Clinomics TrioDx RT-PCR COVID-19 Test

("TrioDx")

Catalog number TR-US-01

Instructions for Use (IFU)

Version 1.7 June 22, 2021

For *in vitro* diagnostic use

For Emergency Use Authorization (EUA) Only

Prescription Use Only

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1. Intended Use

Clinomics TrioDx RT-PCR COVID-19 Test is an *in vitro* diagnostic real-time RT-PCR test intended for the qualitative detection of nucleic acid from SARS-CoV-2 in nasopharyngeal, oropharyngeal, mid-turbinate, and anterior nasal swabs collected from individuals suspected of COVID-19 by their healthcare provider. Testing authorized by the U.S. FDA is limited to laboratories that are certified under the Clinical Laboratory Improvement Amendments of 1988 (CLIA), 42 U.S.C. §263a, and 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 upper 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 infection status. Positive results do not rule out bacterial infection or co-infection with other viruses. The agent detected may not be the definite cause of disease. Laboratories within the United States and its territories are required to report all 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 TrioDx is intended for use by qualified and trained clinical laboratory personnel specifically instructed in the techniques of real-time PCR and *in vitro* diagnostic procedures. The TrioDx is only for use under the Food and Drug Administration's Emergency Use Authorization.

2. Description:

Coronavirus Disease 2019 (COVID-19) is an infectious respiratory disease that initially outbroke in Wuhan, Hubei Shen, China in late 2019 and quickly spread around the world, causing the World Health Organization (WHO) to declare a pandemic on March 11th, 2020.

Clinomics TrioDx RT-PCR COVID-19 Test (TrioDx) is a real-time reverse transcription polymerase chain reaction (Real-time RT-PCR) test for detection of novel coronavirus (SARS-CoV-2) in upper respiratory specimens from individuals suspected of COVID-19 by their health-care provider. The SARS-CoV-2 primer and probe sets included in the test detect three genes present in SARS-CoV-2 viral genome RNA: RdRp (RNA-dependent RNA polymerase), E (Envelope) and N (Nucleoprotein).

3. Product Overview

The TrioDx is a multiplex real-time reverse transcription polymerase chain reaction test (real-time RT-PCR). The SARS-CoV-2 Primer and Probe sets are designed to detect RNA of the 2019-SARS nCoV in upper respiratory specimens from patients suspected of COVID-19 disease by their healthcare provider. The nucleic acid sequences of primers and probes and their target genes used in the test are listed in the following table.

Table 1. Primer and probe sets and target genes

Target	Fluorescent dye	Quencher
RdRp	HEX	ZEN/IBFQ
E	TEXAS RED	ZEN/IBFQ
N	FAM	IBRQ
GAPDH (Internal Control)	Cy5	TAO/IBRQ

4. Test Principle

RNA extraction is performed using a manual process using QIAamp Viral RNA Mini Kit or an automated process using either the Maxwell RSC Viral Total Nucleic Acid Multi-Pack Kit with the Maxwell RSC 48 instrument or MagMAX Viral/Pathogen II Nucleic Acid Isolation Kit with KingFisher Flex with the 96 Deepwell Head Instrument. In each process, the RNA is extracted from 140 µL of Viral Transport Medium (VTM) or Universal Transport Medium (UTM) containing upper respiratory specimens. An elution volume of 50 µL is used to obtain the purified RNA. If the manual process is used, the standard procedure in the user manual provided by the kit manufacturer, Qiagen, is followed. If the RNA extraction is performed with an automated process using the Maxwell RSC Viral Total Nucleic Acid Multi-Pack Kit with Maxwell RSC 48 Instrument, the standard procedure in the user manual provided by the kit manufacturer, Promega, is followed.
If the RNA extraction is performed with an automated process using the MagMAX Viral/Pathogen II Nucleic Acid Isolation Kit with the KingFisher Flex with 96 Deepwell Head Instrument, the standard procedure in the user manual provided by the kit manufacturer, Thermo Fisher, is followed.

5 µL of purified RNA is reverse transcribed using 2X One Step Real-Time RT-PCR enzyme mix into cDNA, which is subsequently amplified in a QuantStudio 6 Real-Time PCR Detection System. In the process, each of the 4 probes with unique reporter dyes anneals to their specific target sequences located between the corresponding forward and reverse primers. During the extension phase of the PCR cycle, the 5' exonuclease activity of Taq polymerase hydrolyzes the probe, causing the reporter dye to separate from the quencher dye, generating a fluorescent signal. The signals from the 4 uniquely labeled probes (each targeting RdRp, E, N or GAPDH) are detected in 4 distinct fluorescent channels. With each cycle, additional reporter dye molecules are cleaved from their respective probes, increasing the fluorescence intensity. The increase in fluorescence intensity is monitored and recorded at each PCR cycle by the QuantStudio 6 Real Time PCR Detection System. The result of the amplification can be viewed through QuantStudio Real-Time PCR software. The user must interpret the results manually based on the suggested Ct values in **Section 12** (**Interpretation of Results**).

Figure 1. Overview of workflow

5. Kit Contents (Materials provided)

The reagents included in one TrioDx kit are sufficient for testing 100 specimens.

The Kit Catalog number is TR-US-01.

Table 2. Components included with the test kit

Name	Specifications	Quantity	Sub-components	Storage upon receipt
2X Real-Time RT-PCR Mix	1 mL	1	RT enzyme, Taq Polymerase, dNTPs, PCR buffer, Mg2+	-20°C
10X Primer & Probe Mix (PPM)	200 µL	1	SARS-nCoV-19 RdRp Primers+ HEX Probe, E gene Primers + Texas Red Probe, N gene Primers+ FAM Probe, GAPDH (IC) Primers+ Cy5 Probe	-20°C
SARS-CoV-2 Positive Control (1×10^4 copies/µL)	20 µL	1	Synthetic RNA fragments spanning RdRp, E, N and GAPDH target regions	-20°C
RNase Free H_2O	1.5 mL	1	RNase Free H_2O water provided for: 1. Negative Control 2. Final Diluted Positive Control 3. RT-PCR Mix	-20°C

6. Required Materials Not Provided

The following table is a list of required components not included with the test but required. It is highly recommended to use the items specified (catalog # and supplier name provided).

Table 3. Required materials not provided

Required Materials not included (name)	Units included per package	Units needed per run	Recommended Supplier	Catalog #/ID
Swab specimen in VTM/UTM	1	1	Provided by healthcare provider	n/a
QIAamp Viral RNA Mini Kit	250	1	Qiagen	52906
Maxwell RSC 48 Instrument	1	1	Promega	AS8500
Maxwell RSC Viral Total Nucleic Acid Kit	144	1	Promega	ASB1330
KingFisher Flex with 96 Deepwell Head Instrument	1	1	Thermo Fisher	5400630
MagMAX Viral/Pathogen II Nucleic Acid Isolation Kit	2,000	1	Thermo Fisher	A48383
KingFisher Deepwell 96 Plate	50	1	Thermo Fisher	95040450
KingFisher 96 tip comb for Deepwell Magnets	100	1	Thermo Fisher	97002534
KingFisher 96 KF microplate	48	1	Thermo Fisher	97002540
1.5 mL microtubes	250 tubes	2	Corning (Axygen)	MCT-150-C-S
MicroAmp™ Optical 96-Well Reaction Plate	500 plates	1 (for 94 samples)	Thermo Fisher Scientific (Applied Biosystems™)	4316813
MicroAmp™ Optical 8-Cap Strips	300 strips	96 (for 94 samples)	Thermo Fisher Scientific (Applied Biosystems™)	4323032
MicroAmp™ Optical Adhesive Film	100 covers	1 (for 94 samples)	Thermo Fisher Scientific (Applied Biosystems™)	4311971
10 µL Filter Tips	96 Tips/Rack	Many	Corning (Axygen)	TXLF-10-R-S
200 µL Filter Tips	96 Tips/Rack	Many	Corning (Axygen)	TF-200-R-S
1000 µL Filter Tips	100 Tips/Rack	Many	Corning (Axygen)	TF-1000-R-S
1-10 µL Pipet	1	1	Eppendorf	3123000020
20-200 µL Pipet	1	1	Eppendorf	3120000054
100-1000 µL Pipet	1	1	Eppendorf	3123000063
Micro Centrifuge	1	1	Eppendorf	5424R

Mini centrifuge	1	1	Benchmark	C1012
Vortex Mixer	1	1	Scientific Industries	SI-0236
Cold Block	1	1	-	-
Racks for 1.5 mL microcentrifuge tubes	1	1	-	-
Racks for 96-Well Plate	1	1	-	-
Powder-free gloves	1	Changing gloves often is recommended	-	-
UV-protective goggles	1	1	-	-
Laboratory coat	1	1	-	-
Decontamination solution	1	1	Thermo Fisher Scientific	AM9890
Heat Block	1	1	-	-
Nuclease-free Water	1	1	Thermo Fisher Scientific	AM9938

7. Real-time PCR Instrument

The TrioDx is to be used with the ABI QuantStudio 6 Flex (Thermo Fisher, Product No. 4485692, Software version 1.3). The instrument requires qualification prior to use with the Clinomics TrioDx. Please refer to the instrument qualification protocol in Appendix A for the required protocol and acceptance criteria. Please refer to Appendix B of this IFU for EUO labeling that should be affixed to select instruments.

TEST TIME AND THROUGHPUT

The QuantStudio 6 Flex instrument capability alone, as well as paired with manual and automated extraction steps is shown below for one lab technician working an 8 hour shift. The testing capability can be increased by optimizing lab technician schedules and incorporating automated extraction into the process.

	QuantStudio 6 Flex alone	QuantStudio 6 Flex and QIAamp manual extraction	QuantStudio 6 Flex and Maxwell automated extraction	QuantStudio 6 Flex and Kingfisher automated extraction
1 lab tech, 8 hour shift**	564	94	282	282

** The lab technician "8 hour" shift is 9 total hours including breaks

PCR Instrument

A total of 94 specimens plus controls is run per RT-PCR plate, and with a run time of approximately 1.5 hours per plate for the QuantStudio 6 Flex instrument.

Using Manual RNA extraction:

The *hands-on* time is approximately 4 hours for RNA extraction with manual preparation for 96 samples using QIAamp Viral RNA Mini Kit. The *hands-on* time for PCR reaction mix preparation is 30 minutes and the real time PCR *instrument* time is approximately 1.5 hours, which is followed by 30 minutes of result analysis and interpretation. If running 94 samples (plus 2 controls) in a 96-well plate at a time, two trained lab technicians with overlapping 8 hour shifts can finish testing up to 282 patient samples per day.

Using Automated RNA extraction:

For automated preparation using Maxwell RSC Viral Total Nucleic Kit with Maxwell RSC 48 Instrument, the *hands-on time* is approximately 30 minutes for 48 samples (or 1 hour for 96 samples) for RNA extraction. The extraction *instrument* time is 30 minutes per 48 samples (or 1 hour for 96 samples). The *hands-on* time for PCR reaction mix preparation is 30 minutes and the real time PCR *instrument* time is approximately 1.5 hours, which is followed by 30 minutes of result analysis and interpretation. If running 94 samples (plus 2 controls) in

a 96-well plate at a time, two trained lab technicians with overlapping 8 hour shifts can finish testing up to 658 patient samples per day.

