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Comparison of three molecular diagnostic assays for SARS-CoV-2 detection: Evaluation of analytical sensitivity and clinical performance

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Abstract

Background: Currently, SARS-CoV-2 RNA detection using real-time reverse-transcription PCR (rRT-PCR) is the standard diagnostic test for COVID-19 infection. Various rRT-PCR assays are currently used worldwide, targeting different genes of the SARS-CoV-2. Here, we compared the analytical sensitivity and clinical performance (sensitivity and specificity) of Allplex SARS-CoV-2/FluA/FluB/RSV assay (Seegene), Standard M nCoV real-time detection kit (SD Biosensor), and U-TOP COVID-19 detection kit (Seasun Biomaterials) for SARS-CoV-2 detection.

Methods: Two hundred and forty-nine nasopharyngeal swab samples were evaluated to compare the clinical performance of the rRT-PCR assays. For the analytical performance evaluation, two RNA controls with known viral loads—SARS-CoV-2 RNA control and SARS-COV-2 B.1.351 RNA control—were used to investigate the potential impact of SARS-CoV-2 variants, particularly the B.1.351 lineage.

Results: Limits of detection ranged from 650 to 1300 copies/ml for rRT-PCR assays, and the mean differences in cycle threshold (C_t) values of the two RNA controls were within 1.0 for each target in the rRT-PCR assays (0.05–0.73), without any prominent C_t value shift or dropouts in the SARS-COV-2 B.1.351 RNA control. Using the consensus criterion as the reference standard, 89 samples were positive, whereas 160 were negative. The overall clinical performance of rRT-PCR assays was comparable (sensitivity 98.88%–100%; specificity 99.38%–100%), whereas the sensitivities of each target gene were more variable.

Conclusions: The three rRT-PCR assays showed comparable analytical sensitivity and clinical performance. The analytical and clinical sensitivities of each target gene were influenced more by the primer and probe design than the target gene itself.

KEYWORDS
COVID-19, PCR, performance evaluation, SARS-CoV-2

1 | INTRODUCTION

Coronavirus disease 2019 (COVID-19) is caused by the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2).[1] Prompt and accurate detection of SARS-CoV-2 is crucial to prevent its transmission and administer the appropriate treatment. SARS-CoV-2 RNA detection using real-time reverse-transcription PCR (rRT-PCR) is the standard diagnostic test for current infections of COVID-19.[2] Various rRT-PCR assays are currently used worldwide, and the assays target different genes of the SARS-CoV-2, such as the envelope (E), ORF1ab/RNA-dependent RNA polymerase (*RdRp*), nucleocapsid (*N*), and spike (*S*) genes.[3-5] rRT-PCR assays targeting more than one position of the SARS-CoV-2 genome are recommended because of potential genetic variation in SARS-CoV-2, which may result in false-negative results.[2]

Since the emergence of SARS-CoV-2, sets of mutations have been noted, and these genetic mutations can affect the performance of the rRT-PCR assay. Mutation analysis of the SARS-CoV-2 genome collected worldwide showed that mutations occurred in all SARS-CoV-2 diagnostic targets.[6,7] Moreover, novel strains with two amino acid (H69 and V70) deletion within the *S* gene, which includes the alpha variant of SARS-CoV-2 B.1.1.7 lineage, have been characterized by the *S* gene dropout in SARS-CoV-2 rRT-PCR assays targeting the *S* gene.[8,9] Several novel mutations in either the *E* or *N* gene have been shown to affect SARS-CoV-2 rRT-PCR results.[10,11] The beta variant of SARS-CoV-2 B.1.351 lineage, initially detected in South Africa,[12] is rapidly taking over the wild-type SARS-CoV-2 globally, with increased transmissibility.[12-14] The impact of the beta variant of SARS-CoV-2 B.1.351 lineage on the performance of SARS-CoV-2 rRT-PCR assays has not been fully elucidated. Because the primer and probe sequence information of commercial SARS-CoV-2 rRT-PCR assays are not available, performance evaluation using various clinical samples is important.

