ACCELERATED EMERGENCY USE AUTHORIZATION (EUA) SUMMARY BMC-CREM COVID-19 TEST (TWO-STEP SINGLEPLEX AND ONE-STEP SINGLEPLEX TESTS) (BOSTON MEDICAL CENTER)

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

(The BMC-CReM COVID-19 Test will be performed at the Boston Medical Center/ Department of Pathology and Laboratory Medicine located at 1 Boston Medical Center Place, Boston, MA 02118, certified under the Clinical Laboratory Improvement Amendments of 1988(CLIA), 42 U.S.C. §263a, as per the Standard Operating Procedure that was reviewed by the FDA under this EUA.)

INTENDED USE

The BMC-CReM COVID-19 Test is a real-time, reverse transcription polymerase chain reaction (RT-PCR) test intended for the qualitative detection of nucleic acid from SARS-CoV-2 in nasopharyngeal, oropharyngeal, anterior nasal and mid-turbinate nasal swabs, nasopharyngeal washes/aspirates or nasal aspirates, and bronchoalveolar lavage specimens from individuals suspected of COVID-19 by their healthcare provider.

Testing is limited to the Boston Medical Center/ Department of Pathology and Laboratory Medicine located at 1 Boston Medical Center Place, Boston, MA 02118 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 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.

Testing with the BMC-CReM COVID-19 Test 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 BMC-CReM COVID-19 Test is intended for use only under the Food and Drug Administration’s Emergency Use Authorization.

Boston Medical Center/Department of Pathology and Laboratory Medicine has validated two versions of the BMC-CReM COVID-19 Test including a two-step singleplex test and a one-step singleplex test.

DEVICE DESCRIPTION AND TEST PRINCIPLE FOR BOTH THE TWO-STEP AND ONE-STEP SINGLEPLEX TESTS

The BMC-CReM COVID-19 Test is a real-time, reverse transcription polymerase chain reaction (RT-PCR) test. Both the two-step and one-step singleplex tests use two primer and probe sets to detect two regions in the SARS-CoV-2 nucleocapsid (N) gene (N1 and N2), and one primer and probe set to detect human RNase P (RP) in a clinical sample (Integrated DNA Technologies, Cat # 10006606). The primer and probe sets are identical to the N1, N2, and RNase P oligonucleotides used in the CDC 2019-Novel Coronavirus (2019-CoV) Real-Time RT-PCR Diagnostic Panel (EUA200001).

RNA is isolated from respiratory specimens including nasopharyngeal, oropharyngeal, anterior nasal and mid-turbinate nasal swabs, nasopharyngeal washes/aspirates or nasal aspirates, and bronchoalveolar lavage using the Qiagen RNeasy Mini Kit (Cat # 74106) For the two-step singleplex test, extracted RNA is reverse transcribed to cDNA via an independent, reverse transcription (RT) reaction using the High Capacity cDNA Reverse Transcription Kit at 37°C for 2 hours on the Mastercycler Nexus X2. cDNA is subsequently amplified using the TaqMan Fast Advanced Master Mix with N1, N2, or RNase P primer/probe sets from IDT. Separate master mixes are prepared for each assay target. For the one-step singleplex test, extracted RNA is transferred into a multi-well format and subsequently converted into cDNA in an RT reaction included within the qRT-PCR protocol ran on the QuantStudio 6 Flex Real-Time PCR System using software version 1.3.

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) to separate from the quencher dye (BHQ-1), generating a fluorescent signal. Fluorescence intensity is monitored at each PCR cycle. Two technical replicates per probe are run with each patient sample (6 total replicates for 1 sample).

INSTRUMENTS USED WITH THE TWO-STEP AND ONE-STEP SINGLEPLEX TESTS

The two-step singleplex BMC-CReM COVID-19 Test is to be used with the Eppendorf Mastercycler Nexus X2 with software version 3.5.1 for cDNA synthesis as well as the Applied Biosystems QuantStudio 6 Flex Real-Time PCR System with software version 1.3 for PCR amplification. For the one-step singleplex test, both cDNA synthesis and PCR amplification are completed on the Applied Biosystems QuantStudio 6 Flex Real-Time PCR System with software version 1.3.

