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Performance evaluation of four rapid antibody tests for the detection of severe acute respiratory syndrome coronavirus 2

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Abstract

Background: The prompt detection of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) is important in the therapeutic management of infected patients. Rapid diagnostic tests are widely used for this purpose. This study aimed to evaluate the clinical performance of four SARS-CoV-2 immunoglobulin IgG/IgM rapid diagnostic tests in the detection of SARS-CoV-2.

Methods: Nasopharyngeal and oropharyngeal swabs and/or sputum were collected from 30 patients infected with SARS-CoV-2 and 30 healthy volunteers. All specimens were tested using four SARS-CoV-2 IgG/IgM rapid diagnostic tests and real-time polymerase chain reaction. We assessed the clinical sensitivity and specificity of the tests.

Results: The clinical sensitivity of FREND™, SsmarTest™, BIOCREDIT™, and IVDLAB™ was 96.67%, 100.00%, 100.00%, and 96.67%, respectively, compared to real-time polymerase chain reaction. The clinical specificity was 96.67%, 100.00%, 86.67%, and 96.67%, respectively.

Conclusion: These findings could expedite the detection of SARS-CoV-2 and thus reduce the risk of further transmission of the virus.

KEYWORDS
rapid antibody test, rapid diagnostic test, SARS-CoV-2, SARS-CoV-2 IgG/IgM antibody, sensitivity, specificity

1 | INTRODUCTION

After severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infection was first detected in Wuhan city, China, in December 2019, the World Health Organization (WHO) proclaimed the coronavirus disease (COVID-19) outbreak a global pandemic in March 2020.[1] Before this, six coronaviruses infected humans; four (229E, OC43, NL63, and HKU1) caused common cold-like symptoms. The remaining two, severe acute respiratory syndrome coronavirus (SARS-CoV) and middle-east respiratory syndrome coronavirus (MERS-CoV), caused serious illness and death in 2003 and 2015, respectively.[2]

In January 2020, a seventh member of the coronaviruses family to infect humans was defined and named SARS-CoV-2.[3]

SARS-CoV-2 infection is a continuing issue worldwide despite the rigorous preventive measures adapted to prevent widespread transmission. Four main methods are used to confirm a SARS-CoV-2 infection: virus culture, sequencing, antibody testing, and quantitative real-time polymerase chain reaction (qRT-PCR). However, sequencing is time-consuming, and viral culture, which is more appropriate for research use, has the potential to infect laboratory staff.[4] Additionally, viral culture requires the organism to be viable and is a lengthy process. Therefore, qRT-PCR, a molecular genetic

test, is now considered the gold standard for SARS-CoV-2 detection in Korea despite the potential of false negatives.[5,6]

Additional limitations of qRT-PCR are that it takes several hours to provide results, and it requires well-trained personnel and expensive equipment to perform. Rapid diagnostic tests (RDTs), which use a capillary technique, are widely used for the timely detection of various pathogens.[7] An RDT is a simple procedure that requires a very small sample size and provides results within 15 min. The several commercially developed RDTs that have been approved for emergency use in the detection of SARS-CoV-2 (http://www.fda.gov/medical-devices/coronavirus-disease-2019-covid-19-emergency-use-authorizations-medical-devices/eua-authorized-serology-test-performance) are developed to detect SARS-CoV-2 antigens or SARS-CoV-2 immunoglobulin IgG/IgM antibodies.

This study aimed to determine the clinical performance of four SARS-CoV-2 immunoglobulin IgG/IgM RDTs used to detect SARS-CoV-2 and compare the results with qRT-PCR data.

2 | MATERIALS AND METHODS

2.1 | Sample collection

Between February 28th and May 6th, 2020, nasopharynx swabs, oropharyngeal swabs, and sputum were collected from 30 patients infected with SARS-CoV-2 and 30 healthy volunteers. All collected samples were stored at −80°C.

All specimens were tested for SARS-CoV-2 using four SARS-CoV-2 IgG/IgM antibody tests: FREND™ COVID-19 IgG/IgM Duo (NanoEntek®), SmarTest™ COVID-19 IgG/IgM detection Kit (SLSBio®), BIOCREDIT™ COVID-19 IgG/IgM Combo (Rapigen®), and IVDLAB™ COVID-19 IgG/IgM Test (IVDLAB®). qRT-PCR (PowerChek™ 2019-nCoV Real-time PCR Kit) was used as a reference.

