MiCo BioMed

Veri-Q PCR 316

Coronavirus disease 2019(COVID-19) Detection Kit

nCoV-QS

Cat. No. 7K105 (50 test/kit)

Cat. No. 7K111 (100 test/kit)

IVD

(BMM-E2242F-0-1, 2021.02)

Contents

1. Intended Use

This Real-time PCR kit is an automated system for qualitative detection of ORF3a and N genes of SARS-CoV-2 RNA from sputum, nasopharyngeal or oropharyngeal swabs specimens from patients with signs and symptoms suggestive of COVID-19(e.g., fever and/or symptoms of acute respiratory illness). This kit is optimized to be used in Veri-Q PCR 316 system (Cat. No.9R501, MiCo BioMed Co., Ltd. Korea). This Kit is designed as a professional use In Vitro diagnostic medical device with trained and specifically trained in the techniques of real-time PCR and in vitro diagnostics Therefore, the Veri-Q PCR 316, nCoV-QS Kit is for aiding to diagnose infections of individual suspected of coronavirus disease 2019.

Positive results are indicative of SARS-CoV-2 RNA detection, but may not represent the presence of transmissible virus. 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. 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 designed for Veri-Q PCR 316 system and is based on TaqMan detection method and designed for chip type plastic ware (LabChip) unlikely real-time PCR using PCR tube. TaqMan® chemistry is the key feature of detection system. TaqMan® probe contains a reporter fluorescent dye on the 5'-end and a quencher dye on the 3'-end. 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. Materials Provided

3.1. Kit Contents & Volume

Cap Color	Components name	Model name	Volume	Quantity		Description
				50 test/kit	100 test /kit	
Purple	2X One-Step RT-PCR Master mix	MMGR06	500μL	1	2	Polymerase, reverse transcriptase, buffer and stabilizer
Brown	Primer/Probe Mixture1	nCoV-PPM1	50μL	1	2	Specific primer & probe mixture
Brown	Primer/Probe Mixture2	nCoV-PPM2	50μL	1	2	Specific primer & probe mixture
Red	Positive Control DNA	nCoV-PC	200μL	1	1	Positive Control DNA
Yellow	Internal Positive Control	IPC5104	100μL	1	1	Internal Positive Control DNA
Green	Nuclease Free Water	DW	300μL	1	1	Ultra-pure water

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

※ This kit provides sufficient volume for 50 or 100 reactions when using 10μL per reaction

3.2. Materials Required but Not Provided

- 0.2 mL or 1.5 mL tube
- Micro pipette, sterilized filtered pipette tips
- Table top centrifuge
- Powder-free gloves
- Lab coats
- Goggle
- Heating block
- Vortex mixer
- Clean bench, Bio Safety Cabinet (BSC)
- Sterile containers for collection of sputum specimens
- UTM with frangible tipped swab(Universal Transport Medium, Noble bioscience, UTNFS-3B-2)
- Veri-Q PREP M16 / Device (Cat. No. 9S101, MiCo BioMed. Co., Ltd. Korea)
- Veri-Q PREP M16 - 16TU-RDSP(Cat. No. 7A131, MiCo BioMed. Co., Ltd. Korea)
- Veri-Q PCR 316(Cat. No. 9R501, MiCo BioMed. Co., Ltd. Korea)/ Device
- Veri-Q PCR 316 - LabChip (Cat. No. 8R002, MiCo BioMed. Co., Ltd. Korea)

3.2.1. Materials for pre-treatment

Item	Source
Equipment	
Pipette aid	
Heat block	
Vortex	
Micro-12 centrifuge (1.5 mL tube)	
Pipette (20-200 μL / 200-1,000 μL)	
Timer	
Plastics ware	
Rack (1.5 mL tube, 15 mL / 50 mL conical tube)	
1.5 mL tube	Disposable
15 mL / 50 mL conical tube	
Filtered pipette tip (20-200 μL / 200-1,000 μL)	
Disposable pipette (10 mL / 25 mL)	
Powder-free latex gloves	

