Letter to the Editor
Diagnostic Immunology

Ann Lab Med 2023;43:120-123
https://doi.org/10.3343/alm.2023.43.1.120
ISSN 2234-3806 • eISSN 2234-3814

ANNALS OF LABORATORY MEDICINE

Clinical Evaluation of Two Rapid Antigen Tests for Severe Acute Respiratory Syndrome Coronavirus 2 Detection

Kyunghee Yu, M.D.[1,2], Junhyup Song, M.D.[1,2], Dokyun Kim, M.D., Ph.D.[2], Yongjung Park, M.D., Ph.D.[2], and Seok Hoon Jeong, M.D., Ph.D.[2]

[1]Department of Laboratory Medicine, Severance Hospital, Yonsei University College of Medicine, Seoul, Korea; [2]Department of Laboratory Medicine, Gangnam Severance Hospital, Yonsei University College of Medicine, Seoul, Korea

Dear Editor,

Rapid antigen detection tests (RADTs) based on immunochromatographic assays (ICAs) or fluorescence immunoassays (FIAs) are now widely used for severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) detection [1-4]. Despite their advantages of speed, lower cost, and simplicity compared to real-time PCR (RT-PCR), concern has been raised regarding the variable sensitivities and specificities of RADTs, which range 0%–94%, making evaluation of RADTs using clinical samples essential [1-5]. To assess the usefulness of RADTs as a rapid detection tool, we evaluated an ICA and FIA (STANDARD Q COVID-19 Ag and STANDARD F COVID-19 Ag, respectively; SD Biosensor Inc., Suwon, Korea).

Nasopharyngeal (NP) swabs were collected from 554 patients with symptoms of COVID-19 and/or a history of contact with confirmed COVID-19 patients who visited Gangnam Severance Hospital between July 1 and October 10, 2021. This study was approved by the Institutional Review Board of Yonsei University Gangnam Severance Hospital, Seoul, Korea, approved this study (3-2021-0113) and waived the need for informed consent since the study was conducted on residual samples. The swabs were placed in AB Transport Medium (AB MEDICAL, Seoul, Korea) and tested for SARS-CoV-2 with PowerChek SARS-CoV-2 and Influenza A&B Multiplex Real-Time PCR Kits (Kogenebiotech Co., Seoul, Korea). All samples were tested concurrently using the two RADTs. Among the 554 samples, 219 were PCR-positive, including 150 from patients reporting any COVID-19–related symptoms (Table 1), and 335 were PCR-negative, including 167 from symptomatic patients. For ICA, a mixture of medium containing NP swabs and extraction buffer was applied to the solid device and incubated for 15 minutes. The results were read with the naked eye. For FIA, the fluorescence signals were measured using STANDARD F2400 (SD Biosensor) and expressed as cut-off indices (COIs). According to the manufacturer, both RADTs can detect the SARS-CoV-2 nucleocapsid antigen.

Statistical analyses were performed using Analyse-it v5.68 (Analyse-it Software Ltd., Leeds, UK). Numerical values were summarized as median and range. The sensitivity, specificity, and 95% confidence intervals (CIs) were calculated. Spearman's rank test was applied to calculate correlation coefficients between results. The area under the receiver operating characteristic curve (AUC) values were calculated by comparing with the RT-PCR results.

Among the PCR-positive patients, 150 with any COVID-19–

Received: April 3, 2022
Revision received: June 3, 2022
Accepted: August 11, 2022

Corresponding author: Yongjung Park, M.D., Ph.D.
Department of Laboratory Medicine, Gangnam Severance Hospital, Yonsei University College of Medicine, 211 Eonju-ro, Gangnam-gu, Seoul 06273, Korea
Tel: +82-2-2019-3533, Fax: +82-2-2057-8926, E-mail: YPARK119@yuhs.ac

Table 1. Results of COVID-19 rapid antigen detection tests according to grouping by real-time PCR Ct values

