DIAGNOVITAL

SARS-CoV-2 Real-Time PCR Kit

Qualitative RT-PCR-based detection of SARS-CoV-2

Instructions for Use

For Use under Emergency Use Authorization

For *in vitro* diagnostic use. For professional use only. For Rx use only.

IVD

REF Σ

09065025 25 tests

09065050 50 tests

09065100 100 tests

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Intended Use

DIAGNOVITAL® SARS-CoV-2 Real-Time PCR Kit is a real-time RT-PCR assay intended for the qualitative detection of nucleic acid from SARS-CoV-2 in anterior nasal and mid-turbinate nasal swabs, nasopharyngeal and oropharyngeal swabs, nasopharyngeal wash/aspirates or nasal aspirates and bronchoalveolar lavage (BAL) specimens from individuals suspected of COVID-19 by their healthcare provider.

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

Results are for the identification of SARS-CoV-2 RNA which is generally detected in respiratory specimens 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 are 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 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 **DIAGNOVITAL® SARS-CoV-2 Real-Time PCR Kit** is intended for use by qualified clinical laboratory personnel specifically instructed and trained in the techniques of real-time PCR and in-vitro diagnostic procedures. The **DIAGNOVITAL® SARS-CoV-2 Real-Time PCR Kit** is only for use under the Food and Drug Administration's Emergency Use Authorization.

Product Description

Explanation of the Test

DIAGNOVITAL® SARS-CoV-2 Real-Time PCR Kit is a qualitative test for the detection of the 2019 novel coronavirus (SARS-CoV-2) in nasopharyngeal and oropharyngeal swab samples collected in Copan Universal Transport Medium System (UTM-RT) (Becton, Dickinson, USA, Cat. No. 220220) or BD Universal Viral Transport System (UVT) (Becton, Dickinson, USA, Cat. No. 220220) and is run on the BIO-RAD CFX96-IVD (Bio-Rad Laboratories, Inc.) and QuantStudio™ 5 Dx, (Applied Biosystems). The RNA Internal Control, used to monitor the entire sample preparation and PCR amplification process, is introduced into each specimen during sample processing. In addition, the test utilizes external controls (low titer positive control and a negative control).

Principles of the Procedure

The **DIAGNOVITAL® SARS-CoV-2 Real-Time PCR Kit** is based on conventional RT-PCR technology including extraction and purification of the nucleic acid genome of SARS-CoV-2 followed by PCR amplification and detection. The test is run on BIO-RAD CFX96-IVD or QuantStudio™ 5 Dx platform. Nucleic acid from patient samples and controls are extracted in parallel using the RTA Viral Nucleic Acid Isolation Kit. Nucleic acid is released by the lysis reagent and bound to the silica columns. Unbound substances and impurities, such as denatured protein, cellular debris and potential PCR inhibitors, are removed with subsequent wash steps and purified nucleic acid is eluted through silica columns with elution buffer. External controls (positive and negative) are processed in the same way with each run.

Selective amplification of target nucleic acid from the sample is achieved using target- specific forward and reverse primers and probes specific to the SARS-CoV-2 envelope gene (E-gene) and the polymerase gene (RdRP). The RdRP gene target is detected by one probe, unique to SARS-CoV-2. Additionally, a conserved region in the structural protein envelope E-gene was chosen also for the pan-Sarbecovirus detection. The pan-Sarbecovirus detection sets will also detect SARS-CoV-2 virus.

Selective amplification of RNA Internal Control is achieved by the use of non-competitive, sequence specific forward and reverse primers and a probe which have no homology with the coronavirus genome. A thermostable DNA polymerase enzyme is used for amplification.

The **DIAGNOVITAL® SARS-CoV-2 Real-Time PCR Kit** master mix contains detection probes for the two SARS-CoV-2 targets and one for the internal RNase P gene. Each of the targets is amplified in a separate reaction. Probes are each labeled with fluorescent dyes that act as a reporter. Each probe also has a second dye which acts as a quencher. When not bound to the target sequence, the fluorescent signals of the intact probes are suppressed by the quencher

dye. During the amplification process, probes anneal to each of the amplified sequences located between the forward and reverse primers for each target. During the extension phase of the PCR cycle, the 5′ nuclease activity of Taq polymerase degrades the bound probe, causing the reporter dye to separate from the quencher dye, generating a fluorescent signal.

REAL TIME PCR-BASED DETECTION OF SARS-CoV-2

The first step in the detection of SARS-CoV-2 is the conversion of viral RNA into cDNA. Afterwards, the target sequences unique for SARS-CoV-2 are specifically amplified with amplification monitored in real time through the use of fluorescently labelled probes: upon incorporation into the newly amplified DNA strands, the fluorophore (FAM™) is released and an increase in fluorescence signal can be observed.

Due to the intrinsic mutation rate of coronaviruses, it is possible that mutations in the target sequence occur and accumulate over time. This can lead to false-negative results with a PCR-based detection approach. **DIAGNOVITAL® SARS-CoV-2 Real-Time PCR Kit** addresses this issue by using 3 detection assays on 3 different target sequences to minimize the chance of false-negative results caused by an altered target sequence.

If samples are tested negative in one or more assays, additional complementary testing may be required. The original target sequences for SARS-CoV-2 are included as a non-infectious target positive control (TPC) to check the integrity of the detection assays.

Materials Provided

	Reagents	Quantity and Volume (25 tests)	Quantity and Volume (50 tests)	Quantity and Volume (100 tests)
1	50X VitaScript™ Reverse Transcriptase	1 × 83 µl	1 × 165 µl	1 × 330 µl
2	Diagnovital® 2X qPCR Mastermix E	1 × 420 µl	1 × 825 µl	1 × 1650 µl
3	Diagnovital® 2X qPCR Mastermix RdRP	1 × 420 µl	1 × 825 µl	1 × 1650 µl
4	Diagnovital® 2X qPCR Mastermix HEC	1 × 420 µl	1 × 825 µl	1 × 1650 µl
5	SARS-CoV-2 Target Positive Control (TPC)	1 × 45 µl	1 × 75 µl	1 × 150 µl
6	Nuclease-free dH_2O	1 × 1000 µl	1 × 1000 µl	1 × 1000 µl

Additional Materials Required

- RTA Viral RNA Isolation Kit (RTA Laboratories, Cat #09010100)
- BioRad CFX-96 IVD marked instrument with BioRad CFX Manager Software version 3.0 or QuantStudio™ 5 Dx (Applied Biosystems) with QuantStudio™ 5 Dx TD Software v 1.0
- BioRad CFX-96 IVD nuclease free 96 well plates: Hard-Shell Thin-Wall 96- Well Skirted PCR Plates (BIO-RAD, Cat#: HSP-9655)
- BioRad sealing tape: Microseal 'B' Adhesive Seals, optically clear (BIO-RAD, Cat#: MSB-1001)
- MicroAmp™ Optical 96-Well Reaction Plate with Barcode & Optical Caps (Thermo Fischer Scientific Cat# 403012)
- MicroAmp™ Optical Adhesive Film (Thermo Fischer Scientific Cat# 4311971)
- Adjustable pipettes & fitting filtered pipette tips
- Appropriate personal protective equipment & workspaces for working with potentially infectious samples
- Surface decontaminants such as DNAZap™ (Life Technologies), DNA Away™ (Fisher Scientific), RNAse Away™ (Fisher Scientific), 10% bleach (1:10 dilution of commercial 5.25-6.0% sodium hypochlorite)
- Nuclease-free tubes, strips, plates to prepare dilutions and master mixes, such as Eppendorf colorless 1.5 ml Microtubes, Cat. No. Z606340
- Suitable storage options for reagents and specimen (4°C, -20°C, -70°C)

Storage

- Store all components at -20°C and avoid repeated freeze and thaw cycles.
- Protect the 2X qPCR master mixes from light as prolonged exposure can diminish the performance of the fluorophores.
- If the kit components have been damaged during transport, contact RTA Laboratories. Do not use as performance may be compromised.
- Keep reagents separate from sample material to avoid contamination.
- Do not use reagents after the designated expiry date.

