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Analysis of diagnostic performance and factors causing nonspecific reactions in SARS-CoV-2 rapid antigen detection tests

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ABSTRACT

Introduction: Early diagnosis and appropriate infection control are important to prevent the transmission of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2). In this study, we aimed to assess the diagnostic performance of SARS-CoV-2 rapid antigen detection (RAD) tests and the factors that cause nonspecific reactions.

Methods: Nasopharyngeal swab specimens (n = 100), sputum specimens (n = 10), and lithium-heparin plasma samples (n = 100) were collected. We evaluated Espline®SARS-CoV-2 (Espline) and SARS-CoV-2 Rapid Antigen Test that also known as STANDARD Q® (STANDARD Q), with reverse transcription-polymerase chain reaction (RT-PCR) and Lumipulse® Presto SARS-CoV-2 Ag as reference tests. In addition, we investigated the effects of inadequate pretreatment methods and five potential causes of nonspecific reactions.

Results: The sensitivities of Espline and STANDARD Q were 60% and 57%, respectively, and their specificity was 100%. It was confirmed that the judgment line for the positive insufficiently mixed specimens was faint. A false-positive result was observed with STANDARD Q when sputum was used as a specimen to investigate judgment the effect of viscosity.

Conclusions: Espline and STANDARD Q show good sensitivity for specimens with Ct values less than 25, but specimens collected within 9 days of symptom onset may still give false negatives. The test should be performed carefully, and the results should be judged comprehensively, taking into account clinical symptoms and patient background.

1Introduction

Early diagnosis and appropriate infection control are important to prevent the transmission of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) [1]. Real-time reverse transcription-polymerase chain reaction (RT-PCR), quantitative antigen test, and qualitative antigen test are currently used to diagnose SARS-CoV-2 infection. RT-PCR is a standard test with extremely high sensitivity. However, the processes of specimen collection, gene extraction, amplification, and detection are complicated, and most require expert techniques. In addition, RT-PCR commonly requires an expensive analyzer. The Lumipulse presto®SARS-CoV-2 Ag (Lumipulse; Fujirebio Inc., Tokyo, Japan), as a quantitative antigen test (qAgT), can measure SARS-CoV-2 antigen with high sensitivity within an assay time of 30 min, but requires a larger-sized analyzer [2–6]. As with other examination methods, many rapid antigen detection (RAD) tests are being developed. Most RAD tests do not require exclusive analyzers and can be easily performed at the patient’s bedside because the equipment is small and lightweight. However, it has been reported that RAD tests are inferior to RT-PCR in terms of sensitivity and due to a higher incidence of false positives [2,7].
2. Materials and methods

2.1. Sample collection

We collected 70 nasopharyngeal specimens from patients diagnosed with COVID-19 and 30 negative nasopharyngeal specimens from patients with suspected COVID-19. All 100 samples were collected using a nylon-flocked nasopharyngeal swab and a tube containing a universal transport medium (UTM; Copan Diagnostics, Murrieta, CA, USA) and stored at -80°C until testing. RT-PCR or qAgT was performed at the time of collection to confirm whether the samples were positive or negative. Nasopharyngeal swabs were collected from November 1, 2020, to March 29, 2021. In addition, 10 sputum specimens and 100 lithium-heparin (He-Li) plasma specimens were collected to investigate nonspecific reactions at Sapporo Medical University Hospital.

Five volunteers were recruited from our hospital staff. Among those willing to participate in the study, the negative group consisted of those with body temperature below 37°C, with no obvious signs of any respiratory infection, and not prescribed any medication. A total of 6 mL of blood was collected in serum collection tubes (5 mL) and sterile spits (1 mL). In addition, a total of seven nasopharyngeal swabs were collected from each of the five volunteers.

Informed consent was obtained in the form of opt out on the website. The details of this study were published on the website to provide an opportunity for patients to refuse. Those who rejected were excluded. This study was approved by the Institutional Review Board of Sapporo Medical University Hospital (reference number 332-64) and ethical committee of Sapporo Medical University Hospital (reference number 33-1-45).

