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
For use with the GWU COVID-19 Test Home Collection Kit for individuals 16 years of age and older

(The GWU COVID-19 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, as described in 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 anterior nasal, mid-turbinate, nasopharyngeal and oropharyngeal swab specimens, nasal washes and aspirates and bronchoalveolar lavage (BAL) specimens collected from individuals suspected of COVID-19 by their healthcare provider.

This test is also for use with anterior nasal swab specimens that are self-collected at home by individuals 16 years of age and older using the GWU COVID-19 Test Home Collection Kit when determined to be appropriate by a healthcare provider. Anterior nasal swab specimens collected using the GWU COVID-19 Test Home Collection Kit can be transported at ambient temperature for testing.

Testing is limited to GWU Public Health Laboratory, located at 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 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 Test 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 Test is only for use under the Food and Drug Administration's Emergency Use Authorization.

DEVICE DESCRIPTION AND TEST PRINCIPLE

1. GWU COVID-19 RT-PCR Test

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 anterior nasal, mid-turbinate, nasopharyngeal and oropharyngeal swab specimens, nasal washes and aspirates and bronchoalveolar lavage (BAL) specimens from individuals suspected of COVID-19 by their healthcare provider. 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.

2. GWU COVID-19 RT-PCR TEST HOME COLLECTION KIT

a. Device Description:

The GWU COVID-19 Test Home Collection Kit is a non-invasive alternative for collecting samples by/from individuals 16 years of age and older who are suspected of COVID-19 by a healthcare provider for use with the GWU COVID-19 RT-PCR Test.

The GWU COVID-19 Test Home Collection Kit collection device consists of a nylon flocked nasal swab, an empty specimen transport tube, sealable biohazard bag with absorbent pad, barcoded sample label, crushproof box, opaque waterproof envelope, and instructions for use.

The GWU COVID-19 Test Home Collection Kit collects virus from anterior nasal swab specimens; it can also be used for the transportation and short-term ambient storage of a sample.

b. Home Collection Kit Ordering and Processing:

Ordering and self-collection

Individuals within the George Washington University (GWU) community may request the GWU COVID-19 Test Home Collection Kit collection device based on the results of an online COVID-19 questionnaire or by telephone or online video appointment with a healthcare provider. The screening performed by healthcare provider will be performed following CDC recommendations for testing prioritization.

Upon prescription of COVID-19 test by a healthcare provider, individuals will either: (1) be provided an email or text containing a secure link to enter name, email, and personal identifier into a secure online database to request the home test kit or (2) be asked to enter into an electronic health record system (EHR) to enter name and email to request the home test kit. Kits will be distributed to individuals following prescription at a designated location.

Individuals will receive the GWU COVID-19 Test Home Collection Kit and a confirmation email. In the confirmation email, individuals will be asked to claim their test kit by confirming/entering the Sample ID barcode present on the pre-printed sample label, entering the collection date and time, as well as complete the sample label by writing their date of birth (DOB), collection date / time, and an additional identifier (e.g., GW ID), and affixing the label to the specimen transport tube immediately prior to self-collection.

The individual using the GWU COVID-19 Test Home Collection Kit self-collects the anterior nasal swab specimen according to the GWU COVID-19 Home Collection Kit Instructions.

Sample packaging instructions

Self-collection samples are packaged per the GWU COVID-19 Test Home Collection Kit, and sealed sample packages are dropped off at a designated drop-off location on the George Washington University campus within 24 hours of collection. Samples are collected in bulk from the drop-off locations at least once a day.

Accessioning

Specimens received at the clinical laboratory for testing with the GWU COVID-19 PCR Test undergo the following accessioning prior to acceptance for testing:

- Labeling and information
    - Samples must have the label affixed to the swab tube and be fully filled out with GWID, DOB, date, and time of sample collection.
    - Improperly/inadequately labeled specimens that cannot be resolved are rejected.
- Expired shipping time

  - The time of sample collection should be inspected.
  - Specimens must be processed ≤56 hours from being collected.
  - Any specimen that is received >56 hours from the collection date/time, must be rejected.
- Improper return of sample packaging
  - Samples should be inspected on arrival to ensure that packaging is intact and not compromised.
  - Samples should be checked that they have been returned in the supplied packing materials, if they have not the sample must be rejected.
  - Samples not in the correct collection/transport tube must be rejected.
  - Samples whose integrity appears compromised should be rejected.
  - Results for rejected samples are reported as invalid and the specimen discarded.