The target gene of SARS-CoV-2 rRT-PCR varies widely. The guidelines regarding target genes for SARS-CoV-2 detection differ worldwide. With the emergence of SARS-CoV-2, the World Health Organization recommended protocols targeting the *E* gene for screening and the *RdRp* gene for confirmation testing.[15] The US Centers for Disease Control and Prevention designed a 2019-nCoV rRT-PCR diagnostic panel targeting different regions of the *N* gene.[16] Among the target genes of the developed SARS-CoV-2 rRT-PCR assays, the *N* gene is the most frequently selected target gene apart from *ORF1ab*, whereas the *S* gene is the least frequently selected target gene.[5]

Recently, the Allplex SARS-CoV-2/FluA/FluB/RSV assay (Seegene), targeting the *S*, *RdRp*, and *N* genes for SARS-CoV-2 detection, have been developed. The previously introduced SARS-CoV-2 rRT-PCR assay by Seegene, namely, Allplex 2019-nCoV assay, which was approved for emergency use with authorization from the US Food and Drug Administration, has been designed to include the *E* gene instead of the *S* gene as the target gene for SARS-CoV-2 detection. Here, we report the analytical sensitivity and clinical performance (sensitivity and specificity) of the Allplex SARS-CoV-2/FluA/FluB/RSV assay in comparison with two commercially available kits: the Standard M nCoV real-time detection kit (SD Biosensor, Osong, Korea) and U-TOP COVID-19 detection kit (Seasun Biomaterials). We also examined the potential impact of the SARS-CoV-2 variant of concern, particularly the B.1.351 lineage, on the analytical and clinical performance.

2 | MATERIALS AND METHODS

2.1 | Clinical samples and viral RNA

This study was performed using residual nasopharyngeal swab (NPS) specimens collected from patients who visited Korea University Guro Hospital between December 2020 and February 2021. The residual specimens were stored at −70°C until analysis and used for the performance evaluation of the three commercial kits. This study was approved by the Institutional Review Board of the Korea University Guro Hospital (2021GR0086).

For analytical sensitivity evaluation, viral RNA controls with known RNA loads, Amplirun SARS-CoV-2 RNA control (Vircell) derived from a Spanish clinical isolate (GISAID accession ID: EPI_ISL_429256) and Amplirun SARS-CoV-2 B.1.351 RNA control (Vircell) derived from a Spanish clinical isolate (GISAID accession ID: EPI_ISL_848199) were used. The two SARS-CoV-2 RNA controls were diluted to obtain eight concentrations as follows: 13, 130, 650, 1300, 13,000, 130,000, 1,300,000, and 13,000,000 copies/ml to analyze the linearity and amplification efficiency of each target of the rRT-PCR assay. The preliminary limit of detection (LoD) was determined with three replicates over five concentrations ranging from 13 to 13,000 copies/ml. At the concentration level spanning the possible LoD, each concentration was replicated five times.

2.2 | RNA extraction and SARS-CoV-2 rRT-PCR

RNA extraction from clinical samples was performed using the nucleic acid extraction platform Microlab STARlet (Hamilton). Allplex SARS-CoV-2/FluA/FluB/RSV assay, Standard M nCoV real-time detection kit, and U-TOP COVID-19 detection kit were used in this study. PCRs for all three rRT-PCR assays were performed using the CFX96 system (Bio-Rad).

The Allplex SARS-CoV-2/FluA/FluB/RSV assay has three targets for SARS-CoV-2, namely, the *S*, *RdRp*, and *N* genes. PCR was performed in a total volume of 20 μl (10 μl of extracted RNA and 10 μl of master mix). A cycle threshold (C_t) value equal to or below 38 was interpreted as positive for each target gene. The standard M nCoV real-time detection kit detects two targets—*E* and *RdRp*—and the reaction volume was 30 μl (10 μl of extracted RNA and 20 μl of master mix). A C_t value equal to or below 36 was interpreted as positive for each target gene. For the U-TOP COVID-19 detection kit, four genes, namely, *ORF1ab*, *N*, *S*, and *E*, were targeted. PCR was performed in a total volume of 30 μl (10 μl of extracted RNA and 20 μl of master mix), and a C_t value equal to or below 38 was interpreted as positive for each target gene.

