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Clinical Evaluation of the Rapid STANDARD Q COVID-19 Ag Test for the Screening of Severe Acute Respiratory Syndrome Coronavirus 2

Hyung Woo Kim, M.D., Mikyoung Park, M.D., Ph.D., and Jong Ho Lee, M.D.
Department of Laboratory Medicine, Yeungnam University College of Medicine, Daegu, Korea

Standard tests for severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) detect the presence of viral RNA using real-time reverse transcription (rRT)-PCR. Recently, convenient, rapid, and relatively inexpensive SARS-CoV-2 antigen (Ag) detection methods have been developed. The STANDARD Q COVID-19 Ag test (SD Biosensor, Inc., Suwon, Korea) is a rapid immunochromatography test that qualitatively detects the nucleocapsid protein of SARS-CoV-2 using gold conjugated antibodies. We evaluated its performance in comparison with that of Allplex 2019-nCoV Assay (Seegene, Seoul, Korea) in a retrospective case-control study using residual samples. The sensitivity and specificity of the STANDARD Q COVID-19 Ag test were 89.2% (58/65) and 96.0% (96/100), respectively. Cycle threshold (Ct) values for the three target SARS-CoV-2 genes (envelope, RNA-dependent RNA polymerase, and nucleocapsid genes) included in Allplex 2019-nCoV Assay were significantly lower in Ag test-positive patients than in Ag test-negative patients ($P<0.001$). The Ag test sensitivity was higher in samples with Ct≤30 and those collected one to five days post symptom onset. In conclusion, the STANDARD Q COVID-19 Ag test can serve as an alternative in high-prevalence settings, when the low sensitivity is compensated or when rRT-PCR tests are limited.

Key Words: Severe Acute Respiratory Syndrome Coronavirus 2, STANDARD Q COVID-19 Ag test, Allplex 2019-nCoV Assay, Antigen, Immunochromatography, Nucleocapsid protein, Performance

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Corresponding author: Jong Ho Lee, M.D.
Department of Laboratory Medicine, Yeungnam University College of Medicine, 170 Hyunchoong-ro, Nam-gu, Daegu 42415, Korea
Tel: +82-53-640-6704
Fax: +82-53-640-6712
E-mail: leejongho@ynu.ac.kr, ae4207@naver.com

In December 2019, a case of unexplained, atypical pneumonia was reported in Wuhan, Hubei Province, China. This atypical pneumonia was caused by a novel coronavirus, severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) [1]. The World Health Organization (WHO) named the disease caused by SARS-CoV-2 as coronavirus disease 2019 (COVID-19) [2]. As of July 14, 2021, SARS-CoV-2 had infected 187,296,646 patients and caused 4,046,470 deaths in 210 countries, and COVID-19 remained a global pandemic [3].

Current standard tests for SARS-CoV-2 detect the presence of viral RNA using real-time reverse transcription (rRT)-PCR [4]. As COVID-19 exponentially spreads across the globe, a rapid method that allows on-field testing and yields immediate results is urgently needed. Compared with rRT-PCR, rapid diagnostic tests (RDTs) do not require complex technology and equipment and are convenient and relatively inexpensive. Therefore, an RDT capable of diagnosing SARS-CoV-2 with excellent clinical performance may serve as an alternative when rRT-PCR testing is limited, though currently the testing capacity in Korea is sufficient [5-7].

Tests for detecting the SARS-CoV-2 antigen (Ag) are commercially available [6], and their performance needs to be validated.

The STANDARD Q COVID-19 Ag test (SD Biosensor, Inc., Suwon, Korea) is an RDT that detects the nucleocapsid protein (NP) of SARS-CoV-2 via immunochromatography, and it is the first SARS-CoV-2 Ag-based RDT approved by the Korea Ministry of Food and Drug Safety, as an alternative when PCR testing is limited. NP is the predominantly expressed structural protein of SARS-CoV-2, and an NP Ag-based test has proven useful in the early diagnosis of COVID-19 [8]. We evaluated the clinical usefulness of the STANDARD Q COVID-19 Ag test, and compared its performance with that of Allplex 2019-nCoV Assay (Seegene, Seoul, Korea), a molecular diagnostic test approved by the Korea Ministry of Food and Drug Safety and the United States Food and Drug Administration for emergency use. Performance evaluation studies of the STANDARD Q COVID-19 Ag test have been conducted both abroad [9-11] and in Korea [12]. This study was conducted using residual samples after COVID-19 testing was performed during hospitalizations and drive-through screenings at a single tertiary hospital at the time of the outbreak in Daegu. The Institutional Review Board (IRB) of Yeungnam University Hospital, Daegu, Korea, approved this study (IRB No. YUMC 2020-03-070). The IRB approved the collection of residual samples under a waiver of consent.

