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

(Capstone Healthcare)

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 the Clinical Virology Laboratory at Capstone Healthcare located at, 8601 Dunwoody Pl. Ste 444, Sandy Springs, GA 30350 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 as per Laboratory Instructions for Use that was reviewed by the FDA under this EUA.)

INTENDED USE

The Genus 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 and oropharyngeal swabs collected from individuals suspected of COVID-19 by their healthcare provider. Testing is limited to the Clinical Virology Laboratory at Capstone Healthcare located at 8601 Dunwoody Pl. Ste. 444, Sandy Springs, GA 20250 which is certified under the Clinical Laboratory Improvement Amendments of 1988 (CLIA), 42 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.

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 oligonucleotide primers and probes for detection of 2019-nCoV were selected from regions of the virus nucleocapsid (N) gene. The panel is designed for specific detection of the 2019-nCoV (two primer/probe sets). An additional primer/probe set to detect the human RNase P gene (RP) in control samples and clinical specimens is also included in the panel. RNA isolated and purified from nasopharyngeal and oropharyngeal swab specimens is reverse transcribed to cDNA and subsequently amplified in the Applied Biosystems QuantStudio 12K Flex Real Time PCR Instrument with QuantStudio Real-Time PCR System software version 1.3. In the 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 probe, causing the reporter dye to separate from the quencher dye, generating a fluorescent signal. With each cycle, additional reporter dye molecules are cleaved from their respective probes, increasing the fluorescence intensity. Fluorescence intensity is monitored at each PCR cycle by. Detection of viral RNA not only aids in the diagnosis of illness but also provides epidemiological and surveillance information.

The assay is based on the principles of the procedure outlined below:

- Total nucleic acid (TNA) is isolated using the Thermofisher MVP or Omega Biotek Mag-Bind DNA/RNA kit. Extraction can be manual or automated on the KingFisher Flex platform.
- Extracted RNA is added to a 384 well plate containing master mix for the conversion of RNA to cDNA and labeling of targeted amplicon regions.
- The 384 well plate is loaded on to the instrument for real-time thermal cycling and real-time data capture.
- The results are then analyzed in the Quant Studio software and imported in to a LIMS system for report generation.

INSTRUMENTS USED WITH TEST

The Genus SARS-CoV-2 real-time RT-PCR assay is to be used with either manual extraction or extraction on the Kingfisher platform. The thermocycler used with the assay is the Applied Biosystem QuantStudio12K Flex instrument with QuantStudio Real-Time PCR System software version 1.3.

REAGENTS AND MATERIALS

Reagent	Vendor/Manufacturer	Catalogue Number
2019-nCoV CDC EUA kit	Integrated DNA Technologies (IDT)	10006770
2019-nCoV N Positive Control	Integrated DNA Technologies (IDT)	10006625
Hs RPP30 Positive Control	Integrated DNA Technologies	10006626

	(IDT)	
TaqPath™ I-Step RT-qPCR Master	ThermoFisher	A15300
MicroAmp® Optical 384 Reaction	ThermoFisher	4343370
Optical Adhesive Film	ThermoFisher	4311971
1.5mL microcentrifuge tubes (DNase/RNase	ThermoFisher	3457IW
Distilled Water (Ultrapure)	ThermoFisher	10977015
Foil seals	ThermoFisher	AB0626
Bleach -0.1	ThermoFisher	N/A
DNAZap™	ThermoFisher	AM9890
RNAse Away™	ThermoFisher	7005-11
Aerosol barrier pipette tips	ThermoFisher	VARIABLE BASED ON PIPET SIZE
Ethanol	Mercedes Scientific	1117274000
Powder-free gloves / Nitrile	Mercedes Scientific	55082
Laboratory-grade wipes	Mercedes Scientific	34155

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

SARS-CoV-2 Positive Template Control (PTC):
A positive template control is needed to monitor substantial reagent failure including primer and probe integrity. The PTC is a plasmid (2019 nCoV_N Positive control) that contains the target regions for the nucleocapsid gene.

Human Specimen Control (HSC) (Negative Control and Extraction Control)
A negative human extraction control is extracted concurrently with the test samples. This provides a nucleic acid extraction procedural control and a secondary negative control. The HSC monitors for failure in lysis and the extraction procedure as well as potential contamination during extraction. The human extraction control consists of previously confirmed negative patient samples.

Internal Control
An internal control is needed to verify that nucleic acid is present in every sample and is used for every sample processed. A primer/probe set detecting the human housekeeping gene RNase P is included in every patient sample reaction.

NTCs (No template control):
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. The NTC is added during rRT-PCR reaction setup and consists of RNase/DNase free water.

INTERPRETATION OF RESULTS

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

All test controls should be examined prior to interpretation of patient results. If the positive and negative controls are not valid, the patient results cannot be interpreted and the assay run must be repeated.

Positive template control: The positive control should have a fluorescence growth curve within 40 cycles (Ct <40) for both the N1 and N2 targets.

HSC: The HSC should be not detected for the N1 and N2 targets but detected for RNase P at a Ct <40.

Internal Control: All clinical samples should exhibit fluorescence growth curves in the RNase P reaction that cross the threshold within 40 cycles. If RNase P is not detected, and there is no detection for the N1 or N2 targets, the result is invalid and should be repeated from extraction. If the repeated result is invalid, consider collecting a new specimen from the patient.

NTC: The NTC should have no detection for any of the targets for the assay

If any of the above controls do not exhibit expected performance as described, the assay may have been set up and/or executed improperly, or reagent or equipment malfunction could have occurred. The run should be invalidated and re-tested.