2.2. RAD tests

RAD tests were performed using the Espline and STANDARD Q. The manufacturer’s swab and UTM were used according to the manufacturer’s protocol. The test was interpreted as positive when both the reference and the judgment lines could be visually confirmed and negative when only the reference line could be confirmed; otherwise, no judgment could be made. Positive and negative results were determined visually by each of the three laboratory technicians to avoid any discrepancy.

2.3. Quantitative antigen test

The specimens were analyzed for SARS-CoV-2 Ag using Lumipulse® Presto SARS-CoV-2 Ag on a fully automated Lumipulse® L2400 analyzer (Fujirebio Inc., Tokyo, Japan). All nasopharyngeal swabs were judged as either positive, negative, or judgment pending based on the antigen levels (negative: less than 1.34 pg/mL, judgment pending: from 1.34 to less than 10.00 pg/mL, and positive: over 10.00 pg/mL) according to the manufacturer’s protocol [2,3]. Nasopharyngeal specimens with pending judgment were excluded because RT-PCR re-tests were necessary.

2.4. RT-PCR

RT-PCR was performed on a LightCycler480 System (Roche, Basel, Switzerland) using the Ampdirect™ 2019 Novel Coronavirus Detection Kit (Shimadzu Corporation, Kyoto, Japan) [9]. All assays were performed according to the manufacturer’s protocol, and samples were judged as positive or negative based on the cycle threshold (Ct) value. When the measured Ct value of the sample was 40 or less, it was judged as positive. The RNA copies by RT-PCR were normalized with the reference materials.

2.5. Statistical analysis

Sensitivity was defined as the proportion of samples identified as SARS-CoV-2 positive by RAD tests initially categorized as positive by RT-PCR or qAgT. Specificity was defined as the proportion of samples identified as negative by the RAD tests initially categorized as negative by RT-PCR or qAgT. All calculations were performed using EZR (Saitama Medical Center, Jichi Medical University, Saitama, Japan), a graphical user interface for R (The R Foundation for Statistical Computing, Vienna, Austria).

2.5.1. Comparison with RT-PCR and qAgT

We evaluated 70 positive and 30 negative specimens. Sensitivity and specificity were calculated for the entire group and for each of the three groups (<25, 25–30, >30) divided according to Ct values when compared to RT-PCR.

Sensitivity and specificity were calculated for the entire group and for each of the four groups (≥1000, 200–1000, 100–200, <100) divided according to antigen values when compared to qAgT.

2.5.2. Comparison based on the date of onset

The 48 specimens for which the onset date could be determined, were classified into two groups: those collected within 9 days of onset (32 specimens) and those collected after 10 days of onset (16 specimens), and the sensitivity was calculated.

2.5.3. Analysis of the effect of different pretreatment methods

The specimen is diluted and becomes less viscous when it is mixed with the treatment solution of RAD tests. In addition, the surfactant in the treatment solution destroys the structure of SARS-CoV-2, exposing the N protein that binds to the antibodies. Since inadequate pretreatment may induce false positives or false negatives, we investigated the effect of different pretreatment methods. Nasopharyngeal swabs (manufacturer’s specific swabs and UTM) were soaked in the treatment solution and then pretreated under the following conditions.

8. Espline® SARS-CoV-2 (Espline, Fujirebio Inc., Tokyo, Japan) utilizes samples collected in a viral transport medium. Therefore, RT-PCR and quantitative antigen testing can be performed in the same sample when a re-test is necessary. The SARS-CoV-2 Rapid Antigen Test® that also known as STANDARD Q®, SD Biosensor Inc. Mannheim, Germany (STANDARD Q) can also be performed on samples collected in a viral transport medium, with a short measurement time of 15 min. In this study, we evaluated the diagnostic performance and analyzed the factors that cause non-specific reactions using Espline and STANDARD Q.
2.6 Manufacture’s specific swabs

2.6.1 Espline

1. Standard method: Rotate the swab 10 times while holding the cotton ball part between fingers from around the tube, and pull it out.
2. Omission method: Rotate the swab 5 times and pull it out.
3. Full omission method: Pull it out immediately.