We conducted a retrospective diagnostic case-control study with a reversed-flow design [13]. Patients who visited Yeungnam University Hospital for COVID-19 drive-through screening or who were hospitalized between February 28, 2020 and April 30, 2020 were included. All samples were collected using nasopharyngeal swabs, transferred to the laboratory in viral transport medium (VTM), and stored at 4°C until and after testing with Allplex 2019-nCoV Assay in accordance with the guidelines reported by Hong, *et al*. [14]. All tests of the Allplex 2019-nCoV Assay were performed on the day of sample collection. The next day, residual samples were stored at -80°C. Samples collected in eNAT (Copan Diagnostics Inc., Brescia, Italy) were excluded, whereas those collected in REST UTM (Noble Biosciences, Inc., Hwaseoung, Korea) or ESwab (Copan Diagnostics) were included for the STANDARD Q COVID-19 Ag test as per the manufacturer's recommendation. Among serial samples of patients with a positive Allplex 2019-nCoV Assay result, the ones tested at initial diagnosis (N=206) were included in this study. Sixty-five samples collected in eNAT and 25 samples with insufficient residual volume for the STANDARD Q COVID-19 Ag test were excluded. Finally, 65 positive samples were selected consecutively (Table 1). In addition, 100 negative samples were collected during the same period. Overall, the study included 75 male and 90 female patients (age range, 12–95 years). STANDARD Q COVID-19 Ag tests were conducted using residual samples in May 2020, in a randomized order, by a laboratory technician who was blinded to the Allplex 2019-nCoV Assay results.

Table 1. Basic characteristics of COVID-19 patients

Characteristic	Total (N=65)	Ag (+) (N=58)	Ag (–) (N=7)
Age (yr)	56 (48–63)	58 (48–64)	51 (49–56)
Sex			
Male	24 (36.9)	22 (37.9)	2 (28.6)
Female	41 (63.1)	36 (62.1)	5 (71.4)
Patient location			
Hospitalization	1 (1.5)	1 (1.8)	0 (0.0)
Drive-through screening	64 (98.5)	57 (98.2)	7 (100.0)
Clinical symptom			
Cough	28 (21.1)	23 (19.6)	5 (31.3)
Productive cough	24 (18.1)	22 (18.8)	2 (12.5)
Fever	22 (16.5)	21 (18.0)	1 (6.3)
Sore throat	19 (14.3)	17 (14.4)	2 (12.5)
Myalgia	11 (8.3)	7 (6.0)	4 (25.0)
Chills	5 (3.8)	5 (4.3)	0 (0.0)
Rhinorrhea	4 (3.0)	4 (3.4)	0 (0.0)
Headache	3 (2.3)	3 (2.6)	0 (0.0)
Dyspnea	3 (2.3)	3 (2.6)	0 (0.0)
Nasal obstruction	3 (1.5)	3 (2.6)	0 (0.0)
Diarrhea	2 (0.8)	2 (1.7)	0 (0.0)
Chest discomfort	1 (0.8)	0 (0.0)	1 (6.3)
Cold sweating	1 (0.8)	0 (0.0)	1 (6.3)
Anorexia	1 (0.8)	1 (0.9)	0 (0.0)
Parosmia	1 (0.8)	1 (0.9)	0 (0.0)
Fatigue	1 (0.8)	1 (0.9)	0 (0.0)
Asymptomatic	4 (3.0)	4 (3.4)	0 (0.0)
Symptom onset to diagnosis			
Asymptomatic	4 (6.1)	4 (6.9)	0 (0.0)
1–5 days	41 (63.1)	39 (67.2)	2 (28.6)
6–10 days	15 (23.1)	11 (19.0)	4 (57.1)
11–21 days	5 (7.7)	4 (6.9)	1 (14.3)

Values are expressed as median (interquartile range) or number (%).
Abbreviations: COVID-19, coronavirus disease 2019; Ag, antigen.

Allplex 2019-nCoV Assay is a multiplex rRT-PCR test that simultaneously detects three target SARS-CoV-2 genes in a single tube. The test is designed to detect the RNA-dependent RNA polymerase (*RdRP*) and nucleocapsid (*N*) genes specific to SARS-CoV-2 and the envelope (*E*) gene that is common to all sarbecoviruses, including SARS-CoV-2. According to the manufacturer's

recommendations, the result was interpreted as positive only if the cycle threshold (CT) values of all three target genes were within the cutoff (<40) and negative if all were outside the cutoff or if there was no amplification; otherwise, the result was interpreted as inconclusive [15, 16].