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

The Genus SARS-CoV-2 assay qualitatively detects the presence or absence of the SARS-CoV-2 RNA virus. Results are reported out for each target as "Detected" or "Not Detected". The patient sample report is cumulatively reported as " Positive" or "Negative" based on the analysis matrix below (published by the CDC).

1. Positive Specimens:
   Specimens with Ct values of **<40.0** in **both N1 and N2 targets**, with or without an acceptable RNAse P, are reported as “Detected” for SARS-CoV-2 RNA.

2. Negative Specimens:
   Specimens undetectable for N1 and N2 but with an acceptable RNAse P Ct value (Ct <40) are reported as “Not Detected” for SARS-CoV-2 RNA.

3. Inconclusive Results:
   Specimens where **either** N1 or N2 is positive (not both) are reported as “Inconclusive.” Repeat testing of nucleic acid with three replicates or re-extraction should occur for inconclusive specimens. If 2 of the 3 replicates from the retest are inconclusive and one is positive, report as positive and recommend

recollection of specimen for confirmation. If the repeat is also inconclusive the lab will report as inconclusive and ask to recollect the specimen.

4. Invalid Results:
   Specimens with no detection for any target (N1, N2, RNase P) are considered invalid. Repeat extraction and rRT-PCR. If the repeated result remains invalid, consider collecting a new specimen from the patient.

Interpretation of Patient Results

2019 nCoV_N1	2019 nCoV_N2	RP	Result Interpretation	Report	Actions
+	+	±	2019-nCoV detected	Positive 2019-nCoV	Report results to CDC and sender
+	-	±	Inconclusive Result	Inconclusive	Repeat testing of nucleic acid with three replicates or re-extract. If 2/3 replicates from the retest are inconclusive and one is positive, report as positive and recommend recollection for confirmation. If the repeat is also inconclusive the lab will report as inconclusive and ask to recollect the specimen.
-	+	±			
-	-	+	2019-nCoV not detected	Not Detected	Report results to sender. Consider testing for other respiratory viruses.
-	-	-	Invalid Result	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 limit of detection (LoD) of the Genus SARS-CoV-2 r-RT-PCR panel at Capstone Healthcare was determined by running 6 dilutions in quadruplicate across two different days. Dilutions were made by performing a 1:10 serial dilution from 200,000 down to 2

copies/uL. Samples were prepared by spiking whole viral genomic RNA into pooled sputum samples. The samples were extracted using the Omega Biotek Mag-Bind Viral DNA/RNA 96 Kit on the KingFisher Flex extraction system and then tested on the QuantStudio 12K Flex thermocycle. To further narrow down the LoD, samples were run in replicates of 20 at 10, 20, 30, 40, 50, and 60 copies/uL. All replicates down to 40 copies/uL showed 100% concordance. Therefore, a preliminary LoD of 40 copies/ul was established.

To confirm the LOD, an additional 20 replicates at a concentration of 40 copies/ul were tested. The RNA was extracted using two different extraction kits (Thermofisher MagMax Viral/Pathogen Nucleic Acid Isolation Kit and Omega Biotek Mag-Bind Viral DNA/RNA 96 kit) on the KingFisher Flex extraction system. The samples were tested on the QuantStudio 12K Flex thermocycler. The LoD of 40 copies/ul was confirmed for both extraction methods based on 100% positivity (20/20 replicates).

2) Analytical Inclusivity/Cross Reactivity

The sequences for the N1 and N2 primers and probe used in this assay are identical to the N1 and N2 primer/probe sequences used in the FDA authorized CDC SARS-CoV-2 assay. Please refer to EUA200001/A004 for an updated *in silico* analysis of the primers/probes used with the CDC assay.

3) Clinical Evaluation:

The clinical performance of the Genus SARS-CoV-2 Assay was assessed by comparing patient specimen results to results obtained using an EUA authorized assay. In this evaluation, a total of 71 patient specimens (43 positive and 28 negative) obtained from nasopharyngeal and oropharyngeal swabs were tested using the Genus SARS-CoV-2 assay and the EUA authorized CDC assay. All positive samples were concordant, yielding a positive percent agreement of 100% (43/43). All negative specimens were also concordant, yielding a negative percent agreement of 100% (28/28).

		CDC Assay	
		Pos	Neg
Genus Assay	Pos	43	0
	Neg	0	28

PPA 100% (43/43)

NPA 100% (28/28)

Genus SARS-CoV-2 Assay Ct values:

Target	Mean Ct	Range
N1	27.93	14.16 - 37.39
N2	28.09	14.26 - 38.37
RNaseP	27.57	22.52 - 36.32

A separate evaluation was conducted with IPSUM Diagnostics using 12 samples (6 positive and 6 negative) previously tested at Capstone Healthcare. Each patient sample contained an oropharyngeal swab and nasopharyngeal swab in one molecular transport media (MTM) vial. All positive and negative results were 100% concordant between Capstone Healthcare and IPSUM Diagnostic's EUA authorized test.

WARNINGS:

- This test has not been FDA cleared or approved;
- This test has been authorized by FDA under an EUA for use by the Clinical Virology Laboratory at Capstone Healthcare located at 8601 Dunwoody Pl. Ste. 444, Sandy Springs, GA 20250 which is certified under the Clinical Laboratory Improvement Amendments of 1988 (CLIA), 42 42 U.S.C. §263a and meets requirements to perform high complexity tests;
- 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