KarmaCare™

COVID-19 MDx RT-PCR

REF BT800

For the qualitative detection of human coronavirus SARS-CoV-2 viral RNA extracted from respiratory tract specimens.

Package Insert (Instructions for Use)

For use under an Emergency Use Authorization (EUA) only

For *in vitro* diagnostic use only

Rx Use only

Intended Use

The *KarmaCare*™ COVID-19 MDx RT-PCR is a real-time reverse transcription polymerase chain reaction (RT-PCR) test intended for the qualitative detection of nucleic acid from SARS-CoV-2 in upper respiratory specimens (such as nasopharyngeal, oropharyngeal and nasal swabs, and nasopharyngeal wash/aspirate or nasal aspirate) and bronchoalveolar lavage 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 the patient's infection status. Positive results do not rule out bacterial infection or co-infection with other viruses. The agent detected may not be the definite cause of the disease. 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.

Testing with the *KarmaCare*™ COVID-19 MDx RT-PCR is intended for use by qualified clinical laboratory personnel specifically instructed and trained in the techniques of real-time PCR and *in vitro* diagnostic procedures. The *KarmaCare*™ COVID-19 MDx RT-PCR is only for use under the Food and Drug Administration's Emergency Use Authorization (EUA).

Summary and Explanation of the Test

There is an outbreak of respiratory disease caused by a novel coronavirus that was first found in Wuhan City, Hubei Province, China. The virus has been named "SARS-CoV-2" and the disease it causes has been named "Coronavirus Disease 2019" (COVID-19). SARS-CoV-2 has been designated as a pandemic by the World Health Organization (WHO) and has been detected worldwide. SARS-CoV-2 is highly contagious and has a severe impact on healthcare systems and economy, globally and to the United States. To effectively control the spread of the SARS-CoV-2, rapid detection of cases and contacts is critical.

The *KarmaCare*™ COVID-19 MDx RT-PCR is intended for qualitative detection of SARS-CoV-2 RNA in nasopharyngeal, oropharyngeal,
and nasal swab specimens, nasopharyngeal wash/aspirate or nasal aspirate, and bronchoalveolar lavage from individuals suspected of COVID-19 by their healthcare provider. The specimen can be collected in Universal Transport (UTM-RT®, Copan Diagnostics, Inc), Universal Viral Transfer (UVT, BD™ Diagnostics) or equivalent. The collection media are not included in the test kit.

Principles of the Test

The *KarmaCare*™ COVID-19 MDx RT-PCR is a qualitative *in vitro* diagnostic assay consisting of reagents for RT-PCR amplification, detection of nucleic acids from SARS-CoV-2, and controls. The test is intended to be used with RNA that has been extracted from respiratory tract specimens obtained from patients who meet the CDC SARS-CoV-2 clinical criteria (e.g., fever, cough and shortness of breath may appear 2-14 days after exposure) in conjunction with the CDC SARS-CoV-2 epidemiological criteria (a history of travel from affected geographic areas within 14 days of their symptom onset, documented COVID-19 infections in a jurisdiction and known community transmission or other epidemiologic criteria for which SARS-CoV-2 testing may be indicated).

The nucleic acids are extracted from respiratory tract specimens using QIAamp® Viral RNA Mini Kit.

Selective amplification of target nucleic acid from the sample is achieved by using target-specific forward and reverse primers for the N gene and RdRP gene that are unique to SARS-CoV-2.

The internal control (MS2 Phage Control) provided in this kit is added to the sample during nucleic acid extraction process and the probe of internal control (MS2 Phage Control) conjugated to ROX included in 4x 1 Step RT-PCR Mix to monitor internal process of nucleic acid extraction. The internal control should be positive in a negative sample however it may be negative or positive for the positive sample. The internal control passes if it meets the validated acceptance criteria.

External positive and negative controls are provided and included in each test run to monitor the instrument's process.

Workflow

Collect and transport the nasopharyngeal and oropharyngeal swab specimen using the Universal Transport (UTM-RT®, Copan Diagnostics, Inc), Universal Viral Transfer (UVT, BD™ Diagnostics) or equivalent.

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Extract RNA from patient sample using the Qiagen QIAamp® Viral RNA Mini Kit

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Perform RT-PCR using the Applied Biosystems™ 7500/7500 Fast Real-Time PCR instrument
Or
Perform RT-PCR using CFX96 Touch™ Real-Time PCR Detection System

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Analyze data using the Applied Biosystems™ 7500 System SDS v1.4 21 CFR Part 11 Module
Or
Analyze data using CFX Manager™ Software v3.1

▼

Review run control results

▼

Review results interpretation for patient samples

Reagents and Materials Provided

The following table outlines reagents supplied in the kit and their storage conditions.

Components	Description	Amount	Storage
4x 1 Step RT-PCR Mix	TaqPath™ 1-Step Multiplex Master Mix (No ROX)	500 µL /vial	–30°C to –10°C
SARS-CoV-2 primer/probe Mix	SARS-CoV-2 Real-Time PCR Assay Multiplex (N gene, RdRP gene, and MS2)	500 µL /vial	–30°C to –10°C
MS2 Phage Control	Internal process control for nucleic acid extraction	2 x 500 µL /vial	–30°C to –10°C
External Positive Control	*In vitro* transcribed RNAs of N gene and RdRP gene	500 µL /vial	–30°C to –10°C
External Negative Control	Nuclease-free Water	500 µL /vial	–30°C to –10°C

Materials Required but not Provided

"MLS" indicates that the material is available at major laboratory suppliers.

