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Note

Does the SARS-CoV-2 rapid antigen test result correlate with the viral culture result?

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ABSTRACT

Rapid antigen tests (RATs) for severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) have advantages over viral culture in terms of cost and rapidity of testing, but they have low sensitivity. In addition, RATs tend to be negative from approximately 11 days after symptom onset. To determine whether the antigen-negative state indicates a lack of infectiousness, we assessed the association between viral culture and RAT results. Viral culture, reverse transcription-quantitative polymerase chain reaction (RT-qPCR), and rapid antigen testing were performed on stored nasopharyngeal samples with threshold cycle values < 30, based on previous RT-qPCR testing. SARS-CoV-2 was isolated by viral culture from nine samples (45%) and one sample (17%) with positive and negative RAT results, respectively. The RAT and viral culture results were both associated with the viral load level and their cutoffs were similar, but the associations were not statistically significant. RAT might be a useful indicator of infectiousness, which can be helpful to control infection. However, further studies with larger sample size are warranted to confirm this observation.

Immunochromatographic rapid antigen tests (RATs) for severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) are simple to perform and have a relatively low cost but they have a low sensitivity [1–3]. A potential advantage of the low sensitivity of RATs is that they do not remain positive during the long period of low viral excretion after infectiousness has passed [4]. Espline® SARS-CoV-2 (Fujirebio, Tokyo, Japan) was approved in Japan as a RAT for diagnosing COVID-19 on May 13, 2020, the first approval of this kit worldwide. Previous studies have shown that RATs have a low sensitivity at low viral load levels (67%), but a relatively high sensitivity (93%) at high viral load levels (>400 copies/test) [5]. In a previous study, we determined that the concordance with reverse transcription-quantitative polymerase chain reaction (RT-qPCR) in the early stage of COVID-19 (within 9 days after symptom onset) was 72% for positive and 100% for negative RAT results. Moreover, in samples with $>10^3$ copies/test (>85 copies/μL), the positive concordance was 85% [1]. We hypothesized the RAT results might be useful for determining whether an individual is still infectious during the convalescent phase when there is no fever and the virus is presumed to be no longer infectious. This study compared SARS-CoV-2 RAT results to viral culture results to test this hypothesis.

Abbreviations: AUC, area under the curve; RAT, rapid antigen test; RT-qPCR, reverse transcription-quantitative polymerase chain reaction; SARS-CoV-2, severe acute respiratory syndrome coronavirus 2.

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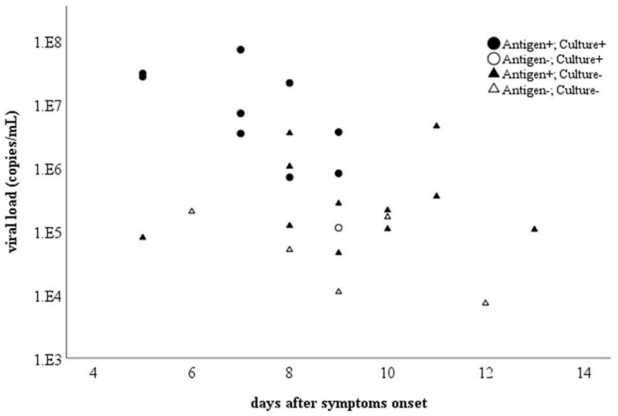

Fig. 1. Results of the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) rapid antigen test and viral culture according to the viral load level and time since disease onset. All tests were conducted on nasopharyngeal samples. Viral load was determined using reverse transcription-quantitative polymerase chain reaction. Espline® SARS-CoV-2 was used as the rapid antigen test.

