INSTRUCTIONS FOR USE

QuantiVirus™ SARS-CoV-2 Test Kit

For Real Time RT-qPCR test

Rx Only

IVD

For Emergency Use Authorization (EUA) only

CATALOG NUMBER

DC-11-0007 (24 Reactions)
DC-11-0008 (48 Reactions)
DC-11-0009 (480 Reactions)

COMPANY

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CONTENTS

PART 1. INTENDED USE

QuantiVirus SARS-CoV-2 Test Kit a real-time RT-PCR test intended for the qualitative detection of nucleic acid from the SARS-CoV-2 in upper respiratory specimens (such as nasal, mid-turbinate, nasopharyngeal, and oropharyngeal swab specimens) and sputum from individuals suspected of COVID-19 by their healthcare provider. Testing is limited to laboratories certified under the Clinical Laboratory Improvement Amendments (CLIA) of 1988, 42 U.S.C. §263a, that meet requirements to perform high complexity tests.

Results are for the identification of SARS-CoV-2 RNA. The SARS-CoV-2 RNA is generally detectable 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 is necessary to determine patient infective 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 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 QuantiVirus SARS-CoV-2 Test Kit is intended for use by qualified trained clinical laboratory personnel specifically instructed and trained in the techniques of real-time PCR and *in vitro* diagnostic procedures. The QuantiVirus SARS-CoV-2 Test Kit is only for use under the Food and Drug Administration's Emergency Use Authorization.

PART 2. PRODUCT DESCRIPTION

The QuantiVirus™ SARS-CoV-2 Test Kit is a real-time reverse transcription polymerase chain reaction (RT -qPCR) test that includes the assays and controls for the qualitative detection of RNA from SARS-CoV-2 in upper respiratory specimens (such as nasal, mid-turbinate, nasopharyngeal, and oropharyngeal swab specimens) and sputum from patients who are suspected of COVID-19.

Extracted RNA is reverse-transcribed and amplified in a single reaction. The Orf1ab, N, and E genes of the SARS-CoV-2 genome are targeted in the RT-PCR assay (Figure 1). Primers and TaqMan probes designed for conserved regions of the SARS-CoV-2 virus genome allow specific amplification and detection of the viral RNA from all strains of SARS-CoV-2 from respiratory specimens. The Human RNase P gene is used as Internal control to monitor viral RNA extraction efficiency and assess amplifiable RNA in the samples to be tested. The assay is a multiplex RT-PCR assay consisting of one reaction with primers and probes for the viral targets (Orf1ab, N and E genes) and internal control in one tube thus with increased assay throughput and ease of use and other advantages as a multiplex assay.

Figure 1. SARS-CoV-2 Genome Structure

PART 3. COMPONENTS AND STORAGE

3.1. Kit Components

QuantiVirus™ SARS-CoV-2 Test Kit includes the following components:

- One step RT-qPCR Master mix
- One set of Primers/ Probes specific to the Orf1ab, N and E SARS-CoV-2 genomic regions and primers/probe for human RNase P gene.
- A Positive control (PC), Extraction control (EC) and a No Template control (NTC)

The QuantiVirus™ SARS-CoV-2 Test Kit is available in 3 pack sizes – 24-reactions kit, 48-reaction kit and 480-reaction kit. Individual components and their descriptions are listed in Table 1 below.

3.2. Shelf-Life

Final storage of kits is proposed at -25°C to -15°C. Based on individual component shelf life, the approximate shelf life of the kit is estimated to be 12 months. Do not use expired reagents from the kit. *Note: EC can be stored at 2 - 8°C after first use and between uses.

Table 1. Kit components

Table 1a. Pack-Size: 24 Reactions

Name of Component	Part #	Description	Pack Size: 24 reactions kit	Label Volume for each vial	Storage Temp
5x Primer/Probe Mix (Multiplex)v2	1010212	Primer/probe Mix (Orf1ab, N and E genes & Human RNase P gene primers and probes)	1 vial	48 µL	-25°C to -15°C
One Step RT-PCR Master Mix	1010222	1-step Multiplex Master mix	1 vial	60 µL	-25°C to -15°C
Positive Control	1010242	Synthetic DNA templates (Positive control PC) for Orf1ab, N and E genes	1 vial	10 µL	-25°C to -15°C
Extraction Control (EC)	1010232	Template material with target sequences for the human RNase P gene)	1 vial	40 µL	-25°C to -15°C*
No Template Control	1010252	Nuclease-Free Water	1 vial	50 µL	-25°C to -15°C

Table 1b. Pack-Size: 48 Reactions

Name of Component	Part #	Description	Pack Size: 48 reactions kit	Label Volume for each vial	Storage Temp
5x Primer/Probe Mix (Multiplex)v2	1010213	Primer/probe Mix (Orf1ab, N and E genes & Human RNase P gene primers and probes)	1 vial	96 µL	-25°C to -15°C
One Step RT-PCR Master Mix	1010223	1-step Multiplex Master mix	1 vial	120 µL	-25°C to -15°C
Positive Control	1010243	Synthetic DNA templates (Positive control PC) for Orf1ab, N and E genes	1 vial	20 µL	-25°C to -15°C
Extraction Control (EC)	1010233	Template material with target sequences for the human RNase P gene	1 vial	60 µL	-25°C to -15°C*
No Template Control	1010253	Nuclease-Free Water	1 vial	100 µL	-25°C to -15°C

Table 1c. Pack-Size: 480 Reactions

Name of Component	Part #	Description	Pack Size: 480 reactions kit	Label Volume for each vial	Storage Temp
5x Primer/Probe Mix (Multiplex)v2	1010214	Primer/probe Mix (Orf1ab, N and E genes & Human RNase P gene primers and probes)	2 vials	480 µL	-25°C to -15°C
One Step RT-PCR Master Mix	1010224	1-step Multiplex Master mix	2 vials	600 µL	-25°C to -15°C
Positive Control	1010234	Synthetic DNA templates (Positive control PC) for Orf1ab, N and E genes	1 vial	100 µL	-25°C to -15°C
Extraction Control (EC)	1010244	Template material with target sequences for the human RNase P gene	1 vial	100 µL	-25°C to -15°C*
No Template Control	1010254	Nuclease-Free Water	1 vial	500 µL	-25°C to -15°C

PART 4. MATERIALS REQUIRED BUT NOT PROVIDED WITH THE KIT

A. Reagents for Viral RNA Isolation

The following commercial kits are recommended for the isolation of viral RNA from clinical samples.

- Thermo Fisher PureLink viral RNA/DNA mini kit (Cat# 122800500)
- MGISP-960 High-throughput Automated Sample Preparation System (Extraction kit used: MGIEasy Magnetic Beads Virus DNA/RNA Extraction Kit (Cat# 1000020261).

Follow manufacturer's Instructions for Use.

