MiCo BioMed

VERI-Q

COVID-19 Multiplex Detection Kit

nCoV-OM / nCoV-QM

Cat. No. 7K107 / 7K108

The test has been validated,

but FDA's independent review of this validation is pending

℞Only

For *In Vitro* Diagnostic Use

Contents

1 Intended Use

This Real-time PCR kit is qualitatively detecting coronavirus disease 2019 (COVID-19) by using the extracted RNA from sputum, nasopharyngeal swab or oropharyngeal swab of suspected patients. This Kit is designed as a professional use In Vitro diagnostic medical device with experience in molecular diagnosis experiments. This test can help diagnose infections of individual suspected of coronavirus disease 2019. Consultation with a medical specialist is required for final diagnosis.
Calibration of the system is traceable to SARS-CoV-2 RNA NCCP 43326

2 Principle of the Procedure

Coronavirus disease 2019 detection kit is based on TaqMan detection method. TaqMan® chemistry is the key feature of detection system. TaqMan® probe contains a reporter fluorescent dye on the 5'-end and a quencher dye. The probe is designed to bind specific target sequence between forward and reverse primers. In every cycle, reporter dye is cleaved by binding to specific target and fluorescent intensity increased as a result. The intensity of fluorescence represents the amount of target genome in certain specimen.

3 Material Provided

3.1 Kit Contents & Volume

3.1.1 nCoV-QM

Mark	Components name	Model name	Volume	Quantity 100 test/kit	Description
MM	2X One-Step RT-PCR Master mix	nCoV-MMGR06BI2	500 µL	1	Polymerase, reverse transcriptase, buffer and stabilizer
PPM	Primer/Probe Mixture	nCoV-M-PPM	100 µL	1	Specific primer & probe mixture
PC	Positive Control	nCoV-PC	200 µL	1	Positive Control DNA
IPC	Internal Positive Control	nCoV-IPC	100 µL	1	Internal Positive Control DNA
DW	Nuclease Free Water	nCoV-DW	300 µL	1	Ultra-pure water

3.1.2 nCoV-OM

Mark	Components name	Model name	Volume	Quantity 100 test/kit	Description
MM	2X One-Step RT-PCR Master mix	nCoV-MMGR06BI2	500 µL	2	Polymerase, reverse transcriptase, buffer and stabilizer
PPM	Primer/Probe Mixture	nCoV-M-PPM	100 µL	1	Specific primer & probe mixture
PC	Positive Control	nCoV-PC	200 µL	1	Positive Control DNA
IPC	Internal Positive Control	nCoV-IPC	100 µL	1	Internal Positive Control DNA
DW	Nuclease Free Water	nCoV-DW	300 µL	1	Ultra-pure water

※ Please avoid light when storing or using the Primer & Probe Mixture.

※ This kit provides sufficient volume for 100 reactions when using 20 µL per reaction.

※ Please use "nCoV-QM" with "Veri-Q PCR 316 QD-P100".

※ Please use "nCoV-OM" with "CFX96 Real-time PCR Detection system" or "Applied Biosystems® 7500 Real-time PCR system".

3.2 Material Required but Not Provided

- 0.2 mL or 1.5 mL tube
- Micro pipette and sterilized pipette tips
- Table top centrifuge
- Powder-free gloves
- Heating block and Vortex mixer
- Clean bench and Bio Safety Cabinet (BSC)
- Ethanol and Isopropyl alcohol
- Veri-Q PCR 316 - LabChip (Cat. No. 8R002, MiCo BioMed. Co., Ltd. Korea)

3.2.1 Compatible methods for RNA extraction

Product	Description	Catalog No.
Veri-Q PREP M16 16TU-CV19	Manufacturer : MiCo BioMed Instrument : Veri-Q PREP M16(Cat No. 9S101)	7A133
Veri-Q ManuPrep Viral DNA/RNA kit	Manufacturer : MiCo BioMed Manual method	7A231
QIAamp MinElute Virus Spin Kit	Manufacturer : QIAGEN Manual method This kit can be on the QIAcube(Cat No. 9001293)	57704

3.2.2 Compatible instruments for Real-time PCR

Detection Kit	Compatible instruments	Description	Catalog No.
nCoV-OM	CFX96 Real-time PCR Detection system	-Manufacturer: Bio-rad -96-well format, five fluorescence detection	1845096
	Applied Biosystems® 7500 Real-time PCR system	-Manufacturer: Thermofisher -96-well format, five fluorescence detection	4345241
nCoV-QM	Veri-Q PCR 316 QD-P100	-Manufacturer: MiCo BioMed -LapChip 16 channel, four fluorescence detection	9R501

4 Warning and Precaution

Please read the instruction for use thoroughly before using the kit and check integrity of all components in the kit before use.