For automated preparation using MagMAX Viral/Pathogen II Nucleic Acid Isolation Kit with KingFisher Flex with 96 Deepwell Head Instrument, the *hands-on* time is approximately 1 hour for 96 samples for RNA extraction. The extraction *instrument* time is 30 minutes for 96 samples. The *hands-on* time for PCR reaction mix preparation is 30 minutes and the real time PCR *instrument* time is approximately 1.5 hours, which is followed by 30 minutes of result analysis and interpretation. If running 94 samples (plus 2 controls) in a 96-well plate at a time, two trained lab technicians with overlapping 8 hour shifts can finish testing up to 658 patient samples per day.

8. Warnings and Precautions

Use Statements

- Prescription use only. Federal Law restricts this device to sale by or on the order of licensed practitioner.
- For *in-vitro* diagnostic use (IVD)
- For use under Emergency Use Authorization (EUA) only
- The TrioDx has not been FDA cleared or approved; but has been authorized by FDA under an EUA for use in authorized laboratories.
- The TrioDx has been authorized only for use for the detection of nucleic acid from SARS-CoV-2 and not for any other viruses or pathogens.
- The emergency use of TrioDx 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) o the Federal Food, Drug, and Cosmetic Act, 21 U.S.C. § 360bbb-3(b)(1), unless the declaration is terminated or authorization is revoked sooner.
- Laboratories within the United States and its territories are required to report all results to the appropriate public authorities.
- The TrioDx must be used for its intended use only.

Safety & Hazards

- Follow standard precautions. All patient specimens should be considered potentially infectious and handled accordingly.
- Do not eat, drink, or smoke in testing areas where reagents and human specimens are handled.
- Proper personal protective equipment including lab coats, gowns, gloves, eye protection, and biological safety cabinet are recommended for manipulation of clinical specimens. Refer to Biosafety in Microbiological and Biomedical Laboratories (BMBL) 5th Edition – CDC and World Health Organization, Laboratory Biosafety Manual 3rd Edition, WHO/CDS/CSR/LYO/2004.11.
- Handle all specimens as if infectious using safe laboratory procedures. Refer to Interim Laboratory Biosafety Guidelines for Handling and Processing Specimens Associated with 2019-nCoV https://www.cdc.gov/coronavirus/2019-nCoV/lab-biosafety-guidelines.html. Dispose of hazardous or biologically contaminated materials according to the practices of your institution.
- Please read the package insert carefully prior to operation. Each step of operation, from specimen collection, storage and transportation, and laboratory testing, should be strictly conducted in line with relevant biosafety regulations and molecular laboratory management.

- Reliability of the test results depends on adequate specimen collection, storage, transport, and processing procedure.
- Repeated freeze-thaw cycles of the reagents can negatively affect the sensitivity of the assay. Do not freeze and thaw more than 5 times.
- If not tested immediately, store extracted RNA at -80 ^{o}C until use and keep on ice or cold block during testing.
- Keep the reagents on ice or cold block during testing.
- Pre-PCR procedures must be taken place in a clean room separated from PCR or post-PCR area to avoid contamination with amplified products.
- Wear disposable gloves and change them frequently to avoid cross-contamination.
- Use of sterilized filter pipette tips is highly recommended to avoid contamination between reagents.
- Regularly clean the work surfaces using 0.5 % sodium hypochlorite.
- Avoid exposure to light of the 10 X Primer & Probe Mix (PPM).

9. Reagent Storage and Handling

- Upon receipt of the kit, aliquot the reagents to appropriate amount and store at -20^{o}C
- Limit the number of freeze-thaw cycles to 5.
- Do not use expired reagents. Refer to the manufactured date and expiry date printed on the package.
- Dispose unused or left-over reagents and waste in accordance with country, federal, state, and local regulations.

10. Test Procedure

Specimen Collection, Transport and Storage

➔ Collection:

(1) Specimen collection should be performed by a qualified healthcare professional (HCP).

(2) Refer to the CDC Interim Guidelines for Collecting, Handling, and Testing Clinical Specimens for COVID-19 (https://www.cdc.gov/coronavirus/2019-nCoV/lab/guidelines-clinical-specimens.html). Proper collection of specimens is the most crucial step in the molecular diagnosis of COVID-19, and a specimen collected inadequately may lead to false negative test results[1]

(3) Specimen collection device is not included with this test. It is recommended that upper respiratory specimens are collected using flocked swabs, such as nylon or Dacron. Calcium alginate swabs and cotton swabs with wooden shafts are not recommended, as they may contain interferent substances.

(4) Follow the manufacturer's instructions included with the specimen collection device. After collection, put swab into sterile tube containing Viral Transport Media (VTM) or Universal Transport Media (UTM).

➔ Transport and Storage:

(1) For transport, the swab specimens collected in VTM or UTM can be stored at 2-8 ^{o}C up to 72 hours.

(2) For long-term storage, store at -70^{o}C or below in accordance with the CDC Interim Guidelines for Collecting, Handling, and Testing Clinical Specimens for COVID-19.

(3) Specimens must be packaged, shipped, and transported according to the International Air Transport Association (IATA) Dangerous Goods Regulation. Follow shipping regulations for UN3373 Biological Substance, Category B when sending potential SARS-CoV-2 specimens.

RNA Extraction

Nucleic acids (RNA) are isolated and purified from upper respiratory specimens using any one of the following:

- QIAamp Viral RNA Mini Kit (Qiagen, Product No. 52906) for manual extraction
- Maxwell RSC Viral Total Nucleic Acid Kit (Promega, Product No. ASB1330) with Maxwell RSC 48 Instrument (Promega, Product No. AS8500, Software version 3.0) for automated extraction
- MagMAX Viral/Pathogen II Nucleic Acid Isolation Kit (Thermo Fisher, Product No. A48383) with KingFisher Flex with 96 Deepwell Head Instrument (Thermo Fisher, Product No. 5400630, Software version 1.01.0) for automated extraction.

[1] Centers for Disease Control and Prevention, Interim Guidelines for Collecting, Handling, and Testing Clinical Specimens from Persons for Coronavirus Disease 2019 (COVID-19)

(1) Transfer 140 µL of RNase-free H_2O to a sterile 1.5 mL microcentrifuge tube to serve as the Negative Control throughout the remainder of the procedure. (see recommended product in **Section 6**).
(2) Transfer 140 µL of Viral Transport Medium (VTM) containing upper respiratory swab specimens to a sterile 1.5 mL microcentrifuge tube.
(3) Follow the standard procedures in the user manual provided by the appropriate kit manufacturer (Qiagen, Promega, or ThermoFisher)
(4) Using an elution buffer volume of 50 µL, follow the standard procedures in the user manual provided by the appropriate kit manufacturer (Qiagen, Promega, or ThermoFisher) to elute sample RNA and Negative Control.
(5) Proceed to RT-PCR without performing RNA quantification.

Real-Time RT-PCR

(1) Prepare the reaction mixture as described in Table 4. Vortex for 5 sec. Centrifuge to collect droplets.
(2) Prepare a sterile 96-well plate for real-time PCR (see recommended supplier info in **Section 6**). Pipette **15 µL** of the reaction mixture into each well.
➔ Per one 96-well plate, use at least one negative control and one positive control (PC).
(3) Negative Control: **5 µL** of the Negative Control obtained from the RNA extraction step + **15 µL** of reaction mixture. Always add and securely seal/cap negative control before proceeding to specimen RNA handling.
(4) Specimens: **5 µL** of purified RNA in each well + **15 µL** of reaction mixture. Securely seal the wells/tubes.

Table 4. Real-Time RT-PCR Reaction Mixture

Components	Volume required for single reaction	Final Concentration	Volume used for N<15 reactions	Volume used for N≥15 reactions
2X real-time RT-PCR Mix	10 µL	1X	(N+1) x 10 µL	(N+3) x 10 µL
10X Primer & Probe Mix (PPM)	2 µL	1X	(N+1) x 2 µL	(N+3) x 2 µL
RNase Free H_2O	3 µL	N/A	(N+1) x 3 µL	(N+3) x 3 µL
RNA sample or Final Diluted Positive Control or Negative Control	5 µL			
Total reaction volume	20 µL/rxn			
Note	N = number of specimens including positive and negative controls.			

WARNING! Make sure the positive control is prepared in an area that is separate from the RT-PCR area.

(5) Positive Control: In an area separated from the RT-PCR assembly area, first prepare Final Diluted Positive Control (see below) and then use 5 µL of Final Diluted Positive Control + **15 µL** of reaction mixture. Securely seal/cap.

To prepare Final Diluted Positive Control:

Dilute SARS-CoV-2 Positive Control (1×10^4 copies/µL) shown in Table 2 to a working stock of 2.5 copies/µL:

a. First dilution: Add 198 µL of RNase-free H_2O into a first 1.5 mL microcentrifuge tube, then add 2 µL of SARS-CoV-2 Positive Control. Gently mix well, then briefly centrifuge.

b. Second (final) dilution: Add 195 µL of RNase-free H_2O into a second 1.5 mL microcentrifuge tube, then add 5 µL of dilution created in substep 5a. Gently mix well, then briefly centrifuge. (This is Final Diluted Positive Control)

IMPORTANT! When applying the Optical Adhesive Film, ensure that pressure is applied across the entire plate and that there is a tight seal across every individual well. Failure to do so runs the risk of an improperly sealed well, leading to potential well-to-well contamination during vortexing and PCR.

(6) Vortex the plate at the highest setting speed for 15 seconds with medium pressure.

(7) Move the plate around to ensure equal contact on the vortex mixer platform.

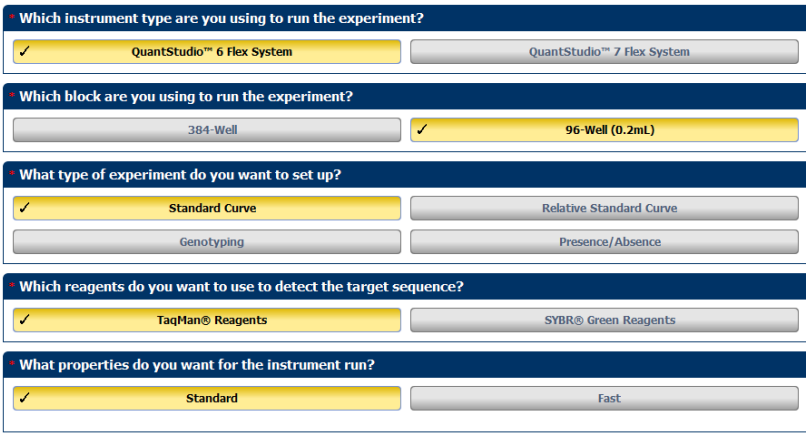
IMPORTANT! Vortex for 15 seconds to ensure proper mixing.

(8) Centrifuge the plate/tubes at 1,500 x g for 30 seconds.

(9) Start the QuantStudio Real-Time PCR Software v1.3.

