EMERGENCY USE AUTHORIZATION (EUA) SUMMARY
BOSTON HEART COVID-19 RT-PCR TEST
(BOSTON HEART DIAGNOSTICS)

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

(The Boston Heart COVID-19 RT-PCR Test will be performed at Boston Heart Diagnostics laboratory located at 200 Crossing Blvd., Framingham, MA 01702, 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 per the laboratory procedures that were reviewed by the FDA under this EUA.)

INTENDED USE

The Boston Heart COVID-19 RT-PCR Test is a real-time RT-PCR assay intended for the qualitative detection of nucleic acid from SARS-CoV-2 in upper respiratory specimens (such as nasal, mid-turbinate, nasopharyngeal, and oropharyngeal swab specimens), nasopharyngeal aspirate and bronchoalveolar lavage specimens from individuals suspected of COVID-19 by their healthcare provider. Testing is limited to the Boston Heart Diagnostics laboratory, located at 200 Crossing Blvd., Framingham, MA 01702, which is certified under 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 upper respiratory specimens and bronchoalveolar lavage 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 infective 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 Boston Heart 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 Boston Heart COVID-19 RT-PCR Test is only for use under the Food and Drug Administration's Emergency Use Authorization.

DEVICE DESCRIPTION AND TEST PRINCIPLE

The Boston Heart COVID-19 RT-PCR Test is a multiplex real-time reverse transcription polymerase chain reaction (RT-PCR) test which includes three primer and probe sets designed to detect RNA from the SARS-CoV-2 in upper respiratory specimens (such as nasal, mid-turbinate, nasopharyngeal, and oropharyngeal swab specimens) and bronchoalveolar lavage specimens from individuals suspected of COVID-19 by their healthcare provider. In addition, Boston Heart COVID-19 RT-PCR Test contains MS2, an internal positive control which serves as an extraction, reverse transcription, and PCR amplification positive control for each well.

INSTRUMENTS USED WITH TEST

The Boston Heart COVID-19 RT-PCR Test is to be used with the following PCR and nucleic acid extraction instrument:

- QuantStudio 12K Flex Real-Time PCR System (Software version 1.4)
- ThermoFisher MagMax Viral/Pathogen Nucleic Acid Isolation Kit
- KingFisher Flex 96 Deep-Well Magnetic Particle Processor (Applied Biosystems #11314008)

REAGENTS AND MATERIALS

The Boston Heart COVID-19 RT-PCR Test has been validated using only the components referenced in this submission.

- ThermoFisher MagMax Viral/Pathogen Nucleic Acid Isolation Kit (#A48383, #A48310)
- TaqPath COVID-19 Combo Kit (#A47814) for RT-PCR.
- KingFisher Flex 96 Deep-Well Instrument (Applied Biosystems, #11314008)

CONTROLS TO BE USED WITH THE BOSTON HEART COVID-19 RT-PCR TEST

Controls that provided with the test kit are as follows:

Positive Amplification Control (PC): To monitor the RT-PCR reaction, Boston Heart COVID-19 RT-PCR uses the Positive Control included with the TaqPath COVID-19 Combo kit. The RNA control contains *in vitro* transcribed (IVT) RNA, which is specific to N, S and ORF1ab regions of SARS-CoV-2 and ensures that the RT-PCR reaction is correctly detecting the SARS-CoV-2 targets. The positive amplification control is performed at 25 c/µL and 200 c/µL; Ct results for each target and each concentration for the controls are tracked for each plate.

MS2 Phage Control: This control is a bacteriophage that acts as a full process control. It is added to each sample and control well at the beginning of the extraction and serves as an internal standard to monitor for extraction inconsistencies and amplification issues. The expected Ct for

the MS2 control is <31 (determined during validation). For samples with no Ct for the SARS-CoV-2 targets, an MS2 Ct <31 indicates proper nucleic acid extraction and RT-PCR.

Positive Extraction Control (PEC): This control is included on every extraction plate and is used to monitor the entire process. The control produces a specific Ct range to ensure reproducibility of the test across the whole process on different days and instruments. Pooled positive clinical samples are used as the material for the PEC and are tested before use to establish the expected Ct.

Negative Extraction Control (NEC): This control is included on each batch of extracted samples. The NEC is used to monitor for contamination in the extraction reagents or of the template nucleic acid. Phosphate buffered Saline (PBS) or TE buffer matrix is used in place of patient samples and goes through the entire extraction and RT-PCR process. MS2 Phage control is added to patient samples. The eluted sample is then added to the RT-PCR plate to simulate a patient sample. The only target that should be present in this sample is MS2.

