ACCELERATED EMERGENCY USE AUTHORIZATION (EUA) SUMMARY

SARS-CoV-2 (E, N and RdRP gene detection) Test

(Exact Sciences Laboratory)

For *In vitro* Diagnostic Use
Rx Only
For use under Emergency Use Authorization (EUA) only

(The SARS-CoV-2 RT-PCR assay will be performed at the Exact Sciences Laboratories laboratory, 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 (E, N and RdRP gene detection) Test* is a real-time RT-PCR assay intended for the qualitative detection of nucleic acid from SARS-CoV-2 in nasal, nasopharyngeal, and oropharyngeal swab specimens, from individuals suspected of COVID-19 by a healthcare professional. Testing is limited to Exact Sciences Laboratories, certified under the Clinical Laboratory Improvement Amendments of 1988 (CLIA), 42 U.S.C. § 263a, to perform high complexity tests.

Results are for the detection 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 SARS-CoV-2 (E, N and RdRP gene detection) Test uses the Allplex (Novaplex) 2019-nCoV Assay Kit real-time reverse transcription polymerase chain reaction (rRT - PCR) test developed by Seegene. The primer and probe sets used with the test are designed to detect three regions of the SARS-CoV-2 single stranded RNA genome:
E gene (an envelope protein) for which the primer/probe set is Sarbecovirus specific and N gene (a nucleocapsid protein) and RdRP (RNA dependent RNA polymerase) both of which are specific to SARS-CoV-2. All probes are labeled with unique fluorophores that are detected and distinguished within the same reaction. RNA isolated from upper respiratory specimens (i.e., nasopharyngeal, oropharyngeal or nasal swab) is reverse transcribed to cDNA and subsequently amplified using the QuantStudio 5 (QS5) real-time PCR system (Applied Biosystems) with Design and Analysis Software version 1.5.1. During the amplification process, the probe anneals to a specific target sequence 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 dyes (FAM, HEX, Quasar 670, and Cal Red 610) to separate from the quencher generating a fluorescent signal. Fluorescence intensity is monitored at each PCR cycle by the QS5.

INSTRUMENTS USED WITH TEST

RNA extraction is conducted using the Maxwell RSC Viral Total Nucleic Acid Purification Kit on the Maxwell RSC 48 Instrument (Promega) for nucleic acid extraction and the QuantStudio 5 real-time PCR system (Applied Biosystems) for cDNA synthesis and PCR amplification of the target sequences.

EQUIPMENT, REAGENTS AND MATERIALS

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

1. Maxwell RSC 48 Instrument (Promega) for nucleic acid extraction
2. QuantStudio 5 real-time PCR system (Applied Biosystems) for cDNA synthesis and PCR amplification of the target sequences.
3. Maxwell RSC Viral Total Nucleic Acid Purification Kit (Promega Cat No. AS1330)
4. AllPlex (Novaplex) 2019-nCoV Assay Kit (Seegene Cat No. RP10243X)
5. 0.2 mL PCR Reaction plates (Applied Biosystems; catalog #4346906 or #4366932)
6. MicroAmp Optical 8-Cap Strips (Applied Biosystems; catalog #4323032)
7. 1.5 mL microcentrifuge tubes (DNase/RNase free)
8. Micropipettes (2 or 10 µL, 200 µL, and 1000µL)
9. Multichannel pipette (2-20 µL)
10. ST-40Aerosol barrier pipette tips Centrifuge Package
11. Molecular grade water, nuclease-free
12. Vortex mixer
13. Microcentrifuge

14. Surface disinfectant (10% bleach wipes and alcohol wipes)

CONTROLS TO BE USED WITH THE COVID-19 RT-PCR

- An Internal Control (Seegene PR-V IC) is included in each clinical sample at the time of extraction and is coextracted and co-amplified with the sample. The IC controls for specimen quality and demonstrates that nucleic acid was generated by the extraction process.

- A No Template (Negative) Control (NTC) (Seegene RNAse-free water) is included on every assay plate and is used to monitor non-specific amplification, cross-contamination during experimental setup, and nucleic acid contamination of reagents. NTC is included in the PCR reaction only.

- A Positive Control (Seegene 2019-nCoV PC) is included in each assay run and is used to verify that the assay is performing as intended. The PC contains E, N and RdRP genomic regions targeted by the test and the IC. The PC is included in the PCR run only.

- A Negative Extraction Control (NEC) is a blank extraction control spiked with internal control. It serves both as a negative extraction control to monitor for any cross contamination that occurs during the extraction process, as well as an extraction control to validate extraction reagents and successful RNA extraction as it is used in combination with IC.

