EMERGENCY USE AUTHORIZATION (EUA)

EUA INTERACTIVE REVIEW TEMPLATE FOR MOLECULAR-BASED TESTS FOR SARS-CoV-2 THAT CAUSES CORONAVIRUS DISEASE 2019 (COVID-19)

EUA200339
BioCore

A. PURPOSE FOR SUBMISSION

Emergency Use Authorization (EUA) request for distribution and/or use of the BioCore 2019-nCoV Real Time PCR Kit for the in vitro qualitative detection of RNA from the SARS-CoV-2 in sputum, oropharyngeal and nasopharyngeal specimens from patients with signs and symptoms of infection who are suspected of COVID-19. Additional testing and confirmation procedures should be performed in consultation with public health and/or other authorities to whom reporting is required. Positive results should also be reported in accordance with local, state, and federal regulations. Performance is unknown in asymptomatic patients.

B. MEASURAND

Specific nucleic acid sequences from the ***N* and *RdRp* gene** of the SARS-CoV-2 genome.

C. APPLICANT

Manufacturer

Company Name:	BioCore Co., Ltd.
Company Address:	Room 605 ACE Technotower 5th, 20, Digital-ro 31-gil, Guro-gu, Seoul 08380, Republic of Korea
TEL:	+82-2-2205-9907
FAX:	+82-2-790-3929
Contact Point:	Hyo Jung Choi (Ms.), Assistant Manager, QA
e-mail:	hjchoi@bio-core.com

U.S Distributor

Company Name:	BCP LLC
Company Address:	695 Town Center Dr Suite # 230 Costa Mesa CA 92626, USA
TEL:	714-844-8900
Contact Point:	Mark Smith
e-mail:	Mark@bestchanceproducts.com

D. PROPRIETARY AND ESTABLISHED NAMES

Proprietary Name –	BioCore 2019-nCoV Real Time PCR Kit (Catalog No. BC01-0099)
Established Name –	Reverse transcription Real Time PCR kit for detection of 2019-nCoV RNA

E. REGULATORY INFORMATION

Approval/Clearance Status:
The BioCore 2019-nCoV Real Time PCR Kit is not cleared, CLIA waived, approved, or subject to an approved investigational device exemption.

Product Code:
QJR

F. PROPOSED INTENDED USE

1) Intended Use:
BioCore 2019-nCoV Real Time PCR Kit is a reverse-transcription Real time PCR test intended for qualitative detection of nucleic acid from the 2019-nCoV in upper respiratory specimens (such as nasal, mid-turbinate, oropharyngeal, and nasopharyngeal swabs) and lower respiratory specimens (such as sputum, bronchioalveolar lavage, and tracheal aspirates) from patients suspected of COVID-19 by their healthcare provider. Testing is limited to laboratories certified under the Clinical Laboratory Improvement Amendments of 1988 (CLIA), 42 U.S.C. §263a, to perform high complexity tests, or by similarly qualified non-U.S. laboratories.

Results are for the identification of 2019-nCoV RNA. The 2019-nCoV 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 2019-nCoV 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 BioCore 2019-nCoV Real Time PCR Kit is intended for use by trained clinical laboratory personnel specifically instructed and trained in the techniques of real-time PCR and in vitro diagnostic procedures. The BioCore 2019-nCoV Real Time PCR Kit is only for use under the Food and Drug Administration's Emergency Use Authorization.

2) Special Conditions for Use Statements:
For under Emergency Use Authorization only
For prescription use only
For in vitro diagnostic use only

3) Special Instrument Requirements:
The BioCore 2019-nCoV Real Time PCR Kit is to be used with the following RT-PCR instrument:

- SLAN 96P (Shanghai Hongshi Medical Technology Co., Ltd)
- CFX96 Dx PCR System (Bio-Rad Inc.)
- Biosystems 7500 Real-Time PCR Instrument System (Applied Biosystems, USA)

The BioCore 2019-nCoV Real Time PCR Kit is to be used with the QIAamp DSP Viral RNA Mini Kit (Qiagen; catalog #61904) for RNA extraction.

G. DEVICE DESCRIPTION AND TEST PRINCIPLE

1) ***Product Overview/Test Principle:***

The BioCore 2019-nCoV Real Time PCR Kit is a real-time reverse transcription polymerase chain reaction (rRT-PCR) test. The 2019-nCoV primer and probe sets are designed to detect RNA from the *N* and *the RdRp* genes from the 2019-nCoV genome in upper and lower respiratory specimens from patients with signs and symptoms of infection who are suspected of COVID-19. The sequence of N-gene and the RdRp gene is specific for SARS-CoV-2.

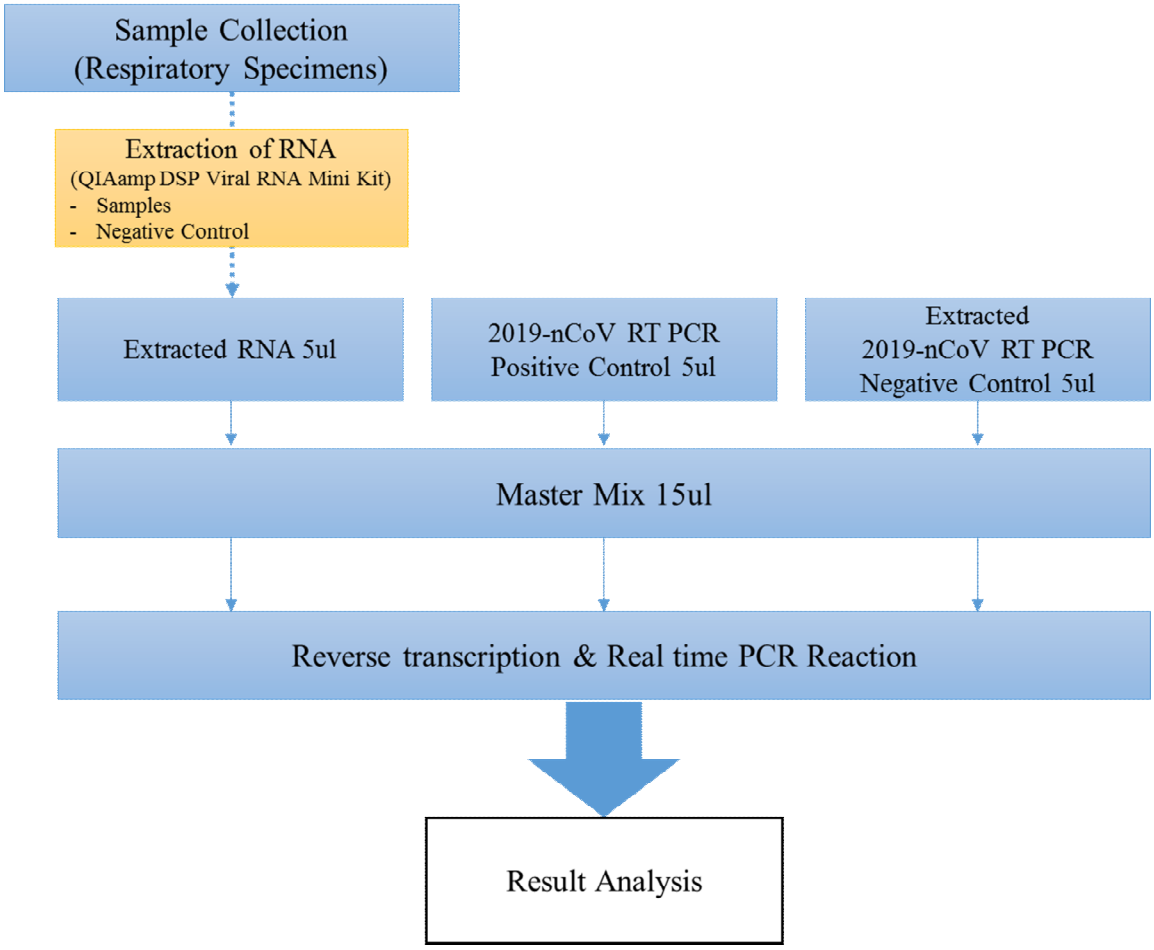
BioCore 2019-nCoV Real Time PCR Kit uses rRT-PCR and Taqman chemistry. The target specific probes are labeled with different. During PCR, a reaction product is formed by a specific primer at the target gene region of the 2019-nCoV, and at the same time, the specific Taqman probe is generating fluorescence signals specific to each gene. This kit is used together with the CFX96 Dx system (Bio-Rad Inc.), Applied Biosystems 7500 Real-Time PCR Instrument System (Thermo Fisher Scientific Inc.), or SLAN-96P (Shanghai Hongshi Medical Technology Co. Ltd). The RNA sample is extracted by using the RNA extraction kit, QIAamp DSP Viral RNA Mini Kit (Qiagen; catalog #61904).

2) ***Description of Test Steps:***

Nucleic acids are isolated and purified from respiratory specimens using QIAamp DSP Viral RNA Mini Kit. Specimen input volume for extraction is 200 µl, the nucleic acid elution volume is 50 µl and isolation/purification is performed according to the kit's instruction manual.

The purified nucleic acid is reverse transcribed and targets amplified using 2019-nCoV RT-PCR Master Mixture in a one-step reaction. The input volume of purified nucleic acid into the cDNA/amplification reaction is 5 µl. In the 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 probe, causing the reporter dye to separate from the quencher dye, generating a fluorescent signal. With each cycle, additional reporter dye molecules are cleaved from their respective probes, increasing the fluorescence intensity. Fluorescence intensity is monitored at each PCR cycle by one of the validated Real-Time PCR Instrument System.

