Validation of Real-Time RT-PCR for Detection of SARS-CoV-2 in the Early Stages of the COVID-19 Outbreak Without Emergency Use Authorization Reagents in the Republic of Korea

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Research

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Abstract

Background: A real-time reverse transcription polymerase chain reaction (RT-PCR) assay for detecting severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) was developed to rapidly diagnose coronavirus disease 2019 (COVID-19). Early diagnosis of COVID-19 enables timely treatment and the implementation of public health measures. We validated the sensitivity, specificity, precision, linearity, accuracy, and robustness of a real-time RT-PCR-based assay for SARS-CoV-2 detection and compared the sensitivity and specificity of Emergency Use Authorization (EUA)-approved reagents for COVID-19 with those of the real-time RT-PCR method.

Methods: The real-time RT-PCR-based assay was performed to specifically amplify genomic markers of SARS-CoV-2.

Results: The real-time RT-PCR assay was highly specific for SARS-CoV-2, and did not amplify genomic fragments of 13 other viruses that cause respiratory diseases. The assay showed high linearity when conducted with a viral isolate from a patient with COVID-19 together with plasmids containing the target genes. The assay showed good repeatability and reproducibility, with a coefficient of variation of 3%, and detected SARS-CoV-2 with a limit of detection of 1 PFU/mL.

Conclusions: The present real-time RT-PCR-based assay can diagnose COVID-19 with high accuracy and sensitivity. This approach is highly effective and can facilitate the early diagnosis of COVID-19 without the use of EUA reagents in the Republic of Korea.

Background

Coronavirus disease 2019, officially named COVID-19 by the World Health Organization (WHO) is a severe acute respiratory syndrome (SARS) caused by a novel coronavirus (SARS-CoV-2). COVID-19 was first reported as an idiopathic pneumonia in Hubei Province, Wuhan, China, in December 2019 [1]. SARS-CoV-2 is more contagious than SARS-CoV, which was first reported in China in 2002, and Middle East respiratory syndrome coronavirus (MERS-CoV), which first emerged in the Middle East in 2012 [2]. SARS-CoV-2 has since spread to several countries outside of China, and has affected populations worldwide. On March 11, 2020, the WHO declared COVID-19 to be a pandemic, and by April 24, 2,653,573 confirmed cases of COVID-19 and 189,658 deaths had been reported across 181 countries. In South Korea, the first confirmed case was reported on January 19, followed by a surge in confirmed cases on February 19; by April 24, 10,718 confirmed cases and 240 deaths were reported nationwide [3–6].

SARS-CoV-2 belongs to the subfamily Orthocoronavirinae, which is a member of the family Coronaviridae [7]. This beta coronavirus has a 30-kb genome, sharing 96% sequence identity with the bat coronavirus RaTG13, 88% with bat coronaviruses ZC45 and ZXC21, 80% with SARS-CoV, and 50% with MERS-CoV [8]. Coronaviruses are enveloped, positive-sense, single-stranded RNA viruses, with at least six open reading frames. The coronavirus genome encodes the following structural proteins: spike (S) glycoprotein, membrane (M) glycoprotein, envelope (E) glycoprotein, and nucleocapsid (N) glycoprotein [8].

Real-time reverse transcription polymerase chain reaction (RT-PCR) is used to detect gene expression levels, facilitating the rapid diagnosis of acute respiratory viral infections [9]. COVID-19 can be diagnosed in the laboratory by detecting SARS-CoV-2 genes in clinical samples collected from suspected patients, followed by viral isolation and culture [10]. Real-time RT-PCR is commonly used worldwide to diagnose COVID-19 in the laboratory setting [9, 11, 12].

A real-time RT-PCR-based assay for detecting SARS-CoV-2 was first developed at the Charité Institute of Virology in Germany, and introduced by the WHO on January 13, 2020 [9]. Additional protocols were subsequently reported by the Chinese Center for Disease Control and Prevention, University of Hong Kong, and the Centers for Disease Control and Prevention of the United States [13]. The assay targets the RNA-dependent RNA polymerase (RdRp) gene, the envelope (E) gene, and the nucleocapsid (N) gene of SARS-CoV-2 [14]. Published protocols differ based on the target genes, primer and
probe sequences, mixture composition, amplification cycles and conditions, and sensitivity. Thus, a standardized, validated assay with highly accurate laboratory performance for detecting SARS-CoV-2 is essential. In this study, the analytical sensitivity, specificity, precision, linearity, accuracy, and robustness of a real-time RT-PCR-based assay for SARS-CoV-2 detection were assessed.