3.2.2. Materials for Real-time PCR

Additional materials for PCR	
Equipment	
Veri-Q PCR 316	9R501, MiCo BioMed. Co., Ltd. Korea
Pipette (1-10 μL / 2-20 μL / 20-200 μL)	
Vortex	
Micro-12 centrifuge (0.2 mL or 8-strip tube / 1.5 mL tube)	
Plastics ware	
Veri-Q PCR 316 LabChip	8R002, MiCo BioMed. Co., Ltd. Korea
Rack (8-strip / 1.5 mL tube)	
Veri-Q PCR 316 - LabChip	Cat. No. 8R002, MiCo BioMed. Co., Ltd. Korea
8-strip tube, 1.5 mL tube	Disposable
Filtered pipette tip (1-10 μL / 2-20 μL / 20-200 μL)	
Powder-free latex gloves	

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 validated to use with Veri-Q PCR 316 system and it couldn't guaranteed performance excepting the system.
3) This assay needs to be carried out by trained and competent personnel.
4) Performance of the product can not be guaranteed if the testing protocol is modified.
5) Treat all specimens as potentially infectious and dispose of them as per local regulations.
6) Wear protective disposable powder-free latex gloves, 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 exchange the components from different lots or reagent kits, or pooling reagents (e.g. buffer bottles from different lots should not be exchanged across lots)
10) Repetitive thawing and freezing of reagents may decrease test sensitivity, so limit to 10 times or less.
11) All reagents have to be sufficiently thawed, mix well and centrifuge briefly before use.
12) Always use sterile filtered tips and dedicated pipettes for each area of work. Avoid moving equipment against the unidirectional process flow.
13) Use always calibrated equipment.
14) Equipment and work benches must be disinfected after the procedure to avoid contamination.
15) Avoid exposing the Primer and Probe Mixture to light, for avoid damage of fluorescence property of probes.
16) In order to get the valid results, always use Positive control and Negative control.
17) After testing, all wastes should be processed with fulfillment of regulation of each country/region.
18) Do not expose the product to heat and keep it at the specified temperature, as there is a risk of performance degradation.

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 10 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) It has to be made sure that the system used for NA extraction is compatible with the Veri-Q PREP M16 – 16TU-CV19 (Cat. No. 7A133, MiCo BioMed.Co., Ltd. Korea)
4) 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.
5) Please refer to IFU of PREP M16 instrument and 16TU-CV19 reagent.
   *If specimen type is sputum, it should refer to 'Appendix1. Viral DNA/RNA extraction from Sputum..

6.2. 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 specimens. 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.

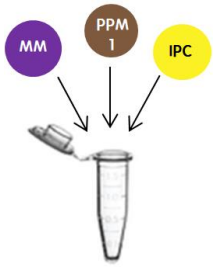
⚠ Completely protect the reagent from light.

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 PPM1 mixture by mixing each component No.1 to No.3 in a 1.5 mL tube. (Refer to the table 'PPM1 Mixture') And then prepare the PPM2 mixture by mixing each component No.1 to No.3 in another 1.5 mL tube (Refer to the table 'PPM2 Mixture')

[PPM1 Mixture]

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

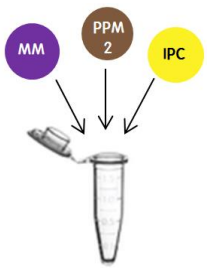
No.	Components name	Model name	PPM1	9 reaction
1	2X One-Step RT-PCR Master mix	MMGR06	5 µL	45 µL
2	Primer/Probe Mixture1	nCoV- PPM1	1 µL	9 µL
3	Internal Positive Control	IPC5104	1 µL	9 µL
	Total		7 µL	63 µL



[PPM2 Mixture]