The test steps are illustrated as follows:



Sample Collection

1) Sampling is conducted by a medical professional.
2) Both, oropharyngeal and nasopharyngeal swabs are collected from the same patient using cotton swabs and inserted both in the same collection tube with viral transport medium (VTM medium)
3) Keep the sample refrigerated at 2~8 ºC when storing it for a short period (not longer than one day) and at -20 ºC or lower when storing it for a long time.

RNA extraction from the specimen

Sputum specimens need to be pretreated with NaOH as follows to reduce the viscosity of the sample.

1. Mix the sputum sample with an equal amount of 4% NaOH solution (40mg/ml nuclease free water)
2. Vortex for 30 seconds, then leave it for 1 minute at 15 ~ 30 °C
3. Please repeat the step 2. 4 times.
4. Centrifuge for 10 minutes at 13,000 rpm and remove the supernatant.
5. Resuspend the remaining pellet using 1XPBS and use it for RNA extraction.

RNA is then extracted from 200µl sample (for sputum use the resuspended pellet from step 6 above), using the QIAamp DSP Viral RNA Mini Kit (Qiagen; catalog #61904) with a manual procedure as described in the manufactures package insert. RNA is eluted in 50µl AVE buffer included in QIAamp DSP Viral RNA Mini Kit.

The Extraction must include a negative control using 200 µl DEPC water or nuclease free TE buffer that is taken through the entire extraction, cDNA and amplification steps.

Reverse Transcription and Real Time PCR Reaction

The Master Mixture of BioCore 2019-nCoV RT PCR Kit is prepared as follows:

1) For each sample, add 5µl of 2019-nCoV RT PCR Primer/Probe Mixture into 10µl of 2019-nCoV RT PCR Reaction Mixture (Table 1).

Table 1. 2019-nCoV RT PCR Master Mixture

Solution \ Number of Samples	1	3
2019-nCoV RT PCR Reaction Mixture	10	30
2019-nCoV RT PCR Primer/Probe Mixture	5	15
Total (µl)	15	45

2) Add 5µl of extracted RNA into 15µl of the Master Mixture (made from step 1)) and mix well by pipetting.

3) Also, add 5µl of 2019-nCoV RT PCR Positive Control (PC) and 5 ul of an extracted 2019-nCoV RT PCR Negative Control (extracted NC) each to 15µl of Master Mixture (made from step 1)). Mix well by pipetting.
Note: Be sure to mix and spin down the sample because it affects the baseline formation unless it is mixed.

4) Place the tubes or plate in a Real Time PCR machine and start the machine using following condition (Table 2).

Table 2. Real Time PCR condition

Temperature	Time	Cycles
50 ºC	30 min	1
95 ºC	15 min	1
95 ºC	15 sec	45
60 ºC	**30 sec**	
→ Fluorescence acquiring		
N gene → FAM RdRp gene → CalRed610 (TexasRed) IC → Cy5		

Note: Check the fluorescence selectable by each device and the fluorescence of the product. The CalRed 610 is interchangeable with TexasRed.

Data Analysis

For data analysis, the threshold of three instruments should be manually set up according to the instrument manuals as follows:

Table 3. Thresholds of SLAN-96P

Channel	Item	Manual Threshold	Negative Threshold	Analysis Type
1	*N* gene	0.1	40	Qualitative
3	*RdRp* gene	0.1	40	Qualitative
4	IC	0.1	40	Qualitative

Table 4. Threshold of CFX96 Dx System

Channel	Item	Manual Threshold
1	*N* gene	200
3	*RdRp* gene	200
4	IC	200

Table 5. Threshold of Applied Biosystems 7500 Real-Time PCR Instrument System

Channel	Item	Manual Threshold
1	*N* gene	10,000
3	*RdRp* gene	10,000
4	IC	10,000

Note of Table 5.
1) When setting the experimental program named "Setup", set the "Select the dye to use as the passive reference" to "None", in "Assign Targets and Samples" of "Plate Setup"
2) Deselect "Auto Baseline" of "Amplification Plot" when analyzing results in "Analysis" section

3) Control Material(s) to be Used with BioCore 2019-nCoV Real Time PCR Kit:

The following control materials are provided with the test kit:

Table 6. Control Materials

No.	Control	Components
1	2019-nCoV RT PCR Positive Control	Human β globin (Internal control) SARS CoV-2 specific *N* gene and *RdRp* gene (circular plasmid at 5xLoD)
2	2019-nCoV RT PCR Negative Control	DEPC treated water

Controls that will be provided with the test kit include:

a) **2019-nCoV RT PCR Negative Control (Extraction and PCR)**
The negative control is needed to monitor contamination of extraction, reverse transcription and amplification reagents and is comprised of nuclease-free water. The negative control must be included in the extraction step and tested one time per every batch of specimen extraction and yield a negative result for each target in the BioCore 2019-nCoV Real Time PCR Kit.

b) **2019-nCoV RT PCR Positive Control (PCR)**
The positive control is needed to confirm functionality of the PCR reagents and is comprised of circular plasmid with the target sequences for the *N* gene, *RdRp* gene of SARS-CoV-2, and Human β globin gene. The positive control should be tested once per PCR run and yield a positive result for each target in the BioCore 2019-nCoV Real Time PCR Kit.

c) **Internal control (every sample)**
The internal control targeting Human β globin is needed to verify that nucleic acid is present in every sample and is used for every sample processed. Because the positive control is a DNA template (circular plasmid), this serves as a control to ensure that the reverse transcription step is proceeding as intended. This also serves as the extraction positive control to ensure that samples resulting as negative for SARS-CoV-2 RNA contain nucleic acid for testing.

H. 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.

1) BioCore 2019-nCoV Real Time PCR Kit Controls – Positive and Negative

Each extraction run must include the 2019-nCoV RT-PCR negative Control (NC). Each PCR test run must include 2019-nCoV RT PCR Positive control, and the test results should be within the following specification:

Table 7. Acceptance Criteria of Controls (Valid)

Control	Ct value			Result
	***N* gene (FAM)**	***RdRp* gene (CalRed610)**	**IC (Cy5)**	
2019-nCoV RT PCR Positive Control	≤ 40	≤ 40	≤ 40	Positive Control (valid)
2019-nCoV RT PCR Negative Control (NC)	No Ct or > 40	No Ct or > 40	No Ct or > 40	Negative Control (valid)

2) Examination and Interpretation of Patient Specimen Results:

Assessment of clinical specimen test results must only be performed if all controls have been determined to be valid. If the negative and/or positive control included in the run is invalid, the entire run is invalid and patient results cannot be interpreted. In this case a root cause analysis needs to be performed, and all patient specimens need to be retested after the root cause has been identified and eliminated. The criteria for interpretation of test results are as follows:

Table 8. Result interpretation for samples tested with the BioCore 2019-nCoV Real Time PCR Kit

No.	Ct value			Result	Action
	***N* gene (FAM)**	***RdRp* gene (CalRed610)**	**IC (Cy5)**		
1	No Ct or > 40	No Ct or > 40	≤ 40	2019-nCoV Negative	Report Result
2	≤ 40	≤ 40	Any [a]	2019-nCoV Positive	Report Result
3	No Ct or > 40	≤ 40			
4	≤ 40	No Ct or > 40			
5	No Ct or > 40	No Ct or > 40	No Ct or > 40	Invalid	Invalidate sample. Do NOT report. Re-test

[a] If the concentration of SARS CoV-2 is high, the amplification of the IC may not occur.

I. PRODUCT MANUFACTURING

The ***BioCore 2019-nCoV Real Time PCR Kit*** has been validated using only the components referenced in this submission and shall not be changed without prior concurrence from the FDA.

1) Overview of Manufacturing and Distribution:

The product will be manufactured at BioCore Co. Ltd. by BioCore Co. Ltd. personnel consistent with practices for the production of In Vitro Diagnostic Reagent Kits for PCR, Real-Time PCR and digital PCR testing based on the quality management system established according to the requirements of 21 CFR 820 Quality System Regulation (So far, no inspection received), K-GMP (Korea GMP, certified by MFDS, Korea health authority) and ISO 13485 (Certified by TÜV SÜD Product Service GmbH, Germany).

The quality management system includes the reporting procedure for *adverse events, as per 21 CFR Part 803, for authorized devices* ***(see Section P).***

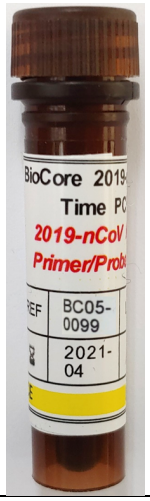
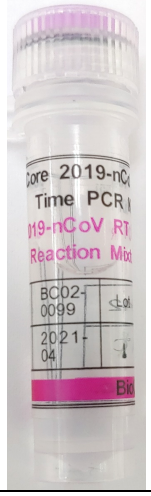
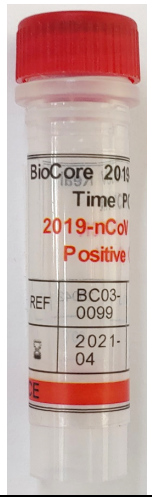
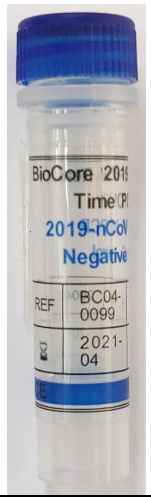
The current manufacturing capabilities include the ability to manufacture approximately 1,000 kits (for 100,000 tests) per day, 5,000 kits (for 500,000 tests) per week, however in the event of a surge in demand this could be increased to twice of normal capacity: 2,000 kits (200,000 tests) per day and 10,000 kits (1,000,000 tests) per week.

The product will be distributed to the U.S. market by designated distributors. The candidate distributors are now under search and negotiation.