B. Consumables

- White 0.2 mL DNase-free PCR tubes or plates (96 well) recommended by the instrument manufacturer
- Nuclease-free, low-binding microcentrifuge tubes
- Nuclease-free pipet tips with aerosol barriers

C. Other Reagents

- Molecular grade DNase/ RNase free water

D. Equipment

- Applied Biosystems™ QuantStudio 5 Real-Time PCR Instrument (QuantStudio™ Design and Analysis Software v1.4), ABI 7500 Fast DX (SDS Software v1.4), Bio-Rad CXF384 Real-Time PCR Instrument (BioRad CFX Manager v3.1) and Roche LightCycler 480 II (LightCycler® 480 SW 1.5.1).
- Dedicated pipettes* (adjustable, 10-100 µL, 100-200 µL, 1000 µL) for sample preparation
- Dedicated pipettes* (adjustable, 1-20 µL, 10-100 µL, 100-200 µL, 1000 µL) for PCR Master Mix preparation
- Dedicated pipettes* (adjustable, 1-20 µL, 10-100 µL) for dispensing of template RNA/DNA
- 12-channel multichannel pipettor (P-10) for transferring reactions to PCR plates.
- Microcentrifuge
- Benchtop centrifuge* with rotor for 1.5 mL tubes
- Benchtop mini centrifuge with rotor for PCR strips
- Benchtop plate centrifuge
- Vortex instrument
- Compatible 96-well PCR plate
- Compatible clear PCR plate sealer
- Reagent reservoir (holding 25 ml liquid or more)
- Spectrophotometer

Note: * Prior to use, ensure that instruments and equipment have been maintained and calibrated according to the manufacturer's recommendations.

PART 5. WARNING AND PRECAUTIONS

5.1. Warnings and Precautions

- For *in vitro* diagnostic use.
- For prescription use 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, and meet the requirements 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 Federal Food, Drug and Cosmetic Act, 21 U.S.C. § 360bbb-3(b)(1), unless the authorization is terminated or revoked sooner.
- Laboratories within the United States and its territories are required to report all results to the appropriate public health authorities.
- Handle all specimens as if infectious using safe laboratory procedures. Refer to CDC Interim Laboratory Biosafety Guidelines for Handling and Processing Specimens Associated with SARS-CoV-2 https://www.cdc.gov/coronavirus/2019-nCoV/lab-biosafety-guidelines.html
- Use extreme caution to prevent contamination of PCR reactions with the positive and negative controls provided.
- Minimize exposure of the 4X PCR Master Mix to room temperature for optimal amplification.
- Avoid over exposure of the primer-probe mixes to light for optimal fluorescent signal.
- Use of non-recommended reagent volumes may result in a loss of performance and may also decrease the reliability of the test results.
- Use of non-recommended volumes and concentrations of the RNA/ DNA sample may result in a loss of performance and may also decrease the reliability of the test results.
- Use of non-recommended consumables with instruments may adversely affect test results.
- Do not re-use any remaining reagents after PCR amplification is completed.
- Additional validation testing by user may be necessary when using non-recommended instruments.
- Perform all experiments under proper sterile conditions using aseptic techniques.
- Perform all procedures using universal precautions.
- Wear personal protective apparel, including disposable gloves, throughout the assay procedure.
- Do not eat, drink, smoke, or apply cosmetics in areas where reagents or specimens are handled.
- Dispose of hazardous or biologically contaminated materials according to the practices of your institution.
- Discard all materials in a safe and acceptable manner, in compliance with all legal requirements.
- Dissolve reagents completely, then mix thoroughly by pipetting up and down several times or vertexing if needed.
- If exposure to skin or mucous membranes occurs, immediately wash the area with large amounts of water. Seek medical advice immediately.
- Do not use components beyond the expiration the date printed on the kit boxes.
- Do not mix reagents from different lots.
- Return all components to the appropriate storage condition after preparing the working reagents.
- Do not interchange vial or bottle caps, as cross-contamination may occur.
- Keep all the materials on ice when in use.
- Do not leave components out at room temperature for more than 2 hours.

- Reagents supplied are formulated specifically for use with this kit. Make no substitutions in order to ensure optimal performance of the kit. Further dilution of the reagents or alteration of incubation time and temperature may result in erroneous or discordant data.

The product contains no substances which at their given concentration, are considered to be hazardous to health or environment.

HMIS

Health	0
Flammability	0
Reactivity	0

5.2. Handling and Storage

This kit is shipped on dry ice. If any component of the kit is not frozen on arrival, the outer packaging has been opened during transit, or the shipment does not contain a packaging note or the reagents, please contact DiaCarta or the local distributors as soon as possible.

The kit should be stored at -20 °C immediately upon receipt at -15°C to -25°C in a constant-temperature freezer and must be protected from light. When stored under the specified storage conditions, the kit is stable until the stated expiration date. It is recommended to store the PCR reagents in a pre-amplification area and the controls in a postamplification (DNA template-handling) area. The kit can undergo up to 6 freeze-thaw cycles without affecting performance.

All reagents must be thawed at ambient temperature for a minimum of 30 minutes before use. Do not exceed 2 hours at ambient temperature. The primer and probe mixes contain fluorophore labeled probes and should be protected from light. It is recommended that all reagents should be kept on ice when setting up the assay mixes.

Attention should be paid to expiration dates and storage conditions printed in the box and labels of all components. Do not use expired or incorrectly stored components.

5.3. General Considerations

Effective use of qPCR tests requires good laboratory practices, including maintenance of equipment that is dedicated to molecular biology. Use nuclease-free lab ware (pipettes, pipette tips, reaction vials) and wear gloves when performing the assay. Use aerosol-resistant pipette tips for all pipetting steps to avoid cross contamination of the samples and reagents.

Prepare the assay mixes in designated pre-amplification areas using only equipment dedicated to this application. Add template RNA/DNA in a separate area (preferably a separate room). Use extreme precautions to prevent RNase and DNase contamination that could result in degradation of the template RNA/DNA, or PCR carryover contamination, which could result in a false positive signal.

Reagents supplied are formulated specifically for use with this kit. Make no substitutions in order to ensure optimal performance of the kit. Further dilution of the reagents or alteration of incubation times and temperatures may result in erroneous or discordant data.

PART 6. SAMPLES & CONTROLS

Patient samples must be collected according to appropriate CDC guidelines. Positive, Extraction control and No Template Controls must be included in every run to accurately interpret patient test results.

Control	Used to monitor	Assays
Positive Control (Synthetic DNA)	RT-PCR reaction	Target gene assays
No Template Control (DNase/RNase free water)	Cross contamination for assay procedure	Target gene assays
Extraction Control	RNA extraction, reverse transcription and qPCR	RNase P gene assay

A. Positive Control (PC)

A positive control is a mix of synthetic DNA templates for the target sequences for Orf1ab, N and E genes of the SARS-CoV-2 genome. Positive controls must show the appropriate values in FAM (Orf1ab), N (CY5 or Quasar 670) and E (TexasRed or CAL Fluor Red 610) channels for the run to be valid. Positive control monitors the function of each assay component.