2.3 | Data analysis

The clinical performance of the three rRT-PCRs was evaluated using reference standards. The reference standard was defined using the consensus criterion, which was obtained from two out of three rRT-PCR assays.[17,18] The results were considered discordant when one of the rRT-PCR assays did not agree with the other two assays. The difference in C_t values between the targets was analyzed using Student's t test. Linearity was assessed using linear regression analysis, and the amplification efficiency was evaluated as previously described.[4]

2.4 | Statistical analysis

The LoD was determined using a positive-rate analysis and defined as the lowest concentration at which all replicates showed positive results (100% detection rate). Statistical significance was set at $p < 0.05$. All statistical analyses and visualizations were performed using the R software (version 3.4.3).

3 | RESULTS

3.1 | Analytical comparisons of SARS-CoV-2 rRT-PCR assays

Analytical comparison of Allplex SARS-CoV-2/FluA/FluB/RSV kit, Standard M nCoV real-time detection kit, and U-TOP COVID-19 detection kit for linearity, amplification efficiency, and LoD were determined using two RNA controls with known viral loads, SARS-CoV-2 RNA control and SARS-CoV-2 B.1.351 RNA control (Figure 1). For both RNA controls, a high degree of linearity was observed for each target of Standard M nCoV real-time detection kit and U-TOP COVID-19 detection kit and the S gene of Allplex SARS-CoV-2/FluA/FluB/RSV kit ($R^2 \geq 0.98$). A lower degree of linearity was observed in the Allplex SARS-CoV-2/FluA/FluB/RSV kit N gene when using SARS-CoV-2 B.1.351 RNA control than using the SARS-CoV-2 RNA control ($R^2 = 0.99$ versus $R^2 = 0.96$). However, the mean differences in C_t values of the two RNA controls were all within 1.0 for each target in the rRT-PCR assays (0.05–0.73), without any prominent C_t value shift or dropouts in SARS-CoV-2 B.1.351 RNA control. Amplification efficiencies for all targets of rRT-PCR assays were between 80% and 120% for both RNA controls, which was consistent with the criteria for efficient multiplex rRT-PCR (Figure 2).[19]

At the concentration level of 13–13,000 copies/ml, for the SARS-CoV-2 RNA control, the LoD for the target genes was estimated to be 1300 copies/ml in all three assays (Table S1). The LoD of each target gene was the lowest for the S gene when the Allplex SARS-CoV-2/FluA/FluB/RSV kit and $ORF1ab$ gene of the U-TOP COVID-19 detection kit were used. When evaluated using the SARS-CoV-2 B.1.351 RNA control, the LoD of Allplex SARS-CoV-2/FluA/FluB/RSV kit was 650 copies/ml for detection of the target genes, and both the Standard M nCoV real-time detection kit and U-TOP COVID-19 detection kit showed LoD of 1300 copies/ml. For SARS-CoV-2 B.1.351 RNA control, the S gene of Allplex SARS-CoV-2/FluA/FluB/RSV kit, E and $RdRp$ gene of Standard M nCoV real-time detection kit, and the ORF1ab gene were the target genes with the lowest LoD.
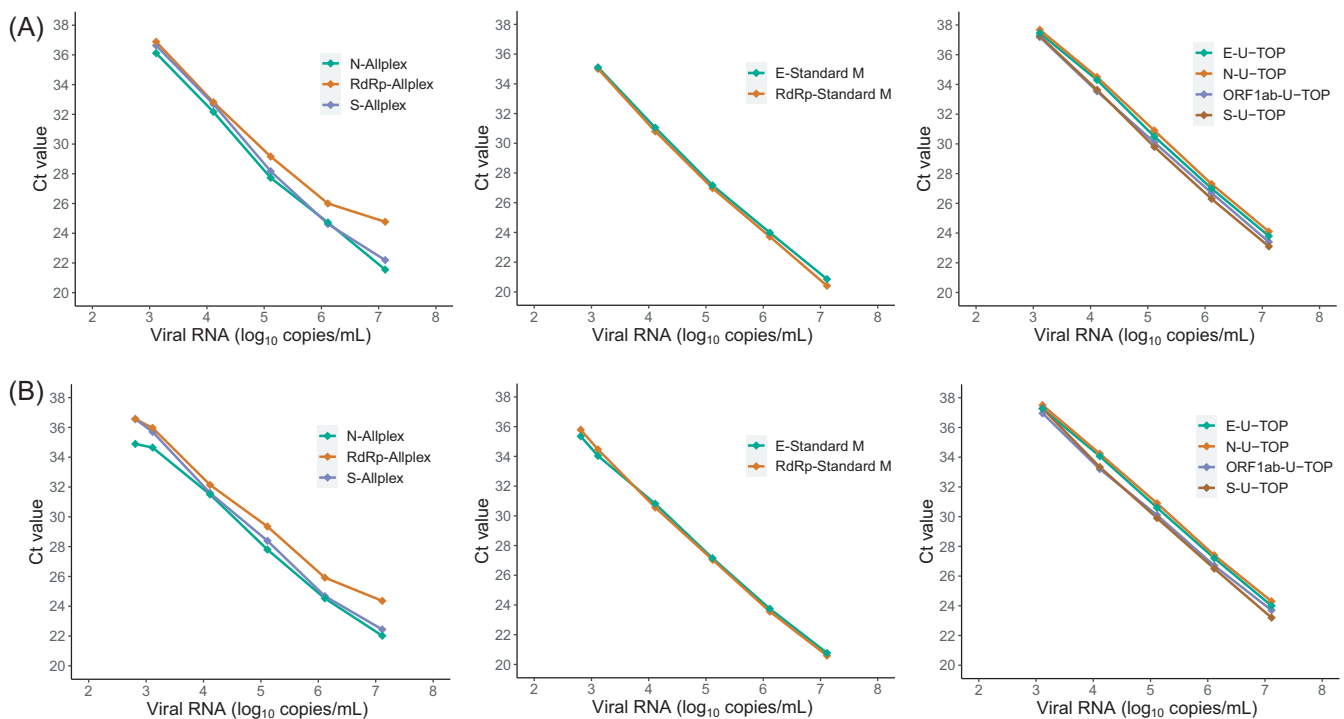