PCR results/PCR *ORF1ab* gene Ct value	Median Ct (1st–3rd quartile)	STANDARD Q Ag (ICA)			STANDARD F Ag (FIA)			$P^{\dagger}$
		Negative, N	Positive, N	% Positive* (95% CI)	Negative, N	Positive, N	% Positive* (95% CI)	
Positive (N=219)	24.9 (19.4–30.6)	76	143	65.3 (58.8–71.3)	72	147	67.1 (60.7–73.0)	0.394
Symptomatic (N=150)‡	22.7 (18.4–28.5)	35	115	76.7 (69.3–82.7)	38	112	74.7 (67.2–81.0)	0.317
Asymptomatic (N=69)	27.5 (22.5–34.1)	41	28	40.6 (29.8–52.4)	34	35	50.7 (39.2–62.2)	0.052
<20.0 (N=61)	17.8 (16.9–18.5)	0	61	100.0 (94.1–100.0)	0	61	100.0 (94.1–100.0)	-
20.0 to <25.0 (N=49)	22.0 (21.0–23.3)	1	48	98.0 (89.3–99.6)	2	47	95.9 (86.3–98.9)	0.564
25.0 to <30.0 (N=47)	26.7 (25.6–27.5)	14	33	70.2 (56.0–81.3)	14	33	70.2 (56.0–81.3)	1.000
30.0 to 38.0 (N=62)	34.1 (32.5–35.2)	61	1	1.6 (0.3–8.6)	56	6	9.7 (4.5–19.5)	0.059
Negative (N=335)	-	332	3	0.9 (0.3–2.6)	299	36	10.7 (7.9–14.5)	<0.001
Symptomatic (N=167)‡	-	166	1	0.6 (0.1–3.3)	147	20	12.0 (7.9–17.8)	<0.001
Asymptomatic (N=168)	-	166	2	1.2 (0.3–4.2)	152	16	9.5 (5.9–14.9)	<0.001
Total (N=554)		408	146	26.4	371	183	33.0	<0.001

*Implies sensitivity of the assay in the PCR-positive groups and '1-specificity' (false-positive rate) in the PCR-negative groups; †McNemar-Mosteller exact test; ‡Includes patients with fever, sore throat, myalgia, any respiratory symptoms, loss of taste or smell, fatigue, headache, and/or gastrointestinal symptoms.
Abbreviations: Ag, antigen; Ct, cycle threshold; ICA, immunochromatographic assay; FIA, fluorescence immunoassay; CI, confidence interval.

