INSTRUCTIONS FOR USE

QuantiVirus™ SARS-CoV-2 Multiplex Test Kit

For Real Time RT-qPCR test

Rx Only

IVD

For Emergency Use Authorization (EUA) only

CATALOG NUMBER

DC-11-0017 (24 Reactions)
DC-11-0018 (48 Reactions)
DC-11-0019 (480 Reactions)

COMPANY

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CONTENTS

PART 1. INTENDED USE

The QuantiVirus™ SARS-CoV-2 Multiplex Test Kit is 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, 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 of 1988 (CLIA), 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 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 Multiplex Test Kit is intended for use by qualified and 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 Multiplex Test Kit is only for use under a Food and Drug Administration's Emergency Use Authorization.

PART 2. PRODUCT DESCRIPTION

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

Extracted RNA is reverse-transcribed and amplified in a single reaction. The Orf1ab gene of the SARS-CoV-2 genome is targeted in the qRT-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-qPCR assay consisting of one reaction with primers and probes for the viral target Orf1ab and internal control in one tube thus with increased assay throughput and ease of use and other advantages as a multiplex molecular diagnostic assay.

Figure 1. SARS-CoV-2 Genome Structure

PART 3. COMPONENTS AND STORAGE

3.1. Kit Components

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

- One step RT-qPCR Master mix
- One set of Primers/ Probe specific to the Orf1ab SARS CoV-2 genomic region 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 Multiplex 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.

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)	1009072	Primer/probe Mix (Orf1ab & Human RNase P gene primers and probes)	1 vial	48 µL	-25°C to -15°C
One Step qRT-PCR Master Mix	1009082	TaqPath 1-step Multiplex Master mix	1 vial	60 µL	25°C to -15°C
Positive Controls	1009102	Synthetic DNA templates (Positive control PC) for Orf1ab	1 vial	10 µL	25°C to -15°C
Extraction Control (EC)	1009092	*In vitro* transcribed RNA ($1x10^5$ copies/µL)	1 vial	10 µL	25°C to -15°C
No Template Control	1009112	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)	1009073	Primer/probe Mix (Orf1ab & Human RNase P gene primers and probes)	1 vial	96 µL	25°C to -15°C
One Step qRT-PCR Master Mix	1009083	TaqPath 1-step Multiplex Master mix	1 vial	120 µL	25°C to -15°C
Positive Controls	1009103	Synthetic DNA templates (Positive control PC) for Orf1ab	1 vial	20 µL	25°C to -15°C
Extraction Control (EC)	1009093	*In vitro* transcribed RNA ($1x10^5$ copies/µL)	1 vial	20 µL	25°C to -15°C
No Template Control	1009113	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)	1009074	**Primer/probe Mix** (Orf1ab & Human RNase P gene primers and probes)	2 vials	480 µL	-25°C to -15°C
One Step qRT-PCR Master Mix	1009084	TaqPath 1-step Multiplex Master mix	2 vials	600 µL	-25°C to -15°C
Positive Controls	1009104	Synthetic DNA templates (Positive control PC) for Orf1ab	1 vial	100 µL	-25°C to -15°C
Extraction Control (EC)	1009094	*In vitro* transcribed RNA ($1x10^5$ copies/µL)	1 vial	100 µL	-25°C to -15°C
No Template Control	1009114	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

- Thermo Fisher PureLink viral RNA/DNA mini kit (cat# 122800500)
- MGIEasy Magnetic Beads Virus DBA/RNA Extraction Kit (Cat# 1000020261)

RNA quality and quantity are critical for the test accuracy. 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) and Bio-Rad CXF 384 Real-Time PCR Instrument (Bio-Rad CFX Manager v3.1).
- MGI MGISP960 High Throughput Automated Sample Preparation System
- 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.
- The QuantiVirus™ SARS-CoV-2 Multiplex Test Kit 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, that meet requirements to perform high complexity tests.
- The QuantiVirus™ SARS-CoV-2 Multiplex Test Kit has been authorized only for the detection of nucleic acid from SARS-CoV-2, not for any other viruses or pathogens.
- The QuantiVirus™ SARS-CoV-2 Multiplex Test Kit 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

6.1. Samples and 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.

Assay controls

Control	Used to monitor	Assays
Positive Control (Synthetic DNA)	RT-PCR reaction	Target gene assay
No Template Control (DNase/RNase free water)	Cross contamination for assay procedure	Target gene assay
Extraction Control (*in vitro* transcribed RNA)	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 gene of the SARS-CoV-2 genome. Positive controls must show the appropriate values in FAM channel for the run to be valid. Positive control monitors the function of each assay component.

b. Extraction control (EC)

