STANDARD M nCoV Real-Time Detection kit

For Use Under the Emergency Use Authorization (EUA) Only

Instructions for Use (IFU)

IVD	**For *in vitro* diagnostic use**
Rx only	**For prescription use only**
EUA	**For Use only under Emergency Use Authorization**
REF	**M-NCOV-01**
Σ	**96 Tests**
	-25℃ ~ -15℃
	SD BIOSENSOR, Inc. Jeungpyeongsandan-Ro, Jeungpyeong-Eup Jeungpyeong-Gun Chungbuk, KR 27915, REPUBLIC OF KOREA

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1. Introduction

Coronavirus is a single-stranded positive-sense RNA virus with an envelope of about 80 to 120 nm in diameter. Its genome is the largest of all RNA viruses and is an important pathogen of many domestic animals, pets, and human diseases. It can cause a variety of acute and chronic diseases. Common signs of a person infected with a coronavirus include respiratory symptoms, fever, cough, shortness of breath, and dyspnea. In more severe cases, infection can cause pneumonia, severe acute respiratory syndrome, kidney failure, and even death. The 2019 new coronavirus, or "2019-nCoV", was discovered because of Wuhan Viral Pneumonia cases in 2019, and was named by the World Health Organization on January 12, 2020, confirming that it can cause colds and the Middle East Respiratory Syndrome (MERS) and more serious diseases such as acute respiratory syndrome (SARS). This kit is helpful for the auxiliary diagnosis of coronavirus infection. The test results are for clinical reference only and cannot be used as a basis for confirming or excluding cases alone.

2. Intended Use

The STANDARD M nCoV Real-Time Detection kit is a real-time reverse transcription-PCR assay for the qualitative detection of SARS-CoV-2 nucleic acids in nasopharyngeal, oropharyngeal, anterior nasal, and mid-turbinate nasal swab, and sputum specimens from individuals who are suspected of COVID-19 by their healthcare provider. Testing is limited to laboratories certified under the Clinical Laboratory Improvement Amendments of 1988 (CLIA), 42 USC §263a, that meet requirements to perform high complexity tests.

Results are for the identification of SARS-CoV-2 RNA. The SARS-CoV-2 RNA is generally detectable in upper respiratory specimens during the acute phase of infection. Positive results are indicative of the presence of SARS-CoV-2 RNA; clinical correlation with patient history and other diagnostic information is necessary to determine patient infection status. Positive results do not rule out bacterial infection or co-infection with other viruses. The agent detected may not be the definite cause of disease. Laboratories within the United States and its territories are required to report all results to the appropriate public health authorities.

Negative results do not preclude SARS-CoV-2 infection and should not be used as the sole basis for patient management decisions. Negative results must be combined with clinical observations, patient history, and epidemiological information.

The STANDARD M nCoV Real-Time Detection kit 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 STANDARD M nCoV Real-Time Detection kit is only for use under the Food and Drug Administration’s Emergency Use Authorization.

3. Principle of the Procedure

STANDARD M nCoV Real-Time Detection kit is designed according to "WHO interim guidance for laboratory testing for 2019 novel coronavirus (2019-nCoV) in humans". This kit is based on TaqMan probe real-time fluorescent PCR technology. Coronavirus RNA is first transcribed into cDNA by reverse transcriptase, and then cDNA is used as a template for PCR amplification. During the PCR reaction, the 5'→ 3' polymerase activity of Taq DNA polymerase and exo-nuclease are simultaneously used. Dicer activity, which causes the degradation of the TaqMan probe, and the separation of the fluorophore and quencher makes the fluorescence signal detectable by the instrument: FAM channel qualitative detection of the new coronavirus (2019-nCoV) ORF1ab (RdRp) gene, JOE (VIC or HEX) channel qualitative detection of the coronavirus E gene, and CY5 channel detection internal reference. The kit uses dUTP and UNG enzymes to prevent contamination of amplification products.

