Clinical Performance Evaluation Report

Coronavirus disease 2019 Detection Kit

	Name	Date	Signature
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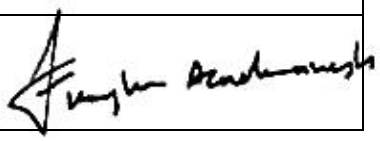
Approved by:	Kayhan Azadmanesh	2020.03.09	

Revision History

Revision No.	Rev. Date	Revision Description
0	2020.03.09	First issue for clinical validation in Head of virology Department Pasteur Institute of Iran (IPI)

Contents

1 Purpose

This study is for clinical validation through comparison equivalence of 'Veri-Q PCR 316 Coronavirus disease 2019 (COVID-19) detection kit'.

2 Device description

2.1 Device name

Common name	Coronavirus disease 2019 (COVID-19) detection kit
Brand name	Veri-Q PCR 316
Model name	nCoV-QS

2.2 Intended use

Veri-Q PCR 316 Coronavirus disease 2019 (COVID-19) detection kit is an in vitro diagnostic reagent for animals using the principle of reverse transcription real-time polymerase chain reaction (PCR). This kit is for qualitative detection of Corona virus disease 19 (COVID-19) using extracted RNA from human specimens (transport medium).

2.3 Principles

nCoV-QS is designed for the Veri-Q316 system and is based on multiplex real-time PCR technology. This system combines Lab-on-a-chip (LabChip) technology with real-time PCR which reduces running time significantly to below 30min (for 45 Cycles). The QuickDetect LabChip Real-time PCR system is based on TaqMan detection method and designed for chip type plastic ware (LabChip) unlike real-time PCR that uses PCR tubes.

TaqMan® chemistry is the key feature of this detection system. The TaqMan® probe contains a reporter fluorescent dye (usually 6-carboxy fluorescein [6-FAM]) on the 5'-end and a quencher dye on the 3'-end. While the probe is intact, the quencher dye greatly reduces the fluorescence emitted by the reporter dye by fluorescence resonance energy transfer (FRET). The probe is designed to bind specific target sequence between traditional forward and reverse primers. If the specific sequence is present, probe binds and is cleaved by the 5' nuclease activity of Taq DNA polymerase during extending nucleic acid.

Cleavage of the probe separates reporter dye from quencher dye, allowing the fluorescent signal to come on. In every cycle, reporter dye molecules are cleaved from their respective probes. Therefore, the fluorescence increases as the number of cycles increases. The intensity of fluorescence represents the amount of target genome in certain specimens.

Using this principle, this kit was designed by pairing FAM of Using this principle, this kit

was designed by FAM, Cy5 and HEX pairing of the COVID-19 detection and internal positive control (IPC) detection. The IPC is for verify the presence of PCR reactions. Consequently, this kit can simultaneously detect genes specific to COVID-19

2.4 Kit components

The kit is consist of 6 components, 2x PCR master mix, primer/probe mixture1, primer/probe mixture2, positive control, internal positive control DNA, nuclease free water filled in a screw tubes, and all together packaged in a cotton box.

No.	Component name	Appearance	Volume	Unit
1	2x one-step RT-PCR Master mix	Colorless, Odorless Liquid in clear tube and purple cap	500 ㎕	1 vial
2	Primer/Probe Mixture 1	Translucent light pink Liquid in brown tube and brown cap	50 ㎕	1 vial
3	Primer/Probe Mixture 2	Translucent light blue Liquid in brown tube and brown cap	50 ㎕	1 vial
4	Positive control DNA	Colorless, Odorless Liquid in clear tube and red cap	200 ㎕	1 vial
5	Internal positive control DNA	Colorless, Odorless Liquid in clear tube and yellow cap	100 ㎕	1 vial
6	Nuclease free water	Colorless, Odorless Liquid in clear tube and green cap	300 ㎕	1 vial

2.5 Instruments and materials required, but not provided

Refer to instructions supplied by manufacturers of the test kits to be used.

