EMERGENCY USE AUTHORIZATION (EUA) SUMMARY FOR THE CLEVELAND CLINIC SARS-COV-2 ASSAY

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

(The Cleveland Clinic SARS-CoV-2 Assay will be performed at the Cleveland Clinic Robert J. Tomsich Pathology and Laboratory Medicine Institute located at 9500 Euclid Ave/LL2, Cleveland, OH 44195, certified under the Clinical Laboratory Improvement Amendments of 1988(CLIA), 42 U.S.C. §263a, as per the Standard Operating Procedure that was reviewed by the FDA under this EUA.)

INTENDED USE

1) Intended Use:

The Cleveland Clinic SARS-CoV-2 Assay is a multi-target, real-time RT-PCR test intended for the qualitative detection of nucleic acid from the SARS-CoV-2 in nasopharyngeal (NP), oropharyngeal (OP), and nasal swabs and bronchoalveolar lavage (BAL), tracheal aspirate (TASP), and sputum from individuals suspected of COVID-19 by their healthcare provider.

Testing is limited to the Cleveland Clinic Robert J. Tomsich Pathology and Laboratory Medicine Institute located at 9500 Euclid Ave/LL2, Cleveland, OH 44195, which is certified under the Clinical Laboratory Improvement Amendments of 1988 (CLIA), 42 U.S.C. §263a and meets requirements to perform high-complexity tests.

Results are for the detection and 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 Cleveland Clinic SARS-CoV-2 Assay is intended for use by qualified clinical laboratory personnel specifically instructed and trained in the techniques of real-time PCR and in-vitro diagnostic procedures. The Cleveland Clinic SARS-CoV-2 Assay is only for use under the Food and Drug Administration's Emergency Use Authorization.

DEVICE DESCRIPTION AND TEST PRINCIPLE

The Cleveland Clinic SARS-CoV-2 Assay is a real-time, reverse transcription polymerase chain reaction (RT-PCR) test. The SARS-CoV-2 primer and probe set(s) are designed to detect RNA from the SARS-CoV-2 in respiratory specimens from patients suspected of COVID-19 by their healthcare provider.

Infectious virus is inactivated by treating the clinical specimen with Roche MagNA Pure External Lysis Buffer. Nucleic acids are extracted with the Roche MagNA Pure 96 DNA and Viral NA Small Volume kit. Primer and probe sets are purchased from Roche as LightMix Modular Assays. LightCycler Multiplex RNA Virus Master, which contains RT and PCR reagents, is added to primer/probe sets followed by template addition. RT-PCR is conducted according to the TIB MOLBIOL cycling parameters on Roche LightCycler 480 and/or cobas z 480 instruments. LightCycler 480 and/or cobas z 480 software generates baseline and cycling threshold values for each primer/probe assay for each sample.

INSTRUMENTS USED WITH THE TEST

The Cleveland Clinic SARS-CoV-2 Assay is to be used with the Roche LightCycler 480 and/or cobas z 480 instruments and software version v1.2.1.62 SP3. Extraction is performed on the Roche MagNA Pure 96 with software version 3.0.

REAGENTS AND MATERIALS

Reagent Description	**Catalog#**	**Manufacturer**
LightMix Modular SARS and Wuhan CoV E gene Kit	53-0776-96	TIB MOLBIOL
LightMix Modular Wuhan CoV RdRP gene Kit (including positive control for RdRP)	53-0777-96	TIB MOLBIOL
LightMix Modular RNAse P Kit	QC-0101-96	TIB MOLBIOL
LightCycler Multiplex RNA Virus Master (Catalog # 06 754 155 001)	06 754 155 001	Roche
SARS-CoV-2 Standard (positive control for E and RP)	COV019	Exact Diagnostics
Hs_RPP30 Human Extraction Control	10006626	IDT
MagNA Pure 96 DNA and Viral NA Small Volume Kit	6543588001	Roche
MagNA Pure 96 External Lysis Buffer	6374913001	Roche
Molecular Biology Grade Water	W4502	Sigma-Aldrich

CONTROLS TO BE USED WITH THE TEST

1) **Positive Control:** A Positive Control for each assay (E gene, RdRP gene, and RP) is included on each run to monitor the performance of the RT-PCR. The RdRP LightMix kit positive control is used for RdRP and a SARS-CoV-2 Standard containing human gDNA is used as a positive control for E and RP.

2) **Negative Control:** A negative template control consisting of PCR grade water to be performed with each primer/probe mix and included on each RT-PCR run.