Target	Channel
ORF1ab (RdRp) gene	FAM
E gene	JOE (VIC or HEX)
Internal control (IC)	CY5

4. Kit Contents

This kit is used for 96 test / kits. The kit contents are as follows;

	Reagent	Quantity	Volume in each reaction
1	2019-nCoV Reaction Solution	750㎕/vial x 2	14 µl
2	RTase Mix	630㎕/vial x 1	6 µl
3	2019-nCoV Positive control	600㎕/vial x 1	-
4	Negative control	600㎕/vial x 1	-
5	Internal control	525㎕/vial x 1	5 µl (as extraction control) 0.5 µl (as internal control)
6	ROX	55㎕/vial x 1	0.5 µl
7	Instructions for use	1	-

①	②	③	④	⑤	⑥
					

5. Storage and Stability Conditions

1. The kit should be shipped and stored at the temperature of -25°C(-13℉) to -15°C(5℉).
2. The components of 2019-nCoV Reaction Solution and Rox should be stored away from light.
3. Kit materials are stable until the expiration date printed on the outer packaging.
4. Freeze-thawing of kit components more than 5 times may lead to inaccurate results.
5. Dispose of unused reagents and waste in accordance with country, federal, state, and local regulations.

6. Compatible Instruments

- CFX96™ Dx System (S/W version 3.1, Bio-Rad)
- Applied Biosystems 7500 Real-Time PCR Instrument System (S/W version 2.0.6, Thermo Fisher Scientific)

7. Additionally Required Materials and Equipment

- Disposable latex gloves
- Sterilized filtered pipette tips
- Pipettes
- Nuclease-free micro centrifuge tube (1.5ml)
- Nucleic acid extraction kit (QIAamp® Viral RNA Mini Kit, Qiagen, Cat.no. 52904)

- Vortex mixer
- Desktop centrifuge
- Clean bench
- RNAse neutralizing agent
- Flake or snow type ice
- Thermal cycler
- 0.2ml PCR strips, plate (DNase, RNase free) and cap or sealing film for each Real-time PCR equipment
- PPE (Personal Protective Equipment)
- Biohazard waste container

8. Description of Symbols

Symbol	Description
	*In vitro* diagnostics
Rx ONLY	For prescription use only
	For Use only under Emergency Use Authorization
	Reference number
	Contains sufficient for <n> tests
	Storage temperature
	Manufaturer
	Expiration date
	Lot (Batch) number
	Date of Manufacture
	Caution
	Instructions for use

9. Warnings and Precautions

- For In Vitro Diagnostic Use Under the FDA Emergency Use Authorization.
- For Prescription Use Only.
- This product has not been FDA cleared or approved, but has been authorized for emergency use by FDA under an EUA for use by authorized laboratories; for use by laboratories certified under CLIA, to perform high complexity tests.
- This product has been authorized only for the detection of nucleic acid from SARS-CoV-2, not for any other viruses or pathogens; and

- The emergency use of this product is only authorized for the duration of the declaration that circumstances exist justifying the authorization of emergency use of in vitro diagnostics for detection and/or diagnosis of COVID-19 under Section 564(b)(1) of the Federal Food, Drug and Cosmetic Act, 21 U.S.C. § 360bbb-3(b)(1), unless the declaration is terminated or authorization is revoked sooner.
- Please read the instructions for use carefully before testing.Do not eat, drink, smoke, apply cosmetics or handle contact lenses in areas where reagents and human specimens are handled.
- Handle all specimens as if infectious using safe laboratory procedures. Refer to Interim Laboratory Biosafety Guidelines for Handling and Processing Specimens Associated with 2019-nCoV https://www.cdc.gov/coronavirus/2019-nCoV/lab-biosafety-guidelines.html. Dispose of hazardous or biologically contaminated materials according to the practices of your institution.
- Improper specimen collection, transfer, storage, and processing may cause erroneous test results.
- Nucleic acid extraction should be performed as soon as possible after specimen collection to avoid viral nucleic acid degradation; if it cannot be performed as soon as possible, it should be stored in accordance with SPECIMEN COLLECTION AND PREPARATION.
- After the operation of the nucleic acid extraction instrument, the used consumables should be sealed. After the instrument is cleaned, turn on the UV lamp for 30 minutes.
- As this test involves extraction of viral RNA and PCR amplification, care should be taken to avoid contamination of the amplification reaction mixture of the kit. Regular monitoring of laboratory contamination is recommended.
- Clinical laboratories should be equipped with equipment and operators in strict accordance with the "Code of Practice for Clinical Gene Amplification Laboratories."
- When using this kit, it should be operated strictly in accordance with the instructions; the specimen processing and specimen addition steps must be performed in a biological safety cabinet or other basic protective facilities, and follow the technical requirements of the clinical gene amplification laboratory.

