ACCELERATED EMERGENCY USE AUTHORIZATION (EUA) SUMMARY FOR THE CSI LABORATORIES SARS-COV-2 ASSAY

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

(The SARS-CoV-2 Assay will be performed at CSI laboratories certified under the Clinical Laboratory Improvement Amendments of 1988(CLIA), 42 U.S.C. §263a, as per the Instructions for Use that were reviewed by the FDA under this EUA.)

INTENDED USE

The SARS-CoV-2 RT PCR Test is a reverse transcriptase real-time RT-PCR test intended for the qualitative detection of nucleic acid from the SARS-CoV-2 upper respiratory specimen (such as nasopharyngeal swabs, nasal, and oropharyngeal swabs), and bronchoalveolar lavage specimens from individuals suspected of COVID-19 by their healthcare provider. Testing is limited to CSI Laboratories that is 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 upper respiratory specimens and bronchoalveolar lavage 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 SARS-CoV-2 RT PCR Test 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 SARS-CoV-2 RT-PCR test is a real-time reverse transcription polymerase chain reaction (rRT-PCR) test based on the EUA FDA issued for the CDC 2019-nCoV Real-Time RT-PCR Diagnostic Panel.

RNA is isolated from upper respiratory and bronchoalveolar lavage specimens utilizing 5073 EPMotion liquid handler with Omega BioTek Viral RNA extraction kit rather than CDC's QIAGEN EZ1 Advanced XL instrument and EZ1 DSP Virus Kit.

Real time RT-PCR is performed on Applied Biosystem Quantstudio 7 instrument (software version 1.3) rather than the Applied Biosystems 7500 Dx Real Time PCR Instrument used for the authorized CDC assay.

The SARS-CoV RT PCR Test is a single-plex real-time RT-PCR which detects the target region on the SARS-CoV-2 viral sequence or an internal control. 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, and 3) simultaneous detection of PCR amplicons by fluorescent dye labelled probes.

The sequences for the N1, N2 primers and probes used in the SARS-CoV2 RT PCR Test are identical to the ones used in the FDA authorized original CDC 2019-Novel Coronavirus (2019-nCoV) real time RT-PCR Diagnostic Panel sourced from IDT (**Table1)**.

Table 1. Oligonucleotide Primer and Probe Sequences

Name	Description	Oligonucleotide Sequence (5' to 3')
SARS-CoV-2_N1-F	2019 nCoV_N1 Forward Primer	GAC CCC AAA ATC AGC GAA AT
SARS-CoV-2_N1-R	2019 nCoV_N1 Reverse Primer	TCT GGT TAC TGC CAG TTG AAT CTG
SARS-CoV-2_N1-P	2019 nCoV_N1 Probe	FAM-ACC CCG CAT TAC GTT TGG TGG ACC-BHQ1
SARS-CoV-2_N2-F	2019-nCoV_N2 Forward Primer	TTA CAA ACA TTG GCC GCA AA
SARS-CoV-2_N2-R	2019-nCoV_N2 Reverse Primer	GCG CGA CAT TCC GAA GAA
SARS-CoV-2_N2-P	2019 nCoV_N2 Probe	FAM-ACA ATT TGC CCC CAG CGC TTC AG-BHQ1
RP-F	Human RNAseP (RP) Forward Primer	AGA TTT GGA CCT GCG AGC G
RP-R	Human RNAseP (RP) Reverse Primer	GAG CGG CTG TCT CCA CAA GT
RP-P	Human RNaseP (RP) Probe	FAM – TTC TGA CCT GAA GGC TCT GCG CG – BHQ-1

INSTRUMENTS USED WITH TEST

The CSI SARS-CoV-2 test is to be used with the following components and equipment:

Table 2A. Instruments for Use with the SARS CoV2 RT PCR Test

Equipment	Manufacturer	Catalog#
Quantstudio 7	ABI/ThermoFisher	4485690
Quantstudio Real-Time PCR Software v1.3	ABI/ThermoFisher	n/a
EPMotion 5073	Eppendorf	5073007411
Thermomixer C	Eppendorf	2231000667