1) Use for in vitro diagnostic only.
2) This kit is optimized to use with recommended system and it couldn't guaranteed performance excepting the system.
3) This assay needs to be carried out by skilled personnel.
4) It couldn't guaranteed performance when used modified protocol.
5) All specimens should be handled as potentially high-harm factor and also it have to trash or disinfection after using of that.
   ***** The handling of high risk infectious materials should be accordant with the law of the relevant country.
6) Wear protective disposable glove, laboratory coat and eye protection goggle when handling specimens and kit reagent.
7) Do not eat, drink or smoke in laboratory areas.
8) Do not use the kit after its expiration date, stated on the label.
9) Do not mix reagents different lot or different tube of the same lot.
10) Avoid repeated thawing and freezing of the reagents because of this may reduce the sensitivity of the test.
11) All reagents have to be sufficiently thawed, mix well and centrifuge briefly before use.
12) Use always sterilized filter tip and recommend use of separating the pipette.
    ***** It should be handled carefully for preventing of contamination by positive control DNA.
    Use always calibrated equipment.
13) It should be disinfected table and the around after experiment.
14) The long-time light exposure of Primer & Probe Mixture should be protected because it can cause the damage of fluorescence property of probes.
15) In order to get the reasonable results, always use Positive control and Negative control.
16) After testing, all wastes should be processed with fulfillment of regulation of each country/region.
17) Do not expose the product to heat and keep it at the specified temperature, as there is a risk of performance degradation.
18) Laboratory Biosafety: Non-propagative diagnostic laboratory work (e.g. sequencing, NAAT) should be conducted at facilities and procedures equivalent to BSL-2
19) If any damage is found at Kit during shipment or before using, please contact the manufacturer or dealer.
20) Contact the manufacturer if the performance of the kit has changed.

5 Reagents Storage, Shelf life and Handling

5.1 Storage

The Kit should be stored at -20±5℃.

5.2 Shelf life

12 months after manufacturing / 20 days after opening.

5.3 Handling

All reagents should be handled on ice during preparation of mixture. Do not repeatedly freeze and thaw more than 5 times and avoid light when store or using the kit.

6 Procedure

6.1 RNA Extraction

1) This kit is not included for Nucleic Acid (NA) extraction reagent.
2) The quality of the extracted NA is important on the performance of the test.
3) If you confirmed the suitability of the NA extraction, alternative NA extraction systems and kits might also be available.

6.1.1 Recommendation for RNA extraction with Veri-Q PREP M16-16TU-CV19

1) The extraction of the NA using the Kit has to be performed following the manufacturer's instructions using at least 500 µL of specimen. For elution of the extracted NA, 50 µL elution buffer should be used.
2) Please refer to IFU of PREP M16 instrument and 16TU-CV19 reagent.

6.1.2 Recommendation for RNA extraction with Veri-Q ManuPrep Viral DNA/RNA kit

1) The extraction of the NA using the Kit has to be performed following the manufacturer's instructions using at least 200 µL of specimen. For elution of the extracted NA, 50 µL elution buffer should be used.
2) Please refer to IFU of Veri-Q ManuPrep Viral DNA/RNA kit.

6.1.3 Recommendation for RNA extraction with QIAamp MinElute Virus Spin Kit

1) The extraction of the NA using the Kit has to be performed following the manufacturer's instructions using 200 µL of specimen. For elution of the extracted NA, 50 µL elution buffer should be used.
2) Please refer to IFU of QIAamp MinElute Virus Spin Kit

6.2 Extraction Internal control (optional)

Extraction Internal control(RNA-EIC) is not include as a kit components. If you want using EIC, you have to request it. The information is below.

No.	Components name	Model name	Volume	Quantity	Cat No.
1	Extraction Internal Control	RNA-EIC	500 µL	100 reaction	7A134

6.3 Sample preparation for Real-time PCR

⚠ The preparation described in this part should be performed within 20 min.