10. Reagent Handling

- Handle all reagents, controls, and samples according to good laboratory practice in order to prevent carryover of samples or controls.
- Before use, visually inspect each reagent tube to ensure that there are no signs of leakage. If there is any evidence of leakage, do not use that material for testing.
- STANDARD M nCoV Real-Time Detection kit Reagent contains guanidine thiocyanate, a potentially hazardous chemical. Do not allow the reagents to come into contact with bleach (sodium hypochlorite) solution because this can cause production of a highly toxic gas.
- Avoid contact of reagents with the skin, eyes, or mucous membranes. If contact does occur, immediately wash with generous amounts of water; otherwise, burns can occur. If these reagents are spilled, dilute with water before wiping dry.
- Dispose of all materials that have come in contact with samples and reagents in accordance with country, state, and local regulations.

11. Good Laboratory Practice

- Do not pipette by mouth.
- Do not eat, drink, or smoke in designated work areas.
- Wear laboratory gloves, laboratory coats, and eye protection when handling samples and reagents. Gloves must be changed between handling samples and STANDARD M nCoV Real-Time Detection kits. Prevent contamination. Avoid contaminating gloves when handling samples and controls.
- Wash hands thoroughly after handling samples and kit reagents, and after removing the gloves.
- Thoroughly clean and disinfect all laboratory work surfaces with a freshly prepared solution of 0.5% sodium hypochlorite in distilled or deionized water (dilute household bleach 1:10). Follow by wiping the surface with 70% ethanol.

12. Procedure

12.1. Specimen collection and preparation

Refer to CDC Interim Guidelines for Collecting, Handling, and Testing Clinical Specimens from Persons Under Investigation (PUIs) for Coronavirus Disease 2019 (COVID-19) (https://www.cdc.gov/coronavirus/2019-nCoV/lab/guidelines-clinical-specimens.html)

[Nasopharyngeal swab]

1. Hold the nasopharyngeal swab close to the nasal septum slowly and deeply to the back of the nasopharynx.
2. Rotate it several times to obtain secretions.
3. Quickly dip the swab into the specimen collection tube, and discard the tail.
4. Tighten the tube cap to seal in case of drying.
5. The swab specimens to be tested can be stored for 1 day at room temperature, 4 days at 2-8°C, and long-term storage below -20° C.
6. If delivery and processing exceed 48 hours, specimens should be transported in dry ice and once in laboratory frozen at -70 °C or colder.

[Oropharyngeal swab]

1. Use moderate swab to wipe the posterior wall of the pharynx and the tonsils on both sides avoiding touching the tongue.
2. Quickly dip the swab into the specimen collection tube, and discard the tail.
3. Tighten the tube cap to seal in case of drying.
4. The swab specimens to be tested can be stored for 1 day at room temperature, 4 days at 2-8°C, and long-term storage below -20°C.
5. If delivery and processing exceed 48 hours, specimens should be transported in dry ice and once in laboratory frozen at -70 °C or colder.

[Nasal mid-turbinate (NMT) swab, also called Deep Nasal Swab]

1. Use a flocked tapered swab. Tilt patient's head back 70 degrees. While gently rotating the swab, insert swab less than one inch (about 2 cm) into nostril (until resistance is met at turbinates).
2. Rotate the swab several times against nasal wall and repeat in other nostril using the same swab.
3. Quickly dip the swab into the specimen collection tube, and discard the tail.
4. Tighten the tube cap to seal in case of drying.
5. The swab specimens to be tested can be stored for 1 day at room temperature, 4 days at 2-8°C, and long-term storage below -20°C.
6. If delivery and processing exceed 48 hours, specimens should be transported in dry ice and once in laboratory frozen at -70 °C or colder.

[Anterior nares specimen (NS)]

1. Using a flocked or spun polyester swab, insert the swab at least 1 cm (0.5 inch) inside the nares and firmly sample the nasal membrane by rotating the swab and leaving in place for 10 to 15 seconds. Sample both nares with same swab.
2. Quickly dip the swab into the specimen collection tube, and discard the tail.
3. Tighten the tube cap to seal in case of drying.
4. The swab specimens to be tested can be stored for 1 day at room temperature, 4 days at 2-8°C, and long-term storage below -20°C.
5. If delivery and processing exceed 48 hours, specimens should be transported in dry ice and once in laboratory frozen at -70 °C or colder.

[Sputum]

1. Collect sputum specimen by inducing a cough into a sterile container.

2. Specimens should be taken carefully to avoid contamination and completely sealed to prevent leakage during transportation (Triple packaging).
3. The sputum specimens to be tested can be stored for 1 day at room temperature, 4 days at 2-8°C, and long-term storage below -20°C.
4. If delivery and processing exceed 48 hours, specimens should be transported in dry ice and once in laboratory frozen at -70 °C or colder.

