ACCELERATED EMERGENCY USE AUTHORIZATION (EUA) SUMMARY
COVID-19 RT-PCR TEST
(LABORATORY CORPORATION OF AMERICA)

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

(The COVID-19 RT-PCR test (LabCorp Laboratory Test Number: 139900) will be performed at the Center for Esoteric Testing in Burlington, North Carolina, or other laboratories designated by LabCorp that are also certified under the Clinical Laboratory Improvement Amendments of 1988(CLIA), 42 U.S.C. §263a as per Laboratory Instructions for Use that was reviewed by the FDA under this EUA.)

INTENDED USE

The COVID-19 RT-PCR test is a real-time reverse transcription polymerase chain reaction (rRT-PCR) test for the qualitative detection of nucleic acid from SARS-CoV-2 in upper and lower respiratory specimens (such as nasopharyngeal or oropharyngeal swabs, sputum, lower respiratory tract aspirates, bronchoalveolar lavage, and nasopharyngeal wash/aspirate or nasal aspirate) collected from individuals suspected of COVID-19 by their healthcare provider. Testing is limited to the Center for Esoteric Testing, Burlington, NC, or other laboratories designated by LabCorp that are also 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.

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

DEVICE DESCRIPTION AND TEST PRINCIPLE

The COVID-19 RT-PCR Test is a real-time reverse transcription polymerase chain reaction (rRT -PCR) test. The test uses three primer and probe sets to detect three regions in the SARS-CoV-2 nucleocapsid (N) gene and one primer and probe set to detect human RNase P (RP) in a clinical sample. RNA isolated from upper and lower respiratory specimens (such as nasopharyngeal or oropharyngeal swabs, sputum, lower respiratory tract aspirates, bronchoalveolar lavage, and nasopharyngeal wash/aspirate or nasal aspirate) is reverse transcribed to cDNA and subsequently amplified using Applied Biosystems QuantStudio7 Flex (QS7) instrument with software version 1.3. 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 dye (FAM) to separate from the quencher dye (BHQ1), generating a fluorescent signal. Fluorescence intensity is monitored at each PCR cycle by QS7.

INSTRUMENTS USED WITH TEST

The COVID-19 RT-PCR test is to be used with the Roche MagNA Pure-96 (MP96) using MagNA Pure 96 DNA and Viral NA Small Volume Kit and Applied Biosystems QuantStudio7 Flex (QS7) instrument with software version 1.3.

REAGENTS AND MATERIALS

Reagent	**Manufacturer**	**Catalog #**
DNA and Viral Small Volume Kit (3x192 purifications)	Roche	06543588001
TaqPath 1-Step RT-PCR Master Mix, GC (2000 reactions)	ThermoFisher	A15300
COVID-19_N1-F Primer	IDT	Custom
COVID-19_N1-R Primer	IDT	Custom
COVID-19_N1-P Probe	IDT	Custom
COVID-19_N2-F Primer	IDT	Custom
COVID-19_N2-R Primer	IDT	Custom
COVID-19_N2-P Probe	IDT	Custom
COVID-19_N3-F Primer	IDT	Custom
COVID-19_N3-R Primer	IDT	Custom
COVID-19_N3-P Probe	IDT	Custom
RP-F Primer	IDT	Custom
RP-R Primer	IDT	Custom
RP-P Probe	IDT	Custom
COVID-19_N_Positive Control	IDT	Custom
Hs_RPP30_Internal Extraction Control	IDT	Custom

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

1) A negative (no template) control is needed to eliminate the possibility of sample contamination on the assay run and is used on every assay plate. This control is molecular grade, nuclease-free water.

2) A positive template (COVID-19_N_P) control is needed to verify that the assay run is performing as intended and is used on every assay plate starting at master mix addition at a concentration of 50 copies/uL. The positive control is made of *in vitro* transcribed and purified viral RNA target that contains one copy each of N1, N2, and N3. The positive template control does not include RNase P target and will result as "undetermined" for that marker.

3) An internal (Hs_RPP30) control targeting RNase P is needed to verify that nucleic acid is present in every sample and is used for every sample processed. This also serves as the extraction control to ensure that samples resulting as negative contain nucleic acid for testing.

4) A negative extraction (NEC) control is a previously characterized negative patient sample. 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.

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.

1) ***COVID-19 RT-PCR test Controls – Positive, Negative, and Internal:***

   Negative (no template control) – negative for all targets detected (Ct Not Detected)
   Positive (COVID-19_N_P) – positive for all targets detected (Ct < 40)
   Internal extraction (Hs_RPP30) – negative for SARS-CoV-2 targets (Ct Not Detected), positive for RNase P (RP) target (Ct < 40)
   Negative extraction (NEC) – negative for SARS-CoV-2 targets (Ct Not Detected), positive for RNase P (RP) target (Ct < 40)

   If any control does not perform as described above, run is considered invalid and all specimens are repeated from extraction step.