(10) Set up the Experiment Properties as follows:

Figure 2. Experiment Properties set-up



(11) Set up the thermal cycling reaction condition in the real-time PCR instrument as follows.

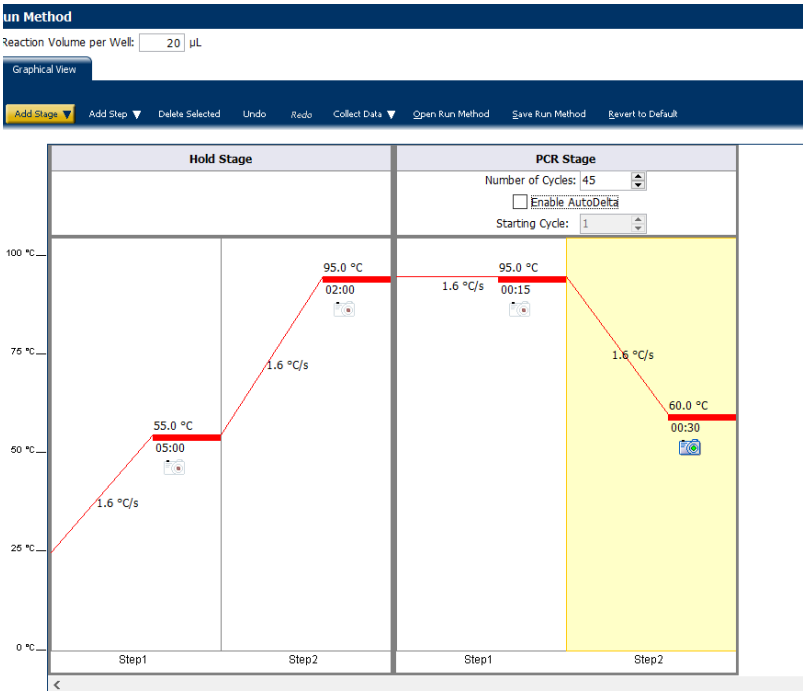
Table 5. Reaction condition set-up

Reaction condition:			
Holding Stage: Reverse Transcription (RT)			
Temp	Time	Cycle	Note
55 °C	5 min	1	Reverse Transcription
95 °C	2 min	1	Inactivation of RT enzyme.
Cycling Stage: Amplification and Detection			
95 °C	15 sec	45	Amplification
60 °C	30 sec		Annealing and **fluorescence detection**

Table 6. Target and fluorescence channel settings:

Target Name	Fluorescence (Reporter)	Quencher
GAPDH (Internal Control)	CY5	None
RdRp gene	VIC (ABI QuantStudio 6)	None
E gene	TEXAS RED	None
N gene	FAM	None

Figure 3. Reaction condition set-up



(12) Insert the 96-well plate into the real-time PCR instrument.

Data Analysis

(1) Select Amplification Plot in Analysis menu

(2) Unselect 'Auto' in threshold setting. Manually adjust the threshold value.

(3) Select 'Baseline Start'. Set start and end values as indicated in the table 7 below.

(4) Repeat for all 4 targets (RdRp, E, N and GAPDH)

(5) Click 'Reanalyze' button to see adjusted plot.

Table 7. Analysis settings

Target Name	Threshold	Baseline	
	ABI QuantStudio 6	Start	End
GAPDH (Internal Control)-CY5	30,000	3	15
RdRp gene -VIC*	30,000	3	15
E gene- TEXAS RED	15,000	3	15
N gene- FAM	30,000	3	15
Note	*The reporting dye used for RdRp detection is HEX dye. For QuantStudio 6 Instrument, select VIC in setting, as they share the same fluorescent channel.		

11. Quality Control

Table 8 provides the list of Controls included with TrioDx.

a) A **negative control** is needed to check for extraneous nucleic acid contamination that can happen during nucleic acid extraction and preparation for real-time RT-PCR and is used through the entire process from sample processing and RNA extraction to real-time RT-PCR reaction. At least one negative control must be included per run. In case of false positives detected in a negative control, the entire process must be repeated starting from extraction step.

b) A **positive control** is needed to verify that the assay is being performed as intended and is used from the RT step of the procedures. At least one positive template control must be included for each run. The Positive Control included in this kit is synthetic RNA fragments spanning the target viral sequences and is used at final 2.5 copies/µL in the PCR reaction mixture for the test.

c) An **internal control**: The 10X primer & probe mix provided in this kit contains a primer & probe set that targets human GAPDH. The purpose of an internal control is to evaluate the real-time PCR instrument performance and is used through the entire process from RNA extraction to data analysis.

Table 8. Controls provided in the test kit

Control Name	Manufacturer of raw materials	Kit label	Specifications	Directions for use
Negative Control	RNase-free H_2O provided by Takara Bio Inc.	Clinomics USA Inc.	1.5 mL per kit	Use 140 µL RNase-free H_2O as Negative Control for RNA extraction step. Add 5 µL of Negative Control obtained from the RNA extraction elution step to 15 µL reaction mix, making the total volume 20 µL. At least one negative control must be included per each real-time RT PCR run.
Positive Control[1]	Synthetic oligo for T7 transcription provided by Twist Biosciences	Clinomics USA Inc.	20 µL per kit	Add 5 µL of Final Diluted Positive Control to 15 µL reaction mix, making the total volume 20 µL. (Final concentration is 625 copies/mL which is approximately 5x the limit of detection) At least one positive control must be included per each real-time RT PCR run.
Internal Control	IDT Inc.	Clinomics USA Inc.	Included in 10X Primer & Probe Mix (PPM) 200 µL per kit	Refer to Interpretation of Results

[1] The positive control included in this kit was generated via *in-vitro* RNA synthesis using HiScribe™ T7 Quick High Yield RNA Synthesis Kit (New England BioLabs). The DNA template used for *in-vitro* RNA synthesis is synthetic dsDNA spanning the target viral sequences (Twist Biosciences). In the process, the template DNA is transcribed by T7 RNA phage polymerase into single-stranded RNA that has 5' to 3' directionality.

Table 9 shows the interpretation guidelines for Controls. Follow the recommended actions based on the result interpretation. Proceed to Section 12: Interpretation of Results only when the quality control results come out to be 'Valid'.

Table 9. Quality control test interpretation

	SARS-CoV-2 RdRp gene	SARS- CoV-2 E gene	SARS-CoV-2 N gene	GAPDH (Internal Control)	Result Interpretation	Recommended Actions
Positive Control	+	+	+	+	Valid	Continue to Patient Specimen Interpretation
	One or more targets detected as negative (-) **				Invalid	Re-run real-time RT-PCR
Negative Control	-	-	-	-	Valid	Continue to Patient Specimen Interpretation
	One or more targets detected as positive (+) ***				Invalid	Re-extraction and re-run real-time RT-PCR. Eliminate any contamination
Note	** Ct value> 38 or Undetermined produces negative (-) result *** Ct value ≤38 produces positive (+) result If any of the controls gives an invalid result interpretation, perform a root cause investigation and re-run all samples starting from extraction once the root cause is identified and eliminated.					

12. Interpretation of Results

- Proceed only when control results (**Section 11**) are valid.
- Refer to the Table 10 to interpret the results for patient samples.
- If the Ct value obtained is ≤ (less than or equal) to 38 then there is a positive (+) result. If the Ct value obtained is > (greater than) 38 then there is a negative (-) result. Follow the recommended actions based on the result interpretation in Table 9.
- Please see details in Table 9 for a result interpretation from the E gene alone.

Table 10. Patient specimen result interpretation

Potential Result Type	RdRp gene (HEX)	E gene (TEXAS RED)	N gene (FAM)	GAPDH Internal Control (CY5)	Result Interpretation	Further interpretation/Actions
Case 1	+	+	+	**+ or -**	Positive SARS-CoV-2	Target results are valid SARS-CoV-2 RNA is detected
Case 2	+	+	-	**+ or -**	Positive SARS-CoV-2	Target results are valid. SARS-CoV-2 RNA is detected Missing detection of individual targets may be due to: 1) a sample at concentrations near or below the limit of detection of the specific target 2) a mutation in the corresponding target region 3) other factors
Case 3	-	+	+	**+ or -**		
Case 4	+	-	+	**+ or -**		
Case 5	+	-	-	**+ or -**		
Case 6	-	-	+	**+ or -**		
Case 7	-	+	-	**+ or -**	Presumptive Positive SARS-CoV-2	Target results are valid. Sarbecovirus RNA is detected but SARS-CoV-2 specific RNA targets cannot be differentiated from other Sarbecovirus such as SARS-CoV-1. Repeat testing. A second positive test result is a reportable result. Further confirmatory testing is recommended if clinically indicated, i.e., If the same result is obtained and it is necessary to determine the Sarbecovirus, request another patient sample and perform this test or perform other confirmatory testing. Missing detection of individual targets may be due to: 1) a sample at concentrations near or below the limit of detection of the specific target 2) a mutation in the corresponding target region 3) other factors
Case 8	-	-	-	+	Negative	Target results are valid. SARS-CoV-2 RNA is NOT detected
Case 9	-	-	-	-	Invalid	Results are invalid.* Repeat test starting from extraction. If result is still invalid, report as Invalid and request new sample.
Note	* Invalid result due to potential handling error (e.g., sample collection, RNA extraction) or inhibition. If either the Negative Control or the Positive Control is Invalid as shown in the Quality Control test in Table 4, the Patient Specimen should not be interpreted. Only a valid result for the Negative Control and Positive Control enables a result interpretation. See notes for Table 4.					

13. Assay Limitations

1. This assay is for in vitro diagnostic use under FDA Emergency Use Authorization only. 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.
2. Use of this assay is limited to personnel who are trained in the procedure. Failure to follow these instructions may result in erroneous results.
3. The performance of TrioDx was established using nasopharyngeal swab. Other swabs (oropharyngeal, mid-turbinate and anterior nasal) are also considered acceptable specimen types for use with the SARS-CoV-2 Test Kit (Real-time PCR) but performance has not been established.
4. 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.
5. PCR product contamination might occur in the laboratory, reagent preparation and cross-contamination of samples and will produce false positive results. The components of the test kit may decline due to improper transportation, storage or inaccurate preparation and will produce false negative results. False negative results may also arise from improper sample collection, degradation of the viral RNA during shipping/storage, using unauthorized extraction or assay reagents, mutation in the SARS-CoV-2 virus and failure to follow instructions for use.
6. Low viral load and excessive degradation in the samples may cause negative results. Thus, a negative result cannot completely exclude the existence of SARS-CoV-2 in the sample and should not be the sole basis of a patient management decision. Follow up testing should be performed according to the current CDC recommendations.
7. Results should be interpreted by a trained professional in conjunction with the patient's history and clinical signs and symptoms, and epidemiological risk factors.
8. Extraction and amplification of nucleic acid from clinical samples must be performed according to the specified methods listed in this procedure. Other extraction approaches and processing systems have not been evaluated.
9. The impacts of vaccines, antiviral therapeutics, antibiotics, chemotherapeutic or immunosuppressant drugs have not been evaluated.
10. Laboratories are required to report all results to the appropriate public health authorities.