Specimens must be packaged and transported in accordance with the International Air Transport Association (IATA) Dangerous Goods Regulation. Follow shipping regulations for UN 3373 Biological Substance, Category B when sending potential SARS-CoV-2 specimens

12.1. Assay Protocol

[Nucleic Acid Extraction]

The QIAamp Viral RNA mini kit (QIAGEN, Cat. No. 52904) is used for nucleic acid extraction of specimens and reference materials.

1. The specimen volume required for nucleic acid extraction is 200㎕.
2. Add 5㎕ of internal control A to each specimen to be extracted (including positive and negative controls)
3. After the nucleic acid extraction is completed, each eluent should be added to a reaction well immediately.

[Reagent Preparation]

1. CFX96™Dx System
   Prepare the PCR mixture according to the table below for N reactions plus the PC and NC and dispense 20㎕ into each PCR reaction tube.

#	Reagents	Dosage in each reaction
1	2019-nCoV Reaction Solution	N x 14㎕
2	RTase Mix	N x 6㎕
Total volume/well		20㎕

2. Applied Biosystems 7500 Real-Time PCR Instrument System

Prepare the PCR mixture according to the table below and dispense 20.5㎕ into each PCR reaction tube.

	Reagents	Dosage in each reaction
1	2019-nCoV Reaction Solution	N x 14㎕
2	RTase Mix	N x 6㎕
3	ROX	N x 0.5㎕
Total volume/well		20.5㎕

[RT-PCR Amplification]

1. Add 10㎕ of each of the negative control, positive control, and patient sample nucleic acid extract to the PCR mixture dispensed in each reaction tube. To the positive and negative control wells, add 0.5㎕ of Internal Control A.
2. Centrifuge at low speed for a few seconds, and place them on the real-time fluorescence quantitative PCR instrument.
3. Set the cycle conditions below on the PCR instrument.

Reaction	Temp. (℃)	Time	Cycle
Reverse transcription	50℃	15 minutes	1
Initial denaturation	95℃	3 minutes	1
Pre-amplification	95℃	5 seconds	5
	60℃	40 seconds	
Amplification	95℃	5 seconds	40
	60℃	40 seconds	
	Collect the signals (FAM/JOE*/Cy5)		

* JOE/VIC/HEX

NOTE In the software operation interface of the Applied Biosystems 7500 real-time PCR instrument, select "ROX" from the Passive Reference pull-down menu.

[Interpretation of Results]

Assessment of clinical specimen test results should be performed after the positive and negative controls have been examined and determined to be valid. If the controls are not valid, the patient results cannot be interpreted.

Open the experiment data with the analysis software and perform the Ct analysis according to the instrument manual. See the table below for the Ct cut-off for each fluorescent channel.

Target	Ct Value	Interpretation
ORF1ab gene (FAM)	Ct≤36	2019-nCov ORF1ab (RdRp) gene positive
E gene (JOE/VIC/HEX)	Ct≤36	Coronavirus E gene positive
IC (CY5)	Ct≤26	Internal control positive

Refer to the table below for the validity and the interpretation of each specimen result according to the results of each channel.

ORF1ab(RdRp) (FAM)	E gene (JOE/VIC/HEX)	IC (Cy5)	Interpretation	Action to be taken
Positive	Positive or Negative	Positive or Negative	• SARS-CoV-2 Positive	Report results to sender and appropriate health authority.
Negative	Positive	Positive or Negative	• SARS-CoV-2 Presumptive Positive.	The extracted RNA should be retested. If residual RNA is not available, re-extract RNA from residual specimen and re-test. If the repeated result remains "PRESUMPTIVE POSITIVE", additional confirmatory testing may be conducted, if it is necessary to differentiate between SARS-CoV-2 and other SARS-like viruses for epidemiological purposes or clinical management.
Negative	Negative	Positive	• SARS-CoV-2 Negative	Report results to sender
Negative	Negative	Negative	• Invalid	The extracted RNA should be retested. If residual RNA is not available, re-extract RNA from residual specimen and re-test. If a second failure occurs, the result is reported to the sender as invalid with a recommendation to recollect if clinically indicated.

NOTE If the target gene signal (FAM, JOE/VIC/HEX) is strong, the CY5 (IC) may be negative.

13. Quality Control

All test controls should be examined prior to interpretation of patient results. If the controls are not valid, the patient results cannot be interpreted. A negative control and a positive control should be set for each batch.