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. Control Result Interpretation

Table 1: Expected Performance of Controls

Control Type	External Control Name	E	RdRP	N	IC	Expected Ct values
Negative Control	NTC	-	-	-	-	No Ct, or Ct ≥ 40 for all targets
Positive Control	Seegene 2019-nCoV PC	+	+	+	+	< 40 Ct for E, RdRP and N targets; < 40 Ct for IC target.
Negative Extraction Control	NEC	-	-	-	+	≥ 40 Ct for N, E and RdRP targets; < 40 Ct for IC target.

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.

Table 2: Interpretation of Patient Results

IC (HEX)	E gene (FAM)	RdRP gene (CalRed 610)	N gene (Quasar 670)	Result	Result interpretation	Patient Report Verbiage
+/-	+	+	+	Positive	All targets are valid. SARS-CoV-2 (COVID-19) RNA detected.	SARS-CoV-2 (COVID-19) RNA detected
+/-	+	-	+	Positive	All targets are valid. SARS-CoV-2 (COVID-19) RNA detected. Any assay with a single viral target detected at Ct<20 should be evaluated by the leadership. Missing amplification of individual targets in this group may due to: 1) Sample concentration near or below the limit of detection of the test, 2) a mutation in the corresponding target region, or 3) other factors	SARS-CoV-2 (COVID-19) RNA detected.
+/-	+	+	-			
+/-	-	+	+			
+/-	-	-	+			
+/-	-	+	-			
+	-	-	-	Negative	All targets are valid. SARS-CoV-2 (COVID-19) RNA **NOT** detected.	SARS-CoV-2 (COVID-19) RNA **NOT** detected. Negative results do not preclude SARS-CoV-2 (COVID-19) infection and should not be used as the sole basis for treatment or other patient management decisions.

IC (HEX)	E gene (FAM)	RdRP gene (CalRed 610)	N gene (Quasar 670)	Result	Result interpretation	Patient Report Verbiage
+/-	+	-	-	Inconclusive	All Target Results are valid. Sarbecovirus RNA is detected but SARS-CoV-2 specific RNA targets are not detected. -Repeat testing -For samples with the same result on a repeated test, additional confirmatory testing will be conducted using an alternative method. (Ship specimen to 650 for testing using the N gene method.)	REPORT FROM ALTERNATIVE TEST WITH THE FOLLOWING COMMENT ADDED: This specimen did not meet the full criteria established for the detection of SARS-CoV-2 (COVID-19) RNA using the SARS-CoV-2 (E, N, RdRP gene detection) assay. The sample was evaluated using an alternative test method.
-	-	-	-	Invalid	Results are invalid. Repeat testing If the result is still invalid, a new specimen should be obtained.	**Invalid** – This specimen exhibited inhibition in the PCR assay or the specimen contained an inadequate amount of clinical material. Repeat testing is suggested if clinically indicated.

*Specimens using alternative test reporting should report the final result first with a comment on why alternative testing was completed. Test reports will include all required information regarding the test and the performing laboratory as outlined by CAP and CLIA.

PERFORMANCE EVALUATION

1) Analytical Sensitivity:

The LoD of the SARS-CoV-2 (E, N and RdRP gene detection) Test was determined using the AccuPlex SARS-CoV-2 Reference Material Kit (SeraCare). AccuPlex recombinant materials are constructed with a replication-deficient mammalian virus producing a safe, non-infectious material. These mammalian virus-based reference materials resemble the complexity of virus targets found in true patient samples, including the viral particle protein coat and lipid bilayer.

a. Tentative LoD

For the tentative LoD, known titer (5 genomic copies/µL) of AccuPlex SARS-CoV-2 Reference Material (SeraCare) were spiked into the extraction lysis buffer. A preliminary LoD was determined by testing dilutions of RNA with 3 replicates/dilution. Spiked samples were tested with the SARS-CoV-2 (E, N and RdRP gene detection) Test following extraction with the Maxwell RSC 48 Instrument and the Maxwell RSC Viral Total Nucleic Acid Purification Kit (Promega). Real-Time RT-PCR assays were performed using the Allplex 2019-nCoV Assay reagents (Seegene) on the QuantStudio 5

system Real-Time PCR Instrument. The RT-PCR assay was setup according to the Allplex 2019-nCOV Assay instructions for use. The lowest concentration of SARS-CoV-2 RNA that yielded a detection rate of ≥ 95% for the E, N and RdRP genes was 0.6 cp/µl (**Table 3**).

