ACCELERATED EMERGENCY USE AUTHORIZATION (EUA) SUMMARY

Thermo Fisher TaqMan 2019-nCoV Assay Kit v1 (singleplex)

(ORF1ab, N, and S gene detection)

Altru Diagnostics, Inc.

Rx Only

For use under Emergency Use Authorization (EUA) only

(Altru Dx SARS-CoV-2 RT-PCR assay will be performed at Altru Diagnostics, Inc, certified under the Clinical Laboratory Improvement Amendments of 1988(CLIA), 42 U.S.C. §263a as per Laboratory Standard Operating Procedure that was reviewed by the FDA under this EUA.)

INTENDED USE

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

Results are for the identification of SARS-CoV-2 RNA. The SARS-CoV-2 RNA is generally detectable in respiratory specimens during the acute phase of infection. Positive results are indicative of the presence of SARS-CoV-2 RNA; clinical correlation with patient history and other diagnostic information is necessary to determine patient infection status. Positive results do not rule out bacterial infection or co-infection with other viruses. The agent detected may not be the definite cause of disease.

Laboratories within the United States and its territories are required to report all positive results to the appropriate public health authorities.

Negative results do not preclude SARS-CoV-2 infection and should not be used as the sole basis for patient management decisions. Negative results must be combined with clinical observations, patient history, and epidemiological information.

The assay is intended for use under the Food and Drug Administration’s Emergency Use Authorization.

DEVICE DESCRIPTION AND TEST PRINCIPLE

The Altru Diagnostics SARS-CoV-2 Test uses the Thermo Fisher Scientific TaqMan 2019-nCoV Assay Kit v1 (singleplex) RUO Kit. The ThermoFisher assay is a Real-Time reverse transcription polymerase chain reaction assay. The primer and probe sets used with the test are designed to amplify and detect three regions of the SARS-CoV-2 single stranded RNA genome: the Orf1ab, N gene, and S gene.

All probes are labeled with FAM and all targets are amplified in individual reactions. RNA isolated from respiratory specimens is reverse transcribed to cDNA using the TaqMan Fast Virus 1-Step Master Mix and subsequently amplified using the QuantStudio 12K Flex Real-Time PCR system (Applied Biosystems) with Software version 1.2.2.

During the amplification process, the probe anneals to the three specific target sequences located between the forward and reverse primers. During the extension phase of the PCR cycle, the 5' nuclease activity of Taq polymerase degrades the bound probe, causing the reporter dye to separate from the quencher dye generating a fluorescent signal.
With each cycle, additional reporter dye molecules are cleaved from their respective probes, increasing the fluorescence. Fluorescence intensity is monitored at each PCR cycle by the QuantStudio 12 Flex.

INSTRUMENTS USED WITH TEST

The Applied Biosystems TaqMan 2019-nCoV Assay Kit v1 (Part No. A47532) is to be used on the QuantStudio 12K Flex Real-Time PCR System (384-well, Block) with Software version: QuantStudio 12K Flex Software V.1.2.2,
Extraction instruments: Thermo Scientific KingFisher Flex Purification System with Applied Biosystems MagMax Viral/Pathogen Ultra and the MagMAX Viral/Pathogen II (MVPII) Nucleic Acid Isolation Kits.

EQUIPMENT, REAGENTS AND MATERIALS

The following main equipment/reagents/materials are required to run this test:

1. Extraction Kits:
   - MagMax Viral/Pathogen Ultra (ThermoFisher #42356)
   - MagMAX Viral/Pathogen II (MVPII; ThermoFisher # A48383)
2. KingFisher Flex Purification system (KingFisher)
3. Thermo Fisher TaqMan 2019-nCoV Assay Kit v1 (singleplex) (Thermo Fisher, # A47532)
4. TaqMan 2019-nCoV Control Kit v1 (Thermo Fisher, #A47533)
5. TaqMan Fast Virus 1-Step MasterQuantStudio 12K Flex

CONTROLS TO BE USED WITH THE SARS-CoV-2 RT-PCR

- The TaqMan 2019-nCoV Control Kit v1 (Cat. No. A47533) serves as an amplification positive control for the Orf1ab, N and S gene amplicon sequences as well as for RNase P. The dilution and reverse transcription of the positive control is performed according to the kit instructions and a single positive control is included in every PCR run. The positive control is used to monitor for failures of RT-PCR reagents and reaction conditions. This control is only included in the RT-PCR reaction.

- The internal control amplifies the human RNase P gene of the sample. This control is used to monitor the RNA extraction, reverse transcription and amplification process.