Item	Source
Real-time PCR instrument and equipment	
Applied Biosystems™ 7500/7500 Fast Real-Time PCR Instrument (SDS Software v1.4. 21 CFR Part 11 Module)	Thermo Fisher: 4351104 (7500 with laptop) 4351105 (7500 with tower) 4359286 (7500 Fast with laptop) 4359284 (7500 Fast with tower)
CFX96 Touch™ Real-Time PCR Detection System (CFX Manager™ Software v3.1)	BioRad: 1855195
Freezers (-80°C ± 15°C and -20°C ± 10°C)	MLS
Refrigerator (2-8°C)	MLS
Microcentrifuge (compatible with 1.5 mL tubes)	MLS
Centrifuge capable of spinning 96 well plates at 3,000 rpm	MLS
Class II biosafety cabinet	MLS
Vortex mixer	MLS
Single and multichannel adjustable pipettors (1 µL to 1,000 µL)	MLS
Specimen collection kit	
Copan Univeral Transport Medium, UTM-RT® BD™ Univeral Viral Transport Medium (UVT) with swabs	Copan: 328C BD: 220221
Nucleic acid extraction system and materials	
QIAamp® Viral RNA Mini Kit	Qiagen: 52904 (for 50 reagents) 52906 (for 250 reagents)
Tubes, plates, and other consumables	
MicroAmp® Fast Optical 96-well Reaction Plate	Thermo Fisher: 4346907
MicroAmp® Optical 96-well Reaction Plate	Thermo Fisher: 4316813
MicroAmp® Optical Adhesive Film	Thermo Fisher: 4311971
MicroAmp® Splash Free 96-well Base	Thermo Fisher: 4312063
MicroAmp® Adhesive Film Applicator	Thermo Fisher: 4333183
Microcentrifuge tubes, sterile, RNase/DNase-free (1.5 mL)	MLS
Hard-Shell® 96-Well PCR Plates	BioRad: HSP-9655
Microseal® 'B' Adhesive Seals	BioRad: MSB-1001
Pipette tips with aerosol resistant barriers, RNase/DNase-free	MLS
Disposable, powder-free gloves	MLS

Precautions

1. For *in vitro* and prescription use only.
2. 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 CLIA of 1988, 42 U.S.C. §263a, that meet requirements to perform high complexity tests.
3. This test has been authorized only for the detection of nucleic acid from SARS-CoV-2, not for any other viruses or pathogens.
4. This test is only authorized for the duration of the declaration that circumstances exist justifying the authorization of emergency use of *in vitro* diagnostics for detection and/or diagnosis of COVID-19 under Section 564(b)(1) of the Act, 21 U.S.C. § 360bbb-3(b)(1), unless the authorization is terminated or revoked sooner.
5. Laboratories within the United States and its territories are required to report all results to the appropriate public health authorities.
6. As with all diagnostic tests, all results must be interpreted together with other clinical information available to the physician.
7. Positive results are indicative of SARS-CoV-2 RNA.
8. Treat all specimens as potentially infectious. Follow universal precautions when handling samples, this kit and its contents.
9. Always use pipette tips with aerosol barriers. Tips that are used must be sterile and free of DNases and RNases. Use only supplied or specified required consumables to ensure optimal test performance.
10. Excess blood on the swab specimen may interfere with test performance and may yield a false positive/negative result. Avoid touching any bleeding areas of the swab when collecting/handling the specimens.
11. All human-sourced materials should be considered potentially infectious and should be handled with universal precautions.
12. Do not use if the package or any assay components are damaged.
13. Other commercial controls have not been validated with this kit and are not recommended.
14. In order to obtain accurate results, the test operator must follow this instruction for use.
15. Perform the procedure given in this package insert as described. Any deviation from the outlined protocols may result in assay failure or cause erroneous results. Modifications to the reagents, protocol, or instrumentation is not permitted, and are in violation of the product Emergency Use Authorization.
16. Do not use the kit contents beyond the expiration date.
17. Use appropriate precautions in the collection, handling, storage, and disposal of patient samples and used kit contents.
18. Do not eat, drink or smoke in the area where the specimens and kit contents are handled.
19. Dispose of used contents as biohazardous waste in accordance with federal, state and local requirements.
20. Nitrile or latex gloves should be worn when performing this test.
21. If the extraction solution contacts the skin or eye, flush with copious amounts of water.
22. Handle all specimens as though they contain infectious agents.
23. Observe established precautions against microbiological hazards throughout the procedure and follow the standard procedures for proper disposal of specimens.
24. Do not interchange kit contents from different kit lots.
25. Do not re-use any contents in the kit.

Limitations

1. The *KarmaCare*™ COVID-19 MDx RT-PCR is for use with respiratory specimens.
2. The *KarmaCare*™ COVID-19MDx RT-PCR testing kit performance was established using nasopharyngeal swab specimens only.While other specimen types listed in the intended use are acceptable specimens (i.e., oropharyngeal swabs, nasopharyngeal wash/aspirate or nasal aspirate, mid-turbinate swabs, and BALs) for testing, performance with the *KarmaCare*™ COVID-19 MDx RT-PCR testing kit has not been established for these specimens.
3. This test may not be able to differentiate newly emerging SARS-CoV-2 subtypes.
4. The detection of viral RNA of SARS-CoV-2 is dependent upon proper specimen collection, handling, transportation, storage, and preparation, including extraction. Failure to observe proper procedures in any one of these steps can lead to incorrect results.
5. Negative results do not rule out SARS-CoV-2 infection and should not be used as the sole basis for treatment or other patient management decisions.
6. Results from the device should be correlated with the clinical history, epidemiological data and other data available to the clinician evaluating the patient.
7. This device has been evaluated for use with human specimen material only.
8. False negative results may occur if the number of copies of target RNA in the clinical specimen is below the detection limits of the device.
9. False negative results may occur if mutations are present in the regions targeted by the test.
10. This device is a qualitative test and does not provide information on the viral load present in the specimen.
11. The performance of this device has not been evaluated for monitoring treatment of SARS-CoV-2 infection.
12. The performance of this device has not been evaluated for the screening of blood or blood products for the presence of SARS-CoV-2.
13. This test cannot rule out diseases caused by other bacterial or viral pathogens.
14. Cross-reactivity with respiratory tract organisms other than those listed in the Analytical Specificity Study may lead to erroneous results.
15. The clinical performance has not been established in all circulating variants but is anticipated to be reflective of the prevalent variants in circulation at the time and location of the clinical evaluation. Performance at the time of testing may vary depending on the variants circulating, including newly emerging strains of SARS-CoV-2 and their prevalence, which change over time.

