SMARTCHEK®

Novel Coronavirus (SARS-CoV-2) Detection Kit

Prepared by

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Contents

1. Introduction

First reported from Wuhan, China, on 31 December 2019, novel coronavirus (SARS-CoV-2) is the cause of coronavirus disease 2019 (COVID-19) and generally spreads through human-to-human contact or respiratory droplet infection from coughs and sneezes and it is thought to have a zoonotic origin. The symptoms of COVID-19 include respiratory symptoms, fever, cough, shortness of breath and breathing difficulties. In severe cases, it can lead to pneumonia, kidney failure and even death. SMARTCHEK® novel coronavirus (SARS-CoV-2) detection kit (Genesystem catalog number : 9799151400) is a real-time polymerase chain reaction (PCR) based detection assay for and intended for use with GENECHECKER® UF-300 real-time PCR platform (Genesystem catalog number : 1399100200) in order to ensure fast amplification of the target sequences of SARS-CoV-2.

1.1 Method Description

SMARTCHEK® novel coronavirus (SARS-CoV-2) detection kit is based on biochip sample format and provides relatively short turn-around-time with simple workflow while it offers key benefits of real-time PCR tests. The kit contains all necessary components for PCR test including primer pairs and probes pre-labeled (dehydrated) in the test chip and premix supplied in a separate tube. A test chip is designed to run 4 tests including positive control and no template control at a time. 5 test chips are included in the package to make the pack size of 20 tests per a kit. This kit is intended for research use only.
This kit adopted probe based real-time PCR for sequence–specific detection of RNA-dependent RNA polymerase (RdRP) gene and N (Nucleocapsid) gene of SARS-CoV-2. This technology merges the polymerase chain reaction chemistry with the use of fluorescent reporter molecules in order to monitor the production of amplification products during each cycle of the PCR amplification.

1.2 Objectives

Evaluation	Objective
Analytical sensitivity test	This test is to check the detection limit of the assay by undertaking 10 fold serial dilution tests (from $1x10^7$ copies to $1x10^0$ copies).
Analytical specificity test	This test is to check the target specificity of the assay by undertaking cross-reactivity tests using 8 different genes of Influenza A virus (H3N2), Influenza A virus (H1N1), Influenza B virus, Human coronavirus NL63, Rhinovirus, Enterovirus, Respiratory syncytial virus (type B), and Respiratory syncytial virus (type A)
Reproducibility test	This test is to check if the assay produces the consistent results under different conditions depending on production lots, testers and places of the tests.
Repeatability test	This test is to check if the assay will produce the same results repeatedly under the same conditions. For evaluation, the tests were undertaken for 8 days by performing two serial tests of 3 repetitions in a day.
Validity test	This test is to evaluate the validity assay by undertaking three repeatable tests following the production standards and the protocol of the final product.
Clinical performance test	This test is to evaluate the performance of the assay by undertaking tests with genomic RNA isolated from upper airway swab of COVID-19 patient. Tests are designed to use genomic RNA in 10 fold serial dilution to check the performance in different concentration of the templates.

1.3 Intended Use

SMARTCHEK® novel coronavirus (SARS-CoV-2) detection kit is intended for qualitative detection of RNA from novel coronavirus (SARS-CoV-2). This product is for research use only and not intended for diagnostic procedures.

1.4 Description on Detection Principle

SMARTCHEK® novel coronavirus (SARS-CoV,-2) detection kit uses one-step reverse transcriptase polymerase chain reaction (RT-PCR) technology and probe chemistry to detect target genes. Complementary DNA (cDNA) is synthesized from a single-stranded RNA template of SARS-CoV-2 through reverse transcriptase in the premix formulation of the detection assay. After synthesis of cDNA, PCR amplification is processed, during which the SARS-CoV-2 specific primers and probes combine with a single strand of cDNA and amplified using DNA polymerase enzymes included in the premix formulation. Real-time amplification curves are displayed on the GENECHECKER® UF-300 real-time PCR platform by the method of monitoring fluorescence signals generated from the reporter dye on 5' end of the probes after exonuclease activities.

1.5 Storage and Stability

PK No.	Contents	Storage	Stability
1	Test Chip	15℃ ~ 30℃	12 months from the date of manufacture
2	Premix	-25℃ ~ -20℃	12 months from the date of manufacture
3	DNA/RNase Free Water	-25℃ ~ -20℃	12 months from the date of manufacture

2. Performance Validation

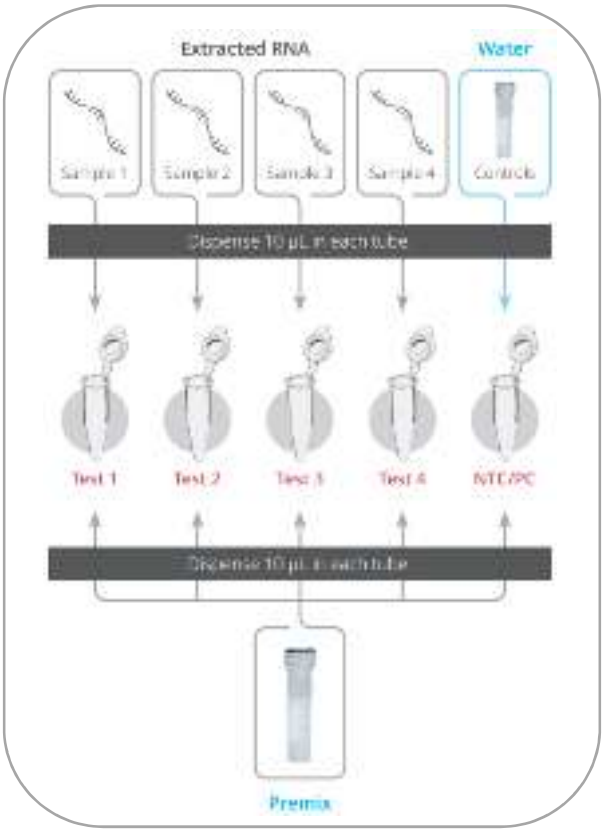
2.1 Validation Summary

2.1.1 Product information

Tested item	SMARTCHEK® Novel Coronavirus (SARS-CoV-2) Detection Kit		
Pack size	20 tests pear a pack (5 test chips per a pack)		
Kit contents	- 5 pieces of test chips (Individually packed) - 5 pieces of chip sealing tapes (Packed in plastic bag in a row) - 5 tubes of premix (Blue label on the cap) - 5 tubes of DNA/RNase free water (White label on the cap) - 25 pieces of microcentrifuge tubes for preparing reaction mixtures		
Lot #	SC200129T	SC200130T	SC200131T
Date of manufacture	January 29, 2020	January 30, 2020	January 31, 2020

2.2 Workflow of the Assay as per the Kit Insert

2.2.1 Preparation of reaction mixture



Reaction Mixture

Component	Volume / Test
Extracted RNA	10 µl
Premix	10 µl
Total	20 µl*

* For dispense into 2 reaction wells in 10 µl. Refer to 2) of this paragraph for detailed instruction.

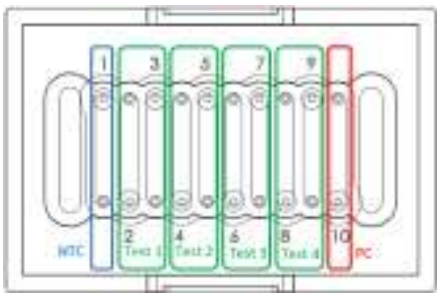
Controls

Component	Volume
DNA/RNase free water	10 µl
Premix	10 µl
Total	20 µl*

* For dispense into the positive control well and no template control well in 10 µl respectively. Refer to 2) of this paragraph for detailed instruction.

1) Prepare 5 microcentrifuge tubes included in PK No. 2.
2) Make aliquot of 10μl of premix and dispense them in 5 different microcentrifuge tubes prepared as 2.1.1.
3) Dispense 10μl of each extracted RNA template in four tubes.
4) Dispense 10μl of DNA/RNase free water in remaining one tube.
5) Vortex and spin-down the tubes.

2.2.2 Test chip configuration



The 20μl of reaction mixtures prepared in 2.1 above is dispensed into two wells of the test chip corresponding to each target, with 10μl reaction volume. Well number 2 through 9 are used for detecting target genes while well number 1 and 10 are used for controls. Configuration of each well of the test chip is as following table.

Well No.	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	
Test	NTC*	Test 1		Test 2		Test 3		Test 4		PC**	
Target gene	N/A	N***	RdRP	N	RdRP	N	RdRP	N	RdRP	N	RdRP

* No template control ** Positive control *** Nucleocapsid

- Corresponding primer pairs and probes along with internal controls are labeled(dehydrated) in each well of the test chip for reaction. Every well except the wells for controls include internal control. The targets are detected from FAM channel and the internal control is detected from ROX channel of UF-300 real-time PCR system.
- Well number 1 of the test chip is used for no template control of the reaction. This well contains pre-labeled primers and probes. Well number 10 of the test chip is used for positive control of the reaction. This well contains pre-labeled primers and probes along with positive templates. FAM channel is used for detecting N gene of SARS-CoV-2 template and ROX channel is used for detecting RdRP gene of SARS-CoV-2 template in this well.

2.2.3 Loading the reaction mixture into test chip

1) Prepare test chip and detach the sealing tape.

2) Aspirate 10μl of the reaction mixture with micropipette and vertically place the tip in the inlet hole of the test chip. Inlet hole is neighboring with printed well number and the diameter of this hole is a bit wider than that of outlet hole.


3) While sample loading, make sure that the end of the tip is securely fit into the inlet hole of the well and apply slight force downward and then slowly dispense the sample into the chip.

4) After dispensing reaction mixtures in to each well of the test chip, every hole of the wells should be sealed using enclosed precut sealing tapes.

5) Take out one strip of sealing tape, peel one piece of sealing tape from the strip using tweezers.



6) Place one end of sealing tape alongside left end of the test chip and seal entire holes. Then, scrub the surface of sealed points using finger, tweezers or scrubbing cloth enclosed in the package of GENECHECKER® UF-300 real-time PCR system.

2.2.4 Test result interpretation

1) Result criteria

Target	Positive Ct Value	Detection Channel
N gene of SARS-CoV-2	Below 37.00	FAM
RdRP gene of SARS-CoV-2	Below 37.00	FAM
Internal positive control	Below 37.00	ROX
Positive control	Below 37.00	FAM and ROX
Negative control	Below 37.00	FAM

2) Result interpretation

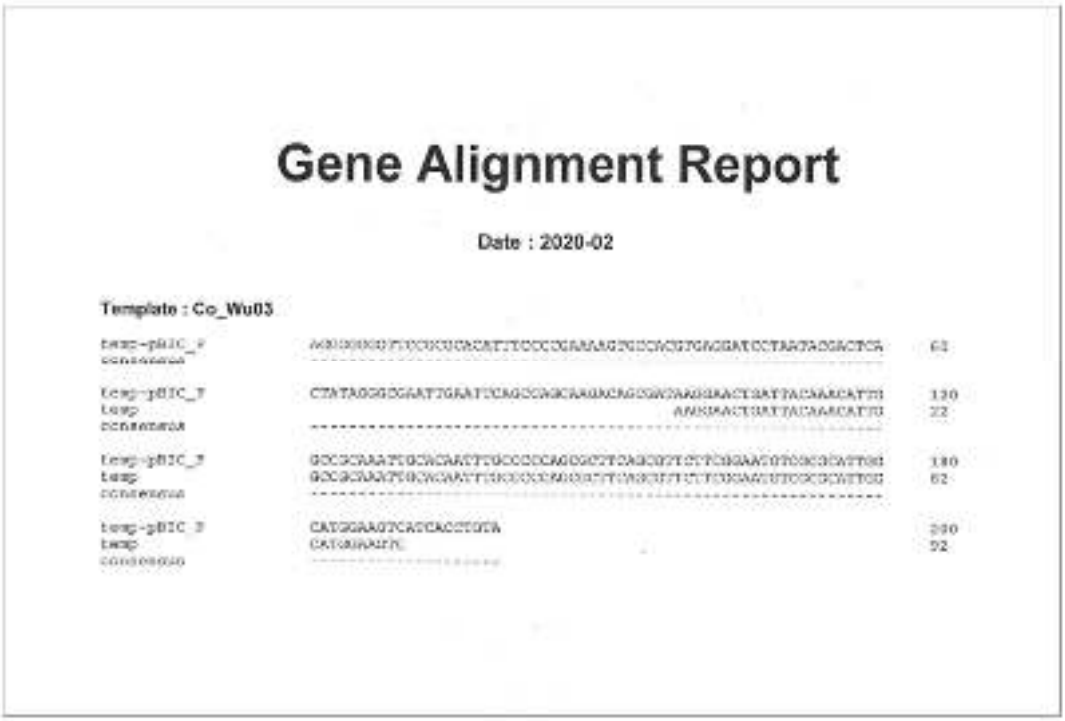
No.	N	RdRP	Internal control	Interpretation	PC	NTC
1	+	+	+ or -	SARS-CoV-2 Positive	Refer to below instruction.	
2	-	-	+	SARS-CoV-2 Negative		
3	-	+	+ or -	Retest		
4	+	-	+ or -	Retest		
5	-	-	-	Retest		

- Positive control should be positive and no template control should be negative in order to make the test valid. All other cases make the test invalid.
- Internal positive control may not be amplified due to primer competitions.