FIGURE 1 Mean C_t values of the target genes for each rRT-PCR assay were tested using serially diluted SARS-CoV-2 wild-type RNA control (A) and SARS-CoV-2 B.1351 lineage RNA control (B)
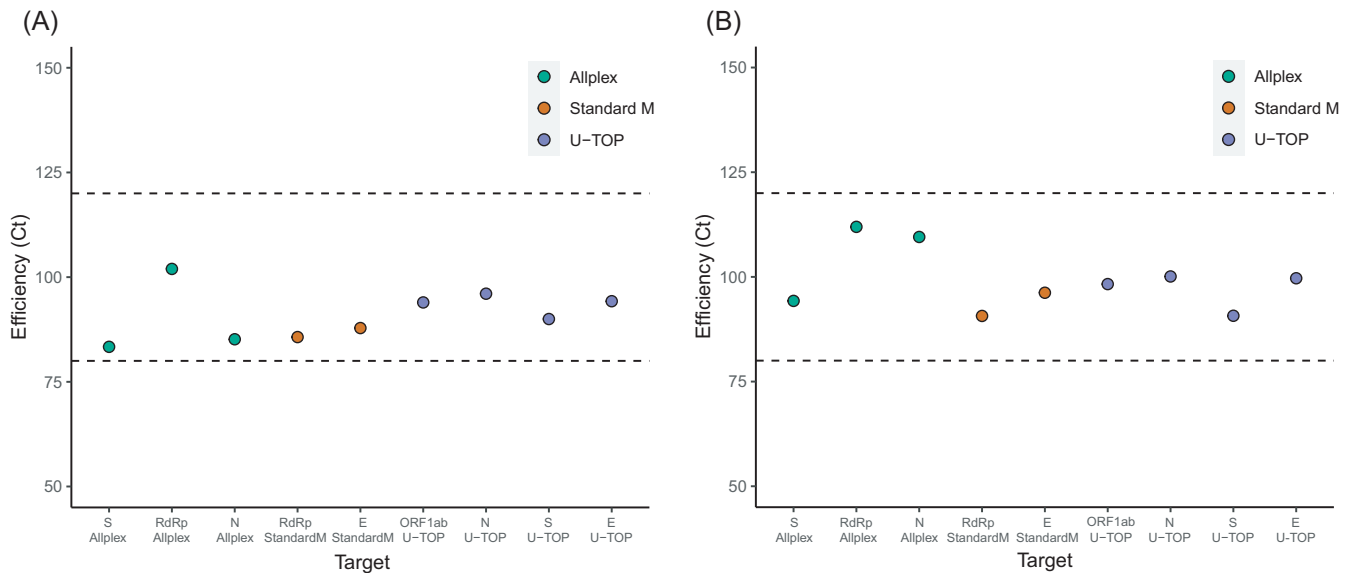
FIGURE 2 PCR efficiencies of nine target genes of three rRT-PCR assays tested using SARS-CoV-2 wild-type RNA control (A) and SARS-CoV-2 B.1351 lineage RNA control (B)