REAGENTS AND MATERIALS

Table 2B. Reagents and Materials for Use with the SARS CoV2 RT PCR Test

Reagents	Manufacturer	Catalog#
Omega Bio-Tek Mag-Bind Viral DNA/RNA 96 Kit	Omega BioTek	M6246-03
TaqPath qPCR master mix	ThermoFisher	A15297
2019-nCov CDC EUA Kit	IDT	10006606
2019-nCoV_N_Positive Control	IDT	10006625
Optical adhesive plate cover	ThermoFisher	4311971
96 well PCR plate	Eppendorf	0030129644
96-well deep well plates	Eppendorf	951033502
Molecular grade water	--	--
Isopropanol	Sigma	--

CONTROLS TO BE USED WITH THE SARS-CoV-2 RT-PCR Test:

The controls to be used with the CSI SARS CoV-2 RT-PCR test are described in **Table 3**

Table 3-Controls to be Used with the CSI SARS CoV-2 RT-PCR Test

Control Type	Description	Purpose	Frequency of Testing
Negative (no template NTC)	Molecular grade; nuclease-free water	To monitor for cross-contamination during RNA extraction and RT-PCR	Every assay plate
Positive [A positive template (CoV+_N_P)]	Positive Control Plasmid supplied by the CDC kit which contains one copy each of N1, N2 and RNase P (IDT). The final load of the plasmid is equivalent to 1,000 copies.	To monitor the integrity of the RT-PCR reagents and process	Every RT-PCR assay plate
Negative Extraction Control (NEC)	previously characterized negative patient sample or cell line. cell line.	To monitor for any cross-contamination that occurs during the extraction and RT-PCR process. This is also an extraction control to validate extraction reagents and successful RNA extraction.	Every extraction and RT-PCR plate
Internal Control (Endogenous Control)	Human RNAse P assay Targeting endogenous RNase P gene	To monitor the integrity of nucleic acid extraction and RT-PCR for each human respiratory tract specimen	Every collected human respiratory tract specimen

[1]It also has RNaseP present

INTERPRETATION OF RESULTS

1) Interpretation of SARS-CoV-2 RT-PCR Test Controls

All test controls should be examined prior to interpretation of patient results. If the controls are not valid, the patient results cannot be interpreted. The run should be invalidated and re-tested with previously extracted RNA. The results from the controls are interpreted according to the criteria shown in **Table 4**

Table 4. Expected Cycle Thresholds (Ct) for SARS-CoV2 RT PCR Test Controls

Control	2019 nCoV N1	2019 nCoV N2	RNAseP
Positive	+ (<40 Ct[1])	+ (<40 Ct)	+ (<40 Ct)
Negative (NEC)	- (>40 Ct)	- (>40 Ct)	+ (<40 Ct)
NTC	-(>40)	-(>40)	-(>40)
Internal Control	-(>40)	-(>40)	+(<40)

[1]Cycle threshold is defined as the number of cycles required for the fluorescent signal to cross the threshold.

Table 4A. Interpretation of SARS-CoV2 RT PCR Test Controls: Positive, Negative, Internal Controls

Control Type	Control Name	Used to Monitor	SARS-CoV-2_N1	SARS-CoV-2_N2	RP
Positive	Positive Control	Reagent integrity	+	+	+
Negative	NTC	Reagent and or environmental contamination	-	-	-
Extraction	NEC	Problems/failure in NA extraction procedure	-	-	+
Internal Control (Endogenous Control)	IC	Ensure ever sample was properly collected and assess for sample integrity	-	-	+

2) Examination and Interpretation of Patient Specimen Results

Assessment of clinical specimen test results should be performed after the positive/negative/extraction controls have been examined and determined to be valid and acceptable. If the controls are not valid, the patient results cannot be interpreted.
The results from testing of patient samples are interpreted according to the criteria described in T**able 5.**