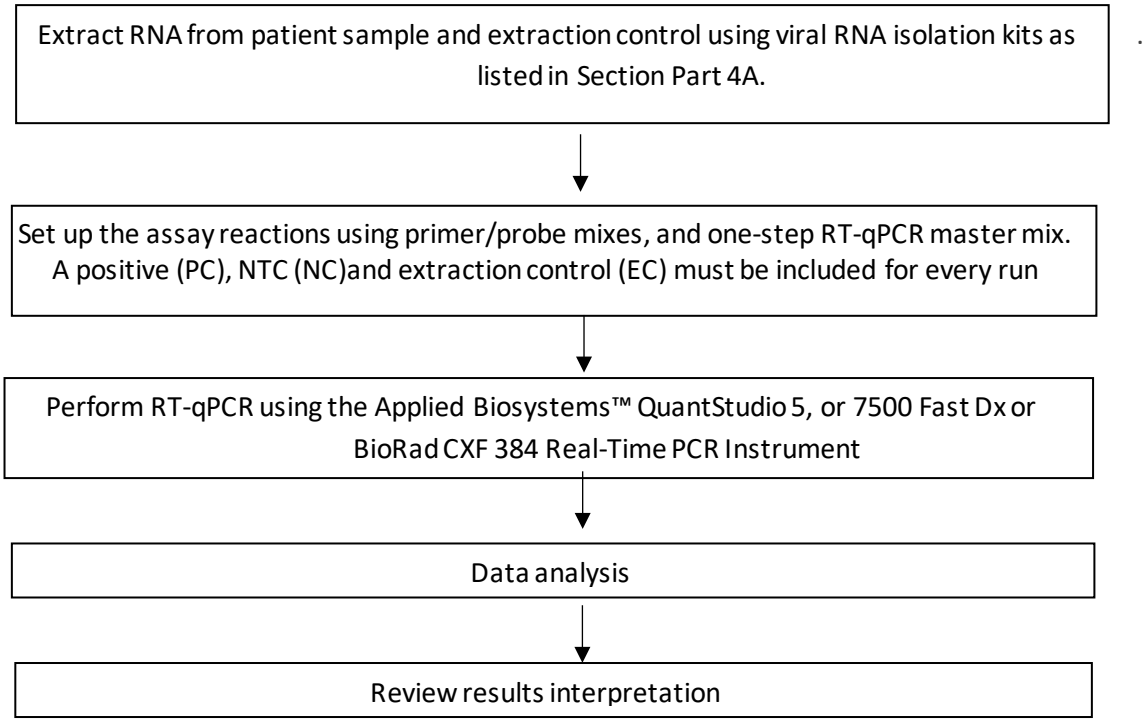
Extraction Control is a human RNase P (RP) gene *in vitro* transcribed RNA. The extraction control RP RNA undergoes the full extraction procedure. As the Extraction Control, there should be amplification for RP gene, but no amplification for the viral gene (ORF 1ab). This control should be run with every 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 respiratory 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 using the one step QuantiVirus™ SARS-CoV-2 Multiplex Test Kit `and the Applied Biosystems™ QuantStudio 5, 7500 Fast Dx or Bio-Rad CXF 384 Real-Time PCR instrument. In the process, the probes anneal to the specific target sequences located between one pair of unique forward and reverse primers for the ORF1ab gene 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

Once the swabs have been collected as per the CDC guidelines above, it is recommended to use Universal Transport Medium (UTM) System and Viral Transport Medium (VTM) for transportation/temporary storage of nasopharyngeal and oropharyngeal swabs. Specimen collected in the UTM or VTM 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 Multiplex Test Kit uses Thermo PureLink™ Viral RNA/DNA Mini Kit (Cat. 12280050), MGIEasy Magnetic Beads Virus DBA/RNA Extraction Kit (Cat# 1000020261) for MGI SP-960 High-throughput Automated Sample Preparation System suggested for RNA isolation (follow manufacturer's IFU for details). It is recommended to use 200 µL starting material for RNA isolation. For each batch of clinical samples to be tested, spike 2 µL of Extraction control (EC) from the QuantiVirus™ kit into 198 µL sterile RNase-free water (or E3 from Thermo PureLink™ Viral RNA/DNA Mini Kit) 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 30-50 µL of E3 in the elution step.

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 µL 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 - 5.5 µL 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

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	Software version
Bio-Rad	CFX384	Bio-Rad CFX Manager v3.1
Thermo Fisher (ABI)	QuantStudio 5	QuantStudio™ Design and Analysis Software v1.4
Thermo Fisher (ABI)	7500 Fast Dx	SDS Software v1.4

Table 5a. Plate 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. 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 and Bio-Rad CFX 384 Real-Time PCR Instrument 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", and RNase P (Internal control) as "HEX", respectively.
b. For Bio-Rad CFX 384, select all channel

7.5.2. Setup the thermocycling parameters for QuantStudio 5 Real-Time PCR Instrument, ABI 7500 Fast Dx and BioRadCFX384 as Shown in Table 6a and Table 6b

Table 6a. RT-qPCR Cycling Parameters on ABI QS5 and ABI 7500 Fast Dx

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	X45	OFF
Annealing and Extension	60	30	1		FAM, HEX

Table 6b. RT-qPCR Cycling Parameters on Bio-Rad CFX 384

RT-qPCR thermocycling parameters for Bio-Rad CFX 384	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