The internal control A is a pseudovirus that contains RNA target, detected by the IC primers/probe set in the STANDARD M nCoV Real-Time Detection kit. Internal control A can be added to patient specimens, prior to extraction, to serve as a total process control The internal control A should also be added to the positive control tube and negative control tube to confirm RT-PCR amplification in each tube. Check the instrument, reagents and amplification conditions for errors and repeat the experiment.

Control	QC requirements		
	ORF1ab(RdRp) gene	E gene	IC

	(FAM)	(JOE/VIC/HEX)	(Cy5)
2019-nCoV Positive control	Ct≤26	Ct≤26	Ct≤26
Negative control	Ct>36.	Ct>36.	Ct≤26
Extraction control* (Internal control A)	-	-	Ct≤26

* If IC is only used without specimen as extraction control, Ct value of ORF1ab(RdRp) gene and E gene do not appear.

14. Limitations of the Kit Protocols

This product has not been FDA cleared or approved, but has been authorized for emergency use by FDA under an Emergency Use Authorization (EUA) for use by laboratories certified under the Clinical Laboratory Improvement Amendments of 1988 (CLIA), 42 U.S.C. § 263a, to perform high complexity tests.

Use of the STANDARD M nCoV Real-Time Detection kit is limited to personnel who are trained in the procedure. Failure to follow these instructions may result in erroneous results.

Laboratories are required to report all results to the appropriate public health authorities.

The performance of this test was established based on the evaluation of a limited number of clinical specimens. Clinical performance has not been established with all circulating variants but is anticipated to be reflective of the prevalent variants in circulation at the time and location of the clinical evaluation. Performance at the time of testing may vary depending on the variants circulating, including newly emerging strains of SARS-CoV-2 and their prevalence, which change over time.

Specimens 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 specimens 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:

- o Improper specimen collection
- o Degradation of the viral RNA during shipping/storage
- o Using unauthorized extraction or assay reagents
- o The presence of RT-PCR inhibitors
- o Mutation in the SARS-CoV-2 virus
- o Failure to follow instructions for use

False-positive results may arise from:

- o Cross contamination during specimen handling or preparation
- o Cross contamination between patient samples
- o Specimen mix-up
- o RNA contamination during product handling

The effect of vaccines, antiviral therapeutics, antibiotics, chemotherapeutic or immunosuppressant drugs have not been evaluated.

Negative results do not preclude infection with SARS-CoV-2 virus and should not be the sole basis of a patient management decision.

A positive result indicates the detection of nucleic acid from SARS-CoV-2.

Nucleic acid may persist even after the virus is no longer viable.

Based on the in silico analysis, SARS-CoV may cross-react with the STANDARD M nCoV Real-Time Detection kit. SARS-CoV is not known to be currently circulating in the human population, therefore is highly unlikely to be present in patient specimens.

15. Conditions of Authorization for Laboratories

The STANDARD M nCoV Real-Time Detection kit assay's Letter of Authorization, along with the authorized Fact Sheet for Healthcare Providers, and Labeling are available on FDA website: https://www.fda.gov/medical-devices/coronavirus-disease-2019-covid-19-emergency-use-authorizations-medical-devices/in-vitro-diagnostics-euas-molecular-diagnostic-tests-sars-cov-2#individual-molecular
To assist clinical laboratories using the STANDARD M nCoV Real-Time Detection kit, the relevant Conditions of Authorization are listed below.

a) Authorized laboratories[1] using your product must include with result reports of your product, all authorized Fact Sheets. Under exigent circumstances, other appropriate methods for disseminating these Fact Sheets may be used, which may include mass media.
b) Authorized laboratories using your product must use your product as outlined in the Authorized Labeling. Deviation from the authorized procedures, such as the authorized instruments, authorized extraction methods, authorized clinical specimen types, authorized control materials, authorized other ancillary reagents and authorized materials required to use your product are not permitted.
c) Authorized laboratories that receive your product must notify the relevant public health authorities of their intent to run your product prior to initiating testing.
d) Authorized laboratories using your product must have a process in place for reporting test results to healthcare providers and relevant public health authorities, as appropriate.
e) Authorized laboratories must collect information on the performance of your product and report to DMD/OHT7-OIR/OPEQ/CDRH (via email: CDRH-EUA-Reporting@fda.hhs.gov) and SD Biosensor(via email: sales@sdbiosensor.com) any suspected occurrence of false positive or false negative results and significant deviations from the established performance characteristics of your product of which they become aware.
f) All laboratory personnel using your product must be appropriately trained in RT-PCR techniques and use appropriate laboratory and personal protective equipment when handling this kit and use your product in accordance with the authorized labeling.
g) SD Biosensor, authorized distributors, and authorized laboratories using your product must ensure that any records associated with this EUA are maintained until otherwise notified by FDA. Such records will be made available to FDA for inspection upon request.