No Template Control/Negative Amplification Control (NTC): Added to every RT-PCR plate, the NTC is used to monitor for RT-PCR reagent contamination. There are no components from the extraction included in these wells and instead of template include DNase/RNase free water along with the assay mix (primers/probes, master mix).

INTERPRETATION OF RESULTS

The controls are assessed after the run is complete prior to analyzing patient results. If any control group does not meet the expected criteria, it is investigated for a root cause, the run entire and/or extraction may have to be repeated and results cannot be interpreted or reported. The criteria for the controls used in the process are shown in the table below.

Table 1: Interpretation of Results for Quality Controls

Control	Valid Result[a]	Invalid result[b]
Internal control (MS2)	Ct <31	Ct ≥ 31
No template control	No amplification signal detected	Amplification detected for any target
Negative extraction control	No amplification signal detected for the SARS-CoV-2 targets, MS2 Ct <31	Amplification detected or MS2 Ct ≥31
Positive Amplification Control (25 c/µL)	Ct <32 for all targets	Ct ≥32 for all targets
Positive Amplification Control (200 c/µL)	Ct <29 for all targets	Ct ≥29 for all targets
Positive Extraction Control (PEC)	Ct ≤31[c] for all targets	C_t >31 for all targets

[a] The patient results can only be reviewed and interpreted if the valid controls results are generated.
[b] If control results are invalid, the plate may be rejected, and patient results may not be analyzed and reported.
[c] Expected Ct changes from lot to lot

Assessment of clinical specimen test results should 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.

Table 2: Result Interpretation for the Boston Heart COVID-19 RT-PCR Test

ORF1ab	N gene	S gene	MS2	Control Status	Result	Action
NEG (ND or Ct ≥37)	NEG (ND or Ct ≥37)	NEG (ND or Ct ≥37)	NEG (ND or Ct ≥31)	Invalid	Invalid	Repeat test. If the repeat result remains invalid, consider collecting a new specimen.
NEG (ND or Ct >37)	NEG (ND or Ct >37)	NEG (ND or Ct >37)	POS Valid Ct <31	Valid	SARS-CoV-2 Not Detected	Report results to healthcare provider. Consider testing for other viruses
One target=POS[1] (Valid Ct <37)			POS (Valid Ct <31)	Valid	SARS-CoV-2 Presumptive Positive[1]	Repeat test. If the repeat result is confirmed to match the first result, the results is interpreted as Positive, Report results to healthcare provider and appropriate public health authorities
Two or more SARS-CoV-2 targets = POS (Valid Ct<37)			POS (Valid Ct <31) or NEG (ND or Ct≥31)	Valid	SARS-CoV-2 Detected	Report results to healthcare provider and appropriate public health authorities.

[1]Samples with a result of SARS-CoV-2 Inconclusive shall be retested one time.
ND = Not Detected

LIMITATIONS

- The use of this assay as an *in vitro* diagnostic under the FDA Emergency Use Authorization (EUA) is limited to laboratory that 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.
- Use of this assay is limited to personnel who are trained in the procedure. Failure to follow these instructions may result in erroneous results.
- The performance of Boston Heart COVID-19 RT-PCR Test was established using nasopharyngeal swabs. Nasal swabs, mid-turbinate nasal swabs, oropharyngeal swabs and BAL specimens are also considered acceptable specimen types for use with the Boston Heart COVID-19 RT-PCR Test but performance has not been established. Please refer to FDA's FAQs on Diagnostic Testing for SARS-CoV-2 for additional information.
- 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 approaches and processing systems have not been evaluated.
- False-negative results may arise from:
  - Improper sample collection
  - Degradation of the viral RNA during shipping/storage
  - Using unauthorized extraction or assay reagents
  - The presence of RT-PCR inhibitors
  - Mutation in the SARS-CoV-2 virus
  - Failure to follow instructions for use
- False-positive results may arise from:
  - Cross contamination during specimen handling or preparation
  - Cross contamination between patient samples
  - Specimen mix-up
  - RNA contamination during product handling
- The effect of vaccines, antiviral therapeutics, antibiotics, chemotherapeutic or immunosuppressant drugs have not yet been evaluated.
- Please note, Negative results do not preclude infection of SARS-CoV-2 virus and should not be the sole basis of a patient management decision. A positive result indicates detection of nucleic acid from the relevant virus. Nucleic acid may persist even after the virus is no longer viable.
- Laboratories are required to report all positive results to the appropriate public health authorities.