11. Result for the E gene is Presumptive Positive. Sample should be retested. For samples with a repeated Presumptive Positive result, additional confirmatory testing may be conducted, if it is necessary to differentiate between SARS-CoV-2 and SARS-CoV-1 or other Sarbecovirus currently unknown to infect humans, for epidemiological purposes or clinical management.
12. This assay cross reacts with SARS-CoV. Additional testing is recommended if the exact coronavirus needs to be identified.
13. Positive results are indicative of the presence of SARS-CoV-2 RNA, but clinical correlation with patient history and other diagnostic information is necessary to determine patient infection status. Positive results do not rule out bacterial infection or co-infection with other viruses. The agent detected may not be the definite cause of disease.
14. 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.

14. Troubleshooting

Table 11. Troubleshooting guidelines

Issue types	Possible causes	Recommended actions
No fluorescence detected in any samples, including positive control.	Reagent degradation	Dispose the current reagent aliquots and use new reagents.
	Components missing in the reaction mixture	Ensure that all necessary reagents are added. Repeat the real-time RT PCR reaction mix preparation.
	Instrument/software settings error	Verify the real-time PCR instrument and software are set up as described in the procedures (**Section 10**).
Fluorescence detected in Negative Control.	Carry-over contamination	Perform a root cause investigation into the Quality control procedures in your laboratory. Disinfect pipettes. Make sure to use filter tips. Separate areas of sample receiving activities, control material preparation areas, sample extraction areas and RT-PCR set up.
	Wells/tubes not properly sealed	Ensure plates and tubes are tightly sealed.
	Contamination in the extraction or preparation area	Clean surfaces and instruments with RNase/DNase. Separate areas for sample receiving, sample extraction and positive control material handling from RT-PCR assembly areas.
Fluorescent signal curve for the endogenous human control does not show sigmoidal characteristic in the patient specimens.	Poor RNA quality	Re-extract RNA using extraction methods recommended in the IFU. Keep at -70^{o}C for long-term storage.
	Not enough volume of RNA added	Repeat RT-PCR using appropriate volume of RNA (5 µL)
Fluorescent signal curve does not show sigmoidal characteristic in positive control (PC).	Positive control quality degradation	Dispose the current PC tube and use a new one.

15. Conditions of Authorization for the Laboratory

The Clinomics TrioDx RT-PCR COVID-19 Test (TrioDx) 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.

To assist clinical laboratories using the TrioDx, the relevant Conditions of Authorization are listed below.

1. Authorized laboratories[1] using the TrioDx must include with test result reports of the TrioDx, all authorized Fact Sheets. Under exigent circumstances, other appropriate methods for disseminating these Fact Sheets may be used, which may include mass media.
2. Authorized laboratories using the TrioDx must perform the TrioDx 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 perform the TrioDx are not permitted.
3. Authorized laboratories that receive the TrioDx must notify the relevant public health authorities of their intent to run the test prior to initiating testing.
4. Authorized laboratories using the TrioDx must have a process in place for reporting test results to healthcare providers and relevant public health authorities, as appropriate.
5. Authorized laboratories must 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 Clinomics USA Inc. (markd@clinomics.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.
6. All laboratory personnel using the test must be appropriately trained in RT-PCR techniques and use appropriate laboratory and personal protective equipment when handling this kit, and use the test in accordance with the authorized labeling.
7. Clinomics, its authorized distributor(s) and authorized laboratories using the Clinomics TrioDx RT-PCR COVID-19 Test must 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] For ease of reference, the letter refers to, "laboratories certified under the Clinical Laboratory Improvement Amendments of 1988 (CLIA), 42 U.S.C. §263a, to perform high complexity tests" as "authorized laboratories."

16. Performance Evaluation

- Limit of Detection (Analytical Sensitivity)

The LoD is defined as the lowest concentration of SARS-CoV-2 that can be reliably detected at least 95% of the time. Three LoD studies were performed to determine the analytical sensitivity of the TrioDx: (1) a preliminary study with AccuPlex materials, (2) a confirmatory study with AccuPlex materials, and (3) a study with heat-inactivated virus (BEI Resources). The materials used for this study are shown in Table 12 below.

Table 12. Materials used in Limit of Detection Study

	Required Material	Product Name	Catalog #	Manufacturer	Concentration
1	Recombinant virus with reference RNA sequence (SARS-CoV-2)	AccuPlex™ SARS-CoV-2 Reference Material Kit	0505-0126	Seracare	4.23×10^3 cp/mL
2	Heat-inactivated virus	SARS-Related Coronavirus 2, Isolate USA-WA1/2020	NR-52286	BEI Resources:	1.16×10^9 cp/mL
3	NP and OP swabs	Nasopharyngeal/Oropharyngeal Swabs + 2mL CTM (Clinical Viral Transport Medium)	UTNFS-3B-2	Noble Biosciences Inc.	n/a

(a) *LoD studies with Recombinant Virus (Accuplex)*

The **preliminary** study was conducted using the AccuPlex™ SARS-CoV-2 Reference Material, which is a recombinant virus containing SARS-CoV-2 sequences, to evaluate the sensitivity of the TrioDx test. Reference material was serially diluted (2-fold) and spiked into 140 uL VTM/UTM containing negative nasopharyngeal swab specimen to create 5 different viral copy-number concentrations. Each dilution was tested in 5 replicates. The nucleic acids were purified using each of the following:

(1) QIAamp Viral RNA Mini Kit (manual)
(2) Maxwell RSC Viral Total Nucleic Acid Kit with the Maxwell RSC 48 instrument
(3) MagMAX Viral/Pathogen II Nucleic Acid Isolation Kit with KingFisher Flex with 96 Deepwell Head Instrument.

Tables 13, 14, and 15 show the detection rate at 5 tentative LoD concentrations for each extraction method.

The positive rate was determined per sample dilution based on the test interpretation, i.e., a positive result in any gene is positive. Based on these results, the tentative LoD was determined to be 0.125 copies/µL. Although each gene at this concentration, did not have 5/5 positive replicates, for each replicate one of the genes was positive and therefore the sample is positive.

Table 13. Preliminary LoD study results from RNA extraction using QIAamp Viral RNA Mini Kit (Manual Prep) - AccuPlex

Concentration RNA copies/uL	Assay 1 (RdRp gene)			Assay 2 (E gene)			Assay 3 (N gene)			Using all 3 genes
	Mean Ct	Standard Deviation (SD)	Detection Rate	Mean Ct	Standard Deviation (SD)	Detection Rate	Mean Ct	Standard Deviation (SD)	Detection Rate	**Detection Rate**
2	32.6	0.2	5/5 (100%)	32.5	0.2	5/5 (100%)	31.7	0.2	5/5 (100%)	**5/5 (100%)**
1	33.6	0.3	5/5 (100%)	33.8	0.2	5/5 (100%)	33.3	0.7	5/5 (100%)	**5/5 (100%)**
0.5	34.9	0.5	5/5 (100%)	34.9	0.7	5/5 (100%)	34.4	0.9	5/5 (100%)	**5/5 (100%)**
0.25	36.2	0.9	5/5 (100%)	36.8	1.5	4/5 (80%)	36.1	1.6	4/5 (80%)	**5/5 (100%)**
0.125	36.5	0.8	5/5 (100%)	37.9	1.3	3/5 (60%)	36.0	0.3	5/5 (100%)	**5/5 (100%)**

Table 14. Preliminary LoD study results from RNA extraction using Maxwell RSC Viral Total Nucleic Acid Kit with Maxwell RSC 48 instrument (Auto Prep) - AccuPlex

Concentration RNA copies/uL	Assay 1 (RdRp gene)			Assay 2 (E gene)			Assay 3 (N gene)			Using all 3 genes
	Mean Ct	Standard Deviation (SD)	Detection Rate	Mean Ct	Standard Deviation (SD)	Detection Rate	Mean Ct	Standard Deviation (SD)	Detection Rate	**Detection Rate**
2	30.7	0.7	5/5 (100%)	32.0	0.1	5/5 (100%)	31.0	0.5	5/5 (100%)	**5/5 (100%)**
1	32.3	0.5	5/5 (100%)	33.1	0.4	5/5 (100%)	31.4	0.2	5/5 (100%)	**5/5 (100%)**
0.5	33.1	0.5	5/5 (100%)	34.1	0.5	5/5 (100%)	33.0	0.4	5/5 (100%)	**5/5 (100%)**
0.25	34.6	0.5	5/5 (100%)	35.9	0.8	5/5 (100%)	34.3	1.0	5/5 (100%)	**5/5 (100%)**
0.125	35.2	0.7	5/5 (100%)	36.5	1.1	4/5 (80%)	33.8	0.6	3/5 (60%)	**5/5 (100%)**

Table 15. Preliminary LoD study results from RNA extraction using MagMAX Viral/Pathogen II Nucleic Acid Isolation Kit with KingFisher Flex instrument (Auto Prep) - AccuPlex

Concentration RNA copies/uL	Assay 1 (RdRp gene)			Assay 2 (E gene)			Assay 3 (N gene)			Using all 3 genes
	Mean Ct	Standard Deviation (SD)	Detection Rate	Mean Ct	Standard Deviation (SD)	Detection Rate	Mean Ct	Standard Deviation (SD)	Detection Rate	**Detection Rate**
2	31.6	0.5	5/5 (100%)	32.5	0.7	5/5 (100%)	31.2	1.0	5/5 (100%)	**5/5 (100%)**
1	33.4	2.2	5/5 (100%)	34.0	1.2	5/5 (100%)	31.6	1.2	5/5 (100%)	**5/5 (100%)**
0.5	33.7	0.5	5/5 (100%)	34.0	0.6	5/5 (100%)	32.6	0.3	5/5 (100%)	**5/5 (100%)**
0.25	34.6	0.3	5/5 (100%)	35.5	0.5	5/5 (100%)	33.8	0.6	5/5 (100%)	**5/5 (100%)**
0.125	36.5	2.5	4/5 (80%)	37.1	1.8	3/5 (60%)	34.5	0.9	4/5 (80%)	**5/5 (100%)**

Confirmatory LoD Study with Accuplex Reference

A Confirmatory LoD study was then performed on 20 replicates for each extraction method. The LoD for each target was then determined to be the lowest concentration at which at least 19/20 replicates were detected since the FDA defines LoD as the lowest concentration at which 19/20 replicates are positive. The detection rate is shown for *each* extraction method individually, and the result is shown in the last column the detection rate using *all three genes* is provided as discussed above.

The final LoD (see Tables 16) was confirmed to be 0.125 copies/µL for all three extraction methods. At least one of the three target genes (RdRp, E or N) was successfully detected in 20 out 20 replicates (100%). Note that the E gene alone was detected at the lowest concentration in just 1 of the 20 samples for two of the extraction methods in Tables 16.