Storage and Stability

- Store kit at -30 ~ -10°C. Avoid multiple freeze-thaw cycles.
- The *KarmaCare*™ COVID-19 MDx RT-PCR kit reagents are stable until the expiration date printed on the outer packaging.

Quality Control

Internal Process Control:

- The *KarmaCare*™ COVID-19 MDx RT-PCR contains an internal procedural control, MS2 Phage Control, that will be added during nucleic acid extraction process.

External Positive and Negative Controls:

- An External Positive Control is used to monitor the RT-PCR instrument reaction setup and reagent integrity. All PCR reactions must include at least one positive control reaction. (The positive control is prepared by *in vitro* transcription kit, MEGAscript® Kit (cat. AM1333), from the cloned DNAs of N gene, and RdRP gene.)
- An External Negative Control is "No Template Control" and used to monitor any potential cross-contamination and reaction setup of the PCR mixture. All PCR reactions must include at least one negative control reaction. (The negative control is prepared using Nuclease-free Water.)

Specimen Type

Acceptable specimen type for testing with the *KarmaCare*™ COVID-19 MDx RT-PCR are respiratory specimens, including oropharyngeal swabs, nasopharyngeal swabs/wash/aspirate or nasal aspirate, mid-turbinate swabs, and BALs, from individuals suspected of COVID-19 by their healthcare provider. It is essential that correct specimen collection, handling, and transportation methods are followed. Inadequate specimen collection, improper specimen handling and/or transport may yield a false negative result; therefore, specimen collection requires specific training and guidance due to the importance of specimen quality to obtain accurate test results.

Specimen Collection, Handling, and Storage

NOTE: Handle all samples and controls as if they are capable of transmitting infectious agents.

Refer to Interim Guidelines for Collecting, Handling, and Testing Clinical Specimens from Patients Under Investigation (PUIs) for 2019 Novel Coronavirus (SARS-CoV-2) https://www.cdc.gov/coronavirus/2019-nCoV/guidelines-clinical-specimens.html. Follow specimen collection device manufacturer instructions for proper collection methods.

Swab specimens should be collected using the sterilized foam, flocked, nylon, or rayon swab with plastic shaft. Calcium alginate swabs are unacceptable and cotton swabs with wooden shafts are not recommended.

Specimens must be packaged, shipped, and transported according to the current edition of the International Air Transport Association (IATA) Dangerous Goods Regulation. Follow shipping regulations for UN 3373 Biological Substance, Category B when sending potential SARS-CoV-2 specimens. Store specimens at 2-8°C and ship overnight to laboratory on ice pack. If a specimen is frozen at -70°C or lower, ship overnight to laboratory on dry ice.

Specimens can be stored at 2-25°C for up to 48 hours after collection. If delivery and processing exceed 48 hours, specimens should be transported in dry ice and once in the laboratory, frozen at -70°C or lower. Extracted nucleic acid should be stored at -70°C or lower.

Nucleic Acid Extraction Methods

The *KarmaCare*™ COVID-19 MDx RT-PCR does not include reagents for isolating nucleic acids from samples. The assay has been validated for use with the Qiagen QIAamp Viral RNA Mini Kit. The user is responsible for following all procedures recommended by the manufacturer of the sample preparation method. The minimum specimen volume needed for purification processing is 140 µL, eluted in 60 µL buffer. The MS2 RNA Phage Control must be added to the sample prior to extraction. Add 5 µL of the MS2 RNA Phage Control to each sample to monitor extraction procedure, reagent integrity, and presence of inhibitors in the specimens. The negative control is not required for the extraction process.The extracted RNA sample should be used immediately after the extraction. The extracted RNA sample can be stored at -70°C or lower for storage.

Description of Symbols

- Consult instructions for use — Indicates the need for the user to consult the instructions for use.
- Manufacturer — Indicates the medical device manufacturer.
- REF Catalog number — Indicates the manufacturer's catalog number so that the medical device can be identified.
- Caution — Indicates the need for the user to consult the instructions for use for important cautionary information such as warnings and precautions that cannot, for a variety of reasons, be presented on the medical device itself.
- LOT Batch code — Indicates the manufacturer's batch code so that the batch or lot can be identified.
- Use by date — Indicates the date after which the medical device is not to be used.
- Contains sufficient for <n> tests — Indicates the total number of IVD tests that can be performed with the IVD.
- IVD For *In Vitro* Diagnostic Use
- Temperature limit — Indicates the temperature limits to which the medical device can be safely exposed.
- Do not use if the package is damaged — Indicates a medical device that should not be used if the package has been damaged or opened.
- Keep away from sunlight — Indicates a medical device that needs protection from light sources.
- Keep dry — Indicates a medical device that needs to be protected from moisture.

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Test Procedures

Perform RT-PCR using the Applied Biosystems™ 7500/7500 Fast Real-Time PCR instrument

Prepare the RT-PCR Reactions

1. If frozen, thaw the purified nucleic acid samples and reagents on ice.
2. Gently vortex the samples and reagents, then centrifuge briefly to collect liquid at the bottom of the tube or sample plate.
3. Prepare the Pre-Reaction Mix and Final Reaction Mix followed by the Table below. If replicates for each sample are desired, the volume of reagents should be adjusted accordingly. Make sure to include both External Positive Control (EPC), and External Negative Control (ENC) when calculating the Primer/Probe Mix volumes and when assigning wells on the thermal cycler.

Final Reaction Mix	
Components	**Volume per sample**
Pre-Reaction Mix	**10.0 µL**
• Primer/Probe Mix	5.0 µL
• 4x 1 step RT-PCR	5.0 µL
Sample, EPC, or ENC	**10.0 µL**
Total Volume per sample	**20.0 µL per sample**

NOTE: In order to cover the potential pipetting loss when preparing the Pre-Reaction Mix/Final Reaction Mix, it is recommended to calculate sufficient sample volume.

4. Set up the reaction plate—Pipette 20.0 µL of the Final Reaction Mix prepared in step 3 into each well of a MicroAmp® Fast Optical 96- Well Reaction Plate.
5. Cover the plate with MicroAmp® Optical Adhesive Film.