The STANDARD Q COVID-19 Ag test qualitatively detects SARS-CoV-2 NP using gold-conjugated antibodies. It comprises a nitrocellulose membrane with control ("C") and test ("T") lines that are coated with mouse monoclonal anti-chicken IgY antibodies and mouse monoclonal anti-SARS-CoV-2 antibodies, respectively. The extraction buffer containing mouse monoclonal anti-SARS-CoV-2 antibodies conjugated with gold interact with SARS-CoV-2 antigens in the samples to form antigen–antibody complexes. These complexes migrate on the membrane until they reach the T line, where they are captured by the mouse monoclonal anti-SARS-CoV-2 antibodies. In this study, nasopharyngeal swab samples in VTM (100 μL) were mixed with the extraction buffer (100 μL) provided with the test kit.

The sensitivity and specificity of the STANDARD Q COVID-19 Ag test were evaluated and compared with those of Allplex 2019-nCoV Assay. Confidence intervals (CIs) for sensitivity and specificity were "exact" Clopper-Pearson CIs. Ct values for the target SARS-CoV-2 genes were compared between Ag test-positive and Ag-negative samples using the Mann–Whitney test. All statistical analyses were performed using MedCalc Statistical Software version 19.5.3 (MedCalc Software Ltd., Ostend, Belgium).

Based on the analysis of the 65 positive samples, the sensitivity and specificity of the STANDARD Q COVID-19 Ag test were 89.2% (95% CI, 79.1%–95.6%) and 96.0% (95% CI, 90.1%–98.9%), respectively (Table 2). Ct values for the target SARS-

Table 2. Performance of the STANDARD Q COVID-19 Ag test for the detection of SARS-CoV-2

Groups	Allplex 2019-nCoV Assay							
	rRT-PCR† (+) (N=65)		rRT-PCR† (–) (N=100)		Sensitivity % (95% CI)	Specificity % (95% CI)	Positive predictive value % (95% CI)	Negative predictive value % (95% CI)
	Ag (+), N (%)	Ag (–), N (%)	Ag (+), N (%)	Ag (–), N (%)				
Overall	58 (89.2)	7 (10.8)	4 (4.0)	96 (96.0)	89.2 (79.1–95.6)	96.0 (90.1–98.9)	93.6 (84.7–97.4)	93.2 (87.2–96.5)
Ct*≤30	51 (78.4)	3 (4.6)	-	-	94.4 (84.6–98.8)	-	-	-
Ct*>30	7 (10.8)	4 (6.2)	-	-	63.6 (30.8–89.0)	-	-	-
Asymptomatic	4 (6.9)	0 (0.0)	-	-	100 (39.8–100.0)	-	-	-
1–5 days	39 (67.2)	2 (28.6)	-	-	95.1 (83.5–99.4)	-	-	-
6–10 days	11 (19.0)	4 (57.1)	-	-	73.3 (44.9–92.2)	-	-	-
11–21 days	4 (6.9)	1 (14.3)	-	-	80.0 (28.4–99.5)	-	-	-

*Ct value for the envelope gene in Allplex 2019-nCoV Assay; †Interpreted as described in the instruction of Allplex 2019-nCoV Assay.
Abbreviations: Ag, antigen; COVID-19, coronavirus disease 2019; CI, confidence interval; Ct, cycle threshold; rRT-PCR, real-time reverse transcription PCR; SARS-CoV-2, severe acute respiratory syndrome coronavirus 2.