Table 5. Interpretation of Patient Results Using the SARS-CoV-2 RT-PCR Test

SARS-CoV-2 N1	SARS-CoV-2 N2	RNase P	Result Interpretation	Report	Actions
+	+	+/-	SARS-CoV-2 Detected	Positive	Send report to client and CDC
If one of the two targets is positive		+/-	Inconclusive Results	Indeterminate	Repeat testing of NA and/or re-extract and repeat RT-PCR. If the repeated result remains inconclusive, contact your State Public Health Laboratory or CDC for further instructions.
-	-	+	SARS-CoV-2 Not Detected	Negative	Report results to sender. Consider testing for other respiratory viruses.
-	-	-	Invalid result	Invalid	Sample is repeated once (extraction and RT-PCR). If 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 was determined using heat-inactivated SARS-CoV-2 (SARS-Related Coronavirus 2, Isolate USA-WA1/2020 heat inactivated from BEI catalog No. NR-52286) spiked into pooled negative nasopharyngeal swabs. The RNA was then extracted from the spiked milieu using the EPMotion 5073 liquid handler and then tested using the 2019 nCoV-2 CDC EUA assay.

The Applied Biosystems QuantStudio 7 Real-Time PCR System equipped with a standard (Std) 96 well block was used to evaluate replicates at each of four different target levels: 18.5, 6.25, 3.12 and 1.56 copies/µL and known negative samples.

The LoD was defined as the lowest concentration (genome copies/µL) that can be detected by the SARS-CoV2 RT PCR Test at least 95% of the time. The LoD of the SARS-CoV-2 RT PCR Test was 6.25 copies/µL (24/24 positive).

The results for 6.25 copies/µl meet the acceptable criteria to confirm the LoD (**Table 6).**

Table 6. Limit of Detection Experiment Using Contrived Samples

Viral RNA Copies/µL	Number Tested	2019 SARS-N1			2019 SARS-N2			RNaseP		
		Positive	Ct[1](avg)	SD	Positive	Ct(avg)	SD	Positive	Ct(avg)	SD
18.5	24	24 (100%)	31.94	.810	24 (100%)	33.96	1.34	24 (100%)	18.92	.216
6.25	**24**	**24 (100%)**	**33.51**	**.896**	**24 (100%)**	**34.73**	**.966**	**24 (100%)**	**19.03**	**.346**
3.13	20	16 (80%)	n/a	n/a	8 (40%)	n/a	n/a	20 (100%)	19.13	.338
1.56	20	3 (15%)	n/a	n/a	2 (10%)	n/a	n/a	20 (100%)	19.01	.295
0	20	0 (0%)	n/a	n/a	0 (0%)	n/a	n/a	20 (100%)	19.34	.386

[1]Cycle threshold is defined as the number of cycles required for the fluorescent signal to cross the threshold.

Block Type Equivalence Study

To validate the use of the Applied Biosystems Quantstudio 7 (QS7) Real-Time PCR System equipped with a 96-fast block for future use with the SARS-CoV-2 RT PCR Test, an equivalency study was performed at 1X LoD.

Twelve nasopharyngeal specimens spiked with heat-inactivated SARS-CoV2 (sourced from BEI) at 6.25 copies/ µl were tested with QS7 Real-Time PCR system equipped with a standard block versus QS Real Time PCR instrument equipped with a 96 well fast block.

Comparable results were obtained for each of the samples for both target genes and the RP internal control (<1 Ct difference), demonstrating that the QS7 Real-Time PCR system equipped with fast block performed similarly to the QS7 Real-Time PCR system equipped with the standard block (**Table 6**).