2) Components Included with the Test

The BioCore 2019-nCoV Real Time PCR Kit (Cat No. BC-01-0099) consists of the following components (Table 9) that are manufactured by ***BioCore Co., Ltd.*** and supplied with the test: 2019-nCoV RT PCR Primer/ Probe Mixture, 2019-nCoV RT-PCR Reaction Mixture, 2019-nCoV RT-PCR Positive control, and 2019-nCoV RT-PCR Negative control.

Table 9. Components of BioCore 2019-nCoV Real Time PCR Kit

Component name	2019-nCoV RT PCR Primer/ Probe Mixture	2019-nCoV RT PCR Reaction Mixture	2019-nCoV RT PCR Positive control	2019-nCoV RT PCR Negative control
Photo				
Description	liquid in self-standing screw tube with brown-colored cap	liquid in self-standing screw tube with violet-colored cap	liquid in self-standing screw tube with red-colored cap	liquid in self-standing screw tube with blue-colored cap
Composition	Primer: 2.0 µM, Probe marked with fluorescent material: 0.4 µM, DW *(see the following table10. for sequence information)*	Reverse transcriptase: 2,750 unit, DNA polymerase: 165 unit, RNase Inhibitor: 550 unit, DW	N gene RdRp gene Human β globin TE buffer	DEPC treated DW *(Please include the NC in extraction step for monitoring contamination of extraction)*
Packaging Unit (for 100 Tests)	550 µl/tube (5 µl/PCR reaction)	1100 µl/tube (10 µl/PCR reaction)	50 µl/tube (5 µl/PCR reaction)	500 µl/tube (in extraction step, use 200 µl)

The Sequence Information for Primer & Probe is as follows (Table 10); both SARS-CoV-2 targets are specific for SARS-CoV-2 and do not detect other sarbeco-viruses:

Table 10. Primer and Probe information

Primer & Probe	Detect for	Sequence	Concentration
N3-F	*N* gene *(specific for SARS-CoV-2)*		
N_R1			
N_P1			
R1-F	*RdRp* gene *(specific for SARS-CoV-2)*		
R1-R			
R1-P			
HBG-F2	Human β Globin gene (Internal Control)		
HBG-R2			
HBG-P2			

3) ***Components Required But Not Included with the Test***

Components required but not included with the test are as follows:

Table 11. Components required but not included with the test

	Product name	Manufacturer	Cat. No.
Instruments	CFX96 Dx System	Bio-Rad	BR185-5484
	Applied Biosystems 7500	Thermo Fisher Scientific Inc	4351104 4351105
	SLAN-96P	Shanghai Hongshi Medical Technology Co.,Ltd	RM98000
	Bench top centrifuge	-	-
	Vortex mixer	-	-
Nuclease free water	To be used as negative control	-	-
4% NaOH	4% NaOH in nuclease free water; used for sputum pretreatments	User Prepared	-
Extraction Kit (Manual)	QIAamp DSP Viral RNA Mini Kit	Qiagen	61904
	Pipet (adjustable)	-	-
Consumables	Sterile pipet tips with filters	-	-
	Sterile 1.5 ml microtube	-	-
	MicroAmp Optical 8-Tube Strip	Applied Biosystems	4316567
	MicroAmp Optical 8-Cap Strip	Applied Biosystems	4323032
	MicroAmp Optical Adhesive Film	Applied Biosystems	4311971

	MicroAmp optical 96-well Reaction Plate	Applied Biosystems	N8010560
	Low-Profile PCR tubes 8-tube Strip, White	Bio-rad	TCS0851
	Optical Flat 8-Cap Strips for 0.2ml tube strips/plate	Bio-rad	TCS0803
	Microseal 'B' PCR Plate Sealing Film, adhesive, optical	Bio-rad	MSB-1001
	Multiplate 96-Well PCR Plates, low profile, unskirted, white	Bio-rad	MLL9651
	Biofact™ 0.2 ml PCR tube	BioFact	PW211-120

Note: Every instrument requires qualification as well as periodic maintenance and calibration. Always read and understand the manufacturer's manual before using them.

4) ***Testing Capabilities***

From sampling to obtaining the final result the test takes approximately four (4) hours:

- Specimen collection: 30 minutes
- RNA Extraction: 1 hour per 10 specimens
- rRT-PCR process including preparation for PCR: 2 hours 30 minutes (PCR process: 2 hours)

One PCR instrument can process a total of 94 specimens and controls at a time (based on 96-well plate). PCR operation cycle time is two (2) hours, and therefore total four (4) cycles could be run per day for eight (8) hours - total 376 tests can be performed

5) ***Reagent Stability:***

The product stability is claimed as follows:

Table 12. Condition of Reagent stability

	BioCore 2019-nCoV Real Time PCR Kit
Storage condition	-20°C
Shelf life	12 months
Open-Kit Stability (after first opening when stored as indicated)	4 months, total 3 times Freezing-Thawing allowed.
Transportation process	Transport Simulation Study performed under stress condition at 40°C, for 7 days.

In order to validate the stability claims, the following studies were performed according to pre-designed study protocol, according to EN ISO 23640:2015 and CLSI Guideline, EP25-A:

- Long Term Stability study

- In-Use Stability study
- Reagent Freezing-Thawing study
- Transportation Simulation study

Note: Please refer to Line data that describe all test replicates with Ct values for each target. Excel file: Line data_BioCore_2020.05.21, Sheet name: Long Term stability, In-Use stability, Freezing-Thawing stability, Transport Simulation

For estimating the stability of Biocore 2019-nCoV Real Time PCR Kit, three (3) study panel members (nCoVRTRP1, 2, 3 and nCoVRTRN) were made as described in Table 13. Then, three Lots (Lot #1, Lot #2, Lot #3) of Biocore 2019-nCoV Real Time PCR Kit were used in the stability studies. In the long-term stability study and Reagent Freezing-Thawing study, the Applied Biosystem 7500 Real-time PCR instrument was used. In the In-Use Stability study and Transportation Simulation study, CFX96 Dx System was used to progress the test.

The study panel members for positive (nCoVRTRP1, 2 and 3) are comprised of two (2) *in vitro* transcript RNA (*N* gene and *RdRp* gene) which are mixed 1:1 with each genes and specific at SARS-CoV-2.
The study panel members for internal control (nCoVRTRN) is comprised with Human β-globin.

Table 13. Information of Stability Study Panel Members

	nCoVRTRP1	**nCoVRTRP2**	**nCoVRTRP3**	**nCoVRTRN**
origin	SARS-CoV-2 specific N gene & RDRP gene (*in vitro* transcript RNA)			Human β-globin (*in vitro* transcript RNA)
concentration	10^4copies/ul	10^2copies/ul	50copies/ul	10^3copies/ul

Table 14. Acceptance Criteria for Stability Study Panel Members (Ct value)

Panel Member	**Ct Value**		
	N gene	**RDRP gene**	**IC**
nCoVRTRP1	24.7 ± 1.0	23.8 ± 1.0	No Ct
nCoVRTRP2	31.6 ± 1.0	30.9 ± 1.0	No Ct
nCoVRTRP3	32.2 ± 1.0	31.9 ± 1.0	No Ct
nCoVRTRN	No Ct	No Ct	27.3 ± 1.0

a. Accelerated Shelf Life

The long term stability of BioCore 2019-nCoV Real Time PCR Kit was estimated through an accelerated shelf life test, with the product samples stored at 40 ºC, at the time points 0, 23, 46, 69, 92, 115, 138 and 173 hours from manufacturing date, which are corresponding to 0, 2, 4, 6, 8, 10, 12, 15 months stored at -20 ºC using the Arrhenius equation with Q10 value, 2.0 applied.

At all-time points (after 0, 23, 46, 69, 92, 115, 138, 173 hours from manufacturing) in accelerated storage temperature condition, concordance rate of all tests was 100%. In this study, the three Lots were used for evaluation and each Lot was tested with 2 replicates for each time points. The Mean Ct values across the 6 replicates are summarized in Table 15 below. Based on the Mean Ct values, the results were detected stable upon storage at 40 ºC (stressed condition) up to 173 hours. Based on the Arrhenius Equation it was concluded that the test device is stable until 15 months under intended storage conditions (i.e., -20ºC). **Accordingly, the shelf life was determined as "12 months" when stored at -20 ºC with safety margin**.

Nevertheless, the real-time stability study is planned and now on-going status, starting from April 6, 2020. If some deviations are found during the study process, the products already on the market will be proper action-taken, according to the internal procedure for correction and removal from the market, if the products are already available on the market.