2.3 Material Information

For the validation tests of this assay, standard positive templates were synthesized by cloning target sequences on the plasmid in accordance with the sequences of N gene and RdRP gene of severe acute respiratory syndrome coronavirus 2 (GenBank accession number : MN908947).
Successful synthesis of the standard positive templates were validated by Sanger sequencing method from which it was confirmed that the sequence of the standard positive templates were 100% identical to the sequences of target genes (GenBank accession number : MN908947).

Confirmed sequence of synthesized plasmid DNA of N gene

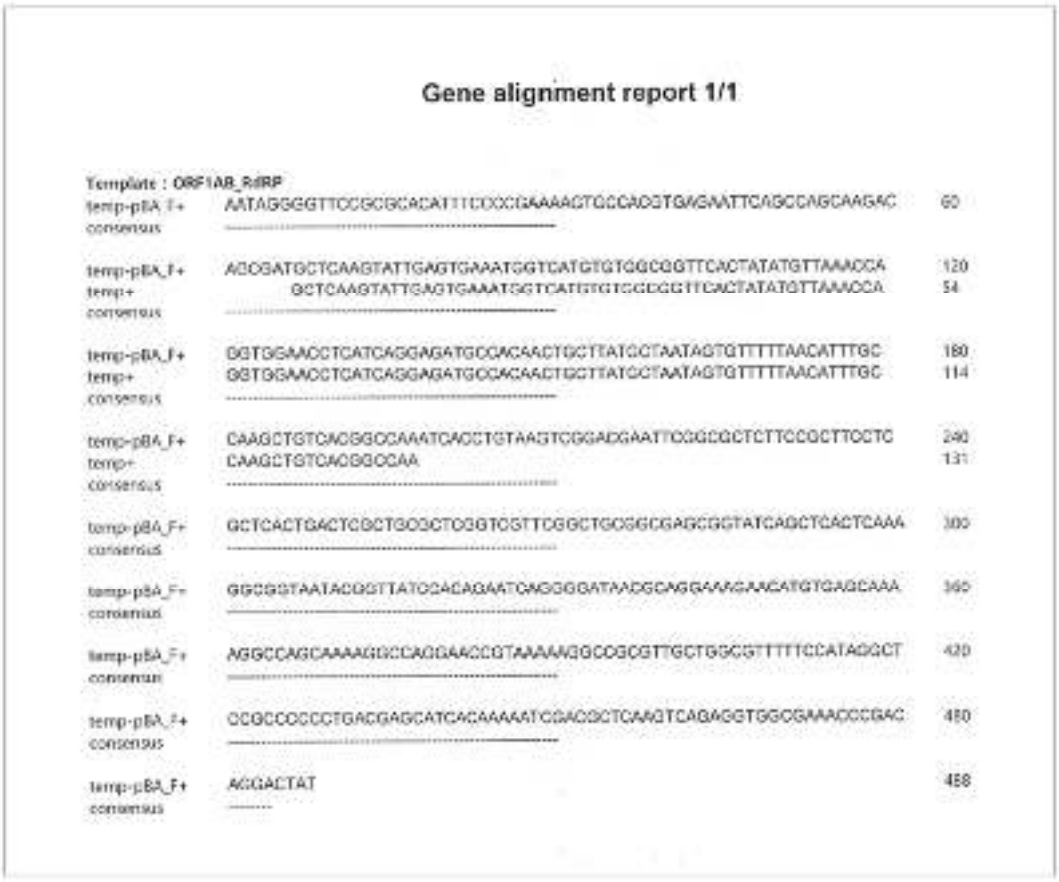


Gene Alignment Report

Date : 2020-02

Template : Co_Wu03

Confirmed sequence of synthesized plasmid DNA of N gene



Gene alignment report 1/1

Template : ORF1AB_RdRP

temp-pBA_F+	AATAGGGGTTCCGCGCACATTTCCCCGAAAAGTGCCACCTGACGAATTCAGCCAGCAAGAC	60
consensus	--	
temp-pBA_F+	ACCGATGCTCAAGTATTGAGTGAAATGGTCATGTGTGGCGGTTCACTATATGTTAAACCA	120
temp+	GCTCAAGTATTGAGTGAAATGGTCATGTGTGGCGGTTCACTATATGTTAAACCA	54
consensus	--	
temp-pBA_F+	GGTGGAACCTCATCAGGAGATGCCACAACTGCTTATGCTAATAGTGTTTTTAACATTTGC	180
temp+	GGTGGAACCTCATCAGGAGATGCCACAACTGCTTATGCTAATAGTGTTTTTAACATTTGC	114
consensus	--	
temp-pBA_F+	CAAGCTGTCACGGCCAAATCAGCTGTAAGTCGGATGAATTCGGCGCTCTTCCGCTTCCTC	240
temp+	CAAGCTGTCACGGCCAA	131
consensus	----------------	
temp-pBA_F+	GCTCACTGACTCGCTGCGCTCGGTCGTTCGGCTGCGGCGAGCGGTATCAGCTCACTCAAA	300
consensus	--	
temp-pBA_F+	GGCGGTAATACGGTTATCCACAGAATCAGGGGATAACGCAGGAAAGAACATGTGAGCAAA	360
consensus	--	
temp-pBA_F+	AGGCCAGCAAAAGGCCAGGAACCGTAAAAAGGCCGCGTTGCTGGCGTTTTTCCATAGGCT	420
consensus	--	
temp-pBA_F+	CCGCCCCCCTGACGAGCATCACAAAAATCGACGCTCAAGTCAGAGGTGGCGAAACCCGAC	480
consensus	--	
temp-pBA_F+	AGGACTAT	488
consensus	--------	

2.4 Analytical Sensitivity Test

2.4.1 Summary of the test

In order to determine the limit of detection(LOD) of SMARTCHEK® novel coronavirus (SARS-CoV-2) detection kit, the positive standard templates were produced by cloning target sequences on the plasmid. The positive standard materials were prepared in 10 fold serial dilution using TE buffer to make $1x10^7$copies, $1x10^6$ copies, $1x10^5$ copies, $1x10^4$ copies, $1x10^3$ copies, $1x10^2$ copies, $1x10^1$copies and $1x10^0$ copies of target DNA respectively. 1μL of the templates in each concentration was used.

2.4.2 Method of the test

The reaction mixtures for analytical sensitivity test were prepared in accordance with the following composition.

◆ Composition of reaction mixture

Component	Volume
Template (10 fold diluted standard materials)	1 μL
Nuclease free water	4 μL
2x Premix	5 μL
Total	10 μL

Prepared reaction mixtures including the template in each concentration were loaded into each well of test chip where primers and probes of each target are prelabeled. Then, PCR tests were performed in accordance with the following reaction program with GENECHECKER® UF-300 real-time PCR platform.

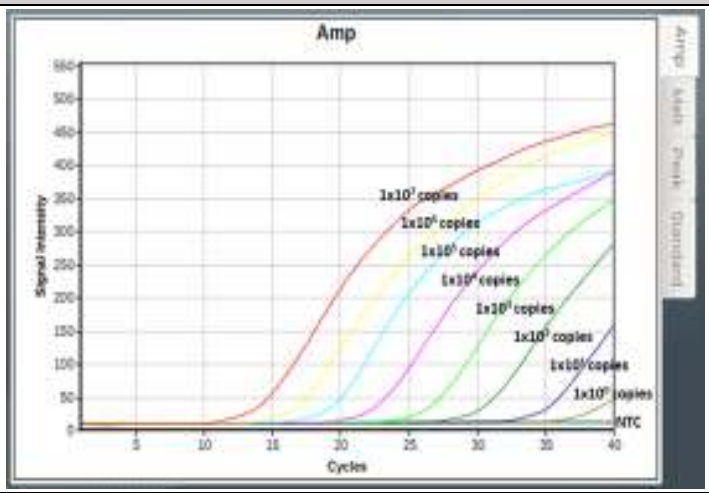
◆ Reaction program with GENECHECKER® UF-300 real-time PCR platform

Step of reaction	Temperature	Time	Cycles
Reverse transcription	50℃	600 sec	1
Pre-denaturation	95℃	30 sec	1
Denaturation	95℃	5 sec	40
Annealing	58℃	20 sec	
Extension	72℃	5 sec	

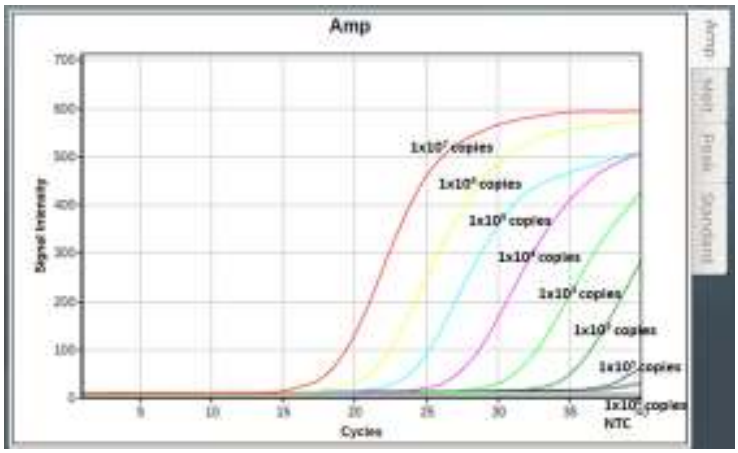
2.4.3 Result of the test

From the analytical sensitivity tests, it was verified that LOD of Nucleocapsid gene of SARS-CoV-2 which differentiates the positive result from no template control of the test was $1x10^1$ copies and the LOD of RdRP gene of SARS-CoV-2 which differentiates the positive result from no template control of the test was $1x10^2$. Accordingly, the LOD of SMARTCHEK® novel coronavirus (SARS-CoV-2) detection kit was determined to be $1x10^2$ copies.

◆ Result of LOD test for Nucleocapsid gene

Concentration	Ct	Result	Displayed chart after completion of the test
$1x10^7$ copies	15.33	Positive	
$1x10^6$ copies	18.08	Positive	
$1x10^5$ copies	20.49	Positive	
$1x10^4$ copies	23.73	Positive	
$1x10^3$ copies	27.58	Positive	
$1x10^2$ copies	31.46	Positive	
$1x10^1$ copies	36.10	Positive	
$1x10^0$ copies	39.69	Negative	
NTC	0.00	Negative	

◆ Result of LOD test for RdRP gene

Concentration	Ct	Result	Displayed chart after completion of the test
1x10^7 copies	18.54	Positive	
1x10^6 copies	21.42	Positive	
1x10^5 copies	24.20	Positive	
1x10^4 copies	27.44	Positive	
1x10^3 copies	31.31	Positive	
1x10^2 copies	35.14	Positive	
1x10^1 copies	39.32	Negative	
1x10^0 copies	0.00	Negative	
NTC	0.00	Negative	

2.5 Analytical Specificity Test

2.5.1 Summary of the test

In order to verify the analytical specificity of SMARTCHEK® novel coronavirus (SARS-CoV-2) detection kit, the RNA templates of the viruses in below table were tested.

◆ List of cross-reactivity tested genes

No.	Target	Sample Type	Sample Source	NCCP* No.
1	Influenza A virus (H3N2 subtype)	Extracted RNA	KCDC**	43230
2	Influenza A virus (H1N1 subtype)	Extracted RNA	KCDC	42004
3	Influenza B virus	Extracted RNA	KCDC	43028
4	human Coronavirus NL63	Extracted RNA	KCDC	43214
5	Rhinovirus	Extracted RNA	KCDC	43225
6	Enterovirus	Extracted RNA	KCDC	43165
7	Respiratory Syncytial virus (type B)	Extracted RNA	KCDC	43181
8	Respiratory Syncytial virus (type A)	Extracted RNA	KCDC	43179

* National Culture Collection for Pathogens ** Centers for Disease Control and Prevention of South Korea

2.5.1 Method of the test

The reaction mixtures for analytical specificity test were prepared in accordance with the following composition.

