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Clinical Performance of the STANDARD M10 SARS-CoV-2 Rapid RT-PCR Assay in an Emergency Department

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Abstract

Background: In emergency departments, rapid screening of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) is important for arranging limited isolation resources and patient care during the coronavirus disease 2019 (COVID-19) pandemic. STANDARD M10 SARS-CoV-2 (SD Biosensor) is a recently developed cartridge-based RT-PCR that provides a turnaround time of 1 h, which is shorter than that for conventional RT-PCR. This study evaluated the clinical performance of STANDARD M10 in an emergency department. **Methods:** From March to June 2022, two pairs of nasopharyngeal and oropharyngeal swabs were collected from patients visiting an emergency department. Respective specimens underwent rapid RT-PCR using STANDARD M10 and conventional RT-PCR using Allplex SARS-CoV-2 (Seegene). When discordant results occurred, specimens undergoing the STANDARD M10 were retested with the Allplex to exclude specimen variations. Retest results replaced initial results of the Allplex. Clinical performance of STANDARD M10 was compared with Allplex. **Results:** The study enrolled 1,971 patients. COVID-19 prevalence was 6.2% based on the Allplex. Compared with the Allplex, overall agreement, positive percent agreement, and negative percent agreement of STANDARD M10 were 99.5% (95% CI: 99.1–99.8%), 95.9% (95% CI: 90.8–98.3%), and 99.8% (95% CI: 99.4–99.9%), respectively. Discordant results between STANDARD M10 and Allplex were observed only in specimens with Ct >33 using the Allplex. **Conclusions:** The STANDARD M10 showed reliable diagnostic performance for detecting SARS-CoV-2 in emergency departments and is a useful tool in emergency healthcare systems because of its easy-to-use cartridge-based assay and short resulting time for detecting SARS-CoV-2.

Introduction

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), which causes coronavirus disease 2019 (COVID-19), has rapidly spread worldwide, and accurate diagnosis of SARS-CoV-2 infection is crucial for reducing its transmission and optimizing timely patient management. The standard method for detecting SARS-CoV-2 is real-time reverse-transcription polymerase chain reaction (RT-PCR) assay with nasopharyngeal and oropharyngeal swabs.[1] The RT-PCR assay ensures high sensitivity and specificity but generally requires specialized laboratory personnel and long turnaround times. Allplex SARS-CoV-2 (Seegene, Seoul, Korea; Allplex) is one of the most commonly used conventional RT-PCR assay in Korea and worldwide.[2,3] While it provides reliable results, it takes approximately 4 h to report results including more than 1 h of hands-on time.[4] Rapid detection of SARS-CoV-2 is a key component to the appropriate and timely allocation of limited national or institutional isolation supplies; furthermore, rapid detection results directs the next step in providing appropriate medical care for patients and preventing spread of the virus in emergency departments; conventional RT-PCR assay is inadequate in meeting the urgent medical process of emergency departments.

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Rapid RT-PCR systems for SARS-CoV-2, such as the Xpert Xpress SARS-CoV-2 assay (Cepheid, CA, USA), have received Emergency Use Authorization from the U.S. and Korea in the early phase of the COVID-19 pandemic.[5] The rapid RT-PCR platform provides accurate results within 1 h; hence, the method was useful, especially in emergency departments. However, the method could not be used appropriately in Korea because the supply of reagents was insufficient, and the Korean health insurance system strictly limited test indication.

Hence, the STANDARD M10 SARS-CoV-2 assay (SD Biosensor, Suwon, Korea; STANDARD M10), which can rapidly detect SARS-CoV-2 based on a point-of-care RT-PCR assay targeting the envelope (*E*) and open reading frame 1ab gene (*ORF1ab*) was introduced in the early phase of the Omicron surge in Korea.[6] However, because only a few reports have validated the clinical performance of STANDARD M10 in a clinical setting, this study aimed to conduct an on-field evaluation with a large cohort of patients to estimate the clinical performance of STANDARD M10 in an emergency department compared with that of a standard RT-PCR method using Allplex.

Materials and Methods

Patients and specimens

This study enrolled patients who visited Hanyang University Guri Hospital's emergency department from March to June 2022. RT-PCR assays were performed for patients who decided to be admitted for further specific treatment. Patients with SARS-CoV-2-positive results in the RT-PCR assay within 45 days before the emergency department visit were excluded. Repeated visits by the same patient were considered individual cases. The Korean Triage and Acuity Scale (KTAS) classification was performed for all patients visiting the emergency department.[7] Disease severity and urgency of treatment increased from KTAS level 5 to level 1. COVID-19-associated symptoms were fever, cough, sputum, rhinorrhea, dyspnea, or throat pain. This study was approved by the Institutional Review Board of Hanyang University Guri Hospital (2022-11-011), which waived the requirement for informed consent to participate in the study.