We also compared the sensitivity and specificity of COVID-19 emergency use authorization (EUA)-approved reagents with those of the real-time RT-PCR method investigated in this study. To determine the accuracy of the results obtained using each reagent, evaluation and verification were carried out using five EUA approved products and respiratory samples from suspected patients.

**Methods**

**Cells and viruses**

The SARS-CoV-2/Korea/KCDC03/2020 virus, isolated by the Korean Center for Disease Control, was used in the experiment. Vero E6 cells were first inoculated with the viruses and cultured for four days. The culture medium was then centrifuged, aliquoted, and stored at −70 °C, and the virus titers were measured using a plaque assay. Viral culture was performed in a biosafety level (BSL)-3 laboratory.

**Extraction of viral RNA**

RNA was extracted from the culture medium using a QIAamp viral RNA mini kit (QIAGEN, Hilden, Germany) according to the manufacturer's instructions. Viral lysis was performed in a BSL-3 laboratory, whereas procedures involving RNA were performed in a BSL-2 laboratory. We extracted RNA from 140 µL of the sample using Qiagen viral RNA mini kits, in accordance with the manufacturer's instructions.

**Real-time RT-PCR for SARS-CoV-2**

Information regarding the primers provided by the WHO and used in real-time RT-PCR [2] is shown in Table 1. AgPath-ID™ one-step RT-PCR reagents (Applied Biosystems, Foster City, CA, USA) were used in accordance with the manufacturer's instructions. One microliter of the primers (10 pmol) and 0.5 µL of probes (10 pmol) were added to the reagents. Primer/probe sets for detecting the RdRp and E genes were added to different tubes. The RNA sample (5 µL) was mixed with the PCR mixture, and PCR was performed at 50 °C for 30 min, 95 °C for 10 min, 95 °C for 15 s, and 60 °C for one min, for 40 cycles; ROX (carboxyrhodamine) was used as a passive reference dye. The Applied Biosystems™ 7500 Fast Real-Time PCR System was used for real-time RT-PCR with the extracted RNA, and the cycle threshold (Ct) value of the SARS-CoV-2 target gene was ascertained (Table 1).
Table 1
Primers and probes in this study for detection of SARS-CoV 2

| Primer/Probe | Sequence (5’–3’) | 
|-------------|-----------------|
| RdRp gene   |                 | 
| RdRp_SARSr-F2 | GTGARATGGTATGTGTGGCGG | 
| RdRp_SARSr-R1 | CARATGTTGGAACACACTAGCATA | 
| RdRp_SARSr-P2 | FAM-CAGGTCACCTACTCAGGAGATGC-BHQ | 
| E gene      |                 | 
| E_Sarbeco_F1 | ACAGGTCAGTTAATAGTTAATAGCGT | 
| E_Sarbeco_R2 | ATATTGACGACGTACGACACA | 
| E_Sarbeco_P1 | FAM-ACACAGCCATCCTGACTGCGCTTCG-BHQ | 

R is G/A; FAM, 6-carboxyfluorescein; BHQ, black hole quencher

Determination of specificity and sensitivity

To assess the specificity of real-time RT-PCR, 23 virus strains—human coronavirus 229E, NL63, OC43, HKU1, MERS-CoV, influenza virus A/H1N1 pdm09, A/H3N2, B, adenovirus type 5, rhinovirus, parainfluenza virus 1/2/3, respiratory syncytial virus A/B, metapneumovirus, bocavirus, measles virus, mumps virus, rubella virus, enterovirus, varicella-zoster virus, and hantavirus (Table 2)—and five samples showing negative results for a known respiratory virus were used. Sensitivity was measured by real-time RT-PCR using plasmids containing the target genes, which were diluted 10-fold from different initial concentrations. To examine the responsivity of the assay to RNA, RT-PCR was performed using 10-fold diluted RNA extracted from a lower respiratory tract sample of the first patient who tested positive for COVID-19 in South Korea.