◆ Composition of reaction mixture

Component	Volume
RNA template from each sample	5 μL
2x Premix	5 μL
Total	10 μL

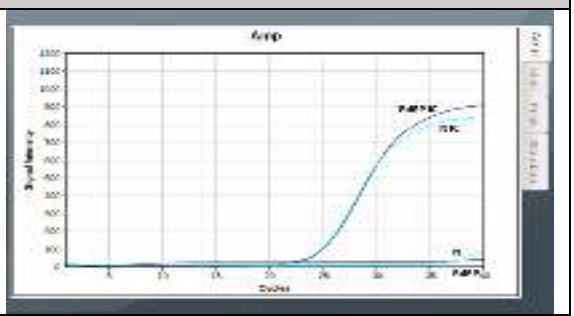
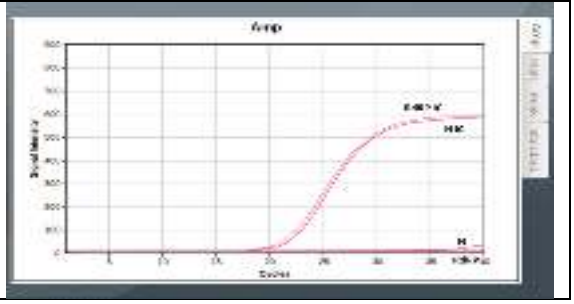
◆ The PCR condition for SARS-CoV-2 Rapi:chip™

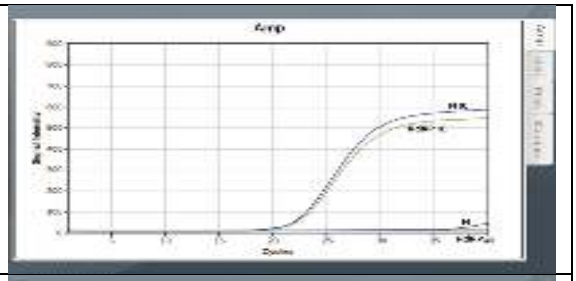
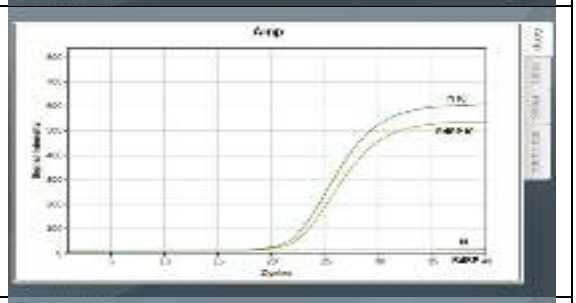
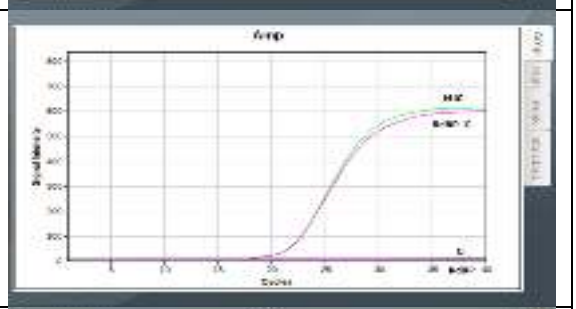
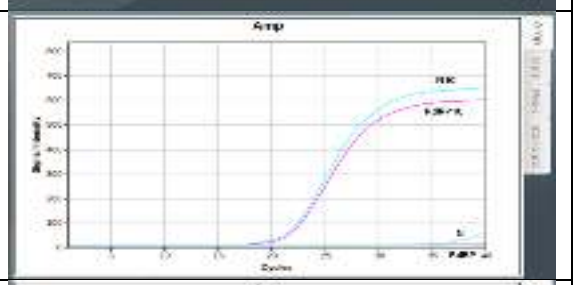
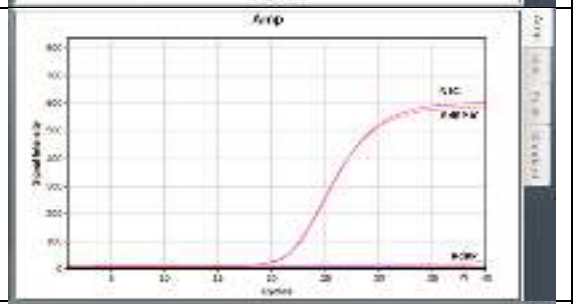
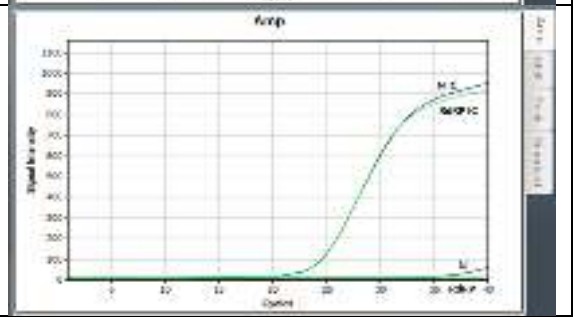
Step of reaction	Temperature	Time	Cycles
Reverse Transcription	50℃	600 sec	1
Pre-Denaturation	95℃	30 sec	1
Denaturation	95℃	5 sec	40
Annealing	58℃	20 sec	
Extension	72℃	5 sec	

2.5.2 Result of the test

From the analytical specificity tests, it was verified that there was no cross reaction of the kit with all the tested targets such as Influenza A virus (H3N2 subtype), Influenza A virus (H1N1 subtype), Influenza B virus, human Coronavirus NL63, Rhinovirus, Enterovirus, Respiratory syncytial virus (type B) and Respiratory syncytial virus (type A).

◆ Result of cross reactivity for Nucleocapsid and RdRP gene of SARS-CoV-2

No.	Virus	Ct (N)	Ct (RdRP)	Result	Displayed chart after completion of the test
1	Influenza A virus (H3N2 subtype)	37.94	0.00	SARS-CoV-2 Negative	
2	Influenza A virus (H1N1 subtype)	0.00	0.00	SARS-CoV-2 Negative	

3	Influenza B virus	0.00	0.00	SARS-CoV-2 Negative	
4	Human coronavirus NL63	0.00	0.00	SARS-CoV-2 Negative	
5	Rhinovirus	0.00	0.00	SARS-CoV-2 Negative	
6	Enterovirus	0.00	39.31	SARS-CoV-2 Negative	
7	Respiratory Syncytial virus (type B)	0.00	0.00	SARS-CoV-2 Negative	
8	Respiratory Syncytial virus (type A)	39.25	0.00	SARS-CoV-2 Negative	

2.6 Reproducibility Test

2.6.1 Summary of the test

For the evaluation of the reproducibility of the assay, cloned DNA of target genes was used as standard template for the tests. Using TE buffer, the standard positive sample in medium concentration ($1x10^6$ copies) and the one in low concentration ($1x10^3$ copies) was respectively prepared for the tests. Prepared standard positive samples in 5μL volume were used for the tests to evaluate the reproducibility of the assay. The same volume of Nuclease free water was used as negative control for each test.

2.6.2 Method of the test

In order to evaluate the reproducibility of the assay, reaction mixtures were prepared in accordance with the following recipe. Prepared reaction mixtures were loaded in the test chip.

◆ Composition of reaction mixture

Component	Volume
Templates in different concentrations	5 μL
2x Premix	5 μL
Total	10 μL

Following reaction program was used for the test with GENECHECKER® UF-300 real-time PCR system

◆ Reaction program with GENECHECKER® UF-300 real-time PCR platform

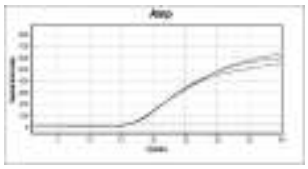
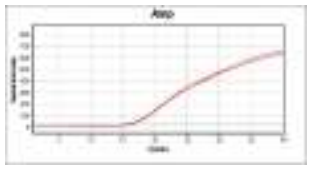
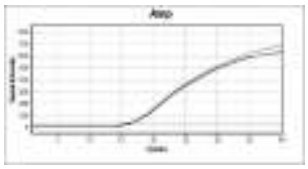
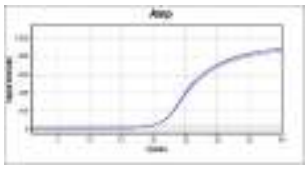
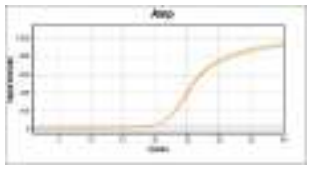
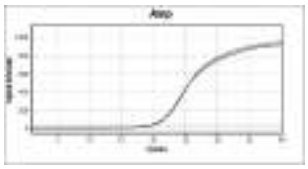
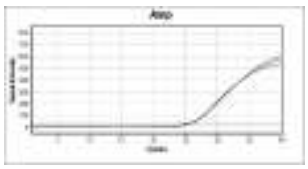
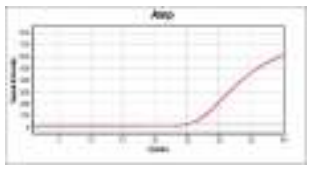
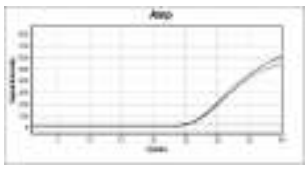
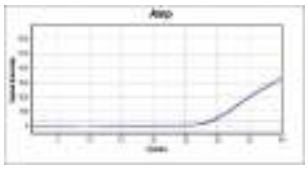
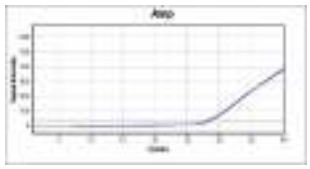
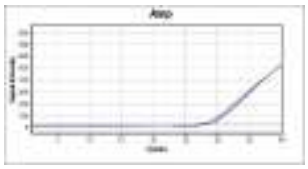
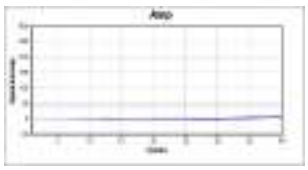
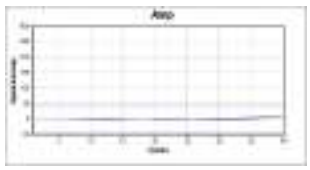
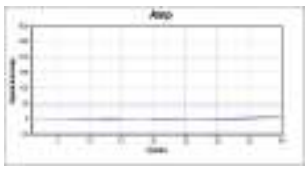
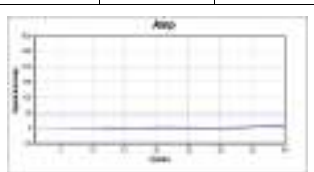
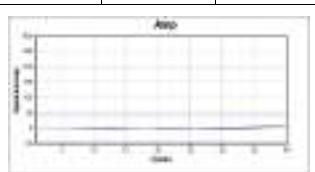
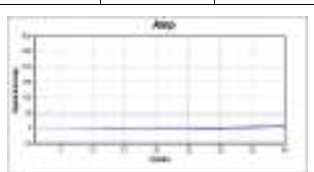
PCR Step	Temperature	Time	Cycles
Reverse Transcription	50℃	600 sec	1
Pre-Denaturation	95℃	30 sec	1
Denaturation	95℃	5 sec	40
Annealing	58℃	20 sec	
Extension	72℃	5 sec	

2.6.3 Result of the test

2.6.3.1 Reproducibility test depending on the production lots

Same tester performed three repeatable tests using the assays from three different production lots along with the standard positive templates in medium and low concentrations. From the tests, it was confirmed that all the standard positive templates were successfully amplified while negative controls were not amplified. From the tests with the standard positive templates in medium concentration, it was confirmed that the lot-to-lot variation of N gene detection primers/probe and RdRP gene detection primers/probe was same at 0.23 Ct. From the tests with the standard positive templates in low concentration, it was confirmed that the lot-to-lot variation of N gene detection primers/probe was 0.18 Ct and the one of RdRP gene detection primers/probe was 0.40 Ct.

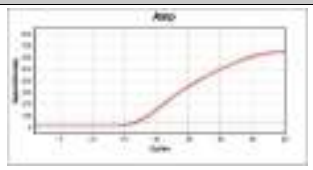
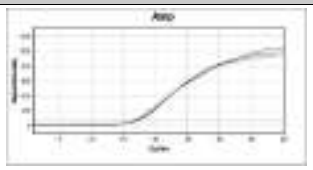
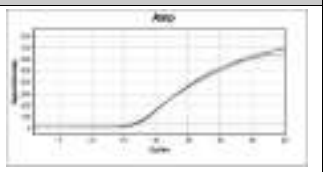
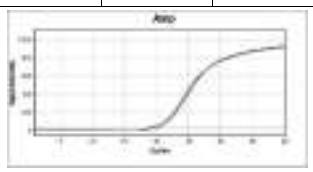
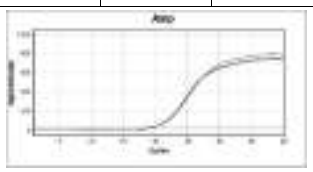
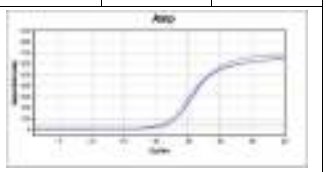
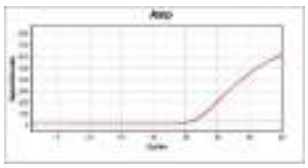
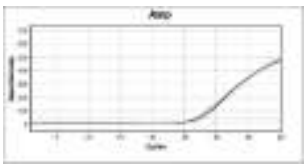
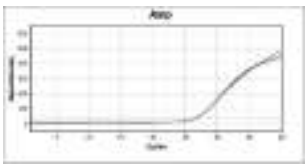
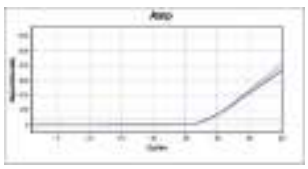
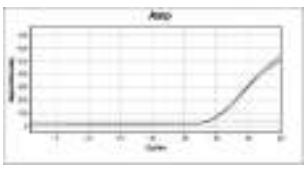
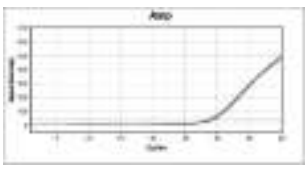
◆ Reproducibility test result depending on the production lots

