detection of the kit.

Table 2: Analytical Sensitivity

		ABI 7500 Results				
Dilution Level	RNA Concentration (copies/μL)	Replicates Detected	Detection Rate	Mean Ct (FAM)	Ct SD	Ct % CV
1	400	36	100%	26.5	.092	0.3%
2	40	36	100%	29.7	.084	0.3%
3	4	36	100%	33.2	.361	1.1%
4	2	36	100%	34.6	.722	2.1%
5	**1**	**35**	**97%**	**35.3**	**.625**	**1.8%**
6	0.5	31	86%	36.6	.938	2.6%
7	0.2	18	50%	36.4	.616	1.7%

10.3 Precision

Assay precision is expressed in the form of the Ct standard deviation (SD) and Ct coefficient of variation (%CV) for multiple wells. Precision for the OPTI SARS CoV-2 RT-PCR Test was determined by the repeated testing of SARS-CoV-2 RNA template dilutions prepared to represent 3 viral load levels:

- High- 2000 copies/reaction (400 copies/μL)
- Mid- 200 copies/reaction (40 copies/μL)
- Low- 20 copies/reaction (4 copies/μL)

10.4 Repeatability

Repeatability was measured by analyzing 12 replicates each of the High, Mid and Low RNA copy number dilutions on a single plate. Results are shown in table 3, below.

Table 3: Repeatability

		ABI 7500 Results				
Dilution Level	RNA Concentration (copies/μL)	Replicates Detected	Detection Rate	Mean Ct (FAM)	Ct SD	Ct % CV
High	400	12	100%	26.5	.086	0.3%
Mid	40	12	100%	29.7	.099	0.3%
Low	4	12	100%	33.1	.463	1.4%

10.5 Inter-instrument and Operator Reproducibility

Inter-Instrument and Operator reproducibility was measured by analyzing 12 replicates each of the High, Mid and Low RNA copy number samples on a single plate per instrument, with a different operator conducting each test. Results are shown in table 4 below.

Table 4: Instrument/operator Reproducibility

		Instrument/Operator Mean Ct (FAM)				Ct (FAM)	
Dilution Level	RNA (copies/μL)	ABI 7500 Operator-1	ABI QS-5 Operator-2	LC480 Operator-3	Detection Rate	Mean	% CV
High	400	26.5	27.2	28.0	100%	27.2	2.3%
Mid	40	29.7	29.8	30.7	100%	30.1	1.6%
Low	4	33.1	33.4	33.5	100%	33.3	1.2%

10.6 Limit of Detection (LoD)

Limit of detection (LoD) is defined as the lowest concentration of SARS-CoV-2 RNA at which > 95% of replicates test positive. LoD for the OPTI SARS-CoV-2 RT-PCR Test was determined from dilutions of synthetic SARS-CoV-2 RNA prepared in nasopharyngeal (NP) swab or sputum sample matrix. 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 following the standard protocol. Extracted RNA was tested on the Applied Biosystems® 7500 PCR instrument. To confirm the LoD, 20 replicates of each sample matrix spiked with SARS-CoV-2 RNA at the initial LoD concentration and a further 3-fold dilution were extracted and tested in the same manner as the initial LoD evaluation. The LoD was confirmed to be 1/μL (5 copies per reaction) in both NP swab and sputum sample matrices. Results are shown in Table 5 below.

An additional study showed comparable LoD results when using the OPTI DNA/RNA Magnetic Bead Kit (OPTI Medical Systems 99-58015) on the Thermo Scientific™ KingFisher™ Duo Prime with extracted RNA tested on the Applied Biosystems® 7500 FAST PCR instrument.

Table 5: LoD Testing in NP Swab and Sputum

	NP Swab Matrix				Sputum Matrix			
RNA (copies/μL)	Mean Ct (FAM)	Mean Ct (VIC/HEX)	Detection Rate	% Detection	Mean Ct (FAM)	Mean Ct VIC/HEX)	Detection Rate	% Detection
1	37.6	25	19/20	95%	38.0	20.4	20/20	100%
0.2–0.3	38.5	25.1	12/20	60%	40.1	20.3	2/20	10%

10.7 Additional Instrument LoD Evaluation

An additional study was conducted to determine the LoD for the OPTI SARS-CoV-2 RT-PCR Test on multiple PCR instruments. Applied Biosystems QuantStudio 5, Agilent Mx3005P, Roche LightCycler® 480, and Bio Molecular Systems Mic PCR instruments were included in this study. The LoD was evaluated by testing 20 replicates of pooled nasopharyngeal (NP) swab and sputum matrix spiked with SARS-CoV-2 synthetic RNA at 1 copy/μL, which had been previously confirmed as the LoD for the ABI 7500 PCR instrument. Detection of 95%of the replicates (19/20) was considered confirmation of the LoD for the instrument. The LoD of 1 copy/μL was confirmed for each of the sample types on all the instruments tested. Sputum samples were not tested on the LC480 or BSM MIC instruments. These results are shown in table 6 below.