Table 16. Confirmatory LoD study results from three different RNA extraction methods - Accuplex

Concentration RNA copies/uL	Assay 1 (RdRp gene)		Assay 2 (E gene)		Assay 3 (N gene)		**Using all 3 genes**
	Mean Ct	Detection Rate	Mean Ct	Detection Rate	Mean Ct	Detection Rate	**Detection Rate**
Extraction using QIAamp Viral RNA Mini Kit (Manual Preparation)							
0.25	35.4	20/20 (100%)	36.1	20/20 (100%)	34.8	20/20 (100%)	20/20 (100%)
0.125	**37.0**	**17/20 (85%)**	**37.8**	**14/20 (70%)**	**36.3**	**15/20 (75%)**	**20/20 (100%)**
Extraction using Maxwell RSC Viral Total Nucleic Acid Kit with Maxwell RSC 48 instrument (Auto Preparation)							
0.25	34.9	20/20 (100%)	35.6	20/20 (100%)	34.5	20/20 (100%)	20/20 (100%)
0.125	**35.6**	**19/20 (95%)**	**36.6**	**19/20 (95%)**	**34.8**	**16/20 (80%)**	**20/20 (100%)**
Extraction using MagMAX Viral/Pathogen II Nucleic Acid Isolation Kit with KingFisher Flex instrument (Auto Preparation)							
0.25	35.7	19/20 (95%)	36.0	20/20 (100%)	34.6	20/20 (100%)	20/20 (100%)
0.125	**37.4**	**16/20 (80%)**	**36.3**	**18/20 (90%)**	**35.9**	**15/20 (75%)**	**20/20 (100%)**

Confirmatory LoD Study with Heat-inactivated Virus - Contrived Clinical Specimen

A bridging study was performed using whole heat inactivated virus (BEI Resources Product # NR-52286: SARS-Related Coronavirus 2, Isolate USA-WA1/2020) to ensure that the LoD of the assay is not significantly different with intact SARS-CoV-2 virus.

The BEI Resources NR-52286 Reference Material was serially diluted and spiked into 140uL VTM containing negative nasopharyngeal swab specimen (item 2 in Table 12). All specimens were isolated using 50-µL elution buffer. The nucleic acids were purified using the QIAamp Viral RNA Mini Kit. Five replicates were performed at each of the following: 1/3X LoD, 1X LoD, and 3X LoD, where the 1X LoD is taken as based on the observed 0.125 copies/uL.

The results are summarized in Table 17. 5/5 replicates were detected at the 1X LoD of 0.125 copies/uL. Consistent with the results with the Accuplex reference material, there was no detection at 1/3X LoD with the BEI reference material. The E gene alone was detected in one of the five replicates at 1X LoD.

Table 17. Confirmatory LoD study results using BEI reference. RNA extraction using QIAamp Viral RNA Mini Kit

LoD	copies/uL	RdRp gene			E gene			N gene			**Using all three genes**
		Mean Ct	SD	Rate	Mean Ct	SD	Rate	Mean Ct	SD	Rate	**Rate**
3X	0.375	35.2	0.5	5/5	34.5	0.9	5/5	33.8	0.3	5/5	**5/5**
1X	0.125	36.9	1.0	4/5	36.4	0.7	5/5	35.2	1.3	3/5	**5/5**
1/3X	0.042	Undetermined	-	0/5	37.9	-	1/5	Undetermined	-	0/5	**1/5**

Final Reported LoD- summary of LoD for all three extraction methods

The final reported LoD is summarized below in Table 18. The LoD based on the test algorithm is in the bottom row of the table and shows the LoD is the same, 0.125 copies/µL, for all three extraction methods.

Table 18. Final reported LoD for each target for three different extraction methods

Gene	QIAamp Viral RNA Mini Kit (Manual Prep)	Maxwell RSC Viral Total Nucleic Acid Kit with Maxwell RSC 48 instrument (Auto Prep)	MagMAX Viral/Pathogen II Nucleic Acid Isolation Kit with KingFisher Flex instrument (Auto Prep)
RdRp gene	0.25 copies/µL	0.125 copies/µL	0.25 copies/µL
E gene	0.25 copies/µL	0.125 copies/µL	0.25 copies/µL
N gene	0.25 copies/µL	0.25 copies/µL	0.25 copies/µL
Final reported LoD using all 3 genes	**0.125 copies/µL**	**0.125 copies/µL**	**0.125 copies/µL**

- Inclusivity (Analytical Sensitivity)

The inclusivity of the TrioDx was evaluated *in silico* by comparing the primer/probe sequences separately with each of the 18,927 Severe acute respiratory syndrome coronavirus 2 sequences published in NCBI and GISAID databases. A total of 9,467 publicly available SARS-CoV-2 sequences accessed via NCBI Virus SARS-CoV-2 data hub, and 9,460 sequences from GISAID (out of 64,286 sequences available as of July 14th, 2020) that are different from the NCBI database. Each was separately used for alignment with the TrioDx assay.

The SARS-CoV-2 primers and probes for RdRp, E and N gene targets showed 100% homology with reference sequence (NC_045512.2). The inclusivity % calculated are summarized in Table 19.

Table 19. Inclusivity test results

Database	Target Genes	Homology					
		Forward Primer	Number of sequences in databases without 100% homology	Probe	Number of sequences in databases without 100% homology	Reverse Primer	Number of sequences in databases without 100% homology
NCBI (n=9467)	RdRp gene	99.92 %	8	99.89 % (CASE 1)	10	99.98 %	2
	E gene	99.96 %	4	99.85 %	14	99.94 %	6
	N gene	99.73 %	26	99.66 %	32	99.82 %	17
GISAID (n=9460)	RdRp gene	99.86 %	13	99.57 %	41	99.98 %	2
	E gene	99.92 %	8	99.76 %	23	99.88 %	11
	N gene	99.75 %	24	99.53 % (CASE 2)	44	99.81 %	18

There was a total of 297 strains that did not demonstrate 100% homology. However, while the primers and probes in Table 19 do not all show 100% homology there was no case in which *all* of the RdRp, E, and N sequences showed less than 100% homology. Specifically:

- 289 strains contained single base pair mismatches in one of the primers and probes that would not impact amplification of the target due to its position. Note in general, one sequence mismatch does not critically impact primer amplification unless such a mismatch occurs in 3', the binding efficiency of the primer may decrease; however, such a case was *not* found.

- 6 strains contained one mismatch in more than one primer and probe that would not impact the amplification of the target due to its position.
- 2 strains contained multi-base pair mismatches that may impact the amplification of the target.
    - **sequence MT372483.1 (Case 1 in Table 19), a total of 4 mismatches were found in RdRp Probe target region.**
      In this case, four base pair mismatches were confirmed in the RdRp gene probe of a total length 24 oligonucleotide sequence. While the RdRp gene may not be detected, these sequences had 100% homology with the N gene and E gene, and a sample with this sequence would consequently still be detected as positive**.**
    - **sequence "Iceland/603/2020" (Case 2 in Table 19; strain name used since GenBank accession number is unavailable), a total of 2 mismatches were found in N gene Probe target region.**
      In this case, two base pair mismatches were confirmed in the N gene probe of a total length 24 oligonucleotide sequence. While the N gene may not be detected, these sequences had 100% homology with the RdRp gene and E gene, and a sample with this sequence would consequently still be detected as positive**.**

Therefore, the multi target design of the test mitigates false negative results due to mismatches in an individual target. Thus, when all three SARs-CoV-2 targets were checked according to the sponsors algorithm, there were no strains that could not be detected by PCR in the 18,927 strains evaluated.

Note: The consensus sequences generated from alignment with the SARS-CoV-2 genome sequences contain ambiguity characters (W, S, M, K, R, Y-refer to IUPAC notation) to represent two positional base variations present in the region. These were not counted as mismatches, as one of the two positional variations demonstrated by the ambiguity characters always matched with the TrioDx primer/probe nucleotide sequences.

Inclusivity for new variants as of January 25, 2021

Additional inclusivity of the TrioDx was evaluated *in silico* by comparing the primer/probe sequences separately with each of 9,275 new variant SARS-CoV-2 sequences published in GISAID databases (through January 25th, 2021).

Three representative Emerging Variants lineages were used from: https://www.cdc.gov/coronavirus/2019-ncov/more/science-and-research/scientific-brief-emerging-variants.html. This included 31 cases in P.1 (Brazil), 8,573 cases in B.1.1.7 (United Kingdom), and 671 cases in B.1.351 (South Africa). Each new variant SARS-CoV-2 sequence was separately used for alignment with the TrioDx assay.

The SARS-CoV-2 TrioDx primers and probes for RdRp, E and N gene targets showed 100% homology with reference sequence (NC_045512.2). The inclusivity is summarized in Table 20.

Table 20. Inclusivity test results for new variants: P.1 (Brazil), B.1.1.7 (United Kingdom), B.1.351 (South Africa)

Database	Target Genes	Homology					
		Forward Primer	Number of sequences in databases without 100% homology	Probe	Number of sequences in databases without 100% homology	Reverse Primer	Number of sequences in databases without 100% homology
GISAID (n=9275)	RdRp gene	99.99 %	1	99.81 %	18	99.96 %	4
	E gene	99.36 %	59	99.92 %	7	100 %	0
	N gene	99.62 %	35	98.71 % (CASE 3)	120	99.83 %	16

P.1 (Brazil) showed 100% homology in all Primer & Probe oligonucleotide sequences.

B.1.1.7 (United Kingdom) showed 237 strains without 100% homology.

B.1.351 (South Africa) showed 20 strains without 100% homology.

Thus, there was a total of 257 strains that did not demonstrate 100% homology. However, while the primers and probes in Table 19 do not all show 100% homology, there was *no new variant* for which *all* of the RdRp, E, and N sequences showed less than 100% homology. Specifically:

- 254 strains contained single base pair mismatches in one of the primers and probes that would not impact amplification of the target due to its position. Note that, in general, one sequence mismatch does not critically impact primer amplification unless such a mismatch occurs in 3', the binding efficiency of the primer may decrease; however, such a case was *not* found.
- 2 strains contained one mismatch in more than one primer and probe that would not impact the amplification of the target due to its position.
- 1 strain contained multi-base pair mismatches that may impact the amplification of the target.
    - **sequence EPI_ISL_856362 (Case 3 in Table 19), a total of 2 mismatches were found in N gene Probe target region.**

      In this case, four base pair mismatches were confirmed in the N gene probe of a total length 24 oligonucleotide sequence. While the N gene may not be detected, these sequences had 100% homology with the RdRp gene and E gene, and a sample with this sequence would consequently still be detected as positive**.**

Therefore, the multi target design of the test mitigates false negative results due to mismatches in an individual target. In summary, when all three SARs-CoV-2 targets were checked according to the algorithm, there were no strains that could not be detected by PCR in the new variant strains evaluated.

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 and instrument used were the Promega Maxwell RSC Viral Total Nucleic Acid Kit with the Promega Maxwell RSC 48 Instrument and the QuantStudio 6 Flex Instrument, respectively. The results are summarized in Table 21.

Table 21. Summary of LoD confirmation result using FDA SARS-CoV-2 reference Panel

Reference Materials Provided by FDA	Specimen Type	Product LoD	Cross-Reactivity
SARS-CoV-2	Nasopharyngeal Swab	6 x 10^3 NDU/mL	N/A
MERS-CoV		N/A	ND

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

Cross-reactivity (Analytical Specificity)

The Cross-Reactivity Studies (see Table 22) have been performed by running BLAST against 32 non-target microorganisms/ closely related virus strains on 7/14/20. No homology ≥ 80% homology was found in any tested organisms, except in SARS-coronavirus (SARS-CoV).