3) **RNA Extraction Control:** The Human Specimen RPP30 control is purchased from IDT and contains a portion of the RPP30 gene which is present in the human genome. The extraction control is included with each extraction run. Extracted control material is tested with each primer/probe mix and is included on each assay run.

4) **Internal Control:** The assay includes primers and a probe for detection of endogenous RNase P nucleic acid that is extracted and amplified from every patient sample.

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.

Table 1. Expected Results of Controls for the Cleveland Clinic SARS-CoV-2 Assay

Control	Control Type	Control Name	Expected Results			Expected Ct values
			E	RdRP	RP	
E Gene/RNase P Positive Control	Synthetic RNA/Human gDNA	SARS-CoV-2 Standard	Positive	Negative	Positive	<40
RdRP Gene Positive Control	Synthetic RNA	RdRP RNA Positive Control	Negative	Positive	Negative	<40
Extraction Control	Plasmid	Hs_RPP30	Negative	Negative	Positive	<40
Negative Control	Water	NTC	Negative	Negative	Negative	No Ct

Assessment of clinical specimens should be performed after the positive, negative, RNA extraction control, and internal controls have been examined and determined to be valid and acceptable. If the controls are not valid, the patient results cannot be interpreted. The interpretation and reporting of clinical specimens is summarized in Table 2.

Table 2. Interpretation of Clinical Results for the Cleveland Clinic SARS-CoV-2 Assay

2019- nCoV E	**2019-nCoV RdRP**	**RP**	**Result Interpretation**	**Actions**	**Report**
Positive	Positive	Positive or Negative	Detected	Report results to Public Health and Submitter	Positive for COVID-19 (SARS-CoV-2) by PCR.
Positive (Ct≤35)	Negative	Positive	Presumptive positive	Report results to Public Health and Submitter	Positive by single pan SARS-CoV target. SARS-CoV-1 cannot be excluded, but it is not currently circulating in North America.
Positive (Ct>35)	Negative	Positive	Indeterminate Result	Repeat extraction and RT-PCR with alternative confirmed method	Indeterminate for COVID19 (SARS CoV2), the test has been repeated with similar results. Please submit a new specimen if clinically indicated.
Positive	Negative	Negative	Indeterminate Result	Repeat extraction and RT-PCR with alternative confirmed method	Indeterminate for COVID19 (SARS CoV2), the test has been repeated with similar results. Please submit a new specimen if clinically indicated.
Negative	Positive	Positive or Negative	Indeterminate Result	Repeat extraction and RT-PCR with alternative confirmed method	Indeterminate for COVID19 (SARS CoV2), the test has been repeated with similar results. Please submit a new specimen if clinically indicated.
Negative	Negative	Positive	Not Detected	Report results to submitter.	Negative for COVID-19 (SARS CoV2) by PCR.
Negative	*Negative*	Negative	Invalid Result	Repeat extraction and RT-PCR. If repeat result remains invalid, suggest recollection.	Invalid for COVID-19 (SARS CoV-2); the specimen was inhibitory to amplification. The test has been repeated with similar results. Please submit a new specimen if clinically indicated.

PERFORMANCE EVALUATION OF THE CLEVELAND CLINIC SARS-COV-2 ASSAY

1) Limit of Detection (LoD) -Analytical Sensitivity:

A. Precision/Reproducibility:

Prior to LoD analysis, studies were conducted to determine precision and reproducibility from serially diluted samples. The SeraCare AccuPlex SARS-CoV-2 Verification Panel (pseudovirus) at starting concentration 100,000 copies/ml was serially diluted in negative patient NP/UTM matrix and tested in triplicate for 2 runs. A blank consisting of negative patient NP/UTM matrix was also tested in triplicate for 2 runs.

Results of Precision/Reproducibility with Pseudovirus:

Tube	Concentration (copies/µl)	Positive for nCoV E	Positive for nCoV RdRP	Negative for COVID-19	Indeterminate or Invalid for COVID-19	Presumptive Positive for COVID-19	Detected (Positive for COVID-19)	% Concordance
1	100	6/6	6/6	0/6	0/6	0/6	6/6	100
2	10*	6/6	6/6	0/6	0/6	0/6	6/6	100
3	1	6/6	2/6	0/6	3/6	1/6	2/6	50
4	0.1	2/6	0/6	4/6	2/6	0/6	0/6	0
6	Blank	0/6	0/6	6/6	0/6	0/6	0/6	100

* RdRP is inefficiently detected below 10 copies/µl.