NOTE: Make sure to handle the MicroAmp® Optical Adhesive Film using MicroAmp® Adhesive Film Applicator. Do not touch the middle part of the cover.

6. Briefly centrifuge the plate to collect the reactions at the bottom of the wells and to eliminate any air bubbles.
7. Take the covered reaction plate to the Applied Biosystems™ 7500/7500 Fast Real-Time PCR Instrument.

Set-Up and Run the Applied Biosystems™ 7500/7500 Fast Real-Time PCR Instrument

NOTE: The *KarmaCare*™ COVID-19 MDx RT-PCR should be performed on the Applied Biosystems™ 7500/7500 Fast operating in Standard Emulation mode. See the Applied Biosystems™ 7500/7500 Fast Real-Time PCR Instructions for Use for detailed instructions. The instrument should be calibrated for each dye before starting the assay following the manufacturer's instructions, as described in Applied Biosystems™ 7500/7500 Fast Real-Time PCR System Maintenance Guide.

1. From the laptop or tower computer, open the "7500 System Software" program on the laptop or tower computer. A new window will appear.
2. Select "**Absolute quantification**" from the "**Assay Type**" drop-down menu. Select "**96- Well Clear**" from the "**Container**" drop-down menu. Select "**ABI COVID19 RNA**" from the "**Template**" menu. Click "**Finish**" at the bottom of the window.

NOTE: Download "ABI COVID19 RNA" template file from the following website: www.accessbio.net

3. Confirm the run settings as the Table below.

Analyte	Target Gene	Probe Fluorophore	Absorbance Peak	Emission Peak
SARS-CoV-2 (COVID-19)	N	FAM	495 nm	520 nm
SARS-CoV-2 (COVID-19)	RdRP	Cy5	651 nm	670 nm
Internal Control	MS2	ROX	575 nm	602 nm

4. Set up the instrument parameters of Applied Biosystems™ 7500/7500 Fast Real-Time PCR as shown below.

Stage Number	Stage Name	Temperature Setting	Time	Number of Cycle
1	UNG* Incubation	25°C	2 min	1 Cycle
2	cDNA Synthesis	55°C	10 min	1 Cycle
3	Pre-Denaturation	94°C	3 min	1 Cycle
4	Amplification	94°C	15 sec	45 Cycles
		58°C	30 sec	

**UNG: Uracil-N-glycosylase which is provided in the 4x 1 step RT-PCR mixture.*

- **Total volume per sample: 20 µL per sample**
- Choose **Standard** for correct thermal profile parameters

NOTE: Make sure all the settings and parameters are correct.

5. Refer to the manufacturer's user manual for details on how to run the plate on the Applied Biosystems™ 7500/7500 Fast Real-Time PCR systems.

Analyzing the Run Data

1. Click "**OK**" from the window once the system run is finished.
2. Enter the Analysis window by clicking "**Analysis**" under the experiment menu. Set the analysis parameters as below. Refer to **Figures 1 & 2** below for the steps.
   ① Select "**ΔRn vs Cycle**" for "**Plot Type**" and "**Log**" for "**Graph Type**" (#1).
   ② Click on "**Analysis Setting**" (#2). This will open the "**Analysis Settings**" window (Figure 2).
   ③ From the "**Analysis Setting**" window, highlight all of the targets under "**Select a Target**" (#3) by dragging.
   ④ In the window "**Ct Settings for the 3 Selected Targets**" (#4), un-check all of the boxes after all targets have been highlighted. Set all baseline value to "**5**" for "**Start Cycle**" and "**15**" for "**End Cycle**" by clicking the up/down arrow.
   ⑤ Click "**Apply Analysis Settings**" (#5). This will close the "**Analysis Settings**" window and return to "**Analysis**" window.
   ⑥ In the Analysis Window (Figure 1) identify the "**Options**" tab (#6), located below the Amplification Plot. Select each "**Target**" individually then highlight all control wells (i.e. Positive Controls and Negative Controls on the plate by clicking on each well. Select multiple wells by holding down the "**Ctrl**" key while clicking on each well.).
   ⑦ In "**Options**" tab (#6), set the "**Threshold**" for each target above all background noise, including that observed within the earliest cycles (cycles 1~4). This is accomplished by dragging the Threshold line (arrow; in Amplification Plot window) up/down, to the appropriate setting.
   ⑧ Click the green "**Analyze**" button (#2) to apply the changes and analyze the run data.

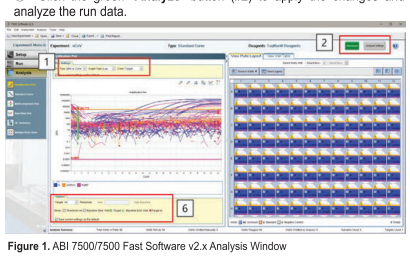

Figure 1. ABI 7500/7500 Fast Software v2.x Analysis Window

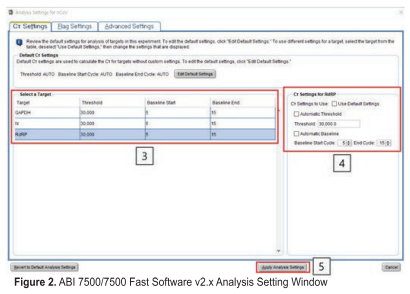

Figure 2. ABI 7500/7500 Fast Software v2.x Analysis Setting Window

Perform RT-PCR using the CFX96 Touch™ Real-Time PCR Detection System

Prepare the RT-PCR Reactions

1. If frozen, thaw the purified nucleic acid samples and reagents on ice.
2. Gently vortex the samples and reagents, then centrifuge briefly to collect liquid at the bottom of the tube or sample plate.
3. Prepare the Pre-Reaction Mix and Final Reaction Mix followed by the Table below. If replicates for each sample are desired, the volume of reagents should be adjusted accordingly. Make sure to include both External Positive Control (EPC), and External Negative Control (ENC) when calculating the Primer/Probe Mix volumes and when assigning wells on the thermal cycler.