⚠ Filter tips and gloves must be used to prevent splashing and potential cross-contamination of specimen. Use extreme care to ensure selective amplification.

⚠ Completely thaw the reagent on ice.

⚠ Briefly centrifuge the reagent tubes to remove drops from the inside of cap.

⚠ Completely protect the reagent from light.

⚠ Use RNA extracted from fresh samples.

1) Centrifuge the Kit components at 3,000 rpm for 5 sec.
   *At this time, centrifuge the other components first and then centrifuge the positive control to prevent contamination between positive control and others.
2) Vortex for 3 sec and then centrifuge at 3,000 rpm for 2 sec.
   * Positive control should be centrifuged separately to prevent contamination.
3) Prepare the PCR mixture by placing each reagent No.1 to No.3 in a 1.5 mL tube. (Refer to the table 'PCR Mixture'.)

6.3.1 Use of CFX96 Real-time PCR Detection system or Applied Biosystems® 7500 Real-time PCR system

Total number of reaction = n sample + 1 positive control +1 negative control +1=n+3

No.	Components name	Model name	1 reaction	Ex) 17 reaction
1	2X One-Step RT-PCR Master mix	nCoV-MMGR06BI2	10 µL	170 µL
2	Primer/Probe Mixture	nCoV-M-PPM	1 µL	17 µL
3	Internal Positive Control*	nCoV-IPC	1 µL	17 µL
4	Template		8 µL	-
	Total		20 µL	

* If you are using' internal extraction control', nCoV-DW should be used instead of IPC.

4) Vortex for 3 sec and centrifuge at 3,000 rpm for 2 sec.
5) Aliquot 12 µL of reaction mixture in each well. (Not provided)
6) Add 8 µL to each well in the order negative control, template, and positive control.
   * Be careful contamination.
7) Mix the PCR mixture and centrifuge at 1,000 rpm for 30 sec.
8) Set up the time and temperature of instrument as shown in the table 'Real-time PCR condition'.

[Real-time PCR condition]

Step	Temperature	Time	Cycle
1	50℃	10 min	1
2	95℃	3 min	1
3	95℃	9 sec	45
4	58℃	30 sec	

*Refer to the appendix1 for run and drive of instrument.

6.3.2 Use of Veri-Q PCR 316 QD-P100

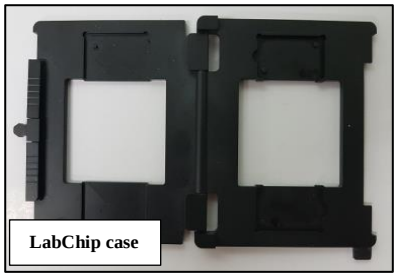
[PCR Mixture]

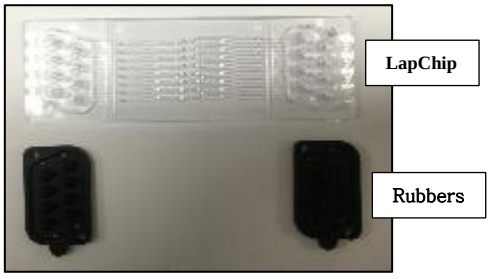
Total number of reaction = n sample + 1 positive control +1 negative control +1=n+3

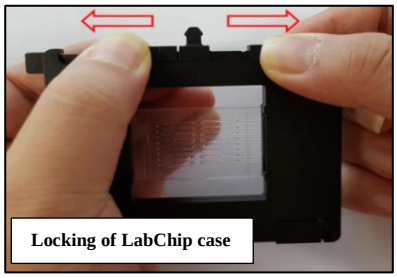
No.	Components name	Model name	PPM	Ex) 17 reaction
1	2X One-Step RT-PCR Master mix	nCoV-MMGR06BI2	5 µL	85 µL
2	Primer/Probe Mixture	nCoVM-PPM	1 µL	17 µL
3	Internal Positive Control*	nCoV-IPC	1 µL	17 µL
4	Template		3 µL	-
	Total		10 µL	

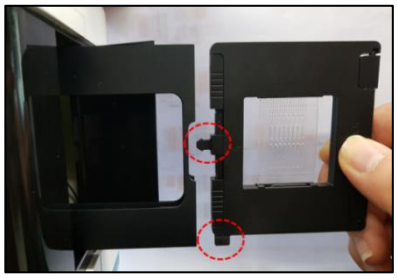
* If you are using' internal extraction control', nCoV-DW should be used instead of IPC.