B. Extraction control (EC)

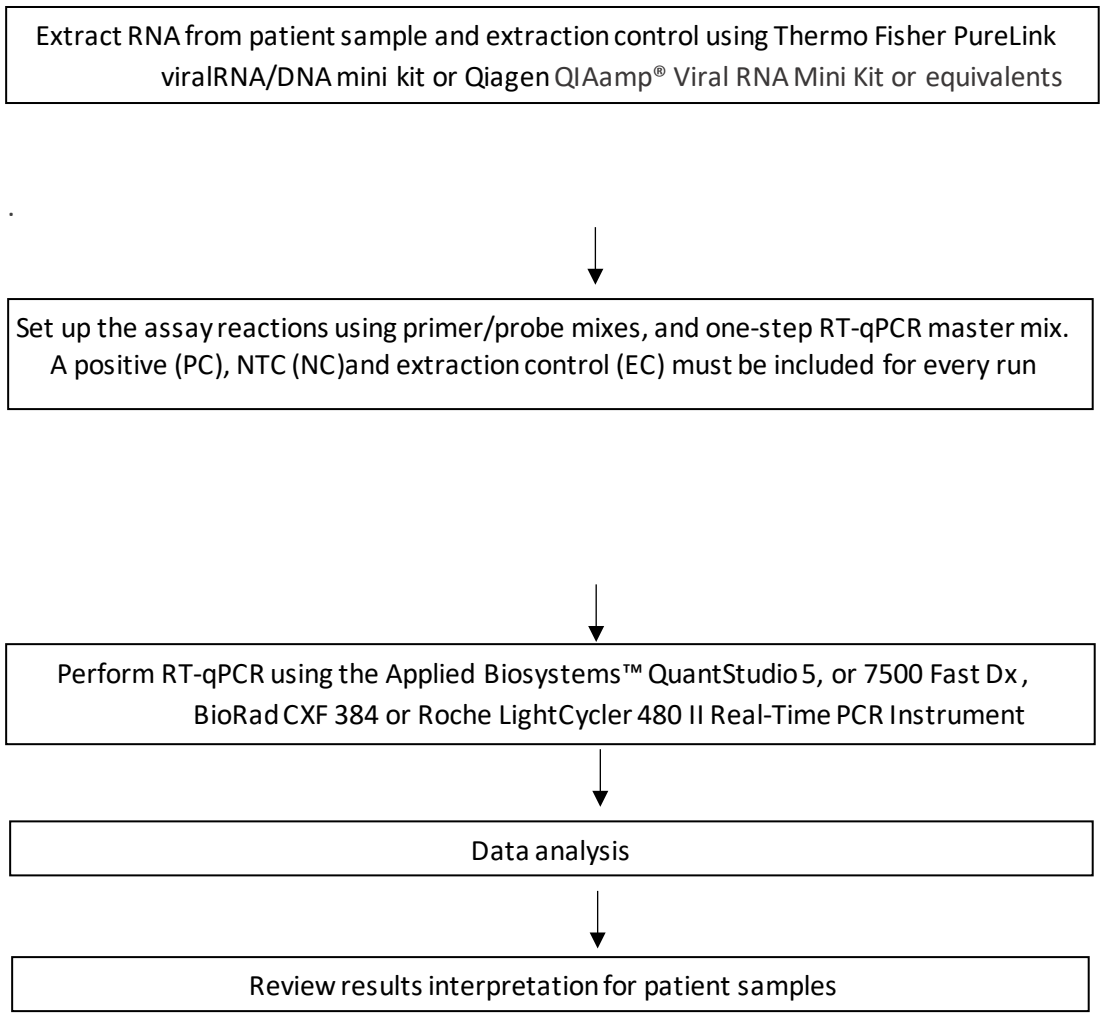
Extraction Control is template material with target sequences for the human RNase P (RP) gene. The extraction control RP RNA undergoes the full extraction procedure. As the Extraction Control, there should be amplification for RP gene (HEX or VIC), but no amplification for the viral genes (ORF 1ab, N and E). This control should be run with every batch of extraction.

C. No Template Control (NTC)

Nuclease free water is used in place of template. No amplification should be observed in all channels, assuring the absence of contamination during assay set-up.

PART 7. WORKFLOW

The brief procedure for performing the assay include the following steps:



The workflow begins with nucleic acid extraction from upper respiratory specimens (such as nasal, mid-turbinate, nasopharyngeal, and oropharyngeal swab specimens) and sputum specimens. RNA is isolated and purified from the specimens using the appropriately chosen viral RNA extraction method, please refer to the above list in section 4.A. The purified nucleic acid is reverse transcribed into cDNA and amplified and detected using the one step QuantiVirus™ SARS-CoV-2 Test Kit and the Applied Biosystems™ QuantStudio 5*, 7500 Fast Dx* or Bio-Rad CXF 384 * or Roche LightCycler 480 II* Real-Time PCR instrument. In the process, the probes anneal to the specific target sequences located between pairs of unique forward and reverse primers for the ORF1ab, N and E genes in the SARS-CoV-2 genome.

The RNase P primers and probe target the human RNase P housekeeping gene to monitor successful RNA extraction. During the extension phase of the PCR cycle, the 5' exo-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. Fluorescence intensity is monitored at each PCR cycle by the PCR instrument.

**Refer to manufacturer's Instructions for use*

7.1. Sample Collection and Handling

Sample collection device is not a part of the assay kit. All testing for COVID-19 should be conducted in consultation with a healthcare provider. We recommend using CDC guidelines for sample collection and storage available at link below -
https://www.cdc.gov/coronavirus/2019-ncov/lab/guidelines-clinical-specimens.html

Nasal, Mid-turbinate, Nasopharyngeal, and Oropharyngeal Swab Collection

Once the swabs have been collected as per the CDC guidelines above, it is recommended to use Universal Transport Medium (UTM) System (for transportation/ temporary storage of nasopharyngeal and oropharyngeal swabs. Specimen collected in the UTM should be processed within 48 hours from collection and stored at 2-25°C during that time as per the manufacturer's instructions.

7.2. Viral RNA Isolation

The QuantiVirus™ SARS-CoV-2 Test Kit uses Thermo PureLink™ Viral RNA/DNA Mini Kit (Cat. 12280050) or Magnetic Beads Virus DNA/RNA Extraction Kit (Cat# 1000020261). (follow manufacturer's IFU for details). It is recommended to use 180 µL (MGISP system) or 200 uL (Thermo PureLink) starting material for RNA isolation. For each batch of clinical samples to be tested, spike 20 µL of Extraction control (EC) from the QuantiVirus™ kit into 180 µL sterile RNase-free water (or E3 from Thermo PureLink™ Viral RNA/DNA Mini Kit) or 160 µL sterile RNase-free water (MGISP automatic extraction system) prior to RNA extraction. Process the spiked Extraction control and clinical sample for viral RNA isolation according to the manufacturer's instructions. It is suggested to elute RNA in 15-25 µL of E3 in the elution step for manual extraction using the Thermo PureLink kit and 40 µL of RNase free water according to the MGISP automatic extraction instructions.

5.5 µL of the extracted RNA can be used in 1 reaction. After RNA isolation, use spectrophotometer to check the RNA concentration, make sure the A260/A280 value is ~ 2.0. Use extreme precautions to handle RNA samples to prevent RNA degradation caused by RNases, follow general lab safety protocol and use precautions for handling RNA. Use DEPC treated water, containers and consumables. Store extracted RNA at -80°C if not using immediately.

7.3. Preparation of Reagents and Assay Mixes

1) Thaw the primer and probe mix, Positive Control, Nuclease-Free Water and 4X qRT-PCR Master Mix provided.
2) Thaw all reaction mixes at room temperature for a minimum of 30 minutes.
3) Keep all thawed reagents on ice.
4) Vortex all components except the PCR Master Mix and 5X Primer and Probe Mix for 5 seconds and perform a quick spin.
5) The RT-qPCR Master Mix and Primer/probe mix should be mixed gently by inverting the tube a few times.