Final Reaction Mix	
Components	**Volume per sample**
Pre-Reaction Mix	**10.0 µL**
• Primer/Probe Mix	5.0 µL
• 4x 1 step RT-PCR	5.0 µL
Sample, EPC, or ENC	**10.0 µL**
Total Volume per sample	**20.0 µL per sample**

NOTE: In order to cover the potential pipetting loss when preparing the Pre-Reaction Mix/Final Reaction Mix, it is recommended to calculate sufficient sample volume.

4. Set up the reaction plate—Pipette 20.0 µL of the Final Reaction Mix prepared in step 3 into each well of a Hard-Shell® 96-Well PCR Plates (Cat. HSP9655).
5. Cover the plate with Microseal® 'B' Adhesive Seals (Cat. MSB-1001).

NOTE: Make sure to handle the Microseal® 'B' Adhesive Seals (Cat. MSB-1001) using Film Sealing Roller. Do not Touch™ the middle part of the cover.

6. Briefly centrifuge the plate to collect the reactions at the bottom of the wells and to eliminate any air bubbles.
7. Take the covered reaction plate to the CFX96 Touch™ Real-Time PCR Detection System.

Set-Up and Run CFX96 Touch™ Real-Time PCR Detection System

NOTE: The *KarmaCare*™ COVID-19 MDx RT-PCR has been validated CFX96 Touch™ Real-Time PCR Detection System operating. See the CFX96 Touch™ Real-Time PCR Detection System PCR Instructions for Use for detailed instructions.

1. From the laptop or tower computer, open up the "CFX Manager™ Software v3.1" program on the laptop or tower computer. A new window will appear.
2. The Startup Wizard automatically appears when CFX Manager software is first opened.
   ① Select "File" menu. Select "Protocol…" from the "Open" drop-down menu.
   ② Select "**ABI COVID19 RNA**" of protocol files from protocol folder and click "Open(O)"

NOTE: Download "ABI COVID19 RNA" protocol file from the following website: www.accessbio.net

   ③ Check the instrument parameters of CFX96 Touch™ Real-Time PCR Detection System as follows.

Stage Number	Stage Name	Temperature Setting	Time	Number of Cycle
1	UNG* Incubation	25°C	2 min	1 Cycle
2	cDNA Synthesis	55°C	10 min	1 Cycle
3	Pre-Denaturation	94°C	3 min	1 Cycle
4	Amplification	94°C	15 sec	45 Cycles
		58°C	30 sec	

**UNG: Uracil-N-glycosylase which is provided in the 4x 1 step RT-PCR mixture.*

   ④ Click "OK" at the right bottom of the window.
   ⑤ Click "Next≫" at the right bottom of the window.
   ⑥ Click "Select Exiting…" at the left top of the window
   ⑦ Click "**ABI COVID19 RNA-plate**" of plate files from plate folder and click "Open(O)"
   - Click "Edit Selected…" and confirm the run settings as the Table below.

NOTE: Download "ABI COVID19 RNA-plate" template file from the following website: www.accessbio.net

Analyte	Target Gene	Probe Fluorophore	Absorbance Peak	Emission Peak
SARS-CoV-2 (COVID-19)	N	FAM	510 nm	530 nm
SARS-CoV-2 (COVID-19)	RdRP	Cy5	675 nm	690 nm
Internal Control	MS2	ROX	610 nm	650 nm

   ⑧ "Next" at the right bottom of the window.
   ⑨ Select one or more blocks, edit run parameters (if necessary), and then click the "Start Run" button to begin the run.
   - When you click the Start Run button, CFX Manager software prompts you to save the name of the data file and then opens the Run Details window.
   ⑩ Refer to the manufacturer's user manual for details on how to run the plate on the CFX96 Touch™ Real-Time PCR Detection System.

Analyzing the Run Data

1. From the laptop or tower computer, open up the "CFX Manager™ Software v3.1" program on the laptop or tower computer. A new window will appear.
2. The Startup Wizard automatically appears when CFX Manager software is first opened.
   ① Select "File" menu. Select "Data File…" from the "Open" drop-down menu.
   ② Select the file you want to analyze from data folder and click "Open(O)"
3. Analysis setting

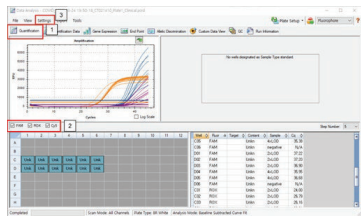

Figure 3. CFX Manager™ Software v3.1

① In the "Quantification"(#1) Window (Figure 3), select only one fluorophore (#2) located below the "Amplification". Select "Protocol…" from the "Open" drop-down menu.
② Select "Baseline Threshold…" from the "Settings" (#3) drop-down menu.

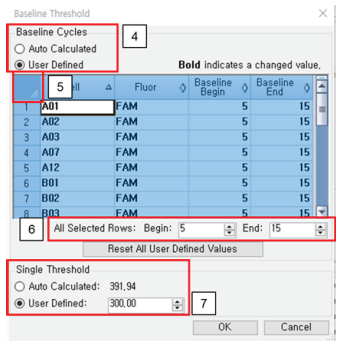

Figure 4. CFX Manager™ Software v3.1 Baseline Threshold Window

③ Select "User Defined"(#4) (Figure 4) in "Baseline Cycles" menu.
④ Click (#5).
⑤ In "All Selected Rows" menu(#6), select 15 for "End" and selects 5 for "Begin".
⑥ In "Single Threshold" menu(#7), select 300 for "User Defined"
⑦ Click "OK"
⑧ Analyze data in "Quantification" menu(#8) (Figure 5).