Table 2
COVID-19 Nucleic detection kit EUA approved in ROK

| Product name                                      | Approval Date | Target gene | Manufacture  |
|--------------------------------------------------|---------------|-------------|--------------|
| PowerCheckTM2019-nCoV                             | 2.4.2020      | RdRp, E     | Kogenbiotech |
| AllplexTM2019-nCoVAssay                          | 2.12.2020     | RdRp, E, N  | Seegene      |
| DiaPlexQTMNovel Coronavirus (2019-nCoV) Detection kit | 2.27.2020    | ORF1a, N    | Solgent      |
| STANDARD M nCoV Real-Time Detection kit           | 2.27.2020     | RdRp, E     | SD biosenser |
| Real-Q 2019-nCoV Detection kit                    | 3.13.2020     | RdRp, E     | Bioseum      |

Plaque assay for virus titration

Vero E6 cells were seeded into 12-well plates at 2.0 × 10^5 cells per well. After 24 h, these cells were infected with 50 µL of 10-fold serial dilutions of the virus samples, and incubated for 1 h to facilitate viral adsorption. The cells were then covered with a basal MEM-α agar overlay containing 0.02% (w/v) diethylaminoethyl-dextran, 0.1% (w/v) glucose, 0.7% (w/v) SeaKem® LE Agarose (LONZA, Basel, Switzerland), 30 mM MgSO₄, and 4 µg/mL trypsin (Gibco, Grand Island, NY, USA). The cells were incubated at 37 °C for three days to facilitate infection. Two days after viral infection, a 0.03% (w/v) crystal violet overlay was added to each well to stain viable cells.

Determination of intra-assay and inter-assay reproducibility and efficiency
To determine the limit of detection (LOD), the titers of the isolated viruses were measured in plaque-forming units (PFU). The virus culture medium was diluted from $3.45 \times 10^6$ PFU/mL to $1 \times 10^5$ PFU/mL. Ten-fold dilutions of the medium were prepared until a concentration of $1 \times 10^{-2}$ PFU/mL was obtained. RNA was extracted from each diluent and used for real-time RT-PCR. The RdRp and E genes were targeted for detection. Real-time RT-PCR was performed in triplicate to assess the assay reproducibility. The assay was repeated three days later, using RNA extracted from the diluted virus culture media to assess repeatability.

**Determination of accuracy of EUA reagents**

To investigate the accuracy of the COVID-19 EUA-approved reagents in the Republic of Korea (Table 2), 55 positive samples (selected five-step positive samples based on the distribution of Ct values of the RdRp gene) and 50 negative samples were used to confirm the matching rate (sensitivity and specificity) of the results between the methods used in this study and each EUA product.

**Statistical analysis**

Inter-assay and intra-assay variations in the Ct value were determined for the triplicate real-time RT-PCR reactions and for the repeat assay three days later. The reliability of each experiment was determined from the F and $P$ values.

**Results**

**Specificity and sensitivity**

Viral RNA detected by PCR or RT-PCR, which was specific to the viral genes, was used for the assay. Human coronaviruses 229E, NL63, OC43, and HKU1, SARS-CoV, MERS-CoV, influenza virus, adenovirus, rhinovirus, parainfluenza virus, respiratory syncytial virus, metapneumovirus, and bocavirus were not detected by this assay. The Ct value could not be determined for measles virus, mumps virus, rubella virus, enterovirus D68, or the nasopharyngeal swab specimens. These results indicate that the assay was highly specific for both target regions, the RdRp and E genes, in SARS-CoV-2 (Table 3).
Table 3
Tests of known respiratory viruses and respiratory specimens for cross-reactivity in SARS-CoV 2 RdRp and E gene real-time PCR