Sample Collection and Handling

- Samples are to be collected in UTM/VTM using Copan/BD swabs (Cat # 220220). Washes/aspirates/BALs can be collected into sterile, nonpyrogenic and DNase-/RNase-free Corning 15 mL centrifuge tubes (Sigma, Cat # CLS430766).
- Recommended storage conditions for swabs resuspended in UTM specimens are refrigerate at 4°C for 2 days; for longer storage times, store at -70°C.
- Residual specimens and extracted nucleic acids can be stored at -70°C for at least 8 weeks without any deterioration of the viral RNA.
- If frozen specimens are used, only thaw the number of specimen extracts that will be tested in a single day.

Do not freeze/thaw extracts more than once before testing as each freeze/ thaw cycle will decrease the RNA quality.

Do not use specimens if:

- they were not kept at 4°C (≤ 2 days) or frozen at -70°C or below.
- they are insufficiently labelled or lack documentation.
- they are not suitable for this purpose (see above for validated sample types).
- the specimen volume is insufficient (i.e., a minimum volume of 150 µl of sample is needed for testing).

Warnings

Biosafety

- For in vitro diagnostic use
- For Prescription Use Only (Rx only)
- This test has not been FDA cleared or approved; the test has been authorized by FDA under an Emergency Use Authorization (EUA) for use by laboratories certified under the Clinical Laboratory Improvement Amendments (CLIA) of 1988, 42 U.S.C. §263a, to perform high complexity tests.
- This test has been authorized only for the detection of nucleic acid from SARS-CoV-2, not for any other viruses or pathogens.
- This test is only authorized for the duration of the declaration that circumstances exist justifying the authorization of emergency use of in vitro diagnostic tests for detection and/or diagnosis of COVID-19 under Section 564(b)(1) of the Act, 21 U.S.C. § 360bbb-3(b)(1), unless the authorization is terminated or revoked sooner.
- Handle all specimens as if infectious using safe laboratory procedures. 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
- Wear appropriate personal protective equipment (e.g. gowns, powder-free gloves, eye protection) when working with clinical specimen.
- Specimen processing should be performed in a certified class II biological safety cabinet following biosafety level 2 or higher guidelines.
- Biosafety in Microbiological and Biomedical Laboratories 5th edition available at http://www.cdc.gov/biosafety/publications/.

- The use of **DIAGNOVITAL® SARS-CoV-2 Real-Time PCR Kit** is restricted to trained laboratory personnel only.
- Amplification technologies such as PCR are sensitive to accidental introduction of PCR product from previous amplifications reactions. Incorrect results could occur if either the clinical specimen or the real-time reagents used in the amplification step become contaminated by accidental introduction of amplification product (amplicon). Workflow in the laboratory should proceed in a unidirectional manner.
- Do not eat, drink, smoke, apply cosmetics or handle contact lenses in areas where reagents and human specimens are handled.
- Dispose of unused kit reagents and human specimens according to local, state, and federal regulations.
- Do not use reagents past their expiration date.

Sample Preparation

- The performance of RT-PCR assays strongly depends on the amount and quality of sample template RNA. It is strongly recommended to qualify and validate RNA extraction procedures for recovery and purity before testing specimens.
- The validated nucleic acid extraction system used in combination with **DIAGNOVITAL® SARS-CoV-2 Real-Time PCR Kit** is the RTA Viral RNA Isolation Kit (RTA Laboratories, Cat #09010100). Refer to the instructions for use for the extraction procedure.
- Store and keep residual specimens and extracted nucleic acids at -70°C.If necessary, extracted nucleic acid can be stored at -80°C for a maximum of 8 months.
- Only thaw the number of specimen extracts that will be tested in a single day.
- Do not freeze/thaw extracts more than once before testing as each freeze/thaw cycle will decrease the RNA quality.

Reaction Setup

- Make sure that all necessary equipment and devices are suitable, calibrated and functional before starting the experiments.

- Decontaminate equipment and workspace and prepare everything needed for the following experiment to keep the workflow short and repeatable.

- Switch on the PCR detection system and program it to avoid delays after setting up the reactions.

- Thaw all components of **DIAGNOVITAL® SARS-CoV-2 Real-Time PCR Kit** on ice and mix gently but thoroughly to ensure even distribution of components. Centrifuge the tubes at 3000 rpm for 5 seconds to collect liquid at the bottom of the tubes.

1. For each of the targets (i.e., E, RdRP and RNase P), prepare enough master mix for all planned reactions (n) according to your sample size. Each target is amplified separately. Therefore, for each master mix, 1 negative control must be included. Additionally, for both SARS-CoV-2 targets (E and RdRP), the TPC positive control must be included. It is recommended to prepare master mix for 2 additional reactions to compensate for pipetting inaccuracies. Find the total volume by multiplying the volume per reaction (see table below) by n+2 ("n" being the number of total samples, including controls). Prepare 3 master mixes, i.e., one for each of the following targets: E, RdRP, and RNase P. When calculating the pipetting volumes for each master mix, please use the volume table below. We recommend calculating 2 additional reactions for pipetting errors. For example, for 10 samples + 1 positive control + 1 negative control, the volumes should be multiplied by 14 (12+2). Then prepare the master mix for each target - E, RdRP and RNase P. Aliquot 16 µl of each master mix into separate wells and add 4 µl of sample /negative control onto the master mix. Each patient specimen will have 3 separate wells. The TPC should be added only to the E and RdRP master mixes.

   The pipetting amounts for a single reaction, as given below:

Component	**Volume**
50X VitaScript™ Reverse Transcriptase	1 µl

Diagnovital® 2X qPCR Mastermix (E / RdRP / HEC)	15 µl
isolated sample RNA / TPC / NTC	4µl

2. Distribute 16 µL of the master mix to each well of your PCR plate.

3. Transfer the Master mix Plate to a separate workspace to add the sample material. Preparing reagents and final reaction setup in separate workspaces helps to avoid contamination of equipment and reagents with sample material.

4. Prepare negative reactions first and seal them before handling positive samples. It is recommended to only bring potentially positive sample material and the included target positive control to the workspace once the NTC is prepared and sealed.

5. Add 4 µl Sample or Control to the respective sample and control wells and seal the plate. Keep reactions on ice until transferring them to the PCR device.

   An example setup is given in (**Fig 1**).

Figure 1: Example pipetting scheme for the distribution of master mixes with the individual assay mixes

	1	2	3	4	5	6	7	8	9	10	11	12
A	E	E	E	E	E	E	E	E	E	E	E	E
B	RdRP	RdRP	RdRP	RdRP	RdRP	RdRP	RdRP	RdRP	RdRP	RdRP	RdRP	RdRP
C	HEC	HEC	HEC	HEC	HEC	HEC	HEC	HEC	HEC	HEC	HEC	HEC
D												
E												
F												
G												
H												

An example pipetting scheme for the addition of samples is given in (**Fig 2**).