REAGENTS AND MATERIALS USED FOR THE TWO-STEP SINGLEPLEX TEST

Reagent Manufacturer and Description	Catalog #	Manufacturer
Qiagen RNeasy Mini Kit (250)	74106	Qiagen
TaqMan Fast Advanced Master Mix	4444557	ThermoFisher Scientific
High Capacity cDNA Reverse Transcription Kit (1000 rxn)	4368814	ThermoFisher Scientific
0.2 ml PCR 8-tube strip with individual attached dome caps	USA Scientific	1402-2900
COVID-19_N1-F Primer (forward primer)	10006606	Integrated DNA Technologies
COVID-19_N1-R Primer (reverse primer)	10006606	Integrated DNA Technologies
COVID-19_N1-P Probe (N1 probe)	10006606	Integrated DNA Technologies
COVID-19_N2-F Primer (forward primer)	10006606	Integrated DNA Technologies
COVID-19_N2-R Primer (reverse primer)	10006606	Integrated DNA Technologies
COVID-19_N2-P Probe (N2 probe)	10006606	Integrated DNA Technologies
RP-F Primer (forward primer)	10006606	Integrated DNA Technologies
RP-R Primer (reverse primer)	10006606	Integrated DNA Technologies
RP-P Probe (RNase P probe)	10006606	Integrated DNA Technologies
Synthetic SARS-CoV-2 RNA	VR-3276SD	ATCC

REAGENTS AND MATERIALS USED FOR THE ONE-STEP SINGLEPLEX TEST

Reagent Manufacturer and Description	Catalog #	Manufacturer
Qiagen RNeasy Mini Kit (250)	74106	Qiagen
TaqPath qPCR Master Mix, CG	A15297	ThermoFisher Scientific
MicroAmp Optical 384-Well Reaction Plate with Barcode	ThermoFisher	43-098-49
MicroAmp Optical Adhesive Film	ThermoFisher	4311971
COVID-19_N1-F Primer (forward primer)	10006606	Integrated DNA Technologies
COVID-19_N1-R Primer (reverse primer)	10006606	Integrated DNA Technologies
COVID-19_N1-P Probe (N1 probe)	10006606	Integrated DNA Technologies
COVID-19_N2-F Primer (forward primer)	10006606	Integrated DNA Technologies
COVID-19_N2-R Primer (reverse primer)	10006606	Integrated DNA Technologies
COVID-19_N2-P Probe (N2 probe)	10006606	Integrated DNA Technologies
RP-F Primer (forward primer)	10006606	Integrated DNA Technologies
RP-R Primer (reverse primer)	10006606	Integrated DNA Technologies
RP-P Probe (RNase P probe)	10006606	Integrated DNA Technologies
Synthetic SARS-CoV-2 RNA	VR-3276SD	ATCC

CONTROLS TO BE USED WITH BOTH THE TWO-STEP AND ONE-STEP SINGLEPLEX TESTS

1) A no template control (NTC) is needed to check for contamination of assay reagents. Molecular grade, nuclease-free water is used in place of sample nucleic acid for this control. The NTC is used on every assay plate.

2) A positive SARS-CoV-2 control is needed to verify proper assay set-up and SARS-CoV-2 reagent integrity. The positive control is commercially supplied

from ATCC (Cat # VR-3276SD) and is made of synthetically engineered RNA containing fragments of ORF1ab, N (encompassing N1 and N2 probe sequences), and E genes. Prior to utilizing this control in the two-step singleplex assay, positive control RNA is first made into cDNA via reverse transcription. For each run of patient samples tested, cDNA generated from the positive control RNA is added to each plate at 1X, 5X and 10X the LoD (i.e., 10, 50, and 100 genome copies (gc)/µL. In the one-step singleplex assay, for each run of patient samples tested, positive control RNA is directly added to each plate at 1X, 5X and 10X the LoD (i.e., 10, 50, and 100 genome copies (gc)/µL.)