Two pairs of nasopharyngeal and oropharyngeal swabs from each patient were used for STANDARD M10 and Allplex assays. Specimens were obtained and transferred using a universal viral transport medium of RM Life Science (Seoul, Korea) or GeneTM gene transport medium (SG Medical, Seoul, Korea). STANDARD M10 assay was performed immediately after sample collection, and Allplex assay was performed within 12 h after sample collection. When discordant results occurred between STANDARD M10 and Allplex assays, the specimen subjected to STANDARD M10 assay was retested with Allplex assay to correct the influence that occurred from different specimens. The retest was performed within 48 h after sample collection, and the initial results of Allplex assay were replaced by the retest results. Samples were stored at 4 before subsequent tests.

STANDARD M10 SARS-CoV-2 assay

In total, 600 μL of viral transport medium of the specimen was loaded into the sample chamber of the cartridge of STANDARD M10 SARS-CoV-2.[8] After placing the cartridge in the STANDARD M10 instrument (SD biosensor), all RT-PCR processes were automatically performed within 1 h. STANDARD M10 SARS-CoV-2 targets the*E* gene and *ORF1ab* gene, and the test reports a positive result if both genes' cycle thresholds (Ct) values are within 40.0 cycles. All experiments were performed according to the manufacturer's instructions.

Allplex SARS-CoV-2 assay

This assay is a conventional RT-PCR assay for detecting SARS-CoV-2; it targets the *E* gene, nucleocapsid (*N*) gene, RNA-dependent RNA polymerase (*RdRP*) region of the *orf1ab* gene, and spike (*S*) gene, with the latter two detected on the same fluorescence channel. The RT-PCR test was performed in the Hanyang University Guri Hospital or Seegene Medical Foundation (Seoul, Korea) laboratory. Nucleic acid extraction for Allplex SARS-CoV-2 assay was performed using Seeprep 32 (Seegene Inc., Korea) at the Hanyang University Guri Hospital or MagNA Pure 96 (Roche, Switzerland) at Seegene medical foundation

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according to the manufacturer's instructions. Amplification and detection were performed on a Bio-Rad CFX96 thermocycler (BioRad Laboratories, The Netherlands). The results were interpreted using Seegene Viewer data analysis software. When the Ct values of all three fluorescence channels (E , N , and $RdRP$ /Sgenes) were within the cutoff of 40.0 cycle, the result was considered positive.[4]

Statistical analysis

All statistical analyses were performed using MedCalc (version 20.027; MedCalc, Ostend, Belgium). Overall agreement, positive percent agreement (PPA), and negative percent agreement (NPA) of the STANDARD M10 were calculated based on the results of the Allplex assay. The agreement level was determined based on Cohen's kappa coefficient values. Inconclusive results were considered negative results. Ct values of the two assays were analyzed using Wilcoxon signed rank test. All tests were two sided, and results with P <0.05 were considered significant.

Results

In total, 1,971 patients underwent RT-PCR assays for SARS-CoV-2 in the emergency department during the study period (Table I). The patients included 1,082 female patients (54.9%) and 889 male patients (45.4%). The median age of all patients was 61 years (0–100 years). KTAS levels were distributed as follows: KTAS 1: 4 (0.2%), KTAS 2: 177 (9.0%), KTAS 3: 899 (45.6%), KTAS 4: 254 (12.9%), and KTAS 5: 637 (32.3%). COVID-19-associated symptoms were observed in 547 patients (27.8%).

The results of the STANDARD M10 and Allplex assays are shown in Table II. A total of 123 specimens were positive using the Allplex and 122 specimens were positive using STANDARD M10. Overall agreement with the STANDARD M10 and Allplex assays was 99.5% (95% confidence interval CI: 99.1–99.8%), and the kappa value was 0.96 (95% CI: 0.94–0.99). PPA and NPA values of STANDARD M10 were 95.9% (95% CI: 90.8–98.3%) and 99.8% (95% CI: 99.4–99.9%), respectively, based on the Allplex.