- 0.2 ml, 1.5 ml tube and LapChip
- Micro pipette, sterilized pipette tips
- Table top centrifuge
- Powder-free gloves
- Heating block, Vortex mixer
- Clean bench, Bio Safety Cabinet (BSC)
- Nucleic Acid extraction Kit or Extraction System
- Veri-Q PREP M16, G2-16TU/ Machine (MiCo BioMed Co.,Ltd. Korea)

- Veri-Q PREP M16, 16TU-CV19/ Reagent (MiCo BioMed Co.,Ltd. Korea)
- Veri-Q PCR 316, QD-P100/ Machine (MiCo BioMed Co.,Ltd. Korea)

2.6 Storage condition and shelf life

2.6.1 Storage condition

Store at -20±5 ℃

2.6.2 Shelf life

1 year from the date of manufacturing. 20 days after opening.

3 Organization & responsibilities

Organization	Name	Roles
MiCo BioMed	Dai Ho Jang	Operator training and monitoring, Setting instrument Writing of protocol & report
MiCo BioMed	Hyun-Sun Kim	Approval of protocol & Report
MiCo BioMed	Dai Ho Jang	Monitor Data treatment Support to prepare protocol and report
Head of virology Department Pasteur Institute of Iran (IPI)	Kayhan Azadmanesh	Overall responsibility for evaluation
Head of virology Department Pasteur Institute of Iran (IPI)	Soheila Ajdary	Kit operating Data treatment

4 Assay method

	Test method	Comparison method
Device name	Veri-Q PCR 316 2019 Novel coronavirus(COVID-19) detection kit, nCoV-QS	Novel coronavirus (2019-nCoV) Nucleic Acid diagnostic kit
Analyte	SARS-CoV-2 RNA	SARS-CoV-2 RNA
Method	Real-time PCR	Real-time PCR
Manufacturer	MiCo BioMed	Sansure Biotech, Inc (China)
Instrument	Veri-Q PCR316, QD-P100	Corbett RG6000
Tested by	Dai Ho, Jang	Dr. Riazirad
Extraction Kit	Veri-Q PREP M16 16TU-CV19	Veri-Q PREP M16 16TU-CV19

4.1 Learner evaluation

- Blind theory examination
- Blind assay test

5 Test procedure

5.1 Test kit

5.1.1 Materials

nCoV-QS (For clinical evaluation test use only)

5.1.2 Specimen

- Coronavirus disease 2019 virus (COVID-19) was isolated through clinical samples from patient samples showing symptoms of COVID-19 infection in Iran.
- This was provided clinical sample by IPI and extracted of RNA from the clinical samples using Veri-Q PREP M16.
- COVID-19 diagnosis of all samples was confirmed by IPI.
- Sample type : nasopharyngeal swab in transport medium
- Positive sample : 7 samples
- Negative sample : 5 samples

5.1.3 Test procedure

1) Prepare the two kinds of mixture for Real-time PCR reaction. <Total number of reaction = n sample + 1 positive control +1 negative control +1>

Material	Volume / reaction	Ex) 9 reaction
nCoV-QS-MMGR06 (2X One-step RT-PCR Master Mix)	5 ㎕	45 ㎕
nCoV-QS PPM1 or nCoV-QS PPM2 (Primer/Probe Mixture)	1 ㎕	9 ㎕
nCoV-QS-IPC (Internal Positive Control DNA)	1 ㎕	9 ㎕
Template (Sample)	3 ㎕	-
Total	10 ㎕	-

2) Aliquot 7 ㎕ of reaction mixture in each tube (Not provide).

3) Add 3 ㎕ of Samples RNA and positive control DNA into each tube.

4) Add 3㎕ of Nuclease free water negative control to check contamination of the PCR reaction.

5) Mix the template and reaction mixture by tapping the tube. And quick spin to remove extra bubbles in the solution.

⚠	Be careful with contamination.

6) Load 8㎕ of each mixture on each channel of LabChip after preparing the mixture.

⚠	Be careful with bubbles. Please avoid make bubbles in the each channel.

7) Install LabChip into the instrument (Veri-Q PCR 316) after combine prepared LabChip with Rubbers and LabChip case.

⚠	Please assemble the Labchip and Rubber tightly.

8) Insert the LapChip case into instrument (QD-P100).

9) Set up the instrument with specific condition of Real-time PCR as below.

Step	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
	Annealing and scan	56℃	13 sec	

5.1.4 Result analysis

All the results are based on Ct values that automatically calculated by software.
Set the threshold line referring the table below.