Figure 2: Example pipetting scheme for the addition of samples. The bottom half of the plate could be used for replicates with an identical setup

	1	2	3	4	5	6	7	8	9	10	11	12
A	NTC	S1	S2	S3	S4	S5	S6	S7	S8	S9	S10	TPC
B	NTC	S1	S2	S3	S4	S5	S6	S7	S8	S9	S10	TPC
C	NTC	S1	S2	S3	S4	S5	S6	S7	S8	S9	S10	TPC
D												

E												
F												
G												
H												

Setting up RT-PCR Program:

1. Switch on the PCR detection system BioRad CFX-96 with BioRad CFX Manager Software version 3.0 or QuantStudio™ 5 Dx with QuantStudio™ 5 Dx TD Software vl.0, and create the following thermal protocol:

Step	**Cycles**	**Temperature**	**Duration**
Reverse Transcription	1	45°C	20 minutes
Initial Denaturation	1	95°C	10 minutes
Amplification	45	95°C	15 seconds
		58°C*	45 seconds

*Enable Data Collection for **FAM™**.

2. Transfer the RT-PCR plate to the PCR device, then cycle according to the program described above. The instrument and software only use default settings for running and analyzing the samples; no additional programming or adjustments (beyond the basic reaction program above) are made by the end user.

Data Collection and Analysis

By the end of the thermal protocol the data is collected automatically by the integrated software CFX Manager Software version 3.0 of BioRad CFX-96 IVD or QuantStudio™ 5 Dx TD Software v 1.0 of QuantStudio™ 5 Dx.

Once the run is finished, do not open the reaction tubes to avoid contamination, and discard according to local guidelines and regulations. Do not autoclave as this may contaminate laboratory equipment with amplicons.

Control Materials to be Used with DIAGNOVITAL® SARS-CoV-2 Real-Time PCR Kit

1. A "no template" (negative) control (NTC) is included in each run and taken through the full sample processing procedure starting with extraction. It is needed to monitor for potential contamination of reagents or samples with nucleic acid containing the target sequence and it should be added once for each master mix. Since there are 3 targets separately amplified in each run (E, RdRP and RNase P) the NTC should be added 3 times in each run (once for each master mix). The NTC consists of molecular grade dH20.

2. A positive template control is needed to control functionality of all RT-PCR enzymes and reagents in the test kit. The positive control consists of synthetic RNA templates for E and RdRP genes, and it should be included for the E and RdRP master mixes. - in other words, twice in a run. The positive control only controls the RT-PCR reaction of the E and the RdRP genes but not the extraction. The Ct value for the TPC must be ≤35 cycles

3. An internal control is needed to ensure correct processing of each sample and to monitor for potential inhibitors that may be present in the sample; and it is used with each sample. The internal control consists of the endogenous human RNase P gene, which is extracted together with the viral RNA from each patient specimen and is run as a separate PCR reaction. The endogenous internal control is amplified with the

RNase P Mix, which contains the forward and reverse primers and a FAM-labeled probe specific for the human RNase P gene.

Interpretation of the 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. For both controls and patient specimens, the threshold for RNase P to be called positive is a Ct of ≤35 For the SARS-CoV-2 targets E and RdRP to be called positive, the cutoff is a Ct of ≤35.

Diagnovital SARS-CoV-2 Test Controls: Positive, Negative, and Internal:

If any control does not perform as described above, the run is considered invalid and all specimens must be repeated starting from the extraction step after a root cause is identified and eliminated.

- NTC: dH20 controls (NTC) taken through extraction and the PCR run for each master mix must not give a positive Ct for any assay. If they do, sample results cannot be reported as the reaction was contaminated with sample RNAIDNA. Decontaminate equipment and workspace and repeat all sample reactions starting from the extraction step.

- Internal Control (RP): All reactions containing samples must give positive Ct values for the internal RNase P (RP) target. The Ct values should be ≤35 cycles. Failure to amplify the RNase P within 35 Ct values indicates inadequate RNA extraction or loss of RNA isolate due to RNase contamination. A sample result without RNase P amplification cannot be interpreted and needs to be repeated starting from the extraction step.

- TPC: Both E and RdRP targets must be observed with Ct values of ≤35 cycles for the TPC control to be valid. If the Ct value is >35 or not all, SARS-CoV-2- targets are tested positive and PCR was compromised. Check the reaction setup and PCR device settings and repeat the reactions with residual nucleic acids. If any of the targets in the positive control is negative, the run is invalid.

  *Avoid repeated freeze thaw cycles, aliquot the components of the kit if the contents of the kit are used for more than 3 times.

Table 1: Expected Performance of DIAGNOVITAL SARS-CoV-2 Test Controls

Control Type	External Control Name	E	RdRP	RP (IC)	Expected Ct values
Positive Control	TPC	+	+	-	≤35 Ct for E, RdRP targets
Negative Control	NTC	-	-	-	No detectable Ct
Internal Control	RNase P (IC)	-	-	+	≤35 Ct for RP (IC)

If any of the above controls does not exhibit the expected performance as described, the assay may have been improperly set up and/or executed improperly, or reagent or equipment malfunction could have occurred. Invalidate the run, perform a root cause analysis, and re-test after the root cause has been eliminated.

Examination and Interpretation of Patient Specimen Results:

- For a sample to be considered positive for SARS-CoV-2, both targets (E and RdRP) AND the RNase P target must give positive Ct values of ≤35. If the RNase P target fails to amplify within ≤35 Ct, but both SARS-2 specific targets are amplified, the sample is considered valid positive.

- For a sample to be considered negative for SARS-CoV-2, none of the two SARS-CoV-2 targets (E and RdRP) must give positive Ct values AND the RNase P must give a positive Ct value (≤35 cycles) to ensure that sample material of suitable quality was present.
- A sample result is invalid if the detection of RNase P in the sample fails and the sample also fails to show amplification of both SARS-CoV-2 targets (E and RdRP) within ≤35 Ct. Invalid results cannot be interpreted. These samples should be repeated from the extraction step. If both SARS-CoV-2 targets are detected in the sample in the absence of the RNase P target, the sample is valid positive (see above).

Table 2: Result Interpretation of DIAGNOVITAL SARS-CoV-2 (Samples)

E	RdRP	RNase P (IC)	Interpretation	Report	Actions
-	-	+	Only the target sequence for the Internal Control was amplified. The sample is considered negative for SARS-CoV-2.	Negative	Report results
+	+	+/-	Both target sequences for SARS-CoV- 2, and the Internal Control were amplified. The sample is considered positive for SARS-CoV-2.	Positive	Report results
-	+	+	SARS-CoV-2 specific RdRP target sequence is detected, and sample is considered positive for SARS-CoV-2. A positive SARS-CoV-2 RdRP result and a negative Sarbecovirus (E-gene) result is suggestive of low concentration of viral RNA, or mutation in the target region of Sarbeco sequence.	Positive	Report results
+	-	+	A negative RdRP result and a positive E result is suggestive of low concentration of viral RNA, a mutation in the SARS-CoV-2 specific RdRP target sequence, or an infection with other Sarbecovirus (e.g., SARS-CoV-2 or some other Sarbecovirus previously unknown to infect humans).	Presumptive positive	Repeat test once starting with the extraction step. If sample is repeat reactive with identical result, additional confirmative testing consistent with public health guidelines must be conducted
-	-	-	PCR was inhibited; results are invalid.	Invalid	Sample is repeated once starting with the extraction step. If the result is again invalid, it is reported to the sender as invalid and collection of a new sample is recommended.