Prior to use, ensure that any precipitate in the RT-qPCR Master Mix is re-suspended by pipetting up and down multiple times. Do not leave kit components at room temperature for more than 2 hours. The PCR reactions are set up in a total volume of 10 µL/reaction. Table 2 shows the component volumes for each 10 ul reaction.

Table 2. Assay components and reaction volume

Components	Volume/Reaction
4X RT - qPCR Master Mix	2.5 µL
5X Primer and Probe Mix	2 µL
RNA sample or Controls*	Sample and EC - 5.5 µL PC and NTC Controls - add 2 µL of controls and add 3.5 µL of nuclease free water to make 5.5 µL volume
Total Volume	10 µL

*Extraction Control (EC) is processed the same as clinical sample after extraction

For accuracy, 4X PCR Master Mix, 5X primers and probes should be pre-mixed into assay mixes as described in Table 3 below.

Preparation of Assay Mixes

Assay mixes should be prepared just prior to use. Label a microcentrifuge tube (not provided) for each reaction mix, as shown in Table 3. For each control and virus detection reaction, prepare sufficient working assay mixes for the RNA samples, one Positive Control, one extraction control and one Nuclease-Free Water for No-Template Control (NTC), according to the volumes in Table 3. Include reagents for 1 extra sample to allow sufficient overage for the PCR set-up. The assay mixes contain all of the components needed for PCR except the templates (sample or controls).

Table 3. Preparation of assay mixes

	Volume of 4X PCR Master Mix	Volume of 5X Primer and probe Mix
Assay Mix	2.5 µL x (n+ 3+ 1)	2 µL x (n+ 3+ 1)

n = number of reactions (RNA samples), + 3 is for 3 controls. Prepare enough for 1 extra sample (+1) to allow for sufficient coverage for the RT-qPCR set-up.

A reaction mix containing all reagents, except for the RNA sample or control templates, should be prepared for the total number of samples and controls to be tested in one run. The Positive Control (PC), Extraction Control (EC) and No Template Control (NTC) should be included in each run.

7.4. Suggested Run Layout

For each reaction, add 4.5 µL of the appropriate assay mix to the plate or tubes. Add up to 5.5 µL of template.

The assay has been validated on the following PCR instruments:

Table 4. Validated PCR Instruments

Company	Model
Bio-Rad	CFX384
Thermo Fisher (ABI)	QuantStudio 5
Thermo Fisher (ABI)	7500 Fast Dx
Roche	Light Cycler 480 II

Table 5a. Suggested late layout for 384-Well Plate

Sample set-up for a single experiment analyzing up to **381 unknown samples**.

		1	3	5	7	9	11	13	15	17	19	21	23
A	Assay Mix	NTC	EC	S1	S2	S3	S4	S5	S6	S7	S8	S9	PC
B	Assay Mix	S10	S11	S12	S13	S14	S15	S16	S17	S18	S19	S20	S21
C	Assay Mix	S22	S23	S24	S25	S26	S27	S28	S29	S30	S31	S32	S33
D	Assay Mix	S34	S35	S36	S37	S38	S39	S40	S41	S42	S43	S44	S45
E	Assay Mix	S46	S47	S48	S49	S50	S51	S52	S53	S54	S55	S56	S57
F	Assay Mix	S58	S59	S60	S61	S62	S63	S64	S65	S66	S67	S68	S69
G	Assay Mix	S70	S71	S72	S73	S74	S75	S76	S77	S78	S79	S80	S81
H	Assay Mix	S82	S83	S84	S85	S86	S87	S88	S89	S90	S91	S92	S93
I	Assay Mix	S94	S95	S96	S97	S98	S99	S100	S101	S102	S103	S104	S105
J	Assay Mix	S106	S107	S108	S109	S110	S111	S112	S113	S114	S115	S116	S117
K	Assay Mix	S118	S119	S120	S121	S122	S123	S124	S125	S126	S127	S128	S129
L	Assay Mix	S130	S131	S132	S133	S134	S135	S136	S137	S138	S139	S140	S141
M	Assay Mix	S142	S143	S144	S145	S146	S147	S148	S149	S150	S151	S152	S153
N	Assay Mix	S154	S155	S156	S157	S158	S159	S160	S161	S162	S163	S164	S165
O	Assay Mix	S166	S167	S168	S169	S170	S171	S172	S173	S174	S175	S176	S177
P	Assay Mix	S178	S179	S180	S181	S182	S183	S184	S185	S186	S187	S188	S189

PC: Positive Control, EC: Extraction Control, NTC: No-Template Control (water), S1-S189: Samples 1-189, up to 381 unknown samples

After all reagents have been added to the plate, tightly seal the plate to prevent evaporation. Spin at 1000 rpm for 1 minute to mix and collect all the reagents at the bottom of the plate wells. Place in the real-time PCR instrument immediately.

Table 5b. Suggested Plate Layout for 96-well Plate.

Sample set-up for a single experiment analyzing up to 93 **unknown samples**.

		1	2	3	4	5	6	7	8	9	10	11	12
A	Assay Mix	NTC	EC	S1	S2	S3	S4	S5	S6	S7	S8	S9	PC
B	Assay Mix	S10	S11	S12	S13	S14	S15	S16	S17	S18	S19	S20	S21
C	Assay Mix	S22	S23	S24	S25	S26	S27	S28	S29	S30	S31	S32	S33
D	Assay Mix	S34	S35	S36	S37	S38	S39	S40	S44	S42	S43	S44	S45
E	Assay Mix	S46	S47	S48	S49	S50	S55	S52	S53	S54	S55	S56	S57
F	Assay Mix	S58	S59	S60	S61	S62	S63	S64	S65	S66	S67	S68	S69
G	Assay Mix	S70	S71	S72	S73	S74	S75	S76	S77	S78	S79	S80	S81
H	Assay Mix	S82	S83	S84	S85	S86	S87	S88	S89	S90	S91	S92	S93

PC: Positive Control, EC: Extraction Control, NTC: No-Template Control (water), S1-S93: Samples 1-93, up to 93 unknown samples

After all reagents have been added to the plate, tightly seal the plate to prevent evaporation. Spin at 1000 rpm for 1 minute to mix and collect all the reagents at the bottom of plate wells. Place in the real-time PCR instrument immediately.

7.5. Instrument Set-Up

Set up the PCR reaction thermocycling conditions on ABI QuantStudio 5, ABI 7500 Fast Dx, Bio-Rad CFX384 Real-Time PCR Instrument, and Roche Light Cycler 480 II as follows.

7.5.1. Selection of Detectors

a. For ABI QuantStudio 5 and ABI 7500 Fast Dx, assign the target Orf1ab in the assay Mix as “FAM”, N as “CY5”, E as “ROX” or “TexasRed” and RNase P (Internal control) as “VIC”, respectively. Note: Please select (**none**)* for **Passive Reference** on ABI 7500 Fast Dx and ABI QuantStudio 5.
b. For Bio-Rad CFX 384, select all channel
c. Use ‘QuantiVirus™ 4 Color’ as the Detector on Roche Light Cycler 480 II

*For ABI QuantStudio 5, click **Plate** tab, select **(none)** for **Passive Reference** at the bottom of the **Plate** tab. For ABI 7500 Fast Dx, click **View** tab, then click **Well inspector**, select (**none**) for **Passive Reference** at the right bottom in the **Well inspector** window.

