EMERGENCY USE AUTHORIZATION (EUA) SUMMARY
BU-SARS-COV-2 TEST
(The HFI Laboratory at Boston University)

For *in vitro* Diagnostic Use
Rx Only
For Use Under Emergency Use Authorization (EUA) Only

The BU-SARS-CoV-2 Test will be performed at the HFI Laboratory at Boston University (dba the BU Clinical Testing Laboratory), located at 610 Commonwealth Avenue, Boston, MA 02215, 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 described in the Laboratory Standard Operating Procedures that were reviewed by the FDA under this EUA.

INTENDED USE

The BU SARS-CoV-2 Test is a High Throughput Real-Time Quantitative Reverse Transcription PCR (qRT-PCR test) intended for the qualitative detection of nucleic acid from SARS-CoV-2 in anterior nares swab specimens from individuals suspected of COVID-19 by their healthcare provider.

Testing is limited to the HFI Laboratory at Boston University (dba the BU Clinical Testing Laboratory), located at 610 Commonwealth Avenue, Boston, MA 02215, 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.

Results are for the identification of SARS-CoV-2 RNA. The SARS-CoV-2 RNA is generally detectable in anterior nares swab 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 definitive 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 BU SARS-CoV-2 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 BU SARS-CoV-2 Test is only for use under the Food and Drug Administration's Emergency Use Authorization.

DEVICE DESCRIPTION AND TEST PRINCIPLE

Device Description

The BU SARS-CoV-2 Test is a real-time reverse transcription polymerase chain reaction (rRT - PCR) test. The SARS-CoV-2 primer and probe set(s) is designed to detect RNA from the SARS-CoV-2 in respiratory specimens from patients as recommended for testing by public health authority guidelines.

The BU SARS-CoV-2 Test uses primer and probe sets (IDT Custom) targeting 2 SARS-CoV-2 specific genetic sequences and one human cellular material control (RNase P (RP)) per sample. Overall performance of the assay requires incorporation of specific external run controls, including a positive template control (2019-nCov, nucleocapsid gene), a negative extraction control (NEC) and a no template control (NTC). External run controls must be valid to report results. Nucleic acid from respiratory samples is extracted using a MagMAX™ Viral/Pathogen II (MVP II) Nucleic Acid Isolation Kit on a Hamilton Microlab STAR instrument followed by qPCR setup on a Hamilton Microlab STAR instrument. Amplification and detection of the targets performed on the Quant Studio 7 Flex Real-Time PCR system (Applied Biosystems).

Description of Test Steps:

1. Specimen Collection

Anterior nares (AN) swabs will be self-collected under observation from both nostrils with an ORACollect RNA (OR-100, DNAGenotek, Inc.) kit or with a Puritan 6" Sterile Standard Foam Swab w/polystyrene handle and placed into a Puritan U-100 BU sample collection tube with 1ml sterile saline solution for transport to the laboratory.

2. Specimen Testing

Upon receipt at the lab, samples are inactivated by heat treatment. Nucleic acid from respiratory samples are extracted using a MagMAX™ Viral/Pathogen II (MVP II) Nucleic Acid Isolation Kit on a Hamilton Microlab STAR instrument followed by qPCR setup on a Hamilton Microlab STAR instrument. Prior to extraction and detection steps, samples are transferred from the initial sample collection vials using a Hamilton Microlab STAR instrument. Inactivated samples are transferred from sample collection vials into deep-well plates for processing.

The QuantStudio 7 Flex (QS7) Real-Time PCR System is used for amplification and real time detection of purified nucleic acid. During the run, the instrument collects data at each cycle of the PCR. The resulting data provides a chronological series of measurements of the fluorescence resulting from the reactions.

INSTRUMENTS USED WITH THE TEST

Table 1. Instruments and Software for Use with the BU SARS-CoV-2 Test

Instrument	Manufacturer
Microlab STAR liquid handling systems	Hamilton
QuantStudio 7 Flex (QS7) Real-Time PCR System	Applied Biosystems (Thermo Fisher)

REAGENTS AND MATERIALS

Table 2. Reagents and Materials Used for Sample Preparation and to Perform the BU SARS-CoV-2 Test