In addition, a positive patient NP sample was diluted in UTM and tested in triplicate for 2 runs.

Results of Precision/Reproducibility with Patient Sample:

Tube	Concentration (copies/µl)[+]	Positive for nCoV E	Positive for nCoV RdRP	Negative for COVID-19	Indeterminate or Invalid for COVID-19	Presumptive Positive for COVID-19	Detected (Positive for COVID-19)	% Concordance
1	20,000	6/6	6/6	0/6	0/6	0/6	6/6	100
2	2,000	6/6	6/6	0/6	0/6	0/6	6/6	100
3	200	6/6	6/6	0/6	0/6	0/6	6/6	100
4	20*	6/6	6/6	0/6	0/6	0/6	6/6	100
5	2	6/6	4/6	0/6	2/6	0/6	4/6	67
6	0.2	2/6	1/6	4/6	1/6	0/6	1/6	17
7	0.02	2/6	0/6	4/6	2/6	0/6	0/6	0

* RdRP is inefficiently detected below 20 copies/µl.

[+]Concentration was estimated with the CDC EUA assay by comparing Ct values of samples and controls at known concentration (diluted in clinical matrix (NP/UTM)).

B. Limit of Detection Studies:

Based on reproducibility testing, 20 replicates were tested to confirm the LoD. LoD studies were performed by diluting the SeraCare AccuPlex SARS-CoV-2 Verification Panel at a starting concentration of 100 copies/µl diluted in 20 negative patient NP/UTM matrix and 20 negative sputum samples to a concentration of 10 copies/µl.

Result of LoD in negative clinical matrix:

SARS-CoV-2 gene	Clinical Matrix	Concentration (copies/µl)	Ratio Confirmed	Avg. Ct	SD
E gene	NP /UTM	10	20/20	32.2	0.24
	Sputum	10	20/20	33.0	0.17
RdRP gene	NP /UTM	10	20/20	36.4	0.28
	Sputum	10	20/20	36.3	0.27

2) Inclusivity (analytical sensitivity):

This SARS-CoV-2 test utilizes LightMix Modular assays for Wuhan CoV E and RdRP genes designed and evaluated by TIB MOLBIOL. An *in silico* analysis was performed using genes E and RdRP primers and probe sequences in literature referenced by TIB MOLBIOL[1]. The primers and probes for each assay were submitted for Basic Local Alignment Search Tool (BLAST) analysis and manually reviewed. The results revealed 100% detection of all 2019-nCoV strains as of July 16, 2020.

Of 7,195 US sequences, 7 had RdRP primer mismatches, 1 had RdRP probe mismatch, 3 had E gene primer mismatches, and 1 had an E gene probe mismatch.

In addition, the analysis of the RP gene primers (detect both DNA and RNA) and probe demonstrated hybridization with only these targeted sites in humans and other organisms. There were no hybridizations detected with any coronaviruses. The final analysis indicated that the RP assay will function as an amplification control and will not detect any coronaviruses.

3) Cross-reactivity (Analytical Specificity)

Cross reactivity testing was performed by assessing clinical samples known to contain target and ATCC strains of organisms as noted below. A panel of organisms closely related to the target and/or commonly found in respiratory specimen types was tested, i.e., organisms that represent the normal flora of the biological specimens to be used in testing, and those that commonly cause similar disease. A 1:100 dilution of a 0.5 MacFarland of each bacteria or yeast obtained from ATCC (nominally equivalent to 3 x 10^6 CFU/mL for bacteria and 1 to 5 x 10^4 CFU/mL for yeast) was

[1] Corman et al., Detection of 2019 novel coronavirus (2019-nCoV) by real-time RT-PCR, https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6988269/.

prepared in upper respiratory matrix and tested for cross-reactivity with the SARS-CoV-2 assays.