1) Vortex for 3 sec and centrifuge at 3,000 rpm for 2 sec.
2) Aliquot 7 µL of reaction mixture in each well (Not provided)
3) Add 3 µL to each well in the order negative control, template, and positive control.
   * Be careful contamination.
4) Mix the PCR mixture and centrifuge at 1,000 rpm for 30 sec.
5) Align the end of the pipette tip vertically to the inlet hole of the LabChip with gravity pressure and gently load 8 µL of each mixture into each channel of LabChip. Load the prepared mixture into LabChip in order negative control, template, and positive control. * Be careful not to make bubbles when loading the mixture.
6) Assemble LabChip with Rubbers and LabChip case and insert it into the instrument.
   * Be careful not to touch the projection of the Rubber with your hands.









7) Set up the time and temperature of instrument as shown in the table 'Real-time PCR condition'.

[Real-time PCR condition]

Step	Temperature	Time	Cycle
1	50℃	10 min	1
2	95℃	3 min	1
3	95℃	9 sec	45
4	58℃	30 sec	

6.4 Quality control

6.4.1 Controls that are provided in the assay kit include:

Reagent	Description	Quantity
Nuclease free water (nCoV-DW)	Molecular grade, DNase and RNase-free water.	300 µL/tube
Positive control (nCoV-PC)	DNA fragments that contains the ORF3a and N real-time RT-PCR amplicon sequences.	200 µL/tube
Internal positive control (nCoV-IPC)	Synthetic DNA that contains the SSIIb of soybean real-time RT-PCR amplicon sequence.	100 µL/tube

6.4.2 Control that is not provided in the assay kit include:

Reagent	Description	Quantity
RNA Extraction Internal Control (RNA-EIC)	Synthetic **RNA** that contains the SSIIb of soybean real-time RT-PCR amplicon sequence	500 µL/tube

The nuclease-free water is used in 'no template' (negative) control that confirms no contamination. The positive template control is needed to confirm that PCR is correctly worked and is used for determining the validity of the test. Our positive control (PC, nCoV-PC) is a mix of synthetic DNA of ORF3a and N gene target fragments of the SARS-CoV-2 genome at concentration of 1 x 10^4 copies/3 µL. Whenever we open the PC tube, we must be extremely careful for cross-contamination. The internal positive control (IPC) is needed to makes sure that each reaction worked properly and used for reference during the PCR in each reaction.

7 Results Analysis

All the results are based on Ct values that automatically calculated by software.

7.1 Fluorophore and cut-off value

Target	Fluorophore	Cut-off of Ct value
ORF3a	FAM	< 40
N	Cy5	< 40
IPC	Texas red	< 40

*Refer to the appendix2 for the appropriate threshold line for each instrument.

7.2 Interpretation of sample results

Sample	ORF3a	N	IPC	Result
	FAM	Cy5	Texas red	
Negative Control	-	-	+	Valid
	One positive		+/-	Invalid, re-test
Positive Control	+	+	+	Valid
	One Negative		+/-	Invalid, re-test
Case 1	-	-	+	Negative
Case 2	+	+	+/- [c]	COVID-19
Case 3	+	-	+/- [c]	COVID-19 [a]
Case 4	-	+	+/- [c]	Potential COVID-19 [b]
Case 5	-	-	-	Invalid, re-test

* Cut off: < 40 Ct

** Quality control is performed using PC (Positive Control) and IPC (Internal Positive Control).

[a] If ORF3a alone is detected, the test result is counted as a COVID-19 positive.

[b] If N alone is detected, the test result is counted as a Potential COVID-19. At the low concentration of viral RNA, only one of the two targets may be detected. In this case, we recommend to repeat the test from the sample preparation for further clear confirmation. If the same result still comes out, we strongly recommend to repeat the test using newly collected sample.

*** Here, we count "Potential COVID-19" as invalid with strong recommendation to repeat the test again for clear confirmation.

[c] High amplification of the sample signals may cause to decrease or removal of the IPC signals.