7.5.3. Start the Run

PART 8. DATA ANALYSIS

8.1. Assessment of qPCR Results

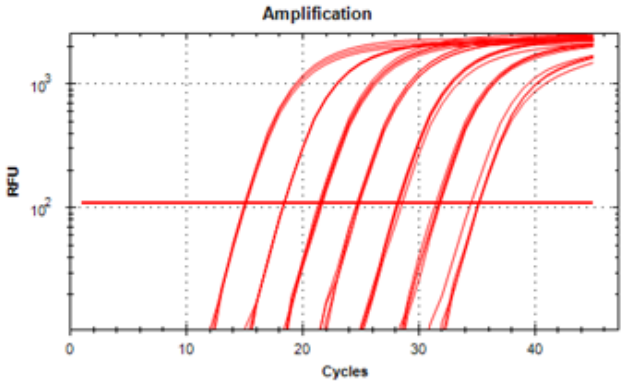
Save and analyze the data following the instrument manufacturer 's instruction.

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

Channel	ABI 7500 Fast Dx	ABI Quant Studio 5	Bio-Rad CFX 384
FAM	10000 ± 1000	20000 ± 2000	200 ± 20
HEX	15000 ± 1500	3000 ± 300	200 ± 20

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 Multiplex 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	<38	Valid
Positive control	Orf1 ab 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 Ct values, according to the criteria outlined in Table 7b below.

Table 7b. Individual Assay Results

Target	Cut-Off	Result
Target Virus Gene (Orf1 ab)	Ct < 40	SARS CoV-2 detected
Target Virus Gene (Orf1 ab)	Ct ≥ 40	SARS CoV-2 not detected
RNase P (IC/EC)	Ct <36	Viral RNA input OK
RNase P (IC/EC)	Ct ≥36	Viral RNA input fail

* IC: Internal Control; EC: Extraction Control

8.2.2. ABI 7500 FAST Dx

A. Ct Values for Controls

The QuantiVirus™ SARS-CoV-2 Multiplex 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	<39	Valid
Positive control	Orf1 ab gene	<21	Valid
Non-template control		⩾45	Valid

B. Ct Values for Samples

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

Table 8b. Individual Assay Results

Target	Cut-Off	Result
Target Virus Gene (Orf1 ab)	Ct < 40	SARS CoV-2 detected
Target Virus Gene (Orf1 ab)	Ct ≥ 40	SARS CoV-2 not detected
RNase P (IC/EC)	Ct <36	Viral RNA input OK
RNase P (IC/EC)	Ct ≥36	Viral RNA input fail

* IC: Internal Control; EC: Extraction Control

8.2.3. Bio-Rad CFX 384

A. Ct Values for Control

The QuantiVirus™ SARS-CoV-2 Multiplex 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	<39	Valid
Positive control	Orf1 ab 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 Ct values, according to the criteria outlined in Table 9b below

Table 9b. Individual Assay Results

Target	Cut-Off	Result
Target Virus Gene (Orf1 ab)	Ct < 40	SARS CoV-2 detected
Target Virus Gene (Orf1 ab)	Ct ≥ 40	SARS CoV-2 not detected
RNase P (IC/EC)	Ct <36	Viral RNA input OK
RNase P (IC/EC)	Ct ≥36	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 and 9a 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 gene	RNase P (IC&EC) *	Status	Result	Action
NEG	NEG	Invalid	NA	Repeat test one more time. If the repeat result remains invalid, consider collecting new specimen.
NEG	POS	Valid	SARS-CoV-2 Not detected	Report results to healthcare provider. Consider testing for other respiratory pathogens.
POS	POS or NEG	Valid	SARS-CoV-2 Detected	Report results to healthcare provider and CDC.

* IC: Internal Control; EC: Extraction Control

PART 10. ASSAY LIMITATIONS

a) The performance of the QuantiVirus™ SARS-CoV-2 Multiplex Test Kit was established using nasopharyngeal swab and sputum specimens. Oropharyngeal swabs, anterior nasal swabs and midturbinate nasal swabs are also considered acceptable specimen types for use with the QuantiVirus™ SARS-CoV-2 Multiplex 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 2019-nCoV have 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.

Conditions of Authorization for the Laboratory

The QuantiVirus™ SARS-CoV-2 Multiplex Test Kit Letter of Authorization, along with the authorized Fact Sheet for Healthcare Providers, the authorized Fact Sheet for Patients and other 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.

Use of the QuantiVirus™ SARS-CoV-2 Multiplex Test Kit must follow the procedures outlined in these manufacturer's Instructions for Use and the conditions of authorization outlined in the Letter of Authorization. Deviations from the procedures outlined are not permitted under the Emergency Use Authorization (EUA). To assist clinical laboratories running the QuantiVirus™ SARS-CoV-2 Multiplex Test Kit, the relevant Conditions of Authorization are listed verbatim below, and are required to be met by laboratories performing the EUA test.

