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 Genus SARS-CoV-2 Assay will be performed at laboratories designated by Capstone Healthcare that are certified under the Clinical Laboratory Improvement Amendments of 1988 (CLIA), 42 U.S.C. §263a and meet 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 laboratories designated by Capstone Healthcare that are certified under the Clinical Laboratory Improvement Amendments of 1988 (CLIA), 42 42 U.S.C. §263a and meet 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 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 Quant Studio 12K Flex Real Time PCR Instrument with Quant Studio 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 into a LIMS system for report generation.

INSTRUMENTS USED WITH TEST

The Genus SARS-CoV-2 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	10006625

	(IDT)	
Hs RPP30 Positive Control	Integrated DNA Technologies (IDT)	10006626
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.

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 corroborate the LoD. The extraction method used was the Omega Biotek Mag-Bind DNA/RNA kit utilizing the KingFisher Flex platform, and amplification was carried out on the Applied Biosystems QuantStudio 12K Flex Real Time PCR Instrument. The results are summarized in the following Table.

Summary of LoD Confirmation Result Using the FDA SARS-CoV-2 Reference Panel

Reference Materials Provided by FDA	Specimen Type	Product LoD	Cross-Reactivity
SARS-CoV-2	Nasopharyngeal Swab	$1.8x10^4$ NDU/mL	N/A
MERS-CoV		N/A	ND

NDU/mL: RNA NAAT detectable units/mL

N/A: Not Applicable

ND: Not Detected

LIMITATIONS

- All users, analysts, and any person reporting diagnostic results should be trained to perform this procedure by a competent instructor. They should demonstrate their ability to perform the test and interpret the results prior to performing the assay independently.
- Performance of the CDC 2019-nCoV Real-Time RT-PCR Diagnostic Panel has only been established in upper and lower respiratory specimens (such as nasopharyngeal or oropharyngeal swabs, sputum, lower respiratory tract aspirates, bronchoalveolar lavage, and nasopharyngeal wash/aspirate or nasal aspirate).
- Negative results do not preclude 2019-nCoV infection and should not be used as the sole basis for treatment or other patient management decisions. Optimum specimen

types and timing for peak viral levels during infections caused by 2019-nCoV have not been determined. Collection of multiple specimens (types and time points) from the same patient may be necessary to detect the virus.

- A false negative result may occur if a specimen is improperly collected, transported or handled. False negative results may also occur if amplification inhibitors are present in the specimen or if inadequate numbers of organisms are present in the specimen.
- Positive and negative predictive values are highly dependent on prevalence. False negative test results are more likely when prevalence of disease is high. False positive test results are more likely when prevalence is moderate to low.
- Do not use any reagent past the expiration date.
- If the virus mutates in the rRT-PCR target region, 2019-nCoV may not be detected or may be detected less predictably. Inhibitors or other types of interference may produce a false negative result. An interference study evaluating the effect of common cold medications was not performed.
- Test performance can be affected because the epidemiology and clinical spectrum of infection caused by 2019-nCoV is not fully known. For example, clinicians and laboratories may not know the optimum types of specimens to collect, and, during the course of infection, when these specimens are most likely to contain levels of viral RNA that can be readily detected.
- Detection of viral RNA may not indicate the presence of infectious virus or that 2019-nCoV is the causative agent for clinical symptoms.
- The performance of this test has not been established for monitoring treatment of 2019-nCoV infection.
- The performance of this test has not been established for screening of blood or blood products for the presence of 2019-nCoV.
- This test cannot rule out diseases caused by other bacterial or viral pathogens.
- The clinical performance has not been established in all circulating variants but is anticipated to be reflective of the prevalent variants in circulation at the time and location of the clinical evaluation. Performance at the time of testing may vary depending on the variants circulating, including newly emerging strains of SARS-CoV-2 and their prevalence, which change over time.

WARNINGS:

- This product has not been FDA cleared or approved, but has been authorized by FDA under an EUA for use by authorized Laboratories; laboratories designated by Capstone Healthcare that are certified under the Clinical Laboratory Improvement Amendments of 1988 (CLIA), 42 42 U.S.C. §263a and meet requirements to perform high complexity tests;
- This product has been authorized only for the detection of nucleic acid from SARSCoV-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 diagnostic tests for detection and/or diagnosis of COVID-19 under Section 564(b)(1) of the Act, 21U.S.C. § 360bbb-3(b)(1), unless the authorization is terminated or revoked sooner