Limitations

- For reliable results, it is essential to adhere to the guidelines given in this manual. Changes in reaction setup or cycling protocol may lead to failed experiments and is not allowed under the Emergency Use Authorization.
- Spontaneous mutations within the target sequence may result in failure to detect the target sequence. While this risk is mitigated in the test's design, if failure to detect the target is expected it is recommended to test the specimen with a different test that detects different target sequences from the SARS-CoV-2 genome.
- Based on the in silico analysis, SARS-CoV and other SARS-like coronaviruses in the same subgenus (Sarbecovirus) as SARS-CoV may cross react with the E and RdRP primer sets of the DIAGNOVITAL SARS-CoV-2 Real-Time PCR Kit. SARS-CoV is not known to be currently circulating in the human population, therefore it is highly unlikely to be present in patient specimens.
- Results must always be interpreted in consideration of all other data gathered from a sample. Interpretation must be performed by personnel trained and experienced with this kind of experiment.
- Users should be trained to perform this assay and competency should be documented.
- Negative results do not preclude SARS-CoV-2 infection and should not be used as the sole basis for treatment or other patient management decisions.
- 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.
- This test cannot rule out diseases caused by other bacterial or viral pathogens.

Conditions of Authorization for the Laboratory

The DIAGNOVITAL SARS-CoV-2 Real-Time PCR Kit Letter of Authorization, along with the authorized Fact Sheet for Healthcare Providers, the authorized Fact Sheet for Patients, and authorized labeling are available on the FDA website: https://www.fda.gov/medical-devices/emergency-situations-medical-devices/emergency-use-authorizations#covid19ivd.

However, to assist clinical laboratories using the DIAGNOVITAL SARS-CoV-2 Real-Time PCR Kit the relevant Conditions of Authorization are listed below:

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

B. Authorized laboratories using your product will use your product as outlined in the Instructions for Use. Deviations from the authorized procedures, including the authorized instruments, authorized extraction methods, authorized clinical specimen types, authorized control materials, authorized other ancillary reagents and authorized materials required to use your product are not permitted.

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

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

E. Authorized laboratories will collect information on the performance of your product and report to DMD/OHT7-OIR/OPEQ/CDRH (via email: CDRH-EUA-Reporting@fda.hhs.gov) and RTA Laboratories (via email: rta@rtalabs.com.tr any suspected occurrence of false positive or false negative results and significant deviations from the established performance characteristics of your product of which they become aware.

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

G. You, authorized distributors, and authorized laboratories using your product will ensure that any records associated with this EUA are maintained until otherwise notified by FDA. Such records will be made available to FDA for inspection upon request.

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

Performance Characteristics

Analytical Sensitivity (Limit of Detection)

The LoD study established the lowest concentration of SARS-CoV-2 (genome copies(cp)/mL) that can be detected by the **DIAGNOVITAL® SARS-CoV-2** test at least 95% of the time. The preliminary LoD was established by testing 10-fold dilutions of a positive patient NP swab sample extracted using the RTA Viral RNA Isolation Kit and quantitated by Droplet Digital PCR (QX200 Droplet Digital PCR System, BioRad). Three extraction replicates were tested per dilution by the **DIAGNOVITAL® SARS-CoV-2 Kit** for the initial LoD range finding study.

The concentration range between 100 cp/ml and 10 cp/ml was further broken down to confirm the LoD by testing 24 individual extraction replicates of 100 cp/mL, 30 cp/mL, and 10 cp/mL. The nasopharyngeal samples were prepared by spiking the quantified SARS-CoV-2 RNA into VTM. Using RTA Viral RNA Isolation Kit, 150 µl of VTM was extracted. The extracted RNA was tested by BIO-RAD CFX96-IVD Real -Time PCR Detection System and QuantStudio™ 5 Dx.

The final LoD for the **DIAGNOVITAL® SARS-CoV-2** is calculated to be 38 copies/mL by probit analysis, which is the lowest concentration at which 95% of replicates were detected (i.e., 24/24 for the E gene and 24/24 for the RdRP gene). This LoD is applicable to both the CFX-96 and QuantStudio 5 Dx platforms.

Table 3: LoD Confirmatory Study Results Using the CFX-96 Platform

SARS-CoV-2 - Confirmatory LoD (BIO-RAD CFX96-IVD)										
Target Level	Valid results	E-Gene			RdRP			RNase P		
		n	Mean Ct	Detection Rate	n	Mean Ct	Detection Rate	n	Mean Ct	Detection Rate
100 cp/mL	24	24	33.85	100%	24	33.92	100%	24	33.40	100%
30 cp/mL	24	18	33.91	75%	20	34.64	83%	24	34.04	100%
10 cp/mL	24	3	35.81	12.5%	6	35.73	25%	10	35.19	42%

Table 4: LoD Confirmatory Study Results Using the QuantStudio 5 Dx Platform

SARS-CoV-2 - Confirmatory LoD (QuantStudio™ 5 Dx)										
Target Level	Valid results	E-Gene			RdRP			RNase P		
		n	Mean Ct	Detection Rate	n	Mean Ct	Detection Rate	n	Mean Ct	Detection Rate
100 cp/mL	24	24	33.52	100%	24	33.58	100%	24	33.51	100%
30 cp/mL	24	19	34.01	79%	20	34.23	83%	20	34.44	83%
10 cp/mL	24	4	35.20	17%	8	35.37	33%	17	34.84	70%

Inclusivity (Analytical Reactivity):

Primer/probe inclusivity was evaluated by BLAST analysis against 4617 publicly available SARS-CoV-2 sequences in the Beta-coronavirus database on May 29, 2020. The Primers E_Sarbeco_F1, E_ Sarbeco_R2, RdRP_SARSr-F2, RdRP_ SARSr-R1 and probes E_Sarbeco_P1 and RdRP_SARSr-P2 exhibited 100% homology with all the available sequences.

The Primers and Probes from WHO were used during the studies. Please refer to the following link: https ://doi.org/10.2807 11560-79 l 7.ES.2020.25.3 .2000045).

Cross-reactivity (Analytical Specificity):

Wet Testing

In this study, the specificity of the **DIAGNOVITAL® SARS-CoV-2** Kit was evaluated by testing the organisms listed in the table below. In the absence of SARS-CoV-2, 20 reference organisms were tested. The potential cross-reactive organisms were tested at concentrations between $1x10^3$ – $1x10^5$ copies/ml. Cross-reactivity with other coronaviruses cell culture supernatants containing human coronaviruses (HCoV)-229E, -NL63, -OC43, and -HKU1, as well as MERS-CoV, were tested against the RdRP, E, and RNase P primers/probes of the **DIAGNOVITAL® SARS-CoV-2** Kit.

For the non-cultivable HCoV-HKU1, a supernatant from human airway culture was used. Virus RNA concentration in all samples was determined by specific real-time RT-PCRs and in-vitro transcribed RNA standards designed for absolute viral load quantification.