- Negative control: No-template control (NTC): A negative no template extraction control (molecular-grade, nuclease-free water) is added to each KingFisher extraction run and carried through to RT-PCR. The negative control verifies that no contamination of sample or reagents occurred during either the extraction or the PCR step.

INTERPRETATION OF RESULTS

All test controls should be examined prior to interpretation of patient results. If the controls are not valid, the patient results cannot be interpreted.

a. Interpretation of Controls – Internal Positive, Positive and Negative Controls

- The Internal Control in a sample indicates that PCR amplification occurred in the well. The presence of RNaseP and no detectable SARS-CoV-2 during the analysis indicates that proper RNA extraction and amplification occurred. In this case, the amplified SARS-CoV-2 indicates proper RNA extraction and amplification.
- TaqMan COVID-19 Control Kit (External Positive Control) must be positive for all four SARS-CoV-2 targets, i.e., the ORF1ab, the N Gene, the S Gene genes and RNaseP and amplification must have a Ct <37 in order for the test result to be valid. The Positive control does not contain MS2.
- Nuclease-Free Water (Negative No Template Control) must be negative for all four targets, i.e., the ORF1ab, the N Gene, the S Gene genes and RNaseP in order for the test result to be valid.

Table 1: Expected Performance of Controls

	ORF1ab	N gene	S gene	RNase P	Status	Result
No Template control (NTC)	All target genes Negative (Ct > 37)			Neg	Valid	Run Passed
TaqMan COVID-19 Positive Control	All target genes Positive (Ct ≤37)			Pos Ct ≤37	Valid	Run Passed

If any of the above controls do not exhibit the expected performance as described, the assay may have been improperly set up and/or executed improperly, or reagent or equipment malfunction could have occurred. Invalidate the run and re-test.

b. Examination and Interpretation of Patient Specimen Results:

Assessment of clinical specimen test results should be performed after the positive and negative controls have been examined and determined to be valid and acceptable. If the controls are not valid, the patient results cannot be interpreted. Assessment of the clinical specimen test results are performed as follows using a Ct 37 as a cutoff: Any target with a Ct ≤37 is positive and any target with a Ct >37 is negative.

Table 2. Interpretation of Patient Samples

ORF1ab	N gene	S gene	RNaseP	Controls Status	Result	Action
NEG	NEG	NEG	NEG	Invalid	Invalid	Re-extract the specimen or request re-collection
NEG	NEG	NEG	POS	Valid	SARS-CoV-2 Not Detected	Release the report on the portal as negative
Only one SARS-CoV-2 target = POS			POS	Valid	SARS-CoV-2 Inconclusive	Re-extract the specimen. If consistently inconclusive, suggest recollection or additional testing, if the results improve, report accordingly
Two or more SARS-CoV-2 targets = POS			POS / NEG	Valid	SARS-CoV-2 detected	Release the report on the portal as positive, call provider and report to public health department

PERFORMANCE EVALUATION

1) Analytical Sensitivity:

Leftover negative deidentified clinical specimens (NP swabs) from prior to the COVID-19 pandemic (i.e., prior to Dec. 2019) were pooled to create the negative matrix used in this study to generate positive samples for the tentative and the confirmatory LoD study.

Using genomic RNA from BEI Resources (NR-52285; v2019-nCoV/USA-WA1/2020 lot# 70033700 quantitated as 5.5x10^7 genome equivalents/mL), 3 replicates of each dilution were created in this negative sample matrix with concentrations in the range of 0.175 to 5 cp/µL. Samples were extracted according to the laboratory SOP using the MagMAX Viral Pathogen Ultra Kit. The lowest concentration that yielded 100% of positivity was 0.625 cp/µL (tentative LoD).

To confirm the tentative LoD 20 replicates containing 0.625 cp/µL were generated by spiking the BEI material into negative nasal swab samples. All 20 replicates produced valid positive results for S and Orf1ab, but not for the N gene (all replicates were RNAse P positive). However, based on the result interpretation of two positive targets, all 20 replicates would therefore be positive at 0.625 copies/µl.