Reagent/Material	Manufacturer/ Supplier	Catalogue/Part Number
Boston University 1500 Rxn Assay (COVID 19) - 1500 Reactions	Integrated DNA Technologies	Custom
Target specific positive controls	Integrated DNA Technologies	Various
MagMAX™ Viral/Pathogen II Nucleic Acid Isolation Kit	LifeTech	A48383
MagMAX™ Viral/Pathogen Proteinase K	LifeTech	A42363
NUCLEASE FREE WATER, 1000 Ml	LifeTech	AM9932
1 X 10ML TAQPATH 1STEP Master Mix, NO ROX	LifeTech	A28523
Puritan U-100 BU sample collection tube with 1ml sterile saline solution	Puritan	Custom
Puritan 6” Sterile Standard Foam Swab w/ polystyrene handle	Puritan	Custom
ORACollect- RNA sample collection kit	DNAGenotek	OR-100
MicroAmp™ EnduraPlate™ Optical 96-Well Clear Reaction Plates with Barcode	LifeTech	4483352
MicroAmp™ EnduraPlate™ Optical 384-Well Clear Reaction Plates with Barcode	LifeTech	4483273
Masterblock 96 DeepWell Sterile 1 Barcode	Greiner Bioone	Custom
MICROAMP OPTICAL ADHESIVE FILM, 100 PC	LifeTech	4311971

CONTROLS

Controls are included on each 96 well extraction plate. The three controls are transferred to the qPCR plate during the PCR set up steps. Each 384 well qPCR plate will contain one (1) Positive Addition Control, four (4) Negative Extraction Controls and four (4) No Template Controls. The one (1) human cellular material control, RNase P (RP), should be present in all valid samples. RP acts as both an extraction control and an internal control. Every testing batch will include all controls

Table 3. Assay Controls Used with the BU SARS-CoV-2 Test

Control Type	Description	Expected Results	Purpose
Positive Addition Control (CoV-PAC)	In vitro transcribed RNA, Plasmid-based DNA or Previous Positive patient	All SARS-CoV-2 targets: Detected	To make sure that extraction and amplification has occurred.

Control Type	Description	Expected Results	Purpose
Negative Extraction Control (NEC)	Previously characterized pooled negative patient	All SARS-CoV-2 targets Not Detected, RP: Detected	To make sure extraction has occurred and contamination has not occurred.
No Template Control (NTC)	Molecular Grade Water	All targets + RP: Not Detected	To make sure that nonspecific amplification or contamination have not occurred.

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.

Table 4. Interpretations of patient sample results

N1	N2	RP	Interpretation	Report
+	+	+/-	Positive for SARS-CoV-2019	Positive
If only 1 of 2 N gene targets are positive.	If only 1 of 2 N gene targets are positive.	+/-	Positive for SARS-CoV-2019	Positive
-	-	+	Negative for SARS-CoV-2019	Negative
-	-	-	Invalid	Invalid - Recollect

PERFORMANCE EVALUATION

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

A preliminary LoD study of the BU SARS-CoV-2 Test was completed using quantified SARS-CoV-2 (USA-WA1/2020) inactivated virus obtained from ZeptoMatrix (NATSARS(COV2)-ST). The preliminary LoD was determined using serial dilutions in triplicate samples from the virus stock diluted in pooled negative anterior nasal (AN) swab specimen matrix to a starting concentration of 1,000 copies/ml (cp/ml). This was used to produce 2-fold serial dilutions to determine the preliminary LoD range. Based on this preliminary data the LoD would be 250 cp/ml.

Table 5. Preliminary LoD results

Concentration (cp/mL)	N1 Detected	N2 Detected
1000	3/3	3/3
500	3/3	3/3
250	3/3	3/3
125	3/3	0/3
62.5	1/3	2/3
31.3	2/3	0/3
15.6	0/3	0/3

An LoD confirmation study of the BU SARS-CoV-2 Test was completed by running 20 replicate samples at 1000 cp/ml, 500 cp/ml, and 250 cp/ml for the BU SARS-CoV-2 Test. This confirmation testing required >95% (19/20) of the samples test positive for the concentration designated as the final LoD. The table below provides the data for the 20 samples resulting in a final LoD for the BU CTL of 500 cp/ml.

Table 6. LoD Confirmation Results

Concentration (cp/mL)	N1 Detected	N2 Detected
1000	20/20	20/20
500	19/20	19/20
250	17/20	20/20

2) Inclusivity (Analytical Reactivity):

The BU SARS-CoV-2 Test uses primer and probe sequences for the detection of the spike protein and nucleocapsid genes that were authorized for use in the CDC nCoV Real-TIME RT PCR Diagnostic Panel (EUA200001). The CDC has provided a blanket Right of Reference to the performance data contained in the EUA request for the CDC nCoV Real-TIME RT PCR Diagnostic Panel, including the *in silico* analysis of SARS-CoV-2 inclusivity.