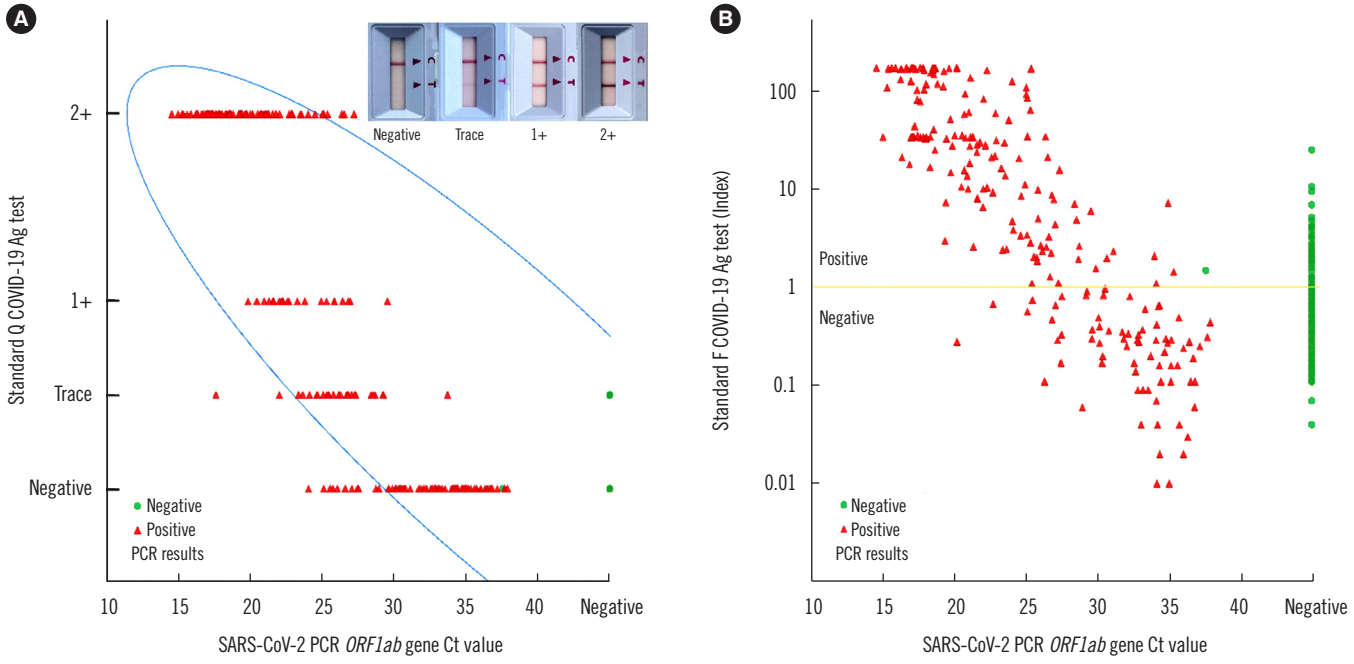

Fig. 1. Correlation between the results of rapid antigen tests for the detection of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) antigen and the cycle threshold (Ct) values of real-time PCR (RT-PCR). Correlation coefficients were calculated by Spearman's rank test. Red triangles represent samples with positive RT-PCR results; and green circles indicate those with negative RT-PCR results. Although the manufacturer does not recommend semi-quantitative reading of the immunochromatographic assay, the results were classified as follows for study purposes only: trace, if paler than the control line; 1+, if similar to the control; 2+, if stronger than the control. (A) Correlation between the results of the immunochromatographic assay and RT-PCR Ct values (Spearman ρ=–0.848, 95% CI –0.870 to –0.822; P<0.001). (B) Correlation between the results of the fluorescence immunoassay and RT-PCR (Spearman ρ=–0.583, 95% CI –0.637 to –0.523; P< 0.001). The yellow line indicates the default cut-off index (1.0) of the fluorescence immunoassay.

related symptoms showed lower PCR cycle threshold (Ct) values compared with those of patients without such symptoms (N=69) (P<0.001). The ICA and FIA showed 65.3%, 67.1% sensitivities and 99.1%, 89.3% specificities, respectively. Both RADTs showed high sensitivity in groups with Ct<25.0 (Table 1). The AUC value of the ICA was 0.824 (95% CI 0.792–0.856), which was higher than that of the FIA at 0.759 (95% CI 0.710–0.809) (P<0.001). Both RADTs revealed better sensitivities in symptomatic patients (N=317), with 76.7% sensitivity and 99.4% specificity for the ICA, and 74.7% sensitivity and 88.0% specificity for the FIA. In asymptomatic patients (N=237), the ICA and FIA showed 40.6%, 50.7% sensitivities and 98.8%, 90.5% specificities, respectively. A significant negative correlation between RT-PCR Ct values and ICA results or COIs from the FIA was noted (Fig. 1). The results of the two RADTs also correlated with each other (Spearman ρ=0.713, 95% CI 0.668–0.753; P<0.001). The weighted kappa coefficient (K_W) between the qualitative results of the ICA and FIA was 0.755 (95% CI 0.696–0.814), demonstrating good agreement.

The two RADTs showed acceptable performance in detecting SARS-CoV-2, with high sensitivity in samples with Ct<25.0 in RT-PCR. Since patients with COVID-19–related symptoms tend to have Ct<25.0, both RADTs can be useful for symptomatic patients, who presumably have high viral loads and are likely to be contagious [6-9]. Compared with the FIA, the ICA, which tends to be simpler and does not require additional equipment, showed slightly lower sensitivity but significantly higher specificity and AUC values. The FIA showed better sensitivity than the ICA, which is an important feature of a screening assay. As this assay provides automated detection of fluorescence signals, less inter-tester variability is expected [8]. We did not consider the potential effects of variants of SARS-CoV-2, including Omicron, on the diagnostic abilities of the RADTs; this suggests the need for further studies on the effects of variants [10]. We evaluated both RADTs using NP swab samples; because many RADTs are intended for self-testing with nasal swabs, our results cannot be applied to self-test kits' performance.