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

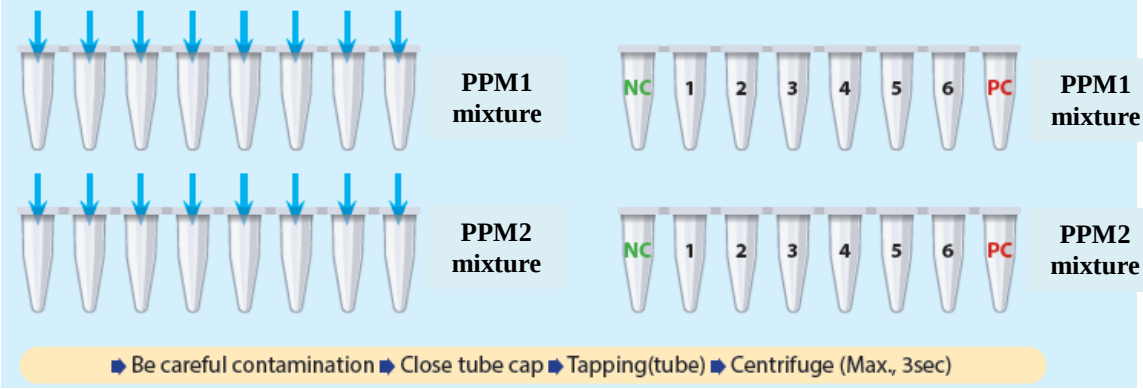
No.	Components name	Model name	PPM2	9 reaction
1	2X One-Step RT-PCR Master mix	MMGR06	5 µL	45 µL
2	Primer/Probe Mixture2	nCoV- PPM2	1 µL	9 µL
3	Internal Positive Control	IPC5104	1 µL	9 µL
	Total		7 µL	63 µL



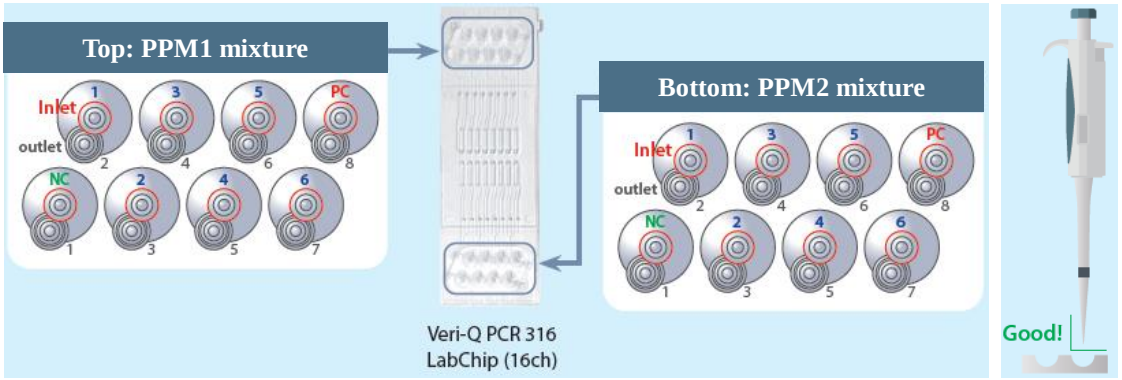
4) Vortex for 3 sec and centrifuge at 3,000 rpm for 2 sec.
5) Aliquot 7 µL of PPM1 mixture prepared above into each 0.2 mL tubes. And aliquot 7 µL of PPM2 mixture prepared above into each 0.2 mL tubes.

6) Prepare negative control by adding 3 μL of nuclease-free water into a PPM1 mixture and a PPM2 mixture tube. (Refer to the figure 'Preparation of Samples and controls')
7) Add 3 μL of extracted RNA from a sample into each PPM1 mixture tubes and PPM2 mixture tubes. (Refer to the figure 'Preparation of Samples and controls')
8) Add 3 μL of positive control into a PPM1 mixture and a PPM2 mixture tube. (Refer to the figure 'Preparation of Samples and controls') * Be careful to avoid contamination.