2.7 STANDARD Q

1. Standard method: Rotate the swab from side to side at least five times, move it up and down to agitate and pull it out.
2. Omission method: Rotate the swab 3 times and pull it out.
3. Full omission method: Pull it out immediately.

2.8 UTM

2.8.1 Espline

1. Standard method: Dip the swab soaked in UTM into the treatment solution and rub the tube well to mix.
2. Omission method: Do not rub, but rotate the swab to mix.
3. Full omission method: Do not mix.

2.9 STANDARD Q

1. Standard method: Transfer UTM to the treatment solution and mix.
2. Omission method: Do not rub, but rotate the swab to mix.
3. Full omission method: Do not mix.

2.10 Analysis of nonspecific reactions causing false-positive results

Blood may adhere to the swab when nasopharyngeal swabs are collected. Fibrin and abnormal protein, which have been reported as causes of nonspecific reactions, with Chemiluminescent Enzyme Immunoassay and Chemiluminescent immunoassay, the same principle immobilized cell. Since reports on nonspecific reactions in SARS-CoV-2 RAD tests have shown viscosity, and we examined mucin, a mucus substance, the effect of mucin was studied. RAD tests were performed after pretreatment by soaking the manufacturer’s specific swab into fibrin-deposited serum, and the test was performed after this pretreatment.

2.11 Addition of blood

Blood samples collected from the volunteers were allowed to stand at room temperature for at least 1 h to allow coagulation. Two pretreatment methods were used. One was followed the manufacturer’s protocol, while the other involved dipping the sample in the treatment solution and immediately removing it.

2.12 Addition of fibrin

Blood samples were centrifuged immediately after sampling, and fibrin was precipitated. The manufacturer’s specific swab from each RAD test was first dipped into fibrin-deposited serum, and the test was performed after this pretreatment.

2.13 Addition of abnormal proteins

The test was performed after pretreatment by soaking the manufacturer’s specific swab of each RAD test in HeLa-Li added plasma from 75 patients with abnormal immunoglobulins (IgA in 30 patients, IgG in 29 patients, and IgM in 16 patients) and rheumatoid factor in 25 patients. The median and interquartile range were 565 (507–594) mg/dL, 2884 (2576–3316) mg/dL, 572 (474–1003) mg/dL, and 306 (175–383) IU/mL, respectively.

Table 1

| Sensitivity (% [95% CI]) | N | Espline STANDARD Q |
|-------------------------|---|-------------------|
| Ct value of RT-PCR      |   |                   |
| <25                     | 27 | 96.3 (81.0–99.9)  | 96.3 (81.0–99.9) |
| 25–30                   | 23 | 65.2 (42.7–83.6)  | 69.0 (38.5–80.3) |
| >30                     | 20 | 5.0 (0.10–24.9)   | 0.0 (0.0–23.8)   |
| All Ct values           | 70 | 60.0 (47.6–71.5)  | 57.1 (44.7–68.9) |

Specificity (% [95% CI])

- 25: 100.0 (83.3–100.0)
- 20: 100.0 (83.3–100.0)

2. Example of test results for each sample

The overall sensitivity of Espline and STANDARD Q were 60.0% (95% CI: 48–72%) and 57.1% (95% CI: 45–69%), respectively, and the specificity was 100.0% (95% CI: 83–100%). The sensitivity of each RAD test was higher in the group with lower Ct values (Table 1).

3. Conclusion

The results of the RAD test, RT-PCR, and qAgT for each sample are presented in Supplementary Table 1.

3.1 Sensitivity and specificity

The overall sensitivity of Espline and STANDARD Q were 64.6% (95% CI: 52–76%) and 61.5% (95% CI: 49–73%), respectively, and the specificity was 100.0% (95% CI: 83–100%). The sensitivity of each RAD test was higher in the group with higher antigen levels (Table 2).

3.2 Comparison with RT-PCR

The overall sensitivity of Espline and STANDARD Q were 60.0% (95% CI: 48–72%) and 57.1% (95% CI: 45–69%), respectively, and the specificity was 100.0% (95% CI: 83–100%). The sensitivity of each RAD test was higher in the group with lower Ct values (Table 1).