8 Trouble shooting

Problems	Probable cause	Recommendation
Cannot see any signal in all channel including positive control	Wrong operation of instrument	Please check Real-time PCR condition and run the assay under correct setting.
	Incorrect preparation of mixture	Please check all components and repeat assay.
	Not available storage condition	Repeat the assay using fresh reagents.
False positive at the negative control	Carry-over contamination	Discard all the components of assay. Repeat the assay using new components.
Not acceptable positive control	Degradation of positive control	Aliquot when thaw positive control. Avoid repeated freezing and thawing.
	Incorrect preparation	Please confirm the protocol and repeat assay.
No appearance or high Ct value of IPC	High concentration of sample	Retest after diluting the DNA using nuclease free water.

9 Limitation

- It must be kept at the storage temperature until expiry date.
  (Storage temperature -20±5℃, expiry date 12 month after manufacturing, 20 days after opening)
- It should be kept away from light.
- Use on ice during the test.
- Use of this product is limited to personnel specially instructed and trained in the techniques of real-time PCR and in vitro diagnostic procedures.
- Good laboratory practice is essential for proper performance of this assay. Extreme care should be taken to preserve the purity of the components of the kit and reaction setups. All reagents should be closely monitored for impurity and contamination. Any suspicious reagents should be discarded.
- Appropriate specimen collection, transport, storage and processing procedures are required for the optimal performance of this test.
- This assay is not to be used on the specimen directly. Appropriate nucleic acid extraction methods have to be conducted prior to using this assay.
- The presence of PCR inhibitors may cause false negative or invalid results.
- As with any diagnostic test, results of the nCoV-QM, nCoV-OM should be interpreted in consideration of all clinical and laboratory findings.

10 Performance Characteristics

10.1 Analytical Sensitivity (LoD)

The analytical sensitivity (Limit of Detection, LoD) of nCov-OM/nCov-QM defines each target gene as 95% detectable concentration (copies/mL). The nasopharyngeal samples were prepared by spiking the viral SARS-CoV-2 genomic RNA (NCCP #43326 from Korea Center for Disease Control) into UTM(Universal Transport Medium). This test was replicated 24 times of each concentration using three instruments. As a result, the analytical sensitivity is shown in the table below.

	ORF3a gene	N gene
ABI7500	21.6 copies/rxn (270.6 copies/mL)	21.9 copies/rxn (273.8 copies/mL)
Bio-Rad, CFX96	6.0 copies/rxn (75.4 copies/mL)	10.1 copies/rxn (126.1 copies/mL)
Veri-Q PCR 316 QD-P100	8.9 copies/rxn (297.8 copies/mL)	9.0 copies/rxn (301.1 copies/mL)

10.2 Analytical Specificity

10.2.1 Cross-reactivity

The analytical specificity was tested against 45 organisms including bacteria and virus that can be isolated from the reference material DNA or RNA and cultured medium samples. (See table below)

Each isolated sample was tested at a concentration at least 5 x10^5 copies/reaction.

			Laboratory testing		In silico analysis	
Organism		**Test**	**ORF3a**	**N**	**ORF3a**	**N**
Specific target						
SARS-CoV-2		O	Detected	Detected	100%	100%
Other high priority pathogens from the same genetic family						
Human coronavirus 229E		O	No reactive	No reactive	N/A	N/A
Human coronavirus OC43		O	No reactive	No reactive	N/A	N/A
Human coronavirus HKU1		O	No reactive	No reactive	N/A	N/A
Human coronavirus NL63		O	No reactive	No reactive	N/A	N/A
SARS-coronavirus ¶		O	No reactive	Detected	80.60%	96.55%
MERS-coronavirus		O	No reactive	No reactive	N/A	N/A
High priority organisms likely in the circulating area						
Adenovirus	B	O	No reactive	No reactive	N/A	N/A
	C	O	No reactive	No reactive	N/A	N/A
Human Metapneumovirus (hMPV)		O	No reactive	No reactive	N/A	N/A