Samples were extracted by RTA Viral RNA Isolation Kit according to the RTA Viral RNA Isolation Kit Handbook. Starting sample volumes were 150 µl and elution volumes were 50 µl. PCR reactions were setup according to the recommendations in the **DIAGNOVITAL® SARS-CoV-2** Kit Handbook. BIO-RAD CFX96-IVD Real-Time PCR Detection System was used for amplification, detection, and analysis. Specifically, the RdRP and E assays of the **DIAGNOVITAL® SARS-CoV-2** Kit did not show any cross-reactivity with potential cross-reactive viruses at the tested concentration for the organisms listed in the table below.

Table 5: Potential Cross-Reactive Markers Tested in the Study

Sample	Source	Sample ID	Replicates Detected/Total	Result
Human Adenovirus	NIBSC	16/324	0/3	Negative
Parainfluenza virus	ATCC	VR-93	0/3	Negative
Influenza A	ATCC	VR-95	0/3	Negative
Influenza A H5N1	ATCC	VR-1609	0/3	Negative
Influenza A H1N1	ATCC	VR-1672	0/3	Negative
Influenza A H3N2	ATCC	VR-822	0/3	Negative
Influenza A H7N7	ATCC	VR-1641	0/3	Negative
Influenza B	ATCC	VR-101	0/3	Negative
Parainfluenza 1	ATCC	VR-94	0/3	Negative
Parainfluenza 2	ATCC	VR-92	0/3	Negative
Parainfluenza 3	ATCC	VR-93	0/3	Negative
Parainfluenza 4	ATCC	VR-579	0/3	Negative
Human Metapneumovirus (hMPV)	ATCC	VR-3250SD	0/3	Negative
Human Enterovirus V71	ATCC	VR-1432	0/3	Negative
Human respiratory syncytial virus	ATCC	VR-154	0/3	Negative
Human Coronavirus NL63	ATCC	VR-3263SD	0/3	Negative
Human Coronavirus HKU1	ATCC	VR-3262SD	0/3	Negative
Human Coronavirus 229E	ATCC	VR-740	0/3	Negative
Beta-coronavirus 1 OC43	ATCC	VR-1558D	0/3	Negative
MERS Coronavirus	ATCC	VR-3248SD	0/3	Negative

TPC			0/3	Positive
NTC			0/3	Negative

In Silico Analysis:

BLAST analysis showed no homology with primers and probes of the **DIAGNOVITAL® SARS-CoV-2 Real-Time PCR Kit** for the organisms listed in the table below.

The in-silico analysis for possible cross-reactions with all the organisms listed in Table 6 was conducted by mapping primers in **DIAGNOVITAL® SARS-CoV-2 Real Time PCR Kit** individually to the sequences downloaded from NCBI database. No potential cross reactivity was observed with analyzed pathogens.

Table 6: In-Silico Analysis for Primers and Probes

Pathogen	Strain	GenBank Accession #	% Homology Forward E Primer	% Homology Reverse E Primer	% Homology E Probe	% Homology Forward RdRP Primer	% Homology Reverse RdRP Primer	% Homology RdRP Probe
SARS-CoV-2	Severe acute respiratory syndrome coronavirus 2 isolate Wuhan-Hu-1, complete genome	NC_045512.2	100%	100%	100%	100%	100%	100%
Human coronavirus 229E	Human coronavirus 229E strain 229E/human/USA/932-72/1993, complete genome	KF514432.1	<50%	<50%	<50%	<50%	<50%	<50%
	Human coronavirus 229E strain 229E/human/USA/933-40/1993, complete genome	KF514433.1	<50%	<50%	<50%	<50%	<50%	<50%
Human coronavirus OC43	Human coronavirus OC43 strain OC43/human/USA/971-5/1997, complete genome	KF530099.1	<50%	<50%	<50%	<50%	<50%	<50%
	Human coronavirus OC43 isolate LRTI_238, complete genome	KX344031.1	<50%	<50%	<50%	<50%	<50%	<50%
Human coronavirus HKU1	Human coronavirus HKU1 strain HKU1/human/USA/HKU1-18/2010, complete genome	KF430201.1	<50%	<50%	<50%	<50%	<50%	<50%
	Human coronavirus HKU1 isolate SI17244, complete genome	MH940245.1	<50%	<50%	<50%	<50%	<50%	<50%
Human coronavirus NL63	Human coronavirus NL63 strain NL63/human/USA/905-25/1990, complete genome	KF530113.1	<50%	<50%	<50%	<50%	<50%	<50%
	Human coronavirus NL63 strain NL63/human/USA/891-4/1989, complete genome	KF530114.1	<50%	<50%	<50%	<50%	<50%	<50%
SARS-coronavirus	SARS coronavirus CUHK-AG01, complete genome	AY345986.1	100%	100%	100%	100%	100%	52%
	SARS coronavirus A022, complete genome	AY686863.1	100%	100%	100%	100%	100%	52%
MERS-Coronavirus	Middle East respiratory syndrome-related coronavirus strain HCoV-EMC, complete genome	MH013216.1	<50%	<50%	<50%	<50%	78%	<50%
Adenovirus	Human adenovirus type 1, complete genome	AC_000017.1	<50%	<50%	<50%	<50%	<50%	<50%
Human Metapneumovirus (hMPV)	Human metapneumovirus strain HMPV/Homo sapiens/PER/FPP00726/2011/A, complete genome	KJ627437.1	<50%	<50%	<50%	<50%	<50%	<50%
Parainfluenza 1	Human parainfluenza virus 1 isolate NM001, complete genome	KX639498.1	<50%	<50%	<50%	<50%	<50%	<50%
Parainfluenza 2	Human parainfluenza virus 2 isolate VIROAF10, complete genome	KM190939.1	<50%	<50%	<50%	<50%	<50%	<50%
Parainfluenza 3	Human parainfluenza virus 3 strain HPIV3/AUS/3/2007, complete genome	KF530243.1	<50%	<50%	<50%	<50%	<50%	<50%
Parainfluenza 4	Human parainfluenza virus 4a isolate HPIV4_DK(459), complete genome	KF483663.1	<50%	<50%	<50%	<50%	<50%	<50%
Influenza A	Influenza A virus (A/New York/PV305/2017(H1N1))	MH798556.1	<50%	<50%	<50%	<50%	<50%	<50%