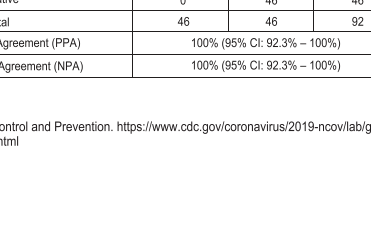

Figure 5. CFX Manager™ Software v3.1 Quantification Data Window

Interpretation of Results and Reporting

Expected Results of External Positive / Negative Controls

All test controls should be examined prior to interpretation of patient results.
If the controls are not valid, the patient results cannot be interpreted or reported, and testing of patient samples should be repeated.
- A positive signal is defined as a Ct value of less than or equal to 43 cycles (Ct ≤ 43 cycles).
- A negative signal is defined as a Ct value of greater than 43 cycles (Ct > 43 cycles).

Validity Criteria

Valid Assay Run: An assay run is determined to be valid when all of the following criteria are met:
- External Positive Control returns a positive signal for the target sequence present in the Control.
  – N gene (FAM channel) – RdRP gene (Cy5/Quarsar 670 channel)
- External Negative Control is negative for all targets.

Invalid Assay Run: An assay run is determined to be invalid when any of the following criteria are met:
- External Positive Control returns a negative signal for the target sequence present in the control.
  – Negative in N gene (FAM) and/or RdRP gene (Cy5/Quarsar 670)
- External Negative Control (No Template Control) is positive for any target signal (FAM, Cy5/Quarsar 670 channel, and/or ROX).

NOTE: A sample that does not return a positive signal for any SARS-CoV-2 RNA target must return a positive signal for internal control (ROX channel). The positive signal observed for any of the SARS-CoV-2 RNA targets show either positive or negative signal for internal control (ROX channel).

Examination and Interpretation of Patient Specimen Results:
- A positive signal is defined as a Ct value of less than or equal to 43 cycles (Ct ≤ 43 cycles).
- A negative signal is defined as a Ct value of greater than 43 cycles (Ct > 43 cycles).

N gene (FAM)	RdRP gene (Cy5/ Quarsar 670)	Internal Control (Rox)	Status	Result	Action
Positive	Positive	Positive	Valid	COVID-19 Positive	Report results to healthcare provider
Positive	Positive	Negative	Valid[a]	COVID-19 Positive	Report results to healthcare provider
Positive	Negative	Positive	Valid	COVID-19 Positive	Report results to healthcare provider
Positive	Negative	Negative	Valid[a]	COVID-19 Positive	Report results to healthcare provider
Negative	Positive	Positive	Valid	COVID-19 Positive	Report results to healthcare provider
Negative	Positive	Negative	Valid[a]	COVID-19 Positive	Report results to healthcare provider
Negative	Negative	Positive	Valid	COVID-19 Negative	Report results to healthcare provider; Consider testing for other other viruses
Negative	Negative	Negative	Invalid	NA	Repeat test. If the repeat result remains invalid, consider collecting a new specimen.

[a] If the positive signal is strong, this affects the amplification of the internal control, and the internal control may appear negative.

Conditions of Authorization for the Laboratory

The *KarmaCare*™ COVID-19 MDx RT-PCR 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/vitro-diagnostics-euas.
However, to assist clinical laboratories using the *KarmaCare*™ COVID-19 MDx RT-PCR ("your product" in the conditions below), the relevant Conditions of Authorization are listed below:
A. Authorized laboratories[1], using your product will include with test result reports, all authorized Fact Sheets. Under exigent circumstances, other appropriate methods for disseminating these Fact Sheets may be used, which may include mass media.
B. Authorized laboratories using your product will use your product as outlined in the authorized labeling. 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 Access Bio, Inc. (info@accessbio.net) any suspected occurrence of false positive or false negative results and significant deviations from the established performance characteristics of your product of which they become aware.
F. All laboratory personnel using your product must be appropriately trained in PCR techniques and use appropriate laboratory and personal protective equipment when handling this kit, and use your product in accordance with the authorized labeling.
G. Access Bio, Inc., 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."*

Performance characteristics

Limit of Detection

The SARS-CoV-2, Isolate USA-WA1/2020 heat-inactivated (BEI Cat# NR-52286, Lot# 70033548), obtained from BEI resources was tested at varying concentrations to determine and confirm the Limit of Detection (LoD). Dilutions were prepared by spiking the SARS-CoV-2 heat-inactivated virus in this negative confirmed swab eluate pool. The extracted sample was tested following the product instructions for testing.

Testing concentration (copies/ reaction)	Replicate	Applied Biosystems™ 7500/7500 Fast Real-Time PCR		CFX96 Touch™ Real-Time PCR	
		N gene FAM (Ct score / result)	RdRP gene Cy5/Quasar670 (Ct score / result)	N gene FAM (Ct score / result)	RdRP gene Cy5/Quasar670 (Ct score / result)
40	1	33.09 / Positive	33.61 / Positive	31.95 / Positive	32.61 / Positive
	2	32.70 / Positive	33.35 / Positive	31.97 / Positive	32.20 / Positive
	3	33.19 / Positive	32.99 / Positive	32.00 / Positive	33.00 / Positive
20	1	33.53 / Positive	35.28 / Positive	33.06 / Positive	34.60 / Positive
	2	34.17 / Positive	35.16 / Positive	34.19 / Positive	36.48 / Positive
	3	34.15 / Positive	35.39 / Positive	34.67 / Positive	35.80 / Positive
10	1	35.19 / Positive	37.47 / Positive	36.82 / Positive	37.90 / Positive
	2	34.47 / Positive	37.63 / Positive	36.74 / Positive	37.62 / Positive
	3	35.05 / Positive	37.43 / Positive	36.12 / Positive	39.30 / Positive
5	1	NA / Negative	NA / Negative	NA / Negative	40.35 / Positive
	2	42.93 / Positive	NA / Negative	NA / Negative	NA / Negative
	3	NA / Negative	NA / Negative	NA / Negative	NA / Negative

The confirmed LoD was defined as the lowest testing concentration level that was detected ≥95% of the time, for contrived SARS-CoV-2 positive sample tested, were presented in the Table below:

