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, and meets requirements to perform high-complexity tests, 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 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 (HCP).

The Cleveland Clinic SARS-CoV-2 Assay is also for use with nasal swab specimens self-collected at home, using the SelfCheck COVID-19 Swabbing Kit, by individuals (18 years of age and older) suspected of COVID-19 when home collection is determined to be appropriate by an HCP.

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 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 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

SARS-CoV-2 Assay

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 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 assay:

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.

Assay 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	6543588001	Roche

Reagent Description	Catalog#	Manufacturer
Volume Kit		
MagNA Pure 96 External Lysis Buffer	6374913001	Roche
Molecular Biology Grade Water	W4502	Sigma-Aldrich

Self-Collection Kit

The SelfCheck COVID-19 Swabbing Kit enables the self-collection of nasal swab specimens at home by individuals (18 years of age and older) when determined to be appropriate by a healthcare provider. The SelfCheck COVID-19 Swabbing Kit collects viral RNA from nasal swab specimens and is used for the short-term room temperature storage and same-day transportation of a sample to the Cleveland Clinic Robert J. Tomsich Pathology and Laboratory Medicine Institute where it is received and processed using the Cleveland Clinic SARS-CoV-2 Assay.

Self-Collection Kit Materials:

The SelfCheck COVID-19 Swabbing Kit consists of a nasal swab, saline in a screw-capped collection tube, biohazard bag with absorbent sheet, padded envelope, test order requisition and SelfCheck instructions. Additional details about each of the individual components are provided in the table below.

Component	Description
Nasal Swab	Sterile, nylon floss (flocked) tip nasal swab
Saline tube	3 ml screw-capped tube with sterile saline; patient label is added at time of pickup
Biohazard bag	Specimen transport bag, Clear
Absorbent sheet	Sheet desiccant
Recloseable bag	Recloseable Bag, containing kit contents
Padded envelope	Gold Self-seal padded mailer with label
Test Order Requisition	Paper with orders printed from EPIC HIS
SelfCheck Instructions	Instruction sheet, full color, double-sided print

MEDICAL OVERSIGHT AND PROCESS TO BE USED FOR SAMPLE COLLECTION

Individuals have to be qualified by a healthcare provider based on either a telemedicine visit or an in-person visit with a healthcare provider for their eligibility to receive and use the SelfCheck COVID-19 Swabbing Kit to self-collect nasal swab specimens at home.

Individuals qualify for testing if: (i) they are exhibiting signs and symptoms consistent with COVID-19; or (ii) they have a recent known or suspected exposure to SARS-CoV-2. Only patients ≥18 years of age with an order for the test placed in the EPIC electronic hospital information system (HIS) may receive the kit. Patients may receive the collection kit during the in person visit or may pick-up the kit at a designated Cleveland Clinic Pharmacy location after evaluation via telemedicine. An overview of the process is described below.

At-Home Unsupervised Collection Kit Workflow

- A patient is determined by a healthcare provider to be eligible for testing with the SelfCheck COVID-19 Swabbing Kit via an in-person or telemedicine visit.
- The healthcare provider places an order for the test via the Cleveland Clinic EPIC electronic hospital information system (HIS).
- The patient is either sent home with a SelfCheck COVID-19 Swabbing Kit, or picks up their kit at the designated Cleveland Clinic Pharmacy locations. The kit is pre-labeled with the patient information.
- The patient using the SelfCheck COVID-19 Swabbing Kit collects the nasal sample and packages the sample into a return envelope following the kit's included instructions which contain step-by-step images of the procedure. The instructions also contain a link to a video where patients can go to watch the procedure prior to collecting their sample.
- On the day of collection, the patient brings the sealed envelope to a Cleveland Clinic drop box located inside a designated Cleveland Clinic Pharmacy or Express Care facility building. Specimens will be picked-up by Cleveland Clinic or contracted couriers on established routes and transported in cars at ambient temperature to the Robert J. Tomsich Pathology and Laboratory Medicine facility where the Cleveland Clinic SARS-CoV-2 Assay is performed. Couriers use electronic scanning to track time of pickup and delivery. Specimens will not be received through the U.S. mail or by a shipping service.
- Specimens received at the clinical laboratory for testing with the Cleveland Clinic SARS-CoV-2 Assay undergo review and accessioning prior to acceptance for testing.
- Positive or indeterminate/invalid -test results are communicated back to the patient via a follow-up phone call from the ordering provider. Patients are also notified by email when their results (positive, negative or indeterminate/invalid) are available electronically in MyChart. Patients who do not have the ability to use MyChart or email, are contacted by telephone.
- When a patient receives a positive result, the patient is contacted by phone and offered enrollment in the Cleveland Clinic Home Monitoring Program, which provides further daily guidance on how to manage their symptoms and care.