PERFORMANCE EVALUATION

1) Analytical Sensitivity:

The Limit of Detection (LOD) was determined for the Boston Heart COVID-19 RT-PCR Test as the lowest detectable concentration of SARS-CoV-2 at which greater or equal to 95% of all replicates test positive.

A preliminary LOD study was performed to determine the LoD of the Boston Heart COVID-19 RT-PCR Test using a previously characterized positive nasopharyngeal swab patient sample for SARS-CoV-2 analyte (quantified by Viracor Eurofins, calculated from the linear regression equation derived from a standard curve). The SARS-CoV-2 positive nasopharyngeal swab sample was spiked in SARS-CoV-2 confirmed-negative nasopharyngeal swab specimens at concentrations ranging from 1000, 500, 250, and 125 copies/mL. Nucleic acid was extracted from the contrived samples using the MagMax Viral/Pathogen Nucleic Acid Isolation Kit and the reverse transcription RT-PCR was performed using two QuantStudio 12K Flex Real-Time PCR Systems and tested in triplicate. The preliminary LoD was determined to be 125 copies/mL.

The LOD was confirmed by spiking 20 replicates of 125 and 250 copies/mL of SARS-CoV-2 positive nasopharyngeal swab sample into nasopharyngeal swab matrix previously confirmed to be negative for SARS-CoV-2. Nucleic acid was extracted from the contrived samples using the MagMax Viral/Pathogen Nucleic Acid Isolation Kit and the reverse transcription RT-PCR was performed using two QuantStudio 12K Flex Real-Time PCR Systems.

The established LOD (125 copies/mL) was achieved on instrument 1, but not on instrument 2. One replicate on instrument 2 had an N gene Ct of 37.27, marginally missing the expected cutoff of <37, S and ORF1 targets were Not Detected and the replicate was resulted as Not Detected. At 250 copies/mL, 19/20 (95%) and 20/20 (100%) replicates were interpreted as Detected on instrument 2 and instrument 1, respectively, (based on the requirement for detection of one of the three targets), which meets the expected acceptance criteria. The LOD was adjusted to 250 copies/mL to reflect the observed performance of NP matrix on both QuantStudio 12K Instruments.

Table 3: LoD Confirmation Study Summary Instrument 1

Target	MS2	N-Gene	S Gene	ORF1ab	% Positive
Concentration (copies/mL)	250 copies/mL	250 copies/mL	250 copies/mL	250 copies/mL	20/20 100%
Mean Ct	25.89	34.42	34.48	34.5	
Standard Deviation	0.19	1.43	2.22	1.31	
CV%	0.74%	4.17%	6.38%	3.99%	

Table 4: LoD Confirmation Study Summary Instrument 2

Target	MS2	N-Gene	S Gene	ORF1ab	% Positive
Concentration (copies/mL)	250 copies/mL	250 copies/mL	250 copies/mL	250 copies/mL	19/20 95%
Mean Ct	24.9	33.64	35.25	33.36	
Standard Deviation	0.1	1.3	1.73	1.85	
CV%	0.40%	3.87%	4.91%	5.54%	

The LoD the Boston Heart COVID-19 RT-PCR Test for was confirmed to be 250 copies/mL based on a positivity rate of ≥95% for 19/20 replicates.

2) ***Analytical Inclusivity:***

The Boston Heart COVID-19 RT-PCR Test utilizes the identical oligonucleotide sequences as those used in the FDA authorized ThermoFisher TaqPath COVID-19 Combo Kit. *In silico* testing of the COVID-19 ORF1ab, S gene, and N gene assays showed 100% homology to all SARS-CoV-2 isolates analyzed, with one exception. EPI_ISL_407084 Beta Coronavirus/Japan/AI/I-004/2020 which showed a mismatch at position 7 from the 5' end of the reverse primer corresponding to 95.6% homology. The mismatch was located at the 5'end of the primer, and therefore is unlikely to have an effect on the assay performance. Life Technologies (a part of Thermo Fisher Scientific Inc.) has granted a right of reference to the performance data contained in the Life Technologies EUA request (FDA submission number EUA200010) to Boston Heart Diagnostics.