Table 6. Comparison of QS 7 equipped with Standard Block vs. Fast Block

Quantstudio 7	**SARS-N1**		**SARS-N2**		**RNaseP**	
Block Type	**Std[2]**	**Fast[3]**	**Std**	**Fast**	**Std**	**Fast**
Mean Ct (6.25 cp/ul)	33.42	33.41	34.64	34.59	19.01	19.15
SD	0.64	1.10	0.91	0.95	0.15	0.36

[2]Standard Block

[3]Fast Block

2) Analytical Inclusivity-In-Silico Analysis:

The CSI laboratories uses the CDC primer and probe kit that targets specific genomic regions of the SARS-CoV-2 nucleocapsid (N) gene that are unique to SARS-CoV-2. The sequences for the N1, N2 primers/probes used in this assay are identical to the N1, N2 primers/probes sequences used in the FDA authorized original CDC 2019-Novel Coronavirus (2019-nCoV) real time RT-PCR Diagnostic Panel. Inclusivity was

demonstrated in the original EUA by mapping the primers and probes to all SARS-CoV-2 genomes available

Given the dramatic increase in available genomic information pertaining to SARS-CoV-2 in publicly available databases, an updated *in-silico* inclusivity analysis was performed May 29, 2020 , aimed at determining the potential alignment capability of the CDC-defined primers relative to the numerous publicly available SARS-CoV-2 sequences.

The in-silico study was performed by doing a BLAST search using blastn suite software on the NCBI website for Betacoronavirus database (https://blast.ncbi.nlm.nih.gov/Blast.cgi?PAGE_TYPE=BlastSearch&BLAST_SPEC=Betacoronavirus) with the sequences for the N1 and N2 primers and probes of the CDC SARS-CoV-2 RT-PCR kit. The analysis included 12,662 sequences.

For each primer and probe there was 100% identity and 100% query coverage with no mismatches found.

3) Cross-reactivity (Analytical Specificity)

The analytical specificity of the CSI Laboratories SARS-CoV2 RT-PCR Test was demonstrated in silico under the original EUA for the 2019-nCov CDC EUA Kit from IDT. The analysis included evaluation of the primer and probe homology with the 43 organisms and viruses listed in recommend list of organisms below. Based on this analysis, significant amplification of non-target sequences that could result in cross-reaction (false-positive results) or interference (false-negative results) was considered unlikely to occur.

4) Clinical Evaluation:

Performance of the CSI laboratories SARS-CoV-2 assay was evaluated using both contrived positive and negative samples as well as confirmed natural clinical positive and negative nasopharyngeal swabs.

Contrived Testing

Performance of the CSI Laboratories test was evaluated using pooled nasopharyngeal swab specimens that were spiked with whole viral SARS-CoV-2 (Isolate USA—WA1/2020 from BEI). In total, 30 negative clinical matrix samples and 48 positive contrived samples were tested. Of the 48 contrived positive clinical samples, 24 were prepared with concentrations 1X LoD (6.25 copies/µl), the remaining half of the positive contrived samples were prepared at 3X the assay LoD (18.75 copies/ µl). Prepared samples were randomized, blinded and run with the Applied Biosystem QS7 instrument equipped with the standard block. Results of the contrived study are summarized **Table 7**.

The results at all tested levels for spiked positives in clinical matrix demonstrated 100% agreement with expected results and all negative samples were non-reactive.

Table 7. Summary of Contrived Sample Testing

	SARS-CoV-2 concentration	# of NP swabs	SARS-N1			SARS-N2			RNaseP		
			Pos	%	Ct avg	Pos	%	Ct avg	Pos	%	Ct avg
SARS-CoV-2 PCR test	1x LoD	24	24	100%	33.51	24	100%	34.73	24	100%	18.92
	3x LoD	24	24	100%	31.94	24	100%	33.95	24	100%	19.03
	Negative	30	0	0%	n/a	0	0%	n/a	30	100%	19.35

Clinical Study With Previously Confirmed Positive and Negative Samples:
In addition to the contrived clinical study, the clinical performance of the CSI Laboratories SARS-CoV-2 RT-PCR Test was evaluated using leftover, frozen nasopharyngeal swabs (NPS) obtained from a local hospital that uses the FDA authorized Cepheid Xpert Xpress SARS-CoV-2 test.