Table 15. Accelerated Shelf Life of Lot #1

Lot #1	Target	Ct value (Mean)							
		After 0 hour	After 23 hour	After 46 hour	After 69 hour	After 92 hour	After 115 hour	After 138 hour	After 173 hour
nCoVRTRP1	N	24.9	24.7	25.4	25.0	25.5	24.8	24.6	25.3
	RDRP	24.5	24.2	24.3	24.7	24.6	24.5	24.2	24.5
	IC	-	-	-	-	-	-	-	-
nCoVRTRP2	N	31.8	32.3	31.7	31.6	32.1	31.8	31.7	32
	RDRP	31.4	31.9	31.2	31.6	31.7	31.4	31.5	31.7
	IC	-	-	-	-	-	-	-	-
nCoVRTRP3	N	32.5	32.8	32.4	32.2	32.9	32.5	32.4	32.7
	RDRP	32.5	32.4	33.2	32.9	32.6	32.5	32.6	32.5
	IC	-	-	-	-	-	-	-	-
nCoVRTRN	N	-	-	-	-	-	-	-	-
	RDRP	-	-	-	-	-	-	-	-
	IC	27.5	27.8	27.9	27.5	27.2	27.3	27.4	27.1

Table 16. Accelerated Shelf Life Result of Lot #2

Lot #2	Target	Ct value (Mean)							
		After 0 hour	After 23 hour	After 46 hour	After 69 hour	After 92 hour	After 115 hour	After 138 hour	After 173 hour
nCoVRTRP1	N	25.6	25.4	24.7	25.4	25.6	24.5	25.4	24.3

	RDRP	24.6	24.4	24.3	24.4	24.6	24.0	24.3	24.3
	IC	-	-	-	-	-	-	-	-
nCoVRTRP2	N	32.3	32.5	32.5	31.2	32.2	32.2	31.9	32.0
	RDRP	32.0	31.7	31.6	30.9	31.8	31.5	31.3	31.7
	IC	-	-	-	-	-	-	-	-
nCoVRTRP3	N	33.0	33.0	32.1	32.6	32.9	32.7	32.6	32.9
	RDRP	32.7	32.5	32.8	32.6	32.7	32.8	32.4	32.5
	IC	-	-	-	-	-	-	-	-
nCoVRTRN	N	-	-	-	-	-	-	-	-
	RDRP	-	-	-	-	-	-	-	-
	IC	27.9	28.0	27.5	27.3	27.5	27.2	27.1	27.1

Table 17. Accelerated Shelf Life Result of Lot #3

Lot #3	Target	Ct value (Mean)							
		After 0 hour	After 23 hour	After 46 hour	After 69 hour	After 92 hour	After 115 hour	After 138 hour	After 173 hour
nCoVRTRP1	N	24.8	24.6	25.2	25.0	24.9	25.1	24.5	24.5
	RDRP	24.4	24.4	24.4	24.5	24.4	24.1	24.4	24.2
	IC	-	-	-	-	-	-	-	-
nCoVRTRP2	N	32.2	31.8	32.4	31.9	32.1	31.7	31.2	31.7
	RDRP	31.9	31.7	31.9	31.8	31.5	31.3	31.4	31.3
	IC	-	-	-	-	-	-	-	-
nCoVRTRP3	N	33.3	33.1	33.0	32.2	33.0	32.9	32.6	32.9
	RDRP	32.6	32.9	32.5	32.5	32.8	32.3	32.6	32.9
	IC	-	-	-	-	-	-	-	-
nCoVRTRN	N	-	-	-	-	-	-	-	-
	RDRP	-	-	-	-	-	-	-	-
	IC	27.8	27.7	27.2	26.9	27.5	26.9	26.9	27.2

Table 18-1. Accelerated Shelf Life Result of 3 Lots

	Target	Ct value (Mean)											
		After 0 hour			After 23 hour			After 46 hour			After 69 hour		
		Lot #1	Lot #2	Lot #3	Lot #1	Lot #2	Lot #3	Lot #1	Lot #2	Lot #3	Lot #1	Lot #2	Lot #3
nCoVRTRP1	N	24.9	25.6	24.8	24.7	25.5	24.6	25.4	24.7	25.2	25.0	25.4	25.0
	RDRP	24.5	24.6	24.4	24.2	24.4	24.4	24.3	24.3	24.4	24.7	24.4	24.5
	IC	-	-	-	-	-	-	-	-	-	-	-	-
nCoVRTRP2	N	31.8	32.3	32.2	32.3	32.5	31.8	31.7	32.5	32.4	31.6	31.2	31.9
	RDRP	31.4	32.0	31.9	31.9	31.7	31.7	31.2	31.6	31.9	31.6	30.9	31.8
	IC	-	-	-	-	-	-	-	-	-	-	-	-
nCoVRTRP3	N	32.5	33.0	33.3	32.8	33.0	33.1	32.4	32.1	33.0	32.2	32.6	32.2
	RDRP	32.54	32.7	32.6	32.4	32.5	32.9	33.2	32.8	32.5	32.9	32.6	32.5
	IC	-	-	-	-	-	-	-	-	-	-	-	-
nCoVRTRN	N	-	-	-	-	-	-	-	-	-	-	-	-
	RDRP	-	-	-	-	-	-	-	-	-	-	-	-
	IC	27.5	27.9	27.8	27.8	28.0	27.7	27.9	27.5	27.2	27.5	27.3	26.9

Table 18-2. Accelerated Shelf Life Result of 3 Lots

	Target	Ct value (Mean)											
		After 92 hour			After 115 hour			After 138 hour			After 173 hour		
		Lot #1	Lot #2	Lot #3	Lot #1	Lot #2	Lot #3	Lot #1	Lot #2	Lot #3	Lot #1	Lot #2	Lot #3
nCoVRTRP1	N	25.5	25.6	24.9	24.8	24.5	25.1	24.6	25.4	24.5	25.3	24.3	24.5
	RDRP	24.6	24.6	24.4	24.5	24.0	24.1	24.2	24.3	24.4	24.5	24.3	24.2
	IC	-	-	-	-	-	-	-	-	-	-	-	-
nCoVRTRP2	N	32.1	32.2	32.1	31.8	32.2	31.7	31.7	31.9	31.2	32	32.0	31.7
	RDRP	31.7	31.8	31.5	31.4	31.5	31.3	31.5	31.3	31.4	31.7	31.7	31.3
	IC	-	-	-	-	-	-	-	-	-	-	-	-
nCoVRTRP3	N	32.9	32.9	33.0	32.5	32.7	32.9	32.4	32.6	32.6	32.7	32.9	32.9
	RDRP	32.6	32.7	32.8	32.5	32.8	32.3	32.6	32.4	32.6	32.5	32.5	32.9
	IC	-	-	-	-	-	-	-	-	-	-	-	-
nCoVRTRN	N	-	-	-	-	-	-	-	-	-	-	-	-
	RDRP	-	-	-	-	-	-	-	-	-	-	-	-
	IC	27.2	27.5	27.5	27.3	27.2	26.9	27.4	27.1	26.9	27.1	27.1	27.2

b. In-Use stability test

For in-use stability establishment, an accelerated aging experiment was performed as well with same condition for the shelf-life study design above. Also, the three Lots were used for evaluation and each Lot was tested 2 replicates for each time points.

All of the test results tested at 0 hour (immediately after manufacturing), 23 hours (corresponding to 2 months) and 46 hours (corresponding to 4 months) were obtained within the acceptance criteria. Therefore, it can be concluded that the performance is maintained up to four (4) months from the time point of product opening. **Accordingly, the 'In-Use stability' of this product is established at three (3) months after opening, when stored at -20 ºC with** safety margin.

Table 19. In-Use Stability Result of three Lots

	Target	**Lot #1 - Ct value (Mean)**			**Lot #2 - Ct value (Mean)**			**Lot #3 - Ct value (Mean)**		
		After 0 hour	After 23 hour	After 46 hour	After 0 hour	After 23 hour	After 46 hour	After 0 hour	After 23 hour	After 46 hour
nCoVRTRP1	N	24.6	24.6	24.6	24.5	24.9	24.7	24.3	24.0	24.8
	RDRP	23.1	22.9	23.4	23.1	23.4	23.5	23.0	23.5	23.5
	IC	-	-	-	-	-	-	-	-	-
nCoVRTRP2	N	31.4	31.8	32.0	31.5	31.3	31.4	31.1	31.9	31.5
	RDRP	30.6	30.5	30.8	31.0	30.1	30.6	30.4	30.7	31.0
	IC	-	-	-	-	-	-	-	-	-
nCoVRTRP3	N	32.2	31.6	32.6	32.7	32.7	32.1	32.5	32.5	31.9
	RDRP	31.2	30.9	31.3	32.4	31.5	31.1	31.9	32.3	31.7
	IC	-	-	-	-	-	-	-	-	-
nCoVRTRN	N	-	-	-	-	-	-	-	-	-
	RDRP	-	-	-	-	-	-	-	-	-
	IC	26.7	27.1	27.0	26.7	27.4	26.8	26.4	26.7	26.6

c. Reagent freezing-thawing stability

To verify the effect of the freezing-thawing cycles to the product performance during in-use, the test was performed once every cycle, up to five (5) cycles of freezing- thawing. Also, one Lot (Lot No. #3) was used for evaluation and tested 3 replicates for each time points. The test results were found same in every cycle up to five (5) times. Therefore, it was confirmed that the BioCore 2019-nCoV Real Time PCR Kit is stable even if the freezing-thawing condition repeated for 5 times (Claimed as 3 times freezing-thawing allowed.)

Table 20. Result of Reagent Freezing-Thawing stability

Study Materials	Target	0 times	1 times	2 times	3 times	4 times	5 times
nCoVRTRP1	N	24.8	25.2	24.5	25.3	24.7	24.8
	RDRP	24.4	24.4	24.3	24.2	24.2	24.4
	IC	-	-	-	-	-	-
nCoVRTRP2	N	31.8	32.4	31.9	32.1	32.2	31.5
	RDRP	31.5	31.9	31.4	31.6	31.3	31.3
	IC	-	-	-	-	-	-
nCoVRTRP3	N	32.7	33.1	32.7	33.0	32.9	32.2
	RDRP	32.9	32.5	32.4	32.3	32.3	32.2
	IC	-	-	-	-	-	-
nCoVRTRN	N	-	-	-	-	-	-
	RDRP	-	-	-	-	-	-
	IC	27.0	27.2	27.1	27.2	26.7	26.9

d. Transport Simulation Study

In order to validate the transport stability, the kits were exposed to temperature stress conditions that simulate the "worst case" transport conditions the kit may experience before it is put under intended storage conditions by the consumer. (CLSI guideline EP25-A, 4.2.3). A total of three (3) lots of kit were tested on day 0 (immediately after manufacture). The kits were then subjected to "high temperature" stress at 40 ºC for 7 days to predict a transport stability for 7 days at -20ºC or less.