Results from cross reactivity study:

Laboratory Tested Organism List	Source	COVID-19 Result
Mycoplasma pneumonia	Patient Sample	Negative
Coronavirus HKU-1	Patient Sample	Negative
Human Metapneumovirus	Patient Sample	Negative
Chlamydophila pneumoniae	Patient Sample	Negative
Coronavirus 229E	Patient Sample	Negative
Coronavirus NL63	Patient Sample	Negative
Influenza A H1	Patient Sample	Negative
Respiratory Syncytial Virus A	Patient Sample	Negative
Parainfluenza Type 1	Patient Sample	Negative
Parainfluenza Type 3	Patient Sample	Negative
Adenovirus	Patient Sample	Negative
Rhinovirus	Patient Sample	Negative
Coronavirus OC43	Patient Sample	Negative
Respiratory Syncytial Virus B	Patient Sample	Negative
Influenza A H3	Patient Sample	Negative
Influenza B	Patient Sample	Negative
Parainfluenza Type 4	Patient Sample	Negative
Parainfluenza Type 2	Patient Sample	Negative
Staphylococcus aureus	ATCC 29213	Negative
Pseudomonas aeruginosa	ATCC27853	Negative
Candida albicans	ATCC10231	Negative
Staphylococcus epidermidis	ATCC12228	Negative
Haemophilus influenzae	ATCC49247	Negative
Streptococcus pneumoniae	ATCC49619	Negative
Streptococcus pyogenes	ATCC19615	Negative
Mycoplasma pneumoniae	Patient Sample	Negative
Coronavirus HKU-1	Patient Sample	Negative

Note: The concentration of organisms/virus present in the above patient samples is not known.

In addition, *in silico* analysis of the SARS-CoV-2 genes E and RdRP primers and probes was performed for all organisms recommended for testing per FDA including the following organisms that were not available for empirical assessment: MERS-coronavirus, *Mycobacterium tuberculosis*, *Streptococcus salivarius*, *Pneumocystis jiroveci*, *Bordetella pertussis*, and Enterovirus.

The analysis did not show cross reactivity with any analyzed organisms. *In silico* cross-reactivity is defined as greater than 80% homology between one of the primers/probes and any sequence present in the targeted microorganism.

According to the TIB MOLBIOL IFU, the E gene assay will detect SARS and Wuhan 2019 CoV pneumonia virus as well as other bat-associated SARS-related viruses (Sarbecovirus); there is no cross reactivity with common human respiratory viruses CoV NL63, 229E, HKU, OC43 or MERS.

4) Matrix Equivalency

An accuracy study was performed with Amies transport medium, sterile saline, and PBS with the SARS-CoV-2 assay. For each medium, 20 samples (10 positives and 10 negatives) were tested. The positive samples were contrived by spiking each medium with patient samples previously tested by a CDC 2019-nCoV EUA assay performed at the Cleveland Clinic (CDC EUA). Results demonstrated 100% (20/20) concordance for each sterile saline, PBS, and Amies. One sample in Amies was presumptive positive. In addition, 10 samples for each sterile saline and PBS were contrived by spiking a positive patient sample at 2x LoD and tested. Results demonstrated 100% (10/10) concordance for sterile saline, and 100% (10/10) concordance for PBS.

Results from Accuracy study:

Matrix	**SARS-CoV-2 Gene E Concordance**	**SARS-CoV-2 Gene RdRP Concordance**	**Overall Concordance**
Amies	20/20	19/20*	100%
Sterile Saline	20/20	20/20	100%
Sterile Saline at 2x LoD	10/10	10/10	100%
PBS	20/20	20/20	100%
PBS at 2x LoD	10/10	10/10	100%

*One sample in Amies was presumptive positive.

An LoD confirmation study was also performed with sterile saline by diluting the SeraCare AccuPlex SARS-CoV-2 Verification control (starting concentration 100 copies/µl) in negative patient NP/saline matrix to a concentration of 10 copies/µl. Thirty total specimens were tested (20 positive and 10 negative). There was 100% positive/negative agreement with the expected results for both SARS-CoV-2 targets.

Results from sterile saline LoD study:

SARS-CoV-2 gene	**Clinical Matrix**	**Concentration (copies/µl)**	**Expected/Tested**	**Avg. Ct**	**SD**
E gene	NP/saline	10	20/20	32.4	0.22
E gene	NP/saline	0	10/10	NA	NA
RdRP gene	NP/saline	10	20/20	37.7	0.25
RdRP gene	NP/saline	0	10/10	NA	NA

5) Clinical Evaluation:

Performance of the Cleveland Clinic SARS-CoV-2 Assay was evaluated by blinding and testing 60 NP specimens (30 positives and 30 negatives) previously characterized by the CDC EUA assay. After testing, specimens were unblinded and assessed to determine agreement. The Cleveland Clinic SARS-CoV-2 Assay results demonstrated 97% (29/30) positive percent agreement (PPA) and 100% (30/30) negative percent agreement (NPA).