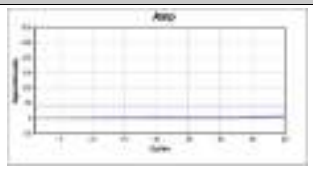
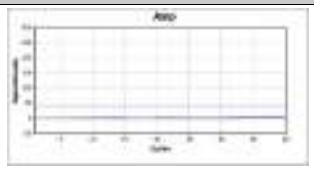
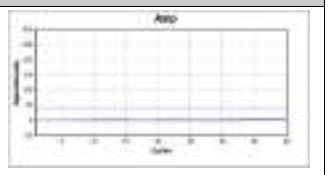
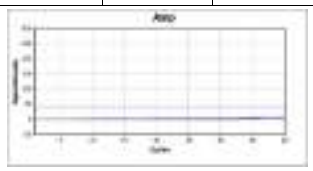
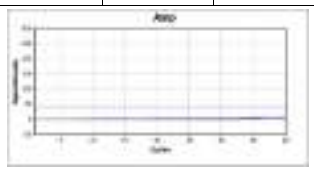
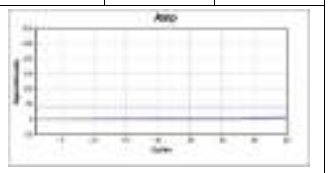
Template	Target	Lot 1			Lot 2			Lot 3		
$1x10^6$ copies	N									
		16.67	16.92	16.69	16.45	17.01	16.98	16.54	16.80	16.31
	RdRP									
		20.04	20.11	20.02	20.13	20.03	19.90	19.63	19.93	19.39
	Result	Positive	Positive	Positive	Positive	Positive	Positive	Positive	Positive	Positive
$1x10^3$ copies	N									
		25.72	25.99	26.07	25.81	25.67	25.97	25.97	25.49	25.85
	RdRP									
		28.76	29.32	29.51	28.36	28.74	28.68	28.27	29.18	29.03
	Result	Positive	Positive	Positive	Positive	Positive	Positive	Positive	Positive	Positive
NTC	N									
		Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative
	RdRP									
		0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	Result	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative

2.6.3.2 Reproducibility test depending on the testers

Three different testers performed three repeatable tests using the assays from the same production lot along with the standard positive templates in medium and low concentrations. From the tests, it was confirmed that all the standard positive templates were successfully amplified while negative controls were not amplified. From the tests with the standard positive templates in medium concentration, it was confirmed that the tester-to-tester variation of N gene detection primers/probe was 0.39 Ct and the one of RdRP gene detection primers/probe was 0.64 Ct. From the tests with the standard positive templates in low concentration, it was confirmed that the lot-to-lot variation of N gene detection primers/probe was 0.40 Ct and the one of RdRP gene detection primers/probe was 0.22 Ct.

◆ Reproducibility test result depending on the testers

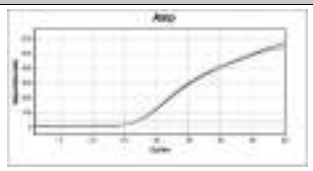
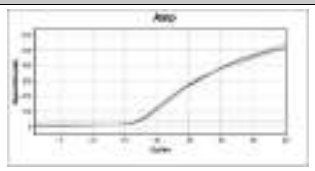
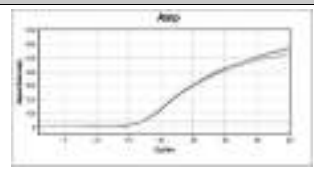
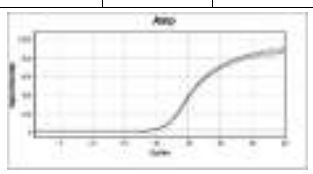
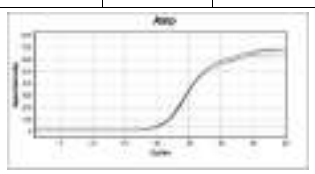
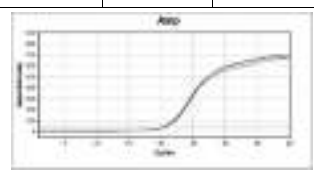
Sample	Target	Tester 1			Tester 2			Tester 3		
$1x10^6$ copies	N									
		16.82	16.15	16.39	16.93	17.09	17.36	16.35	16.57	17.12
	RdRP									
		20.03	20.19	19.37	20.03	19.84	19.77	21.33	21.35	20.13
	Result	Positive	Positive	Positive	Positive	Positive	Positive	Positive	Positive	Positive
$1x10^3$ copies	N									
		25.92	25.85	26.17	26.44	26.61	27.11	26.75	26.77	26.67
	RdRP									
		28.52	28.50	28.68	28.67	28.92	28.79	28.85	28.56	29.25
	Result	Positive	Positive	Positive	Positive	Positive	Positive	Positive	Positive	Positive

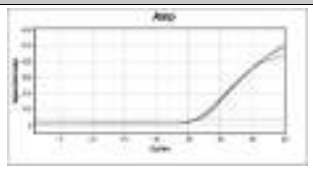
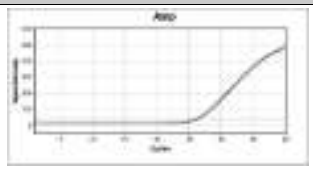
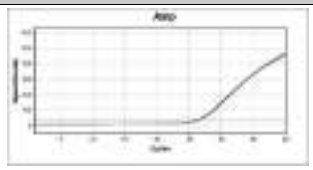
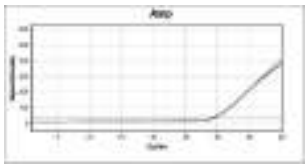
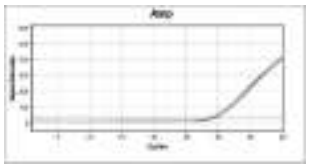
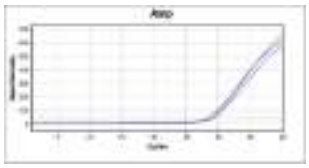
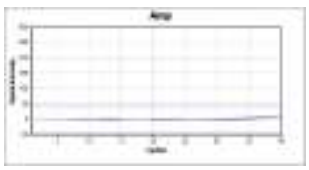
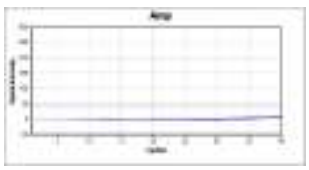
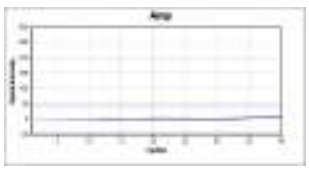
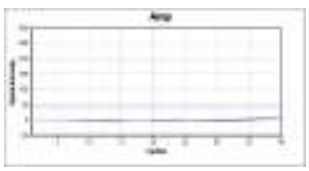
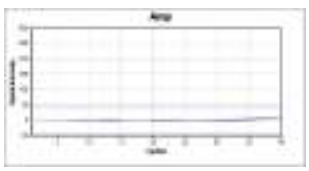
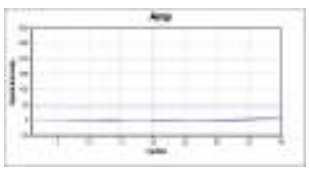
Sample	Target	Tester 1			Tester 2			Tester 3		
NTC	N									
		0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	RdRP									
		0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	Result	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative

2.6.3.3 Reproducibility test depending on the places of the tests

At three different places of tests, repeatable tests were performed by one tester using the assays from the same production lot along with the standard positive templates in medium and low concentrations. From the tests, it was confirmed that all the standard positive templates were successfully amplified while negative controls were not amplified. From the tests with the standard positive templates in medium concentration, it was confirmed that the place-to-place variation of N gene detection primers/probe was 0.17 Ct and the one of RdRP gene detection primers/probe was 0.15 Ct. From the tests with the standard positive templates in low concentration, it was confirmed that the place-to-place variation of N gene detection primers/probe was 0.24 Ct and the one of RdRP gene detection primers/probe was 0.57 Ct.

◆ Reproducibility test result depending on the laboratory

Template	Target	Place 1			Place 2			Place 3		
$1x10^6$ copies	**N**									
		16.90	16.92	16.94	17.05	17.21	17.33	16.83	16.80	16.86
	RdRP									
		20.28	19.93	20.06	20.17	19.82	20.00	20.28	20.08	20.24
	Result	Positive	Positive	Positive	Positive	Positive	Positive	Positive	Positive	Positive

Template	Target	Place 1			Place 2			Place 3		
$1x10^3$ copies	N									
		26.11	26.60	26.47	25.89	26.36	26.37	26.63	26.42	26.66
	RdRP									
		29.46	29.70	29.66	28.94	29.75	29.69	28.05	28.84	28.62
	Result	Positive	Positive	Positive	Positive	Positive	Positive	Positive	Positive	Positive
NTC	N									
		0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	RdRP									
		0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	Result	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative

2.7 Repeatability Test

2.7.1 Summary of the test

For the evaluation of the repeatability of the assay, cloned DNA of target genes was used as standard template for the tests. Using TE buffer, the standard positive sample in medium concentration ($1x10^6$ copies) and the one in low concentration ($1x10^3$ copies) was respectively prepared for the tests. Prepared standard positive samples in 5µL volume were used for the tests to evaluate the repeatability of the assay. The same volume of Nuclease free water was used as negative control for each test.

2.7.2 Method of the test

In order to evaluate the repeatability of the assay, reaction mixtures were prepared in accordance with the following recipe. Prepared reaction mixtures were loaded in the test chip.

◆ Composition of reaction mixture

Component	Volume
Templates in two different concentrations	5 μL
2x Premix	5 μL
Total	10 μL

Following reaction program was used for the test with GENECHECKER® UF-300 real-time PCR system

◆ Reaction program with GENECHECKER® UF-300 real-time PCR platform

PCR Step	Temperature	Time	Cycles
Reverse Transcription	50℃	600 sec	1
Pre-Denaturation	95℃	30 sec	1
Denaturation	95℃	5 sec	40
Annealing	58℃	20 sec	
Extension	72℃	5 sec	

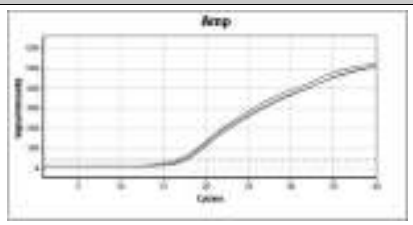
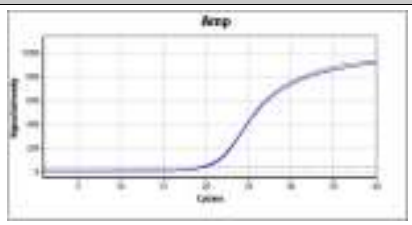
2.7.3 Result of the test

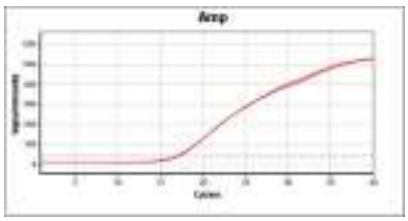
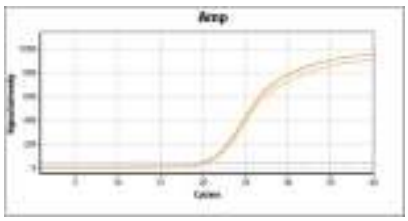
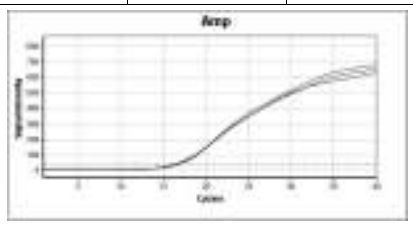
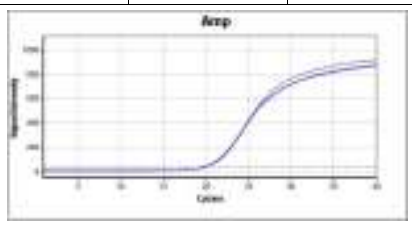
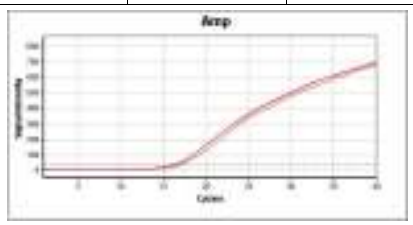
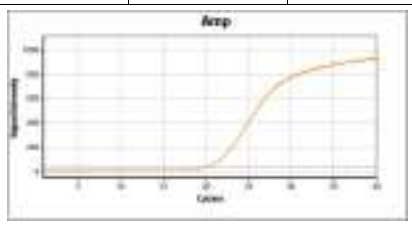
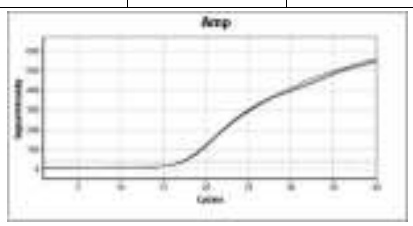
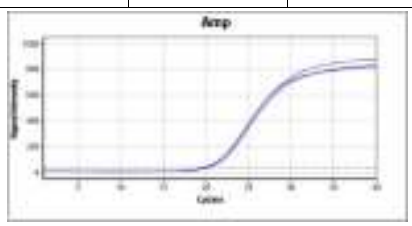
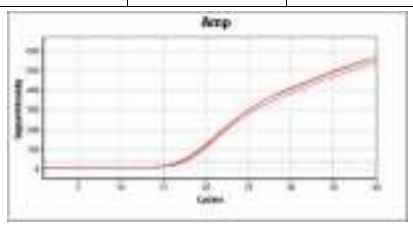
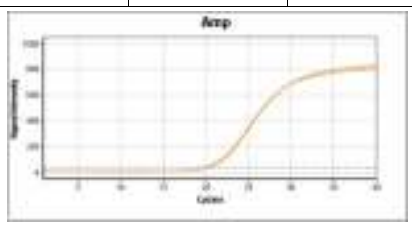
Repeatability test by time point was performed for 8 different days using standard positive templates in medium and low concentrations. From the tests, it was confirmed that all the standard positive templates were successfully amplified while negative controls were not amplified.

