ACCELERATED EMERGENCY USE AUTHORIZATION (EUA) SUMMARY SARS-CoV-2 RT-PCR Assay

(Cedars-Sinai Medical Center)

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 Cedars-Sinai Medical Center Department of Pathology and Laboratory Medicine, 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 SARS-CoV-2 assay is a real-time reverse transcription polymerase chain reaction (rRT-PCR) test intended for the qualitative detection of nucleic acid from SARS-CoV-2 in nasopharyngeal swabs collected from individuals suspected of COVID-19 by their healthcare provider. Testing is limited to the Cedars-Sinai Medical Center Department of Pathology and Laboratory Medicine certified under the Clinical Laboratory Improvement Amendments of 1988 (CLIA), 42 42 U.S.C. §263a 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.

The assay is intended for use by qualified and trained clinical laboratory personnel specifically instructed and trained in the techniques of real-time PCR and in vitro diagnostic procedures. The assay is only for use under the Food and Drug Administration's Emergency Use Authorization.

DEVICE DESCRIPTION AND TEST PRINCIPLE

The Cedars-Sinai Medical Center SARS-CoV-2 RT-PCR assay is based on the real-time reverse transcriptase polymerase chain reaction (rRT-PCR) technology for the qualitative detection of SARS-CoV-2 specific RNA. This test uses the A* STAR FORTITUDE KIT 2.0 for the qualitative detection of SAR-CoV-2 specific RNA in nasopharyngeal swab samples. The primer/probe sets are designed to target a single region, the NSP-3 gene, which is specific to SARS-CoV-2.

RNA is isolated from nasopharyngeal swab specimens utilizing either the Qiagen QIA Connect or QIAsymphony extraction platforms. Real-time RT-PCR technology utilizes reverse transcriptase (RT) to convert RNA into complimentary DNA (cDNA). The cDNA then undergoes the polymerase chain reaction (PCR), where amplification of specific targeted sequences and detection of the amplified DNA occurs. Detection is facilitated by probes that are labelled with fluorescent reporter and quencher dyes.

The assay is a duplex real-time RT-PCR which simultaneously detects the target region on the SARS-CoV-2 viral sequence and an internal control in one single reaction. The probe specific for the SARS-CoV-2 RNA are labelled with the fluorophore FAM. The probe specific for the Internal Control (IC) is labelled with the fluorophore HEX. Using probes linked to distinguishable dyes enables the parallel detection of SARS-CoV-2 specific RNA as well as the detection of the Internal Control in corresponding detector channels of the real-time RT-PCR instrument.

The test consists of three processes in a single assay: 1) reverse transcription of target RNA to cDNA, 2) PCR amplification of target and Internal Control DNA, and 3) simultaneous detection of PCR amplicons by fluorescent dye labelled probes.

INSTRUMENTS USED WITH TEST

The Cedars-Sinai Medical Center SARS-CoV-2 RT-PCR Qualitative Assay is to be used with the following:

RNA extraction is conducted using either of the following validated extraction instruments and extraction kits:

- Qiagen QIA connect extraction system - QIAamp RNA Viral kit
- Qiagen QIAsymphony - QIAsymphony DSP Virus/Pathogen Midi kit.

Real-time reverse transcription polymerase chain reaction was validated using the following analyzers:

- Applied Biosystems QuantStudio 6 Flex instrument with version 1.3 and
- ABI 7500 Fast Real Time PCR system with version 1.4

REAGENTS AND MATERIALS

Reagent/Material	Catalog Number
A*STAR FORTITUDE KIT 2.0	Accelerate Technologies, NFIH001
Qiagen QIAmp Viral Mini QIAcube kit	Qiagen, 52925 (50 rxn), 52926 (240 rxn)
Ethanol (96-100%)	Pharmco Product Inc., 111000200
QIAsymphony DSP Virus/Pathogen Kit	Qiagen,937055 (96 rxn)
8-Rod Covers	Qiagen, 997004
2 mL screw cap tubes	Sarstedt, 72.693
Sample Prep Cartridges, 8-well	Qiagen, 997002
Filter tips, 200 ul and 1500 ul	Qiagen, 990332, 997024
MicroAmp Fast Optical 96-well Reaction Plate with Barcode	Applied Biosystems, 4346906
MicroAmp Optical Adhesive Film	Applied Biosystems, 4311971
Rainin Pipettes- P1000, P200, P20, P10	Mettler Toledo, 17014382,17014391, 17014392,17014388
Aerosol resistant tips- P1000, P200, P20 (Rainin)	Mettler Toledo, 30389223,30389242, 6064515
Microcentrifuge tubes	Qiagen, 19588

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

Positive Control:
One positive control is run with each assay on each plate. This control is designed to assess the integrity of the PCR run. Positive control is provided with the A*STAR FORTITUDE KIT 2.0.