The "no amplicon formed" designation shown in Table 22 means that there is less 80% homology in the *in silico* analysis and hence the amplicon will not be generated during the PCR amplification.

Table 22. List of microorganisms tested for cross-reactivity via *in silico*

Category	Microorganisms	Taxid	NCBI database	RdRp gene	E gene	N gene
Other high priority pathogens from the same genetic family	Human coronavirus 229E	11137	NC_002645.1	No amplicon formed	No amplicon formed	No amplicon formed
	Human coronavirus OC43	31631	NC_006213.1	No amplicon formed	No amplicon formed	No amplicon formed
	Human coronavirus NL63	277944	NC_005831.2	No amplicon formed	No amplicon formed	No amplicon formed
	Human coronavirus HKU1	290028	NC_006577.2	No amplicon formed	No amplicon formed	No amplicon formed
	SARS-coronavirus*	694009	NC_004718.3	>80% homology found in Probe sequence	>80% homology found Forward and Reverse Primers and Probe	>80% homology found in Forward Primer and probe
	Middle East respiratory syndrome-related coronavirus (MERS-CoV)	1335626	NC_019843.3	No amplicon formed	No amplicon formed	No amplicon formed

Category	Microorganisms	Taxid	NCBI database	RdRp gene	E gene	N gene
High priority organisms likely present in a respiratory specimen	Human Adenovirus C1	10533	AC_000017.1	No amplicon formed	No amplicon formed	No amplicon formed
	Human Metapneumovirus (hMPV)	162145	NC_039199.1	No amplicon formed	No amplicon formed	No amplicon formed
	Human parainfluenza virus 1	12730	NC_003461.1	No amplicon formed	No amplicon formed	No amplicon formed
	Human parainfluenza virus type 2	1979160	NC_003443.1	No amplicon formed	No amplicon formed	No amplicon formed
	Human parainfluenza virus type 3	11216	NC_001796.2	No amplicon formed	No amplicon formed	No amplicon formed
	Human Parainfluenza virus 4a	11224	NC_021928.1	No amplicon formed	No amplicon formed	No amplicon formed
	Human orthopneumovirus (Human respiratory syncytial virus A)	11250	NC_038235.1	No amplicon formed	No amplicon formed	No amplicon formed
	Influenza A virus (A/PR 8/1934 (H1N1))	211044	NC_002022.1	No amplicon formed	No amplicon formed	No amplicon formed
	Influenza virus type B	11520	NC_002204.1	No amplicon formed	No amplicon formed	No amplicon formed
	Human Enterovirus EV68	42789	NC_038308.1	No amplicon formed	No amplicon formed	No amplicon formed
	Human orthopnemovirus (Human respiratory syncytial virus B)	11250	NC_001781.1	No amplicon formed	No amplicon formed	No amplicon formed
	Human rhinovirus A1	573824	NC_038311.1	No amplicon formed	No amplicon formed	No amplicon formed
	Chlamydia pneumoniae TW-183	182082	NC_005043.1	No amplicon formed	No amplicon formed	No amplicon formed
	Haemophilus influenzae	727	CP007805.1	No amplicon formed	No amplicon formed	No amplicon formed
	Legionella pneumophila NCTC12273	446	NZ_LR134380.1	No amplicon formed	No amplicon formed	No amplicon formed
	Mycobacterium tuberculosis H37Rv	83332	NC_018143.2	No amplicon formed	No amplicon formed	No amplicon formed
	Streptococcus pneumoniae	1313	NZ_LN831051.1	No amplicon formed	No amplicon formed	No amplicon formed
	Streptococcus pyogenes	1314	NZ_LN831034.1	No amplicon formed	No amplicon formed	No amplicon formed
	Bordetella pertussis 18323	568706	NC_018518.1	No amplicon formed	No amplicon formed	No amplicon formed
	Mycoplasma pneumoniae FH	722438	NZ_CP010546.1	No amplicon formed	No amplicon formed	No amplicon formed
	Pneumocystis jirovecii	42068	NW_017264778 1	No amplicon formed	No amplicon formed	No amplicon formed
	Candida albicans	237561	GCF_000182965 .3 (8chr) (XM_715226.)	No amplicon formed	No amplicon formed	No amplicon formed
	Pseudomonas aeruginosa DSM 50071	1123015	NZ_CP012001.1	No amplicon formed	No amplicon formed	No amplicon formed

Category	Microorganisms	Taxid	NCBI database	RdRp gene	E gene	N gene
	Staphylococcus aureus subsp. Aureus NCTC 8325	93061	NC_007795.1	No amplicon formed	No amplicon formed	No amplicon formed
	Staphylococcus epidermidis	1282	NZ_CP035288.1	No amplicon formed	No amplicon formed	No amplicon formed
	Streptococcus salivarius	1304	NZ_LR134274.1	No amplicon formed	No amplicon formed	No amplicon formed

The *in silico* cross reactivity study (see Table 22) yielded only one species (SARS-CoV, marked with an * in the table above) that shares more than 80% homology with multiple of the primers and probes included in the TrioDx test kit:

- RdRp gene probe
- E gene primers and probe
- N gene forward primer and probe.

The results indicate that TrioDx kit does not cross react to any of non-target microorganisms except for SARS-coronavirus. Therefore, the negative samples do not show any false positives due to cross-reactivity.

Non-target microorganisms also do not interfere with the positive results of the TrioDx COVID-19 test. In the case of SARS-coronavirus no amplicons will be generated from unintended cross reactivity in RdRp and N genes, as both forward *and* reverse primers must match to form amplicons. The RdRp gene shows more than 80% homology with only the probe. The N gene shows more than 80% homology with forward primer and probe.

While the *in silico* results indicate that co-infection with SARS-CoV-1 could theoretically reduce the sensitivity of the test, this is clinical not relevant since the CDC reports that since 2004, there have not been any known cases of SARS-CoV reported anywhere in the world.

Wet testing was not performed for these reasons and the *in silico* analysis was deemed sufficient.

- Clinical Evaluation

The clinical performance of the TrioDx was established by testing retrospective samples using 30 natural, non-contrived positive clinical samples and 30 natural, non-contrived negative clinical samples that were procured from a reference laboratory. Commercially sourced positive clinical samples were used following the FDA's COVID-19 Test Development and Review FAQs. All 60 clinical specimen samples were Nasopharyngeal Swabs (NP) in Aptima Specimen Transport Medium or Multitrans Medium.

Nucleic acids from the 60 clinical samples were purified using QIAamp Viral RNA Mini Kit and the samples were tested using QuantStudio 6 Flex. Since the LoD was the same for both the manual and automated extraction methods, the Clinical Evaluation was not repeated using the automated extraction method.

For the comparator, all 60 clinical samples had been tested and validated as positive or negative at the Reference Laboratory on an EUA authorized assay. The corresponding results from the reference laboratory were used as a comparator with the TrioDx assay. An EUA authorized assay with high sensitivity performed at the reference laboratory on the 60 samples detects two regions of the ORF1ab gene in the same fluorescent channel. The TrioDx detects three SARS-CoV-2 targets, RdRp, E and N gene in three distinct fluorescent channels.

A second study was performed to test seven low positive clinical samples using two different EUA authorized comparator assays. These samples were considered low positive when compared to the Ct values at the mean LoD of each EUA authorized assay. The TrioDx detected all 7 low positive samples with 100% agreement with the comparator assays.

All procedures for the TrioDx clinical evaluation were conducted in accordance with **Section 10 (Test Procedures)** described in this Instruction for Use (IFU).

The results of the clinical evaluation study are summarized in Table 23. Both positive percent agreement (PPA) and negative percent agreement (NPA) came out to be 100%.

Table 23. Clinical evaluation results summary

n = 67		EUA Authorized Comparator Test		Total
		Positive	Negative	
TrioDx	Positive	37	0	37
	Negative	0	30	30
Total		37	30	67

Positive Percent Agreement: 100% (37/37) 95% CI: 90.51% - 100.00%
Negative Percent Agreement: 100% (30/30) 95% CI: 88.43% - 100.00%

17. Symbols

Table 22. Symbols

Symbol	Definition
	*In Vitro* Diagnostic Use device
	Batch lot number
	Catalog number
	Use-By-Date (Expiration Date)
	Temperature Limit
	See Instruction for Use (IFU)
	Manufacturer
	Manufacture date
	Caution
	Contains sufficient <n> tests
	Prescription Use only
	Emergency Use Authorization

18. Contact Information

Clinomics USA Inc.

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P: +1 (619) 261-4321

Email: markd@clinomics.com

Website: http://clinomics.com/en/main

19. Appendix A: Qualification of the ABI QuantStudio 6 Flex Real-Time PCR System using the Clinomics TrioDx RT-PCR COVID-19 Test

1. PURPOSE

This procedure describes a method for verifying the performance of the ABI QuantStudio 6 Flex Real-Time PCR System with the Clinomics TrioDx RT-PCR COVID-19 Test using spiked samples prepared for extraction. The QuantStudio 6 Flex Real-Time PCR System must pass the acceptance criteria described in this procedure prior to being utilized for clinical testing.

2. MATERIALS

Description	Quantity	Provided in the Kit
Viral Transport Media (VTM) or Universal Transport Media (UTM), sterile	~3 mL*	No
SARS-CoV-2 Positive Control	1 vial	Yes
RNase Free H_2O	1 vial	Yes

*Sufficient for preparing two spike-in samples

3. QUALITY CONTROL

3.1. Perform testing only as directed and in accordance with Good Laboratory Practices.

3.2. Ensure instrument calibration and maintenance are current prior to performing qualification.

3.3. Clean work surfaces and equipment with 10% bleach followed by 70% alcohol before and after use.

3.4. Store and use kit components as directed. Keep reagents and other kit components on ice or a cold block during testing, limiting freeze/thaw cycles to five.

3.5. The positive control provided with the TrioDx RT-PCR COVID-19 Test is a non-infectious synthetic oligonucleotide comprised of RNA fragments spanning SARS-CoV-2 genes (E, N, and RdRp) along with human GAPDH.