[1] The letter of authorization refers to, "laboratories certified under the Clinical Laboratory Improvement Amendments of 1988 (CLIA), 42 U.S.C. §263a, that meet the requirements to perform high complexity tests" as "authorized laboratories."

16. Analytical Performance Evaluation

16.1 Analytical Sensitivity - Limit of Detection (LoD)

The LoD study established the lowest concentration of SARS-CoV-2 (genome copies(cp)/μL) that can be detected by the STANDARD M nCoV Real-Time Detection kit at least 95% of the time. The LoD was established by testing twenty replicates of six different dilutions of SARS-CoV-2 viral genomic RNA spiked into both sputum and nasopharyngeal swab specimen (collected in UTM). The study results that are summarized in the tables below show that the LoD for the STANDARD M nCoV Real-Time Detection kit is 0.5 cp/μL for upper and lower respiratory specimens on the ABI 7500; 0.25 cp/μL for upper respiratory specimens and 0.125 cp/μL for lower respiratory specimens on the CFX95; and 0.5 cp/μL for upper respiratory specimens and 0.25 cp/μL for lower respiratory specimens on the LC480

Table 1. Results of the Analytical Sensitivity for the STANDARD M nCoV Real-Time Detection kit on the ABI 7500

Specimen type	Concentration (cp/uL)	ORF1ab Target			E Target		
		Positive replicates	mean Ct value	Standard Deviation	Positive replicates	mean Ct value	Standard Deviation
NP swabs	1	20/20	33.1	0.6	20/20	32.4	0.7
	0.5	20/20	33.7	0.7	20/20	34.0	0.8
	0.25	17/20	35.3	0.9	16/20	35.1	0.7
	0.125	10/20	35.3	0.6	12/20	35.4	0.8
	0.0625	9/20	35.6	0.4	9/20	36.0	0.4
	0.0312	2/20	35.5	0.7	2/20	36.5	0.6
Sputum	1	20/20	31.6	0.3	20/20	32.1	0.7
	0.5	20/20	31.5	0.3	20/20	31.2	0.3
	0.25	18/20	32.9	0.40	19/20	32.8	0.4
	0.125	13/20	34.1	0.8	18/20	33.8	0.6
	0.0625	8/20	35.2	0.6	13/20	35.0	0.8
	0.0312	3/20	35.6	0.4	11/20	35.6	0.6

Table 2. Results of the Analytical Sensitivity for the STANDARD M nCoV Real-Time Detection kit on the CFX96

Specimen type	Concentration (cp/uL)	ORF1ab Target			E Target		
		Positive replicates	mean Ct value	Standard Deviation	Positive replicates	mean Ct value	Standard Deviation
NP swabs	1	20/20	30.9	0.1	20/20	31.2	0.2
	0.5	20/20	32.2	0.3	20/20	32.3	0.3
	0.25	20/20	33.2	0.4	19/20	33.1	0.5
	0.125	18/20	34.3	0.8	18/20	34.1	0.6
	0.0625	16/20	34.5	0.7	15/20	34.9	0.7
	0.0312	11/20	35.3	0.8	8/20	35.8	0.5
Sputum	1	20/20	31.1	0.4	20/20	31.3	0.3
	0.5	20/20	32.1	0.3	20/20	31.9	0.4
	0.25	20/20	33.0	0.5	20/20	32.8	0.4
	0.125	19/20	34.4	0.6	17/20	34.3	0.9
	0.0625	14/20	35.3	0.5	16/20	35.0	0.5
	0.0312	10/20	35.6	0.6	12/20	35.8	0.6

16.2 Analytical Sensitivity - Reactivity/Inclusivity

In silico analysis conducted to the primers and probe showed that STANDARD M nCoV Real-Time Detection kit will detect all SARS-CoV-2 sequences in NCBI and GISAID databases. The sequences of in silico analysis is the full genome sequences of SARS-CoV-2 except partial sequence and miss reading sequence

Table 3. Results of In Silico analysis of ORF1ab(RdRp) primer/probe set

No. of Sequence ID analyzed	**NCBI =1084 and GISAID = 10,198**
No. of Sequence ID of 100% Homology	**11,282**
No. of Sequence ID of less than 100% Homology	**3**

The STANDARD M nCoV Real-Time Detection kit showed 100% homology to 11,279 out of 11,282 sequences against the ORF 1ab(RdRP) primers and probe set. The 3 mismatched sequences were confirmed to single nucleotide mismatch each.