- Authorized laboratories[1] using the QuantiVirus™ SARS-CoV-2 Multiplex Test Kit 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.
- Authorized laboratories will perform the QuantiVirus™ SARS-CoV-2 Multiplex Test Kit as outlined in the authorized labeling. Deviations from the authorized procedures, including authorized extraction methods, authorized clinical specimen types, authorized control materials, authorized other ancillary reagents and authorized materials required to perform the QuantiVirus™ SARS-CoV-2 Multiplex Test Kit are not permitted.
- Authorized laboratories that receive your product will notify the relevant public health authorities of their intent to run your product prior to initiating testing.
- Authorized laboratories will have a process in place for reporting test results to healthcare providers and relevant public health authorities, as appropriate.
- Authorized laboratories will collect information on the performance of the test and report to: DMD/OHT7-OIR/OPEQ/CDRH (via email: CDRH-EUA- Reporting@fda.hhs.gov) and to DiaCarta Inc, (via phone: 510-878-6662 or via email: covid19support@diacarta.com) any suspected occurrence of false positive or false negative results and significant deviations from the established performance characteristics of the test of which they become aware.
- All laboratory personnel using the test must be appropriately trained/experienced in molecular in vitro diagnostic test techniques, use appropriate laboratory and personal protective equipment when handling this kit, and use the test in accordance with the authorized labeling.
- DiaCarta Inc, authorized distributors, and authorized laboratories 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 11. ASSAY PERFORMANCE

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

11.1. 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 Multiplex Test Kit was conducted and verified using SeraCare AccuPlex SARS-CoV-2 Reference Material Kit (Cat# 0505-0126). 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. All RNAs were extracted from spiked samples using MGI SP-960 High-throughput Automated Sample Preparation System with MGIEasy Magnetic Beads Virus DNA/RNA Extraction Kit (Cat# 1000020261) and tested with the QuantiVirus™ SARS-CoV-2 Multiplex Test Kit on different real time PCR instruments (see below) in this LoD study. 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 1x LoD of viral RNA 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.

11.1.1. LoD for ABI QuantStudio 5

The following data confirmed the assay analytical sensitivity was **100 copies/mL** for ABI QuantStudio 5.

Table 10a. Summary of Twenty Replicates for Assay Sensitivity (ABI QuantStudio 5)

Target	RNA (copy/mL)	Total	AVE Ct	SD	CV	Positive	Negative	Call Rate
ORF1ab	100 copies/mL	20	36.24	1.59	0.04	20	0	100%

11.1.2. LoD for ABI 7500 Fast Dx

The data confirmed the assay analytical sensitivity was **50 copies/mL** for ABI 7500 Fast Dx.

Table 10b. 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	50 copies/mL	20	34.54	0.99	0.03	19	1	95%
	100 copies/mL	20	33.35	0.63	0.02	20	0	100%

11.1.3. LoD for Bio-Rad CFX 384

The data confirmed the assay analytical sensitivity was **100 copies/mL** for Bio-Rad CFX 384.

Table 10c. Summary of Twenty Replicates for Assay Sensitivity (Bio-Rad CFX 384)

Target	RNA (copy/mL)	Total	AVE Ct	SD	CV	Positive	Negative	Call Rate
ORF1ab	100 copies/mL	20	35.17	0.74	0.02	20	0	100%

11.2. Nucleic acid Extraction Method Comparison Study

In order to compare Thermo Fisher PureLink and MGI SP960 viral RNA extraction methods, SeraCare AccuPlex SARS-CoV-2 Reference Material (Cat# 0505-0126) was spiked in SARS-CoV-2 confirmed-negative sputum specimens and diluted to a final concentration at 200 copies/mL as extraction material. Twenty samples were extracted with Thermo Fisher PureLink and MGI SP960 method separately. The individually extracted RNA using both methods was tested on the same plate using QuantiVirus SARS-CoV-2 Multiplex Test Kit on ABI QS5. The extraction method comparison data were summarized in Table 11. Data in Table 11 showed that extracted viral RNA from all samples from both methods was detected by SARS-CoV-2 Multiplex Test kit, indicating that these two extraction methods are comparable.