<Preparation of Samples and controls>

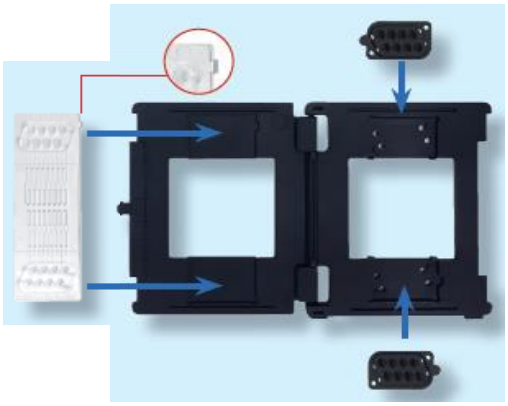


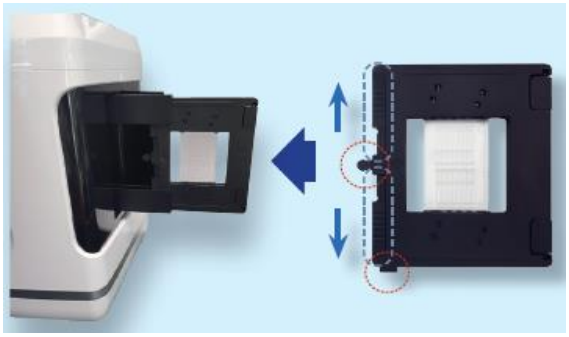
9) Mix the PCR mixture and centrifuge at 3,000 rpm for 2 sec.
10) Align the end of the pipette tip vertically to the inlet hole of LabChip with gravity pressure and gently load 8 μL of each the mixture into each channel of the LabChip. Load the prepared mixtures into the LabChip in order negative control, template (purified nucleic acid samples), and positive control as below. * Be careful not to make bubbles when loading the mixture.



		1	2	3	4	5	6	7	8
Case 1. Sample = 6ea	PP1	NC	S1	S2	S3	S4	S5	S6	PC
	PP2	NC	S1	S2	S3	S4	S5	S6	PC
		9	10	11	12	13	14	15	16
		1	2	3	4	5	6	7	8
Case 2. Sample > 3ea	PP1	NC	S1	S2	S3	S4	TE buffer	TE buffer	PC
	PP2	NC	S1	S2	S3	S4	TE buffer	TE buffer	PC
		9	10	11	12	13	14	15	16
		1	2	3	4	5	6	7	8
Case 3. Sample < 3ea	PP1	NC	S1	S2	S3	PC	TE buffer	TE buffer	TE buffer
	PP2	NC	S1	S2	S3	PC	TE buffer	TE buffer	TE buffer
		9	10	11	12	13	14	15	16

11) 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.





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

[Real-time PCR condition]

Step	Description	Temperature	Time	Cycle
1	Reverse Transcription	50℃	5 min	1
2	Initial denaturation	95℃	8 sec	1
3	Denaturation	95℃	9 sec	45
4	Annealing, extension and detection*	56℃	13 sec	

* The channels for data collection are FAM, HEX, and Cy5.

13) Set up a threshold line for each fluorescence in the (result analysis) software for all samples according to the table below.

	Target	Fluorophore	Threshold line	Cu-off of Ct value
nCoV-QS-PPM1	*ORF3a*	FAM	1000	< 40
	IPC	Cy5	1500	< 40
nCoV-QS-PPM2	*N*	Cy5	1500	< 40
	IPC	HEX	500	< 40