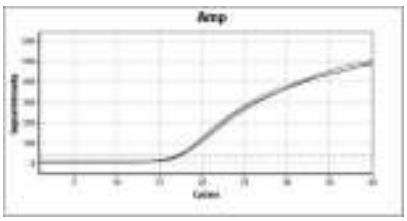
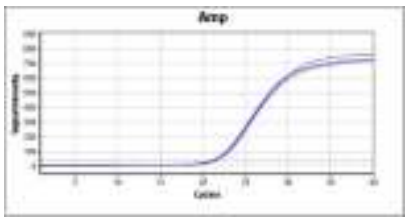
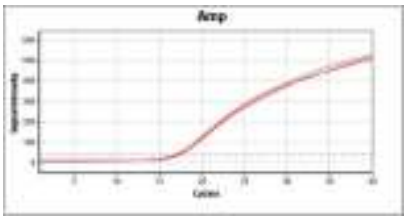
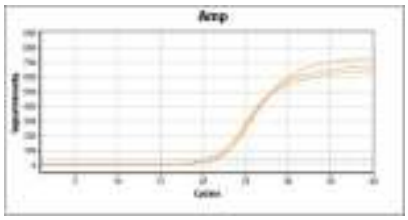
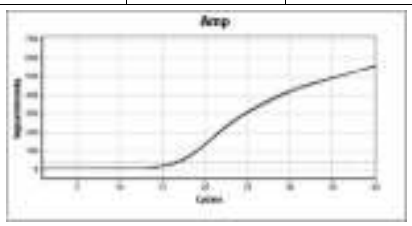
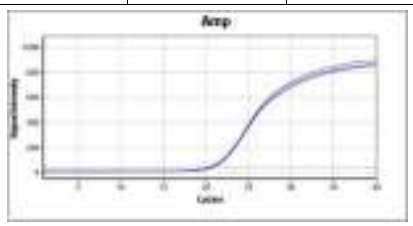
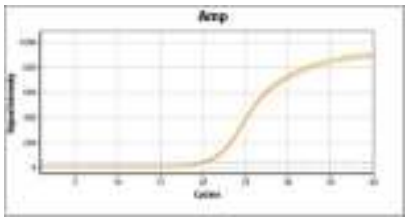
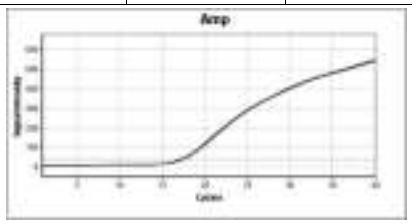
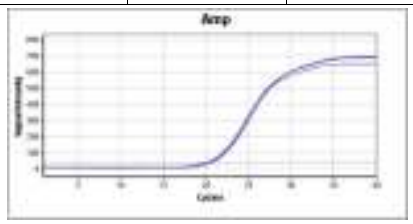
2.7.3.1 Repeatability tests with the templates in medium concentration

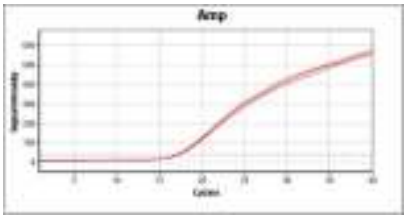
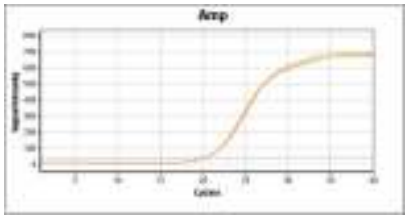
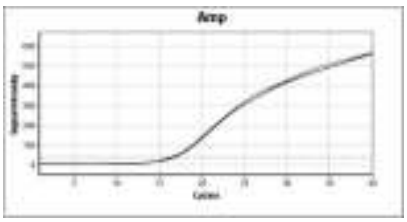
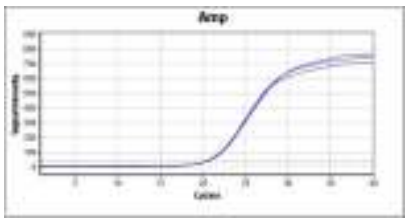
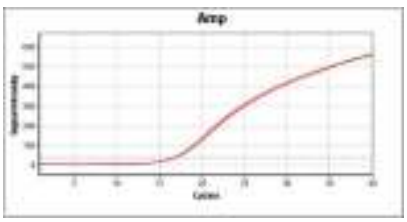
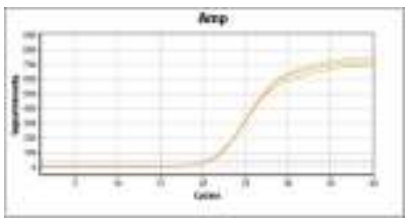
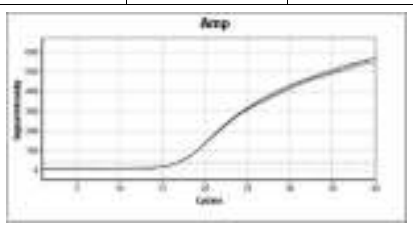
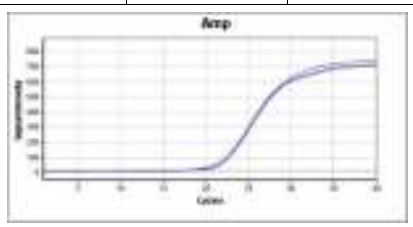
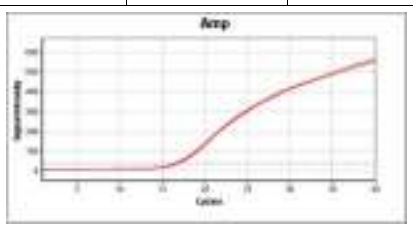
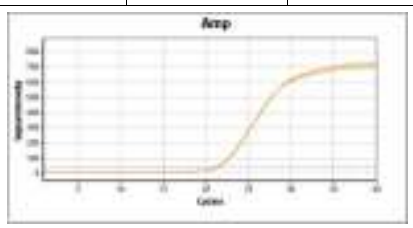
From the tests with the standard positive templates in medium concentration, it was confirmed that the time point variation of N gene detection primers/probe were 0.44 Ct and the one of RdRP gene detection primers/probes was 0.53 Ct.

◆ Repeatability test result with the templates in medium concentration

Time Point	Test	N			RdRP		
Day 1	No. 1						
		17.61	17.22	16.41	19.15	19.67	19.59
	Result	Positive	Positive	Positive	Positive	Positive	Positive

Time Point	Test	N			RdRP		
Day 1	No. 2						
		17.16	16.97	16.93	19.62	20.10	19.63
	Result	Positive	Positive	Positive	Positive	Positive	Positive
Day 2	No. 1						
		16.84	16.31	16.73	19.68	19.78	19.91
	Result	Positive	Positive	Positive	Positive	Positive	Positive
	No. 2						
		17.20	16.55	16.35	20.05	19.94	20.03
	Result	Positive	Positive	Positive	Positive	Positive	Positive
	No. 1						
		17.17	17.25	17.37	19.86	20.29	19.90
	Result	Positive	Positive	Positive	Positive	Positive	Positive
	No. 2						
		17.14	17.56	16.90	20.22	20.34	20.03
	Result	Positive	Positive	Positive	Positive	Positive	Positive

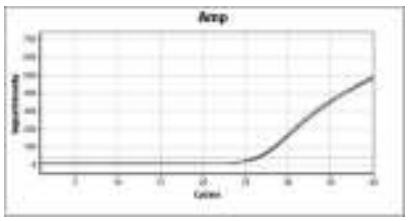
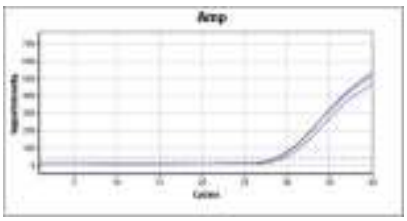
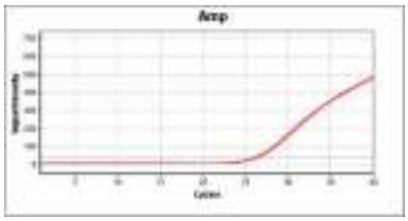
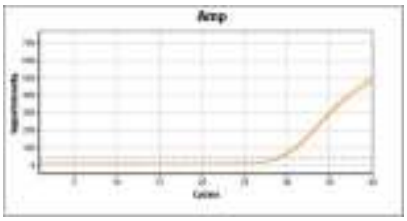
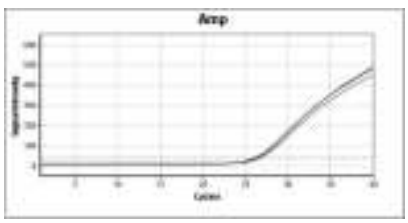
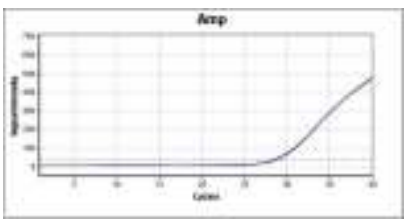
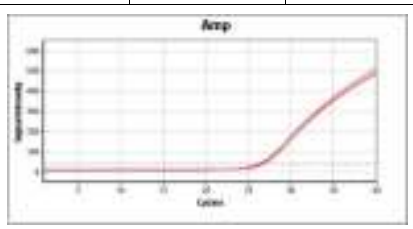
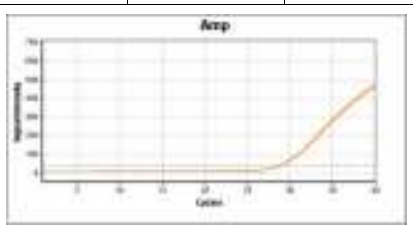
Time Point	Test	N			RdRP		
Day 4	No. 1						
		17.17	17.02	17.52	21.06	21.32	20.99
	Result	Positive	Positive	Positive	Positive	Positive	Positive
	No. 2						
		16.88	17.35	17.05	21.16	21.22	20.13
	Result	Positive	Positive	Positive	Positive	Positive	Positive
Day 5	No. 1						
		17.01	16.56	16.70	20.61	20.21	20.11
	Result	Positive	Positive	Positive	Positive	Positive	Positive
	No. 2						
		17.04	17.08	16.83	19.67	20.04	20.28
	Result	Positive	Positive	Positive	Positive	Positive	Positive
Day 6	No. 1						
		17.09	17.16	17.50	20.08	20.19	20.73
	Result	Positive	Positive	Positive	Positive	Positive	Positive

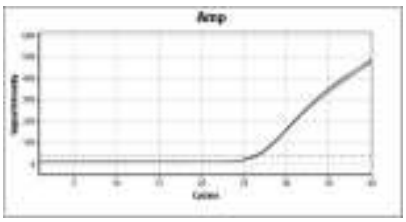
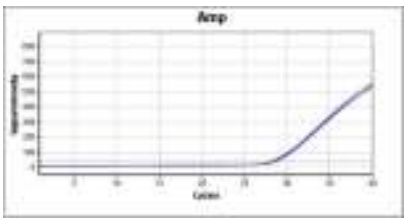
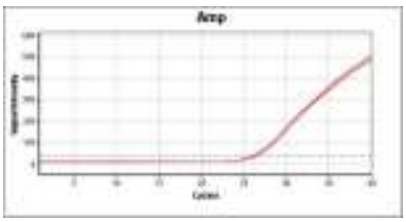
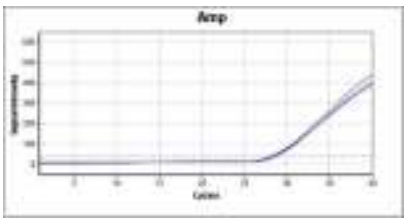
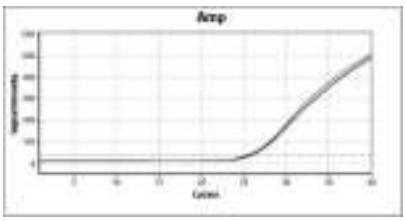
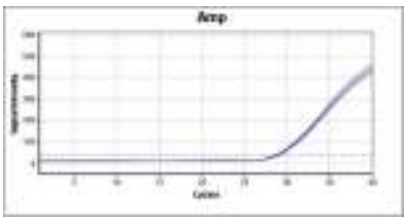
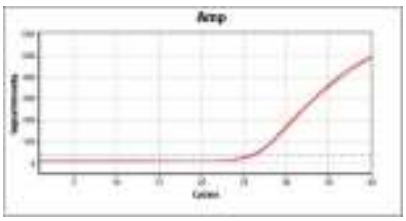
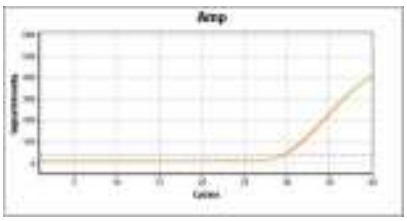
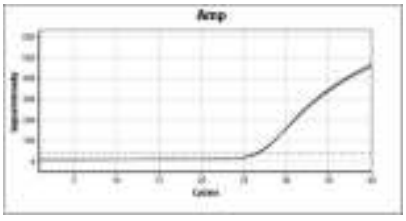
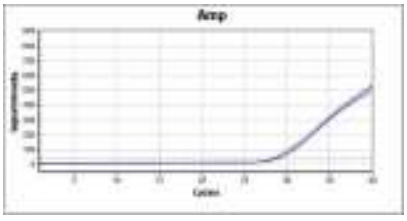
Time point	Test	N			RdRP		
Day 6	No. 2						
		17.06	17.10	17.28	20.22	20.18	20.17
	Result	Positive	Positive	Positive	Positive	Positive	Positive
Day 7	No. 1						
		15.92	15.38	16.33	20.21	20.21	20.33
	Result	Positive	Positive	Positive	Positive	Positive	Positive
	No. 2						
		16.18	16.93	16.23	20.43	20.31	20.28
	Result	Positive	Positive	Positive	Positive	Positive	Positive
Day 8	No. 1						
		16.86	16.81	16.93	20.38	21.15	21.25
	Result	Positive	Positive	Positive	Positive	Positive	Positive
	No. 2						
		17.05	16.46	16.69	21.30	21.25	21.14
	Result	Positive	Positive	Positive	Positive	Positive	Positive