Table 11. Comparison of Thermo Fisher PureLink and MGI SP960 Extraction Methods

Sample	Description	Extraction method	Orf1ab	RNase P	Status	Result
1	AccuPlex SARS-CoV-2 spiked 200 copies/mL	Invitrogen Purelink	34.79	25.49	valid	detected
		MGI SP960	34.55	27.25	valid	detected
2	AccuPlex SARS-CoV-2 spiked 200 copies/mL	Invitrogen Purelink	35.01	24.99	valid	detected
		MGI-automated	36.34	27.07	valid	detected
3	AccuPlex SARS-CoV-2 spiked 200 copies/mL	Invitrogen Purelink	33.80	24.41	valid	detected
		MGI-automated	34.03	26.85	valid	detected
4	AccuPlex SARS-CoV-2 spiked 200 copies/mL	Invitrogen Purelink	33.94	24.27	valid	detected
		MGI-automated	35.49	26.08	valid	detected
5	AccuPlex SARS-CoV-2 spiked 200 copies/mL	Invitrogen Purelink	34.35	25.21	valid	detected
		MGI-automated	34.82	26.97	valid	detected
6	AccuPlex SARS-CoV-2 spiked 200 copies/mL	Invitrogen Purelink	36.05	25.07	valid	detected
		MGI-automated	34.11	25.78	valid	detected
7	AccuPlex SARS-CoV-2 spiked 200 copies/mL	Invitrogen Purelink	33.12	24.59	valid	detected
		MGI-automated	34.71	27.22	valid	detected
8	AccuPlex SARS-CoV-2 spiked 200 copies/mL	Invitrogen Purelink	34.86	24.51	valid	detected
		MGI-automated	33.38	26.74	valid	detected
9	AccuPlex SARS-CoV-2 spiked 200 copies/mL	Invitrogen Purelink	34.41	23.66	valid	detected
		MGI-automated	37.47	26.52	valid	detected
10	AccuPlex SARS-CoV-2 spiked 200 copies/mL	Invitrogen Purelink	34.54	24.82	valid	detected
		MGI-automated	35.11	26.52	valid	detected
11	AccuPlex SARS-CoV-2 spiked 200 copies/mL	Invitrogen Purelink	35.78	25.79	valid	detected
		MGI-automated	35.70	26.52	valid	detected
12	AccuPlex SARS-CoV-2 spiked 200 copies/mL	Invitrogen Purelink	34.77	25.52	valid	detected
		MGI-automated	34.21	26.77	valid	detected
13	AccuPlex SARS-CoV-2 spiked 200 copies/mL	Invitrogen Purelink	33.85	24.69	valid	detected
		MGI-automated	34.13	26.96	valid	detected
14	AccuPlex SARS-CoV-2 spiked 200 copies/mL	Invitrogen Purelink	33.76	24.55	valid	detected
		MGI-automated	35.00	26.92	valid	detected
15	AccuPlex SARS-CoV-2 spiked 200 copies/mL	Invitrogen Purelink	33.43	24.57	valid	detected
		MGI-automated	33.81	26.02	valid	detected
16	AccuPlex SARS-CoV-2 spiked 200 copies/mL	Invitrogen Purelink	34.92	24.52	valid	detected
		MGI-automated	33.28	27.04	valid	detected
17	AccuPlex SARS-CoV-2 spiked 200 copies/mL	Invitrogen Purelink	34.95	24.56	valid	detected
		MGI-automated	34.53	26.73	valid	detected
18	AccuPlex SARS-CoV-2 spiked 200 copies/mL	Invitrogen Purelink	35.47	24.76	valid	detected
		MGI-automated	35.39	26.95	valid	detected
19	AccuPlex SARS-CoV-2 spiked 200 copies/mL	Invitrogen Purelink	35.25	24.00	valid	detected
		MGI-automated	36.99	26.37	valid	detected
20	AccuPlex SARS-CoV-2 spiked 200 copies/mL	Invitrogen Purelink	33.41	25.30	valid	detected
		MGI-automated	32.21	25.56	valid	detected

11.3. Precision

Precision studies include intra-run, inter-run, instrument and operator variability evaluation. The assay precision was assessed by the repeated testing of samples with three different template concentrations.

- Inter-assay %CV was established for same lot of reagents tested on the same instrument by the same user.

- Intra-assay %CV was established through performance of kit on reference samples run in replicates of nine.
- Operator variability was evaluated with one lot of reagents by two operators.

Reproducibility is demonstrated based on %CV of Ct values.

11.3.1. Intra-Assay Reproductivity

Each assay at three sample template concentrations was repeated 10 times and run on the sample plate. Average Ct and CV were calculated.

Table 12. Intra assay of the target for SARS-Cov-2 detection kit

SARS-COV-2 - Orf1ab Gene (FAM)				Reference RP (HEX)		
Sample concentration (copies/mL)	**Mean Ct**	**% Replicate Detection**	**Coefficient of Variation (%)**	**Mean Ct**	**% Replicate Detection**	**Coefficient of Variation (%)**
50	34.39	100	2.72%	27.24	100	1.83%
100	33.51	100	1.43%	27.21	100	0.78%
200	32.39	100	2.37%	27.25	100	0.66%
300	31.58	100	1.73%	27.34	100	0.80%
500	30.50	100	0.76%	27.26	100	1.05%

The Intra assay overall CV was <3% for this assay.

11.3.2. Operator Reproducibility

The assay reactions were set up by two operators using the same lot of reagent and run on the same instrument. Average Ct and CV were calculated.