Swab Rehydration

Specimens collected using the GWU COVID-19 Test Home Collection Kit and deemed acceptable will be rehydrated in the laboratory prior to processing in 1 mL of DNA/RNA Shield (Zymo Research, R1100), vortexed, allowed to incubate for at least five (5) minutes at room temperature, and vortexed again.

Result Communication

Test results are communicated back to individuals that used the GWU COVID-19 Test Home Collection Kit via secure notifications from the EHR or secure online testing database. Individuals receiving positive test result will first be contacted directly by a healthcare provider. Individuals with an inconclusive or invalid test result will be contacted directly by lab staff to encourage re-testing as soon as possible.

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.
GWU COVID-19 RT-PCR Test			
Viral Transport Media (VTM)	Sample collection and transport media	Prepared at GWU according to CDC protocol	n/a

Reagent	Description	Vendor	Catalog No.
GWU COVID-19 RT-PCR Test			
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-CoV-2 RNA	Synthetic RNA transcript contains SARS-CoV-2 N1 and N2 RT-PCR amplicon sequences; supplied at 290,000 copies/µL	BEI Resources	NR-53258
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

Table 2: GWU COVID-19 RT-PCR Test Home Collection Kit

Name	Description	Quantity	Material Supplier
Swab	Copan Diagnostics Nylon Flocked Dry Swab w/ 80mm breakpoint	1	Fisher Scientific Cat. No.: 23-600-951
Collection tube	Greiner Bio-One Serum No Additive Tubes	1	Fisher Scientific Cat. No.: 22-040-163
Sample label	Zebra Z-Perform 2000D Direct Thermal Label, 1" x 2", White	2	Staples Cat. No.: 10010028
Biohazard Specimen Bags	Minigrip Biohazard Bag	1	Fisher Scientific Cat. No: 01-824
Absorbent pad	Therapak 3x4" Absorbent Sheet	1	VWR Cat. No: 89170-926
Crushproof box	7" x 3" x 1" Corrugated Mailers	1	Staples Cat. No.: M731
Resealable Envelope	6 X 9, 2.5 Mil Poly Mailers with Perforation	1	RoyalBag PMP0609

COLLECTION DEVICE STABILITY

The shelf-life stability of the home kit is defined by the shortest shelf-life of each of the components in the kit, as assigned by the manufacturer, and printed clearly in the outside of the home kit box.

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

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)

Negative extraction control:
An RNA extraction performed with transport media in place of the specimen and used as a full process Negative Control that monitors for contamination of RNA extraction kit and RT-PCR reagents.

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) and a full process negative control for N1 and N2 targets monitoring contamination and carryover.

NTC (No template control; nuclease-free water; OPTIONAL):
Negative control for assay or RT-PCR reagent contamination that can be included in the RT-PCR step only.

SARS-CoV-2 synthetic RNA (BEI NR-52358; OPTIONAL):
Contains the N1 and N2 viral genome fragments as defined in the CDC EUA protocol. Positive control for RT-PCR (N1 and N2 targets).

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.

1. Control Result Interpretation

If any of the controls do not exhibit the expected performance as described in Table 3 below, 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.

Table 3. Controls and Expected Results for Valid Controls used with the GWU COVID-19 RT-PCR Test

CONTROL TYPE	N1	N2	RP
Positive extraction control	Ct ≤ 40.0	Ct ≤ 40.0	Ct ≤ 40.0
Negative human specimen control	No Ct	No Ct	Ct ≤ 40.0
Negative extraction control	No Ct	No Ct	No Ct
NTC	No Ct	No Ct	No Ct
SARS-CoV-2 synthetic RNA	Ct ≤ 40.0	Ct ≤ 40.0	No Ct

2. 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 4: 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 5. 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 6: 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**

c. Limit of Detection (LoD) for Dry Anterior Nasal Swabs

To determine the Limit of Detection (LoD) for dry anterior nasal swab, anterior nasal swabs were collected from negative individuals as dry swabs using the GWU COVID-19 RT-PCR Test Home Collection Kit. A tentative LoD experiment was performed based on the previously established LoD of the GWU COVID-19 PCR Test (i.e., 12.5 copies/µl). Heat-inactivated virus (BEI Resources, NR-52286) was pre-diluted in PBS and spiked directly onto the collected negative anterior nasal swabs at different concentrations close to the initial LoD of 12.5 copies/µL. Three swabs were mad for each concentration.