The BU IRB determines which variants are predominant or emerging on campus by deep sequencing of residual positive samples that are biobanked in the BU CTL. This includes identifying all variants that have been deemed "variants of interest" and "variants of concern," or VOC by the CDC (Alpha, Beta, Gamma, Delta, Lambda, Mu, and Omicron). BU reports these VOC to the city and state as they are identified under their expanded authority during this public health emergency.

Sequencing of the positive samples identified in the BU CTL in began in January of 2021 and will continue to ensure that BU identifies prevalent and/or clinically significant variants in the population to validate the performance of our assay over time. BU will also continue monitoring of assay robustness in the event of the emergence of any new VOC.

3) Cross-Reactivity (Analytical Specificity), Microbial Interference:

The N1 and N2 primers, purchased from Integrated DNA Technologies and utilized in the BU SARS-CoV-2 Test are identical to those of the CDC nCoV Real-TIME RT PCR Diagnostic Panel. The CDC performed both an *in silico* analysis and wet-testing to evaluate cross-reactivity. The CDC has granted right of reference to using the performance data submitted to the FDA as part of EUA200001.

4) Interfering Substances

Potentially interfering substances for upper respiratory samples were selected for testing based on the FDA Template for Developers of Molecular Diagnostics Recommendations. The recommended substances shown below were tested at the suggested concentrations in 3x LoD (1,500 cp/ml) samples (spiked quantitated inactivated virus in pooled negative AN specimens) and without inactivated virus (pooled negative AN specimens) in triplicate. Interference was not detected with any of the potential interfering substances tested.

Table 7. Interfering Substances Results

Substance	Concentration	Inactivated Virus	N1 Detected	N2 Detected	Result Match
Afrin Original Nasal Spray	15% v/v	Yes	3/3	3/3	100%
Afrin Original Nasal Spray	15% v/v	No	0/3	0/3	100%
Cepacol Lozenges	3 mg/ml	Yes	3/3	3/3	100%
Cepacol Lozenges	3 mg/ml	No	0/3	0/3	100%
Chloraseptic Sore Throat Spray	5% V/V	Yes	2/3	3/3	100%
Chloraseptic Sore Throat Spray	5% V/V	No	0/3	0/3	100%
Cough Syrup	5%	Yes	3/3	3/3	100%
Cough Syrup	5%	No	0/3	0/3	100%
Mucin, bovine submaxillary gland, type I-S	2.5 mg/ml	Yes	3/3	3/3	100%
Mucin, bovine submaxillary gland, type I-S	2.5 mg/ml	No	0/3	0/3	100%
Nicotine	0.03 mg/ml	Yes	3/3	3/3	100%
Nicotine	0.03 mg/ml	No	0/3	0/3	100%

5) Specimen Stability:

Sample storage for specimens in the BU SARS-CoV-2 Test follow the CDC guidance for respiratory samples.

6) Clinical Evaluation:

Clinical performance of the BU SARS-CoV-2 Test evaluated concordance of specimen results with those of an EUA authorized molecular comparator test. Thirty-six (36) positive (prospective and retrospective) and 171 negative (prospective) anterior nasal (AN) swab specimens collected from symptomatic individuals by the BU Clinical Testing Laboratory (BU CTL) were evaluated by the candidate and comparator test. All samples were run under the standard BU CTL protocol as outlined in SOP SARS-CoV-2 PCR and followed the standard manufacturer protocols for the Cepheid assays.

All negative samples were negative for both the candidate and comparator test. One discordant result between the candidate and comparator test occurred. The PPA was 97% and the NPA was 100%.

Table 8. Clinical Evaluation Results

		EUA Authorized Comparator Test	
		Positive	**Negative**
BU SARS-CoV-2 Test	**Positive**	35	0
	Negative	1	171
Positive Agreement		97% (35/36)	
Negative Agreement		100% (171/171)	

Limitations

- 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.
- Detection of RNase P indicates that human nucleic acid is present and implies that human biological material was collected and successfully extracted and amplified. It does not necessarily indicate that the specimen is of appropriate quality to enable detection of SARS-CoV-2.

WARNINGS

- For in vitro diagnostic use
- Rx Only
- For use under Emergency Use Authorization (EUA) only
- 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; 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 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.