Pathogen	Strain	GenBank Accession #	% Homology Forward E Primer	% Homology Reverse E Primer	% Homology E Probe	% Homology Forward RdRP Primer	% Homology Reverse RdRP Primer	% Homology RdRP Probe
	segment 2 polymerase PB1 (PB1) gene, complete cds; and nonfunctional PB1-F2 protein (PB1-F2) gene, complete sequence							
Influenza B	Influenza B virus (B/Nicaragua/8689_13/2017) segment 2 polymerase PB2 (PB2) gene, complete cds	MK969560.1	<50%	<50%	<50%	<50%	<50%	<50%
Enterovirus	Human enterovirus 68 isolate EV68_NL_201013421 VP1 protein gene, partial cds	JF896312.1	<50%	<50%	<50%	<50%	<50%	<50%
Respiratory syncytial virus	Respiratory syncytial virus strain B/WI/629-Q0190/10, complete genome	JN032120.1	<50%	<50%	<50%	<50%	<50%	<50%
Rhinovirus	Human rhinovirus 14, complete genome	NC_001490.1	<50%	<50%	<50%	<50%	<50%	<50%
Chlamydia pneumoniae	Chlamydia pneumoniae genome assembly PB2, chromosome : 1	NZ_LN847241.1	<50%	77%	50%	<50%	<50%	52%
Haemophilus influenzae	Haemophilus influenzae PittGG, complete genome	CP000672.1	<50%	59%	<50%	<50%	<50%	<50%
Legionella pneumophila	Legionella pneumophila strain Philadelphia_1_CDC, complete genome	CP015928.1	<50%	54%	50%	59%	50%	56%
Mycobacterium tuberculosis	Mycobacterium tuberculosis DNA, complete genome, strain: HN-506	AP018036.1	<50%	63%	50%	59%	<50%	<50%
Streptococcus pneumoniae	Streptococcus pneumoniae strain D39V chromosome, complete genome	CP027540.1	<50%	<50%	54%	<50%	50%	56%
Streptococcus pyogenes	Streptococcus pyogenes MGAS8232, complete genome	AE009949.1	53%	59%	<50%	<50%	50%	64%
Bordetella pertussis	Bordetella pertussis strain B3921, complete genome	CP011448.1	<50%	63%	<50%	<50%	<50%	52%
Mycoplasma pneumoniae	Mycoplasma pneumoniae strain 14-637 chromosome, complete genome	CP039772.1	<50%	54%	<50%	<50%	<50%	<50%
Pneumocystis jirovecii	Pneumocystis jirovecii isolate SW7_full mitochondrion, complete genome	MH010446.1	<50%	<50%	<50%	<50%	<50%	<50%
Candida albicans	Candida albicans strain L757 mitochondrion, complete genome	NC_018046.1	<50%	<50%	<50%	<50%	<50%	<50%
Pseudomonas aeruginosa	Pseudomonas aeruginosa UCBPP-PA14, complete genome	CP000438.1	50%	77%	<50%	59%	<50%	<50%
Staphylococcus epidermidis	Staphylococcus epidermidis strain SP3 16S ribosomal RNA gene, partial sequence	KY750253.1	<50%	<50%	<50%	<50%	<50%	<50%
Streptococcus salivarius	Streptococcus salivarius strain LAB813 chromosome, complete genome	CP040804.1	65%	54%	<50%	59%	50%	<50%

Endogenous Interference Substances Studies:

Potential endogenous and exogenous interfering substances that could interfere with the performance of the **DIAGNOVITAL® SARS-CoV-2 Real-Time PCR Kit** were evaluated. The substances were tested at the concentrations indicated in the table below, both with and without spiked positive (quantified) patient RNA. UTM was spiked with the substances indicated below. In the sampled matrixes, RNA was extracted using the RTA RNA Viral Isolation Kit. The extracted RNA was tested in triplicate using the **DIAGNOVITAL® SARS-CoV-2 Real-Time PCR Kit** on the Bio-Rad CFX-96 instrument.

In the table below, the results show that the PCR was not affected by the potential endogenous interfering substances.

Table 7: Interference Study

Potential Interfering Substance	Conc.	Positive Samples		Negative Samples
		Viral Strain Level	Results	Results
Mucin: bovine submaxillary gland, type I-S	2.5 mg/ml	2.5X LoD	3/3	0/3

Blood (human)	2.5% v/v	2.5X LoD	3/3	0/3
Afrin Original nasal spray	15% v/v	2.5X LoD	3/3	0/3
Basic Care allergy relief nasal spray (Gluococorticoid)	5% v/v	2.5X LoD	3/3	0/3
NeilMed Nasal gel	1.25%	2.5X LoD	3/3	0/3
GoodSense All Day Allergy, Cetirizine HCl Tablets 10 mg	1mg/mL	2.5X LoD	3/3	0/3
Cepacol Sore Throat (benzocaine/menthol lozenges)	5 mg/mL	2.5X LoD	3/3	0/3
Zanamivir	3.3 mg/mL	2.5X LoD	3/3	0/3
Tamiflu	2.2 µg/mL	2.5X LoD	3/3	0/3
Mupirocin ointment	5mg/mL	2.5X LoD	3/3	0/3
Tobramycin	4ug/mL	2.5X LoD	3/3	0/3

Clinical Evaluation

Specificity

Clinical specimens that were characterized as negative for SARS-CoV-2 by the Roche Cobas SARS-CoV-2 Test were used in clinical evaluation studies. They were collected from patients with signs and symptoms of an upper respiratory infection and by qualified personnel according to the package insert of the collection device of the Copan swabs and Copan UTM. Specimens were handled as described in the package insert of the collection device and were stored frozen until use. Samples were tested to be negative also for common upper respiratory tract infections. The following samples were obtained: 30 oropharyngeal, 10 nasal, 30 nasopharyngeal swabs, and 30 bronchoalveolar lavage (BAL) specimens. Aliquots of the samples were extracted and tested in a blinded manner together with the positive spiked samples described below and according to the **DIAGNOVITAL® SARS-CoV-2 Real-Time PCR Kit** Instructions for Use using the BIO-RAD CFX96-IVD Real-Time PCR Detection System and QuantStudio™ 5 Dx for amplification, detection, and analysis.

Table 8: Diagnostic Specificity Study

		BIO-RAD CFX96-IVD			QuantStudio™ 5 Dx		
Nasopharyngeal Swabs							
	Sample ID	**Target Gene E Cq**	**Target Gene RdRP Cq**	**RNase P**	**Target Gene E Cq**	**Target Gene RdRP Cq**	**RNase P**
1	**SARS-CoV-2_1**	N/A	N/A	25.14	N/A	N/A	25.08
2	**SARS-CoV-2_2**	N/A	N/A	25.48	N/A	N/A	25.89
3	**SARS-CoV-2_3**	N/A	N/A	25.60	N/A	N/A	25.93
4	**SARS-CoV-2_4**	N/A	N/A	25.69	N/A	N/A	26.75
5	**SARS-CoV-2_5**	N/A	N/A	25.41	N/A	N/A	26.46
6	**SARS-CoV-2_6**	N/A	N/A	25.32	N/A	N/A	25.30
7	**SARS-CoV-2_7**	N/A	N/A	25.66	N/A	N/A	26.44
8	**SARS-CoV-2_8**	N/A	N/A	25.60	N/A	N/A	25.59
9	**SARS-CoV-2_9**	N/A	N/A	25.34	N/A	N/A	26.62
10	**SARS-CoV-2_10**	N/A	N/A	25.20	N/A	N/A	25.37
11	**SARS-CoV-2_11**	N/A	N/A	25.78	N/A	N/A	26.59
12	**SARS-CoV-2_12**	N/A	N/A	25.59	N/A	N/A	25.57
13	**SARS-CoV-2_13**	N/A	N/A	25.39	N/A	N/A	26.15
14	**SARS-CoV-2_14**	N/A	N/A	25.48	N/A	N/A	25.49
15	**SARS-CoV-2_15**	N/A	N/A	25.37	N/A	N/A	25.00
16	**SARS-CoV-2_16**	N/A	N/A	25.35	N/A	N/A	25.30
17	**SARS-CoV-2_17**	N/A	N/A	25.25	N/A	N/A	25.04
18	**SARS-CoV-2_18**	N/A	N/A	25.29	N/A	N/A	26.67
19	**SARS-CoV-2_19**	N/A	N/A	25.55	N/A	N/A	25.47
20	**SARS-CoV-2_20**	N/A	N/A	25.35	N/A	N/A	25.47
21	**SARS-CoV-2_21**	N/A	N/A	25.37	N/A	N/A	25.35
22	**SARS-CoV-2_22**	N/A	N/A	25.21	N/A	N/A	26.01
23	**SARS-CoV-2_23**	N/A	N/A	25.43	N/A	N/A	26.41