Negative Control:
One negative control is run with each assay on each plate. This control is needed to eliminate the possibility of sample contamination on the assay run. This control is provided with the A*STAR FORTITUDE KIT 2.0.

Extraction Control:
A positive extraction control consisting of a pool of previously characterized positive patient samples is run with each batch. This serves as an extraction control to validate extraction reagents and verify RNA extraction efficiency.

Internal Control:
An internal control is needed to verify that nucleic acid is present in every sample and is used for every sample processed. The internal control is one of the components in the preparation of the Master Mix and added to each specimen during the RT-PCR set-up. The internal control (IC) is provided with the A*STAR FORTITUDE KIT 2.0 and is labeled with the fluorophore dye HEX.

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 (please see Table 1).

1) ***SARS-CoV-2 RT-PCR test Controls – Negative, Positive, Internal, and Extraction:***

- Negative Control - this control must be NOT DETECTED, showing an absence of fluorescence growth curve in the SARS-CoV-2 channel but DETECTED on the IC channel. If this control has a detectable Ct in the SARS-CoV-2 channel, this indicates contamination of the PCR run and it is considered Invalid. Testing must be repeated.

- Positive Control - the positive control must be DETECTED, showing an exponential growth curve in the SARS-CoV-2 channel and IC channel. The Ct value on the SARS-CoV-2 channel must be detected within the specified Ct range 33 ± 2 CT. Results from PCR runs with a negative result for the positive control cannot be reported and must be repeated.

- Internal Control - the internal control must be DETECTED for every patient sample at a Ct value < 40 CT. Samples that do not have a detected internal control result should be repeated. If the internal control remains undetected, the sample is reported as Invalid.

- Extraction Control - this control should have a detectable Ct within the specified Ct range for a positive sample. If this control has no detectable Ct in the reaction well, the run is considered Invalid and all samples are re-extracted.

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

The positive and negative controls must yield valid expected results for patient results to be reported. If unexpected control results are obtained, results are not reported, and the samples must be retested after investigation into the source of error. All clinical samples should yield positive results for the IC at < 40 Ct. Samples that fail to show detection of the IC target is invalid and should be repeated from the extracted product. If the extraction control yields a negative result, the whole batch extraction batch must be re-extracted and retested.

Table 1: Interpretation of patient results

Sample	SARS-CoV-2 (FAM)	IC (HEX)	Interpretation	Actions
Positive Control (PC)	1. CT values < 35; AND 2. Presence of fluorescence growth curves in the PCR amplification step.	CT values < 40	QC passed	Run Valid
Negative Control (NC)	1. CT values undetermined; AND 2. Absence of fluorescence growth curves in the PCR amplification step.	CT values < 40		
Test Sample	1. CT values < 40; AND 2. Presence of fluorescence growth curves in the PCR amplification step.	CT values < 40 or CT values undetermined	Detected for SARS-CoV-2	Report result in LIS and to State Dept. of Public Health (ELR)
	1. CT values undetermined; AND 2. Absence of sigmoidal fluorescence growth curves in the PCR amplification step.	CT values < 40	Not detected for SARS- CoV-2	Report result in LIS and to State Dept. of Public Health (ELR)
	CT values undetermined	CT values undetermined or values > 40	Invalid	Repeat extraction and rRT-PCR. If the result remains invalid, consider collecting a new specimen from the patient.

PERFORMANCE EVALUATION

1) ***Analytical Sensitivity:***

Limit of Detection (LoD):
The LoD study established the lowest concentration of SARS-CoV-2 that can be detected at least 95% of the time using the SARS-CoV-2 assay. The LoD study was conducted using the RNA positive control included in the A*STAR FORTITUDE KIT 2.0. A preliminary LoD was first established and then confirmed in pooled negative nasopharyngeal clinical specimens. First, the positive RNA control was tested at 250 copies/reaction, 25copies/reaction, and 12.5 copies/reaction with 10 replicates per dilution to establish a preliminary LoD. The samples were extracted using the QIAsymphony DSP Virus/Pathogen

Midi kit and run on both the QuantStudio 6 and ABI 7500 Fast thermocyclers. The preliminary LoD of 25 copies/reaction (equivalent to 10 copies/µl) was then confirmed based on a positivity rate of 95% (19/20) when testing of 20 replicates.