Testing concentration (copies/reaction)	Replicate	Applied Biosystems™ 7500/7500 Fast Real-Time PCR		CFX96 Touch™ Real-Time PCR	
		N gene FAM (Ct score / result)	RdRP gene Cy5/Quasar670 (Ct score / result)	N gene FAM (Ct score / result)	RdRP gene Cy5/Quasar670 (Ct score / result)
10	1	36.05 / Positive	37.39 / Positive	35.72 / Positive	37.91 / Positive
	2	36.01 / Positive	37.24 / Positive	39.70 / Positive	38.16 / Positive
	3	36.43 / Positive	37.06 / Positive	36.91 / Positive	38.88 / Positive
	4	37.29 / Positive	38.10 / Positive	38.10 / Positive	37.72 / Positive
	5	38.42 / Positive	41.47 / Positive	36.64 / Positive	39.33 / Positive
	6	38.51 / Positive	39.14 / Positive	36.72 / Positive	37.81 / Positive
	7	37.98 / Positive	39.55 / Positive	38.50 / Positive	39.85 / Positive
	8	36.26 / Positive	37.06 / Positive	36.07 / Positive	38.18 / Positive
	9	36.18 / Positive	37.11 / Positive	37.55 / Positive	38.33 / Positive
	10	37.13 / Positive	40.07 / Positive	39.08 / Positive	39.80 / Positive
	11	36.00 / Positive	37.64 / Positive	36.99 / Positive	37.80 / Positive
	12	36.38 / Positive	38.27 / Positive	36.04 / Positive	39.36 / Positive
	13	40.26 / Positive	41.87 / Positive	39.50 / Positive	37.04 / Positive
	14	41.45 / Positive	40.64 / Positive	37.45 / Positive	39.64 / Positive
	15	37.21 / Positive	37.35 / Positive	38.36 / Positive	39.09 / Positive
	16	37.00 / Positive	37.23 / Positive	38.09 / Positive	38.02 / Positive
	17	36.41 / Positive	37.31 / Positive	37.48 / Positive	37.00 / Positive
	18	36.87 / Positive	37.51 / Positive	37.57 / Positive	39.24 / Positive
	19	36.05 / Positive	37.33 / Positive	37.77 / Positive	36.02 / Positive
	20	37.18 / Positive	40.00 / Positive	36.13 / Positive	39.94 / Positive
Result percentile % (# of positive / # of replicate)		100% (20/20)	100% (20/20)	100% (20/20)	100% (20/20)

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 corroborate the LoD. The extraction method used was the Qiagen QIAamp® Viral RNA Mini Kit. The results are summarized in Table A.

Table A: Summary of LoD Confirmation Result Using the FDA SARS-CoV-2 Reference Panel

Real-time PCR instrument	Reference Materials Provided by FDA	Specimen Type	Product LoD	Cross-Reactivity
CFX96 Touch™ Real-Time PCR	SARS-CoV-2	NPS	1800 NDU/mL	N/A
	MERS-CoV		N/A	ND
Applied Biosystems™ 7500/7500 Fast Real-Time PCR	SARS-CoV-2	NPS	5400 NDU/mL	N/A
	MERS-CoV		N/A	ND

NPS: Nasopharyngeal Swabs NDU/mL: RNA NAAT detectable units/mL N/A: Not Applicable ND: Not Detected

Analytical Reactivity (Inclusivity)

***In silico* analysis**

The analytical reactivity (inclusivity) of *KarmaCare*™ COVID-19 MDx RT-PCR was evaluated using *in silico* analysis of the assay primers and probes in relation to the total of 8715 SARS-CoV-2 sequences available in the GenBank and GISAID gene database as of May 28, 2020 for two targets including N and RdRP gene.
The primer/probe set for the N and RdRP gene had a 100% match to all sequences except for 8 and 5 sequences respectively. The mismatch results are as follows;

- 1 mismatch in the forward primer for the N gene (GenBank Accession ID; MT292570, GISAID Accession ID; EPI_ISL_445126, EPI_ISL_427439)
- 1 mismatch in the reverse primer for the N gene (GenBank Accession ID; MT370876)
- 1 mismatch in the probe for the N gene (GenBank Accession ID; MT371035, GISAID Accession ID; EPI_ISL_444772, EPI_ISL_424951)
- 1 mismatch in the forward primer for the RdRP gene (GenBank Accession ID; MT451726)
- 1 mismatch in the reverse primer for the RdRP gene (GenBank Accession ID; MT461651, GISAID Accession ID; EPI_ISL_444703)
- 1 mismatch in the probe for the RdRP gene (GenBank Accession ID; MT325588, GISAID Accession ID; EPI_ISL_424870)

Despite the mismatches identified above, the *KarmaCare*™ COVID-19 MDx RT-PCR test is still expected to detect all the SARS-CoV-2 strains included in the *in silico* analysis, because every mismatch identified above is based on only one of the two targets that are amplified during the test. Even if a single mutation affects amplification/detection of one of the targets, the presence of the other target can still generate a valid positive result.

Analytical Specificity – Cross Reactivity (Exclusivity)

***In silico* analysis**

In silico analysis was performed on all potential cross-reactive microorganisms described in Table below. The *in silico* blast analysis evaluated whether there is any significant amplification of non-target sequences that could either result is cross-reactive or potentially interfere to the detectability of the actual target analyte.
The blast analysis showed ≥ 80% homology for one of assay component (forward primers, reverse primers, or probes) for forty-nine (49) indicated organisms. Despite ≥ 80% homology of one assay component for forty-nine (49) indicated organisms, there is no anticipated amplification because the hybridization of all three assay components is necessary to generate a signal. The *in silico* analysis indicates that significant amplification of non-target sequences that result in cross-reactivity or potentially interfere with the detection of SARS-CoV-2 is not likely to occur.