TABLE 1 Detailed results of clinical samples analyzed using three rRT-PCR assays

	Allplex SARS-CoV-2/FluA/FluB/RSV kit	Standard M nCoV real-time detection kit	U-TOP COVID-19 detection kit
Target genes	*S*, *RdRp*, and *N* genes	*E* and *RdRp* genes	*ORF1ab*, *N*, *S*, and *E* genes
Positive results			
Any target genes	90	89	88
All target genes	85	87	84
S and *RdRP* genes	1		
S gene	3		
RdRP gene	1	2	
ORF1ab, *N*, and *E* genes			1
ORF1ab and *N* genes			1
ORF1ab and *E* genes			1
E gene			1
Negative results	159	160	161

Note: The positive results were defined the result of at least two of the three SARS-CoV-2 real-time PCR assays.

3.2 | Clinical samples

Two hundred and forty-nine NPS specimens collected from 215 patients were part of this study. The median age of the patients was 60 years (range, 7–89 years), and 44.19% (*n* = 95/215) patients were male. Among 249 samples, 42 (16.87%), 57 (22.89%), 135 (54.22%), and 15 (6.02%) samples were from inpatients, patients visiting the emergency department, the COVID-19 screening clinic, and outpatients, respectively. All 249 NPS specimens tested negative for influenza A and B and RSV using the Allplex SARS-CoV-2/FluA/FluB/RSV kit.

3.3 | Positivity and C_t value distributions of clinical samples

All samples were analyzed using the Allplex SARS-CoV-2/FluA/FluB/RSV kit, Standard M nCoV real-time detection kit, and U-TOP COVID-19 detection kit for comparison. The overall results of all three SARS-CoV-2 rRT-PCR assays are shown in Table 1. Among the assays, the Standard M nCoV real-time detection kit showed the highest positivity for all target genes, whereas the Allplex SARS-CoV-2/FluA/FluB/RSV kit showed the highest positive results for at least one target gene. For each assay, the *S* gene in the Allplex

TABLE 2 C_t value and clinical performance comparison of each of the target genes for real-time reverse-transcription PCR assays

Genes	Allplex SARS-CoV-2/FluA/FluB/RSV kit			Standard M nCoV real-time detection kit		U-TOP COVID-19 detection kit			
	S	RdRp	N	RdRp	E	ORF1ab	N	S	E
C_t value (mean ± SD)	26.44 ± 6.94	26.52 ± 7.29	25.28 ± 6.72	25.76 ± 6.89	25.65 ± 6.71	27.62 ± 6.66	28.20 ± 6.49	28.34 ± 6.69	27.72 ± 6.53
Sensitivity (95% CI)	98.88 (93.90–99.97)	97.75 (92.12–99.73)	95.51 (88.89–98.76)	100 (95.94–100)	97.75 (92.12–99.73)	97.75 (92.12–99.73)	96.63 (90.46–99.30)	94.38 (87.38–98.15)	97.75 (92.12–99.73)
Specificity (95% CI)	99.38 (96.57–99.98)	100 (97.72–100)	100 (97.72–100)	100 (97.72–100)	100 (97.72–100)	100 (97.72–100)	100 (97.72–100)	100 (97.72–100)	100 (97.72–100)

Note: The clinical performance was evaluated based on the reference standard results, which was defined by the consensus criterion.