Parainfluenza virus 1-4	Type 1	O	No reactive	No reactive	N/A	N/A
	Type 2	O	No reactive	No reactive	N/A	N/A
	Type 3	O	No reactive	No reactive	N/A	N/A
	Type 4	X	-	-	N/A	N/A
Influenza A	H1	O	No reactive	No reactive	N/A	N/A
	H3	O	No reactive	No reactive	N/A	N/A
Influenza B		O	No reactive	No reactive	N/A	N/A
Enterovirus		O	No reactive	No reactive	N/A	N/A
Respiratory syncytial virus	A	O	No reactive	No reactive	N/A	N/A
	B	O	No reactive	No reactive	N/A	N/A
Rhinovirus		O	No reactive	No reactive	N/A	N/A
Chlamydia pneumonia		O	No reactive	No reactive	N/A	N/A
Haemophilus influenzae		O	No reactive	No reactive	N/A	N/A
Legionella pneumophila		X	-	-	N/A	N/A
Mycobacterium tuberculosis		X	-	-	N/A	N/A
Streptococcus pneumonia		O	No reactive	No reactive	N/A	N/A
Streptococcus pyogenes		O	No reactive	No reactive	N/A	N/A
Bordetella pertussis		O	No reactive	No reactive	N/A	N/A
Mycoplasma pneumoniae		X	-	-	N/A	N/A
Pneumocystis jirovecii (PJP)		X	-	-	N/A	N/A
High priority organisms, including organisms commonly found in the clinical matrix						
Influenza C		X	-	-	N/A	N/A
Parechovirus		X	-	-	N/A	N/A
Candida albicans		X	-	-	N/A	N/A
Corynebacterium diphtheriae		X	-	-	N/A	N/A
Legionella non-pneumophila		X	-	-	N/A	N/A
Bacillus anthracosis (Anthrax)		X	-	-	N/A	N/A
Moraxella cararrhalis		X	-	-	N/A	N/A
Neisseria elongata		X	-	-	N/A	N/A
Neisseria miningitidis		X	-	-	N/A	N/A
Pseudomonas aeruginosa		X	-	-	N/A	N/A
Staphylococcus epidermidis		X	-	-	N/A	N/A
Streptococcus salivarius		X	-	-	N/A	N/A
Leptospirosis		X	-	-	N/A	N/A
Chlamydia psittaci		X	-	-	N/A	N/A
Coxiella burneti (Q-Fever)		X	-	-	N/A	N/A
Staphylococcus aureus		O	No reactive	No reactive	N/A	N/A

¶ Plasmid DNA including N gene of SARS-CoV-1 was used (IDT Cat #10006624). The SARS control is the same sequence. Bat SARS-like coronavirus isolates bat-SL-CoVZC45 (GenBank: MG772933.1).

The specificity was determined using both wet-tests and in-silico analysis. Results of in-silico analysis demonstrated that there was significant homology between the SARS-coronavirus

and our assay primer/probes for N gene. When the cross reactivity to N gene was tested by wet laboratory experiments, it appeared at $1.6X10^3$ copies of SARS-coronavirus control (GenBank: MG772933.1).

10.2.2 Interfering substances

Endogenous Interfering Substances Study was designed to evaluate PCR inhibition by interfering substances of the PCR kit according to CLSI guideline, EP7-A: 2002. Control group was prepared by spiking 3X LoD (900 copies/ml) SARS-CoV-2 genomic RNA into universal transport medium (UTM, Noble bioscience, UTNFS-3B-2) while test sample group by spiking the same RNA into the UTM with interfering substances of mucin, saliva, whole blood, and ethanol. There were ± 2 Ct value differences between the control and the test groups. The PCR reaction was not inhibited with these substances.

10.3 Clinical performance evaluation

10.3.1 Result of Clinical evaluation with contrived specimens

Performance of the Veri-Q™ COVID-19 Multiplex Assay kit was evaluated using clinical oropharyngeal and nasopharyngeal swab specimens spiked with SARS-CoV-2 RNA (Korea Center for Disease Control, NCCP Cat #43326). Here, 90 negative and 34 positive contrived clinical matrix samples were tested.

34 positive contrived clinical samples consisted with 16 samples with 1-2X LoD, 12 samples with 4x LoD, and 6 samples with 20x LoD SARS-CoV-2 genomic RNA. Viral RNA was extracted from spiked samples in which blind test was performed using Veri-Q™ COVID-19 Multiplex Assay kit. CDC 2019-Novel Coronavirus (2019-nCoV) Real-Time RT-PCR Diagnostic Panel (CDC., EUA authorized 2-4-2020) was used to confirm the samples where ABI7500 real-time PCR was applied.

The result showed 100 % detection for the spiked samples with 1X – 20X LoD RNA for ABI 7500 and CFX96 real-time PCR. However, there were 94% detection at the 1X -2X LoD and 100% detection at 4X - 20X LoD RNA using Veri-Q PCR316. All 90 samples were negative.