7. Results Analysis

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

7.1. Interpretation of sample results

Sample	nCoV-PPM1		nCoV-PPM2		Result
	ORF3a	IPC	N	IPC	
	FAM	Cy5	Cy5	HEX	
Negative Control	-	+	-	+	Valid
	+/-	-	+/-	-	Invalid, re-test [a]
Positive Control	+	+	+	+	Valid
	+/-	-	+/-	-	Invalid, re-test [a]
Case 1	-	+	-	+	SARS-CoV-2 RNA not detected
Case 2	+	+/- [c]	+	+/- [c]	SARS-CoV-2 RNA detected
Case 3	+	+/- [c]	-	+	SARS-CoV-2 RNA detected
Case 4	-	+	+	+/- [c]	Inconclusive for SARS-CoV-2 RNA[b]
Case 5	+	+/- [c]	-	-	Invalid, re-test [a]
Case 6	-	-	+	+/- [c]	Invalid, re-test [a]
Case 7	-	-	-	-	Invalid, re-test [a]

* Cut off: < 40 Ct

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

[a] In the case of an 'Invalid, re-test' result, all samples must be re-test. And if the second result is in case 'Invalid, re-test', a sample is taken from the patient again.

[b] In the case of an 'Inconclusive for SARS-CoV-2 RNA' result, sample must be re-test. And if the second result is in case 'Inconclusive for SARS-CoV-2 RNA' or 'Invalid, re-test', a sample is taken from the patient again.

[c] Due to the high amplification of the sample, the amplification of IPC could decrease or not be detected.

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.
	Storage of reagents outside of recommended storage conditions	Repeat the assay using fresh reagents.
False positive at the negative control	cross contamination of samples	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. Repetitive thawing and freezing of reagents may decrease test sensitivity, so limit to 10 times or less.
	Incorrect preparation	Please confirm the protocol and repeat assay.
No appearance or low Ct value of IPC	High concentration of sample	Please check the result of Case 2,3,4 (Refer to '7.1 Interpretation of sample results')
	Incorrect preparation	Please check all components and repeat assay.
Abnormal graph	Pipetting error	Make sure to pipette premix solution into LabChip.
	Bubbles in LabChip	Load mixture into LabChip carefully.

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-QS should be interpreted in consideration of all clinical and add epidemiological data.
- The primers & probes have been designed to detect the highly conservative regions of the ORF3a and N genes of the virus. However, due to the high mutation rates of the RNA viruses, low possibility of mutation within the conservative regions still exists, which may lead to false negative results with this kit
- This kit is limited to the detection of SARS-CoV-2 RNA from oropharyngeal swab, nasopharyngeal swab and sputum specimens. Other specimen types were not validated.
- We have confirmed specificity through wet tested and/or analyzed in silico. 2 target sequence were perfect match with SARS-CoV-2 and it have over 80% homology sequence with SARS-coronavirus. But, it were no reactive with SARS-coronavirus in wet-testing. If although, it cannot be make sure that all types of SARS are unresponsive.

10. Performance Characteristics

- **Analytical Sensitivity (LoD)**

  Analytical sensitivity (limit of detection, LoD) of nCoV-QS defines each target gene as 95% detectable concentration (copy/μL).

  This test was repeated 24 times for each concentration using two types of samples.

  The analytical sensitivity analysis results are shown in the table below.

Specimens type	ORF3a gene	N gene
Nasopharyngeal or oropharyngeal swab	137.850 copies/mL (4.14 copies/rxn)	151.028 copies/mL (4.53 copies/rxn)
Sputum	112.175 copies/mL (3.36 copies/rxn)	169.103 copies/mL (5.07 copies/rxn)

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

  - The analytical specificity of the nCoV QS was tested against 42 organisms including bacteria and virus that can be isolated from the reference material DNA or RNA and cultured medium samples.
  - Each isolate d sample was tested at a concentration at least 5 x10^5 copies/reaction.
  - It was confirmed that nCoV-QS was specifically detected in positive control.

- **Interfering substances**

  - The PCR inhibition reaction of the nCoV-QS was tested against 4 interfering substances.

No.	Interfering substances	concentration
1	Mucin	50 ug/mL
2	Saliva	100%
3	Whole Blood	100 μL/mL
4	Ethanol	2%

  - As a result, the difference Ct value was ± 2, between the control and test group at each concentration.
  - The PCR reaction was not inhibited with these substances.