2.2 | Ethical approval

The study protocol was approved by Dankook University Institutional Review Board (IRB approval number 2020-11-013). The study was conducted in conformance with the principles of the Declaration of Helsinki. Patient consent was waived because this study used statistics from tests conducted by medical institutions for diagnosis and did not use the patients' personal information.

2.3 | Antibody testing

To evaluate the tests, their sensitivity (percent positive agreement [PPA]), specificity (percent negative agreement [PNA]), and accuracy (overall percent agreement [OPA]) were measured. The sensitivity of the FREND™ COVID-19 IgG/IgM Duo, SmarTest™ COVID-19 IgG/IgM detection Kit, BIOCREDIT™ COVID-19 IgG/IgM Combo,

and IVDLAB™ COVID-19 IgG/IgM Test, relative to qRT-PCR, was 96.67%, 100.00%, 100.00%, and 96.67%, respectively. The specificity was 96.67%, 100.00%, 86.67%, and 96.67%, respectively, and the accuracy was 96.67%, 100.00%, 93.33%, and 96.67%, respectively.

The Cohen's kappa value for FREND™ COVID-19 IgG/IgM Duo, SmarTest™ COVID-19 IgG/IgM detection Kit, BIOCREDIT™ COVID-19 IgG/IgM Combo, and IVDLAB™ COVID-19 IgG/IgM Test, relative to qRT-PCR, was 0.933, 1.000, 0.867, and 0.933, respectively (Table 1).

2.3.1 | BIOCREDIT™ COVID-19 IgG/IgM combo

The kit components and specimens were equilibrated to room temperature before testing. The test device was removed from the foil pouch and placed on a clean, dry, and level surface. Then 10 μl of serum or plasma, or 20 μl of whole blood, was added to the sample well (S) of the device using a capillary tube or disposable dropper. Three drops of assay buffer were then added to the S. The results were provided within 10–15 min.

2.3.2 | FREND™ COVID-19 IgG/IgM Duo

The tubes and sealed pouches from the kit were thawed to room temperature for 15–30 min before the testing procedure. The sample ID was recorded on the cartridge in the designated area. A 35 μl sample was added to a sample dilution tube and mixed well. This sample was pipetted into the sample inlet on the cartridge using a calibrated micropipette with a fresh tip. The "Test" button was pressed on the "Main" screen of the FREND™ System. The system moved to the Patient ID screen automatically. The Patient ID was entered, and the "Enter" key was pressed to begin the test. The cartridge was inserted into the cartridge slot using the cartridge arrows as a guide. When the reaction in the cartridge was complete, the FREND™ System automatically began the reading process. When the measurements were completed, the cartridge was automatically expelled, and the results were displayed.

2.3.3 | IVDLAB™ COVID-19 IgG/IgM test

The specimens and the test device were equilibrated to room temperature before testing (15–30 min). The sealed pouch was opened, and the device was placed on a clean, dry, and level surface. Using a micropipette or capillary microtip, 10 μl of serum, plasma, or whole blood was added to the sample well. Approximately two to three drops (80–120 μl) of dilution solution were added to the sample wells. The results were provided within 10 min.

2.3.4 | SsmarTest™ COVID-19 IgG/IgM detection kit

The personal identification number of the sample was written on the device. Then, using the enclosed syringe, 10 μl of the sample was

TABLE 1 Sensitivity and specificity of the four rapid diagnostic tests analyzed in this study

Rapid diagnostic tests	PPA (sensitivity)			NPA (specificity)			OPA (accuracy)			Cohen's Kappa value		
		95% CI			95% CI			95% CI			95% CI	
	%	Lower	Upper	%	Lower	Upper	%	Lower	Upper	%	Lower	Upper
BIOCREDIT™	100.0	88.4	100.0	86.7	69.3	96.2	93.3	83.8	98.2	0.9	0.7	1.0
FREND™	96.7	82.8	99.9	96.7	82.8	99.9	96.7	88.5	99.6	0.9	0.8	1.0
IVDLAB™	96.7	82.8	99.9	96.7	82.8	99.9	96.7	88.5	99.6	0.9	0.8	1.0
Ssmar™	100.0	88.4	100.0	100.0	88.4	100.0	100.0	94.0	100.0	1.0	1.0	1.0

Note: CI is the statistical estimate obtained from the observed data.