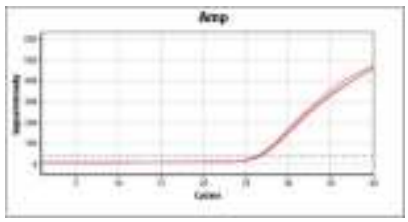
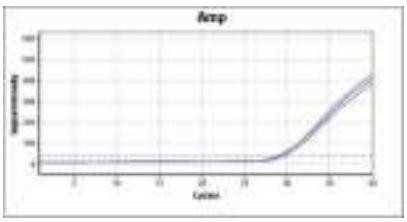
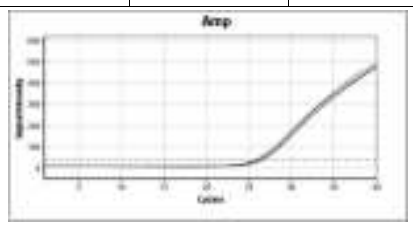
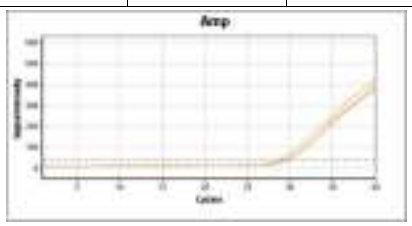
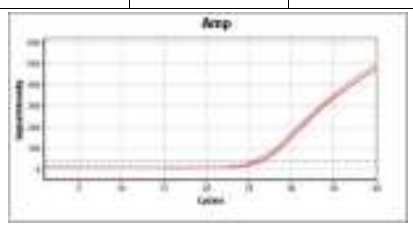
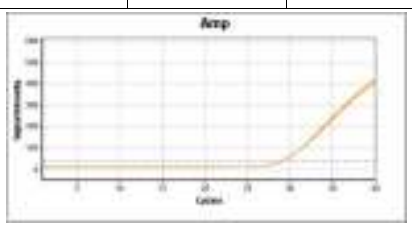
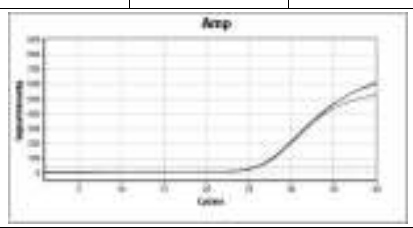
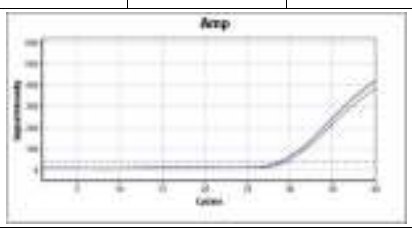
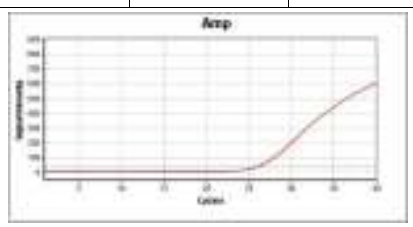
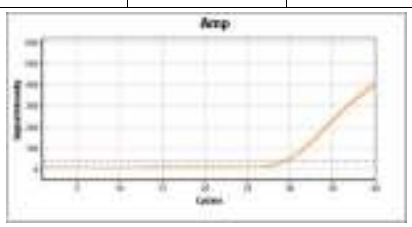
2.7.3.2 Repeatability tests with the templates in low concentration

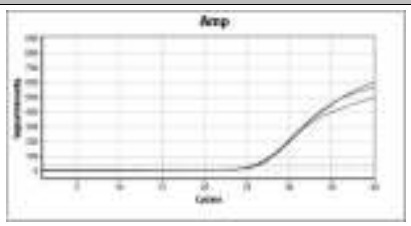
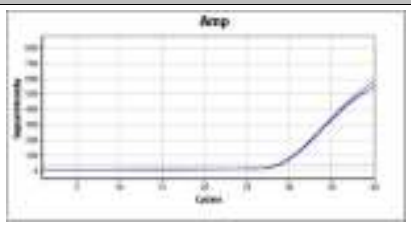
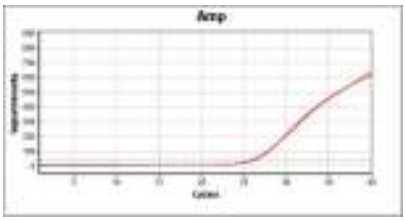
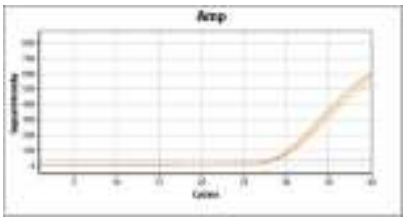
From the tests with the standard positive templates in low concentration, it was confirmed that the time point variation of N gene detection primers/probes was 0.33 Ct and the one of RdRP gene detection primers/probes was 0.44 Ct.

- Repeatability test result with the templates in low concentration

Time point	Test	N			RdRP		
Day 1	No. 1						
		26.57	26.25	26.03	28.61	28.50	29.45
	Result	Positive	Positive	Positive	Positive	Positive	Positive
	No. 2						
		25.96	26.12	26.44	28.87	29.05	28.67
	Result	Positive	Positive	Positive	Positive	Positive	Positive
Day 2	No. 1						
		26.65	26.06	26.30	28.53	28.84	28.50
	Result	Positive	Positive	Positive	Positive	Positive	Positive
	No. 2						
		26.42	26.12	26.75	28.76	29.05	28.85
	Result	Positive	Positive	Positive	Positive	Positive	Positive

Time point	Test	N			RdRP		
Day 3	No. 1						
		26.32	26.25	26.55	28.64	28.35	28.19
	Result	Positive	Positive	Positive	Positive	Positive	Positive
	No. 2						
		26.44	26.33	26.11	28.40	28.52	28.06
	Result	Positive	Positive	Positive	Positive	Positive	Positive
Day 4	No. 1						
		26.09	25.86	25.50	29.08	28.97	28.72
	Result	Positive	Positive	Positive	Positive	Positive	Positive
	No. 2						
		26.06	26.29	25.89	29.59	29.46	29.66
	Result	Positive	Positive	Positive	Positive	Positive	Positive
Day 5	No. 1						
		26.65	26.41	26.38	28.74	28.62	28.20
	Result	Positive	Positive	Positive	Positive	Positive	Positive

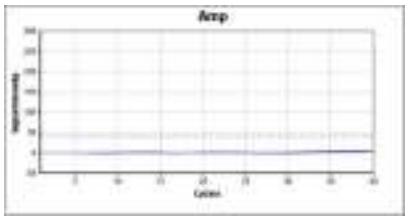
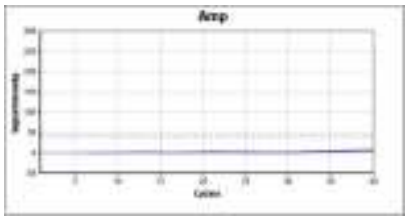
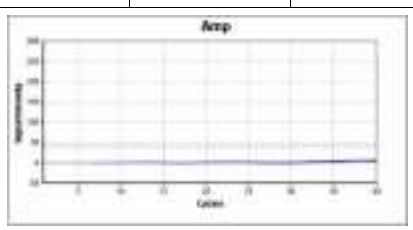
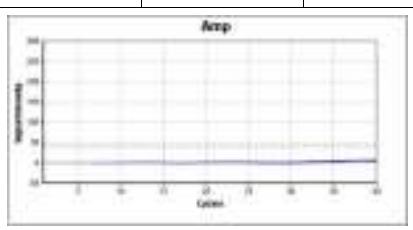
Time point	Test	N			RdRP		
Day 5	No. 2						
		26.47	26.26	26.63	28.99	28.92	29.59
	Result	Positive	Positive	Positive	Positive	Positive	Positive
Day 6	No. 1						
		25.84	26.56	26.18	29.63	28.68	29.46
	Result	Positive	Positive	Positive	Positive	Positive	Positive
	No. 2						
		25.47	26.22	26.59	29.04	29.14	29.04
	Result	Positive	Positive	Positive	Positive	Positive	Positive
Day 7	No. 1						
		25.79	25.68	25.53	28.73	29.53	28.77
	Result	Positive	Positive	Positive	Positive	Positive	Positive
	No. 2						
		26.09	25.96	25.84	29.65	29.63	28.94
	Result	Positive	Positive	Positive	Positive	Positive	Positive

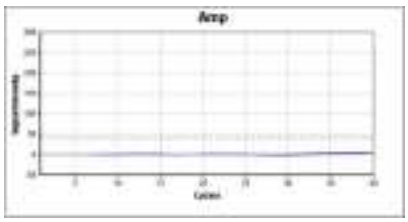
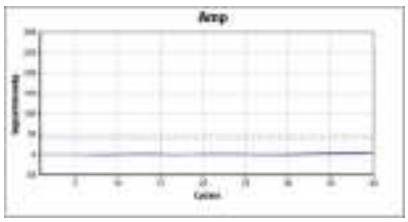
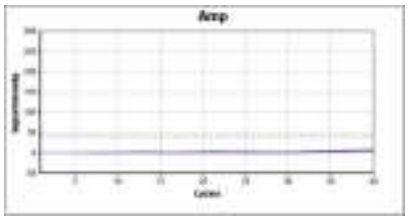
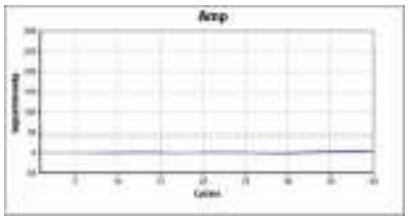
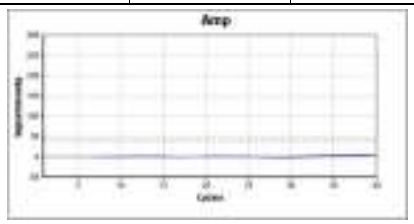
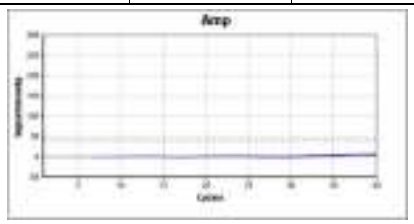
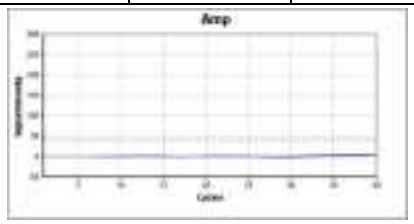
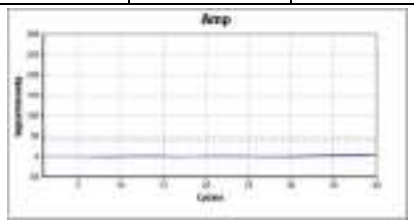
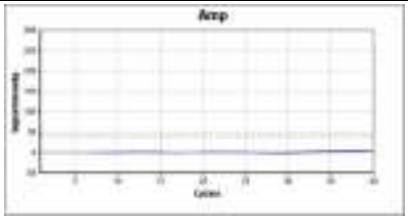
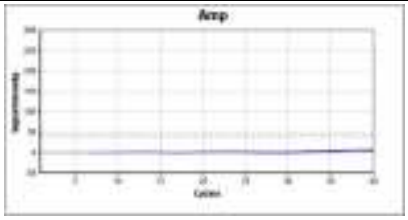
Time point	Test	N			RdRP		
Day 8	No. 1						
		26.01	25.66	26.15	28.77	28.40	28.43
	Result	Positive	Positive	Positive	Positive	Positive	Positive
	No. 2						
		25.67	25.62	26.09	28.61	28.42	28.04
	Result	Positive	Positive	Positive	Positive	Positive	Positive