Stored nasal swab samples from COVID-19 patients admitted to our hospital between March 6 and June 14, 2020 were used in the study. The virus transport media were refrigerated within 24 h after sample collection and were further frozen at −80 °C latest by 72 h after the refrigeration. We used 26 samples in virus transport media (universal transport media, Copan) that had previously been shown to have threshold cycle values less than 30 by RT-qPCR. We performed RT-qPCR, RAT (Espline® SARS-CoV-2), and viral cultures with VeroE6 and VeroE6/TMPRSS2 cells [6]. VeroE6 and VeroE6/TMPRSS2 cells (JCRB1819) were cultured in 1 mL Dulbecco's modified eagle medium "Nissui" (D-MEM, Nissui Pharmaceutical Co., Ltd., Tokyo, Japan) containing 10% heat-inactivated fetal bovine serum (FBS) and 1% penicillin–streptomycin (MP Biomedicals, France). These cells with D-MEM (1 mL) were added to a 24-well plate and incubated for 72 h at 37 °C with 5% CO_2. Once the cells adhered to the base and monolayer culture was obtained in each well, the medium was replaced with 1 mL Eagle's minimum essential medium (Fujifilm, Japan) containing 2% FBS. Further, the wells were inoculated with 100 μL of each clinical specimen in a BSL3 laboratory. After 1 week, the cell denaturation effect (CPE) was observed under a microscope. Further, 50 μL of the culture medium was inoculated into new cells. This process was performed for 2 weeks. After that, RNA was extracted from the supernatant of the cells that showed CPE. RNA was subjected to the SARS-CoV-2 direct detection RT-qPCR kit (Takara Bio Inc., Shiga, Japan) to confirm viral replication. RAT was performed using highly concentrated reagent for virus transport media. This study was approved by the ethical review board of National Center for Global Health and Medicine (NCGM-G-003472-02) and participants provided written consent.

Overall, 26 samples were collected from 21 patients. Of these, 25 samples were taken when the patients had fever, respiratory symptoms such as cough, chest pain, dysgeusia, or dysosmia. The RATs tended to be positive if the viral load was high, regardless of the time since illness onset, whereas viral cultures tended to be positive in samples collected within 10 days of illness onset (Fig. 1). SARS-CoV-2 was isolated by viral culture from nine of 20 samples (45%) with positive RAT results and one of six samples (17%) with negative RAT results, a non-significant difference ($p = 0.23$). From the 10 viral culture-positive samples, SARS-CoV-2 was isolated in three samples with both VeroE6 and VeroE6/TMPRSS2 cells, in six with VeroE6/TMPRSS2 cells only, and in one with VeroE6 cells only. Receiver-operating characteristic curves revealed that high viral load was associated with RAT positivity (area under the curve [AUC]: 0.88, 95% CI: 0.74–1.0, $p = 0.006$) and viral culture (AUC: 0.89, 95% CI: 0.76–1.0, $p = 0.001$) results. Based on Youden's index, a viral load $>2.1 \times 10^5$ copies/mL predicted RAT positivity with a sensitivity of 75% and a specificity of 100%, and a viral load $>5.4 \times 10^5$ copies/mL predicted culture positivity with a sensitivity of 90% and a specificity of 81% (Fig. 2).

Although we were unable to show significant results due to the small sample size, there was an association between the viral load, RAT, and viral culture results, with an optimal cutoff value of 10^5 copies/mL, suggesting a strong association between the viral culture and RAT results. There have been few previous studies in which antigen testing and viral culture were performed simultaneously [3,7]. Yamayoshi et al. [7] compared the RATs (including Espline® SARS-CoV-2) and viral culture results on 18 nasopharyngeal samples. Of these, seven of eight RAT-positive samples (88%) and three of ten RAT-negative samples (30%) were positive on viral culture [7]. There were fewer samples that were positive based on viral culture in our study than in the study by Yamamoto et al. [1] because a higher proportion of the samples in our study were collected >9 days after symptom onset. Positive viral culture results are known to be associated with the number of days since the

