EMERGENCY USE AUTHORIZATION (EUA) SUMMARY

GWU COVID-19 RT-PCR test

(George Washington University Public Health Laboratory)

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

(The GWU SARS-CoV-2 RT-PCR test will be performed at the George Washington University Public Health Laboratory, located at Science and Engineering Hall 800 22nd St. NW, Washington, DC 20052, which is certified under the Clinical Laboratory Improvement Amendments of 1988 (CLIA), 42 U.S.C. §263a, and meets the requirements to perform high complexity tests. The Laboratory Standard Operating Procedure was reviewed by the FDA under this EUA.)

INTENDED USE

The GWU COVID-19 RT-PCR test is a real-time (rt) reverse transcriptase (RT) polymerase chain reaction (PCR) intended for the qualitative detection of nucleic acid from the SARS-CoV-2 in upper respiratory specimens (such as nasal, mid-turbinate, nasopharyngeal and oropharyngeal swabs) and BAL collected from individuals suspected of COVID-19 by their healthcare provider. Testing is limited to GWU Public Health Laboratory, 800 22nd St. NW, Washington, DC 20052, which is certified under the Clinical Laboratory Improvement Amendments of 1988 (CLIA), 42 U.S.C. §263a, and meets the requirements 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 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 GWU COVID-19 RT-PCR 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 GWU COVID-19 RT-PCR is only for use under the Food and Drug Administration's Emergency Use Authorization.

DEVICE DESCRIPTION AND TEST PRINCIPLE

The GWU COVID-19 RT-PCR test is a real-time reverse transcription polymerase chain reaction (rRT -PCR) test. The SARS-CoV-2 primer and probe set is designed to detect RNA from the SARS-CoV-2 in upper respiratory specimens from patients as recommended for testing by public health authority guidelines. The test measures the presence or absence of RNA for the nucleocapsid protein of the SARS-CoV-2 using CDCs N1 and N2 primers. The test also co-extracts and amplifies sequences from the human RNase P gene detected by a differently labeled fluorophore.

RNA is isolated from claimed specimens, then reverse transcribed to cDNA and subsequently amplified using the LightCycler 480 instrument with Software version 1.5. During the amplification 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 bound probe, causing the reporter dye (FAM and CAL Fluor Red 610) to separate from the quencher dye (BHQ), generating a fluorescent signal. Fluorescence intensity is monitored at each PCR cycle by the LightCycler 480 instrument.

INSTRUMENTS USED WITH TEST

The GWU COVID-19 RT-PCR test is run on the Roche LightCycler 480 using the Roche LightCycler 480 v.1.5 software. Extraction is performed with the MagMAX-96 Viral RNA Isolation Kit (Ambion by Life Technologies) on the Hamilton Microlab STAR liquid-handling system with Hamilton VENUS software.

EQUIPMENT, REAGENTS AND MATERIALS

The following equipment/reagents/materials are required to run this test in additional to the consumables for the extraction and PCR process:

Table 1: Reagents, Material and Equipment required

Reagent	Description	Vendor	Catalog No.
Viral Transport Media (VTM)	Sample collection and transport media	n/a (Prepared according to CDC protocol)	n/a
qRT-PCR kit	1-step quantitative qRT-PCR master mix (qScript XLT)	Quantabio/VWR	#76047-080
Real-time PCR system	Roche LightCycler 480 with software v.1.5	Roche	05015243001
CDC EUA kit	Primer/probe mixes for N1, N2, and RP targets (supplied at working concentrations)	IDT	269057855
RNA isolation and purification kit	MagMAX-96 Viral RNA Isolation Kit	Thermo	AM 1836
Synthetic SARS-	Synthetic RNA transcript contains SARS-	BEI Resources	NR-53258