Abbreviations: CI, confidence interval; C_t, cycle threshold; SD, standard deviation

SARS-CoV-2/FluA/FluB/RSV kit, the *RdRP* gene in the Standard M nCoV real-time detection kit, and the *ORF1ab* and *E* genes in the U-TOP COVID-19 detection kit were the most frequently detected targets with positive results.

We investigated whether the difference in positivity among the targets in each assay was affected by the difference in C_t values. The C_t value distributions of the clinical samples are shown in Table 2 and Figure 3. When comparing the C_t distribution value of each target gene for the Allplex SARS-CoV-2/FluA/FluB/RSV kit, the *N* gene showed the lowest C_t value, followed by the *S* and *RdRp* genes. The difference in C_t values between the targets was statistically significant (*S* and *RdRp* gene, $p = 0.0011$ and others all $p < 0.001$). In the Standard M nCoV real-time detection kit, the *E* gene showed a lower C_t value than that of the *RdRp* gene, but the difference was not statistically significant ($p = 0.9709$). For the U-TOP COVID-19 detection kit, the lowest C_t value was observed in the *ORF1ab* gene followed by the *E*, *N*, and *S* genes, which was the same order as the positivity in clinical samples. The difference between the C_t values of each target was significant (all $p < 0.001$).

When the same target genes of the two different assays were compared, the mean difference in the C_t values of *RdRp* gene between the Allplex SARS-CoV-2/FluA/FluB/RSV kit and the Standard M nCoV real-time detection kit was 0.982. The mean differences in the C_t values of other overlapping genes were −2.179 of *E* gene between the Standard M nCoV real-time detection kit and the U-TOP COVID-19 detection kit, −2.282 of *N* gene and −2.483 of *S* gene between the Allplex SARS-CoV-2/FluA/FluB/RSV kit and the U-TOP COVID-19 detection kit, respectively. The differences between the C_t values of same target genes were all statistically significant ($p < 0.001$).

3.4 | Clinical samples with discordant results

Among the 249 samples, 6 (2.41%) samples showed discordant results between the rRT-PCR assays (Table 3). With the review of medical records, six samples with discordant results were follow-up samples from COVID-19 patients during or after the treatment, favoring the presumptive presence of SARS-CoV-2 RNA. For these six COVID-19 patient follow-up samples, inconclusive results of rRT-PCR assays were interpreted as positive.

3.5 | Clinical performance of SARS-CoV-2 rRT-PCR assays

The clinical performance of SARS-CoV-2 rRT-PCR assays was evaluated for the overall results and for each target gene. As described above, the clinical performance was evaluated according to the reference standard. The consensus criterion was defined as the result of at least two of the three SARS-CoV-2 real-time PCR assays. The Allplex SARS-CoV-2/FluA/FluB/RSV kit showed positive results in one or more targets for all six discordant samples, but one sample
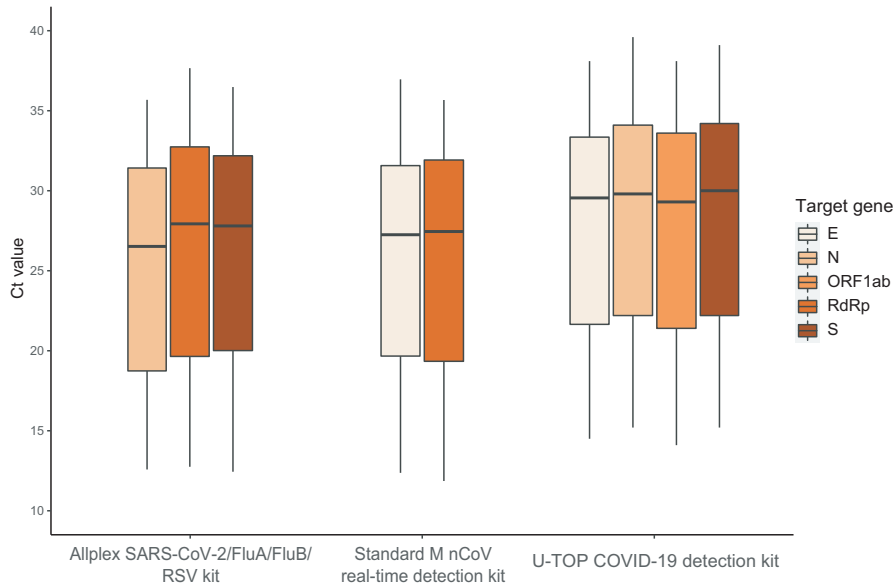
FIGURE 3 C_t value of the distribution of target genes of three real-time reverse-transcription PCRs when analyzing the clinical samples