Table 4. Results of In Silico analysis of E primer/probe set

No. of Sequence ID analyzed	**NCBI =1084 and GISAID = 10,198**
No. of Sequence ID of 100% Homology	**11,282**
No. of Sequence ID of less than 100% Homology	**4**

STANDARD M nCoV Real-Time Detection kit showed 100% homology to 11278 out of 11282 sequences against the E primers and probe set. The 4 mismatched sequences were confirmed to single nucleotide mismatch each.

On March 29, 2022, an *in silico* analysis was conducted to evaluate the effect of twelve SARS-CoV-2 variants on the performance of STANDARD M nCoV Real-time Detection Kit. The following variants were analyzed for mismatches against the STANDARD M nCoV Real-time Detection Kit primers and probes: Alpha (B1.1.7), Beta (B.1.351), Gamma (P.1), Delta (B.1.617.2), Lambda C.37), Mu (B.1.621), Kappa (B.1.617.1), Eta (B.1.525), Iota (B.1.526), Zeta (P.2), Epsilon (B.1.427), Epsilon (B.1.429), Omicron(B.1.1.529), Stealth Omicron(BA.2), Omicron(BA.3), Delta-Omicron(AY.4/BA.1). From this analysis, a single nucleotide mismatch in the forward primer for the ORF1ab target, associated with the Delta (B.1.617.2), Lambda (C.37) and Delta-Omicron(AY.4/BA.1) variants, was identified. Also a single nucleotide mismatch in the forward primer for the E target was found in the Omicron(B.1.1.529), Stealth Omicron(BA.2) and Omicron(BA.3) variants.

Additionally, an LoD study was performed by wet-testing serial dilutions of Delta(NCCP 43390) variant RNA ($7.9x10^7$ copies/ul) spiked into nasopharyngeal swab specimen matrix (collected in UTM). Samples were extracted using the QIAamp Viral RNA Mini Kit and tested on the CFX96 (Bio-Rad). The results show an LoD of 0.25 cp/μL (20/20 replicates detected) with a mean Ct of 34.10 for the ORF1ab target and an LoD of 0.125 cp/μL (19/20 replicates detected) with a mean Ct of 34.98 for the E target. Therefore, this mutation is not predicted to impact the performance of the STANDARD M nCoV Real-time Detection Kit.

A contemporary in silico analysis of inclusivity conducted in December 2022 showed no evidence of significant mismatches that are predicted to affect detection of circulating variants of SARS-CoV-2.

16.3 Analytical Specificity:

a) Cross-Reactivity: Cross-reactivity of the STANDARD M nCoV Real-Time Detection kit was evaluated both *in silico* analysis and by wet-testing whole organisms/viruses purchased from ATCC.

a-1) In Silico analysis

In silico analysis of the STANDARD M nCoV Real-Time Detection kit primers and probes was performed against the organisms and viruses listed in Table 5. The *in silico* analysis did not reveal greater than 80% homology to any of the primers and probes for the ORF1ab and E targets and does not predict cross-reactivity of the STANDARD M nCoV Real-Time Detection kit with the listed organisms and viruses.

Table 5. Organisms and viruses evaluated for cross-reactivity, by in silico analysis, against the primers and probes for SARS-CoV-2 from the STANDARD M nCoV Real-Time Detection kit.

Organism
Human coronavirus OC43
Human coronavirus HKU1
Human coronavirus 229E
Human coronavirus NL63
SARS-coronavirus
MERS-coronavirus
Parainfluenza virus 1
Parainfluenza virus 2
Parainfluenza virus 3
Parainfluenza virus 4
Enterovirus(EV68)
Mycobacterium tuberculosis
Bordetella pertussis
Pneumocystis jirovecii
Influenza C
Parechovirus
Corynebacterium diphtheriae
Neisseria elongata
Neisseria meningitidis
Pseudomonas aeruginosa
Staphylococcus epidermidis
Streptococcus salivarius
Leptospira interrogans
Chlamydia psittaci
Coxiella burnetii (Q-Fever)

***a*-2) Wet Testing**

The 22 organisms and viruses, listed in Table 6, were wet-tested for cross-reactivity with the STANDARD M nCoV Real-Time Detection kit. All organisms and viruses were tested by spiking the organism/virus into NP swab matrix mixed with lysis buffer at the concentrations listed in Table 6. Each organism/virus was tested for cross-reactivity, in triplicate, on the CFX96. No cross-reactivity was observed for the organisms and viruses tested.