Reagent	Description	Vendor	Catalog No.
CoV-2 RNA	CoV-2 N1 and N2 RT-PCR amplicon sequences; supplied at 290,000 copies/µL		
Extraction controls	Prepared in-house from human nasopharyngeal, nasal, or mid-turbinate swab eluent	N/A	N/A
Inactivated SARS-CoV-2 virus	Quantitative inactivated SARS-CoV-2 control used in LoD testing	BEI Resources	NR-53250

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

Table 2. Controls and Expected Results for the 2019-nCoV qRT-PCR Panel

CONTROL TYPE	DESCRIPTION/PURPOSE	N1	N2	RP
Positive extraction control	A human specimen negative for SARS-CoV-2 spiked with 50 copies/µL SARS-CoV-2 synthetic control RNA (BEI NR-53258). Full process Positive Control for RNA extraction and RT-PCR (all targets)	Ct ≤ 40.0	Ct ≤ 40.0	Ct ≤ 40.0
Negative human specimen control	A human specimen negative for SARS-CoV-2. Full process Positive Control for RNA extraction and Reverse Transcription (RP target only); contamination control for N1 and N2 targets	No Ct	No Ct	Ct ≤ 40.0
Negative extraction control	An RNA extraction performed with transport media in place of the specimen. Full process Negative Control for RNA extraction kit reagent contamination, RNA extraction procedures	No Ct	No Ct	No Ct
NTC (No template control; nuclease-free water)	(OPTIONAL CONTROL) Negative control for assay or RT-PCR reagent contamination	No Ct	No Ct	No Ct
SARS-CoV-2 synthetic RNA (BEI NR-52358)	(OPTIONAL CONTROL) Contains the N1 and N2 viral genome fragments as defined in the CDC EUA protocol. Positive control for RT-PCR (N1 and N2 targets)	Ct ≤ 40.0	Ct ≤ 40.0	No Ct

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. Result interpretation for patient samples was established based on a cutoff of 40 Ct for SARS-CoV-2 target.

a. Control Result Interpretation

If any of the controls do not exhibit the expected performance as described in Table 2 above, the assay may have been improperly set up and/or executed improperly, or reagent or equipment malfunction could have occurred. Invalidate the run and re-test.

b. Examination and Interpretation of Patient Specimen Results:

Assessment of clinical specimen test results should only be performed after the positive and negative controls have been examined and determined to be valid and acceptable. If the controls are not valid, the patient results cannot be interpreted, and testing needs to be repeated after a root cause is identified and eliminated.

Table 3: Interpretation of Sample Results

SARS-CoV-2 N1	SARS-CoV-2 N2	RP	Result Interpretation	Report	Actions
+	+	±	SARS-CoV-2 Detected	Positive SARS-CoV-2	Report results to CDC and sender.
If only one of the two targets are positive		±	Inconclusive Result	Inconclusive	Repeat testing of nucleic acid and/or re-extract and repeat qRT-PCR. If the repeated result remains inconclusive, report to the submitter and recommend re-testing.
-	-	+	SARS-CoV-2 Not Detected	SARS-CoV-2 Not Detected	Report results to submitter.
-	-	-	Invalid Result	Invalid	Repeat extraction and qRT-PCR. If the repeated result remains invalid, report as “insufficient sample” and consider collecting a new specimen from the participant.

PERFORMANCE EVALUATION

1) Limit of Detection (LoD) -Analytical Sensitivity:

a. Tentative LoD Study:

To establish the limit of detection (LoD) for the GWU SARS-CoV-2 RT-PCR test, a tentative LoD study was performed. A dilution series was performed with the inactivated SARS-CoV-2 virus samples (BEI) diluted into a pool of nasopharyngeal swab matrix previously tested negative for infection with the SARS-CoV-2 virus. Each concentration was run with three individual extraction replicates.