Table 2: LoD confirmation

Thermocycler	Positivity rate	Mean Ct
QuantStudio 6	20/20	33.89
ABI 7500 FAST	19/20	35.33

2) Analytical Inclusivity

An in silico analysis was conducted by Accelerate Technologies, which included a sequence alignment of the primer and probe sequences with all publicly available complete human-derived SARS-CoV-2 genomes in GenBank as of 4.24.20. This analysis demonstrated acceptable inclusivity for the assay.

3) Cross-Reactivity

Cross-reactivity of the CSMC SARS-CoV-2 PCR Qualitative Assay was evaluated using both *in silico* analysis and wet testing performed by Cedars-Sinai Medical Center Department of Pathology and Laboratory Medicine.

Cross-reactivity wet testing was performed at the Cedars-Sinai Medical Center Department of Pathology and Laboratory Medicine utilizing 29 characterized samples positive for Influenza A and B, seasonal human coronaviruses (OC43 and HKUl) and Respiratory Syncytial Virus (RSV). All 29 samples were extracted using the Qiagen QIAamp Viral RNA Mini Kit/QIAcube Connect extraction protocol. RT-PCR was performed using the QuantStudio 6 analyzer and tested with the SARS-CoV-2 assay. The data are summarized in Table 3 below and demonstrate no false positive detection.

Table 3: Summary of wet-testing for the analytical specificity of the CSMC COVID-19 RT-PCR assay

Organism	Strain	Source	CSMC COVID-19 RT-PCR Result
Human coronavirus - Sample l	HKUI	Clinical specimen	Not Detected
Human coronavirus - Sample 2	HK.U l	Clinical specimen	Not Detected
Human coronavirus - Sample 3	HKU I	Clinical specimen	Not Detected
Human coronavirus - Sample 4	HK.U l	Clinical specimen	Not Detected
Human coronavirus - Sample 5	HKUI	Clinical specimen	Not Detected
Human coronavirus - Sample 6	OC43	Clinical specimen	Not Detected
Human coronavirus - Sample 7	OC43	Clinical specimen	Not Detected
Huma n coronavirus - Sample 8	OC43	Clinical specimen	Not Detected
Human coronavirus - Sample 9	OC43	Clinical specimen	Not Detected

Human coronavirus - Sample l 0	O C43	Clinical specimen	Not Detected
Influenza A - Sample 11	-	Clinical specimen	Not Detected
Influenza A - Sample 12	-	Clinical specimen	Not Detected
Influenza A - Sample 13	-	Clinical specimen	Not Detected
Influenza A - Sample 14	-	Clinical specimen	Not Detected
Influenza A - Sample 15	-	Clinical specimen	Not Detected
Influenza A - Sample 16	-	Clinical specimen	Not Detected
Influenza B - Sample 17	-	Clinical specimen	Not Detected
Influenza B - Sample 18	-	Clinical specimen	Not Detected
Influenza B - Sample 19	-	Clinical specimen	Not Detected
Influenza B - Sample 20	-	Clinical specimen	Not Detected

Additional *in-silico* cross reactivity studies were performed by Accelerate Technologies. Results demonstrate that there were no significant homologies with other coronaviruses or human flora that would predict potential false-positive real-time RT-PCR results. The organisms tested in the cross-reactivity *in silico* analysis are outlined below in Table 4:

Table 4: Cross Reactivity *In silico* analysis:

Pathogen	Strain
Leptospirosis	*Leptospira santarosai* strain UW
Pseudomonas aeruginosa	na
Legionella pneumophila	*Legionella pneumophila*
Legionella non-pneumophila	*Legionella feeleii*
Moraxella catarrhalis	*Moraxella catarrhalis* strain VA5
Neis*seria elongata* and *Neisseria meningitidis*	*Neisseria perflava* strain NSU-per_40
Bordetella pertussis	na
Haemophilus influenzae	na
Coxiella burnetii (Q-Fever)	*Coxiella burnetii*
Staphylococcus aureus	na
Staphylococcus epidermidis	*Staphylococcus epidermidis* strain SR1
Streptococcus salivarius	*Streptococcus salivarius* isolate 24
Streptococcus pneumoniae	na
Streptococcus pyogenes	*Streptococcus* strain DRV1
Bacillus anthracis (Anthrax)	*Bacillus anthracis* strain 9131
Corynebacterium diphtheriae	Corynebac*terium diphtheriae* strain C7(-)
Mycobacterium tuberculosis	na
Mycoplasma pneumoniae	*Mycoplasma pneumoniae* isolate MX17-29
Candida albicans	*Candida albicans* ATCC 266555
Adenovirus (e.g. C1 Ad. 71)	Human adenovirus B3
Human coronavirus 229E	Human coronavirus 229E isolate 507A_2008