Table 21. Summary Transport Simulation Result (across all lots)

Study Materials	Target	Ct value (Mean)							
		0 day (QC)	After 1 day	After 2 days	After 3 days	After 4 days	After 5 days	After 6 days	After 7 days
nCoVRTRP1	N	24.3	24.4	24.3	24.2	24.5	24.2	24.4	24.3
	RDRP	23.2	23.3	23.2	23.0	23.1	23.3	23.3	23.3
	IC	-	-	-	-	-	-	-	-
nCoVRTRP2	N	30.8	30.9	30.9	30.7	30.9	31.4	31.2	31.3
	RDRP	30.1	30.2	29.8	29.6	30.0	30.3	30.3	30.3
	IC	-	-	-	-	-	-	-	-
nCoVRTRP3	N	31.4	31.5	31.6	31.7	31.6	31.5	31.3	31.6
	RDRP	31.4	31.3	30.9	30.6	30.7	31.4	31.3	31.0
	IC	-	-	-	-	-	-	-	-

nCoVRTRN	N	-	-	-	-	-	-	-	-
	RDRP	-	-	-	-	-	-	-	-
	IC	27.7	27.7	27.6	27.6	27.6	27.5	27.5	27.5

The product will be transported to the USA by using a specially designed packaging system, the Intelsius™, where providing the temperature monitoring data. Additional transportation validation is scheduled to be performed in May 2020.

<Intelsius™ packaging system>
- Product/service name: BT020 BioTherm 45
- The BT020 BioTherm 45 Dry Ice shipping service would be used for transport validation. It is part of the BioTherm Insulated Dry Ice range designed to ensure consignments are fully compliant to International Air Transport Association (IATA), Accord européen relatif au transport international des marchandises Dangereuses par Route (ADR) and United States Department of Transportation (DOT, 49 CFR).
The service is qualified to maintain sample integrity for 168 hours against the demanding Intelsius High Performance Dry Ice profile.

Note: Please refer to the 'BT020 BioTherm 45 dry ice QR8003.7' providing the specification of BT020 BioTherm 45 Dry Ice shipping service

Table 22. Profile of The BT020 BioTherm 45 Dry Ice shipping solution

Temperature	Cycle Period	Cycle Period Hours	Total Time Hours
40°C → 25°C	8	12 hours	96 hours
25°C → 40°C	7	12 hours	84 hours

The dry ice shipping solution for the GDI 45 has kept all product probes below the temperature of -60°C for a duration of 167 hours 50 minutes and below -20.0°C for a duration in excess of 168 hours. The system had a total –20.0°C duration of 172hrs 50min with a dry ice fill of 35.9kg of dry ice giving a calculated dry ice loss rate loss rate of 208.1g/hr.

J. PERFORMANCE EVALUATION

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

a. Preliminary LoD Study

This study was conducted to evaluate the LoD of the BioCore 2019-nCoV Real Time PCR Kit using a SARS CoV-2 (COVID-19) RNA distributed by National Culture Collection for Pathogens, Korea (NCCP No. 43326). All sample replicates were prepared by spiking the SARS CoV-2 (COVID-19) RNA into negative clinical sputum matrix. This sputum matrix was pretreated with 4% NaOH as described in the testing procedure to homogenize the viscosity of the sample *(Please refer to page 4 for detail of pretreatment).*

A preliminary LoD study was performed and included eight (8) replicates at each of four (4) different concentrations:
SARS CoV-2 RNA - 2000, 1000, 500, 250 copies/mL

Then, the samples were extracted by QIAamp DSP Viral RNA Mini Kit (Qiagen; catalog #61904). The test was performed with all three (3) claimed instruments to be used together, SLAN-96P, CFX96 Dx System, and Applied Biosystems 7500 Real-time PCR Instrument System. Line data describing all replicated test with Ct values for each target was provided in file: *Line data_BioCore_2020.05.21, Sheet name: LoD*

Table 23. LoD test result by SLAN-96P

Target Level	Valid results (tested)	SARS-CoV-2 N gene Positive			SARS-CoV-2 RdRp gene Positive			Internal Control Positive		
		n*	Mean Ct	Detection Rate	n*	Mean Ct	Detection Rate	n*	Mean Ct	Detection Rate
2000 cp/mL	8	8	36.03	100.0	8	33.20	100.0	8	27.44	100
1000 cp/mL	8	8	38.08	100.0	8	34.69	100.0	8	27.33	100
500 cp/mL	8	3	39.99	37.5	8	35.68	100.0	8	27.42	100
250 cp/mL	8	0	41.31	0.0	7	38.04	87.5	8	27.16	100

n* : The number of results that detected positive signal in target gene.

Table 24. LoD test result by CFX96 Dx System

Target Level	Valid results (tested)	SARS-CoV-2 *N* gene Positive			SARS-CoV-2 *RdRp* gene Positive			Internal Control Positive		
		n*	Mean Ct	Detection Rate	n*	Mean Ct	Detection Rate	n*	Mean Ct	Detection Rate
2000 cp/mL	8	8	36.03	100.0	8	33.20	100.0	8	27.44	100
1000 cp/mL	8	8	38.08	100.0	8	34.69	100.0	8	27.33	100
500 cp/mL	8	3	39.99	37.5	8	35.68	100.0	8	27.42	100
250 cp/mL	8	0	41.31	0.0	7	38.04	87.5	8	27.16	100

n* : The number of results that detected positive signal in target gene.

Table 25. LoD test result by Applied Biosystems 7500 Real-time PCR

Target Level	Valid results (tested)	SARS-CoV-2 *N* gene Positive			SARS-CoV-2 *RdRp* gene Positive			Internal Control Positive		
		n*	Mean Ct	Detection Rate	n*	Mean Ct	Detection Rate	n*	Mean Ct	Detection Rate
2000 cp/mL	8	8	32.46	100.0	8	31.56	100.0	8	26.54	100
1000 cp/mL	8	8	32.84	100.0	8	34.39	100.0	8	26.24	100
500 cp/mL	8	8	36.54	100.0	8	35.83	100.0	8	26.88	100
250 cp/mL	8	4	37.25	50.0	5	38.22	62.5	8	26.89	100

n* : The number of results that detected positive signal in target gene.

As the result of preliminary LoD study with three instruments, the LoD detected is 500 copies/mL.

b. Confirmatory LoD

Additionally, the preliminary LoD was confirmed by testing twenty (20) replicates of three different concentrations (1000, 500, 250 copies/mL) of SARS CoV-2 (COVID-19) RNA in Applied Biosystem 7500 Real-time PCR, four different concentrations (2000, 1000, 500, 250 copies/mL) of SARS CoV-2 (COVID-19) RNA in SLAN 96P and CFX96 Dx System.

Table 26. Confirmatory LoD result of SLAN-96P

Target Level	Valid results (tested)	SARS-CoV-2 *N* gene Positive			SARS-CoV-2 *RdRp* gene Positive			Internal Control Positive		
		n*	Mean Ct	Detection Rate	n*	Mean Ct	Detection Rate	n*	Mean Ct	Detection Rate
2000 cp/mL	20	20	36.21	100.0	20	33.34	100.0	20	27.51	100
1000 cp/mL	20	19	38.24	100.0	20	34.71	100.0	20	27.06	100
500 cp/mL	20	5	39.31	20.0	20	36.20	100.0	20	27.04	100
250 cp/mL	20	4	41.43	20.0	17	37.68	85.0	20	27.05	100

Table 27. Confirmatory LoD result of CFX96 Dx System

Target Level	Valid results (tested)	SARS-CoV-2 *N* gene Positive			SARS-CoV-2 *RdRp* gene Positive			Internal Control Positive		
		n*	Mean Ct	Detection Rate	n*	Mean Ct	Detection Rate	n*	Mean Ct	Detection Rate
2000 cp/mL	20	20	33.33	100.0	20	33.48	100.0	20	27.36	100
1000 cp/mL	20	20	34.56	100.0	20	35.23	100.0	20	26.91	100
500 cp/mL	20	19	36.58	95.0	17	36.23	85.0	20	26.86	100
250 cp/mL	20	6	36.61	30.0	3	38.76	15.0	20	26.86	100

Table 28. Confirmatory LoD result of Applied Biosystems 7500 Real-time PCR

Target Level	Valid results (tested)	SARS-CoV-2 *N* gene Positive			SARS-CoV-2 *RdRp* gene Positive			Internal Control Positive		
		n*	Mean Ct	Detection Rate	n*	Mean Ct	Detection Rate	n*	Mean Ct	Detection Rate
1000 cp/mL	20	20	34.92	100.0	20	35.34	100.0	20	26.47	100
500 cp/mL	20	19	35.61	95.0	20	35.90	100.0	20	26.82	100
250 cp/mL	20	14	37.48	70.0	17	37.73	85.0	20	26.42	100

Note: Please refer to Line data that described all replicated test with Ct values for each target.

Excel file: Line data_BioCore_2020.05.21, Sheet name: Confirmatory LoD

The study results, summarized in Table 29 and 30, establish a LoD for the BioCore 2019-nCoV Real Time PCR Kit measured on the SLAN 96P, CFX96 Dx System, and Applied Biosystem 7500 Real-time PCR were 500 copies/mL.