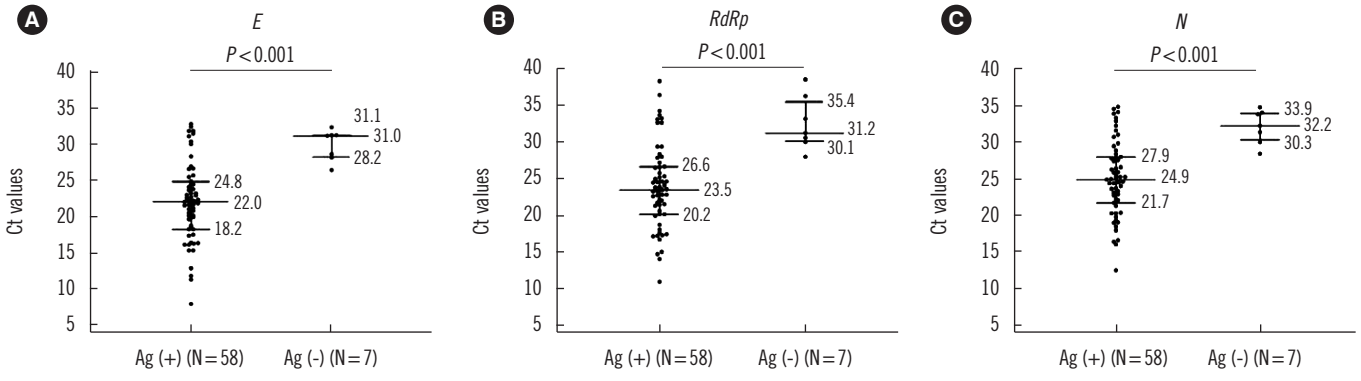

Fig. 1. Comparison of Ct values for the target SARS-CoV-2 genes (A) *E*, (B) *RdRp*, and (C) *N* in Ag test-positive and Ag test-negative samples. Values are expressed as median (interquartile range).
Abbreviations: Ct, cycle threshold; SARS-CoV-2, severe acute respiratory syndrome coronavirus 2; Ag, antigen; *E*, envelope gene; *RdRp*, RNA-dependent RNA polymerase gene; *N*, nucleocapsid gene.

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CoV-2 genes were significantly lower in Ag test-positive samples than in Ag test-negative samples (Fig. 1, $P<0.001$).

The performance of the STANDARD Q COVID-19 Ag test was inferior to that of rRT-PCR (Table 2). However, its sensitivity was higher for samples with a Ct value ≤30 and samples collected one to five days post symptom onset than for those with a Ct value >30 and those collected after six days post symptom onset; the former possibly accounts for a significant proportion of transmission cases. This finding is consistent with the recommendation to use rapid Ag tests by the European Center for Disease Prevention and Control and WHO [17, 18].

Improvement in Ag test performance is required because of the potentially large number of false negatives due to low sensitivity, and false positives despite the high specificity. However, the STANDARD Q COVID-19 Ag test has several benefits over rRT-PCR for SARS-CoV-2 detection, such as simplicity of use, easy availability, low cost, and the short time needed to obtain the results. Therefore, the STANDARD Q COVID-19 Ag test, when used considering the minimal performance requirements set by the WHO, can serve as an effective alternative in high-prevalence settings, when the low sensitivity is compensated or when rRT-PCR tests are limited.

Our study had several limitations. First, the number of samples used in this study was small. More than a half the samples were collected one to five days post symptom onset and yielded a Ct value for *E* of <30 (36/65). Moreover, since only four asymptomatic patients were included, we cannot definitively conclude that the STANDARD Q COVID-19 Ag test shows good performance in asymptomatic patients, despite the 100% sensitivity in this group. Overall, the patient distribution may not reflect the general population with low-prevalence settings, and results of the performance evaluation may vary greatly depending on the patient group [12]. Second, as this was a retrospective study conducted with residual samples, we mixed nasopharyngeal swab samples in VTM with the extraction buffer, while directly mixing nasopharyngeal swab samples with extraction buffer is the primary recommendation. Sample dilution with VTM may have decreased the test sensitivity for samples with high Ct values. Furthermore, the C line of the STANDARD Q COVID-19 Ag test did not appear in preliminary tests of samples collected in eNAT, suggesting that guanidine thiocyanate in this transport medium may have denatured proteins [19]. Therefore, directly mixing the nasopharyngeal swab with the extraction buffer provided with STANDARD Q COVID-19 Ag test kit is required to accurately assess the clinical performance of the test. Third, Ct values of Ag test-positive samples may not accurately indicate the patient status. Ct values can vary depending on the sample quality and may not correlate with the presence of SARS-CoV-2 Ag.

In conclusion, the STANDARD Q COVID-19 Ag test can act as an alternative in high-prevalence settings, when the low sensitivity is compensated or when rRT-PCR tests are limited. It is necessary to evaluate the method further by directly mixing nasopharyngeal swabs with the extraction buffer provided with STANDARD Q COVID-19 Ag test kit. Moreover, analysis of a larger number of samples is needed to evaluate the performance in low-prevalence settings in the general population.

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AUTHOR CONTRIBUTIONS

Lee JH conceived and designed the study and performed the STANDARD Q COVID-19 Ag tests. Kim HW performed data analysis and wrote the manuscript. Lee JH and Park MK reviewed the manuscript.

CONFLICTS OF INTEREST

None declared.

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ORCID

Hyung Woo Kim https://orcid.org/0000-0003-1330-425X
Mikyoung Park https://orcid.org/0000-0002-4815-6958
Jong Ho Lee https://orcid.org/0000-0002-6837-838X

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