TABLE 3 Details of discordant clinical sample results

| Sample No. | Reference standard | Allplex SARS-CoV-2/FluA/FluB/ RSV kit | | | | Standard M nCoV real-time detection kit | | | U-TOP COVID-19 detection kit | | | | |
		Result	S (C_t)	$RdRP$ (C_t)	N (C_t)	Result	$RdRP$ (C_t)	E (C_t)	Result	$ORF1ab$ (C_t)	N (C_t)	S (C_t)	E (C_t)
1	Positive	Target detected	ND	37.3	ND	Target detected	35.6	ND	Target not detected	≥38.0	≥38.0	ND	ND
2	Positive	Target detected	36.0	37.5	35.7	Target detected	35.7	34.1	Target detected	37.0	≥38.0	≥38.0	37.7
3	Positive	Target detected	35.8	36.6	ND	Target detected	34.0	34.4	Target detected	36.8	37.7	≥38.0	≥38.0
4	Positive	Target detected	35.5	36.7	34.5	Target detected	35.6	34.0	Target detected	35.9	36.6	ND	35.4
5	Positive	Target detected	36.5	ND	ND	Target detected	35.1	33.8	Target detected	36.0	37.0	37.6	36.1
6	Negative	Target detected	37.4	ND	ND	Target not detected	ND	ND	Target not detected	≥38.0	≥38.0	ND	≥38.0

Abbreviations: C_t, cycle threshold; ND, not detected.

yielded negative results by both Standard M nCoV real-time detection kit and U-TOP COVID-19 detection kit, which was later considered as true negative based on the consensus criterion. The rest of the five discordant samples were considered as true-positive samples.

The diagnostic performances of the three assays for the overall results are shown in Table 4. Among the 249 samples, 89 were positive and 160 were negative. Both, the Allplex SARS-CoV-2/FluA/ FluB/RSV kit and Standard M nCoV real-time detection kit, showed the highest overall sensitivity (both 100%) for SARS-CoV-2 detection. Regarding specificity, the Standard M nCoV real-time detection kit and U-TOP COVID-19 detection kit showed the highest overall specificity (both 100%). Considering each target gene of all three rRT-PCR assays, the *RdRp* gene of Standard M nCoV real-time detection kit showed the highest sensitivity (100%), followed by the *S*

gene of Allplex SARS-CoV-2/FluA/FluB/RSV kit (98.88%). The specificities of each target gene in the three assays showed high specificities ranging from 99.38% to 100% (Table 2).

4 | DISCUSSION

The overall analytical sensitivity evaluation of all rRT-PCR assays showed comparable performance, whereas the analytical sensitivities of each target gene were more variable. The analytical sensitivity of each target gene appears to be influenced more by the primer and probe design rather than the target gene. The importance of primer design and the optimization of primer sets and detection protocols for SARS-CoV-2 have been previously reported.[4,20] Except for the *ORF1ab* gene in the U-TOP COVID-19 detection kit, other

TABLE 4 Clinical performance comparison of the three rRT-PCRs for detection of SARS-CoV-2

	Results according to reference standards					
SARS-CoV-2 PCR assays	TP	FP	FN	TN	Sensitivity (95% CI)	Specificity (95% CI)
Allplex SARS-CoV-2/FluA/FluB/RSV kit	89	1	0	159	100 (95.94–100)	99.38 (96.57–99.98)
Standard M nCoV real-time detection kit	89	0	0	160	100 (95.94–100)	100 (97.72–100)
U-TOP COVID-19 detection kit	88	0	1	160	98.88 (93.90–99.97)	100 (97.72–100)

Abbreviations: CI, confidence interval; FN, false negative; FP, false positive; TN, true negative; TP, true positive.