INSPECTION OF SPECIMENS RECEIVED AT CLINICAL LABORATORIES FOR TESTING

Specimens from patients using the SelfCheck COVID-19 Swabbing Kit must be checked for the following criteria upon receipt at clinical Laboratories prior to processing according to Cleveland Clinic Standard Operating procedures:

- Identifiers and Orders: The name and date of birth on the specimen label and paper requisition must match. The identifiers on the specimen and requisition are verified in comparison to the electronic orders.
- Specimen acceptability: The source, collection swab type and transport media are verified. (See rejection criteria below.)
- Transport time: The collection date and time on the specimen and received date and time are recorded electronically in the Laboratory Information System. Specimens exceeding stability criteria are rejected.

Rejection criteria for the SelfCheck COVID-19 Swabbing Kit:

- Patient order/specimen identification discrepancy
- Improper swab submitted (only the swab provided with the kit is accepted; wood, calcium alginate and gel swabs are rejected)
- Improper media used (only the saline provided in the kit is acceptable)
- Broken or leaking specimen container (<3 ml volume)
- Specimen outside of established stability (56 hours ambient)
- Note: The specimen will not be automatically rejected if collection date and time are missing on the requisition. The patient will be contacted to provide the information.
- Note: If a test is rejected, the order will be cancelled, and the ordering provider will be contacted.

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.

2019- nCoV E	**2019-nCoV RdRP**	**RP**	**Result Interpretation**	**Actions**	**Report**
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 Public Health and 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

6) Simulated Sample Stability/Shipping Studies for the SelfCheck COVID-19 Swabbing Kit

Sample stability studies were conducted to evaluate the effect of temperature variation on the stability of SARS-CoV-2 RNA during sample transport from the patient's home to the testing laboratory for processing. The study was designed to simulate sample storage before transport and during transport at ambient temperature as well as the extreme temperature conditions that could be experienced during the summer and winter months. See Tables below for summer and winter thermal profiles, respectively, that were evaluated in this study.

Summer temperature excursion:

Temperature	**Cycle Period**	**Cycle Period Hours**	**Total Time Hours**
40°C	1	8	8
22°C	2	4	12
40°C	3	2	14
30°C	4	36	50
40°C	5	6	56

Winter temperature excursion:

Temperature	**Cycle Period**	**Cycle Period Hours**	**Total Time Hours**
-10°C	1	8	8

Temperature	Cycle Period	Cycle Period Hours	Total Time Hours
18°C	2	4	12
-10°C	3	2	14
10°C	4	36	50
-10°C	5	6	56

Simulated sample stability and shipping studies were performed using a total of 40 samples including 20 samples at 2X LoD, 10 samples at 5-10X LoD, and 10 negative samples. The positive samples were contrived by spiking negative nasal specimen matrix in saline with positive clinical specimens, the concentration of which were determined by comparing the Ct values of samples and controls at known concentration tested by CDC EUA assay. Each specimen contained the collection swab used in the kit. After the contrived positive and negative samples underwent the thermal excursions, they were tested with the Cleveland Clinic SARS-CoV-2 Assay. The mean Ct values and percent agreements are presented in Table below.

Summary Results of SelfCheck COVID-19 Swabbing Kit Stability Study:

Sample (N)	Gene	Baseline Ave Ct	Summer Ave ΔCt	Winter Ave ΔCt	Percent Agreement
Negative (10)	E	ND	ND	ND	100%
	RdRP	ND	ND	ND	100%
	RNase P	28.29	-1.00	0.14	100%
5x LoD (10)	E	27.30	-0.61	0.22	100%
	RdRP	33.23	-1.00	-0.03	100%
	RNase P	28.39	-0.84	0.12	100%
2x LoD (20)	E	28.88	-0.92	-0.09	100%
	RdRP	34.85	-1.09	0.09	100%

ND: Not Detected

The results of the SelfCheck COVID-19 Swabbing Kit Stability Study demonstrate that when tested with the Cleveland Clinic SARS-CoV-2 Assay, the contrived positive specimens are stable in saline when exposed to a broad range of temperature conditions. These data support the use of the SelfCheck COVID-19 Swabbing Kit for transport and storage of specimens following self-collection of nasal swabs at home at room temperature for up to 56 hours from the time of collection.