Table 6: LoD Confirmation on Multiple PCR Instruments

		NP Swab Matrix				Sputum Matrix			
PCR Instrument	RNA (copies/μL)	Mean Ct (FAM)	Mean Ct (VIC/HEX)	Detection Rate	% Detection	Mean Ct (FAM)	Mean Ct VIC/HEX)	Detection Rate	% Detection
ABI 7500	1	37.6	25	19/20	95%	38.0	20.4	20/20	100%
ABI QS-5	1	36.5	26.1	19/20	95%	37.7	20.2	20/20	100%
Agilent MX3005P	1	33.7	22.5	19/20	95%	35.1	18.7	19/20	95%
Roche LC480	1	36.2	26.3	20/20	100%	Not Tested			
BMS MIC	1	33.7	25.3	20/20	100%	Not Tested			

10.8 Contrived Positive Clinical Results

An initial clinical evaluation was performed with the OPTI SARS-CoV-2 RT PCR Test, using banked nasopharyngeal (NP) swab and sputum specimens. Contrived positive samples were prepared by spiking known concentrations of SARS-CoV-2 RNA relative to the product LoD into confirmed negative clinical specimens. A total of 30 contrived positive and 30 negative samples were tested for each sample type. RNA was extracted with the RealPCR DNA/RNA Magnetic Bead Extraction Kit, and PCR was performed using the Applied Biosystems® 7500 PCR instrument. All the contrived positive samples were detected as positive, and all negative samples yielded negative results. Table 7 below shows the results for each sample type.

Table 7: Contrived Positive Clinical Results

		Sample Status		
		Pos	Neg	Totals
OPTI SARS-CoV-2	Pos	60	0	60
RT-PCR	Neg	0	60	60
	Totals	60	60	120

		95% Confidence Limits	
		Low CL	High CL
Positive % Agreement (PPA)	100.0%	92.6%	100%
Negative % Agreement (NPA)	100.0%	92.6%	100%

10.9 Diagnostic Sensitivity and Specificity

Diagnostic sensitivity and specificity of the OPTI SARS-CoV-2 RT PCR Test were assessed by testing NP swab and sputum samples for which the SARS-CoV-2 status was confirmed. The reference test methods used to determine sample status were the CDC test[1] or the Institute Pasteur test[2]. Sixty negative samples were defined as negative because they were collected prior to the COVID-19 pandemic. Available details for the testing are listed in Table 8. One laboratory only shared limited information regarding sample types and extraction method. A total of 133 negative and 75 positive clinical samples were included in analysis. Diagnostic sensitivity is defined as the percentage of confirmed positive samples that yield positive test results. Diagnostic specificity is defined as the percentage of confirmed negative samples that yield negative test results.

The details for each of the sample sets are listed in the table below.

Laboratory	Sample types	Extraction Method	Reference PCR Method	Positive	Negative
A French Laboratory	Respiratory samples	Unknown	Institut Pasteur	8	1
A US Laboratory Maine, USA	NP swabs	Roche MagNA Pure 96	CDC	25	8
IDEXX, Maine, USA	NP swabs	RealPCR DNA/RNA MagBeads	CDC	42	64
IDEXX, Maine, USA	NP swabs and sputum	RealPCR DNA/RNA MagBeads	History: Collected prior to COVID-19		60
Total				75	133

Table 8: Clinical Evaluation with Diagnostic Samples

		Sample Status		
		Pos	Neg	Totals
OPTI SARS-CoV-2	Pos	75	0	75
RT-PCR	Neg	0	133	133
	Totals	75	133	208

		95% Confidence Limits	
		Low CL	High CL
Diagnostic Sensitivity	100.0%	94.0%	100%
Diagnostic Sensitivity	100.0%	96.5%	100%

[1]https://www.cdc.gov/coronavirus/2019-ncov/lab/rt-pcr-panel-primer-probes.html

[2]Corman VM, Landt O, Kaiser M, et al. Detection of 2019 novel coronavirus (2019-nCoV) by real-time RT-PCR. Euro Surveill 2020; 25.

For technical assistance on the OPTI SARS-CoV-2 RT-PCR Test:
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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

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