7.5.2. Setup the thermocycling parameters for QuantStudio 5 Real-Time PCR Instrument, ABI 7500 Fast Dx, Bio-Rad CFX384, and Roche Light Cycler 480II as Shown in Table 6a and Table 6b.

Table 6a. RT-qPCR Cycling Parameters on ABI QS5, ABI 7500 Fast Dx, and Roche Light Cycler 480II

Step	Temperature (°C)	Time (Seconds)	Ramp Rate (°C/s)	Cycles	Data Collection
UNG Incubation*	25	120	1.6	1	OFF
Reverse Transcription	53	600	1.6	1	OFF
Polymerase Activation	95	120	1.6	1	OFF
Denaturation	95	3	1	45	OFF
Annealing and Extension	60	30	1		FAM, VIC, CY5, TexasRed

*Note: For the Roche LightCycler 480 II instrument, you can delete the UNG incubation step and start with Reverse Transcription. Just load the plate and let it sit at RT for 2 minutes before starting the run.

Table 6b. RT-qPCR Cycling Parameters on Bio-Rad CFX384

RT-qPCR thermocycling parameters for Bio-Rad CFX384	Temperature (°C)	Time (Seconds)	Cycles	Data Collection
UNG incubation	25	120	1	OFF
Reverse Transcription	53	600	1	OFF
Polymerase activation	95	120	1	OFF
Denaturation	95	3	45	OFF
Annealing and Extension	60	30		FAM, HEX, CY5 and TexasRed

7.5.3. Start the Run

For more detailed instructions of setting-up different qPCR instruments, please refer to the Instrument Setting-up and Data Analysis document. This document is available upon request.

* Note: Color compensation needs to be performed on Roche Light Cycler 480 II before running the assay. There is a separate protocol for color compensation (this document is available upon request). In Detection Formats, select custom filter combinations and name it as QuantiVirus™ 4 Color or other names for the custom detection format for the assay.

PART 8. DATA ANALYSIS

8.1. Assessment of qPCR Results

8.1.1. Data Analysis for Roche Light Cycler 480 II

For the Light Cycler 480 II, open the LightCycler480 SW 1.5.1.61 and select Abs Quant/2nd Derivative Max algorithm to analyze the run file data.

8.1.2. Data Analysis for ABI QuantStudio 5, Bio-Rad CFX384 and ABI 7500 Fast Dx

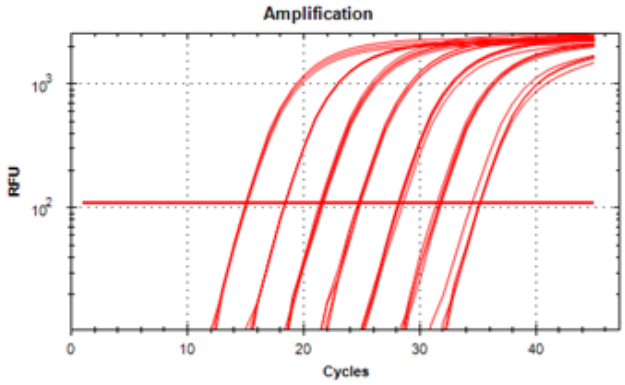
Save and analyze the data following the instrument manufacturer's instruction.

Adjust the threshold above any background signal to around the middle of the exponential phase of the amplification curve in the log view (e.g. Figure 2). It is recommended to set the thresholds according to the table below. The procedure chosen for setting the threshold should be used consistently. Exact threshold setting may be different for individual instruments and can be adjusted based on the amplification curves if needed.

Suggested Threshold setting on the real-time PCR instruments

Instrument \Channel	FAM	HEX or VIC	TexasRed	CY5
ABI QuantStudio 5	15000 ± 1500	10000 ± 1000	30000 ± 3000	30000 ± 3000
BioRadCFX 384	100 ± 10	200 ± 20	400 ± 40	300 ± 30
ABI 7500 Fast Dx	20000 ± 2000	20000 ± 2000	20000 ± 2000	30000 ± 3000

Figure 2. Amplification curve of 10 -fold serial dilution of templates showing the threshold setting



8.2. Assessment of the Assay Run

8.2.1. ABI QuantStudio 5

A. Ct Values for Controls

The QuantiVirus™ SARS-CoV-2 Test Kit protocol dictates that the controls should be analyzed before the analysis of patient samples. The kit positive, extraction and no template control Ct values must meet the acceptance criteria in Table 7a below for the assay to be valid. If kit control(s) fail, the test is invalid and needs to be repeated. Patient sample data is analyzed and interpreted only after all the kit controls pass.

Table 7a. Acceptable Ct Values for Positive Control, Extraction Controls and No Template Control

Control		Acceptable Ct	Test valid/invalid
Extraction control	RNase P gene	<39	Valid
Positive control	Orf1ab gene	<22	Valid
	N gene	<25	Valid
	E gene	<24	Valid
Non-template control		≥45	Valid

B. Ct Values for Samples

Assessment of the results for each individual assay should be based on the Cq values, according to the criteria outlined in Table 7b below.

Table 7b. Assessment of Individual Assay Results

Target	Cut-Off	Result
Target Virus Genes (Orf1ab, N and E)	Cq < 40**	POS
Target Virus Genes (Orf1ab, N and E)	Cq ≥ 40	NEG
RNase P (IC/EC)*	Cq <36	Viral RNA input OK
RNase P (IC/EC)*	Cq ≥36	Viral RNA input fail

* IC: Internal Control; EC: Extraction Control

** For samples showing Ct <40, especially samples with Ct<20, please check the amplification curves (in linear view) to make sure the Ct is generated from real amplification. If a sample shows Ct but the amplification curve looks not real, e.g. a straight line with slope, it is recommended to re-run the sample and assess the assay result from the re-running data.

8.2.2. ABI 7500 FAST Dx

A. Ct Values for Controls

The QuantiVirus™ SARS-CoV-2 Test Kit protocol dictates that the controls be analyzed before the analysis of patient samples. The kit positive, extraction and no template control Ct values must meet the acceptance criteria in Table 8a below for the assay to be valid. If kit control(s) fail, the test is invalid and needs to be repeated. Patient sample data is analyzed and interpreted only after all the kit controls pass.

Table 8a. Acceptable Ct Values for Positive Control, Extraction Control and No Template Control

Control		Acceptable Ct	Test valid/invalid
Extraction control	RNase P gene	<40	Valid
Positive control	Orf1ab gene	<22	Valid
	N gene	<23	Valid
	E gene	<23	Valid
Non-template control		⩾45	Valid

B. Ct Values for Samples

Assessment of the results for each individual assay should be based on the Cq values, according to the criteria outlined in Table 8b below.

Table 8b. Assessment of Individual Assay Results

Target	Cut-Off	Result
Target Virus Genes (Orf1ab, N and E)	Cq < 40	POS
Target Virus Genes (Orf1 ab, N and E)	Cq ≥ 40	NEG
RNase P (IC/EC)	Cq <36	Viral RNA input OK
RNase P (IC/EC)	Cq ≥36	Viral RNA input fail

*IC: Internal Control; EC: Extraction Control

8.2.3. Bio-Rad CFX384

A. Ct Values for Control

The QuantiVirus™ SARS-CoV-2 Test Kit protocol dictates that the controls be analyzed before the analysis of patient samples. The kit positive, extraction and no template control Ct values must meet the acceptance criteria in Table 9a below for the assay to be valid. If kit control(s) fail, the test is invalid and needs to be repeated. Patient sample data is analyzed and interpreted only after all the kit controls pass.