target genes were included in each assay more than once. All rRT-PCR assays were performed using the same RNA extraction system, and the same amount of eluate was added to the reaction (all 10 μl), and therefore, the same effective sample volume was achieved.[21] When we directly compared the analytical sensitivities of each target, the *S* gene in the Allplex SARS-CoV-2/FluA/FluB/RSV assay was approximately 10-fold more sensitive than the *S* gene in the U-TOP COVID-19 detection kit. The *RdRp* and *E* genes in the Standard M nCoV real-time detection kit were approximately 10-fold more sensitive for SARS-CoV-2 B.1.351 detection than the *RdRp* gene in the Allplex SARS-CoV-2/FluA/FluB/RSV assay and the *E* gene in the U-TOP COVID-19 detection kit, respectively.

There are several factors known to cause false-positive SARS-CoV-2 rRT-PCR results such as contamination during sampling and processing, cross-reaction with other viruses, and non-specific low-level reactions in the PCR process.[22] False-negative results can occur due to inadequate sample collection, sample degradation, too early or too late sample collection, and mutations in primer and probe regions of rRT-PCR.[23] In this study, the discordant results between the rRT-PCR assays were observed in follow-up samples from COVID-19 patients. Persistently positive rRT-PCR results in COVID-19 patients' follow-up samples do not indicate replication-competent SARS-CoV-2 virus[24–26]; however, viral RNA shedding has been reported in COVID-19 patients with variable duration.[27,28] The positive targets detected in rRT-PCR assays favored the presence of low number of copies of SARS-CoV-2 RNA in these samples.[29] However, previous history of COVID-19 infection does not eliminate the possibility of false-positive results; therefore, we implemented a consensus criterion as the reference standard. Interestingly, in line with the analytical sensitivity results, the *S* and *ORF1ab* genes were the most sensitive target genes using the Allplex SARS-CoV-2/FluA/FluB/RSV assay and U-TOP COVID-19 detection kit. In the case of the Standard M nCoV real-time detection kit, although the difference in analytical sensitivity was not evident, *RdRp* showed higher sensitivity than the *E* gene.

The analytical comparison using SARS-CoV-2 RNA and SARS-CoV-2 B.1.351 RNA controls showed a low degree of linearity in the *RdRp* gene of the Allplex SARS-CoV-2/FluA/FluB/RSV assay. However, the difference in linearity did not result in analytical sensitivity. Our data showed that the presence of other mutations in B.1.351 did not have an impact on the analytical sensitivity and that the C_t value shift was minimal. Wollschläger et al. have reported a D3L mutation in B.1.1.7-positive samples causing an *N* gene dropout

or C_t value shift in the Allplex SARS-CoV-2/FluA/FluB/RSV assay but not in the Allplex 2019-nCoV assay.[30] As expected, *N* gene dropout or C_t value shift was not observed in the B.1.351 lineage, which does not carry the D3L mutation.

This study had some limitations. The impact of the B.1.351 lineage on the clinical performance of rRT-PCR could not be evaluated. We initially aimed to evaluate the clinical performance of B.1.351-positive samples. Therefore, the clinical performance was evaluated using patient samples collected during December 2020 and February 2021 when COVID-19 infection by the SARS-CoV-2 B.1.351 lineages were reported in Korea. Variant screening using variant-specific PCR (SARS-CoV-2 Variants I and II assay, Seegene) revealed that all 89 SARS-CoV-2 positive samples were negative for the targeted variants, suggesting that B.1.351 was not present in our clinical samples (data not shown). Because of the low prevalence of SARS-CoV-2 B.1.351 lineage in our clinical samples, only the effect of the SARS-CoV-2 B.1.351 lineage on analytical sensitivities was evaluated using RNA controls.

In summary, comparative evaluation of rRT-PCR assays, including the recently developed Allplex SARS-CoV-2/FluA/FluB/RSV assay, showed that all three rRT-PCR assays showed comparable overall analytical and clinical performances. For each target of the rRT-PCR assay, the analytical and clinical sensitivity of each target gene appeared to be influenced more by the primer and probe design than the target gene itself. Additionally, the B.1.351 lineage did not have an impact on the analytical sensitivity, and the C_t value shift was minimal. However, further studies using B.1.351-positive clinical samples are warranted to confirm its impact on clinical performance.

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