Spiked samples were allowed to dry at room temperature for 24 hours at ambient temperature (15-30°C) and were then rehydrated in 1 mL of DNA/RNA Shield according to the validated protocol (see below). The tentative LoD was determined to be between 6.25 and 12.5 copies/µl (see Table 7).

Table 7: Tentative Limit of Detection Data

Copies/µl	Copies/Swab*	**N1 Cp**	N1 Mean Ct ±SD	**N2 Cp**	N2 Mean Ct ±SD	**RP Cp**	RP Mean Ct ±SD	**Result**
125	125,000	31.66	**32.05** ± 0.4	32.2	**32.56** ± 0.41	28.79	**31.09** ± 2.00	Positive
		32.04		32.46		32.04		Positive
		32.45		33.01		32.43		Positive
62.5	62,500	32.44	**32.42** ± 0.37	32.68	**32.76** ± 0.25	32	**30.62** ± 1.79	Positive
		32.04		32.56		28.6		Positive
		32.77		33.04		31.26		Positive
37.5	37,500	34.46	**34.02** ± 0.41	34.71	**34.3** ± 0.43	30.3	**30.42** ± 2.55	Positive
		33.96		33.85		33.02		Positive
		33.65		34.33		27.93		Positive
12.5	12,500	35.28	**35.36** ± 0.35	35.97	**37.26** ± 1.77	29.57	**30.79** ± 1.12	Positive
		35.74		39.28		31.03		Positive
		35.06		36.54		31.76		Positive
6.25	6,250	36.24	**35.85** ± 0.37	0	**36.85** ± 0.52	29.68	**28.97** ± 3.33	Inconclusive
		35.82		36.49		31.89		Positive
		35.5		37.22		25.34		Positive

*Before elution in 1 mL of DNA/RNA Shield

To confirm the preliminary LoD, 20 replicates of negative nasal swabs were spiked with heat-inactivated virus at 12,500 copies/swab and tested after rehydration. 19/20 replicates were positive for both viral targets, with the one replicate testing positive for one of two viral targets (see Table 8).

Table 8: Confirmatory Limit of Detection Data at 12.5 copies/µL

Sample	N1 Cp	N2 Cp	RP Cp	Result
Mean Ct	35.52	35.82	30.94	**19/20 (95%)**
Median Ct	35.44	35.72	30.87	
SD	0.54	0.67	2.08	

2. Analytical 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.

This inclusivity analysis has not identified variants of sufficient frequency (<5%) to affect the performance of the GWU SARS-CoV-2 RT-PCR Test, including the Alpha (B.1.1.7), Beta (B.1.351), Gamma (P.1), Delta (B.1.617.2) and omicron (B.1.1.529) variants.

3. 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.

4. 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 9. 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%)

5. 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 MagMAX-96 Viral RNA Isolation Kit (Ambion by Life Technologies) on the Hamilton Microlab STAR liquid-handling system with Hamilton VENUS software. Amplification was performed on the Roche LightCycler 480 using the Roche LightCycler 480 v.1.5 software. The results are summarized in the following Tables.