Table 9a. Acceptable Ct Values for Positive Control, Extraction Control and No Template Control

Control		Acceptable Ct	Test valid/invalid
Extraction control	RNase P gene	<37	Valid
Positive control	Orf1ab gene	<23	Valid
	N gene	<23	Valid
	E gene	<25	Valid
Non-template control		⩾45	Valid

B. Ct Values for Samples

Assessment of the results for each individual assay should be based on the Cq values, according to the criteria outlined in Table 9b below.

Table 9b. Assessment of Individual Assay Results

Target	Cut-Off	Result
Target Virus Genes (Orf1ab, N and E)	Cq < 40	POS
Target Virus Genes (Orf1ab, N and E)	Cq ≥ 40	NEG
RNase P (IC/EC)	Cq <36	Viral RNA input OK
RNase P (IC/EC)	Cq ≥36	Viral RNA input fail

*IC: Internal Control; EC: Extraction Control

8.2.4. Roche Light Cycler 480II

A. Ct Values for Controls

The QuantiVirus™ SARS-CoV-2 Test Kit protocol dictates that the controls should be analyzed before the analysis of patient samples. The kit positive, extraction and no template control Ct values must meet the acceptance criteria in Table 10a below for the assay to be valid. If kit control(s) fail, the test is invalid and needs to be repeated. Patient sample data is analyzed and interpreted only after all the kit controls pass.

Table 10a. Acceptable Ct Values for Positive Control, Extraction Controls and No Template Control

Control		Acceptable Ct	Test valid/invalid
Extraction control	RNase P gene	<34	Valid
Positive control	Orf1ab gene	<23	Valid
	N gene	<23	Valid
	E gene	<25	Valid
Non-template control		⩾45	Valid

B. Ct Values for Samples

Assessment of the results for each individual assay should be based on the Cq values, according to the criteria outlined in Table 10b below.

Table 10b. Assessment of Individual Assay Results

Target	Cut-Off	Result
Target Virus Genes (Orf1ab, N and E)	Cq < 40	POS
Target Virus Genes (Orf1ab, N and E)	Cq ≥ 40	NEG
RNase P (IC/EC)	Cq <37	Viral RNA input OK
RNase P (IC/EC)	Cq ≥37	Viral RNA input fail

*IC: Internal Control; EC: Extraction Control

PART 9. INTERPRETATION OF RESULTS

The Positive control, Extraction control and the No Template Control in the kit must function as outlined in tables 7a, 8a, 9a and 10a above. If the controls do not function as required, the test is invalid. All the samples need to be retested.

Interpretation of the Results

Orf1ab	N gene	E gene	RNase P (EC)	Status	Result	Action
NEG	NEG	NEG	NEG	Invalid	NA	Repeat test one more time. If the repeat result remains invalid, consider collecting new specimen.
NEG	NEG	NEG	POS	Valid	SARS-CoV-2 Not detected	Report results. Consider testing for other respiratory pathogens.
Two or more positive			POS or NEG	Valid	SARS-CoV-2 Detected	Report results.
One positive			POS or NEG	Valid	SARS-CoV-2 Inconclusive	Repeat test one more time. If the repeat result remains inconclusive, collect new sample.

PART 10. ASSAY LIMITATIONS

a) The performance of QuantiVirus™ SARS-CoV-2 Test Kit was established using nasopharyngeal swab samples. Anterior nasal swabs, mid-turbinate nasal swabs, and oropharyngeal swabs are also considered acceptable specimen types for use with the QuantiVirus™ SARS-CoV-2 Test Kit but performance has not been established.

b) Samples must be collected, transported, and stored using appropriate procedures and conditions. Improper collection, transport, or storage of specimens may hinder the ability of the assay to detect the target sequences.

c) Extraction and amplification of nucleic acid from clinical samples must be performed according to the specified methods listed in this procedure. Other extraction kits have not been evaluated.

d) If the virus mutates in the RT-qPCR target region, SARS-CoV-2 may not be detected or may be detected less predictably.

e) False Positive results may arise from the contamination during specimen handling or preparation, or between patient samples.

f) 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. Optimum specimen types and timing for peak viral levels during infections caused by SARS-CoV-2have not been determined.

g) False Negative results may arise from:
- Improper sample collection
- Degradation of the viral RNA during shipping/storage
- The presence of RT-PCR inhibitors
- Mutation(s) in the sequence of SARS-CoV-2 virus

h) The performance of this test was established based on the evaluation of a limited number of clinical specimens. Clinical performance has not been established with 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.

PART 11. CONDITIONS OF AUTHORIZATION FOR THE LABORATORY

The QuantiVirus™ SARS-CoV-2 Test 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/coronavirus-disease-2019-covid-19-emergency-use-authorizations-medical-devices/in-vitro-diagnostics-euas.

However, to assist clinical laboratories using the QuantiVirus™ SARS-CoV-2 Test Kit ("your product" in the conditions below), the relevant Conditions of Authorization are listed below:

A. Authorized laboratories[1] using your product will include with result reports of your product, 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 You (via email: covid19support@diacarta.com or via phone: 510-878-6662, option 4) 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 RT-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, that meet requirements to perform high complexity tests" as "authorized laboratories."

PART 12. ASSAY PERFORMANCE

The performance characteristics of the SARS-CoV-2 Multiplex Detection assay were established on the Applied Biosystems™ QuantStudio 5, 7500 Fast Dx or Bio-Rad CXF 384 or Roche LightCycler 480II Real-Time PCR instrument.

12.1. Analytic Sensitivity and Limit of Detection (LOD)

To determine the Limit of Detection (LoD) and analytical sensitivity of the kit, studies were performed using serial dilutions of analyte and the LoD was determined to be the lowest concentration of template that could reliably be detected with 95% of all tested positive.

LoD of each target assay in the QuantiVirus™ SARS-CoV-2 Test Kit were conducted and verified using SeraCare AccuPlex SARS-CoV-2 Reference Material Kit (Cat# 0505-0126). Non-infectious viral particles from the AccuPlex SARS-CoV-2 Reference Material Kit were spiked in sputum at various concentrations (50 copies/mL, 100 copies/mL, 150 copies/mL, 200 copies/mL and 300 copies/mL) diluted from the stock concentration of 5000 copies/mL. Real-time RT-PCR assay was performed with the provided kit reagents and tested on. ABI QS5, ABI 7500 Fast Dx and Bio-Rad CFX 384 PCR instruments

The LoD was confirmed by testing viral RNA at preliminary LoD with 20 replicates. The LoD was determined to be the lowest concentration (copies/ml) at which ≥95% (19/20) of the 20 replicates were tested as positive.

12.1.1. LoD for ABI QuantStudio 5

The following data confirmed the multiplex assay analytical sensitivity was 200 copies/mL for ABI QuantStudio 5.