Table 13. Different Operator Reproducibility

		Operator 2			Operator 1			Overall		
Assay targe	sample concentration (copies/ mL)	AVE	SD	CV	AVE	SD	CV	AVE	SD	CV
ORf1 ab	100	35.01	0.76	2.17%	35.89	1.25	3.49%	35.45	0.35	2.83%
	200	34.09	0.83	2.44%	34.63	0.49	1.42%	34.36	0.24	1.93%
	300	33.62	0.66	1.96%	34.07	0.57	1.69%	33.85	0.06	1.83%
	500	32.50	0.38	1.18%	33.06	0.46	1.39%	32.78	0.05	1.28%
RNase P	100	27.29	0.38	1.41%	26.06	0.37	1.41%	26.68	0.01	1.41%
	200	27.43	0.18	0.67%	25.94	0.22	0.83%	26.68	0.02	0.75%
	300	27.43	0.12	0.44%	26.03	0.21	0.79%	26.73	0.06	0.62%
	500	27.59	0.25	0.91%	26.17	0.42	1.59%	26.88	0.12	1.25%

Overall CV for two operators is <3% for this assay.

11.3.3. Inter-Instrument Reproducibility

Assay reactions were set up with three replicates and run on three different qPCR instruments including Bio-Rad CFX 384, ABI QS5 and ABI 7500 Fast Dx. Average Ct and CV were calculated.

Table 14. Instrument Reproducibility

		ABI QS5			BioRadCFX384			ABI 7500 Fast Dx			Overall		
Assay target	sample concentration (copies/ mL)	AVE	SD	CV	AVE	SD	CV	AVE	SD	CV	AVE	SD	CV
ORf1 ab	100	35.89	1.25	3.49%	35.01	0.76	2.17%	33.51	0.48	1.43%	35.45	0.39	2.36%
	200	34.63	0.49	1.42%	34.09	0.83	2.44%	32.39	0.70	2.17%	34.08	0.17	2.01%
	300	34.07	0.57	1.69%	33.62	0.66	1.96%	31.58	0.55	1.73%	33.36	0.06	1.79%
	500	33.06	0.46	1.39%	32.50	0.38	1.18%	30.50	0.23	0.76%	32.38	0.11	1.11%
RNase P	100	26.06	0.37	1.41%	27.29	0.38	1.41%	27.25	0.21	0.78%	27.95	0.09	1.20%
	200	25.94	0.22	0.83%	27.43	0.18	0.67%	27.26	0.18	0.66%	26.87	0.02	0.72%
	300	26.03	0.21	0.79%	27.43	0.12	0.44%	27.32	0.22	0.80%	26.91	0.05	0.68%
	500	26.17	0.42	1.59%	27.59	0.25	0.91%	27.65	0.29	1.05%	27.03	0.09	1.18%

The results indicate that three instruments have <3% CV.

11.4. Inclusivity

The QuantiVirus™ SARS-CoV-2 Multiplex 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 Multiplex Test Kit panel with all publicly available sequences of SARS-CoV-2 in Genbank (about 97 SARS-CoV-2 strains) and 47450 SARS-CoV-2 sequences as of July 13, 2020 to demonstrate the estimated inclusivity of the QuantiVirus™ SARS-CoV-2 Multiplex 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 Multiplex Test Kit to detect 100% of all the SARS-CoV-2 strains.

In summary, *in silico* analysis of the QuantiVirus™ SARS-CoV-2 Multiplex Test Kit assay showed that the assay can detect all SARS-CoV2 virus strains analyzed in this study.

11.5. Cross-Reactivity

The QuantiVirus™ SARS-CoV-2 Multiplex Test Kit has been designed to detect all SARS-CoV-2 strains. At the same time, the primers and probe 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 SARS-CoV-2 assay design was 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 analyzed in silico (see 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 human -coronavirus (NL63), MERS-coronavirus and our assay primer/probes for ORF1ab gene. However, high homology is only seen for either forward primer or reverse primer, and not for both forward and reverse primers at the same time. For NL63, only the forward primer has high homology not the reverse primer. For MERS-coronavirus, homology is only seen for the reverse primer not for the forward primer and probe. The 3‘ ends of primers are SARS-CoV-2 specific. All other homologies were not significant for the pair of primers and probes in order to predict a false positive result *in silico*.

We have tested the cross-reactivity in wet lab. MERS-coronavirus, SARS-CoV coronavirus 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 PFU/mL) and run on the QuantiVirus™ SARS-CoV-2 Multiplex Test Kit in triplicates. The cross-reactivity testing results are summarized in Table 16. All the test controls generated the valid results and passed. The tested organisms generated negative results for the Orf1ab gene of SARS-CoV-2, suggesting that the tested organisms do not cross-react with the QuantiVirus™ SARS-CoV-2 Multiplex Test Kit. The cross reactivity with human coronavirus (NL63 and 229E) and MERS-coronavirus were tested and confirmed that they did not show any cross reactivity at $1X10^5$ PFU/mL.