3.3 Comparison with the quantitative antigen test

The overall sensitivity of Espline and STANDARD Q were 64.6% (95% CI: 52–76%) and 61.5% (95% CI: 49–73%), respectively, and the specificity was 100.0% (95% CI: 83–100%). The sensitivity of each RAD test was higher in the group with higher antigen levels (Table 2).

3.4 Comparison based on the date of onset

The sensitivity of Espline and STANDARD Q was 65.6% (95% CI: 47–81%) and 62.5% (95% CI: 44–79%), respectively, within 9 days from onset.
specimens had a Ct value of less than 29.5, it was inferred that many with Ct values of 25 or less [17]. High concordance rate with RT-PCR in specimens with a high viral load, specimens had a high viral load. RAD tests have been reported to have a vagno_
3.5 Analysis of the effect of different pretreatment methods
3.5.1 SARS-CoV-2 negative specimens
No specimens showed positive results in any of the pretreatment conditions.
3.5.2 SARS-CoV-2 positive specimens
No false negatives were observed in any of the pretreatment conditions for any of the UTM samples. However, in one sample analyzed by Espline, the judgment line appeared faint when the sample was immersed in the treatment solution and immediately removed. In another sample analyzed by STANDARD Q, the judgment line appeared faint under the pretreatment condition of no pipetting.
3.5.3 Analysis of false-positive reactions
In addition to blood, fibrin, abnormal proteins, and mucin, no effects were observed in either RAD test for the two pretreatment methods.
Although sputum sample 5 was negative for Espline, it was positive in STANDARD Q. As RT-PCR was negative, the result of STANDARD Q turned out to be a false positive (Table 3, Fig. 1).
4 Discussion
Using RT-PCR as the reference standard, the sensitivity of Espline and STANDARD Q were found to be 60.0% and 57.1%, respectively. The sensitivity of Espline was reported to be 39.7% by Aoki [15] and 70% by Salvagnon [16]. The median Ct value of specimens used in the report by Aoki was 28.0 for Espline positive specimens, whereas the median Ct value in this study was lower at 23.7. In other words, the sensitivity of Espline in this study was higher than that reported by Aoki because the number of specimens with a higher viral load was greater. As for Salvagnon’s report, the median Ct value was not known, but since the specimens had a Ct value of less than 29.5, it was inferred that many specimens had a high viral load. RAD tests have been reported to have a high concordance rate with RT-PCR in specimens with a high viral load, with Ct values of 25 or less [17-25]. In this study, the sensitivity of both RAD tests was 96.3%, which was good for samples with Ct values less than 25. In contrast, in a previous study, 41.1% of the samples had a Ct value of 29 or less (65.7% in this study), which indicates a lower viral load than that in this study, but the sensitivity was as high as 70%. The reason for this was thought to be that it was intended for dedicated swab specimens. In the present study, the scraped swab was inserted into the UTM, and only a portion of it was used as a specimen, which was considered less sensitive than the manufacturer’s swab. When comparing sensitivity, it is necessary to consider the viral load and the specimen type. On categorizing samples into those collected within 9 days of symptom onset (32/48) and those collected after 10 days of symptom onset (16/48), the sensitivity of Espline were 65.6% and 43.8%, respectively, and that of STANDARD Q were 62.5% and 43.8%, respectively. In a previous report, the incidence of onset within 9 days was 73.3%, and that after 10 days was 29.2%. This difference was larger than that observed in this study [15]. The reason for this is not clear, but it may be because the previous report had 27 and 102 specimens whereas we had 32 and 16 specimens collected within 9 days and after 10 days of onset, respectively. It is thought that the small number of specimens after 10 days may have affected the results. These results suggest that there is a risk of missing SARS-CoV-2 positive individuals, as approximately 30% of the specimens tested negative, even within 9 days of onset. If SARS-CoV-2 infection is suspected based on clinical symptoms or patient background, confirmation by RT-PCR or quantitative antigen testing is necessary, even if the RAD test result is negative.
Although RAD tests are easy to perform, false negatives and false positives are a concern [7,8,26-32]. It has been pointed out that some of these could be caused by examiners who are not familiar with handling RAD tests [6]. We assessed the effects of abnormal proteins, blood, and fibrin as possible causes of false positives, but none of them led to false-positive results. On the other hand, while using sputum as a specimen to investigate the effect of viscosity, a false-positive result was observed with STANDARD Q. We suspected bacterial cross-reactivity as the reason for this false-positive result; however, culture tests showed only indigenous bacteria. The false-positive rate for the rapid antigen test was reported to be 8% (13/172) [7]. They collected nasal swabs using manufacture swabs. In this study, specimens were collected at multiple facilities, and the staff who collected the specimens had only minimal training, which may have resulted in a high false-positive rate due to errors in testing procedures, including pretreatment. Another study reported a false-positive rate of 1% (5/594), and false-positives tended to occur in highly viscous specimens, although the cause was unclear [8]. Samples, mainly nasopharyngeal and throat swabs, were collected and mixed viral transport media. The sputum used in this study was highly viscous, which was consistent with this report. Other reports...
showed that the false-negative rate of STANDARD Q was 0.1% [26], 0.3% [27], and 1% [28]. These samples were all nasopharyngeal swabs collected using manufacturer’s swabs. Therefore, the false-negative rate of STANDARD Q in clinical practice was considered to be approximately 1% at the highest. False positives have been reported to be caused by strong viscosity and cross-reactivity with parainfluenzavirus; however, detailed mechanisms of false-positive reactions have not been identified [29]. False positives have also been reported with Espline, which did not show false positives in this study, suggesting the possibility of cross-reactivity with rhinovirus and HIV as the cause [30–32]. The electrostatic effect of mucin was also speculated to be the cause of the false-positive results. The sputum is composed of approximately 90% charged molecules. If the antibodies solidified on the membrane of STANDARD Q in clinical practice was considered to be approximately 100 g/mL of mucin, which is lower than the mucin concentration of the saliva, the possibility that the results of this study were affected by cross-reactivity cannot be denied. Therefore, we used commercially available mucins, but found no effect and were unable to elucidate the cause of the false positives.