Ct values of positive or inconclusive results from the two RT-PCR assays are described in Table III. Ct values of the E gene and$RdRP$ ($orf1ab$) of concordant positive results were higher for Allplex than for STANDARD M10 ($P < 0.001$ for both). Two specimens, which were positive with Allplex and negative with STANDARD M10, showed median Ct values of 36.75, 37.96, and 36.50 for the$E$, $RdRP$, and N genes, respectively. Three positive specimens with Allplex and inconclusive with STANDARD M10 showed median Ct values of 35.75, 37.33, and 36.85 for the E , $RdRP$, and$N$ genes, respectively. Ct of $orf1ab$ was not detected with STANDARD M10 for these three specimens. The Ct value of inconclusive results of Allplex, which were positive or negative with STANDARD M10, was higher than 38.0. The four specimens which were positive with STANDARD M10 showed median Ct values of 35.15 and 34.59 for the Egene and $orf1ab$, respectively. Nine specimens of STANDARD M10 were inconclusive, and those negative with Allplex showed Ct values of 35.29 and 34.98 for the E gene and $orf1ab$, respectively. No specimens were negative with Allplex and positive with STANDARD M10.

Discussion

The study results demonstrated high agreement between the rapid RT-PCR assay using the STANDARD M10 and the conventional RT-PCR using the Allplex SARS-CoV-2 for detecting SARS-CoV-2 during the Omicron surge in Korea. Discordant results between the two assays were observed in specimens having low viral loads, which were reflected with high Ct values.

Few studies have evaluated the clinical performance of STANDARD M10. Hong et al. estimated the sensitivity of STANDARD M10 among 342 positive samples proven by the conventional RT-PCR using the Allplex SARS-CoV-2 assay.[8] They reported a sensitivity of 87.7% with STANDARD M10 using simulation with a virtual specimen pool comprising specimens having a Ct value of E gene [?]30 as 27.7%. In the present study, STANDARD M10 detected 118 specimens among 123 positive specimens that were positive with Allplex assay (95.9%), although the positive specimens consisted of 34.1% of specimens with a Ct value [?]30 for E gene, which demonstrates acceptable clinical performance based on the criteria by the Korean Ministry of Food and Drug Safety. These findings suggest that clinical performance would be different

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between the evaluation from manipulated laboratory dataset and the data acquired from consecutive patients in the real-world clinical setting.

Discordant results between STANDARD M10 and Allplex were observed in specimens with a Ct value >33 using the Allplex assay. These findings that the limit of detection of RT-PCR is associated with a viral load of the samples estimated by the Ct value are also reported by previous researchers who evaluated the performance of rapid RT-PCR. Jeong et al. found that the PPA of STANDARD M10 was 97.4% of the conventional RT-PCR assays.[9] However, the PPA was decreased to 87.5% in samples with a Ct value of >30 for the *E* gene, similar to that noted in the present study. Recent studies compared well-known cartridge-based rapid RT-PCR assays, Xpert Xpress SARS-CoV-2 (Xpert) and ID NOW COVID-19 (Abbott, IL, USA; ID NOW) with the Roche Cobas SARS-CoV-2 (Roche Molecular Systems, NJ, USA) for samples with low, medium, and high SARS-CoV-2 viral concentrations.[10,11] The two rapid RT-PCR assays showed 100% positive agreement for medium and high viral concentrations, defined as having a Ct value <30. However, for low viral concentrations defined as a Ct value >30, positive agreement for the Xpert was 97.1% (95% CI: 83.4–99.8%), whereas it was 34.3% (95% CI: 19.7–52.2%) for the ID NOW assay.[11]

Before the COVID-19 pandemic, the Korea Disease Control and Prevention Agency prepared a laboratory diagnostic system in response to a potential unknown disease outbreak.[12] This system was successfully applied for laboratory diagnosis based on developing and implementing RT-PCR for the causative pathogen of COVID-19 in the very early phase of the COVID-19 pandemic in Korea. However, there was no reliable domestic rapid RT-PCR method in Korea, and thus, diagnosis depended on imported rapid-RT-PCR methods such as Xpert. The Xpert method yields reliable results, but it has issues with high prices and substantial undersupply of reagents in Korea. STANDARD M10 is a method similar to Xpert, a completely automated cartridge-based point-of-care testing method but less expensive than Xpert and in potentially sufficient supply. After the introduction of STANDARD M10, the emergency healthcare system of Korea could manage the Omicron surge as it can serve as a reliable method in the real-world setting.

This is the first study including consecutive data collection from a large patient cohort for clinical evaluation of STANDARD M10 in an emergency department during the Omicron surge. However, several limitations must be considered. First, because the study was performed during the Omicron surge, the performance of detecting other variants of concern could not be evaluated. Second, analytical performance was not evaluated. Third, specimens used for STANDARD M10 and Allplex in the initial test were different. Fourth, although retesting was performed with the same specimen for specimens showing discordant results, potential specimen effect could remain. Fifth, the ratio of patients with severe medical conditions (KTAS levels 1 and 2 and a portion of KTAS level 3) is lesser in this study than the real-word ratio. Because the patient groups were indicated for the Xpert assay by the Korea health insurance system, these patients underwent Xpert instead of STANDARD M10. Thus, KTAS level 1–3 patients may be underrepresented in this study.