24	**SARS-CoV-2_24**	N/A	N/A	25.45	N/A	N/A	26.99
25	**SARS-CoV-2_25**	N/A	N/A	25.50	N/A	N/A	25.70
26	**SARS-CoV-2_26**	N/A	N/A	25.44	N/A	N/A	26.43
27	**SARS-CoV-2_27**	N/A	N/A	25.42	N/A	N/A	26.70
28	**SARS-CoV-2_28**	N/A	N/A	25.47	N/A	N/A	26.13
29	**SARS-CoV-2_29**	N/A	N/A	25.57	N/A	N/A	27.00
30	**SARS-CoV-2_30**	N/A	N/A	25.48	N/A	N/A	25.86
Mean Ct				25.40			26.08
	TPC	19.58	20.24	N/A	20.23	21.06	N/A
	NTC	N/A	N/A	N/A	N/A	N/A	N/A
		BIO-RAD CFX96-IVD			**QuantStudio™ 5 Dx**		
Oropharyngeal Swabs							
	Sample ID	**Target Gene E Cq**	**Target Gene RdRP Cq**	**RNase P**	**Target Gene E Cq**	**Target Gene RdRP Cq**	**RNase P**
31	**SARS-CoV-2_31**	N/A	N/A	26.03	N/A	N/A	25.89
32	**SARS-CoV-2_32**	N/A	N/A	26.05	N/A	N/A	25.70
33	**SARS-CoV-2_33**	N/A	N/A	26.02	N/A	N/A	26.17
34	**SARS-CoV-2_34**	N/A	N/A	26.07	N/A	N/A	26.79
35	**SARS-CoV-2_35**	N/A	N/A	26.02	N/A	N/A	26.84
36	**SARS-CoV-2_36**	N/A	N/A	25.77	N/A	N/A	26.50
37	**SARS-CoV-2_37**	N/A	N/A	26.07	N/A	N/A	26.71
38	**SARS-CoV-2_38**	N/A	N/A	25.97	N/A	N/A	25.58
39	**SARS-CoV-2_39**	N/A	N/A	25.91	N/A	N/A	26.46
40	**SARS-CoV-2_40**	N/A	N/A	26.07	N/A	N/A	25.05
41	**SARS-CoV-2_41**	N/A	N/A	25.87	N/A	N/A	26.49
42	**SARS-CoV-2_42**	N/A	N/A	25.96	N/A	N/A	25.67
43	**SARS-CoV-2_43**	N/A	N/A	25.92	N/A	N/A	26.40
44	**SARS-CoV-2_44**	N/A	N/A	25.96	N/A	N/A	25.99
45	**SARS-CoV-2_45**	N/A	N/A	26.05	N/A	N/A	25.89
46	**SARS-CoV-2_46**	N/A	N/A	26.04	N/A	N/A	25.62
47	**SARS-CoV-2_47**	N/A	N/A	25.78	N/A	N/A	25.81
48	**SARS-CoV-2_48**	N/A	N/A	25.91	N/A	N/A	25.81
49	**SARS-CoV-2_49**	N/A	N/A	26.01	N/A	N/A	26.25
50	**SARS-CoV-2_50**	N/A	N/A	25.69	N/A	N/A	26.08
51	**SARS-CoV-2_51**	N/A	N/A	26.03	N/A	N/A	26.51
52	**SARS-CoV-2_52**	N/A	N/A	25.90	N/A	N/A	26.71
53	**SARS-CoV-2_53**	N/A	N/A	26.14	N/A	N/A	26.73
54	**SARS-CoV-2_54**	N/A	N/A	26.04	N/A	N/A	25.29
55	**SARS-CoV-2_55**	N/A	N/A	26.10	N/A	N/A	26.04
56	**SARS-CoV-2_56**	N/A	N/A	26.23	N/A	N/A	25.91
57	**SARS-CoV-2_57**	N/A	N/A	25.97	N/A	N/A	26.65
58	**SARS-CoV-2_58**	N/A	N/A	26.09	N/A	N/A	25.66
59	**SARS-CoV-2_59**	N/A	N/A	26.07	N/A	N/A	26.17
60	**SARS-CoV-2_60**	N/A	N/A	25.93	N/A	N/A	25.34
Mean Ct				26.00			26.09
	TPC	19.36	20.84	N/A	20.17	21.22	N/A
	NTC	N/A	N/A	N/A	N/A	N/A	N/A
		BIO-RAD CFX96-IVD			**QuantStudio™ 5 Dx**		
BAL							
61	**SARS-CoV-2_61**	N/A	N/A	26.24	N/A	N/A	25.54
62	**SARS-CoV-2_62**	N/A	N/A	26.31	N/A	N/A	25.85
63	**SARS-CoV-2_63**	N/A	N/A	26.18	N/A	N/A	25.47
64	**SARS-CoV-2_64**	N/A	N/A	26.27	N/A	N/A	25.44
65	**SARS-CoV-2_65**	N/A	N/A	26.36	N/A	N/A	26.98
66	**SARS-CoV-2_66**	N/A	N/A	26.12	N/A	N/A	26.87
67	**SARS-CoV-2_67**	N/A	N/A	26.07	N/A	N/A	26.74
68	**SARS-CoV-2_68**	N/A	N/A	26.11	N/A	N/A	26.60
69	**SARS-CoV-2_69**	N/A	N/A	26.22	N/A	N/A	25.51
70	**SARS-CoV-2_70**	N/A	N/A	26.10	N/A	N/A	25.34