Table 11a. Summary of Twenty Replicates for Assay Sensitivity (ABI QuantStudio 5)

Target	RNA (copy/mL)	Total	AVE Ct	SD	CV	Positive	Negative	Call Rate
ORF1ab gene	200 copies/mL	20	34.09	0.66	1.92%	20	0	100%
N gene	200 copies/mL	20	35.11	1.81	5.14%	20	0	100%
E gene	200 copies/mL	20	34.99	1.68	4.82%	20	0	100%

12.1.2. LoD for ABI 7500 Fast Dx

The data confirmed the assay analytical sensitivity was **200 copies/mL** for ABI 7500 Fast Dx.

Table 11b. Summary of Twenty Replicates for Assay Sensitivity (ABI 7500 Fast Dx)

Target	RNA (copy/mL)	Total	AVE Ct	SD	CV	Positive	Negative	Call Rate
ORF1ab gene	100 copies/mL	20	34.28	1.05	3.08%	20	0	100%
N gene	100 copies/mL	20	35.73	1.12	3.13%	20	0	100%
E gene	200 copies/mL	20	34.24	0.98	2.87%	20	0	100%

12.1.3. LoD for Bio-Rad CFX 384

The data confirmed the assay analytical sensitivity was 100 copies/mL for Bio-Rad CFX384.

Table 11c. Summary of Twenty Replicates for Assay Sensitivity （Bio-Rad CFX384）

Target	RNA (copy/mL)	Total	AVE Ct	SD	CV	Positive	Negative	Call Rate
ORF1ab gene	100 copies/mL	20	33.76	0.97	2.87%	20	0	100%
N gene	100 copies/mL	20	35.97	1.02	2.85%	20	0	100%
E gene	100 copies/mL	20	37.87	0.58	1.52%	20	0	100%

12.1.4. LoD for Roche LightCycler 480II

The data confirmed the assay analytical sensitivity was 200 copies/mL for Roche LightCycler 480 II.

Table 11d. Summary of Twenty Replicates for Assay Sensitivity (Roche LightCycler 480 II)

Target	RNA (copy/mL)	Total	AVE Ct	SD	CV	Positive	Negative	Call Rate
ORF1ab gene	100 copies/mL	20	32.85	0.57	1.7%	20	0	100%
N gene	200 copies/mL	20	35.04	0.58	1.7%	20	0	100%
E gene	100 copies/mL	20	36.13	0.59	1.6%	20	0	100%

12.2. Inclusivity

The QuantiVirus™ SARS-CoV-2 Test Kit has been designed using publicly available SARS-CoV-2 viral RNA sequences for the detection of SARS-CoV-2 strains or isolates. 102 NCBI and 125 GISAID target sequences were retrieved and aligned to identify conserved regions and specific regions of the SARS-CoV-2 genome, where primers and probes were designed for the assay. Alignments were performed with the designed oligonucleotide primer and probe sequences of QuantiVirus™ SARS-CoV-2 Test Kit panel with all publicly available sequences of SARS-CoV-2 in Genbank (about 97 SARS-CoV-2 strains) and 51560 SARS-CoV-2 sequences from GISAID as of August 28, 2020 to demonstrate the estimated inclusivity of the QuantiVirus™ SARS-CoV-2 Test Kit. All the alignments exhibited 100% of identity of design to the available SARS-CoV-2 sequences, suggesting the potential ability of the QuantiVirus™ SARS-CoV-2 Test Kit to detect 100% of all the SARS-CoV-2 strains.

In summary, in silico analysis of the QuantiVirus™ SARS-CoV-2 Test Kit assay design showed that the assay can detect all SARS-CoV2 virus strains and exhibited no cross reactivity with non-SARS-CoV2 species.

12.3. Cross-Reactivity

The QuantiVirus™ SARS-CoV-2 Test Kit has been designed to detect all SARS-CoV-2 strains. At the same time, the primers and probes were designed in the SARS-CoV-2 virus specific genome region ensuring the specific detection of the SARS-CoV-2 viral RNA. *In silico* analysis of the QuantiVirus SARS-CoV2 Test kit design were performed and compared to common respiratory flora and other viral pathogens from the same genetic family as SARS-CoV-2 according to the Recommended List of Organisms to be as shown in Table 15 or by Direct wet lab Testing.

Table 15. List of organisms tested for cross-reactivity by in silico analysis

#	Organism	#	Organism
1	Human coronavirus 229E	14	Rhinovirus
2	Human coronavirus OC43	15	Enterovirus
3	Human coronavirus HKU1	16	*Chlamydia pneumoniae*
4	Human coronavirus NL63	17	*Haemophilus influenzae*
5	SARS-coronavirus	18	*Legionella pneumophila*
6	MERS-coronavirus	19	*Mycobacterium tuberculosis*
7	Adenovirus	20	*Streptococcus pneumoniae*
8	Human Metapneumovirus (hMPV)	21	*Streptococcus pyogenes*
9	Parainfluenza virus 1-4	22	*Bordetella pertussis*
10	Influenza A	23	*Candida albicans*
11	Influenza B	24	*Pseudomonas aeruginosa*
12	Enterovirus	25	*Staphylococcus epidermis*
13	Respiratory Syncytial Virus A	26	*Staphylococcus salivarius*

Results of *in silico* analysis demonstrates that there is significant homology between the SARS-coronavirus (MK062184.1) and our assay primer/probes for N gene and E gene. However, the 3'ends of primers or probes are SARS-CoV-2 specific. All other homologies were not significant for the pair of primers and probes in order to predict a in silico false positive result.

The potential cross-reactivity of the Quantivirus SARS-CoV-2 Test kit was also evaluated in wet lab. MERS coronavirus, SARS-CoV samples were ordered from IDT and NATtrol Respiratory Validation Panel from ZeptoMetrix (cat#NATRVP-3). RNA/DNA were extracted from high titer stocks of the potentially cross-reacting microorganisms (estimated 1.0E+05 unites/mL), RNA/DNA were extracted 200 µL microorganisms' stocks using the Thermo Fisher viral RNA extraction kit (PureLink™ Viral RNA/DNA Mini Kit, cat# 12280050) and Qiagen QIAamp DNA Mini Kit (Cat#. 51304). 5.5 µL of purified RNA/DNA was tested with QuantiVirus™ SARS-CoV-2 Test Kit. The cross-reactivity testing results are summarized in Table 16. The tests were run in triplicates. All the test controls passed The tested organisms all show negative for the three targeted genes of SARS-CoV-2, suggesting there is no cross-reactivity between SARS-CoV-2 detection and the organisms tested. The cross-reactivity with SARS-coronavirus (MG772933.1) was tested and confirmed that it did not show any cross reactivity at 10^5 copies/mL.