In conclusion, the STANDARD M10 SARS-CoV-2 rapid RT-PCR assay showed reliable clinical performance compared with the conventional RT-PCR using Allplex SARS-CoV-2 in an emergency department. STANDARD M10 can be a useful method for emergency healthcare because of its advantage of cartridge-based fully automated system and short reporting time for rapidly detecting SARS-CoV-2 compared with conventional RT-PCR. The diversity of rapid-RT-PCR systems may also be useful to solve problems associated with an imbalance of resources of rapid RT-PCR technology in the world.

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None

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Table I. Clinical and laboratory characteristics of 1,971 patients who underwent RT-PCR for SARS-CoV-2 in the emergency department

Characteristics	Characteristics	Values
Total patients, n	Total patients, n	1,971
Sex, n (%)	Sex, n (%)	
	Male	889 (45.1)
	Female	1,082 (54.9)
Median age, years (range)	Median age, years (range)	61 (0–100)
KTAS, n (%)	KTAS, n (%)	
	Level 1	4 (0.2)
	Level 2	177 (9.0)
	Level 3	899 (45.6)
	Level 4	254 (12.9)
	Level 5	637 (32.3)
COVID-19-associated symptom, n (%)	COVID-19-associated symptom, n (%)	547 (27.8)

COVID-19, coronavirus disease 2019; KTAS, Korean Triage and Acuity Scale.

Table II. Comparison of RT-PCR results using STANDARD M10 and Allplex assays for SARS-CoV-2 detection

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STANDARD M10	Allplex	Allplex	Allplex
	Negative	Positive	Total
Negative	1,844	5	1,849
Positive	4	118	122
Total	1,848	123	1,971
Overall agreement, % (95% CI)	99.5 (99.1–99.7)	99.5 (99.1–99.7)	99.5 (99.1–99.7)
Kappa, % (95% CI)	0.96 (0.93–0.98)	0.96 (0.93–0.98)	0.96 (0.93–0.98)
PPA, % (95% CI)	95.1 (89.8–97.7)	95.1 (89.8–97.7)	95.1 (89.8–97.7)
NPA, % (95% CI)	99.8 (99.4–99.9)	99.8 (99.4–99.9)	99.8 (99.4–99.9)

SARS-CoV-2, severe acute respiratory syndrome coronavirus 2; CI, confidence interval; PPA, positive percent agreement; NPA, negative percent agreement.

Table III. Ct values according to the result combinations determined by two RT-PCR assays for SARS-CoV-2 detection

RT-PCR results	RT-PCR results	Patient No, n	Ct values, median (range)	Ct values, median (range)	Ct values, median (range)	Ct values, median (range)	Ct values, median (range)	Ct values, median (range)	Ct val me (ra
			Allplex	Allplex	Allplex	Allplex	STANDARD M10	STANDARD M10	ST M1
Allplex	STANDARD M10		*E*	*RdRP*	*N*	*E*	*E*	*orf1ab*	
Positive	Positive	118	25.54* (12.80–38.40)	26.57^{+} (15.23–39.37)	25.67 (12.63–38.48)	24.14* (10.09–34.94)	24.14* (10.09–34.94)	24.76^{+} (11.17–34.34)	
Inconclusive	Inconclusive	12	37.22 (30.31–39.19)	34.78 (32.96–39.20)	36.09 (29.25–39.06)	34.47 (31.50–35.95)	34.47 (31.50–35.95)	34.02 (33.68–34.35)	
Discrepant result	Discrepant result								
Positive	Negative	2	36.75 (35.16–36.62)	37.96 (38.06–38.08)	36.85 (36.31–38.87)	-	-	-	
Positive	Inconclusive	3	35.75 (33.21–35.75)	37.33 (34.35–37.33)	36.50 (32.42–36.50)	33.02 (31.04–34.14)	33.02 (31.04–34.14)	-	
Inconclusive	Negative	4	38.18 (36.59–38.23)	-	38.38 (37.79–38.97)	-	-	-	
Inconclusive	Positive	4	38.22 (37.01–38.54)	39.04 (38.77–39.31)	39.31 (39.31)	35.15 (33.40–36.06)	35.15 (33.40–36.06)	34.59 (33.98–35.26)	
Negative	Inconclusive	9	-	-	-	35.29 (34.58–36.30)	35.29 (34.58–36.30)	34.98 (34.40–36.16)	

*P value for comparison between Ct of E gene is <0.001.

$+P$ value for comparison between Ct of *RdRP* (*orf1ab*) is <0.001.

SARS-CoV-2, severe acute respiratory syndrome coronavirus 2; Ct, cycle threshold.

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