Table 4. Results of the Tentative LoD Study

Target Level* [cp/µL]	Valid tested replicates	SARS-CoV-2 N1 Positive			SARS-CoV-2 N2 Positive			Internal Control (Human RNAse P, RP) Positive		
		n	Mean Ct	Detection Rate	n	Mean Ct	Detection Rate	n	Mean Ct	Detection Rate
100	3	3	31.16	100%	3	32.95	100%	3	32.76	100%
50	3	3	32.65	100%	3	34.19	100%	3	32.39	100%
25	3	3	32.18	100%	3	35.50	100%	3	33.21	100%
12.5	**3**	**3**	**32.82**	**100%**	**3**	**36.77**	**100%**	**3**	**33.86**	**100%**
6.25	3	2	32.58	66.7%	2	37.22	66.7%	3	31.55	100%
3.125	3	0	n/a	0%	1	37.88	33.3%	3	31.51	100%

b. Confirmation of the LoD: Nasal swab combined with saline oral rinse

Based on the results of the tentative study, RNA was extracted from 20 NP samples in VTM spiked with 12.5 copies/µL inactivated SARS-CoV-2. All samples were individually extracted and processed per the laboratory SOP. The Limit of Detection for the inactivated virus using the GWU COVID-19 PCR Test is 12.5 copies/µL for samples in NP sample matrix in VTM.

Table 5: Confirmatory LoD study in Nasal Swab in Oral Rinse Matrix

SARS-CoV-2 RNA (copies/µL)	Number Tested	Positive	Mean Ct Target		
			SARS-CoV-2		RNase P IC
			N1	N2	
12.5	**20**	**20 (100%)**	**32.5**	**33.4**	**29.7**

2) ***Analytical Inclusivity/Specificity:***

a. Inclusivity

The GWU SARS-CoV-2 RT-PCR test utilizes primer and probe sets identical to the N1 and N2 SARS-CoV-2 target genes used in the CDC 2019-Novel Coronavirus (2019-CoV) Real-Time RT-PCR Diagnostic Panel. Accordingly, inclusivity of the primer and probe sets used in the SARS-CoV-2 (N gene detection) Test have been evaluated by FDA under EUA200001. CDC has provided right-of-reference to leverage their EUA data. Accordingly, an inclusivity analysis was not repeated.

b. Cross-reactivity

The GWU SARS-CoV-2 RT-PCR test utilizes primer and probe sets identical to the N1 and N2 SARS-CoV-2 target genes used in the CDC 2019-Novel Coronavirus (2019-CoV) Real-Time RT-PCR Diagnostic Panel. CDC has provided right-of-reference to leverage their EUA data. Accordingly, inclusivity of the primer and probe sets used in the SARS-CoV-2 (N gene detection) Test have been evaluated by FDA under EUA200001, and a cross reactivity study was not repeated.

3) ***Clinical Evaluation:***

A total of 82 samples were previously tested with an FDA authorized comparator test. Of the 82 samples, 40 were confirmed positive and 42 confirmed negative nasopharyngeal specimens in viral transport media. These 82 samples were tested in a blinded fashion at GWU per Laboratory SOP. All samples had RP Ct values less than 40, and the N1 and N2 viral targets were not detected in any of the confirmed negatives (100% agreement). Viral targets were detected in 38/40 confirmed positive samples (95% agreement).

Table 6. Clinical Validation Results

		EUA Authorized Comparator			**Total**
		Positive	**Inconclusive**	**Negative**	
GWU SARS-CoV-2 RT-PCR	**Positive**	38	0	0	38
	Inconclusive	0	0	0	0
	Negative	2 *	0	42	44
Total		**40**	**0**	**42**	**82**

* The comparator is using a cutoff of Ct40 but has a mean Ct between 37 and 39 at its LoD, depending on the instrument. One of the two false negative samples had Ct values close to Ct 39 for both comparator targets (E/RdRp), indicating that the sample was close to the comparator LoD. The other

sample had Ct values of 35.8 and 35.5 for E and RdRp, respectively, indicating that the sample was also low positive.