- **Clinical evaluation**

  We performed clinical evaluation with contrived samples and patients' samples as described in the protocol of the IFU. When the contrived samples were compared with CDC Diagnostic Panel (CDC 2019-Novel Coronavirus Real-Time RT-PCR Diagnostic Panel, EUA authorized 2-4-2020), 100% consistent results were obtained without false positives nor false negatives. In addition, the same results were found in the comparative tests of nasopharyngeal swabs and sputum samples using a reference Kit(FDA EUA approved). 109 positive samples and 110 negative samples were used in the clinical

evaluation, showing 100% agreement with the reference.

This represents that the test kit nCoV-QS is equivalent to the reference kit.

Each result is as below.

I. Contrived samples results

		Comparison assay		Total
		CDC 2019-nCoV Panel (+)	**CDC 2019-nCoV Panel (-)**	
Test assay	**Veri-Q nCoV-QS (MiCo BioMed) (+)**	28	0	28
	Veri-Q nCoV-QS (MiCo BioMed) (-)	0	42	42
Total		28	42	70

- ✓ Positive percent agreement (PPA): 100% [95% CI: 87.7,100]
- ✓ Negative percent agreement (NPA): 100% [95% CI: 91.6, 100]
- ✓ Overall percent agreement (OPA): 100% [95% CI: 94.9, 100]
- ✓ Kappa: 1 [95% CI: 1, 1]

II. Clinical samples results

A. Nasopharyngeal swab

		Comparative assay		Total
		Allplex™ 2019-nCoV assay (Seegene) (+)	**Allplex™ 2019-nCoV assay (Seegene) (-)**	
Test assay	**Veri-Q nCoV-QS (MiCo BioMed) (+)**	69	0	69
	Veri-Q nCoV-QS (MiCo BioMed) (-)	0	70	70
Total		69	70	139

- ✓ Positive percent agreement (PPA): 100% [95% CI: 94.8,100]
- ✓ Negative percent agreement (NPA): 100% [95% CI: 94.9, 100]
- ✓ Overall percent agreement (OPA): 100% [95% CI: 97.4, 100]
- ✓ Kappa: 1 [95% CI: 1, 1]

B. Sputum

		Comparative assay		Total
		Allplex™ 2019-nCoV assay (Seegene) (+)	**Allplex™ 2019-nCoV assay (Seegene) (-)**	
Test assay	**Veri-Q nCoV-QS (MiCo BioMed) (+)**	40	0	40
	Veri-Q nCoV-QS (MiCo BioMed) (-)	0	40	40
Total		40	40	80

- ✓ Positive percent agreement (PPA): 100% [95% CI: 91.2,100]
- ✓ Negative percent agreement (NPA): 100% [95% CI: 91.2, 100]
- ✓ Overall percent agreement (OPA): 100% [95% CI: 95.5, 100]
- ✓ Kappa: 1 [95% CI: 1, 1]

C. Nasopharyngeal swab and sputum

		Comparative assay		Total
		Allplex™ 2019-nCoV assay (Seegene) (+)	**Allplex™ 2019-nCoV assay (Seegene) (-)**	
Test assay	**Veri-Q nCoV-QS (MiCo BioMed) (+)**	109	0	109
	Veri-Q nCoV-QS (MiCo BioMed) (-)	0	110	110
Total		109	110	219

- ✓ Positive percent agreement (PPA): 100% [95% CI: 96.7, 100]
- ✓ Negative percent agreement (NPA): 100% [95% CI: 96.7, 100]
- ✓ Overall percent agreement (OPA): 100% [95% CI: 98.3, 100]
- ✓ Kappa: 1 [95% CI: 1, 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

MiCo BioMed Co.,Ltd.

3rd and 4th Floor , 54 Changeop-ro, Sujeong-gu, Seongnam-si, Gyeonggi-do, Korea, 13449 www.micobiomed.com

12.2. Contact

If there is any issue when you use this kit, please contact to MiCo BioMed Co.,Ltd.

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