Table 29. LoD Summary of each target gene

PCR Instrument	Target	Positive Rate	Limit of Detection (copies/mL)
SLAN-96P	N gene	19/20	1000
	RdRp gene	20/20	500
CFX96 Dx System	N gene	19/20	500
	RdRp gene	20/20	1000
Applied Biosystem 7500 Real-time PCR	N gene	19/20	500
	RdRp gene	20/20	500

Table 30. LoD Summary of each Real Time PCR Instruments

PCR Instrument	Limit of Detection (copies/mL)
SLAN-96P	500
CFX96 Dx System	500
Applied Biosystem 7500 Real-time PCR	500

2) ***Inclusivity (analytical sensitivity):***

The inclusivity of the BioCore 2019-nCoV Real Time PCR Kit was evaluated using in silico analysis of the assay primers and probes in relation to 3,007 of SARS-CoV-2 sequences available in the NCBI.
In "Severe acute respiratory syndrome coronavirus 2 data hub" of NCBI, 3,007 of Nucleotide complete (complete DNA) was analyzed which are registered from November 30, 2019 to April 30, 2020.

As the result, 21 cases with homology of '< 100%' in the primer/probe region were identified. All of the 21 cases were just one base pair mismatch.

Table 31. *In silico* Analysis

No.	Accession	Description	Target 1 (*N* gene)			Target 2 (*RdRp* gene)		
			FWD P	REV P	PROBE	FWD P	REV P	PROBE
1	MT365028	Severe acute respiratory syndrome coronavirus 2 isolate SARS-CoV-2/human/HKG/HKU-905a/2020, complete genome	**95% (21/22)**	100%	100%	100%	100%	100%
2	MT114414	Severe acute respiratory syndrome coronavirus 2 isolate SARS-CoV-2/human/HKG/HKU-903a/2020, complete genome	**95% (21/22)**	100%	100%	100%	100%	100%

3	MT114415	Severe acute respiratory syndrome coronavirus 2 isolate SARS-CoV-2/human/HKG/HKU-903b/2020, complete genome	**95% (21/22)**	100%	100%	100%	100%	100%
4	MT370516	Severe acute respiratory syndrome coronavirus 2 isolate SARS-CoV-2/human/TWN/CGMH-CGU-03/2020, complete genome	**95% (21/22)**	100%	100%	100%	100%	100%
5	MT370518	Severe acute respiratory syndrome coronavirus 2 isolate SARS-CoV-2/human/TWN/CGMH-CGU-05/2020, complete genome	**95% (21/22)**	100%	100%	100%	100%	100%
6	MT370904	Severe acute respiratory syndrome coronavirus 2 isolate SARS-CoV-2/human/USA/NY1-PV08001/2020, complete genome	**95% (21/22)**	100%	100%	100%	100%	100%
7	MT374102	Severe acute respiratory syndrome coronavirus 2 isolate SARS-CoV-2/human/TWN/CGMH-CGU-06/2020, complete genome	**95% (21/22)**	100%	100%	100%	100%	100%
8	MT374103	Severe acute respiratory syndrome coronavirus 2 isolate SARS-CoV-2/human/TWN/CGMH-CGU-07/2020, complete genome	**95% (21/22)**	100%	100%	100%	100%	100%
9	MT459922	Severe acute respiratory syndrome coronavirus 2 isolate SARS-CoV-2/human/GRC/264_32497/2020, complete genome	100%	**95% (19/20)**	100%	100%	100%	100%
10	MT450973	Severe acute respiratory syndrome coronavirus 2 isolate SARS-CoV-2/human/AUS/VIC58/2020, complete genome	**95% (21/22)**	100%	100%	100%	100%	100%
11	MT450980	Severe acute respiratory syndrome coronavirus 2 isolate SARS-CoV-2/human/AUS/VIC66/2020, complete genome	**95% (21/22)**	100%	100%	100%	100%	100%
12	MT451007	Severe acute respiratory syndrome coronavirus 2 isolate SARS-CoV-2/human/AUS/VIC97/2020, complete genome	**95% (21/22)**	100%	100%	100%	100%	100%
13	MT451158	Severe acute respiratory syndrome coronavirus 2 isolate SARS-CoV-2/human/AUS/VIC262/2020, complete genome	**95% (21/22)**	100%	100%	100%	100%	100%
14	MT451168	Severe acute respiratory syndrome coronavirus 2 isolate SARS-CoV-2/human/AUS/VIC272/2020, complete genome	**95% (21/22)**	100%	100%	100%	100%	100%
15	MT451176	Severe acute respiratory syndrome coronavirus 2 isolate SARS-CoV-2/human/AUS/VIC280/2020, complete genome	100%	100%	100%	100%	100%	**97% (30/31)**
16	MT451186	Severe acute respiratory syndrome coronavirus 2 isolate SARS-CoV-2/human/AUS/VIC294/2020, complete genome	**95% (21/22)**	100%	100%	100%	100%	100%
17	MT451194	Severe acute respiratory syndrome coronavirus 2 isolate SARS-CoV-2/human/AUS/VIC302/2020, complete genome	**95% (21/22)**	100%	100%	100%	100%	100%
18	MT451197	Severe acute respiratory syndrome coronavirus 2 isolate SARS-CoV-2/human/AUS/VIC305/2020, complete genome	100%	100%	100%	100%	100%	**97% (30/31)**
19	MT459979	Severe acute respiratory syndrome coronavirus 2 isolate SARS-CoV-	100%	100%	**96% (24/25)**	100%	100%	100%

		2/human/SRB/Novi Pazar-363/2020, complete genome						
20	MT461626	Severe acute respiratory syndrome coronavirus 2 isolate SARS-CoV-2/human/USA/WA-UW-4270/2020, complete genome	**95% (21/22)**	100%	100%	100%	100%	100%
21	MT450872	Severe acute respiratory syndrome coronavirus 2 isolate SARS-CoV-2/human/SRB/KV26/2020, complete genome	100%	100%	**96% (24/25)**	100%	100%	100%

Note: Please refer to Line data that describes all replicated test with Ct values for each target.
Excel file: Line data_BioCore_2020.05.21, Sheet name: in silico

Mismatched complete DNAs have never been detected in both *N* gene and *RdRp* gene. Through these results, it is expected that 3,007 of the SARS-COV-2 complete DNAs (reported until 2020.04.30) can be detected as a positive result.

3) ***Cross-reactivity (Analytical Specificity):***

a. Cross-Reactivity - Wet Testing

Cross-reactivity testing was conducted with potentially cross reacting viruses that are likely result in infection of the respiratory tract. Twenty-seven (27) viruses spiked into transport medium at the concentration indicted in Table 32 below were extracted by QIAamp DSP Viral RNA Mini Kit, then tested using the SLAN-96P instrument. All 27 viruses including pooled human nasal wash tested showed negative results, indicating that there was no cross-reactivity with the tested viruses.

Table 32. Cross-reactivity test result (2019-nCoV negative)

No	Strain	Origin	Concentration		Result		
			ng/ul	cp/ul	Test 1	Test 2	Test 3
1	Influenza virus A H1N1	ZeptoMetrix panel	1	6.8×10^7	-	-	-
2	Influenza virus A H3N2	ZeptoMetrix panel	1	6.8×10^7	-	-	-
3	Influenza virus A Pdm2009	ZeptoMetrix panel	1	6.8×10^7	-	-	-
4	Influenza virus B	ZeptoMetrix panel	1	6.4×10^7	-	-	-
5	RSV A2	ZeptoMetrix panel	1	6.2×10^7	-	-	-
6	Rhinovirus 1A	ZeptoMetrix panel	1	1.3×10^8	-	-	-
7	Corona Virus_229E	ZeptoMetrix panel	1	3.4×10^7	-	-	-
8	Corona Virus_OC43	ZeptoMetrix panel	1	3.0×10^7	-	-	-
9	Corona Virus_NL63	ZeptoMetrix panel	1	3.4×10^7	-	-	-
10	Corona Virus_HKU-1	ZeptoMetrix panel	1	3.1×10^7	-	-	-
11	Metapneumovirus	ZeptoMetrix	1	7.0×10^7	-	-	-

		panel					
12	Rhinovirus A	ATCC VR-1131	1	1.3×10^8	-	-	-
13	Rhinovirus B	ATCC VR-284	1	1.3×10^8	-	-	-
14	Parainfluenza virus 1	ZeptoMetrix panel	1	6.2×10^7	-	-	-
15	Parainfluenza virus 2	ZeptoMetrix panel	1	6.0×10^7	-	-	-
16	Parainfluenza virus 3	ZeptoMetrix panel	1	6.2×10^7	-	-	-
17	Parainfluenza virus 4	ZeptoMetrix panel	1	6.0×10^7	-	-	-
18	Adenovirus type 1	ZeptoMetrix panel	1	2.6×10^7	-	-	-
19	Adenovirus type 3	ZeptoMetrix panel	1	2.6×10^7	-	-	-
20	Adenovirus type 31	ZeptoMetrix panel	1	2.7×10^7	-	-	-
21	Coxsackievirus A3	Pathogenic Virus Bank	1	1.3×10^8	-	-	-
22	Coxsackievirus B3	Pathogenic Virus Bank	1	1.3×10^8	-	-	-
23	Echovirus 6	Pathogenic Virus Bank	1	1.3×10^8	-	-	-
24	Echovirus 7	Pathogenic Virus Bank	1	1.3×10^8	-	-	-
25	Enterovirus 68	ATCC VR-1076	1	1.3×10^8	-	-	-
26	Enterovirus 71	Pathogenic Virus Bank	1	1.3×10^8	-	-	-
27	SARS-coronavirus	In vitro transcribed RNA	1	5.3×10^8	-	-	-
28	Pooled human nasal wash #1	Clinical Specimen			-	-	-
29	Pooled human nasal wash #2	Clinical Specimen			-	-	-
30	Pooled human nasal wash #3	Clinical Specimen			-	-	-

Note: Please refer to Line data that describes all replicated test with Ct values for each target. Excel file: Line data_BioCore_2020.05.21, Sheet name: Cross reactivity

b. Cross-reactivity – *In silico* Analysis

Cross-reactivity of the BioCore 2019-nCoV Real Time PCR Kit was evaluated by *in silico* analysis and cross-reactivity was defined as greater than 80% homology between 'oligo set' and any sequence present in the targeted microorganism as Table 33. As a result of analysis, there were no microorganisms with potential cross-reactive sequences.