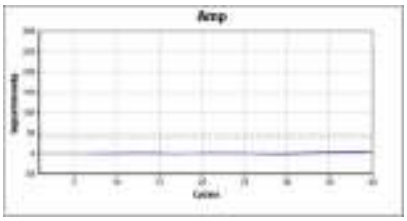
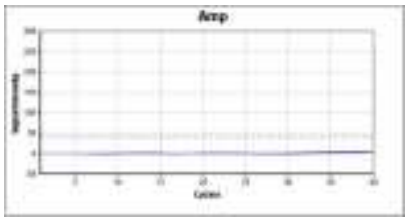
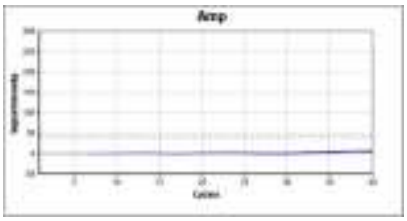
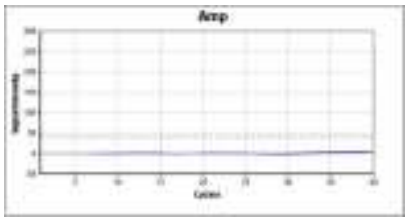
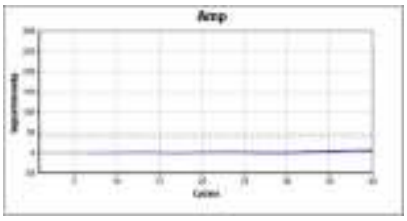
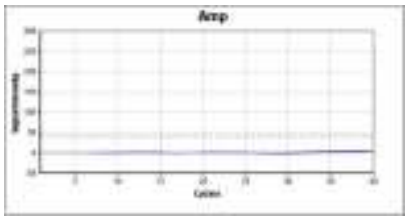
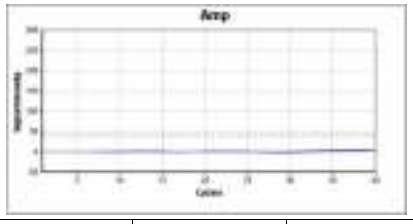
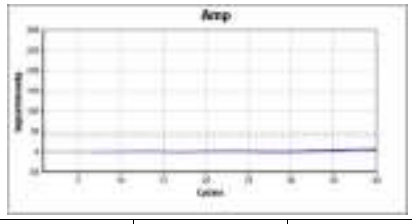
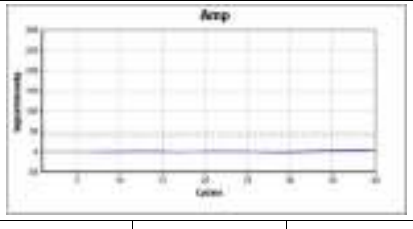
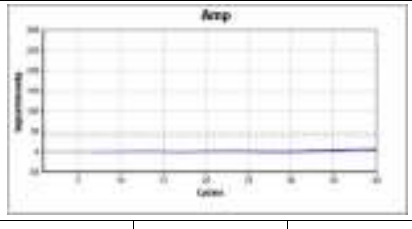
2.7.3.3 Repeatability **test with no template control**

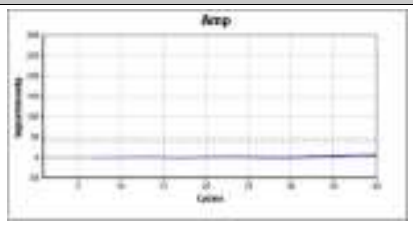
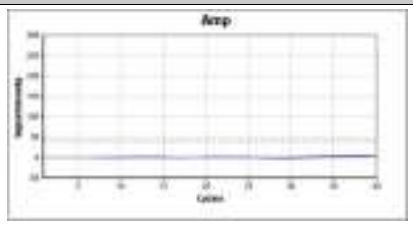
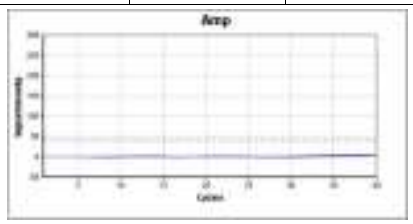
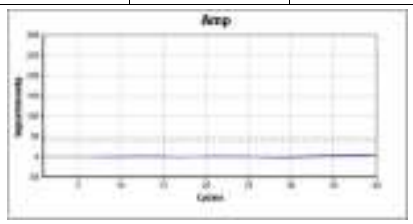
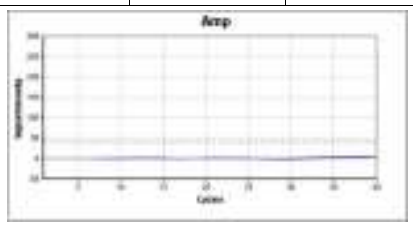
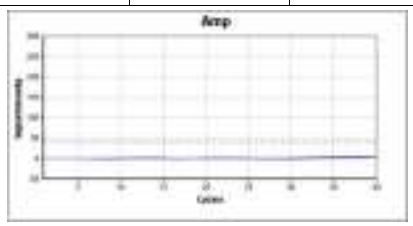
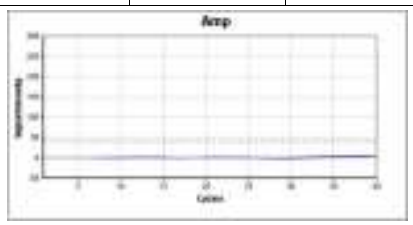
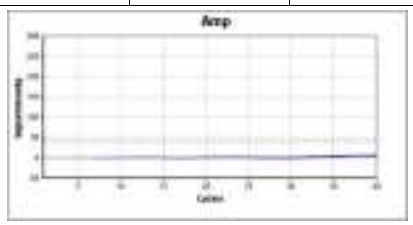
From the tests with the no template control, there was no amplification at all the tests and it was confirmed that there is no time point variation of the tests.

◆ Repeatability test result with no template control

Time point	Test	N			RdRP		
Day 1	No. 1						
		0.00	0.00	0.00	0.00	0.00	0.00
	Result	Negative	Negative	Negative	Negative	Negative	Negative
	No. 2						
		0.00	0.00	0.00	0.00	0.00	0.00
	Result	Negative	Negative	Negative	Negative	Negative	Negative

Time point	Test	N			RdRP		
Day 2	No. 1						
		0.00	0.00	0.00	0.00	0.00	0.00
	Result	Negative	Negative	Negative	Negative	Negative	Negative
	No. 2						
		0.00	0.00	0.00	0.00	0.00	0.00
	Result	Negative	Negative	Negative	Negative	Negative	Negative
Day 3	No. 1						
		0.00	0.00	0.00	0.00	0.00	0.00
	Result	Negative	Negative	Negative	Negative	Negative	Negative
	No. 2						
		0.00	0.00	0.00	0.00	0.00	0.00
	Result	Negative	Negative	Negative	Negative	Negative	Negative
Day 4	No. 1						
		0.00	0.00	0.00	0.00	0.00	0.00
	Result	Negative	Negative	Negative	Negative	Negative	Negative

Time point	Test	N			RdRP		
Day 4	No. 2						
		0.00	0.00	0.00	0.00	0.00	0.00
	Result	Negative	Negative	Negative	Negative	Negative	Negative
Day 5	No. 1						
		0.00	0.00	0.00	0.00	0.00	0.00
	Result	Negative	Negative	Negative	Negative	Negative	Negative
	No. 2						
		0.00	0.00	0.00	0.00	0.00	0.00
	Result	Negative	Negative	Negative	Negative	Negative	Negative
Day 6	No. 1						
		0.00	0.00	0.00	0.00	0.00	0.00
	Result	Negative	Negative	Negative	Negative	Negative	Negative
	No. 2						
		0.00	0.00	0.00	0.00	0.00	0.00
	Result	Negative	Negative	Negative	Negative	Negative	Negative

Time point	Test	N			RdRP		
Day 7	No. 1						
		0.00	0.00	0.00	0.00	0.00	0.00
	Result	Negative	Negative	Negative	Negative	Negative	Negative
	No. 2						
		0.00	0.00	0.00	0.00	0.00	0.00
	Result	Negative	Negative	Negative	Negative	Negative	Negative
Day 8	No. 1						
		0.00	0.00	0.00	0.00	0.00	0.00
	Result	Negative	Negative	Negative	Negative	Negative	Negative
	No. 2						
		0.00	0.00	0.00	0.00	0.00	0.00
	Result	Negative	Negative	Negative	Negative	Negative	Negative

2.8 Validity Test

2.8.1 Summary of the test

For the evaluation of the validity of the assay, cloned DNA of target genes was used as standard template for the tests. Using TE buffer, the standard positive sample in medium concentration ($1x10^6$ copies) and the one in low concentration ($1x10^3$ copies) was respectively prepared for the tests. Prepared standard positive samples in 5µL volume were used for the tests to evaluate the validity of the assay. The same volume of Nuclease free water was used as negative control for each test.

2.8.2 Method of the test

In order to evaluate the validity of the assay, reaction mixtures were prepared in accordance with the following composition. Prepared reaction mixtures were loaded in the test chip.

◆ Composition of reaction mixture

Component	Volume
Templates in different concentrations	5 μL
2x Premix	5 μL
Total	10 μL

Following reaction program was used for the test with GENECHECKER® UF-300 real-time PCR system

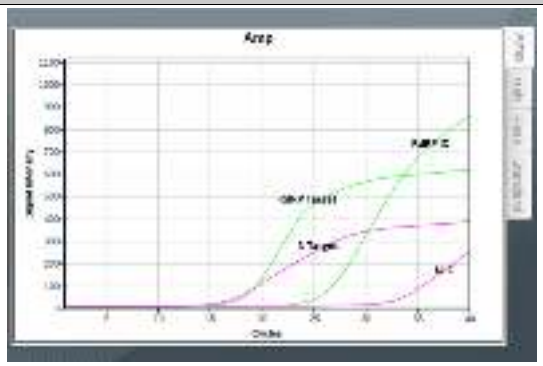
◆ Reaction program with GENECHECKER® UF-300 real-time PCR platform

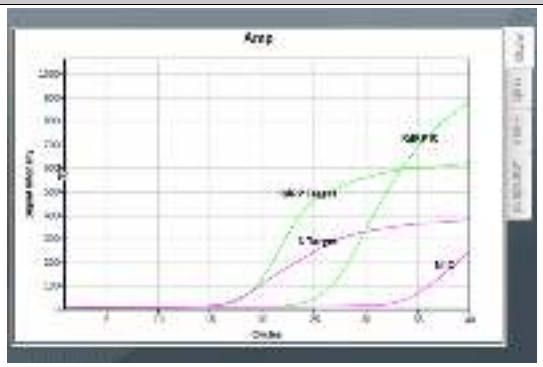
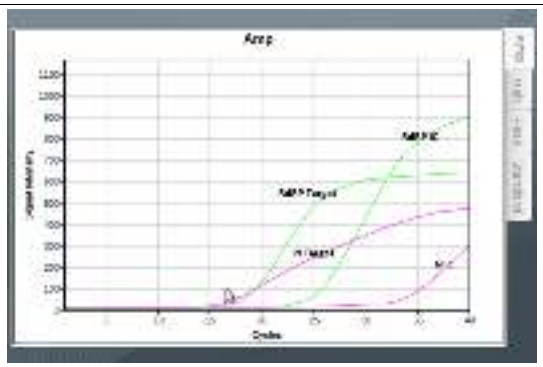
PCR Step	Temperature	Time	Cycles
Reverse Transcription	50℃	600 sec	1
Pre-Denaturation	95℃	30 sec	1
Denaturation	95℃	5 sec	40
Annealing	58℃	20 sec	
Extension	72℃	5 sec	

2.8.3 Result of the Test

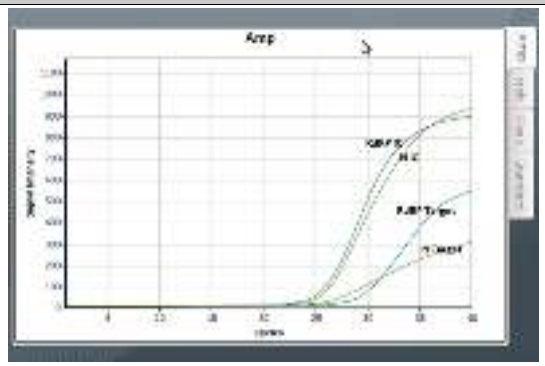
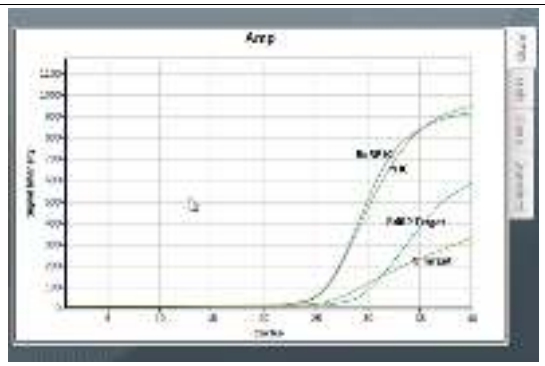
Three repeatable tests using the assay along with the standard positive templates in medium and low concentrations were performed. From the tests, it was confirmed that all the standard positive templates were successfully amplified while negative controls were not amplified.