Abbreviations: CI, Confidence interval; NPA, negative percent agreement; OPA, overall percent agreement; PPA, positive percent agreement.

carefully dispensed into the device to avoid overfilling it. When the sample pad absorbed the entire sample, 20 μl of the enclosed running solution was added. The results were obtained within 15–20 min. Finally, interpretation of the results from the four RDTs was carried out.

The FREND™ System detects SARS-CoV-2 IgG/IgM using a fluorescence immunoassay. The cut-off index (COI) is determined quantitatively by testing the specimens that were collected 8 days from the onset of SARS-CoV-2 infection symptoms. A COI <1.0 indicates a negative result, while a COI ≥1.0 indicates a positive.

The other three RDTs detect SARS-CoV-2 IgG/IgM using a lateral flow immunoassay, and the results can be read by the naked eye. One band in the control line, within the result window, indicates a negative result, while a visible control line and an IgG test line indicate an IgG-positive result. A visible control line and an IgM test line indicate an IgM-positive result. A visible control line, IgG test line, and IgM test line indicate the presence of IgG and IgM antibodies. If the control line fails to appear within the result window, the result is considered invalid.

2.4 | Real-time PCR analysis

The PowerChek™ 2019-nCoV Real-time PCR Kit specifically targets the E gene for beta coronavirus and the RdRp gene for SARS-CoV-2 in sputum, nasopharyngeal swabs, and oropharyngeal swabs. This qRT-PCR assay is based on the WHO and Korea Centers for Disease Control and Prevention reference method. RNA was isolated by the QIAcube (Qiagen) following the manufacturer's instructions. The kit components were thawed on ice, and the tubes were spun down before use. The volumes of template RNA, qRT-PCR premix, and each primer/probe mix were 5, 11, and 4 μl, respectively, bringing the total volume of the PCR mixture to 19 μl. The tubes were briefly centrifuged to thoroughly mix the reagents and remove any air bubbles and drops present inside the cap. The qRT-PCR thermocycling process consisted of one cycle at 50°C for 30 min, one cycle at 95°C for 10 min, 40 cycles at 95°C for 15 s, and 40 cycles at 60°C for 1 min. The sample was positive if the corresponding fluorescence accumulation curve signal crossed the cycle threshold (Ct). A Ct value <35.0 was considered positive. Results were accepted as relevant if the positive and negative amplification controls passed.

2.5 | Statistical analysis

SAS version 9.4 (SAS Institute Inc.) was used to perform all statistical analyses, including descriptive statistical analysis and frequency analysis.

3 | RESULTS

In total, 60 specimens collected between February 28th and May 6th, 2020, were tested for SARS-CoV-2 infection using four SARS-CoV-2 IgG/IgM RDTs. Thirty specimens (50.0%) were confirmed as

positive by these tests. The results were confirmed by qRT-PCR analysis (Table 2).

The IgG and IgM positivity rate detected using the BIOCREDIT™ test was 50.0% (30/60) and 53.3% (32/60), respectively (Table 2); 50.0% (30/60) and 23.3% (14/60), respectively, for the FREND™ test; 41.7% (25/60) and 50.0% (30/60), respectively, for the IVDLAB™ test; and 46.7% (28/60) and 46.7% (28/60), respectively, for the SsmarTest™ test (Table 2).

The lowest SARS-CoV-2 IgG positivity rate of 41.7% (25/60) was detected in the IVDLAB™ analysis, and the lowest SARS-CoV-2 IgM positivity rate of 23.3% (14/60) was detected in the FREND™ analysis (Table 2). The highest SARS-CoV-2 IgG positivity rate (50.0%, 30/60) was detected in the BIOCREDIT™ and FREND™ analysis. The highest SARS-CoV-2 IgM positivity rate (76.7%, 46/60) was detected in the FREND™ analysis (Table 2).

The RDT with the largest positivity rate difference between qRT-PCR analysis and SARS-CoV-2 IgG detection was the IVDLAB™ test with an 8.3% difference. On the other hand, the RDT with the largest positivity rate difference between qRT-PCR analysis and SARS-CoV-2 IgM detection was the BIOCREDIT™ test, with a difference of 30.0%.