In conclusion, these two RADTs could be useful rapid screening tools, especially when RT-PCR is not readily available. However, additional PCR tests are required because of the limited diagnostic performance of RADTs in asymptomatic COVID-19 patients with low viral loads.

ACKNOWLEDGEMENTS

None.

AUTHOR CONTRIBUTIONS

Park Y designed the study. Yu K, Park Y, Song J, and Kim D collected and analyzed the data. Yu K wrote the manuscript. Park Y and Jeong SH reviewed the manuscript. All authors reviewed and approved the final version of the manuscript.

CONFLICTS OF INTEREST

None declared.

RESEARCH FUNDING

None declared.

ORCID

Kyunghee Yu https://orcid.org/0000-0002-6493-0683
Junhyup Song https://orcid.org/0000-0001-6359-1942
Dokyun Kim https://orcid.org/0000-0002-0348-5440
Yongjung Park https://orcid.org/0000-0001-5668-4120
Seok Hoon Jeong https://orcid.org/0000-0001-9290-897X

REFERENCES

1. Dinnes J, Deeks JJ, Adriano A, Berhane S, Davenport C, Dittrich S, et al. Rapid, point-of-care antigen and molecular-based tests for diagnosis of SARS-CoV-2 infection. Cochrane Database Syst Rev 2020;8:CD013705.
2. World Health Organization. Diagnostic testing for SARS-CoV-2: interim guidance. https://apps.who.int/iris/handle/10665/334254 (Update on September, 2020).
3. Hong KH, Kim GJ, Roh KH, Sung H, Lee J, Kim SY, et al. Update of guidelines for laboratory diagnosis of COVID-19 in Korea. Ann Lab Med 2022;42:391-7.
4. Kim HW, Park M, Lee JH. Clinical evaluation of the Rapid STANDARD Q COVID-19 Ag test for the screening of severe acute respiratory syndrome coronavirus 2. Ann Lab Med 2022;42:100-4.
5. World Health Organization. Antigen-detection in the diagnosis of SARS-CoV-2 infection using rapid immunoassays. https://www.who.int/publications/i/item/antigen-detection-in-the-diagnosis-of-sars-cov-2infection-using-rapid-immunoassays (Update on October, 2021).
6. Jaaskelainen AE, Ahava MJ, Jokela P, Szirovicza L, Pohjala S, Vapalahti O, et al. Evaluation of three rapid lateral flow antigen detection tests for the diagnosis of SARS-CoV-2 infection. J Clin Virol 2021;137:104785.
7. Porte L, Legarraga P, Iruretagoyena M, Vollrath V, Pizarro G, Munita J, et al. Evaluation of two fluorescence immunoassays for the rapid detection of SARS-CoV-2 antigen-new tool to detect infective COVID-19 patients. PeerJ 2021;9:e10801.
8. Corman VM, Haage VC, Bleicker T, Schmidt ML, Mühlemann B, Zuchowski M, et al. Comparison of seven commercial SARS-CoV-2 rapid point-of-care antigen tests: a single-centre laboratory evaluation study. The Lancet Microbe 2021;2:e311-9.

9. Pena-Rodriguez M, Viera-Segura O, Garcia-Chagollan M, Zepeda-Nuno JS, Munoz-Valle JF, Mora-Mora J, et al. Performance evaluation of a lateral flow assay for nasopharyngeal antigen detection for SARS-CoV-2 diagnosis. J Clin Lab Anal 2021;35:e23745.
10. Ferre VM, Peiffer-Smadja N, Visseaux B, Descamps D, Ghosn J, Charpentier C. Omicron SARS-CoV-2 variant: What we know and what we don't. Anaesth Crit Care Pain Med 2022;41:100998.