3) An internal control targeting RNase P is needed to verify that nucleic acid is present in every sample and is used for every sample processed. This also serves as a positive extraction control to ensure that samples resulting as negative contain nucleic acid for testing. Detection of the RP gene in patient test samples verifies successful extraction of the sample, proper assay setup, sample integrity, and collection of human biological material.

4) A negative extraction (NEC) control is a previously characterized negative patient sample. The NEC assesses for cross-contamination that could occur during the RNA extraction process. A NEC is used in each extraction batch.

INTERPRETATION OF RESULTS FOR BOTH THE TWO-STEP AND ONE-STEP SINGLEPLEX TESTS

All test controls must be examined prior to interpretation of patient results. If the controls are not valid, the patient results cannot be interpreted (Refer to Table 1 for a summary of control results). Interpretation of test controls is the same for the two-step and one-step singleplex tests.

1) <u>BMC-CReM COVID-19 Test Controls – Positive, Negative, Extraction, and Internal</u>:

- **No Template Control (NTC)**
  The no template control must be negative (Ct Not Detected) for all targets. If NTC wells have a Ct value < 40, this implies contamination of the RT-PCR reaction or that the assay was setup improperly and therefore, the run is not used for diagnostic decisions. The RT-PCR run is invalid. All samples must be re-extracted and re-tested with fresh controls.

- **SARS-CoV-2 Positive Control cDNA**
  The positive control cDNA must have detectable Ct values (< 40 Ct) for at least one of the positive control concentrations for the plate to be valid. If the positive control cDNA fails to yield N1 and N2 Ct values (i.e., undetected) for all included concentrations, the plate/run is not used for diagnostic decisions. In this case, the RT-PCR reaction must be repeated for all samples using residual extraction material. If the repeat test result for N1 and N2 is negative for all concentrations

of this control, all samples should be re-extracted and re-tested using fresh controls, including all concentrations of the positive control.

- **Human RNase P (RP) Gene Internal Control**
  All clinical samples should exhibit fluorescence growth curves in the RNase P reaction that can be detected (Ct < 40), indicating the presence of the human RNase P gene. There exists the possibility that some samples may fail to exhibit RNase P growth curves due to low cell numbers in the original clinical sample. A negative RP signal does not preclude the presence of SARS-CoV-2 virus RNA in a clinical specimen. Samples that have a clear positive result for N1 and/or N2, can be interpreted as positive irrespective of their RP value. If a sample shows no amplification for N1 and N2 and also fails to show detectable levels for RP, the sample is deemed invalid and needs to be re-extracted and re-tested.

- **Negative Extraction Control (NEC)**
  The negative extraction control (negative clinical sample) must be negative for N1 and N2 targets (Ct Not Detected), and positive for the RP target (Ct < 40). If positive results are obtained for N1 or N2 targets, contamination of nucleic acid extraction reagents or cross-contamination of samples may have occurred. The extraction run and the RT-PCR run are invalid and should be repeated using extracted RNA from residual patient samples and fresh controls.

Table 1. Expected Results of Controls for the BMC-CReM COVID-19 Test

Control Type	Control Name	Used to Monitor	Expected Results			Expected Ct Values
			N1	N2	RNase P (RP)	
Negative	NTC	contamination during qRT-PCR process	-	-	-	Undetected
Positive	SARS-CoV-2 RNA	amplification/primer-probe integrity	+	+	-	Ct < 40 (N1, N2,)[a] Undetected (RP)
Extraction	NEC	extraction cross-contamination	-	-	+	Undetected (N1, N2) Ct < 40 (RP)
	Human RP gene	extraction/amplification/sample stability	NA	NA	+	Ct < 40 (RP)

NA; Not Applicable
Undetected (Ct > 40)
[a] At least 1 positive control concentration (i.e., 10, 50, and 100 genome copies (gc)/µL [1X, 5X, 10X LoD]) needs to have a Ct < 40.