A total of 25 positive and 30 negative samples were tested in a blinded study using the Applied Biosystem QS7 instrument equipped with the standard block..

All positive and negative clinical samples tested with the SARS-CoV-2 RT PCR Test were in 100% agreement to results tested with the FDA authorized Cepheid Xpert Xpress SARS-CoV-2 Test (**Table 8**).

Table 8. Performance of SARS-CoV-2 RT-PCR Test vs Cepheid Authorized Test

Patient NP Specimens		Cepheid Xpert Xpress SARS-CoV-2 (FDA authorized test)		
		Positive	**Negative**	**Total**
CSI Laboratories SARS-CoV-2	**Positive**	25	0	25
	Negative	0	30	30
	Total	25	30	55
Positive Agreement		100.0% (25/25), 95% CI: (86.7% - 100%)*		
Negative Agreement		100.0% (30/30), 95% CI: (88.7%-100%)*		

*95% Confidence intervals

An additional comparator was also used to confirm the robustness of the CSI Laboratories SARS-CoV-2 RT-PCR Test. The use of 10 additional, patient nasopharyngeal swabs (4 positive and 6 negative) as determined by the FDA authorized Quest Diagnostics SARS-CoV-2 rRT-PCR assay (EUA200015) were tested with the Applied Biosystem QS7 instrument equipped with the standard block in a blinded format.

All positive and negative samples tested with the SARS-CoV-2 RT PCR Test were in 100% agreement to results tested by Quest Diagnostic Authorized (**Table 9**).

Table 9. Performance of SARS-CoV-2 RT-PCR Test vs Quest Authorized Test

Patient NP Specimens		Quest Diagnostics (FDA Authorized test) EUA200015)		
		Positive	**Negative**	**Total**
CSI Laboratories SARS-CoV-2	**Positive**	4	0	4
	Negative	0	6	6
	Total	4	6	10
Positive Agreement		100% (4/4), 95%CI: (51.0%; 100%)*		
Negative Agreement		100% (6/6), 95%CI: (61.0%; 100%)*		

* Two-sided 95% score confidence intervals

All Clinical study results are acceptable and support use of the CSI SARS-CoV-2 RT-PCR Test for testing clinical specimens.

WARNINGS:

- This test has not been FDA cleared or approved;
- This test has been authorized by FDA under an EUA for use by authorized laboratories;
- This test has been authorized only for the detection of nucleic acid from SARSCoV-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 diagnostic tests for detection and/or diagnosis of COVID-19 under Section 564(b)(1) of the Act, 21 U.S.C. § 360bbb-3(b)(1), unless the authorization is terminated or revoked sooner.

FDA SARS-CoV-2 Reference Panel Testing

The evaluation of sensitivity and MERS-CoV cross-reactivity was performed using reference material (T1), blinded samples and a standard protocol provided by the FDA. The study included a range finding study and a confirmatory study for LoD. Blinded sample testing was used to establish specificity and to confirm the LoD. RNA isolation utilized the 5073 EPMotion liquid handler with Omega BioTek Viral RNA extraction kit. Real time RT-PCR was performed on Applied Biosystem Quantstudio 7 instrument (software version 1.3). The results are summarized in the following Table.

Table 10. Summary of LoD Confirmation Result using the FDA SARS-CoV-2 Reference Panel

Reference Materials Provide d by FDA	**Specimen Type**	**Product LoD**	**Cross-Reactivity**
SARS-CoV-2	Nasal Swab	$1.8x10^3$ NDU/mL	N/A
MERS-CoV		N/A	ND

NDU/mL = RNA NAAT detectable units/mL
N/A: Not applicable
ND: Not Detected