In addition, 20 sputum (10 positive and 10 negative), 22 BAL (11 positive and 11 negative), and 31 tracheal aspirates (TASP) (13 positive and 18 negative) specimens previously characterized by the CDC EUA were tested with the Cleveland Clinic SARS-CoV-2 Assay. Results are shown below. Three specimens (one sputum and two tracheal aspirates) are presumptive positive per the Cleveland Clinic SARS-CoV-2 Assay as RdRP was not detected but they were positive for E gene.

Results from Clinical Evaluation:

Nasopharyngeal Swabs		**Comparator test**		
		SARS-CoV-2 Positive	**SARS-CoV-2 Negative**	**Total**
Cleveland Clinic SARS-CoV-2 Assay Result	Positive	29	0	29
	Negative	1	30	31
	Total	30	30	60
PPA (Positive Percent Agreement): 96.67% (95% CI, 83.33~ 99.41%) NPA (Negative Percent Agreement): 100% (95% CI, 88.65~100%)				

Sputum		**Comparator test**		
		SARS-CoV-2 Positive	**SARS-CoV-2 Negative**	**Total**
Cleveland Clinic SARS-CoV-2 Assay Result	Positive/Presumptive Positive	10*	0	10
	Negative	0	10	10
	Total	10	10	20
PPA (Positive Percent Agreement): 100% (95% CI, 72.25~ 100%) NPA (Negative Percent Agreement): 100% (95% CI, 72.25~100%) *Includes one specimen positive for E gene and negative for RdRP gene (presumptive positive).				

BAL		**Comparator test**		
		SARS-CoV-2 Positive	**SARS-CoV-2 Negative**	**Total**
Cleveland Clinic SARS-CoV-2 Assay Result	Positive/Presumptive Positive	11	0	11
	Negative	0	11	11
	Total	11	11	22
PPA (Positive Percent Agreement): 100% (95% CI, 74.12~ 100%) NPA (Negative Percent Agreement) : 100% (95% CI, 74.12~100%)				

TASP		Comparator test		
		SARS-CoV-2 Positive	**SARS-CoV-2 Negative**	**Total**
Cleveland Clinic SARS-CoV-2 Assay Result	Positive/Presumptive Positive	13*	0	13
	Negative	0	18	18
	Total	13	18	31
PPA (Positive Percent Agreement): 100% (95% CI, 77.19~ 100%) NPA (Negative Percent Agreement): 100% (95% CI, 82.41~100%) *Includes two specimens positive for E gene and negative for RdRP gene (presumptive positive).				

All Sample Types Combined		Comparator test		
		SARS-CoV-2 Positive	**SARS-CoV-2 Negative**	**Total**
Cleveland Clinic SARS-CoV-2 Assay Result	Positive/Presumptive Positive	63*	0	63
	Negative	1	69	70
	Total	64	69	133
PPA (Positive Percent Agreement): 98.44% (95% CI, 91.67~ 99.72%) NPA (Negative Percent Agreement): 100% (95% CI, 94.73~100%) *Includes three specimens positive for E gene and negative for RdRP gene (presumptive positive).				

Summary of Ct values for Detected Samples

	E gene		RdRP gene		RP	
	Median Ct	**Ct range**	**Median Ct**	**Ct range**	**Median Ct**	**Ct range**
NP	23.7	14.4-34.5	30.8	21.7-35.8	25.8	21.8-32.7
Sputum	19.6	18.5-33.8	25.2	23.9-29.5	25.0	18.6-30.3
BAL	20.23	17.8-27.3	26.8	22.1-32.3	23.3	18.9-28.8
TASP	20.9	18.6-33.8	27.5	24.7-32.4	20.7	18.3-23.9

Limitations

- SARS-CoV and other SARS-like coronaviruses in the same subgenus (Sarbecovirus) as SARS-CoV-2 may cross-react with the E-gene target. SARS-CoV is not known to be currently circulating in the human population, therefore is highly unlikely to be present in patient specimens.

WARNINGS:

- This test has not been FDA cleared or approved;
- This test has been authorized by FDA under an EUA for use by the Cleveland Clinic Robert J. Tomsich Pathology and Laboratory Medicine Institute located at 9500 Euclid Ave./LL2, Cleveland, OH 44195;
- This test has been authorized only for the detection of nucleic acid from SARS-CoV-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 diagnostics for detection and/or diagnosis of COVID-19 under Section 564(b)(1) of the Federal Food, Drug and Cosmetics Act, 21 U.S.C. § 360bbb-3(b)(1), unless the authorization is terminated or revoked sooner.