Target	nCoVQS-PPM1		nCoVQS-PPM2	
	ORF3a	IPC	N	IPC
Fluorescent dye	FAM	Cy5	Cy5	HEX
Threshold line	1000	1500	1500	500
Cut-off Ct value	<40	<40	<40	<40

Check the Ct value of the positive control & Negative control as shown in the table below.
If it does not detect within this range, it should be perform again.

Sample	nCoVQS-PPM1		nCoVQS-PPM2		Results
	ORF3a	IPC	N	IPC	
	FAM	Cy5	Cy5	HEX	
Negative control	-	+	-	+	Valid
	+/-	-	+/-	-	Invalid, re-test
Positive control	+	+	+	+	Valid
	+/-	-	+/-	-	Invalid, re-test
Case 1	-	+	-	+	Negative
Case 2	+	+/- [b]	+	+/- [b]	COVID-19
Case 3	+	+/- [b]	-	+	Potential COVID-19 [a]
Case 4	-	+	+	+/- [b]	Potential COVID-19 [a]
Case 5	+	+/-	-	+/-	Invalid, re-test

Case 6	-	+/-	+	+/-	Invalid, re-test
Case 7	-	-	-	-	Invalid, re-test

* Cut off: < 40 Ct

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

[a] If one of two reaction is positive, the test should be recommended re-test and second result is same or 'case2', the case should be assessed to 'potential COVID-19'. In this case, the infection may be low in concentration, so be careful about judgement.

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

5.1.5 Quality control

It should be check the Ct value of PC and NC to confirm for validation of each experiment. The valid ranges are shown in the table below. If it does not appear within the range, it should be re-test.

Type	nCoV Ct Value	IPC Ct Value	Interpretation
Negative control	ND*	< 40	Valid
Positive control	< 40	< 40	Valid

5.2 Comparison kit

5.2.1 Materials

This is according to 'Manual of Novel coronavirus (2019-nCoV) Nucleic Acid diagnostic kit '

Protocol of 'Head of virology Department Pasteur Institute of Iran (IPI)' is the same with Novel coronavirus (2019-nCoV) Nucleic Acid diagnostic kit protocol.

- The test for positive and negative samples performed the comparison kit and the test kit simultaneously.

5.2.2 Test condition

Target	Reporter
N gene	FAM
ORF gene	Cy5

Temperature	Time	Number of cycles
50°C	30 min	1
95°C	1 min	1
95°C	15 sec	45

55°C	30 sec	

5.2.3 Result analysis

Target	Cut-off value
N gene	<35
ORF gene	<35

5.2.4 Quality control

It should be check the Ct value of PC and NC to confirm for validation of each experiment. The valid ranges are shown in the table below. If it does not appear within the range, it should be re-test.

6 Test results

6.1 Criteria of result analysis

The data was compared for the positive and negative values determined by two experimental methods using the clinical samples.

6.2 Raw data

6.2.1 Clinical test result of positive samples and Negative Samples

No.	Sample code	Country	Comparison result	Test Kit		
				ORF Ct value	N Ct value	Result
1	Sample 1	Iran	Negative	N/D	N/D	Negative
2	Sample 2	Iran	Negative	N/D	N/D	Negative
3	Sample 3	Iran	Positive	25.47	24.90	Positive
4	Sample 4	Iran	Positive	30.87	30.37	Positive
5	Sample 5	Iran	Negative	N/D	N/D	Negative
6	Sample 6	Iran	Positive	21.23	20.24	Positive
7	Sample 7	Iran	Negative	N/D	N/D	Negative
8	Sample 8	Iran	Positive	32.74	20.25	Positive
9	Sample 9	Iran	Positive	31.32	N/D	Positive
10	Sample 10	Iran	Positive	35.01	20.13	Positive
11	Sample 11	Iran	Negative	N/D	N/D	Negative
12	Sample 12	Iran	Positive	31.46	N/D	Positive

*N/D : Not detection

7 Statistical methods

nCoV-19		Comparison kit		Total
		Positive	Negative	
Test kit	Positive	7	0	7
	Negative	0	5	5
Total		7	5	12

8 Performance acceptance criteria

8.1 Overall agreement

The kit shall have an overall percentage agreement of at least 95%.

9 Conclusion

9.1 Result summary

The clinical overall percent agreement of nCoV-QS was found to be 100%. As this result satisfies the evaluation criteria (> 95%), it was judged that the test reagent nCoV-QS and the comparative reagent were equivalent.