2) ***Examination and Interpretation of Patient Specimen Results:***

   RP – all clinical samples should yield positive results for RP target at < 40 Ct. Samples that fail to show detection of RP and all three SARS-CoV-2 targets within this range should be repeated from extraction step. If sample detects any of the SARS-CoV-2 targets, the lack of amplification of RP target can be valid.

COVID-19 RT-PCR test results interpretation

SARS-CoV-2 N1	SARS-CoV-2 N2	SARS-CoV-2 N3	RNase P	Result Interpretation	Report	Actions
+	+	+	+/-	SARS-CoV-2 Detected	POSITIVE	Report results to sender and appropriate public health authorities.
If only one or both targets are positive		+/-	+/-	SARS-CoV-2 Detected	POSITIVE	Report results to sender and appropriate health authorities.
-	-	+	+/-	SARS-CoV-2 is Presumptive Positive	PRESUMPTIVE POSITIVE	Sample is repeated once. If the repeated result remains "PRESUMPTIVE POSITIVE", additional confirmatory testing may be conducted, if it is necessary to differentiate between SARS-CoV-2 and other SARS-like viruses for epidemiological purposes or clinical management.
-	-	-	+	SARS-CoV-2 Not Detected	NEGATIVE	Report results to sender.
-	-	-	-	Invalid Result	INVALID	Sample is repeated once. I f a second failure occurs, it is reported to sender as invalid and recommend recollection if patient is still clinically indicated.

PERFORMANCE EVALUATION

1) Analytical Sensitivity:

Limit of Detection (LoD):

The LoD study established the lowest concentration of SARS-CoV-2 (genome copies(cp)/µL) that can be detected by the COVID-19 RT-PCR test at least 95% of the time. The preliminary LoD was established by testing 10-fold dilutions of SARS-CoV-2 synthetic RNA. The preliminary LoD was confirmed by testing 20 replicates of 2-fold dilutions (50 cp/µL, 25 cp/µL, 12.5 cp/µL, 6.25 cp/µL, 3.125 cp/µL, and 1.25 cp/µL). The samples of 2-fold dilutions were prepared by spiking the quantified live SARS-CoV-2 into negative respiratory clinical matrices (NP swabs and BAL). The study results showed that the LoD of the COVID-19 RT-PCR test is 6.25 cp/µL (19/20 positive).

2) ***Analytical Specificity:***

Cross-reactivity of the COVID-19 RT-PCR test was evaluated using both *in silico* analysis and by testing whole organisms or purified nucleic acid from a panel of organisms listed in the table below.

The empirical testing showed that all targets were negative for all tested microorganisms except for the SARS coronavirus which is expected to react with N3 target (target for the universal detection of SARS-like viruses) of the COVID-19 RT-PCR test.

Cross-reactivity test results:

Sample Name	N1 CT	N2 CT	N3 CT	Source (Concentration)
Adenovirus 11	Not detected	Not detected	Not detected	ATCC VR-12D (1e^6)
Adenovirus 5	Not detected	Not detected	Not detected	ATCC VR-5D; Adenoid 75 (1.5e^6)
Bordetella pertussis	Not detected	Not detected	Not detected	Patient Sample (1e^5)
Chlamydophila pneumoniae	Not detected	Not detected	Not detected	ATCC 53592D; AR-39 (5e^6)
Enterovirus 70	Not detected	Not detected	Not detected	ATCC VR-836; J670-71 (1e^6)
Haemophilus influenzae	Not detected	Not detected	Not detected	ATCC 51907D (1e^6)
Human coronavirus	Not detected	Not detected	Not detected	ATCC VR-740; 229E (1e^6)
Human coronavirus	Not detected	Not detected	Not detected	ATCC VR-3263SD; NL63 (7e^5)
Human coronavirus	Not detected	Not detected	Not detected	ATCC VR-3262SD; HKU1 (6e^5)
Human coronavirus	Not detected	Not detected	Not detected	Patient Sample; OC43 (1e^5)
Human metapneumovirus	Not detected	Not detected	Not detected	ATCC VR-3250SD (6e^5)
Human parainfluenza virus 1	Not detected	Not detected	Not detected	ATCC VR-94D; C35 (2e^7)
Human parainfluenza virus 2	Not detected	Not detected	Not detected	ATCC VR-92D; Greer (2e^7)
Human parainfluenza virus 3	Not detected	Not detected	Not detected	ATCC VR-1782; ATCC-2011-5
Human parainfluenza virus 4b	Not detected	Not detected	Not detected	ATCC VR-1377; CH 19503
Human respiratory syncytial virus	Not detected	Not detected	Not detected	ATCC VR-1580; 18537
Human rhinovirus 61	Not detected	Not detected	Not detected	ATCC VR-1171; 6669-CV39
Influenza A	Not detected	Not detected	Not detected	ATCC VR-1679D; H3N2, A/Hong Kong/8/68 (2e^6)
Influenza B	Not detected	Not detected	Not detected	ATCC VR-1735D; B/Taiwan/2/62 (3e^6)
Legionella pneumophila	Not detected	Not detected	Not detected	ATCC 33152D-5; Philadelphia-1 (1.5e^6)
Middle East Respiratory Syndrome coronavirus	Not detected	Not detected	Not detected	ATCC VR-3248SD; MERS (6e^5)
Mycobacterium tuberculosis	Not detected	Not detected	Not detected	ATCC 25177; H37Ra
Mycoplasma pneumoniae	Not detected	Not detected	Not detected	ATCC 15531D; FH of Eaton Agent (3e^6)
Severe Acute Respiratory Syndrome coronavirus	Not detected	Not detected	30.768	BEI NR-3882; SARS
Streptococcus pneumoniae	Not detected	Not detected	Not detected	ATCC 33400D-5 (3e^6)
Streptococcus pyogenes	Not detected	Not detected	Not detected	ATCC 12344D-5; T1 (3e^6)