Human coronavirus, 229E	*Corynebacterium diphtheriae*	*Legionella longbeachae*
Human coronavirus, OC43	*Coxiella burnetii*	*Legionella pneumophila*
Human coronavirus, HKU1	*Enterobacter cloacae*	*Leptospira interrogans*
Human coronavirus, NL63	*Enterococcus faecium*	*Moraxella catarrhalis*
SARS-coronavirus	*Enterovirus, EV-D68*	*Mycobacterium tuberculosis*
MERS-coronavirus	*Escherichia coli*	*Mycoplasma pneumoniae*
Adenovirus, 71	*Fusobacterium massiliense*	*Neisseria elongata*
Bacillus anthracis	*Haemophilus influenzae*	*Neisseria meningitidis*
Bacillus subtilis	*Human Metapneumovirus (hMPV)*	*Pseudomonas aeruginosa*
Bacteroides fragilis	*Human Parainfluenza virus, 4a*	*Rhinovirus*
Bifidobacterium adolescentis	*Human parechovirus 1*	*Ruminococcus champanellensis*
Bordetella pertussis	*Human RSV*	*Staphylococcus aureus*
Candida albicans	*Influenza A*	*Staphylococcus epidermis*
Chlamydia pneumoniae	*Influenza B*	*Staphylococcus salivarius*
Chlamydia psittaci 6BC	*Influenza C*	*Streptococcus pneumoniae*
Clostridium botulinum	*Lactobacillus rhamnosus*	*Streptococcus pyogenes*

Cross Reactivity (Exclusivity) Wet Test

The cross reactivity (exclusivity) study was performed by testing 22 potentially cross-reacting organisms with the *KarmaCare*™ COVID-19 MDx RT-PCR. The negative testing samples were prepared with each bacterial and viral extracted RNA at the testing concentration indicated in the Table below without SARS-CoV-2. The testing samples were tested in triplicate on *KarmaCare*™ COVID-19 MDx RT-PCR. All the triplicates of microorganisms were tested as negative at the testing concentration, indicating none of the tested microorganisms cross-reacted on the *KarmaCare*™ COVID-19 MDx RT-PCR presented in the Table below:

Microorganisms	Strain	Reference#	Testing concentration	Results of triplicate without SARS-CoV-2 (# of positive / # of replicate)
Human Coronavirus	OC43	VR-1558	2.8×10^5 $TCID_{50}$/mL	0/3
Human Coronavirus	NL63	NR-470	1.6×10^6 $TCID_{50}$/mL	0/3
Avian Coronavirus	Massachusetts	NR-43284	2.8×10^7 $CEID_{50}$/mL	0/3
Canine Coronavirus	UCD1	NR-868	1.6×10^6 $TCID_{50}$/mL	0/3
SARS Coronavirus	Urbani	NR-9323	10^5 copies/µL	0/3
MERS Coronavirus	EMC/2012	NR-50171	8.9×10^6 $TCID_{50}$/mL	0/3
Human Astrovirus Type 1	Oxford	NR-51388	2.8×10^7 $TCID_{50}$/mL	0/3
Human Astrovirus Type 2	Oxford	NR-51389	1.6×10^{10} $TCID_{50}$/mL	0/3
Human RSV	A2000/3-4	NR-28530	2.8×10^5 $TCID_{50}$/mL	0/3
Influenza B Virus	B/Florida/4/2006	NR-9696	2.81×10^8 $CEID_{50}$/mL	0/3
Kilbourne F113	A/England/42/1972 (HA, NA) x A/Puerto Rico/8/1934 (H3N2)	NR-3623	1.4×10^7 $CEID_{50}$/mL	0/3
Klebsiella oxytoca	MIT 10-5244	HM-625	10^5 copies/µL	0/3
Klebsiella pneumoniae	Isolate 1	NR-15410	10^5 copies/µL	0/3
Leptospira interrogans	HAI0156	NR-19891	10^5 copies/µL	0/3
Measles Virus	Edmonston	NR-44362	2.8×10^4 $TCID_{50}$/mL	0/3
Mycobacterium abscessus	#103	NR-44261	10^5 copies/µL	0/3
Mycobacterium avium	2285 Smooth	NR-44265	10^5 copies/µL	0/3
Mycobacterium intracellulare	1956	NR-44267	10^5 copies/µL	0/3
Pseudomonas aeruginosa	Boston 41501	27853	10^5 copies/µL	0/3
Staphylococcus aureus	AIS 1000505	NR-46420	32 ug/mL	0/3
Staphylococcus aureus	M0200	NR41881	10^5 copies/µL	0/3
Streptococcus pneumoniae	TCH8431	HM-145	10^5 copies/µL	0/3

Clinical Evaluation

The clinical performance of *KarmaCare*™ COVID-19 MDx RT-PCR was evaluated using 46 negative and 46 positive retrospectively collected clinical NP swab samples from symptomatic patients in the New York area during the 2020 COVID-19 pandemic period. All 92 retrospective samples were collected by nasopharyngeal swab and stored in viral transport medium. The NP swab samples were confirmed by the FDA-EUA cleared RT-PCR as a comparator method.
All the negative and positive samples were tested in a blinded fashion. Each sample was assigned a unique identification number. The expected results of the samples were completely blinded to the operators in this study. All 92 retrospective NP swab samples were extracted individually and tested according to the *KarmaCare*™ COVID-19 MDx RT-PCR testing procedures.
All 46 negative samples were tested as negative. All 46 positive samples were tested as positive in the *KarmaCare*™ COVID-19 MDx RT-PCR. No false positive or false negative result was observed. The results for the negative and positive samples are shown in the Tables below:

KarmaCare™ COVID-19 MDx RT-PCR (Applied Biosystems™ 7500/7500 Fast Real-Time PCR)	Molecular Comparator		
	Positive	Negative	Total
Positive	46	0	46
Negative	0	46	46
Total	46	46	92
Positive Percent Agreement (PPA)	100% (95% CI: 92.3% – 100%)		
Negative Percent Agreement (NPA)	100% (95% CI: 92.3% – 100%)		

KarmaCare™ COVID-19 MDx RT-PCR (CFX96 Touch™ Real-Time PCR)	Molecular Comparator		
	Positive	Negative	Total
Positive	46	0	46
Negative	0	46	46
Total	46	46	92
Positive Percent Agreement (PPA)	100% (95% CI: 92.3% – 100%)		
Negative Percent Agreement (NPA)	100% (95% CI: 92.3% – 100%)		

References

1. Centers for Disease Control and Prevention. https://www.cdc.gov/coronavirus/2019-ncov/lab/guidelines-clinical-specimens.html