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

Organisms	SARS CoV-2 Orf1ab
Coronavirus 229E	Negative
Coronavirus HKU-1	Negative
Coronavirus NL63	Negative
Coronavirus OC43	Negative
Influenza A H1N1pdm	Negative
Influenza AH1	Negative
Influenza AH3	Negative
Influenza B	Negative
Parinfluenza 1	Negative
Parinfluenza 2	Negative
Parinfluenza 3	Negative
Parinfluenza 4	Negative
Adenovirus3	Negative
Metapneumovirus	Negative
Rhinovirus	Negative
RSV A	Negative
B.pertussis	Negative
C.pneumoniae	Negative
M.pneumoniae	Negative
MERS-coronavirus	Negative
SARS-coronavirus	Negative
Pooled nasal swab (CLIA lab DC)	Negative

11.6. Clinical Evaluation

11.6.1. Clinical Evaluation on ABI QuantStudio 5

Clinical evaluation of the QuantiVirus™ SARS-CoV-2 Multiplex Test Kit was conducted with contrived sputum specimens including 60 positive and 30 negative samples (Table 17a). Contrived sputum samples were prepared by spiking in SeraCare AccuPlex SARS-CoV-2 Reference Material Kit, Cat # 0505-0126.

20 sputum samples were contrived at 1x LoD (100 copies/mL), 20 samples at 2x LoD (1x200 copies/mL), 10 samples at 3x LoD (300 copies/mL) and another 10 sputum samples were spiked at the concentration of 5x LoD (500 copies/mL). Viral RNA was extracted using MGIEasy Magnetic Beads Virus DBA/RNA Extraction Kit (Cat# 1000020261) with MGI SP-960 High-throughput Automated Sample Preparation System from spiked samples and tested blindly with the QuantiVirus™ SARS-CoV-2 RT-qPCR.

Data show that there is 100% agreement with the spiked sample at 1x LoD (1x100 copies/mL), and 100% agreement at all other concentrations including 200 copies/mL, 300 copies/mL, and 500 copies/mL (Table 17a). Table 17a also shows that all 30 negative samples were tested negative.

Table 17a. Contrived clinical sample evaluation with viral particles (QuantStudio 5)

Specimen Type	Viral RNA Spiked	SARS-CoV-2			Performance Agreement	95% CI
		Positive	Negative	Total		
viral RNA +sputum	100 copies /mL (1x LoD)	20	0	20	100%	69.9-97.2%
	200 copies/mL (2x LoD)	20	0	20	100%	76.4-99.1%
	300 copies/mL (3x LoD)	10	0	10	100%	72.3-100%
	500 copies/mL (5x LoD)	10	0	10	100%	72.3-100%
H2O + sputum	0 copy/mL	0	30	30	100%	90.6-100%

11.6.2. Clinical Evaluation on ABI 7500 Fast Dx

Clinical evaluation of the QuantiVirus™ SARS-CoV-2 Multiplex Test Kit was conducted with contrived sputum specimens including 80 positive and 30 negative samples (Table 17b). Contrived sputum samples were prepared by spiking in SeraCare AccuPlex SARS-CoV-2 Reference Material Kit, Cat # 0505-0126.

20 sputum samples were contrived at 1x LoD (1x50 copies/mL), 20 samples at 2x LoD (2x50 copies/mL), 20 samples at 4x LoD (4x50 copies/mL), 10 sputum samples at 6x LoD (300 copies/mL) and another 10 sputum samples at 10x LoD (500 copies/mL). Viral RNA was extracted using MGIEasy Magnetic Beads Virus DBA/RNA Extraction Kit (Cat# 1000020261) with MGI SP-960 High-throughput Automated Sample Preparation System from spiked samples and tested blindly with the QuantiVirus™ SARS-CoV-2 Multiplex Test Kit.

Data show that there is 95% agreement with the spiked sample at 1x LoD (1x50 copies/mL), and 100% agreement at all other concentrations including 100 copies/mL (2x LoD), 200 copies/mL (4x LoD), 300 copies/mL (6x LoD). and 500 copies/mL (10x LoD) (Table 17b). All 30 negative samples were tested negative.

Table 17b. Contrived clinical sample evaluation with viral particles (ABI 7500 Fast Dx)

Specimen Type	Viral RNA Spiked	SARS-CoV-2			Performance Agreement	95% CI
		Positive	Negative	Total		
viral RNA +sputum	50 copies /mL (1x LoD)	19	1	20	95%	83.9-100%
	100 copies/mL (2xLoD)	20	0	20	100%	83.9-100%
	200 copies/mL (4x LoD)	20	0	20	100%	83.9-100%
	300 copies/mL (6x LoD)	10	0	10	100%	72.3-100%
	500 copies/mL (10xLoD)	10	0	10	100%	72.3-100%
H2O + sputum	0 copy/mL	0	30	30	100%	90.6-100%

11.6.3. Clinical Evaluation on Bio-Rad CFX 384

Clinical evaluation of the QuantiVirus™ SARS-CoV-2 Multiplex Test Kit was conducted with contrived sputum specimens including 60 positive and 30 negative samples (Table 17c). Contrived sputum samples were prepared by spiking in SeraCare AccuPlex SARS-CoV-2 Reference Material Kit, Cat # 0505-0126.