4. PROCEDURE

4.1. Prepare two spike-in samples for subsequent RNA extraction.

4.1.1. Label three 1.5 mL RNase-free microcentrifuge tubes as “Standard, A, and B”.

4.1.2. Pipette 495 µL of sterile VTM or UTM into Tube Standard and add 5 µL of SARS-CoV-2 Positive Control.

4.1.3. Vortex briefly and centrifuge to collect the contents at the bottom of the tube.

4.1.4. Pipette 990 µL of sterile VTM or UTM into Tube A and add 10 µL of the mixture from Tube Standard.

4.1.5. Vortex briefly and centrifuge to collect the contents at the bottom of the tube.

4.1.6. Pipette 800 µL of sterile VTM or UTM into Tube B and add 200 µL of the mixture from Tube A.

4.1.7. Vortex briefly and centrifuge to collect the contents at the bottom of the tube.

4.2. Extract the spike-in samples (Tubes A and B) and a negative control in triplicate for a total of nine extractions.

4.2.1. For manual extraction using the QIAGEN QIAamp Viral RNA Mini Kit:

4.2.1.1. Prepare enough Buffer AVL-carrier RNA mix for nine samples according to the manufacturer's instructions.

4.2.1.2. Label nine 1.5 mL microcentrifuge tubes as Tubes 1-9.

4.2.1.3. Pipette 560 µL of Buffer AVL-carrier RNA mix into each of Tubes 1-9.

4.2.1.4. Add 140 µL of Tube A into each of Tubes 1-3.

4.2.1.5. Add 140 µL of Tube B into each of Tubes 4-6.

4.2.1.6. Add 140 µL of RNase Free H2O into each of Tubes 7-9.

4.2.1.7. Extract the samples using the QIAGEN QIAamp Viral RNA Mini Kit following the manufacturer's instructions.

4.2.1.8. Elute the samples using 50 uL of Buffer AVE.

4.2.2. For automated extraction using the Promega Maxwell RSC 48 instrument and Maxwell RSC Viral Total Nucleic Acid Kit:

4.2.2.1. Label nine 1.5 mL microcentrifuge tubes as Tubes 1-9.

4.2.2.2. Pipette 140 µL of Tube A into each of Tubes 1-3.

4.2.2.3. Pipette 140 µL of Tube B into each of Tubes 4-6.

4.2.2.4. Pipette 140 µL of RNase Free H2O into each of Tubes 7-9.

4.2.2.5. Extract the samples using the Maxwell RSC Viral Total Nucleic Acid Kit and Maxwell RSC 48 instrument following the manufacturer's instructions.

4.2.2.6. Elute the samples in 50 µL of Nuclease-Free Water provided in the extraction kit.

4.2.3. For automated extraction using the Thermo Fisher KingFisher Flex instrument and MagMAX Viral/Pathogen II Nucleic Acid Isolation Kit:

4.2.3.1. Pipette 5 µL of Proteinase K into each of nine wells of a 96-well deep well plate, designated as wells 1-9.

4.2.3.2. Add 140 µL of Tube A into each of wells 1-3.

4.2.3.3. Add 140 µL of Tube B into each of wells 4-6.

4.2.3.4. Add 140 µL of RNase Free H2O into each of wells 7-9.

4.2.3.5. Extract the samples using the MagMAX Viral/Pathogen II Nucleic Acid Isolation Kit and KingFisher Flex instrument following the manufacturer's instructions.

4.2.3.6. Elute the samples in 50 µL of Elution Buffer.

4.3. Perform RT-PCR and result interpretation following the TrioDx RT-PCR COVID-19 Test IFU.

4.4. Expected Results

4.4.1. The eluates for Tubes 1-3 and wells 1-3 should contain a concentration of SARS-CoV-2 at approximately 5xLoD. The result should be positive for GAPDH, N, E, and RdRp.

4.4.2. The eluates for Tubes 4-6 and wells 4-6 contain a low concentration of SARS-CoV-2 at approximately 1xLoD. The result should be positive for GAPDH, N, E, and RdRp.

4.4.3. The eluates for Tubes 7-9 and wells 7-9 are negative samples that should not contain SARS-CoV-2. The result should be negative for GAPDH, N, E, and RdRp.

4.4.4. Acceptance Criteria:

4.4.4.1. Moderately Positive Samples (Tubes 1-3): 100% (3/3) concordance with the expected results should be observed

4.4.4.2. Low Positive Samples (Tubes 4-6): At least 66% (2/3) concordance with the expected results should be observed

4.4.4.3. Negative samples (Tubes 7-9): 100% (3/3) concordance with the expected results should be observed

4.4.4.4. The ABI QuantStudio 6 Flex Real-Time PCR System is considered qualified for use with the Clinomics TrioDx RT-PCR COVID-19 Test for clinical testing when the above criteria are met.

20. Appendix B: Additional Label for ABI QuantStudio 6 Flex

Please print and place this label on the front panel of the instruments. If the instrument includes labeling indicating "For Research Use Only", please cover with the below "Emergency Use Only" labeling. The instrument should retain this labeling throughout the EUA use of the Clinomics TrioDx RT-PCR COVID-19 Test.

Emergency Use Only

This instrument is authorized for use with Clinomics TrioDx RT-PCR COVID-19 Test

Clinomics TrioDx RT-PCR COVID-19 Test

Product Information Card

Prescription use only
In-vitro Diagnostic use
For Emergency Use Authorization only

Please refer to the full Instructions for Use (IFU), available at http://clinomics.com/en/covid19 for detailed instructions. Requests for a printed version at no additional cost can be sent to the contact information found on this document.

1. Description

Clinomics TrioDx RT-PCR COVID-19 Test (TrioDx) is a real-time reverse transcription polymerase chain reaction (Real-time RT-PCR) test for detection of novel coronavirus (SARS-CoV-2) in nasopharyngeal, oropharyngeal, mid-turbinate, and anterior nasal swabs collected from individuals suspected of COVID-19 by their health-care provider. The SARS-CoV-2 primer and probe sets included in the test detect three genes present in SARS-CoV-2 viral genome RNA: RdRp (RNA-dependent RNA polymerase), E (Envelope) and N (Nucleoprotein).

2. Intended Use

Clinomics TrioDx RT-PCR COVID-19 Test is an *in vitro* diagnostic real-time RT-PCR test intended for the qualitative detection of nucleic acid from SARS-CoV-2 in nasopharyngeal, oropharyngeal, mid-turbinate, and anterior nasal swabs collected from individuals suspected of COVID-19 by their healthcare provider. Testing authorized by the U.S. FDA is limited to laboratories that are certified under the Clinical Laboratory Improvement Amendments of 1988 (CLIA), 42 U.S.C. §263a, and 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 upper 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 infection status. Positive results do not rule out bacterial infection or co-infection with other viruses. The agent detected may not be the definite cause of disease. Laboratories within the United States and its territories are required to report all 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 TrioDx is intended for use by qualified and trained clinical laboratory personnel specifically instructed in the techniques of real-time PCR and *in vitro* diagnostic procedures. The TrioDx is only for use under the Food and Drug Administration's Emergency Use Authorization.

3. Kit Contents (Materials Provided)

Name	Specifications	Quantity	Sub-components
SARS-CoV-2 Positive Control	100 µL	1	Synthetic RNA fragments spanning RdRp, E, N and GAPDH target regions
2X Real-Time RT-PCR Mix	1 mL	1	RT enzyme, Taq Polymerase, dNTPs, PCR buffer, Mg2+
10X Primer & Probe Mix (PPM)	200 µL	1	SARS-nCoV-19 RdRp Primers+ HEX Probe, E gene Primers+ Texas Red Probe, N gene Primers+ FAM Probe, GAPDH (IC) Primers+ Cy5 Probe
RNase Free H2O	500 µL	1	N/A

The reagents included in one TrioDx kit are sufficient for testing 100 samples. Store at -20°C upon receipt.

4. Materials Required but Not Provided

Name	Recommended Supplier	Catalog #/ID
Swab specimen in VTM/UTM	Provided by healthcare provider	n/a
QIAamp Viral RNA Mini Kit	Qiagen	52906
1.5 mL microtubes	Corning (Axygen)	MCT-150-C-S
MicroAmp™ Optical 96-Well Reaction Plate	Thermo Fisher Scientific	4316813
MicroAmp™ Optical 8-Cap Strips	Thermo Fisher Scientific	4323032
MicroAmp™ Optical Adhesive Film	Thermo Fisher Scientific	4311971
10 µL Filter Tips	Corning (Axygen)	TXLF-10-R-S
200 µL Filter Tips	Corning (Axygen)	TF-200-R-S
1000 µL Filter Tips	Corning (Axygen)	TF-1000-R-S
1-10 µL Pipet	Eppendorf	3123000020
20-200 µL Pipet	Eppendorf	3120000054
100-1000 µL Pipet	Eppendorf	3123000063
Micro Centrifuge	Eppendorf	5424R
Mini centrifuge	Benchmark	C1012
Vortex Mixer	Scientific Industries	SI-0236

5. Compatible Real-Time PCR Instruments

The TrioDx is to be used with the ABI QuantStudio 6 Flex (Thermo Fisher, Product No. 4485692, Software version 1.3).

6. Warnings and Precautions

Use Statements

1. Prescription use only. Federal Law restricts this device to sale by or on the order of licensed practitioner.
2. For *in-vitro* diagnostic use (IVD)
3. For use under Emergency Use Authorization (EUA) only
4. The TrioDx has not been FDA cleared or approved; but has been authorized by FDA under an EUA for use in authorized laboratories.
5. The TrioDx has been authorized only for use for the detection of nucleic acid from SARS-CoV-2 and not for any other viruses or pathogens.
6. The emergency use of TrioDx 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) o the Federal Food, Drug, and Cosmetic Act, 21 U.S.C. § 360bbb-3(b)(1), unless the declaration is terminated or authorization is revoked sooner.
7. Laboratories within the United States and its territories are required to report all results to the appropriate public authorities.
8. The TrioDx must be used for its intended use only.

Safety & Hazards

1. Follow standard precautions. All patient specimens should be considered potentially infectious and handled accordingly.
2. Do not eat, drink, or smoke in testing areas where reagents and human specimens are handled.
3. Proper personal protective equipment including lab coats, gowns, gloves, eye protection, and biological safety cabinet are recommended for manipulation of clinical specimens. Refer to Biosafety in Microbiological and Biomedical Laboratories (BMBL) 5th Edition – CDC and World Health Organization, Laboratory Biosafety Manual 3rd Edition, WHO/CDS/CSR/LYO/2004.11.
4. Handle all specimens as if infectious using safe laboratory procedures. Refer to Interim Laboratory Biosafety Guidelines for Handling and Processing Specimens Associated with 2019-nCoV https://www.cdc.gov/coronavirus/2019-nCoV/lab-biosafety-guidelines.html. Dispose of hazardous or biologically contaminated materials according to the practices of your institution.
5. Please read the package insert carefully prior to operation. Each step of operation, from specimen collection, storage and transportation, and laboratory testing, should be strictly conducted in line with relevant biosafety regulations and molecular laboratory management.
6. Reliability of the test results depends on adequate specimen collection, storage, transport, and processing procedure.
7. Repeated freeze-thaw cycles of the reagents can negatively affect the sensitivity of the assay. Do not freeze and thaw more than 5 times.
8. If not tested immediately, store extracted RNA at -80 °C until use and keep on ice or cold block during testing.
9. Keep the reagents on ice or cold block during testing.
10. Pre-PCR procedures must be taken place in a clean room separated from PCR or post-PCR area to avoid contamination with amplified products.
11. Wear disposable gloves and change them frequently to avoid cross-contamination.
12. Use of sterilized filter pipette tips is highly recommended to avoid contamination between reagents.
13. Regularly clean the work surfaces using 0.5 % sodium hypochlorite.
14. Avoid exposure to light of the 10 X Primer & Probe Mix (PPM).