Table 3. SARS-CoV-2 LoD

Target Level [cp/µL]	SARS-CoV-2 N-Gene Positive			SARS-CoV-2 Orf1ab-Gene Positive			SARS-CoV-2 S-Gene Positive			Internal Control RNaseP Positive*		
	n	Mean Ct	Detection Rate	n	Mean Ct	Detection Rate	n	Mean Ct	Detection Rate	n	Mean Ct	Detection Rate
0.625	17	33.8	85%	20	33.0	100%	20	31.0	100%	60*	18.7	100%
0.7	20	33.8	100%	20	33.0	100%	20	31.0	100%	60*	18.7	100%

*Note: RNase P is n=60 because this is a singleplex assay in which each of the SARS-CoV targets is amplified and detected in parallel in the same reaction with RNAseP.

Based on the result interpretation of two positive targets, the final LoD for this test is 0.625 copies/µL.

2) Analytical Inclusivity/Specificity:

a. Inclusivity

Per the manufacturers protocol supplied with the test, the included target assays have undergone bioinformatic selection and analysis to specifically target sequences that are unique to SARS-CoV-2.

b. Cross-Reactivity

Wet testing was performed by preparing contrived samples using the Natrol Respiratory Verification Panel V2 (ZeptoMetrix; Ref# NATRVP2-BIO). The Zeptometrix panel information did not provide concentrations of the individual organisms. However, the presence of sufficient cross-reactive nucleic acid in the ZeptoMetrix material was estimated by analyzing the same pools on the laboratory's respiratory multiplex panel which has similar TaqMan chemistry with FAM labeled probes. The panel produced amplification for all the organisms in the table below with Ct values indicative of moderate to high concentrations of these organism. The prepared spiked samples with the organisms listed in Table 4 were extracted and analyzed per laboratory SOP to determine the cross reactivity for all three assays. All three COVID-19 targets were undetermined.

Table 4. Cross Reactivity with the Natrol Respiratory Verification Panel V2

Pool	Pathogen	Each Pathogen	Ct value
Pool 1 (800 uL VTM + volume for each organism	Adenovirus Type 3	50 uL	Undetermined
	Influenza A 2009 H1N1	50 uL	Undetermined
	Influenza B	50 uL	Undetermined
	Coronavirus OC43	50 uL	Undetermined
Pool 2 (800 uL VTM + volume for each organism	Rhinovirus 1 A	50 uL	Undetermined
	Influenza A subtype H3	50 uL	Undetermined
	Parainfluenza virus Type 1	50 uL	Undetermined
	Parainfluenza virus Type 2	50 uL	Undetermined
Pool 3 (750 uL VTM + volume for each organism	Adenovirus Type 1	50 uL	Undetermined
	Influenza A H1	50 uL	Undetermined
	Parainfluenza virus Type 3	50 uL	Undetermined
	Coronavirus NL63	50 uL	Undetermined
	Bordetella Para pertussis	50 uL	Undetermined
Pool 4 (750 uL VTM + volume for each organism	Adenovirus Type 31	50 uL	Undetermined
	Bordetella pertussis	50 uL	Undetermined
	Chlamydia pneumoniae	50 uL	Undetermined
	Mycoplasma pneumoniae	50 uL	Undetermined
	Coronavirus HKU1	50 uL	Undetermined
Pool 5 (800 uL VTM + volume for each organism	Parainfluenza virus Type 4	50 uL	Undetermined
	Coronavirus 229E	50 uL	Undetermined
	Respiratory Syncytial Virus A	50 uL	Undetermined
	Human Metapneumovirus 8	50 uL	Undetermined

In addition, 14 residual clinical respiratory pathogen panel samples which were detected as positive for Coronavirus HKU1, NL63 or OC43 were analyzed to assess the cross reactivity with related viruses. None of the samples detected any of the three SARS-CoV-2 targets but all produced RNaseP amplicons with Ct values at or significantly below 18.

Table 5. Cross reactivity with Leftover, De-Identified Specimens

Sample ID/Corona Type	Ct Value (S, ORF1ab and N)
200304RM0006- CoV_HKU1-CoV_HKU1	Undetermined
200304RM0060- CoV_NL63-CoV_NL63	Undetermined
200304RM0082- CoV_OC43-CoV_OC43	Undetermined
200303RM0030- CoV_HKU1-CoV_HKU1	Undetermined
200303RM0036- CoV_OC43-CoV_OC43	Undetermined
200303RM0037- CoV_OC43-CoV_OC43	Undetermined
200303RM0067- CoV_OC43-CoV_OC43	Undetermined
200229RM0006- CoV_HKU1-CoV_HKU1	Undetermined
200229RM0021- CoV_NL63-CoV_NL63	Undetermined
200228RM0002- CoV_OC43-CoV_OC43	Undetermined
200228RM0024- CoV_OC43-CoV_OC43	Undetermined
200227RM0047- CoV_NL63-CoV_NL63	Undetermined
200227RM0018- CoV_HKU1-CoV_HKU1	Undetermined
200227RM0022- CoV_NL63-CoV_NL63	Undetermined