Table 3: SARS-CoV-2 Tentative LoD

SARS-CoV-2 - Tentative LoD										
Target Level*	**Valid results**	**N Positive**		**SARS-CoV-2 (N/ORF) Detection Rate**	**E Positive**		**SARS-CoV-2 (E/ORF) Detection Rate**	**RdRP Positive**		**SARS-CoV-2 (RdRP/ORF) Detection Rate**
		n	**Mean Ct**		**n**	**Mean Ct**		**n**	**Mean Ct**	
0.3 cp/µL	2	2	36.20	66%	3	31.88	100%	3	36.98	100%
0.6 cp/µL	3	3	37.59	100%	3	34.56	100%	3	37.87	100%
1.25 cp/µL	3	3	35.83	100%	3	32.34	100%	3	35.80	100%
2.5 cp/µL	3	3	34.61	100%	3	31.34	100%	3	34.63	100%
Negative	2			0%			0%			0%
Tentative LoD: 0.6 cp/µL [lowest target level demonstrating >95% detection rate of SARS-COV-2]										

* Target copy level refers to the amount of copies present per µl of sample input in extraction based on sample input volume of 100 µl. Nucleic acid elution volume is 50 µl.

b. Confirmatory LoD

The LoD was verified using RNA samples from 20 additional replicates. Dilutions were generated using AccuPlex SARS-CoV-2 Reference Material (SeraCare) in a suspension of human A549 cells and VTM to mimic clinical specimens. Results are summarized in Table 4. The E and RdRP genes have an LoD of 0.6 copies/µl. However, the LoD for the N gene is at 1.25 cp/µl as the 0.6 copies/ul only detected 18/20 (90%) positives.

Table 4: Confirmatory LOD

	E gene	**N gene**	**RdRP**	**Internal control**
RNA concentration - 0.6 cp/µl of sample				
Positives	20/20	18/20	20/20	20/20
Mean Ct	30.7	35.1	34.4	28.1
Std dev	0.69	1.27	1.18	1.28
RNA concentration - 1.25 cp/µl of sample				
Positives	20/20	20/20	20/20	20/20
Mean Ct	30.8	35.0	35.1	29.8
Std dev	1.3	1.2	0.8	1.6

2) *Analytical Inclusivity/Specificity:*

a. Inclusivity

The SARS-CoV-2 (E, N and RdRP gene detection) amplification assay was developed by Seegene, Inc (Republic of Korea). As such, the primer and probe sequences utilized with this test were not readily available for in silico analysis. The SARS-CoV-2 (E, N and RdRP gene detection) Test is designed to detect the N and RdRP genes specific for SARS-CoV-2, and the E gene for all of Sarbecovirus including SARS-CoV-2.

b. Cross-Reactivity

i. *In Silico* Analysis

The primers and probes used with the SARS-CoV-2 (E, N and RdRP gene detection) Test were analysed in silico and wet-tested for cross-reactivity to 61 viral pathogens by Seegene and results are available under the following link: http://www.seegene.com/upload/product/Allplex_2019_nCoV_performance_data.pdf

ii. Wet Testing

Additionally, nucleic acid from common respiratory pathogens was extracted and tested with the SARS-CoV-2 (E, N and RdRP gene detection) Test by Exact Sciences Laboratories to further demonstrate analytical specificity and exclusivity. Studies were performed with nucleic acids extracted using the NATtrol Respiratory Pathogen Panel-1 (NATRPP-1) from Zeptometrix that is formulated with purified, intact virus particles and bacterial cells that have been chemically modified to render them non-infectious. Nucleic acids were extracted using Maxwell RSC 48 Instrument (Promega) and the Maxwell RSC Viral Total Nucleic Acid Purification Kit (Promega). No cross-reactivity was observed for the tested organisms.

Table 5. Organisms Tested for Cross-Reactivity

Organism	Replicates	Result (E, RdRP, N)
Influenza A H1N1 (A/NY/02/09)	4	E, RdRP, and N Not detected
Influenza A H1 (A/New Caledonia/20/99	4	E, RdRP, and N Not detected
Influenza A H3 (A/Brisbane/10/07)	2	E, RdRP, and N Not detected
Influenza B (Florida/02/06)	2	E, RdRP, and N Not detected
Respiratory Syncytial Virus A	4	E, RdRP, and N Not detected
Respiratory Syncytial Virus B (CH93(18-18)	2	E, RdRP, and N Not detected
Parainfluenza 1	3	E, RdRP, and N Not detected
Parainfluenza 2	2	E, RdRP, and N Not detected
Parainfluenza 3	2	E, RdRP, and N Not detected
Parainfluenza 4A	3	E, RdRP, and N Not detected
Parainfluenza 4B	3	E, RdRP, and N Not detected
Rhinovirus (1A)	3	E, RdRP, and N Not detected
Adenovirus Type 3	3	E, RdRP, and N Not detected
Human coronavirus NL63	5	E, RdRP, and N Not detected
Human coronavirus OC43	3	E, RdRP, and N Not detected
Human coronavirus HKU1	2	E, RdRP, and N Not detected
Human coronavirus 229E	3	E, RdRP, and N Not detected