In conclusion, the Espline and STANDARD Q demonstrate great sensitivity for specimens with Ct values less than 25, but specimens collected within 9 days of onset may still give false-negative results. The test should be performed carefully, and the results should be judged comprehensively, taking into account clinical symptoms and patient background.

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Declaration of competing interest

The authors declare the following conflicts of interest that may be considered potential competing interests: Satoshi Takahashi received IgM interference in the Abbott iVanclo immunoassay: a case report. Clin Chim Acta 2015;447:52–3. https://doi.org/10.1016/j.cca.2015.05.006.

[12] Hiramatsu K, Tanaka Y, Takeda K, Iida T, Takasaka Y, Mizokami M. False positive serum des-gamma-carboxy prothrombin after resection of hepatocellular carcinoma. Rinsho Byori 2007;55:330–7.

[13] Yamada M, Abe T, Sato N, Sato T, Morikane K. Basic evaluation of HISCLC-2000 assay of hepatitis B virus surface antigen. Japanese Journal of Medical Technology 2016;59:98–102.

[14] Heishima Y. Saliva and health of elderly population -Characteristics of Salovary compress and related factors-. Shikoku Dent Res 2006;19:77–91.

[15] Aoki K, Nagasawa T, Ishii Y, Yagi S, Kashikawa K, Miyazaki T, et al. Evaluation of clinical utility of novel coronavirus antigen detection reagent, Espline® SARS-CoV-2. J Infect Chemother 2021;27:319–22. https://doi.org/10.1007/j. jic.2020.11.015.

[16] Salvagno GL, Nocini R, Gianflliippi G, Fisario G, Pighi L, De Nittis S, et al. Limit of detection in different matrices of 19 commercially available rapid antigen tests for the detection of SARS-CoV-2. Sci Rep 2021;11:18313. https://doi. org/10.1038/s41598-021-97489-9.