Table 16. Summary of Cross-Reactivity Between SARS-CoV-2 Kit and Organisms tested

	N gene			ORF gene			E gene			RP gene		
Organisms	Ct_mean	STD	CI	Ct_mean	STD	CI	Ct_mean	STD	CI	Ct_mean	STD	CI
Coronavirus 229E	45.00	0	0	45.00	0	0	45.00	0	0	39.76	3.72	9.59
Coronavirus HKU-1	41.71	4.65	11.99	45.00	0	0	45.00	0	0	41.86	4.44	11.45
Coronavirus NL63	45.00	0	0	45.00	0	0	45.00	0	0	45.00	0	0
Coronavirus OC43	45.00	0	0	45.00	0	0	45.00	0	0	41.21	2.88	7.44
Influenza A H1N1pdm	45.00	0	0	45.00	0	0	45.00	0	0	45.00	0	0
Influenza AH1	45.00	0	0	45.00	0	0	45.00	0	0	45.00	0	0
Influenza AH3	45.00	0	0	45.00	0	0	45.00	0	0	45.00	0	0
Influenza B	45.00	0	0	45.00	0	0	45.00	0	0	45.00	0	0
Parinfluenza 1	45.00	0	0	45.00	0	0	45.00	0	0	45.00	0	0
Parinfluenza 2	45.00	0	0	45.00	0	0	45.00	0	0	45.00	0	0
Parinfluenza 3	45.00	0	0	45.00	0	0	45.00	0	0	45.00	0	0
Parinfluenza 4	45.00	0	0	45.00	0	0	45.00	0	0	45.00	0	0
Adenovirus3	45.00	0	0	45.00	0	0	44.65	0.49	1.28	33.64	0.35	0.89
Metapneumovirus	45.00	0	0	45.00	0	0	45.00	0	0	44.16	1.19	3.07
Rhinovirus	45.00	0	0	45.00	0	0	45.00	0	0	41.69	3.65	9.41
RSV A	45.00	0	0	45.00	0	0	45.00	0	0	45.00	0	0
B.pertussis	45.00	0	0	41.64	4.75	12.26	45.00	0	0	45.00	0	0
C.pneumoniae	45.00	0	0	45.00	0	0	45.00	0	0	42.04	4.19	10.81
M.pneumoniae	45.00	0	0	45.00	0	0	45.00	0	0	45.00	0	0
H.influenzae	45.00	0	0	45.00	0	0	45.00	0	0	45.00	0	0
P.aeruginosa	45.00	0	0	45.00	0	0	45.00	0	0	45.00	0	0
S.pneumoniae	45.00	0	0	45.00	0	0	45.00	0	0	45.00	0	0
S.pyogenes	45.00	0	0	45.00	0	0	45.00	0	0	45.00	0	0
SARS	42.40	3.68	9.49	45.00	0	0	45.00	0	0	45.00	0	0
MERS	45.00	0	0	45.00	0	0	45.00	0	0	45.00	0	0
RP	45.00	0	0	45.00	0	0	37.56	5.28	13.61	23.93	0.09	0.23
NTC	45.00	0	0	45.00	0	0	45.00	0	0	45.00	0	0
PC	20.06	0.02	0.05	18.30	0.03	0.09	19.13	0.04	0.11	45.00	0	0

*PC- positive control; NTC- no target control; EC-extraction control

12.4. Clinical Evaluation

To evaluate the clinical performance of the QuantiVirus™ SARS-CoV-2 Test Kit, a panel of patient samples with known status including 43 positive samples and 57 negative samples were tested with the QuantiVirus™ SARS-CoV-2 Test Kit using the ABI QuantStudio5 instrument. The clinical samples were all NP samples and the status of these samples was confirmed by FDA-authorized RT-PCR assay. The clinical sample testing data were summarized in Table 17.

Table 17. Clinical sample testing with QuantiVirus™ SARS-CoV-2 Test Kit

Patient samples	N	QuantiVirus SARS-CoV-2 Test			PPA	NPA
		Detected	Inconclusive	Not Detected		
Positive	43	43	0	0	100% (95% CI: 91.8%-100%)	100% (95% CI: 99%-100%)
Negative	57	0	0	57		

12.5. FDA SARS-CoV-2 Reference Panel Testing

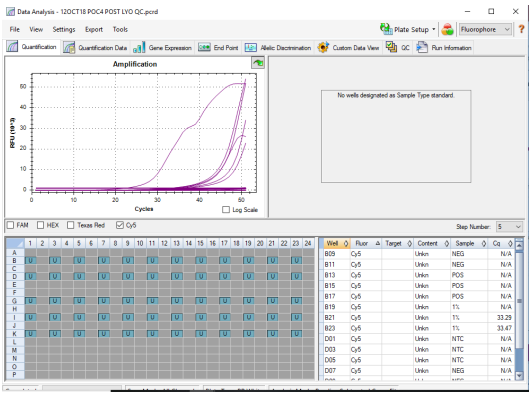
The evaluation of sensitivity and MERS-CoV cross-reactivity was performed using reference material (T1), blinded samples and a standard protocol provided by the FDA. The study included a range finding study and a confirmatory study for LoD. Blinded sample testing was used to establish specificity and to confirm the LoD. The extraction method and instrument used were Thermo Fisher PureLink viral RNA/DNA mini kit (cat# 122800500) and ABI 7500 Fast Dx. The results are summarized in Table 18.

Table 18 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	600 NDU/mL	N/A
MERS-CoV		N/A	ND

NP: nasopharyngeal swab
NDU/mL: RNA NAAT detectable units/mL
N/A: Not applicable
ND: Not detected

PART 13. ASSAY TROUBLESHOOTING

Problem	Cause	Solution
Fluorescence signals in No Template Control (NTC), e.g. Cq <= 40 	The positive signal may be caused by contamination during setting-up of the PCR; Or The signal is not true target amplification, but background curves generated by the software of the qPCR instrument.	Repeat the PCR with new reagents. Follow the general rules of GLP in a PCR lab. It is recommended to set up the qPCR reactions in a separate area, where no DNA is handled and with equipment designated for pre-PCR activities. Make sure the workspace and instruments are decontaminated regularly. Ignore the Ct value of NTC if the amplification curve looks not real but background noise.
The Positive Control did not meet the criteria set for acceptable values of the virus RNA detection kit. The assay is invalid.	Kit was not stored at the recommended conditions; Or Kit shelf-life expired.	Check the kit label for storage conditions and expiration date and use a new kit if necessary.
The edge wells have abnormal amplification curves, resulting in high baseline threshold with incorrect estimation of Ct values. 	Edge wells show high background fluorescence which prevents software from calling Ct values for sample wells.	All wells showing high background fluorescence must be deselected, threshold reset to a lower value and then reanalyzed using user defined threshold setting.

PART 14. CUSTOMER AND TECHNICAL SUPPORT

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Note: For SDSs for reagents and chemicals from other manufacturers, contact the manufacturer.

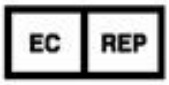
Contact:
Email: covid19support@diacarta.com
Phone: 510-878-6662, option 4 (tech support)

QuantiVirus™ is a pending trademark of DiaCarta, Inc. All other names, logos and other trademarks listed below are the property of their respective owners

1. Thermo Fisher Scientific® QuantStudio™ 5 System
2. Applied Biosystems™ 7500 Real-Time PCR Systems
3. Bio-Rad CFX 384 System
4. Thermo Fisher Scientific® | PureLink™ RNA Mini Kit
5. Applied Biosystems™ TaqPath™ 1-Step Multiplex Master Mix
6. SeraCare AccuPlex™ SARS-CoV-2 Reference Material Kit

PART 15. SYMBOLS USED IN PACKAGING

Symbols used in packaging

Symbol	Definition
	In vitro Diagnostics
	Authorized Representative in the European Community
	Catalog Number
	Manufactured By
	Temperature Limitation
	Batch Code
	Expiration Date
	Contains sufficient for <n> tests
1011-11-17	Date Format (year-month-day)
1011-11	Date Format (year-month)

HMIS

Health	0
Flammability	0
Reactivity	0

The product contains no substances which at their given concentration, are considered to be hazardous to health.

PART 16. REFERENCE

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