2) Examination and Interpretation of Patient Specimen Results:

Assessment of clinical specimen test results should be performed after the positive, NTC, and negative extraction controls have been examined and determined to be valid and acceptable. If the controls are not valid, the patient results cannot be interpreted. The table below (Table 2) provides guidance on interpretation and

reporting of patient results. Interpretation of patient results is the same for the two-step and one-step singleplex tests.

Table 2. Interpretation of Patient Results for the BMC-CreM COVID-19 Test

N1 (Ct < 40)	N2 (Ct < 40)	RNase P (Ct < 40)	Interpretation	Report Result	Actions
+	+	+/-	SARS-CoV-2 **Detected**	**POSITIVE**	Reported to the electronic medical record (EMR) and sender/appropriate public health authorities.
If one or both of these targets are positive (2/2 technical replicates)		+/-	SARS-CoV-2 **Detected**	**POSITIVE**	Reported to the electronic medical record (EMR) and sender/appropriate public health authorities.
If signal is detected, but no target reaches 2/2 detected technical repeats		+	**Inconclusive**	**INCONCLUSIVE**	Sample is repeated at qRT-PCR step once more using 2 technical replicates. If sample is still inconclusive, the result is reported into the EMR as such and it is recommended that a new sample is obtained from the patient
-	-	+	SARS-CoV-2 **Not Detected**	**NEGATIVE**	Reported to the electronic medical record (EMR).
-	-	-	**Invalid test**	**INVALID**	The result is reported into the EMR as such and it is recommended that a new sample is obtained from the patient.

PERFORMANCE EVALUATION OF THE BMC-CREM COVID-19 TEST

1) Analytical Sensitivity:

Limit of Detection (LoD) for the Two-Step Singleplex Test:

The LoD of the two-step singleplex BMC-CReM COV-19 Test was determined using ATCC synthetic SARS-CoV-2 RNA (VR-3276SD) spiked into phosphate buffered saline (PBS). A preliminary LoD was determined by testing serial dilutions (10,000,000 gene copies/µL - 10 gene copies/µL) of RNA in PBS in triplicate. Spiked samples were tested with the two-step BMC-CreM COVID-19 Test following extraction with the Qiagen RNeasy Mini Kit. The initial LoD was 10 gene copies/µL.

The LoD was verified by testing 20 additional extraction replicates consisting of PBS spiked at both 10 gene copies/µL (10^1) and 3.16 gene copies/µL ($10^{0.5}$) (See Table 3). Samples were spiked with RNA prior to extraction with the Qiagen RNeasy Mini Kit. The results of the LoD confirmatory study are summarized below.

Table 3. LoD Verification Study Results for the Two-Step Singleplex Test

Concentration (gene copies/µL)	Average Ct Values		N1, N2 Detection Rate	
	N1	N2	N1	N2
10^{1}; 10	32.92	33.85	20/20	20/20
$10^{0.5}$; 3.16	34.46	35.11	18/20	19/20

Limit of Detection (LoD) for the One-Step Singleplex Test:

The LoD of the one-step BMC-CReM COV-19 Test was determined using ATCC synthetic SARS-CoV-2 RNA (VR-3276SD) spiked into phosphate buffered saline (PBS). A preliminary LoD was determined by testing serial dilutions (10,000 gene copies/µL - 0.32 gene copies/µL) of RNA in PBS in triplicate. Spiked samples were tested with the BMC-CreM COVID-19 Test following extraction with the Qiagen RNeasy Mini Kit. The initial LoD was 1 gene copy/µL.