Table 6. Organisms and viruses evaluated for cross-reactivity by wet-testing

No.	Category	Cross-reactivity substance	Specimen Info.	Spiking Concentration
1	Non 2019-nCoV coronavirus infections	MERS-CoV	ATCC VR-3248SD	1 x 10^5 copy/ml
		HCoV-229E	ATCC VR-740	1 x 10^6 PFU/mL
		HCoV-HKU1	ATCC VR-3262SD	
		HCoV-NL63	ATCC VT-3263SD	

		HCoV-OC43	ATCC VR-1558	
2	Non 2019-nCoV Viral infections	Influenza A virus	ATCC VR-1811	
		Influenza B virus	ATCC VR-1735	
		Respiratory syncytial virus	ATCC VR-1540	
		Rhinovirus	ATCC VR-284	
		Parainfluenza virus	ATCC VR-94	
		Adenovirus	ATCC VR-3	
3	Bacteria	*Legionella pneumophila*	ATCC 33152	1 x 10^6 CFU/mL
		Chlamydia pneumoniae	ATCC VR-2282	
		Mycoplasma pneumoniae	ATCC 15531	
		Haemophilus influenzae	ATCC 10211	
		Moraxella catarrhalis	ATCC 25240	
		Streptococcus pyogenes	ATCC 19615	
		Bowman Animal bacterium	ATCC 19606	
		Klebsiella pneumoniae	ATCC 13883	
		Pseudomonas aeruginosa	ATCC 27853	
		Streptococcus pneumoniae	ATCC 6301	
4	Pooled human nasal wash		Employee	5~50%

17. Clinical Performance Evaluation

The performance of the STANDARD M nCoV Real-Time Detection kit was evaluated in a clinical study using 30 retrospective nasopharyngeal specimens (NP) (collected in UTM) and 30 retrospective sputum samples collected from patients with signs and symptoms of a respiratory infection. All samples were extracted using the QIAamp Viral RNA Mini kit and were tested on the CFX96™ Dx System using the STANDARD M nCoV Real-Time Detection Kit and an EUA-authorized comparator. All samples were tested in randomized and blinded fashion. 53% of the tested sputum and 33% of the tested NPS clinical specimens were weak positive samples. The positive and negative percent agreements between the STANDARD M nCoV Real-Time Detection kit and the comparator are shown below for both sputum and NPS specimens:

Table 7. Result of clinical study

Nasopharyngeal swab		**EUA-authorized Comparator assay**	
		Positive	**Negative**
STANDARD M nCoV Real-time Detection Kit	Positive	15	0
	Negative	0	15
Positive Percent Agreement: 15/15 = 100 % (95% CI, 78.20 - 100.00%) Negative Percent Agreement: 15/15 =100 % (95% CI, 78.20 - 100.00%)			

Sputum		**EUA-authorized Comparator assay**	
		Positive	**Negative**
STANDARD M nCoV Real-time Detection Kit	Positive	15	0
	Negative	0	15
Positive Percent Agreement: 15/15 = 100 % (95% CI, 78.20 - 100.00%) Negative Percent Agreement: 15/15 =100 % (95% CI, 78.20 - 100.00%)			

PPA and NPA were both 100% for nasopharyngeal swabs and sputum. No false positive and false negative results were observed with the STANDARD M nCoV Real-Time Detection Kit.

18. Troubleshooting

1. If the PC and IC are invalid: check for the expiration date indicated on the box label due to the possibility of invalidity of the expiration date or for improper storage conditions.
2. If the IC is invalid: check the results of the other tubes to see if they have been added to the PCR mixture. If the target Ct is ≦ 25Ct, the IC may not be detected due to the overflow of the target amplicon.
3. If NC is invalid: this may be due to contamination of the workplace or the equipment, or improper storage.

19. Reference

1. Clinical management of severe acute respiratory infection when novel coronavirus (nCoV) infection is suspected.

Interim guidance. WHO.2020

2. Diagnostic detection of Wuhan coronavirus 2019 by real-time RT-PCR.2020
3. Diagnosis and treatment of pneumonia caused by new coronavirus (trial version 4) National Health Commission. 2020
4. CDC Interim Guidelines for Collecting, Handling, and Testing Clinical Specimens from Persons Under Investigation (PUIs) for Coronavirus Disease 2019 (COVID-19) https://www.cdc.gov/coronavirus/2019-nCoV/lab/guidelines-clinical-specimens.html