Positive Percent Agreement (PPA): 38/40 = 95.0% (95% CI: 83.5% - 98.6%)
Negative Percent Agreement (NPA): 42/42 = 100% (95% CI: 91.6% - 100%)

WARNINGS:

- For *in vitro* diagnostic use.
- Rx only.
- For use under Emergency Use Authorization (EUA).
- Members of the infectious disease laboratory will be trained to perform this assay and competency will be assessed and documented per CAP regulations.
- The GWU SARS-CoV-2 Test has not been FDA cleared or approved;
- The test has been authorized by FDA under an Emergency Use Authorization (EUA) for use by the George Washington University Public Health Laboratory, located at Science and Engineering Hall, 800 22nd St. NW, Washington, DC 20052;
- The GWU SARS-CoV-2 Test has been authorized only for the detection of nucleic acid from SARS-CoV-2, not for any other viruses or pathogens.
- The GWU SARS-CoV-2 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 Federal Food, Drug and Cosmetic Act, 21 U.S.C. § 360bbb-3(b)(1), unless the authorization is terminated or revoked sooner.
- Laboratories within the United States and its territories are required to report all positive results to the appropriate public health authorities.
- Specimens should always be treated as if infectious and/or biohazardous in accordance with safe laboratory procedures.
- Follow necessary precautions when handling specimens. Use personal protective equipment (PPE) consistent with current guidelines for the handling of potentially infectious sample.
- Always use pipette tips with aerosol barriers. Tips that are used must be sterile and free from DNases and RNases.
- Do not eat, drink, smoke, or apply cosmetic products in work areas.
- Do not use reagents after the expiry date
- Dispose of waste in compliance with local, state, and federal regulations.
- Positive results are indicative of the presence of SARS-CoV-2 RNA
- Handle all samples and controls as if they are capable of transmitting infections agents.
- Proper aseptic technique should always be used when working with RNA.
- Separate areas of sample extraction, positive control material handling, and PCR should be set up to reduce the risk of contamination.
- Hands and dust particles may carry bacteria and molds and are the most common sources of RNase contamination. Always wear powder-free latex, vinyl, or nitrile

gloves while handling reagents, tubes and RNA samples to prevent RNase contamination from the surface of the skin or from dusty laboratory equipment.
- Change gloves frequently and keep tubes closed.
- During the procedure, work quickly and keep everything on cold blocks when possible to avoid degradation of RNA by endogenous or residual RNases.
- Clean working surfaces, pipettes, etc. with 20% bleach or other solution that can destroy nucleic acids and RNases. To eliminate accelerated deterioration of any plastics and metals, wipe down with 70% ethanol after using 20% bleach. Make sure all bleach is removed to eliminate possible chemical reactions between bleach and guanidine thiocyanate which is present in the extraction reagents. (If DNA/RNA Shield is used as the specimen transport medium, do not use bleach at all.)

LMITATIONS:

- The performance of this SARS-CoV-2 assay was established using nasopharyngeal swab specimens. Nasopharyngeal, nasal, oropharyngeal and mid-turbinate swabs and BAL are also considered acceptable specimen types for use with the SARS-CoV-2 assay, but performance has not been established.
- 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.
- This test cannot rule out diseases caused by other bacterial or viral pathogens.
- Samples must be collected, transported, and stored using appropriate procedures and conditions. Improper collection, transport, or storage of specimens may hinder the ability of the assay to detect the target sequences.
- Extraction and amplification of nucleic acid from clinical samples must be performed according the specified methods listed in this procedure. Other extraction kits have not been evaluated.
- Results from the GWU COVID-19 RT-PCR test should be used as an adjunct to clinical observations and other information available to the physician. The result is only for clinical reference, and the clinical management of patients should be considered in combination with their symptoms/signs, history, other laboratory tests and treatment responses.
- Although the detected target sequences of this kit are in conserved regions of the SARS-CoV-2 genome, rare mutations may lead to negative results.
- Negative results do not preclude SARS-CoV-2 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 SARS-CoV-2 have not been determined.