Respiratory syncytial virus	na
Influenza A	na
Influenza B	B/South Australia/11/2014
Influenza C	Influenza C virus (C/Milan/128/2009)
Enterovirus (e.g. EV68) includes Rhinovirus	Echovirus E25 isolate NSW-V58-2010-ECHO25
Parainfluenza virus 1-4	Sendai virus (Harris strain)
Human coronavirus OC43	Human coronavirus OC43 isolate TN97_1-4
Pneumocystis jirovecii (PJP)	*Pneumocystis jirovecii* RU7
Chlamydia psittaci	*C.psittaci* plasmid pCpA1
Chlamydia pneumoniae	type strain of *Chlamydia pneumoniae*
Parechovirus	Human parechovirus 6 isolate HPeV_751939_ADAM_NL07
Human Metapneumovirus (hMPV)	Human metapneumovirus isolate TN/89/7-13
Human coronavirus NL63	Human coronavirus NL63 isolate NL63/FRA-EPI/Caen/2004/01
Human coronavirus HKU1	Human coronavirus HKU1 isolate 13MYKL1997
pooled human nasal wash	Raw sequencing data of the nasal microbiome of a set of medical school students
SARS-coronavirus	SARS coronavirus isolate WHU
MERS-coronavirus	Middle East respiratory syndrome coronavirus isolate Jeddah-air-1-2014

4) ***Clinical Evaluation:***

The clinical evaluation was conducted by testing patient specimens that were confirmed positive and negative with an EUA authorized test by local Public Health authorities and other clinical laboratories.

Comparison to an EUA authorized test:

In this study, 30 confirmed positive and 30 confirmed negative nasopharyngeal swab samples using the EUA authorized test at the Los Angeles County Department of Public Health and two other clinical laboratories were tested against the CSMC SARS-CoV-2 RT-PCR Qualitative Assay. For this clinical evaluation study, the following EUA authorized tests were utilized:

1. Los Angeles County Department of Public Health - CDC 2019-nCoV Real-Time RT-PCR Diagnostic Panel (CDC)
2. 1st Outside Reference Laboratory - Quest SARS-CoV-2 rRT-PCR
3. 2nd Outside Clinical Laboratory - Panther Fusion SARS-CoV-2

The samples were extracted using the QIAmp Viral RNA extraction kit on the QIAcube Connect and then run on both the QuantStudio 6 and ABI 7500 Fast thermocyclers. For both the positive and negative patient specimens, 100% concordance was obtained with the EUA authorized tests (i.e., PPA=100%, NPA=100%), as depicted in the tables below:

Table 5: Confirmed Positive Specimens by EUA authorized Test:

EUA Authorized Test	LDT concordance	LDT mean Ct QuantStudio 6	LDT mean Ct ABI 7500 Fast
CDC 2019 nCoV RT-PCR	4/4	29.37	30.13
Quest SARS-CoV-2 rRT-PCR	21/21	23.97	24.16
Panther Fusion SARS-CoV-2	5/5	21.22	22.78
Total	30/30	24.23	24.73
	PPA: 100%		

Table 6: Confirmed Negative Specimens by EUA authorized Test:

EUA Authorized Test	LDT concordance	LDT mean Ct QuantStudio 6	LDT mean Ct ABI 7500 Fast
CDC 2019 nCoV RT-PCR	4/4	Not detected	Not detected
Quest SARS-CoV-2 rRT-PCR	26/26	Not detected	Not detected
Panther Fusion SARS-CoV-2	N/A	Not detected	Not detected
Total	30/30	Not detected	Not detected
	NPA: 100%		

Confirmation of 5 positive and 5 negative specimens:

Additionally, five positive and five negative patient samples were sent to the Los Angeles County Public Health Laboratory and tested using the CDC assay. All results are concordant, yielding 100% PPA and 100% NPA. This testing fulfills the requirement for evaluation of clinical specimens.