The lowest number of SARS-CoV-2 positive specimens (1/30) was observed <7 days from the onset of symptoms to the date of sample collection (real-time PCR Ct value: E gene 22.2 copies/ml, RdRp gene 19.8 copies/ml) (Table 3). The highest number of SARS-CoV-2 positive specimens (13/30) was observed 14–20 days after the onset of symptoms (real-time PCR Ct value: E gene 26.6 copies/ml, RdRp gene 25.2 copies/ml) (Table 3). In the FRENDTM analysis of SARS-CoV-2, the IgM-positive rate was observed to be the lowest (50.0%, 6/13). For a period of >20 days from sample collection, the RDTs, except the FRENDTM test kit, showed a SARS-CoV-2 IgG/IgM positivity rate of 100% (8/8) (real-time PCR Ct value: E gene 33.1 copies/ml, RdRp gene 31.6 copies/ml) (Table 3).

4 | DISCUSSION

Since the first Korean patient with confirmed SARS-CoV-2 infection was reported on January 20th, 2020, there have been 27,427 more

confirmed cases in Korea. A total of 478 deaths have been recorded (http://ncov.mohw.go.kr/). The estimated virus incubation period is between 2–14 days with 95% confidence.[8]

All four RDTs exhibited excellent performance, with all exceeding the target sensitivity and specificity except for the BIOCREDIT™, which had a lower specificity. With better accuracy and more rapid results, the rapid antibody test can be used for mass screening in areas of high SARS-CoV-2 prevalence and can combat the lack of PCR supply in developing countries. Currently, over 25 antibody tests have been approved for emergency use by the US Food and Drug Administration, and 11 antibody tests are undergoing evaluation by the Korean Food and Drug Administration (http://ncov.mohw.go.kr/).

The target antigens of SARS-CoV-2 for antibody production are viral structural proteins known as the spike (S), envelope (E), membrane (M), and nucleocapsid (N).[2] The SsmarTest™ and IVDLAB™ kits use both the S and N proteins as immobilized antigens to detect SARS-CoV-2 antibodies. The FREND™ kit uses only the N protein, and the BIOCREDIT™ kit uses only the S protein on the solid phase membrane of the rapid antibody test kit. The N protein is abundant in SARS-CoV-2, and the S protein is highly immunogenic.[9] The receptor-binding domain (RBD) of the S protein combines with angiotensin-converting enzyme-2 receptors in the lower bronchial system and lung and mediates infection.[10] The neutralizing antibody blocks this pathway, preventing virus infection in the early phase.[9] Therefore, candidate vaccines for SARS-CoV-2 adopt the RBD of the S protein as a stimulant to the host immune system. Indeed, a vaccine with an RBD of the S protein of SARS-CoV could elicit a neutralizing antibody response and protective activity in vaccinated animals.[11] However, to date, no commercially available serological test has been used to detect neutralizing antibodies, regardless of the antigenic target.[12] Hence, the positive results of an RDT kit should not be used to indicate "immunity passports" because immunity-based licenses can only be introduced if serology testing for the neutralizing antibody is accurate.[13]

According to this study, IgM antibodies are present 6 days after infection. This finding supports those of previous studies.[14,15] Regarding IgG, one study revealed that 40% of asymptomatic individuals and 12.9% of symptomatic individuals were negative for

TABLE 2 Rapid diagnostic test and real-time polymerase chain reaction results

| Rapid diagnostic tests | IgG | | | | | IgM | | | | | Result | |
| | Positive | | Negative | | Total | Positive | | Negative | | Total | Positive | |
	N	%	N	%	N	N	%	N	%	N	N	%
BIOCREDIT™	30	50.0	30	50.0	60	32	53.3	28	46.7	60	34	56.7
FREND™	30	50.0	30	50.0	60	14	23.3	46	76.7	60	30	50.0
IVDLAB™	25	41.7	35	58.3	60	30	50.0	30	50.0	60	30	50.0
Ssmar™	28	46.7	32	53.3	60	28	46.7	32	53.3	60	30	50.0
Total(N)	28.3	47.1	31.8	52.9	60	26	43.3	34	56.7	60	30	51.7
PowerChek™ 2019-nCoV Real-time PCR Kit	30	50.0	30	52.9	60	14	23.3	46	76.7	60	30	50.0

Abbreviations: IgG, Immunoglobulin G; IgM, Immunoglobulin M; PCR, polymerase chain reaction.