71	**SARS-CoV-2_71**	N/A	N/A	26.12	N/A	N/A	25.04
72	**SARS-CoV-2_72**	N/A	N/A	26.06	N/A	N/A	26.16
73	**SARS-CoV-2_73**	N/A	N/A	26.04	N/A	N/A	26.42
74	**SARS-CoV-2_74**	N/A	N/A	26.17	N/A	N/A	25.45
75	**SARS-CoV-2_75**	N/A	N/A	26.23	N/A	N/A	26.42
76	**SARS-CoV-2_76**	N/A	N/A	26.15	N/A	N/A	25.08
77	**SARS-CoV-2_77**	N/A	N/A	26.30	N/A	N/A	25.50
78	**SARS-CoV-2_78**	N/A	N/A	25.98	N/A	N/A	26.62
79	**SARS-CoV-2_79**	N/A	N/A	26.33	N/A	N/A	26.03
80	**SARS-CoV-2_80**	N/A	N/A	26.08	N/A	N/A	25.51
81	**SARS-CoV-2_81**	N/A	N/A	26.19	N/A	N/A	26.05
82	**SARS-CoV-2_82**	N/A	N/A	26.12	N/A	N/A	26.29
83	**SARS-CoV-2_83**	N/A	N/A	26.34	N/A	N/A	25.05
84	**SARS-CoV-2_84**	N/A	N/A	26.24	N/A	N/A	26.89
85	**SARS-CoV-2_85**	N/A	N/A	26.26	N/A	N/A	25.18
86	**SARS-CoV-2_86**	N/A	N/A	26.13	N/A	N/A	26.47
87	**SARS-CoV-2_87**	N/A	N/A	26.17	N/A	N/A	25.64
88	**SARS-CoV-2_58**	N/A	N/A	26.07	N/A	N/A	25.95
99	**SARS-CoV-2_89**	N/A	N/A	26.38	N/A	N/A	25.91
90	**SARS-CoV-2_90**	N/A	N/A	26.27	N/A	N/A	26.99
Mean Ct				26.20			25.97
	TPC	20.03	21.04	N/A	20.75	20.97	N/A
	NTC	N/A	N/A	N/A	N/A	N/A	N/A
		BIO-RAD CFX96-IVD			**QuantStudio™ 5 Dx**		
Anterior Nasal Swab							
91	**SARS-CoV-2_91**	N/A	N/A	29.83	N/A	N/A	29.77
92	**SARS-CoV-2_92**	N/A	N/A	30.11	N/A	N/A	29.97
93	**SARS-CoV-2_93**	N/A	N/A	29.46	N/A	N/A	29.44
94	**SARS-CoV-2_94**	N/A	N/A	30.18	N/A	N/A	29.38
95	**SARS-CoV-2_95**	N/A	N/A	29.74	N/A	N/A	29.95
96	**SARS-CoV-2_96**	N/A	N/A	30.14	N/A	N/A	29.66
97	**SARS-CoV-2_97**	N/A	N/A	30.00	N/A	N/A	30.39
98	**SARS-CoV-2_98**	N/A	N/A	29.78	N/A	N/A	29.04
99	**SARS-CoV-2_99**	N/A	N/A	31.12	N/A	N/A	29.50
100	**SARS-CoV-2_100**	N/A	N/A	29.64	N/A	N/A	29.67
Mean Ct				30.0			29.68
	TPC	19.27	20.91	N/A	20.21	21.03	N/A
	NTC	N/A	N/A	N/A	N/A	N/A	N/A

The negative percent agreement was calculated based on the result obtained from the prior testing using the Roche Cobas SARS-CoV-2 Test. None of the 100 SARS-CoV-2 negative clinical specimens gave positive test result for SARS-CoV-2. Diagnostic specificity of **DIAGNOVITAL® SARS-CoV-2 Real-Time PCR Kit** is 100 % (see combined performance tables below).

Sensitivity

A second aliquot of the negative samples described above was tested in a contrived clinical study. Positive samples were generated by spiking the negative aliquots of the 30 NP swabs and 30 BALs with a quantified clinical specimen positive for SARS-CoV-2 (see LoD above) at 1.5X LOD (20 samples), 2X LOD (5 samples), and 80X LOD (5 samples) SARS-CoV-2 RNA. Positive specimens were tested in a blinded manner with the negative specimen from the specificity section above.

The positive percent agreement was calculated based on the agreement of the **DIAGNOVITAL® SARS-CoV-2 Real-Time PCR Kit** result with the expected spiked results in NP swabs and BALs. Results are shown below.

Table 9: Clinical Performance of the DIAGNOVITAL® SARS-CoV-2 Real-Time PCR Kit either against the expected results (spiking status for NP swab and BALs) or compared to the Roche Cobas SARS-CoV-2 Test (Nasal and OP swabs)

Sample Concentration	n	Target 1 (E Gene)		Target 2 (RdRP Gene)		RNase P	
		% positive (two-sided 95% CI)	Mean Ct	% positive (two-sided 95% CI)	Mean Ct	% positive	Mean Ct
NASOPHARYNGEAL-SWABS							
150 copies/mL 1.5X LoD	20	100 (80.6 – 99.9)	33.8	100 (80.6 – 99.9)	34.3	100 (80.6 – 99.9)	26.1
200 copies/mL 2X LoD	5	100 (65.8 – 99.9)	33.9	100 (65.8 – 99.9)	34.2	100 (65.8 – 99.9)	26.1
8000 copies/mL 80X LoD	5	100 (65.8 – 99.9)	25.0	100 (65.8 – 99.9)	25.2	100 (65.8 – 99.9)	25.5
Negative	30	0 (n/a)	N/A	0 (n/a)	N/A	100 (88.7 - 100)	25.4
Positive Percent Agreement: 30/30 = 100% (95% CI: 88.7% - 100%) Negative Percent Agreement: 10/10 = 100% (95% CI: 72.1% - 100%)							
NASAL-SWABS							
Negative	10	0 (n/a)	N/A	0 (n/a)	N/A	100 (72.1 – 99.9)	26.5
OROPHARYNGEAL SWABS							
Negative	30	0 (n/a)	N/A	0 (n/a)	N/A	100 (88.7 - 100)	25.7
Negative Percent Agreement (Nasal Swabs): 10/10 = 100% (95% CI: 72.1% - 100%) Negative Percent Agreement (Oropharyngeal Swabs): 10/10 = 100% (95% CI: 72.1% - 100%)							
BAL							
150 copies/mL 1.5X LoD	20	100 (80.6 – 99.9)	33.2	100 (80.6 – 99.9)	33.8	100 (80.6 – 99.9)	25.8
200 copies/mL 2X LoD	5	100 (65.8 – 99.9)	33.4	100 (65.8 – 99.9)	33.7	100 (65.8 – 99.9)	25.9
8000 copies/mL 80X LoD	5	100 (65.8 – 99.9)	24.4	100 (65.8 – 99.9)	24.7	100 (65.8 – 99.9)	26.0
Negative	30	0 (n/a)	N/A	0 (n/a)	N/A	100 (88.7 - 100)	26.2
Positive Percent Agreement: 30/30 = 100% (95% CI: 88.7% - 100%) Negative Percent Agreement: 30/30 = 100% (95% CI: 88.7% - 100%)							

Additional Clinical Evaluation

To evaluate the clinical performance of the **DIAGNOVITAL® SARS-CoV-2 Real-Time PCR Kit**, nasopharyngeal swabs that were previously tested by the PhoenixDx 2019-nCoV EUA authorized assay from Trax Management Services Inc. The PhoenixDx assay received US FDA authorization on April 20, 2020. A total of 60 nasopharyngeal swabs were tested retrospectively with the **DIAGNOVITAL® SARS-CoV-2 Real-Time PCR Kit**. Qualitative results of the study are displayed in Table 10. The clinical evaluation of the **DIAGNOVITAL® SARS-CoV-2 Real-Time PCR Kit** demonstrated 100% PPA and NPA compared to the previously authorized test.

		Phoenix Dx 2019-nCoV EUA Authorized Assay		
		Positive	**Negative**	**Total**
	Positive	30	0	30

Diagnovital SARS-CoV-2 Real-Time PCR Kit	**Negative**	0	30	30
	Total	30	30	60
Positive Percent Agreement		30/30; 100% (88.65% - 100.00%)[1]		
Negative Percent Agreement		30/30; 100% (88.65% - 100.00%)		

Table 10: Results from Clinical Study Using the PhoenixDx EUA Authorized Assay as a Comparator

[1]Two-sided 95% score confidence intervals

Symbols


Expiry Date

Lot/Batch

Catalog number


Temperature limitation


Caution; consult accompanying documents


Manufacturer

In Vitro Diagnostic Medical Device


Consult instructions for use


Contains sufficient for (n) amount tests

Ordering Information

For ordering information, contact Protrim Enterprises, Inc. or Parcha LLC

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