Organism	Replicates	Result (E, RdRP, N)
Human Metapneumovirus (Peru6-2003)	2	E, RdRP, and N Not detected
Human Bocavirus	2	E, RdRP, and N Not detected
Mycoplasma pneumoniae (M129)	4	E, RdRP, and N Not detected
Legionella (non-pneumophila)	2	E, RdRP, and N Not detected
Chlamydophila pneumoniae	2	E, RdRP, and N Not detected

3) Clinical Evaluation:

Performance of the SARS-CoV-2 (E, N and RdRP gene detection) Test was evaluated with 40 nasopharyngeal (NP) and oropharyngeal (OP) clinical specimens and 40 contrived specimens prepared as noted below. RNA was extracted from all samples using the Maxwell RSC Viral Total Nucleic Acid Purification Kit (Promega). Testing for detection of the E, N and RdRP genes was performed in a total of seven RT-PCR runs with four controls.

a. Clinical Specimen Testing

A sample panel provided by the Wisconsin State Laboratory of Hygiene (WSLH) was used for clinical evaluation. Samples were tested with the SARS-CoV-2 (E, N and RdRP gene detection). Test and concordance was determined based on results reported by the WSLH after testing specimens with the CDC 2019-nCoV Real-Time RT-PCR Diagnostic Panel. Concordance with the CDC test was based on a Ct value < 40 for specimens considered positive and a Ct value of ≥ 40 for specimens considered negative for the presence of SARS-CoV-2 E, N and RdRP genes. The sample panel consisted of the following:

- Five de-identified positive patient respiratory specimens and 15 de-identified negative respiratory specimens.
- 20 blinded de-identified patient specimens (10 positive and 10 negative).

The Ct values obtained at Exact Sciences Laboratories for the 15 positive specimens include 9 specimens with a Ct value of < 28, 2 specimens with Ct values between 28-30 and 4 specimens with Ct values > 30. Of the 25 negative specimens analyzed, one specimen had Ct values of 37 for the E and N gene. These results are considered inconclusive. Therefore, all the positive and negative specimens are in concordance with the expected outcome (Table 6).

Table 6: Evaluation with Clinical Specimens

		CDC PCR	
		PRESUMPTIVE POSITIVE	NEGATIVE
Exact Sciences Laboratories	PRESUMPTIVE POSITIVE	15	0
	NEGATIVE	0	25

Positive Percent Agreement (PPA): 15/15 = 100% (95% CI: 74.6% -100%)
Negative Percent Agreement (NPA): 25/25 = 100% (95% CI: 83.4% -100%)

In addition, the first five positive and first five negative samples by the SARS-CoV-2 (E, N and RdRP gene detection) Test were also sent to the WSLH for confirmatory testing. All 10 patient specimens yielded concordant results. The testing on these clinical specimens performed at Exact Sciences Laboratories and at the alternate testing laboratory fulfills the requirement for confirmatory testing for at least 5 positive and 5 negative specimens.

b. Contrived Specimen Testing

A series of 16 contrived clinical specimens were prepared by spiking negative NP or OP clinical specimens or Universal Transport Medium (UTM) with high titer positive specimens. In addition, 24 samples were prepared by spiking UTM and 10,000 A549 cells with AccuPlex SARS-nCOV-2019 Reference Material at 1X, 2X and 3X LoD. Samples were tested per the SOP. Performance was compared against the expected result based on the spiking status of the sample. Results of this study are summarized in Table 7.

Table 7: Evaluation with Contrived Specimens

Contrived Specimen Study								
Specimens	**Sample Concentration**	**n**	**N Target**		**E Target**		**RdRP**	
			Positive (n)	**Mean Ct**	**Positive (n)**	**Mean Ct**	**Positive (n)**	**Mean Ct**
AccuPlex™ SARS-nCOV-2019 Reference Material	1.25 copies/µL 1 X LoD	7	7	34.5	7	28.7	7	32.3
	2.5 copies/µL 2 X LoD	9	9	33.5	9	28.3	9	31.4
	3.75 copies/µL 3 X LoD	8	8	33.1	8	27.6	8	30.7
Negative	N/A	8	1	34.6	0	> 40	0	>40

Positive Percent Agreement (PPA): 40/40 = 100% (95% CI: 89.1% - 100%)
Negative Percent Agreement (NPA): 8/8 = 100% (95% CI: % 59.8- 100%)