The LoD was verified by testing 20 additional extraction replicates consisting of PBS spiked at 1 (10^{1}), 0.32 ($10^{-0.5}$), and 0.1 (10^{-1}) gene copies/µL (See Table 4). Samples were spiked with RNA prior to extraction with the Qiagen RNeasy Mini Kit. The LoD of the one-step singleplex BMC-CReM COVID-19 Test was determined to be 1 genome copy/µL.

Table 4. LoD Verification Study Results for the One-Step Singleplex Test

Concentration (gene copies/µL)	Average Ct Values		N1, N2 Detection Rate	
	N1	N2	N1	N2
10^{0}; 1	35.00	35.33	20/20	20/20
$10^{-0.5}$; 0.32	36.60	36.81	11/20	9/20
10^{-1}; 0.10	36.30	36.47	4/20	6/20

2) Analytical Inclusivity/Specificity for BOTH the Two-Step and One-Step Singleplex Tests:

The BMC-CreM COVID-19 Test utilizes identical oligonucleotide sequences for the N1 and N2 target genes as those used in the CDC 2019-Novel Coronavirus (2019-CoV) Real-Time RT-PCR Diagnostic Panel. The inclusivity and cross-reactivity of the CDC EUA assay has been previously evaluated. The CDC has granted a right of reference to the performance data contained in the CDC's EUA request (EUA200001) to any entity seeking an FDA EUA for a COVID-19 diagnostic device. No additional cross-reactivity testing was completed. However, because the inclusivity data was performed by the CDC in February 2020 and additional SARS-CoV-2 sequences have since been deposited in publicly available databases, an updated in silico analysis to assess the inclusivity of the assay was performed.

The sequences of the N1 and N2 primers were blasted (via blastn) against the Betacoronavirus database, consisting of the viral genomes for the entire family of viruses (including, but not limited to, SARS-CoV-2). Each probe was independently queried against the genomic/viruses/Betacoronavirus database consisting of 12773 sequences of mixed DNA last updated on 06/01/2020.

When aligning the N1 probe against the reference SARS-CoV-2 viral genome (NC_045512.2 Severe acute respiratory syndrome coronavirus 2 isolate Wuhan-Hu-1, complete genome), 100% sequence alignment was found. Blasting the N1 probe against the Betacoronavirus database identified 5183/12,773 sequences with alignment using blastn. 4702/5183 hits demonstrated alignment to SARS-CoV-2 (taxid 2697049). The score for these sequences was 48.1. For a query 24 nucleotides long (e.g. the length of N1 probe), a 100% (i.e. 24/24 identical nucleotides) alignment against a SARS-CoV-2 viral target corresponds to a score of 48.1. Scores below the 100% alignment score signify incomplete matches or inferior sequence alignment. Therefore, the N1 probe sequence has 100% alignment for the 4702 SARS-CoV-2 sequences. The N1 probe additionally showed 1 hit with a score of 48.1 with bat coronavirus RaTG13 (taxid 2709072) as expected.

When aligning the N2 probe against the reference SARS-CoV-2 viral genome (NC_045512.2 Severe acute respiratory syndrome coronavirus 2 isolate Wuhan-Hu-1, complete genome), 100% sequence alignment was found. Blasting the N2 probe against the Betacoronavirus database identified 4772/12,773 sequences with alignment using blastn. 4469/4772 hits demonstrated alignment to SARS-CoV-2 (taxid 2697049) with a score of 46.1. Since the N2 probe sequence is shorter compared to N1 (23 nucleotides), the 100% alignment score is denoted as 46.1. The N2 probe sequence shared 100% alignment with 4469 SARS-CoV-2 sequences (taxid 2697049). Similar to the N1 probe, the N2 probe also showed alignment and produced 1 hit out of 4772 sequences with a score of 30.2 to bat coronavirus RaTG13 (taxid 2709072).