Table 33. Cross-reactivity analysis by *in silico* analysis

	Accession	Description	Target 1 (*N* gene)			Target 2 (*RdRp* gene)		
			FWD P	REV P	PROBE	FWD P	REV P	PROBE
1	KT225476.2	Middle East respiratory syndrome coronavirus isolate MERS-CoV/THA/CU/17_06_2015, complete genome	0%	0%	0%	0%	46% (13/28)	0%
2	HV214386.1	Genome sequence of Chlamydia pneumoniae	0%	0%	0%	0%	0%	0%
3	CP000672.1	Haemophilus influenzae PittGG, complete genome	0%	0%	0%	0%	0%	0%
4	FR747827.1	Legionella pneumophila LPS gene cluster, strain ATCC 43736 serogroup 13	0%	0%	0%	0%	39% (11/28)	0%
5	AP018036.1	Mycobacterium tuberculosis DNA, complete genome, strain: HN-506	64% (14/22)	65% (13/20)	60% (15/25)	60% (12/20)	0%	48% (15/31)
6	CP027540.1	Streptococcus pneumoniae strain D39V chromosome, complete genome	55% (12/22)	65% (13/20)	60% (15/25)	65% (13/20)	64% (18/28)	0%
7	AE009949.1	Streptococcus pyogenes MGAS8232, complete genome	55% (13/22)	70% (14/20)	60% (13/25)	75% (15/20)	46% (13/28)	45% (14/31)
8	GCA_000306945.1	Bordetella pertussis 18323, complete genome	50% (11/22)	60% (12/20)	60% (13/25)	65% (13/20)	46% (13/28)	39% (12/31)
9	GCA_001272835.1	Mycoplasma pneumoniae FH (mycoplasmas)	68% (15/22)	55% (11/20)	60% (13/25)	55% (11/20)	43% (12/28)	42% (13/31)
10	GCA_002571455.1	Pneumocystis jirovecii (ascomycetes)	59% (13/22)	60% (12/20)	80% (20/25)	60% (12/20)	50% (14/28)	42% (13/31)
11	GCA_003454735.1	Candida albicans (budding yeasts)	59% (13/22)	65% (13/20)	56% (14/25)	75% (15/20)	61% (17/28)	45% (14/31)
12	CP000438.1	Pseudomonas aeruginosa UCBPP-PA14, complete genome	64% (14/22)	65% (13/20)	60% (15/25)	60% (12/20)	46% (13/28)	39% (12/31)
13	AP008934.1	Staphylococcus saprophyticus subsp. saprophyticus ATCC 15305 DNA, complete genome	59% (13/22)	55% (11/20)	48% (12/25)	55% (11/20)	54% (15/28)	45% (14/31)
14	CP040804.1	Streptococcus salivarius strain LAB813 chromosome, complete genome	59% (13/22)	60% (12/20)	60% (15/25)	55% (11/20)	54% (15/28)	39% (12/31)

c. Interference Substances Study

An interference study was conducted for the BioCore 2019-nCoV Real Time PCR Kit using 2 endogenous potentially interfering substances and 3 exogenous potentially interfering substances at the concentrations indicated in Table 31 below. Substances were tested in 6 SARS-CoV-2 negative specimens (2 of sputum, 2 oropharyngeal swabs, and 2 nasopharyngeal swabs) and 6 SARS-CoV-2 positive specimens (2 of sputum, 2 oropharyngeal swabs, and 2 nasopharyngeal swabs).

The positive specimens were made by spiking Sars-CoV2 (COVID-19, NCCP No. 43326) into the 6 negative specimens. All samples were extracted by QIAamp DSP Viral RNA Mini Kit (Qiagen; catalog #61904). Each specimen was tested with two replicates using the Applied Biosystems 7500 Real-time PCR. No interference was observed for the substances and concentration tested. Line data that descripted all replicated test with Ct values for each target were provided in the file: *Line data_BioCore_2020.05.21*,

Table 334. Interfering Substances Study Results

Interferent	C[1]	Matrix	No SARS-CoV2	SARS-CoV-2 [5000 copies/mL = 10xLoD]				
			Negativity Rate	Positivity Rate	Target	Mean Ct	SD [Ct]	Mean Difference in Ct
Control	-	Sputum	8/8 100%	8/8 100%	N	32.2	0.2	32.2 ± 0.5
					RdRp	33.2	0.2	33.2 ± 2.8
		NP	8/8 100%	8/8 100%	N	32.0	0.2	32.0 ± 1.2
					RdRp	33.4	0.4	33.4 ± 3.8
		OP	8/8 100%	8/8 100%	N	31.8	0.3	31.8 ± 1.8
					RdRp	33.8	0.7	33.8 ± 1.9
Mucin (60ug/ml)	-	Sputum	4/4 100%	4/4 100%	N	32.2	0.3	32.2 ± 0.3
					RdRp	33.3	0.4	33.3 ± 0.6
		NP	4/4 100%	4/4 100%	N	31.8	0.1	31.8 ± 0.1
					RdRp	33.2	0.4	33.2 ± 0.5
		OP	4/4 100%	4/4 100%	N	31.6	0.6	31.6 ± 0.8
					RdRp	34.2	0.5	34.2 ± 0.6
Blood (5% v/v)	60 g/L	Sputum	4/4 100%	4/4 100%	N	32.1	0.5	32.1 ± 0.7
					RdRp	33.1	0.1	33.1 ± 0.2
		NP	4/4 100%	4/4 100%	N	31.7	0.2	31.7 ± 0.3
					RdRp	33.2	0.3	33.2 ± 0.4
		OP	4/4 100%	4/4 100%	N	32.1	0.3	32.1 ± 0.4
					RdRp	34.0	0.7	34.0 ± 0.6
Oxymeta-zoline 0.05% (v/v)	0.2 g/L	Sputum	4/4 100%	4/4 100%	N	32.5	1.0	32.5 ± 0.9
					RdRp	34.6	1.6	34.6 ± 1.7
		NP	4/4 100%	4/4 100%	N	32.1	0.3	32.1 ± 0.4
					RdRp	33.6	0.4	33.6 ± 0.3
		OP	4/4 100%	4/4 100%	N	33.0	0.2	33.0 ± 0.2
					RdRp	34.7	1.0	34.7 ± 1.5
Zanamivir 5mg/ml	0.2 g/L	Sputum	4/4 100%	4/4 100%	N	32.5	1.4	32.5 ± 1.4
					RdRp	34.0	1.3	34.0 ± 1.7
		NP	4/4 100%	4/4 100%	N	32.7	1.4	32.7 ± 1.7
					RdRp	35.3	1.5	35.3 ± 1.4
		OP	4/4 100%	4/4 100%	N	33.1	1.1	33.1 ± 1.4
					RdRp	34.9	1.1	34.9 ± 1.4
Oseltamivir 75mg/ml	2 mg/L	Sputum	4/4 100%	4/4 100%	N	32.5	1.0	32.5 ± 1.4
					RdRp	34.4	1.3	34.4 ± 1.5
		NP	4/4 100%	4/4 100%	N	32.4	0.7	32.4 ± 0.8
					RdRp	33.8	1.0	33.8 ± 1.5
		OP	4/4 100%	4/4 100%	N	33.2	0.2	33.2 ± 0.3
					RdRp	34.6	0.3	34.6 ± 0.4

[1] *C = Concentration*

4) Clinical Evaluation:

Collection and storage of clinical specimens, and initial clinical testing were performed in the Seoul Clinical Laboratories (SCL). The SCL is a client-centered consignment agency in Korea that specializes in diagnostic testing and procures clinical specimens from hospitals and certified lab by Korea CDC.

One hundred twenty (120) retrospective, de-identified clinical samples were tested, of which 60 (20 positive and 40 negative) were lower respiratory tract samples (sputum, pretreated with 4% NaOH per procedure described above) and 60 (20 positive and 40 negative) were upper respiratory tract samples. Upper respiratory sample were oropharyngeal (OP) and nasopharyngeal (NP) swabs that

derived from the same patient and were combined into one VTM tube. Samples were stored at -70ºC inside the deep freezer. After collection samples were extracted by QIAamp DSP Viral RNA Mini Kit and tested with the BioCore 2019-nCoV Real Time PCR Kit (BioCore Co., Ltd. Biotechnology Division) and a comparator device (the Allplex 2019-nCoV Assay from Seegene Inc., EUA authorized by FDA (US) and Korea Authority (MFDS) using the CFX96 Dx System (Bio-rad)). Testing was performed at the Seoul Clinical Laboratories and test results are summarized in Table 35.

Table 35. Results classified with clinical specimen

Sputum		**Comparator assay (Allplex 2019-nCoV Assay)**	
		POSITIVE	NEGATIVE
BioCore 2019-nCoV RT PCR Kit	POSITIVE	20	0
	NEGATIVE	0	40
Positive Percent Agreement: 20/20 = 100% (95% CI: 83.89%-100.00%) **Negative Percent Agreement: 40/40 = 100%** (95% CI: 91.24%-100.00%)			
Combined Oropharyngeal swabs & Nasopharyngeal swabs		**Comparator assay (Allplex 2019-nCoV Assay)**	
		POSITIVE	NEGATIVE
BioCore 2019-nCoV RT PCR Kit	POSITIVE	20	0
	NEGATIVE	0	40
Positive Percent Agreement: 20/20 = 100% (95% CI: 83.89%-100.00%) **Negative Percent Agreement: 40/40 = 100%** (95% CI: 91.24%-100.00%)			

Note: Please refer to Line data that described all replicated test with Ct values for each target. Excel file: Line data_BioCore_2020.05.15, Sheet name: Clinical specimens

Performance was estimated as 100% PPA and 100% NPA for upper and lower respiratory specimen types

No false positive and false negative samples were observed with the 'BioCore 2019-nCoV Real Time PCR Kit'.