| Viruses and specimens | Subtype | Real-time RT-PCR |
|-----------------------|---------|-----------------|
|                       |         | RdRP | E-Sarbeco |
| HCoV 229E             | UD      | UD   | UD       |
| HCoV NL63             | UD      | UD   | UD       |
| HCoV OC43             | UD      | UD   | UD       |
| HCoV HKU1             | UD      | UD   | UD       |
| MERS-CoV              | KCDC    | UD   | UD       |
| Influenza virus A(H1N1) | UD  | UD   | UD       |
| Influenza virus A(H3N2) | UD  | UD   | UD       |
| Influenza virus B      | UD      | UD   | UD       |
| Adenovirus Type 5     | UD      | UD   | UD       |
| Rhinovirus            | UD      | UD   | UD       |
| Parainfluenza virus 1 | UD      | UD   | UD       |
| Parainfluenza virus 2 | UD      | UD   | UD       |
| Parainfluenza virus 3 | UD      | UD   | UD       |
| Respiratory syncytial virus A | UD  | UD   | UD       |
| Respiratory syncytial virus B | UD  | UD   | UD       |
| Human Metapneumovirus | UD      | UD   | UD       |
| Human Bocavirus       | UD      | UD   | UD       |
| Measles virus A       | UD      | UD   | UD       |
| Mumps virus Jerylin   | UD      | UD   | UD       |
| Rubella virus Moraten  | UD      | UD   | UD       |
| Enterovirus D68       | UD      | UD   | UD       |
| Varicella Zoster virus| UD      | UD   | UD       |
| Hantanvirus           | UD      | UD   | UD       |
| Negative specimen 1   | UD      | UD   | UD       |
| Negative specimen 2   | UD      | UD   | UD       |
| Negative specimen 3   | UD      | UD   | UD       |
| Negative specimen 4   | UD      | UD   | UD       |
| Negative specimen 5   | UD      | UD   | UD       |
Analytical sensitivity was assessed by determining the LOD for each gene using plasmid DNA containing the RdRp and E genes of SARS-CoV-2. The assay had a mean LOD of $8 \times 10^0$ copies/µL in triplicate runs (Table 4, Fig. 1). Ct values of 37.96 and 37.19 and CVs of 0.65 and 0.34 were obtained for the RdRp and E genes, respectively, indicating good analytical performance.

### Table 4

| Copies/mL | 1st | 2nd | 3rd | Average | CV |
|-----------|-----|-----|-----|---------|----|
|           | RdRP | E-Sarbeco | RdRP | E-Sarbeco | RdRP | E-Sarbeco | RdRP | E-Sarbeco | RdRP | E-Sarbeco |
| $8 \times 10^4$ | 24.18 | 23.51 | 24.25 | 23.38 | 24.15 | 23.28 | 24.19 | 23.39 | 0.05 | 0.12 |
| $8 \times 10^3$ | 27.68 | 26.73 | 27.78 | 26.79 | 27.62 | 26.88 | 27.69 | 26.80 | 0.08 | 0.07 |
| $8 \times 10^2$ | 31.28 | 30.10 | 31.24 | 30.29 | 31.14 | 30.31 | 31.22 | 30.23 | 0.07 | 0.11 |
| $8 \times 10^1$ | 35.41 | 33.74 | 35.00 | 33.90 | 34.51 | 33.92 | 34.98 | 33.85 | 0.45 | 0.10 |
| $8 \times 10^0$ | 37.21 | 37.56 | 38.26 | 36.88 | 38.41 | 37.13 | 37.96 | 37.19 | 0.65 | 0.34 |
| $8 \times 10^{-1}$ | UD | UD | UD | UD | UD | UD | UD | UD | UD | UD |
| $8 \times 10^{-2}$ | UD | UD | UD | UD | UD | UD | UD | UD | UD | UD |
| Negative Control | UD | UD | UD | UD | UD | UD | UD | UD | UD | UD |

**Evaluation of assay efficacy using a sample from the first patient with confirmed COVID-19 in South Korea**

RNA extracted from a lower respiratory tract mucus sample from the first patient confirmed to have COVID-19 in South Korea was used to evaluate the responsivity of the real-time RT-PCR-based assay for the viral RNA, and to assess its efficacy [4]. Real-time RT-PCR was performed after 10-fold dilution of the RNA. The Ct value of the RdRp gene was 36.62 at a $10^4$-fold dilution, and that of the E gene was 36.97 at $10^{-5}$-fold dilution. Real-time RT-PCR accurately detected the target genes in the patient samples (Table 5, Fig. 2).

### Table 5. Accuracy and Precision of Real-time PCR for SARS-CoV 2 (RdRp and E gene)

**A. RdRP gene**

| SARS-CoV-2 PFU | inter CV% | F-value | P-value | intra CV% |
|----------------|-----------|---------|---------|-----------|
| $1 \times 10^5$ | 2.47 | 0.98 | 0.36 | 0.64 |
| $1 \times 10^4$ | 2.49 | 0.11 | 0.75 | 1.02 |
| $1 \times 10^3$ | 2.56 | 0.61 | 0.46 | 0.75 |
| $1 \times 10^2$ | 2.71 | 0.21 | 0.66 | 1.21 |
| $1 \times 10^1$ | 2.68 | 0.00 | 0.99 | 0.26 |
| $1 \times 10^0$ | 1.88 | 0.35 | 0.57 | 0.74 |