[17] Cubas-Atienzar AI, Kontogianni K, Edwards T, Wooding D, Buist K, Thompson CR, et al. Diagnostic accuracy of two commercial SARS-CoV-2 antigen tests for the detection of SARS-CoV-2. Infection 2021;49:789–90. https://doi.org/10.1007/s15010-02001757-0.

[18] Weiss G, Bellmann-Weiler R. Rapid antigen testing and non-infectious shedding of SARS-Cov2. Infection 2021;49:789–90. https://doi.org/10.1515/dx-2021-0107.

[19] Mina MJ, Parker R, Larremore DB. Rethinking Covid-19 test sensitivity - a strategy for containment. N Engl J Med 2020;383:e120. https://doi.org/10.1056/ NEJMtp2026361.

[20] An agency of the European Union. Options for the use of rapid antigen tests for COVID-19 in the EU/EEA - first update. Technical report. [accessed 4 August, 2022].

[21] Pekosz A, Parvu V, Li M, Andrews JC, Manneh VG, Kodsi S, et al. Antigen-based testing but not real-time polymerase chain reaction correlates with severe acute respiratory syndrome coronavirus 2 viral culture. Clin Infect Dis 2021;73:e2861–6. https://doi.org/10.1093/cid/ciaa1706.

[22] Singanayagam A, Patel M, Charlett A, Lopez Bernal J, Saliba V, Ellis J, et al. Duration of infectiousness and correlation with RT-PCR cycle threshold values in cases of COVID-19, England, January to May 2020. Euro Surveill 2020;25:2001483. https://doi.org/10.2807/1560-7917.ES.2020.25.32.3279447.

[23] Abduljalil JM. Laboratory evaluation of SARS-CoV-2: available approaches and limitations. New Microbes New Infect 2020;36:100711. https://doi.org/10.1016/j. nmni.2020.100713.

[24] Kerenkov M, Poopalasingam N, Madler M, Vandylla K, Eggeling R, Wirtz M, et al. Evaluation of a rapid antigen test to detect SARS-CoV-2 infection and identify potentially infectious individuals. J Clin Microbiol 2021;59:e008921. https://doi.org/10.1128/JCM.00892-21.

[25] Berger A, Nooga MTN, Perez-Rodriguez FJ, Aal YA, Sattornet-Roche P, Gayet-Ageron A, et al. Diagnostic accuracy of two commercial SARS-CoV-2 antigen tests for detecting rapid tests at the point of care in community-based testing centers. PLoS One 2021;16:e0248921. https://doi.org/10.1371/journal.pone.0248921.

[26] Homza M, Zelena H, Janosek J, Tomaskova H, Jezo E, Kloudova A, et al. Five antigen tests for SARS-CoV-2 and their viroviability matters. Viruses 2021;13:684. https://doi.org/10.3390/v13040684.
[29] 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. Lancet Microbe 2021;2:e311–9. https://doi.org/10.1016/S2666-5247(21)00056-2.

[30] Otake S, Miyamoto S, Mori A, Iwamoto T, Kasui M. False-positive results in SARS-CoV-2 antigen test with rhinovirus-A infection. Pediatr Int 2021;63:1135–7. https://doi.org/10.1111/ped.14582.

[31] Itoh K, Kawamitsu T, Osaka Y, Sato K, Suzuki Y, Kiriba C, et al. False positive results in severe acute respiratory coronavirus 2 (SARS-CoV-2) rapid antigen tests for inpatients. J Infect Chemother 2021;27:1089–91. https://doi.org/10.1016/j.jiac.2021.03.011.

[32] Yamanoha K, Kinjo T, Akamine M, Setoguchi M, Tateyama M, Fujita J. False-positive for SARS-CoV-2 antigen test in a man with acute HIV infection. J Infect Chemother 2021;27:1112–4. https://doi.org/10.1016/j.jiac.2021.04.011.

[33] Suzuki C, Yanai K, Nomachi M, Imayasu M, Araki K, Sasaki A, et al. Effects of lysozyme and mucin on amoebicidal activity of lactoferrin against Acanthamoeba sp. AAO14. J Eye 2015;32:551–5.