TABLE 3 The rapid antibody test positivity rates for IgG and IgM according to the period from the onset of symptoms to the date of sample collection among 30 SARS-CoV-2 patients and real-time polymerase chain reaction Ct values

		The period from the onset of symptoms to the date of sample collection															
		<7 days				7–13 days				14–20 days				>20 days			
		Positive				Positive				Positive				Positive			
		IgG		IgM		IgG		IgM		IgG		IgM		IgG		IgM	
Rapid diagnostic tests		N	%	N	%	N	%	N	%	N	%	N	%	N	%	N	%
BIOCREDIT™		0	0.0	1	100.0	7	87.5%	8	100.0%	13	100.0%	13	100.0%	8	100.0%	8	100.0%
FREND™		1	100.0	1	100.0	7	87.5%	4	50.0%	13	100.0%	6	46.2%	8	100.0%	3	37.5%
IVDLAB™		0	0.0	1	100.0	4	50.0%	7	87.5%	13	100.0%	13	100.0%	8	100.0%	8	100.0%
Ssmar™		1	100.0	1	100.0	8	100.0%	6	75.0%	11	84.6%	13	100.0%	8	100.0%	8	100.0%
Total (n)		1	50.0	1	100.0	8	81.3%	8	78.1%	13	96.2%	13	86.5%	8	84.4%	8	84.4%
Real-time PCR Ct value	E gene	22.2				28.6				26.6				33.1			
	RdRP gene	19.8				26.5				25.2				31.6			

Abbreviations: E gene, Envelope gene; IgG, immunoglobulin G; IgM, immunoglobulin; RdRp gene, RNA dependent RNA polymerase.

IgG in the early convalescent phase.[16] However, we did not detect IgG disappearance in the current study. The results of IgM-positive cases were collected 7–13 days after the onset of symptoms. The IgG-positive cases were almost always detected by the FREND™ kit, and these cases comprised samples collected 14–20 days after the onset of symptoms. The FREND™ kit exhibited lower sensitivity for IgM detection than the other kits. With regards to false negatives observed with the FREND™ and IVDLAB™ kits, the Ct values for the E gene and the RdRp gene were 32.04 and 33.64, respectively; however, a Ct value of <35.00 was considered positive. We hypothesized that antibody production is proportional to the severity of the disease. This finding is consistent with that of a previous study.[17]

During the early phase of the pandemic, the utility of antibody testing was negligible; however, this can be used as an effective tool for identifying prior infection in non-hospitalized individuals and for seroprevalence surveys when SARS-CoV-2 infection is ongoing, as in the current situation.[12]

Based on this study, the detection rate in the early phase of the illness is low because antibody production is active approximately 1 week from the onset of symptoms. However, it is known that IgM and IgG ELISAs show positive results from samples collected as early as 4 days after the onset of symptoms, and higher levels occur after 2 weeks of illness.[18] In addition, very few studies have investigated assay performance in asymptomatic patients.[12] Accordingly, antibody tests could be used as complementary assessments and would be particularly useful in patients who exhibit suggestive clinical features (at approximately 14 days after the onset of symptoms) but have negative, indeterminate, or unavailable molecular diagnostic test results.[12]

This study has several limitations. First, the sample size was small. A small sample may be insufficient to effectively evaluate the clinical performance of RDTs to detect SARS-CoV-2, resulting in biased results. Second, it is known that the majority of SARS-CoV-2 contigs have an 85% similarity to a bat SARS-like CoV and a similar sequence to SARS-CoV-1.[19] Therefore, false-positive results may be due to the presence of other beta-coronaviruses. Therefore, additional studies are required to provide a more accurate evaluation of the clinical performance of RDTs. Despite these limitations, we found that the FREND™ kit exhibited a lower sensitivity for IgM detection than the other kits. Therefore, our study provides insight into the clinical performance of four SARS-CoV-2 IgG/IgM antibody RDTs for detecting SARS-CoV-2.

We expect that this study will provide information that can be used to safeguard public health, reduce the incidence of coronavirus disease 2019 (COVID-19) caused by SARS-CoV-2, and provide information that can be used to treat patients.

AUTHOR CONTRIBUTIONS

JS Kim and JK Kim made substantial contributions to the conception and design of the study. SW Ryu and BK Jung made substantial contributions to data acquisition and analysis. All authors agree to be accountable for all aspects of the study and ensure that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

PATIENT CONSENT

Patient consent was waived for this study.

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