7. Reagent Storage and Handling

- Upon receipt of the kit, aliquot the reagents to appropriate amount and store at -20°C
- Limit the number of freeze-thaw cycles to 5.
- Do not use expired reagents. Refer to the manufactured date and expiry date printed on the package.
- Dispose unused or left-over reagents and waste in accordance with country, federal, state, and local regulations.

8. Test Procedure

Specimen Collection, Transport and Storage

➔ Collection:

(1) It is highly recommended that only the qualified healthcare professional (HCP) conduct the specimen collection.
(2) Refer to the CDC Interim Guidelines for Collecting, Handling, and Testing Clinical Specimens for COVID-19 (https://www.cdc.gov/coronavirus/2019-nCoV/lab/guidelines-clinical-specimens.html). Proper collection of specimens is the most crucial step in the molecular diagnosis of COVID-19, and a specimen collected inadequately may lead to false negative test results[1]
(3) Specimen collection device is not included with this test. It is recommended that nasopharyngeal/oropharyngeal swab specimens are collected using flocked swabs, such as nylon or Dacron. Calcium alginate swabs and cotton swabs with wooden shafts are not recommended, as they may contain interferent substances.
(4) Follow the manufacturer's instructions included with the specimen collection device. After collection, put swab into sterile tube containing Viral Transport Media (VTM) or Universal Transport Media (UTM).

➔ Transport and Storage:

(5) For transport, the swab specimens can generally be stored at 2-8 °C up to 72 hours.
(6) For long-term storage, store at -70°C or below.
(7) Specimens must be packaged, shipped, and transported according to the International Air Transport Association (IATA) Dangerous Goods Regulation. Follow shipping regulations for UN3373 Biological Substance, Category B when sending potential SARS-CoV-2 specimens.

RNA Extraction

Nucleic acids (RNA) are isolated and purified from upper respiratory specimens using any one of the following:

- QIAamp Viral RNA Mini Kit (Qiagen, Product No. 52906) for manual extraction
- Maxwell RSC Viral Total Nucleic Acid Kit (Promega, Product No. ASB1330) with Maxwell RSC 48 Instrument (Promega, Product No. AS8500, Software version 3.0) for automated extraction
- MagMAX Viral/Pathogen II Nucleic Acid Isolation Kit (Thermo Fisher, Product No. A48383) with KingFisher

Flex with 96 Deepwell Head Instrument (Thermo Fisher, Product No. 5400630, Software version 1.01.0) for automated extraction

(1) Transfer 140 µL of RNase-free H2O to a sterile 1.5 mL microcentrifuge tube to serve as the Negative Control throughout the remainder of the procedure.
(2) Transfer 140 µL of Viral Transport Medium (VTM) containing nasopharyngeal/oropharyngeal swab specimens to a sterile 1.5-mL microcentrifuge tube.
(3) Follow the standard procedures in the user manual provided by the appropriate kit manufacturer (Qiagen, Promega, or ThermoFisher)
(4) Using an elution buffer volume of 50 µL, follow the standard procedures in the user manual provided by the appropriate kit manufacturer (Qiagen, Promega, or ThermoFisher) to elute sample RNA and Negative Control.
(5) Proceed to RT-PCR without performing RNA quantification.

Real-Time RT-PCR

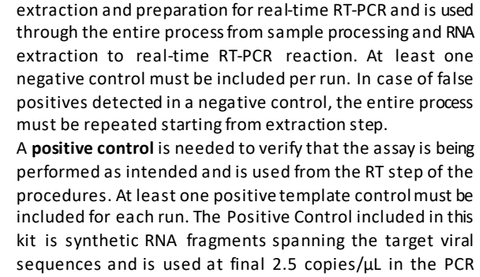
Components	Volume required for single reaction	Final Conc.	Volume used for N<15 reactions	Volume used for N≥15 reactions
2X real-time RT-PCR Mix	10 µL	1X	(N+1) x 10 µL	(N+2) x 10 µL
10X Primer & Probe Mix (PPM)	2 µL	1X	(N+1) x 2 µL	(N+2) x 2 µL
RNase Free H2O	3 µL	N/A	(N+1) x 3 µL	(N+2) x 3 µL
RNA sample	5 µL			
Total reaction volume	20 µL/rxn			
Note	N = number of specimens including positive and negative controls.			

(1) Prepare the reaction mixture as described in the table above. Vortex for 5 sec. Centrifuge to collect droplets.
(2) Prepare a sterile 96-well plate for real-time PCR. Pipette 15 µL of the reaction mixture into each well.
➔ Per one 96-well plate, use at least one negative control and one positive control (PC).
(3) Negative Control: 5 µL RNase-free H2O + 15 µL reaction mixture. Always add and securely seal/cap negative control before proceeding to specimen RNA handling.
(4) Specimens: 5 µL of purified RNA in each well + 15 µL reaction mixture. Securely seal the wells/tubes.
(5) Positive Control: In an area separated from the RT-PCR assembly area, first prepare Final Diluted Positive Control (see below) and then use 5 µL of Final Diluted Positive Control + 15 µL of reaction mixture. Securely seal/cap.
To prepare Final Diluted Positive Control, dilute SARS-CoV-2 Positive Control to a working stock of 2.5 copies/µL:
   a. First dilution: Add 198 µL of RNase-free H2O into a first 1.5 mL microcentrifuge tube, then add 2 µL of SARS-CoV-2 Positive Control. Gently mix well, then briefly centrifuge.
   b. Second (final) dilution: Add 195 µL of RNase-free H2O into a second 1.5 mL microcentrifuge tube, then add 5 µL of dilution created in substep 5a. Gently mix well, then briefly centrifuge. (This is Final Diluted Positive Control)

(6) Centrifuge the plate/tubes at 1,500 x g for 30 seconds.

Software Setting

(1) Start the QuantStudio Real-Time PCR Software v1.3.
(2) Set up the Experiment Properties as follows:



(3) Set up the thermal cycling reaction condition in the real-time PCR instrument as follows.

Reaction condition:			
Holding Stage: Reverse Transcription (RT)			
Temp	Time	Cycle	Note
55 °C	5 min	1	Reverse Transcription
95 °C	2 min	1	Inactivation of RT enzyme.
Cycling Stage: Amplification and Detection			
95 °C	15 sec	45	Activation
60 °C	30 sec		Annealing and fluorescence detection

Please refer to the instrument user guide for detailed instructions on how to use each instrument.

9. Quality Control

The following is the list of Controls included with TrioDx.

a) A **negative control** is needed to check for extraneous nucleic acid contamination that can happen during nucleic acid extraction and preparation for real-time RT-PCR and is used through the entire process from sample processing and RNA extraction to real-time RT-PCR reaction. At least one negative control must be included per run. In case of false positives detected in a negative control, the entire process must be repeated starting from extraction step.
b) A **positive control** is needed to verify that the assay is being performed as intended and is used from the RT step of the procedures. At least one positive template control must be included for each run. The Positive Control included in this kit is synthetic RNA fragments spanning the target viral sequences and is used at final 2.5 copies/µL in the PCR reaction mixture for the test.
c) An **internal control**: The 10X primer & probe mix provided in this kit contains a primer & probe set that targets human GAPDH. The purpose of an internal control is to evaluate the real-time PCR instrument performance and is used through the entire process from RNA extraction to data analysis.

The following table shows the interpretation guidelines for Controls. Follow the recommended actions based on the result interpretation. Proceed to Interpretation of Results only when the quality control results come out to be 'Valid'.

	RdRp gene	E gene	N gene	GAPDH	Result Interpretation
Pos Control	+	+	+	+	Valid
	One or more targets detected as negative (-) **				Invalid
Neg Control	-	-	-	-	Valid
	One or more targets detected as positive (+) ***				Invalid

**Ct value > 38 or Undetermined produces negative (-) result. Re-run real-time RT-PCR when one or more targets detected as negative (-)

***Ct value ≤38 produces positive (+) result. Re-extraction and re-run real-time RT-PCR when one or more targets detected as positive (+). Eliminate any contamination.

If any of the controls gives an invalid result, perform a root cause investigation and re-run all samples starting from extraction once the root cause is identified and eliminated.

10. Interpretation of Results

- Proceed only when control results are valid.
- If the Ct value obtained is ≤ to 38 then there is a positive (+) result. If the Ct value obtained is > 38 then there is a negative (-) result. Follow the recommended actions based on the result interpretation table below.

RdRp gene (HEX)	E gene (TEXAS RED)	N gene (FAM)	GAPDH (CY5)	Result Interpretation
+	+	+	+ or -	Positive SARS-CoV-2
+	+	-		
-	+	+		
+	-	+		
+	-	-		
-	-	+		
-	+	-	+ or -	Presumptive Positive for SARS-CoV-2
-	-	-	+	Negative
-	-	-	-	Invalid*

*Invalid result due to potential handling error (e.g., sample collection, RNA extraction) or inhibition.

11. Assay Limitations

1. This assay is for *in vitro* diagnostic use under FDA Emergency Use Authorization only. 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.
2. Use of this assay is limited to personnel who are trained in the procedure. Failure to follow these instructions may result in erroneous results.
3. The performance of TrioDx was established using nasopharyngeal swab. Other swabs (oropharyngeal, mid-turbinate and anterior nasal) are also considered acceptable specimen types for use with the SARS-CoV-2 Test Kit (Real-time PCR) but performance has not been established.
4. 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.
5. PCR product contamination might occur in the laboratory, reagent preparation and cross-contamination of samples and will produce false positive results. The components of the test kit may decline due to improper transportation, storage or inaccurate preparation and will produce false negative results. False negative results may also arise from improper sample collection, degradation of the viral RNA during shipping/storage, using unauthorized extraction or assay reagents, mutation in the SARS-CoV-2 virus and failure to follow instructions for use.
6. Low viral load and excessive degradation in the samples may cause negative results. Thus, a negative result cannot completely exclude the existence of SARS-CoV-2 in the sample and should not be the sole basis of a patient management decision. Follow up testing should be performed according to the current CDC recommendations.
7. Results should be interpreted by a trained professional in conjunction with the patient's history and clinical signs and symptoms, and epidemiological risk factors.
8. Extraction and amplification of nucleic acid from clinical samples must be performed according to the specified methods listed in this procedure. Other extraction approaches and processing systems have not been evaluated.
9. The impacts of vaccines, antiviral therapeutics, antibiotics, chemotherapeutic or immunosuppressant drugs have not been evaluated.
10. Laboratories are required to report all results to the appropriate public health authorities.
11. Result for the E gene is Presumptive Positive. Sample should be retested. For samples with a repeated Presumptive Positive result, additional confirmatory testing may be conducted, if it is necessary to differentiate between SARS-CoV-2 and SARS-CoV-1 or other Sarbecovirus currently unknown to infect humans, for epidemiological purposes or clinical management.
12. This assay cross reacts with SARS-CoV. Additional testing is recommended if the exact coronavirus needs to be identified.
13. Positive results are indicative of the presence of SARS-CoV-2 RNA, but clinical correlation with patient history and other diagnostic information is necessary to determine patient infection status. Positive results do not rule out bacterial infection or co-infection with other viruses. The agent detected may not be the definite cause of disease.
14. 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.

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