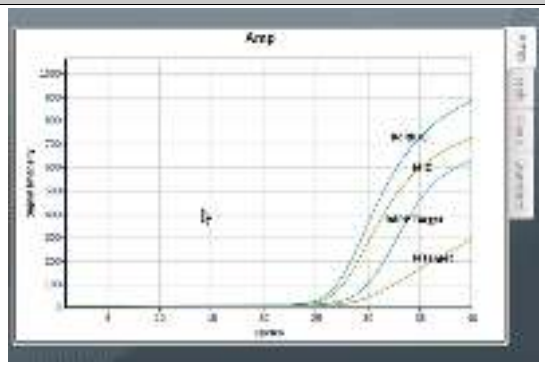
◆ Validity test result with the templates in medium concentration

Template	Test	Displayed chart after completion of the test	Ct (N)	Ct (RdRP)	Result
$1x10^6$ copies.	No.1		18.36	18.77	Positive

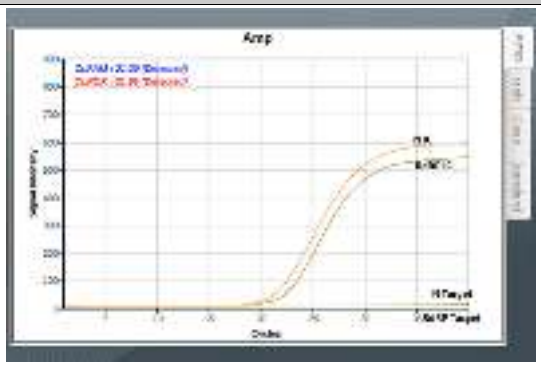
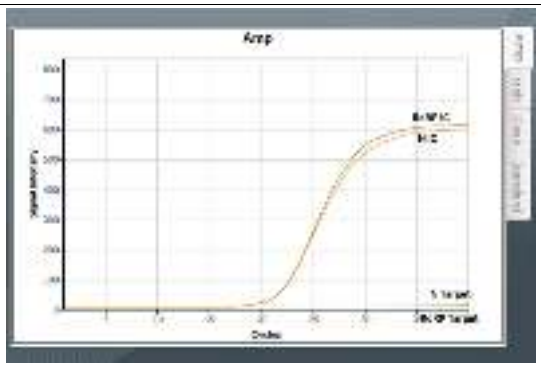
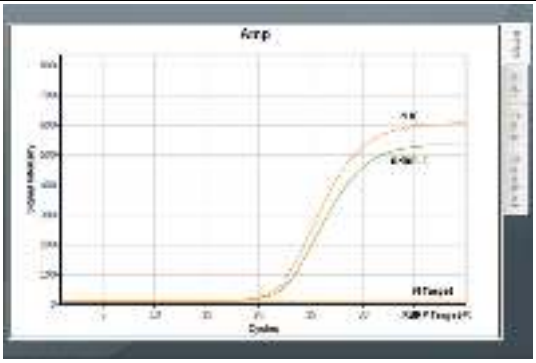
Template	Test	Displayed chart after completion of the test	Ct (N)	Ct (RdRP)	Result
$1x10^6$ copies.	No.2		17.89	18.69	Positive
	No.3		18.12	18.56	Positive

◆ Validity test result of templates in low concentration

Template	Test	Displayed chart after completion of the test	Ct (N)	Ct (RdRP)	Result
$1x10^3$ copies.	No.1		27.65	29.32	Positive
	No.2		27.54	29.21	Positive

Template	Test	Displayed chart after completion of the test	Ct (N)	Ct (RdRP)	Result
	3		30.44	28.82	Positive

◆ Validity test result of negative controls

Template	Test	Displayed chart after completion of the test	Ct (N)	Ct (RdRP)	Result
NTC	1		0.00	0.00	Negative
	2		0.00	0.00	Negative
	3		0.00	0.00	Negative

2.9 Clinical Performance Test

2.9.1 Summary of the test

For the test of clinical performance of the assay, genomic RNA of SARS-CoV-2 which was isolated from the upper airway swab of COVID-19 patient was used. This sample was obtained from National Culture Collection for Pathogen of Centers for Disease Control and Prevention of South Korea. Using TE buffer, the 6 genomic RNA samples of SARS-CoV-2 in 10 fold serial dilution were prepared as follows.

No.	Sample	Sample Type	Sample Concentration	Sample Source	NCCP* No.
1	SARS-CoV-2	Extracted RNA	54 ng/μL	KCDC**	43326
2	SARS-CoV-2	Extracted RNA	5.4 ng/μL	KCDC	43326
3	SARS-CoV-2	Extracted RNA	0.54 ng/μL	KCDC	43326
4	SARS-CoV-2	Extracted RNA	0.054 ng/μL	KCDC	43326
5	SARS-CoV-2	Extracted RNA	0.0054 ng/μL	KCDC	43326
6	SARS-CoV-2	Extracted RNA	0.00054 ng/μL	KCDC	43326

* National Culture Collection for Pathogens ** Centers for Disease Control and Prevention of South Korea

Prepared standard genomic RNA samples in 5μL volume were used for the tests to evaluate the performance of the assay.

2.9.2 Method of the test

In order to evaluate the performance of the assay with genomic RNA isolated from clinical sample, reaction mixtures were prepared in accordance with the following recipe. Prepared reaction mixtures were loaded in the test chip.

◆ Composition of reaction mixture

Component	Volume
Templates in different concentrations	5 μL
2x Premix	5 μL
Total	10 μL

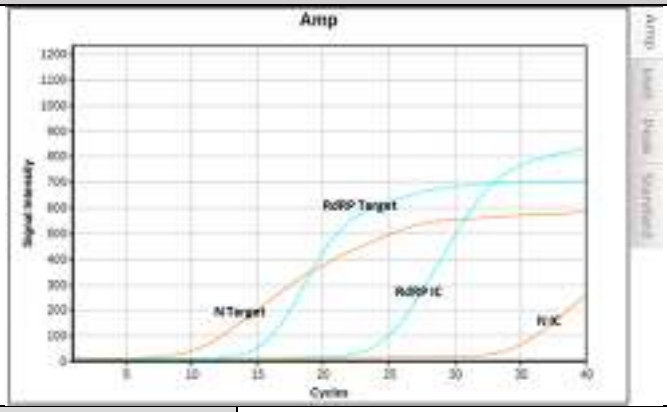
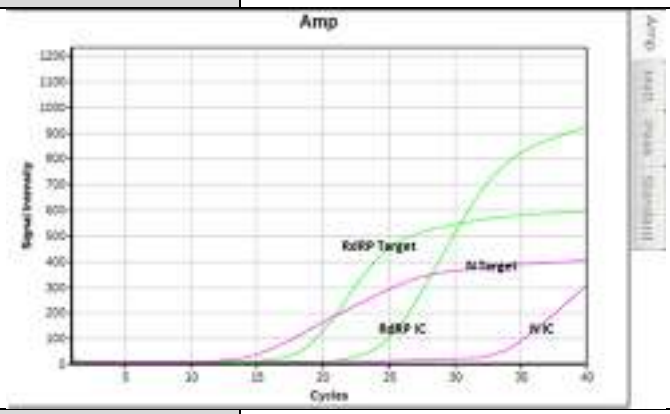
Following reaction program was used for the test with GENECHECKER® UF-300 real-time PCR system

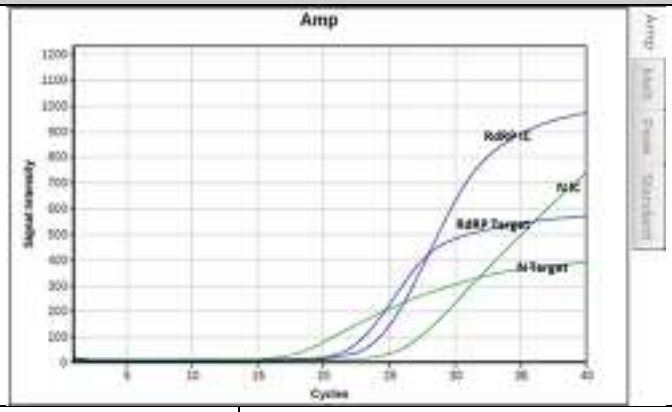
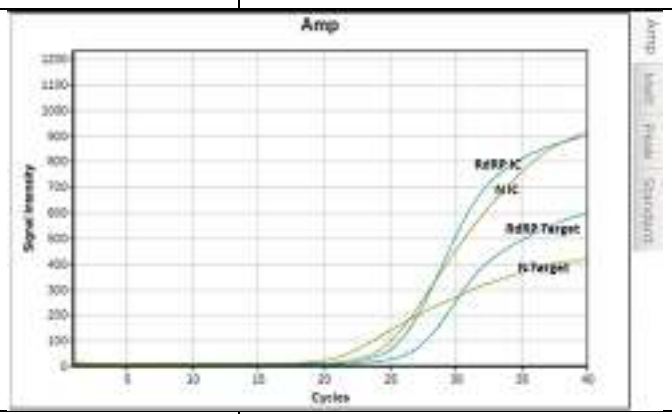
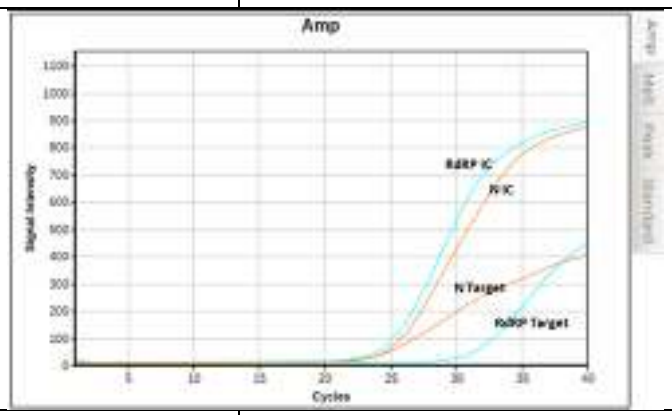
◆ Reaction program with GENECHECKER® UF-300 real-time PCR platform

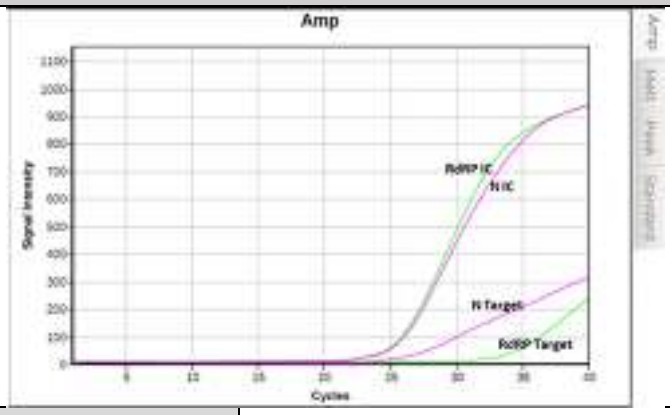
PCR Step	Temperature	Time	Cycles
Reverse Transcription	50℃	600 sec	1
Pre-Denaturation	95℃	30 sec	1
Denaturation	95℃	5 sec	40
Annealing	58℃	20 sec	
Extension	72℃	5 sec	

2.9.3 Result of the test

All the tests using 6 genomic RNA of SAR-CoV-2 samples in 10-fold serial dilution provided positive results as illustrated below.

Sample Concentration	Displayed chart after completion of the test	
54 ng/μL		
	Ct.(N)	11.14 (+)
	Ct.(RdRP)	15.42 (+)
	Result	SARS-CoV-2 positive
5.4 ng/μL		
	Ct.(N)	16.20 (+)
	Ct.(RdRP)	18.71 (+)
	Result	SARS-CoV-2 positive

Sample Concentration	Displayed chart after completion of the test	
0.54 ng/μL		
	Ct.(N)	19.25 (+)
	Ct.(RdRP)	22.23 (+)
	Result	SARS-CoV-2 positive
0.054 ng/μL		
	Ct.(N)	22.23 (+)
	Ct.(RdRP)	26.66 (+)
	Result	SARS-CoV-2 positive
0.0054 ng/μL		
	Ct.(N)	25.19 (+)
	Ct.(RdRP)	31.50 (+)
	Result	SARS-CoV-2 positive

Sample Concentration	Displayed chart after completion of the test	
0.00054 ng/μL		
	Ct.(N)	28.01 (+)
	Ct.(RdRP)	34.63 (+)
	Result	SARS-CoV-2 positive

From the clinical performance test of the assay, it was verified that the assay provides designed analytical performance with genomic RNA of SARS-CoV-2.

3. Conclusion

From the validation study on SMARTCHEK® Novel Coronavirus (SARS-CoV-2) Detection Kit of Genesystem which was conducted by the molecular biologists of diagnostic technology development department from corporate R&D center of Genesystem, following results were obtained.

- The limit of detection of the assay is 100 copies based on the result of the sensitivity tests.

- This assay has no cross reactions with 8 different viruses which are Influenza A virus (H3N2 subtype), Influenza A virus (H1N1 subtype), Influenza B virus, human Coronavirus NL63, Rhinovirus, Enterovirus, Respiratory syncytial virus (type B) and Respiratory syncytial virus (type A).

- This assay offered consistent results from the reproducibility tests under various parameters to check the lot-to-lot variation, tester-to-tester variation, time-to-time variation and space-to-space variation.

- This assay offered consistent results from the repeatability tests performed by three times per a day for 8 consecutive days.

- The assay produced in accordance with the standard operating procedure of Genesystem provided valid test results from the three repeatable tests which was performed in accordance with the final testing protocol.

- The assay provided designed analytical performance from the tests with genomic RNA of SARS-CoV-2 which was isolated from upper airway swab of COVID-19 patient.

In conclusion, from the validation study, it was verified that the sensitivity, specificity, reproducibility, repeatability and validity of SMARTCHEK® Novel Coronavirus (SARS-CoV-2) Detection Kit meets its specification.