K. UNMET NEED ADDRESSED BY THE PRODUCT

Reports of undiagnosed pneumonia cases linked to a seafood market in Wuhan City, Hubei Province, China first appeared in early December 2019. The cause of the respiratory illness was determined in early January 2020 to be a novel (new) coronavirus (originally named "2019-nCoV" – later renamed to SARS-CoV-2), that has continued to expand both within China and Internationally. Cases of COVID-19 have now been identified in over 150 countries. Sustained community spread (human-to-human transmission) of SARS-CoV-2 has also been reported in the United States and globally.

On January 30, 2020, the International Health Regulations Emergency Committee of the World Health Organization declared the outbreak a "public health emergency of international concern" (PHEIC). On January 31, 2020, Health and Human Services Secretary Alex M. Azar II declared a public health emergency (PHE) for the United States to aid the nation's healthcare community in responding to 2019-nCoV. Also, on January 31, the President of the United States signed a presidential "Proclamation on Suspension of Entry as Immigrants and Nonimmigrants of Persons who Pose a Risk of Transmitting 2019 Novel Coronavirus".

Most patients with confirmed 2019-nCoV infection appear to develop fever and/or symptoms of acute respiratory illness (e.g., cough, difficulty breathing). However, limited information is currently available to characterize the full spectrum of clinical illness associated with 2019-nCoV infection. Signs and symptoms may appear any time from 2 to 14 days after exposure to 2019-nCoV virus. Based on preliminary data, the median incubation period is approximately 4 days.

The WHO characterized COVID-19 as a pandemic on March 11, 2020. The WHO COVID-19 Situation Report #119 (May 18, 2020) documented a global total of over 4,618,821 cases confirmed cases, with 311,847 deaths. According to the Centers for Disease Control and Prevention (CDC), as of May 18, 2020, there have been 1,480,349 cases in the United States, with 89,407 deaths. Laboratories in the United States therefore need diagnostic tools for use in the COVID-19 emergency for rapid diagnosis of acute COVID-19.

On February 4, 2020, pursuant to section 564(b)(1)(C) of the Act, the Secretary of the Department of Health and Human Services (HHS) determined that there is a public health emergency that has a significant potential to affect national security or the health and security of United States citizens living abroad, and that involves the COVID-19. Pursuant to section 564 of the Act, and on the basis of such determination, the Secretary of HHS then declared that circumstances exist justifying the authorization of the emergency use of in vitro diagnostics for detection and/or diagnosis of COVID-19 subject to the terms of any authorization issued under section 564(a) of the Act.

This Real-Time Fluorescent RT-PCR Kit for Detecting SARS-2019-nCoV can augment clinically important laboratory evidence for individuals who meet COVID-19 clinical and/or epidemiological criteria for testing.

Under emergency use authorization (EUA), BioCore Co., Ltd. plans to distribute the BioCore 2019-nCoV Real Time PCR Kit for the detection of nucleic acid from SARS-CoV-2 in upper respiratory specimens (nasal swabs, nasopharyngeal swabs [i.e., throat swabs] and oropharyngeal swabs) collected from individuals who meet COVID-19 clinical and/or epidemiological criteria for testing. This EUA request for the BioCore 2019-nCoV Real Time PCR Kit is intended to expand domestic readiness within the United States and its territories by expanding diagnostic testing capabilities for COVID-19 during public health emergency.

FDA consulted with subject matter experts within HHS on the public health needs for diagnostic devices to detect SARS-CoV-2 nucleic acid. It is FDA's conclusion that there currently exists a public health need for such devices, i.e., that there is no adequate, approved (cleared), and available alternative to the BioCore 2019-nCoV Real Time PCR Kit for COVID-19 detection during the public health emergency.

L. APPROVED/CLEARED ALTERNATIVE PRODUCTS

Currently no methods for the detection of the SARS-CoV-2 have been approved/ cleared by FDA.

M. BENEFITS AND RISKS:

Risks

The BioCore 2019-nCoV Real Time PCR Kit has been designed to minimize the likelihood of false test results. However, should false results occur, they may present risks to patients:

- False positive result:
  - A false positive result in the context of the current public health emergency could lead to misallocation of resources used for surveillance and prevention.
  - A false positive result could delay correct diagnosis and initiation of appropriate treatment for the actual cause of patient illness.
- False negative result:
  - Although a negative result does not rule out 2019-nCoV infection, a false negative has the potential to result in initiation of inappropriate treatment and a delay in correct diagnosis.
  - A false negative result can trigger a waste of health care resources, as additional evaluations are pursued in the effort to establish the true diagnosis.
  - A false negative result can contribute to further spread of the disease.

Benefits

The primary benefit of distributing the BioCore 2019-nCoV Real Time PCR Kit for clinical use is that it provides a relatively fast diagnostic method for symptomatic patients, which enables faster intervention.

- True positive test results provide support for the diagnosis of a 2019-nCoV infection. The results can be used in conjunction with clinical and epidemiological information to guide patient management.

- Distribution of the factsheets can help healthcare professionals and the public learn more about 2019-nCoV disease and how to avoid acquiring it.

Risk Assessment

- Negative results may not preclude a 2019-nCoV infection. It is emphasized that the BioCore 2019-nCoV Real Time PCR Kit should not be used as the sole basis for treatment or other patient management decisions. The clinical features of the illness and the type and risk of exposure must be considered pivotal to making patient management decisions. A negative test should not be interpreted as definitive, if other aspects of the patient's clinical presentation or recent epidemiologic exposures indicate such is likely, and diagnostic tests for other diseases are negative.
- False negative results due to failure of PCR are minimized by the presence of controls that monitor nucleic acid extraction and PCR efficiency.
- Risk of patient discomfort or harm during sample collection is minimal.

Risk-Benefit Assessment

The risks posed by use of the BioCore 2019-nCoV Real Time PCR Kit are mitigated by:

- Use of the BioCore 2019-nCoV Real Time PCR Kit in conjunction with other laboratory, epidemiological, and clinical evaluation tools
- Distribution of Healthcare Provider and Patient fact sheets.
- Post-market studies/reporting or conditions of Authorization.

Based on these factors, the potential benefits from the use of the BioCore 2019-nCoV Real Time PCR Kit are expected to outweigh the risks.

N. FACT SHEET FOR HEALTHCARE PROVIDERS AND PATIENTS:

Fact Sheets for Patients and Healthcare Providers - *Attached.*

O. INSTRUCTIONS FOR USE/ PROPOSED LABELING/PACKAGE INSERT:

Instructions for Use:	Instructions for Use; BioCore 2019-nCoV Real Time PCR Kit (Attached)
Box Labels:	EUA200339.Labels BioCore 2019-nCoV RT-2020.05.15- (Attached)

P. RECORD KEEPING AND REPORTING INFORMATION TO FDA:

BioCore Co., Ltd. will track adverse events and report to FDA under 21 CFR Part 803. A website is available to report on adverse events, and this website is referenced in the Fact Sheet for Health Care providers as well as through the ***BioCore Co., Ltd.*** Product Support website: ***http://www.bio-core.com***. Each report of an adverse event

will be processed according to ***BioCore***'s Non-Conformance Reporting Requirements, and Medical Device Reports will be filed with the FDA as required. Through a process of inventory control, ***BioCore Co., Ltd.*** will also maintain records of device usage/purchase. ***BioCore Co., Ltd.*** will collect information on the performance of the test, and report to FDA any suspected occurrence of false positive or false negative results of which ***BioCore Co., Ltd.*** becomes aware. ***BioCore Co., Ltd.*** will maintain records associated with this EUA and ensure these records are maintained until notified by FDA. Such records will be made available to FDA for inspection upon request.

Q. FDA ADMINISTRATIVE INFORMATION:

This request for emergency use authorization EUA200339 was received by the Division on 4/20/2020. Initial feedback was provided to the sponsor by email on May 4th 2020. The initial submission had significant issues. Interactive correspondence is captured in the submissions Docman folder.

Interactive Correspondence History

Email 5/04/2020:	Initial feedback provided to firm with multiple requests
Email 5/06/2020:	Sponsor sent updated template and line data with some requests addressed
Email 5/08/2020:	Lead Reviewer provided a second feedback to the firm
Email 5/13/2020:	Sponsor provided updated template and revised line data
Email 5/14/2020:	Sponsor provided up IFU
Email 5/13/2020:	Lead reviewer provided some minor comments and questions in the template, as well as feedback on the IFU, and test Kit Labels
Email 5/15/2020:	Sponsor provided updated documents
Emails 5/19/2020:	Several emails were exchanged between the lead reviewer and the sponsor to finalize the Instructions for Use.

Issue Summary:

All of the following issues were successfully resolved:

- Clinical Study: contrived only; clinical data was submitted
- LoD: LoD information on study was insufficient to understand the study

Final Conclusions for Interactive Review:

It is FDA's conclusion that there currently exists a public health need for such devices, i.e., that there is no adequate, approved (cleared), and available alternative to the BioCore 2019-nCoV Real Time PCR Kit for the novel SARS-CoV-2 that causes coronavirus disease 2019 COVID-19 detection during the public health emergency. Based on the review of the provided information I recommend authorization of the EUA submission (EUA200339).