Table 10. Summary of LoD Confirmation Results with the FDA SARS-CoV-2 Reference Panel

Reference Materials Provided by FDA	Specimen Type	Product LoD	Cross-Reactivity
SARS-CoV-2	Nasopharyngeal Swabs in VTM	$1.8x10^5$ NDU/mL	N/A
MERS-CoV		N/A	ND

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

Table 11. Summary of LoD Confirmation Results with the FDA SARS-CoV-2 Reference Panel

Reference Materials Provided by FDA	Specimen Type	Product LoD	Cross-Reactivity
SARS-CoV-2	Mid-turbinate in DNA/RNA Shield	$1.8x10^5$ NDU/mL	N/A
MERS-CoV		N/A	ND

NDU/mL:RNA NAAT detectable units/mL N/A: Not Applicable

ND: Not Detected

6. Home Collection Sample Stability Study

To test the stability of dry anterior nasal swabs, 25 negative clinical swabs were collected with the swab provided with the home collection device (Copan Diagnostics Nylon Flocked Dry Swab), spiked with 25,000 copies of heat-inactivated virus (2x LoD), allowed to dry at ambient temperature, rehydrated per SOP and tested after 24, 48, 56, 72, and 96 hours. Five replicates were tested at each timepoint. Cp values were consistent for up to 56 hours. Data shown below in Table 12.

Table 12: Dry Swab Stability Data

	Sample	N1 Cp	N2 Cp	RP Cp	Result
24 Hrs	STBL-24H-1	34.50	34.61	28.26	Positive
	STBL-24H-2	34.02	35.07	29.22	Positive
	STBL-24H-3	34.59	34.86	29.54	Positive
	STBL-24H-4	34.98	35.88	30.99	Positive
	STBL-24H-5	34.98	34.48	30.82	Positive
	Mean±SD	**34.61 ± 0.40**	**34.98 ± 0.55**	**29.77 ± 1.14**	
48 Hrs	STBL-48H-1	35.31	35.92	30.45	Positive
	STBL-48H-2	35.18	35.51	30.03	Positive
	STBL-48H-3	33.99	35.84	31.89	Positive
	STBL-48H-4	35.22	36.24	29.96	Positive
	STBL-48H-5	34.76	36.61	31.57	Positive
	Mean±SD	**34.89 ± 0.55**	**36.02 ± 0.42**	**30.78 ± 0.89**	
56 Hrs	STBL-56H-1	35.28	36.08	31.60	Positive
	STBL-56H-2	34.72	35.70	31.46	Positive
	STBL-56H-3	34.72	34.65	31.25	Positive
	STBL-56H-4	35.6	35.25	31.86	Positive
	STBL-56H-5	35.27	35.97	31.88	Positive
	Mean±SD	**35.12 ± 0.39**	**35.53 ± 0.59**	**31.61 ± 0.27**	
72 Hrs	STBL-72H-1	35.86	39.40	33.94	Positive
	STBL-72H-2	N/D	37.09	33.85	Inconclusive
	STBL-72H-3	36.87	37.28	33.66	Positive
	STBL-72H-4	35.80	37.30	33.01	Positive
	STBL-72H-5	36.88	37.76	34.71	Positive
	Mean±SD	**36.35 ± 0.60**	**37.77 ± 0.95**	**33.83 ± 0.61**	
96 Hrs	STBL-96H-1	34.77	36.11	28.23	Positive
	STBL-96H-2	35.74	37.81	29.96	Positive
	STBL-96H-3	35.98	36.41	29.57	Positive
	STBL-96H-4	35.88	37.94	34.04	Positive
	STBL-96H-5	37.91	37.90	29.08	Positive
	Mean±SD	**36.06 ± 1.14**	**37.23 ± 0.90**	**30.18 ± 2.25**	

To confirm stability of dry anterior nasal swabs for 56 hours, 40 negative clinical swabs were collected. 20 were spiked with 25,000 copies (2x LoD) to simulate low positive samples, 10 were spiked with 62,500 copies (10x LoD) to simulate higher positive samples,

and 10 were tested as negatives to test for false positives. Spiked samples were allowed to dry at ambient temperature for 56 hours before rehydration in DNA/RNA Shield and testing.