BLAST analysis showed no homology with primers and probes of the COVID-19 RT-PCR test for the organisms listed in the table below.

***In silico* analysis:**

Pathogen	Strain	GenBank Acc#	% Homology Test Forward Primer	% Homology Test Reverse Primer	% Homology Test Probe
Candida albicans	All	All	0	0	0
Neisseria meningitidis	All	All	0	0	0
Pseudomonas aeruginosa	All	All	0	0	0
Staphylococcus aureus	All	All	0	0	0

3) Clinical Evaluation:

A contrived clinical study was performed to evaluate the performance of the COVID-19 RT-PCR test. A total of 100 individual clinical respiratory samples, 50 NP swabs and 50 BALs, were used in this study. 100 negatives and 80 contrived positives were tested. Negative samples include 50 NP swabs and 50 BALs. Positive samples were comprised of 40 NP swabs and 40 BALs spiked with quantitated live SARS-CoV-2. 10 samples each were spiked at 8x, 4x, 2x, and 1X LoD. In one contrived BAL sample, prepared at LoD, N3 target was not determined. The positive and negative percent agreements between the COVID-19 RT-PCR test and the expected results in NP swabs and BALs are shown below:

Clinical performance of the COVID-19 RT-PCR test with NP swabs:

	SARS-CoV-2 concentration	Number of NP swabs	N1 target % Positive (95% CIs)	N2 target % Positive (95% CIs)	N3 target % Positive (95% CIs)
COVID-19 RT-PCR test	1x LoD	10	100% (72.25 – 100)	100% (72.25 – 100)	100% (72.25 – 100)
	2x LoD	10	100% (72.25 – 100)	100% (72.25 – 100)	100% (72.25 – 100)
	4x LoD	10	100% (72.25 – 100)	100% (72.25 – 100)	100% (72.25 – 100)
	8x LoD	10	100% (72.25 – 100)	100% (72.25 – 100)	100% (72.25 – 100)
	Negative	50	0 (NA)	0 (NA)	0 (NA)

NA = Not available

Performance of the COVID-19 RT-PCR test against the expected results are:

Positive Percent Agreement 40/40 = 100% (95% CI: 91.24% - 100%)
Negative Percent Agreement 50/50 = 100% (95% CI: 92.87% - 100%)

Clinical performance of the COVID-19 RT-PCR test with BAL specimens:

	SARS-CoV-2 concentration	Number of NP swabs	N1 target % Positive (95% CIs)	N2 target % Positive (95% CIs)	N3 target % Positive (95% CIs)
COVID-19 RT-PCR test	1x LoD	10	100% (72.25 – 100)	100% (72.25 – 100)	90%* (59.59 – 98.22)
	2x LoD	10	100% (72.25 – 100)	100% (72.25 – 100)	100% (72.25 – 100)
	4x LoD	10	100% (72.25 – 100)	100% (72.25 – 100)	100% (72.25 – 100)
	8x LoD	10	100% (72.25 – 100)	100% (72.25 – 100)	100% (72.25 – 100)
	Negative	50	0 (NA)	0 (NA)	0 (NA)

NA = Not available
*One BAL sample had failed detection of N3 target. Since the SARS-CoV-2 specific targets, N1 and N2 were detected, the overall result for this sample was “POSITIVE”.

Performance of the COVID-19 RT-PCR test against the expected results are:

Positive Percent Agreement 40/40 = 100% (95% CI: 91.24% - 100%)
Negative Percent Agreement 50/50 = 100% (95% CI: 92.87% - 100%)

Additionally, five positive and five negative patient samples were sent to the North Carolina Department of Health (NCDOH) and tested on the CDC assay under an EUA. All results were concordant.

Sample	COVID-19 RT-PCR test	NCSLPH Result CDC assay under and EUA
1	Not detected	Not detected
2	Not detected	Not detected
3	Positive	Presumptive Positive
4	Not detected	Not detected
5	Positive	Presumptive Positive
6	Positive	Presumptive Positive
7	Not detected	Not detected
8	Positive	Presumptive Positive
9	Not detected	Not detected
10	Positive	Presumptive Positive