20 sputum samples were contrived at 1x LoD (100 copies/mL), 20 samples at 2x LoD (1x200 copies/mL), 10 sputum samples at 3x LoD (300 copies/mL) and another 10 sputum samples at 5x LoD (500 copies/mL). Viral RNA was extracted from spiked samples and tested blindly with the QuantiVirus™ SARS-CoV-2 Multiplex Test Kit.

Data show that there is 100% agreement with the spiked sample at 1x LoD (1x100 copies/mL), and 100% agreement at all other concentrations including 200 copies/mL, 300 copies/mL, and 500 copies/mL (Table 17c). All the 30 negative samples were tested negative.

Table 17c. Contrived clinical sample evaluation (Bio-Rad CFX 384)

Specimen Type	Viral RNA Spiked	SARS-CoV-2			Performance Agreement	95% CI
		Positive	Negative	Total		
viral RNA +sputum	100 copies /mL (1x LoD)	20	0	20	100%	69.9-97.2%
	200 copies/mL (2x LoD)	20	0	20	100%	76.4-99.1%
	300 copies/mL (3x LoD)	10	0	10	100%	72.3-100%
	500 copies/mL (5x LoD)	10	0	10	100%	72.3-100%
H2O + sputum	0 copy/mL	0	30	30	100%	90.6-100%

11.6.4. Clinical Sample Testing

Further clinical evaluation of this kit was conducted with nasopharyngeal swab (NP) samples including 41 positive samples and 52 negatives samples (Table 18). Out of these 93 clinical samples, 41 were identified as SARS-CoV-2 positive and 52 negative, based on FDA EUA RT-PCR test. Samples were blind-tested with QuantiVirus™ SARS-CoV-2 Multiplex Test Kit on ABI QuantStudio 5, and the data were summarized in Table 18 (see below). The data showed that the positive percent agreement (PPA) is 97.6% (95% CI: 0.91-1.0) and negative percent agreement (NPA) is 100% (95% CI: 0.87-1.0).

Table 18. Clinical Samples Evaluation with ABIQS5 qPCR Instrument by QuantiVirus™ SARS-CoV-2 Multiplex Test

Patients samples	N	QuantiVirus SARS-CoV-2 Multiplex Test			PPA (95% CI)	NPA (95% CI)
		Detected	Inconlusive	Not Detected		
Positive	41	40	1	0	97.6% (0.91-1.0)	100% (0.87-1.0)
Negative	52	0	0	52		

11.6.5. FDA SARS-CoV-2 Reference Panel Testing

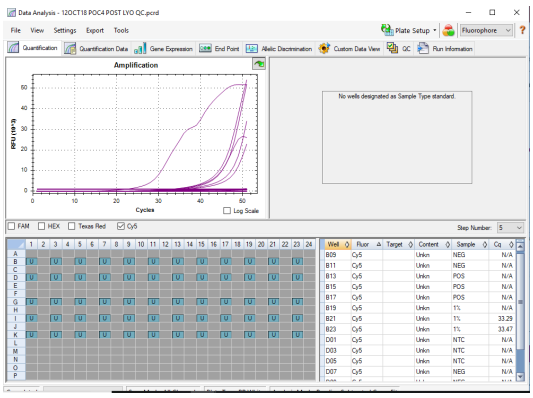
The evaluation of sensitivity and MERS-CoV cross-reactivity was performed using reference material (T1), blinded samples and a standard protocol provided by the FDA. The study included a range finding study and a confirmatory study for LoD. Blinded sample testing was used to establish specificity and 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 19.

Table 19: 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 12. ASSAY TROUBLESHOOTING

Problem	Cause	Solution
Fluorescence signals in No Template Control (NTC), e.g. Ct <= 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 13. CUSTOMER AND TECHNICAL SUPPORT

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  - Safety Data Sheets (SDSs; also known as MSDSs)

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)

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

PART 14. SYMBOLS USED IN PACKAGING

Symbols used in packaging

Symbol	Definition
Rx	Prescription Only
EUA	Emergency Use Authorization
	In vitro Diagnostic Use
	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 15. REFERENCE

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CDC guidelines for Sample collection –
https://www.cdc.gov/coronavirus/2019-ncov/lab/guidelines-clinical-specimens.html

FDA EUA guidance –
https://www.fda.gov/regulatory-information/search-fda-guidance-documents/policy-diagnostic-tests-coronavirus-disease-2019-during-public-health-emergency

Thermo Fisher viral RNA extraction kit PureLink™ Viral RNA/DNA Mini Kit (Cat# 12280050) Kit for RNA isolation
https://www.thermofisher.com/order/catalog/product/12280050#/12280050
Catalog Number 12280050 Publication NumberMAN0000562

QuantStudio 5
https://assets.thermofisher.com/TFS-Assets/LSG/manuals/MAN0017162_QS5HIDInstrument_UG.pdf
Publication Number MAN0017162

ABI 7500 Fast Dx
https://www.thermofisher.com/order/catalog/product/4406985#/4406985
Publication Part Number4406991