7) Human Usability study

A Human Usability Study was conducted to assess user comprehension of the SelfCheck COVID-19 Swabbing Kit for both collection and packaging of the nasal specimens for transport. The study was conducted at a Cleveland Clinic Express Care site to simulate the at-home environment and the participants were observed directly by a care provider during the sample collection and packing process.

Adult patients ≥18 years of age with an order for COVID-19 molecular testing were recruited. Participants were not selected based on age, race, gender, ethnicity or other

demographic characteristics. Fifty-one participants with a variety of ages and education levels were enrolled. Results from 13 patients were excluded from analysis because they reported prior medical or laboratory experience. Of the 38 patients evaluating the SelfCheck COVID-19 Swabbing Kit, 71.1% were female and 28.9% were male. The age distribution was as follows: 18-24 years, 5.3%; 25-34 years, 10.5%; 35-44 years, 2.6%; 45-54 years, 10.5%; 55-64 years, 23.7%; 65-74 years, 36.8%, ≥75 years, 10.5%. Educational level achieved was: did not graduate high school, 2.6%; high school graduate or equivalent, 18.4%; some college, no degree, 39.5%; associate degree 13.2%; bachelor's degree, 21.1%; master's degree, 5.3%; professional degree, 0%.

The study participants read the instructions in the COVID-19 Self-Collection Kit and used the instructions and materials to collect their own sample. Collection was observed by a health care provider. Caregivers did not provide assistance or answer questions during the usability study. After collection, the patient placed the swab in a tube with 3 ml of normal saline and package for delivery to the lab, as described in the kit instructions. Following specimen collection using the kit, a second specimen was collected by the caregiver using a nasal swab and routine practices for COVID-19 testing. Upon the completion of the sample collection, both patients and the caregiver who watched the patient using the SelfCheck COVID-19 Swabbing Kit completed a questionnaire designed by the Cleveland Clinic to evaluate their experience and suggest enhancements. Based on answers from questionnaires, the Instructions for using the SelfCheck COVID-19 Swabbing Kit were modified slightly to provide clarification. Specimens collected by patients were tested with the Cleveland Clinic SARS-CoV-2 Assay and results were compared to nasal swabs collected by the caregiver.

Thirty-seven out of 38 patients were able to successfully collect the nasal swab. All 37 samples were acceptable for SARS-CoV-2 molecular testing based on laboratory assessment. Adequate sampling was determined by the presence of RNase P in all 37 samples and the amount of RNase P detected was similar to that detected with a provider-collected swab (average Δ Ct <0.2), indicating successful collection of human biological material that was extracted and amplified. All patients indicated that they would be comfortable using the SelfCheck COVID-19 Swabbing Kit at home.

Based on the usability study data and feedback, the SelfCheck COVID-19 Swabbing Kit instructions were understandable, the kit was easy to use, and samples were successfully self-collected, which has demonstrated the usability that is acceptable to the FDA.

Additional Requirement:

In addition to validation studies, Cleveland Clinic will submit a report to the FDA (within 30 days of authorization) summarizing any testing performed with the SelfCheck COVID-19 Swabbing Kit including how many kits were requested and sent for home collection. Cleveland Clinic will also document the number of kits that were disseminated and returned to the laboratory according to the instructions, how many

specimens were rejected during accessioning and the reasons for rejection, and the positivity rate of the first SelfCheck COVID-19 Swabbing Kit lot.

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 product has not been FDA cleared or approved, but test has been authorized for emergency use by FDA under an EUA for use by authorized laboratories;
- This product has been authorized only for the detection of nucleic acid from SARS-CoV-2, not for any other viruses or pathogens; and
- The emergency use of this product 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 declaration is terminated or authorization is revoked sooner.