3) ***Cross-Reactivity:***

The Boston Heart COVID-19 RT-PCR Test utilizes identical oligonucleotide sequences as those used in the FDA authorized COVID-19 Combo Kit. Life Technologies has granted a right of reference to the performance data contained in the Life Technologies COVID-19 Combo Kit EUA request (FDA submission number EUA200010) to Boston Heart Diagnostics.

4) ***Sample Stability:***

A study was performed to extend the CDC's recommended specimen storage conditions at 2-8°C for up to 72 hours to 7 days after collection. Nineteen previously tested clinical specimens positive for SARS-CoV-2 were used to determine the stability of SARS-CoV-2 specimens at 2-8°C from the originally determined 4-day stability to 7 days. Samples previously tested and characterized were stored at 2-8°C and re-tested after seven days of storage on the Boston Heart COVID-19 RT-PCR Test. The results are summarized in the Table below:

Table 5:Extended Specimen Stability

Boston Heart COVID-19 RT-PCR Test	Specimen Storage 2-8°C	Total	% Performance Agreement	95% CI
	7 Days			
Detected	19	19/20	95%	76.4-99.1%

Of the nineteen positive samples, 18/19 (95%) matched between the original data. One sample originally resulted as positive, was inconclusive upon repeating after seven days of storage, with only the S-Gene displayed as detected. Results indicate that freshly collected samples stored at 2-8°C and processed within 72 hours is the preferable method for SARS-CoV-2. However, samples stored at 2-8°C for a maximum of 7 days is acceptable.

5) Clinical Evaluation

Clinical Evaluation of the Boston Heart COVID-19 RT-PCR Test

A total of 60 clinical nasopharyngeal swab specimens (30 negatives and 30 positives for SARS-CoV-2) were tested with the Boston Heart COVID-19 RT-PCR Test and the results were compared to results obtained with the EUA authorized Viracor Eurofins assay (EUA200124) performed at the Viracor Eurofins Laboratory. Samples were extracted using the MagMax Viral/Pathogen Nucleic Acid Isolation Kit and the reverse transcription RT-PCR was performed using QuantStudio 12K Flex Real-Time PCR System.

Table 6: Clinical Evaluation of the Boston Heart COVID-19 RT-PCR Test

Boston Heart COVID-19 RT-PCR Test	FDA EUA RT-PCR Assay		Total	% Performance Agreement	95% CI
	Detected	Not Detected			
Detected	29	1	30	PPA 96.7% (29/30)	83.3-99.4%
Not Detected	1	29	30	NPA 96.7% (29/30)	83.3-99.4%
Total	30	30	60		

Clinical Evaluation Bridging Study of the Boston Heart COVID-19 RT-PCR Test

A clinical evaluation bridging study of the Boston Heart COVID-19 RT-PCR Test was performed to access the performance of the Agilent Bravo liquid hander for automated pipetting. 628 nasopharyngeal swab samples (60 positive and 568 negatives for SARS-CoV) were evaluated by both the EUA approved method (EUA 200659) and in duplicate using the Agilent Bravo liquid handler. Nucleic acid was extracted from the contrived samples using the MagMax Viral/Pathogen Nucleic Acid Isolation Kit and the reverse transcription RT-PCR was performed using two QuantStudio 12K Flex Real-Time PCR Systems. The results are presented in the Table below:

Table 7: Clinical Evaluation of the Boston Heart COVID-19 RT-PCR Test

Boston Heart COVID-19 RT-PCR Test	Boston Heart COVID-19 RT-PCR Test with the Agilent Bravo		Total	% Performance Agreement	95% CI
	Detected	Not Detected			
Detected	59	1	60	PPA 100% (59/59)	93.9-100%
Not Detected	0	568	568	NPA 99.8% (568/569)	99-99.97-%
Total	59	569	628		

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 SARS-CoV-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 Federal Food, Drug, and Cosmetic Act, 21 U.S.C. § 360bbb-3(b)(1), unless the authorization is terminated or revoked sooner.

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 confirm the LoD. The extraction method and instrument used were the MagMax Viral/Pathogen Nucleic Acid Isolation Kit (#A48383) on a KingFisher Flex with a Deep-Well 96 head. The results are summarized in the following Table.. The results are summarized in the following Table.

Table 8: 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	$5.4x10^3$ NDU/mL	N/A
MERS-CoV		N/A	ND

NDU/mL = RNA NAAT detectable units/mL
N/A: Not applicable
ND: Not detected