Table 13 – Confirmation of SARS-CoV-2 RNA stability on spiked dry anterior nasal swabs from negative individuals held for 56 hours at ambient temperature prior to rehydration

Concentration	Replicates		N1	N2	RP	Detection Rate (% Agreement with Expected Result)
10xLoD	10	**Mean Ct**	34.33	35.59	31.27	10/10 (100%)
		Median Ct	34.69	36.40	31.75	
		SD	1.84	2.49	1.83	
2xLoD	20	**Mean Ct**	35.51	36.54	29.71	20/20 (100%)
		Median Ct	35.90	36.70	29.96	
		SD	1.43	1.80	1.77	
Negative	10	**Mean Ct**	N/A		30.27	0/10 (100%)
		Median Ct			30.38	
		SD			1.12	

7. Rehydration Protocol for Sample Processing

Specimens collected using the GWU COVID-19 Test Home Collection Kit and deemed acceptable will be rehydrated prior to processing in 1 mL of DNA/RNA Shield (Zymo Research, R1100), vortexed, allowed to incubate for at least five (5) minutes at room temperature, and vortexed again.

To validate this protocol, 15 negative clinical swabs and 15 negative clinical swabs spiked with 25,000 copies (2X LoD) of heat-inactivated virus were allowed to dry for 24 hours at ambient temperature prior to testing. All swabs were positive for RNase P well in-range of acceptance criteria. All negative swabs tested negative for viral targets, and all contrived positive swabs tested positive for both viral targets. Data shown in Table 8 below.

Table 14: Dry Swab Rehydration Validation

Concentration	Replicates		N1	N2	RP	Detection Rate (% Agreement with Expected Result)
2xLoD	15	**Mean Ct**	34.32	34.93	30.93	15/15 (100%)
		Median Ct	34.34	35.05	30.80	
		SD	0.33	0.52	1.42	
Negative	15	**Mean Ct**	N/A		30.62	0/15 (100%)
		Median Ct			30.56	
		SD			1.31	

8. Human Usability Study:

A human usability study was conducted to assess the user-friendliness of the home test kit. 35 participants were recruited from GWU's in-person test site and observed onsite. Following their sample collection, participants were emailed a questionnaire based on a template provided by the FDA and tailored to the kit. Samples were assessed for acceptability upon arrival in the lab and processed for testing with the GWU COVID-19 PCR Test.

a. Demographics:

The Following demographic information on the study participants is representative of the student and staff body of GWU and consistent with the intended user population.

Characteristic	N	Percent of Population
Total population (number)	35	
Age (yr) 4-14	0	0%
15-17	0	0%
>18	35	100%
Gender		
Female	15	42.8%
Male	20	57.1%
Education level:		
High school	5	14.3%
Some college	15	42.8%
College Degree	15	42.8%

b. Observer Assessment:

The observer did not note difficulties regarding the usability of the GWU Covid-19 RT-PCR Test Home Collection Kit and Collection Instructions.

c. RNase P Results:

Table 15: RNaseP Detection Results

# Samples RNAse P detected	Mean Ct Median Ct	Range of Ct	Detection Rate (%)
35	31.2 31.0	29.4 – 35.9	35 /35 **(**100 **%)**

d. Outcome Post Participation Questionnaire

Most if not all participants found that (1) it was not difficult to register the kit, (2) the instructions were clear regarding sample collection and packaging, (3) knew how to adequately label the tube, and (4) felt that they understood how to collect an adequate sample (e.g., how to unpack the swab, how deep the swab should be inserted, how often to rotate the swab in each nostril, how to insert the swab in the tube and break the shaft). As

such the participant Questionnaire did not raise concerns regarding the usability of the GWU Covid-19 RT-PCR Test Home Collection Kit.

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.
- This product has not been FDA cleared or approved but has been authorized for emergency use by FDA under an EUA for use by the authorized laboratory;
- This product has been authorized only for the detection of nucleic acid from SARS-CoV-2, not for any other viruses or pathogens.
- 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 diagnostics 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 declaration is terminated or authorization is revoked sooner.
- Laboratories within the United States and its territories are required to report all 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 use of this assay as an in vitro diagnostic under FDA Emergency Use Authorization (EUA) is limited to a single laboratory that is certified under the Clinical Laboratory Improvement Amendments of 1988 (CLIA), 42 U.S.C. § 263a, and meets requirements perform high complexity tests.
- 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.
- The performance of this test was established based on the evaluation of a limited number of clinical specimens. Clinical performance has not been established with 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.