Reagent model	Instrument	SARS-CoV-2 concentration	Results (Detected/Tested)	% Detection
nCoV-OM	ABI 7500	1X to 2X LoD	16 / 16	100
		4x LoD	12 / 12	100
		20x LoD	6 / 6	100
		NEG	0 / 90	0
	CFX96	1X to 2X LoD	16 / 16	100
		4x LoD	12 / 12	100
		20x LoD	6 / 6	100
		NEG	0 / 90	0

nCoV-QM	Veri-Q PCR 316	1X to 2X LoD	15 / 16	94
		4x LoD	12 / 12	100
		20x LoD	6 / 6	100
		NEG	0 / 90	0

The positive and negative agreements between the Veri-Q™ COVID-19 Multiplex Assay kit is shown in Table.

Reagent model			**Positive**	**Negative**
nCoV-OM	ABI7500	Positive	34	0
		Negative	0	90
	CFX96	Positive	34	0
		Negative	0	90
nCoV-QM	Veri-Q PCR 316	Positive	33	0
		Negative	1	90

		PPA (95% CI)	**NPA (95% CI)**	**OPA (95% CI)**
nCoV-OM	ABI7500	100% (89.7% -100%)	100% (96.0% -100%)	100% (97.1% - 100%)
	CFX96	100% (89.7% -100%)	100% (96.0% -100%)	100% (97.1% - 100%)
nCoV-QM	Veri-Q PCR 316	97% (84.7% -99.9%)	100% (96.0% -100%)	99% (:95.6% - 100%)

10.3.2 Result of Clinical evaluation with Nasopharyngeal swab

A retrospective study was performed using leftover archived RNA from symptomatic patients suspected of COVID-19 infection. The clinical samples were collected at Youngnam University Medical Center (YUMC) in Daegu City of South Korea where all samples were confirmed as COVID-19 positive.

Our Veri-Q™ COVID-19 Multiplex Assay kit was compared with Allplex™ 2019-nCoV Assay kit (Seegene, Inc., EUA authorized 4-21-2020) using BioRad CFX96 real-time PCR for 65 positive and 93 negative specimens at YUMC.

Our kit results were 100% identical to Seegene's ones. Two-sided 95% score confidence intervals for sensitivity and specificity are (94.5%, 100%) and (96.1%, 100%), respectively as shown in the Table below.

nCoV-OM		Comparative assay		Total
		Positive	Negative	
Veri-Q™ COVID-19 Multiplex Assay	Positive	65	0	65
	Negative	0	93	93
Total		65	93	158

- ✓ Positive percent agreement (PPA) : 100% [95% CI: 94.5,100]
- ✓ Negative percent agreement (NPA) : 100% [95% CI: 96.1, 100]
- ✓ Overall percent agreement (OPA) : 100% [95% CI: 97.7, 100]
- ✓ Kappa : 1 [95% CI: 1, 1]

When the same comparison tests were performed using MiCo BioMed's Veri-Q™ PCR316, match ratio was slightly reduced as shown in the Table below.

nCoV-QM		**Comparative assay**		**Total**
		Positive	Negative	
Veri-Q™ COVID-19 Multiplex Assay	Positive	63	0	63
	Potential positive	1	0	1
	Negative	1	93	94
Total		65	93	158

- ✓ Positive percent agreement (PPA) : 96.9% [95% CI: 89.3, 99.6]
- ✓ Negative percent agreement (NPA) : 100% [95% CI: 96.1, 100]
- ✓ Overall percent agreement (OPA) : 98.7% [95% CI: 95.5, 99.8]
- ✓ Kappa : 0.97 [95% CI: 0.94, 1]

11 Reference

- Centers for Disease Control and Prevention (CDC), DEPARTMENT OF HEALTH & HUMAN SERVICES, Division of Viral Diseases '2019-Novel Coronavirus (2019-nCoV) Real-time rRT-PCR Panel Primers and Probes'
- World Health Organization (WHO), Laboratory testing for 2019 novel coronavirus (2019-nCoV) in suspected human cases Interim guidance_ updated 14 January 2020
- Laboratory biorisk management for laboratories handling human specimens suspected or confirmed to contain novel coronavirus: Interim recommendations. Geneva: World Health Organization; 2013.
- WHO laboratory biosafety manual, third edition. Geneva: World Health Organization; 2004.
- Guideline for the collection of clinical specimens during field investigation of outbreaks WHO/CDS/CSR/EDC/200.4

12 Manufacture

12.1 Factory address

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