3) Clinical Evaluation:

Clinical Validation of the Two-Step Singleplex Test:

Performance of the two-step singleplex BMC-CreM COVID-19 Test was evaluated by testing 60 clinical nasopharyngeal swab specimens (30 positive and 30 negative) that were previously confirmed to be positive/negative by the Massachusetts Department of Public Health (MDPH) using the CDC EUA authorized assay or by Beth Israel Deaconess Medical Center (BIDMC) using the Abbott RealTime SARS-CoV-2 EUA authorized assay (authorization March 18, 2020). Twenty out of 30 positives and 20/30 negatives were confirmed by the MDPH using the CDC authorized assay and 10/30 positives and 10/30 negatives were confirmed using the Abbott RealTime EUA assay.

Clinical samples were blinded and randomized for testing, extracted with the Qiagen RNeasy Mini Kit, and ran on the two-step assay. The positive and negative percent agreement between the two assays was 100% for both the N1 and N2 targets. For the positive samples, the average Ct values for N1, N2 and RNase P were 25.25, 25.59, and 31.98, respectively. The average Ct value for RNase P for the negative specimens was 33.17. Results of the study are summarized below (Table 5).

Table 5. Clinical Evaluation Summary Data for Nasopharyngeal Swab Specimens Using the Two-Step Singleplex Test

Two-Step Singleplex		Comparator – CDC EUA Assay OR Abbott RealTime SARS-CoV-2 EUA Assay		
		Positive	**Negative**	**Total**
BMC-CreM COVID-19 Test	**Positive**	30	0	30
	Negative	0	30	30
	Total	30	30	60
Positive Percent Agreement		30/30; 100% (88.65% - 100.00%)[1]		
Negative Percent Agreement		30/30; 100% (88.65% - 100.00%)[1]		

[1]Two-sided 95% score confidence intervals

Clinical Confirmation of the Two-Step Singleplex Test:

The first five positive and first five negative patient samples were sent to the Massachusetts Department of Public Health (MDPH) and tested with the CDC EUA assay (unmodified). All positive and negative results were 100% concordant.

Clinical Validation of the One-Step Singleplex Test:

Performance of the one-step singleplex BMC-CreM COVID-19 Test was evaluated by testing 60 clinical nasopharyngeal swab specimens (30 positive and 30 negative) that were previously confirmed to be positive/negative by Boston Medical Center using the Roche cobas SARS-CoV-2 assay (unmodified) that was authorized on March 12, 2020. Boston Medical Center internally validated the Roche assay per CLIA recommendations.

Clinical samples were blinded and randomized for testing, extracted using the Qiagen RNeasy Mini Kit, and ran on the one-step assay. The positive and negative percent agreement between the two assays was 100% for both the N1 and N2 targets. For the positive samples, the average Ct values for N1, N2 and RNase P were 29.90, 30.12, and 28.31, respectively. The average Ct value for RNase P for the negative specimens was 28.79. Results of the study are summarized below (Table 6).

Table 6. Clinical Evaluation Summary Data for Nasopharyngeal Swab Specimens Using the One-Step Singleplex Test

One-Step Singleplex		Comparator – Roche cobas SARS-CoV-2 Assay (unmodified)		
		Positive	**Negative**	**Total**
BMC-CreM COVID-19 Test	**Positive**	**30**	**0**	**30**
	Negative	**0**	**30**	**30**
	Total	**30**	**30**	**60**
Positive Percent Agreement		30/30; 100% (88.65% - 100.00%)[1]		
Negative Percent Agreement		30/30; 100% (88.65% - 100.00%)[1]		

[1]Two-sided 95% score confidence intervals

Clinical Confirmation of the One-Step Singleplex Test:
The first five positive and first five negative patient samples were tested with the Boston Medical Center's Roche cobas SARS-CoV-2 authorized assay (unmodified) that was validated per CLIA. All positive and negative results were 100% concordant.

WARNINGS:

- This test has not been FDA cleared or approved;
- This test has been authorized by FDA under an EUA for use by the authorized laboratory;
- 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 diagnostics 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.