3) Clinical Evaluation:

a. Testing of Contrived Specimens:

Residual clinical nasal swab specimens from November 2019 (leftover deidentified samples from routine testing with the respiratory panel at Altru Diagnostics) were analyzed as 30 individual samples. The results were expected negative because the specimens were collected before the COVID-19 pandemic in the U.S.; i.e., prior to December 2019. These samples were split in aliquots. One aliquot was tested as a negative sample with the Altru Diagnostic SARS-CoV-2 test according to the instructions for use. A second aliquot was tested as a contrived positive sample. Negative and contrived positive samples were blinded for testing.

Thirty contrived reactive specimens were created by spiking genomic RNA from NR-52285 v2019-nCoV/USA-WA1/2020, lot# 70033700) into the SARS-CoV-2 negative nasal swab matrix (see above). Samples were extracted using the MagMAX Viral Pathogen Ultra Kit and tested per the laboratory SOP.

All negative specimens gave a SARS-CoV-2 not detected result; all spiked samples were detected positive for SARS-CoV-2 for all three targets.

Table 6. Testing of Contrived Clinical Specimens

Clinical Evaluation for Altru Dx COVID 19 RT PCR Test														
Target Level (cp/uL)	**Valid results**	**SARS-CoV-2 (Orf1) Positive**		**SARS-CoV-2 (Orf1) Detection Rate %**	**Valid results**	**SARS-CoV-2 (S) Positive**		**SARS-CoV-2 (S) Detection Rate**	**Valid results**	**SARS-CoV-2 (N) Positive**		**SARS-CoV-2 (N) Detection Rate**	**Rnase P Positive**		**Rnase P Detection Rate**
		n	**Mean Ct**			**n**	**Mean Ct**			**n**	**Mean Ct**		**n**	**Mean Ct**	
Negative	30	30	UND	N/A	30	30	UND	N/A	30	30	UND	N/A	90	23.3	100%
1400 cp/mL (2 X LoD)	20	20	32.1	100%	20	20	30.9	100%	20	20	32.4	100%	60	19	100%
2100 cp/mL (3 X LoD)	5	5	32	100%	5	5	30.6	100%	5	5	32.2	100%	15	18.7	100%
3500 cp/mL (5 X LoD)	5	5	31.2	100%	5	5	29.9	100%	5	5	31.3	100%	15	18.8	100%

Positive Percent Agreement (PPA): 30/30 = 100% (95% CI: 88.7% -100%)
Negative Percent Agreement (NPA): 30/30 = 100% (95% CI: 88.7% -100%)

b. Clinical Sample Testing

The sponsor submitted 5 positive and 6 negative nasal swabs samples to the Houston Health Lab (Houston Department of Health and Human Services (HDHHS)) for the verification of the results. The Houston Health Lab used the Emergency Use authorized CDC 2019-nCoV Real-Time RT-PCR Diagnostic Panel.

Table 7. Clinical Sample Testing

		HDHHS	
		POSITIVE	NEGATIVE
Altru Dx	POSITIVE	5	0
	NEGATIVE	0	6

The testing of these clinical specimens performed at Altru Diagnostics and at the alternate testing laboratory fulfills the requirement for confirmatory testing for at least 5 positive and 5 negative specimens.

LIMITATIONS

- Members of the infectious disease laboratory will be trained to perform this assay and competency will be assessed and documented per CLIA regulations.
- Negative results do not preclude SARS-CoV-2 infection and should not be used as the sole basis for treatment or other patient management decisions.
- A false negative result may occur if a specimen is improperly collected, transported or handled. False negative results may also occur if amplification inhibitors are present in the specimen or if inadequate numbers of organisms are present in the specimen.
- This test cannot rule out diseases caused by other bacterial or viral pathogens.

WARNINGS:

- This test has not been FDA cleared or approved;
- This test has been authorized by FDA under an EUA for use by authorized laboratories;
- This test has been authorized only for the detection of nucleic acid from SARSCoV-2, not for any other viruses or pathogens; and
- This test is only authorized for the duration of the declaration that circumstances exist justifying the authorization of emergency use of in vitro diagnostic tests for detection and/or diagnosis of COVID-19 under Section 564(b)(1) of the Act, 21 U.S.C. § 360bbb-3(b)(1), unless the authorization is terminated or revoked sooner.