**B. E gene**
| SARS-CoV-2 PFU | inter CV% | F-value | P-value | intra CV% |
|----------------|-----------|---------|---------|-----------|
| 1 × 10^5       | 3.47      | 0.01    | 0.93    | 0.43      |
| 1 × 10^4       | 2.10      | 0.01    | 0.91    | 0.39      |
| 1 × 10^3       | 2.42      | 0.00    | 0.95    | 0.16      |
| 1 × 10^2       | 1.55      | 0.00    | 0.98    | 0.20      |
| 1 × 10^1       | 1.39      | 0.00    | 1.00    | 0.74      |
| 1 × 10^0       | 0.71      | 1.36    | 0.29    | 1.02      |

P value>0.05 indicating that there is no difference between days.

F value<5.99 indicating that there is no difference between days

F(1,6;0.05)=5.99

CV(%): Coefficient of Variation

### Linearity of real-time RT-PCR for detecting SARS-CoV-2

Assay performance was assessed using 10-fold dilutions of viruses with known PFU values as standards. Four independent runs were performed, using viruses with known PFU values as standards for the consensus sequence and each isolate of SARS-CoV-2 in South Korea. Linear regression analysis revealed that different curves were linear with correlation coefficients of $R^2 = 0.998$ among viruses with known PFU values (Table 6, Fig. 3).

### Table 6

Sensitivity and specificity of EUA Real-time PCR kits and verified reagent in this study for SARS-CoV 2

| In this study | PowerCheck™2019-nCoV | Allplex™2019-nCoVAssay | DiaPlexQ™Novel Coronavirus | STANDARD MnCoV Real-Time Detection kit | Real-Q 2019-nCoV Detection kit |
|---------------|----------------------|------------------------|---------------------------|---------------------------------------|-------------------------------|
|               | Pos | Neg | Inc | Pos | Neg | Inc | Pos | Neg | Inc | Pos | Neg | Inc |
| Pos           | 54  | 53  | 1*  | 54  | 54  | 54  | 54  | 54  | 54  | 54  | 54  | 54  |
| Neg           | 50  | 50  | 50  | 50  | 50  | 50  | 50  | 50  | 50  | 50  | 50  | 50  |
| Inc           | 1   | 1   | 1   | 1   | 1   | 1   | 1   | 1   | 1   | 1   | 1   | 1   |
| Sensitivity (%) | 98.2 | 98.2 | 98.2 | 98.2 | 98.2 | 98.2 |
| Specificity (%) | 100 | 100 | 100 | 100 | 100 | 100 |

*Non-consistent cases in one reagent were confirmed by further examination as inconclusive cases, not false cases

*95% confidence interval: 90.4 ~ 99.7%

#95% confidence interval: 92.9 ~ 100%

### Limit of detection and limit of quantification (correlation between real-time RT-PCR and virus titration)
To determine the LOD for SARS-CoV-2, plasmid DNA of a known concentration containing each target gene was used. Assay performance was assessed using 10-fold dilutions of the plasmid DNA standards. Three independent runs were carried out using the plasmid DNA standards and each clone of SARS-CoV-2. The analytical detection limit was $8 \times 10^0$ copies/µL for all real-time RT-PCR assays (Table 4, Fig. 1). The viral culture medium, with a known viral titer, was diluted from $10^5$ to $10^{-2}$ PFU/mL, and the RNA was extracted from the medium. Real-time RT-PCR was repeated four times to determine the limit of quantification at each concentration. The LOD was 1 PFU/mL (Table 5, Fig. 3).

**Accuracy and precision**

To assess the accuracy and precision of the detection of the target genes using the assay, real-time RT-PCR was performed using four 10-fold serial dilutions of a virus culture medium with a known virus titer. The experiment was repeated three days later. The inter-assay CV was 1.88–2.71 and 0.71–3.47, whereas the intra-assay CV was 0.26–1.21 and 0.16–1.02 for the RdRp and E genes, respectively. The $P$ value was greater than 0.05, and the $F$ value was smaller than 5.99 for all the experiments, indicating accurate and precise detection (Table 5, Fig. 3).

**Accuracy of EUA reagents**

Based on the nucleic acid detection reagents evaluated in this study, all five EUA products could detect SARS-CoV-2 at a sensitivity of at least 98.2% and a specificity of 100% (95% confidence interval: 90.4–99.7%). Non-consistent results obtained with one reagent were confirmed by further examination as inconclusive cases rather than false cases (Table 6).

**Discussion**

Molecular methods are more rapid, accurate, and sensitive for virus detection than culture methods. In this study, we established a consensus method using molecular tools for detecting SARS-CoV-2. Early diagnosis of SARS-CoV-2-infected patients is essential for controlling the dynamics of the COVID-19 pandemic.

Since its initial emergence in Wuhan, China in late 2019, COVID-19 has rapidly spread worldwide [2]. COVID-19 is caused by SARS-CoV-2, with its clinical symptoms including dyspnea, cough, and mild respiratory symptoms that progress to pneumonia. It is difficult to distinguish SARS-CoV-2 from other common respiratory viruses such as influenza viruses, because of their highly similar symptoms [8]. A genetic assay with high specificity is necessary to detect SARS-CoV-2.

During early viral spread, the WHO reported a protocol for detecting SARS-CoV-2. The assay was developed by the Charité Research Organization in Germany on January 17, 2020 [9, 15]. This was the first genetic assay to be developed and released after the first report of the SARS-CoV-2 genome on January 11, 2020. We used this assay to rapidly detect SARS-CoV-2. The protocol involves a common reporter dye, 6-carboxyfluorescein FAM, together with BlackBerry Quencher; however, as this quencher is not used in Korea, it was replaced with black hole quencher (BHQ) during probe synthesis. We detected the target genes RdRp and E.

To assess the specificity of the assay, which was performed using specifically designed primer probes, real-time RT-PCR was conducted on 23 respiratory viruses, including influenza viruses, and five respiratory tract samples that had tested negative for SARS-CoV-2. No respiratory viruses other than SARS-CoV-2 were detected. The real-time RT-PCR assay showed excellent sensitivity, and had a high LOD.

Studies have shown that it is possible to accurately detect COVID-19 genes using detection reagents that did not receive EUA. The Provincial Institute of Health and Environmental Research carried out regional training programs for COVID-19 diagnosis, which were particularly useful in emergency diagnostic situations in the early stages of the pandemic. COVID-19 diagnostic EUA reagents were approved for private sector use after comparative analysis.
Conclusions
In conclusion, we evaluated a real-time RT-PCR assay, and found that it had high specificity and sensitivity for SARS-CoV-2 and good analytical performance, using gene cloning and viruses isolated from the first patient who tested positive for COVID-19 in South Korea.

List Of Abbreviations
BHQ Black hole quencher
EUA Emergency Use Authorization
LOD Limit of detection
PFU Plaque-forming units
RT-PCR Reverse transcription polymerase chain reaction
SARS Severe acute respiratory syndrome
WHO World Health Organization

Declarations
Ethics approval and consent to participate
This study was approved by the Korea Centers for Diseases Control and Prevention Ethics Committee—KCDC Authority (approval number #2020-03-01-P-A). This study was performed in accordance with the relevant laws and regulations that govern research in the Korea Centers for Diseases Control and Prevention.

Consent for publication
Not applicable

Availability of data and materials
Not applicable

Competing interests
The authors declare that they have no competing interests

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Authors' contributions
All authors have contributed significantly to the creation of this manuscript.

Authors’ individual Contributions: Yoon-Seok Chung: Conceptualization, Methodology, Validation, Formal Analysis, Investigation, Writing, Supervision. Nam-Joo Lee: Methodology, Validation, Formal Analysis, Investigation. Sang Hee Woo: Methodology, Validation, Formal Analysis, Jeong-Min Kim: Methodology, Validation, Formal Analysis, Investigation. Heui
Man Kim: Conceptualization, Methodology, Validation, Hye Jun Jo: Methodology, Validation. Ye Eun Park: Validation, Formal Analysis. Myung-Guk Han: Conceptualization, Project administration.

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Figures
Figure 1

Analysis of linearity of Real-time PCR for SARS-CoV 2 with Plasmid DNA cloned target PCR sequences.

Figure 2

Analysis of linearity Real-time PCR for SARS-CoV 2 with RNA from 1st patient’s nasopharyngeal swab.

Figure 3
Analysis of Linearity of Real-time PCR for SARS-CoV 2 with RNA form virus isolates.