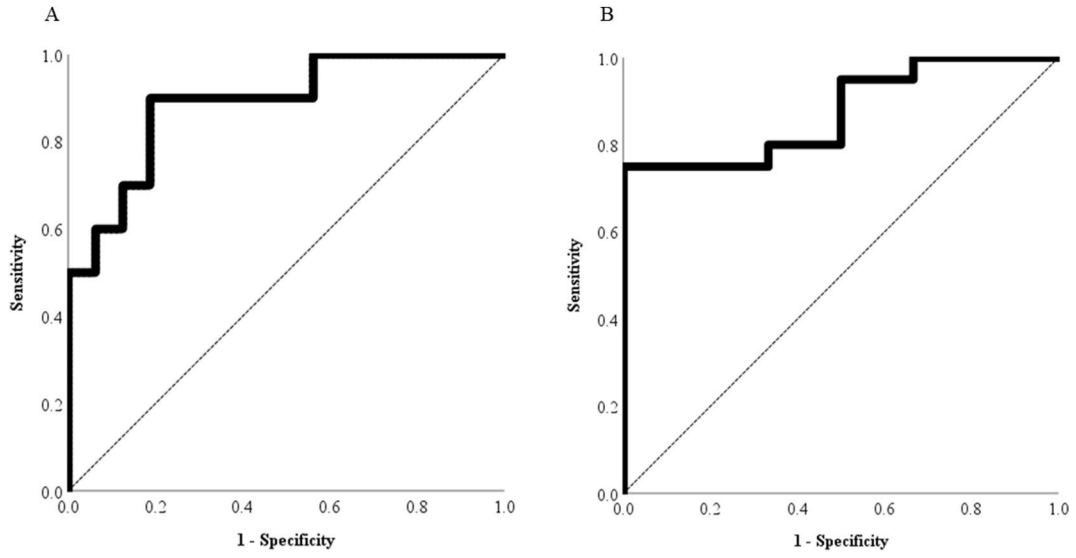

Fig. 2. Receiver-operating characteristic curves of diagnostic tests for severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2). A: Viral culture result according to SARS-CoV-2 viral load; B: rapid antigen test result according to SARS-CoV-2 viral load. Viral load was measured by reverse transcription-quantitative polymerase chain reaction. Espline® SARS-CoV-2 was used as the rapid antigen test.

disease onset [8]. On the other hand, because the antigen test depends on the amount of nucleic acid as it captures nucleoproteins, the results of RAT and viral culture tended to strongly correlate with viral load and duration after the onset of symptoms, respectively, as shown in Fig. 1. The freezing and thawing involved in the aliquoting process may also have contributed to the lower yield on viral culture in our study. Although results of the study by Yamamoto et al. [1] suggest that RATs could be used as a tool to screen for infectiousness, they did not report the relationship between the RAT and the viral culture results. In this study, specimens having Ct below 30 by earlier RT-PCR were used to secure a sufficient amount of virus for virus culture while excluding the possibility of sampling errors. Therefore, the stage of the disease was slightly biased toward the early stage, and one of the limitations was that we could not examine specimens with low viral load. Although our results did not differ significantly due to the small sample size, they suggest that RATs could be used as a tool to screen for infectiousness. If we can show this association, it may be useful for screening test in resource-limited facilities where tests such as nucleic acid amplification tests (NAATs) are not available and for determining viral remnants after infection that were found to be positive by NAATs. Further investigation is required to confirm the association between the RAT and viral culture results.

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Authorship statement

All authors meet the ICMJE authorship criteria as stated here:

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(2) drafting the article or revising it critically for important intellectual content: K. Yamamoto, and M. Nagashima, I. Yoshida, K. Sadamasu, N. Ohmagari.

(3) Final approval of the version to be submitted: All authors.

Declaration of competing interest

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References

[1] Yamamoto K, Suzuki M, Yamada G, Sudo T, Nomoto H, Kinoshita N, et al. Utility of the antigen test for coronavirus disease 2019: factors influencing the prediction of the possibility of disease transmission. Int J Infect Dis 2021;104:65–72. https://doi.org/10.1016/j.ijid.2020.12.079.

[2] Scohy A, Anantharajah A, Bodéus M, Kabamba-Mukadi B, Verroken A, Rodriguez-Villalobos H. Low performance of rapid antigen detection test as frontline testing for COVID-19 diagnosis. J Clin Virol 2020;129:104455. https://doi.org/10.1016/j.jcv.2020.104455.

[3] Mak GC, Cheng PK, Lau SS, Wong KK, Lau CS, Lam ET, et al. Evaluation of rapid antigen test for detection of SARS-CoV-2 virus. J Clin Virol 2020;129:104500. https://doi.org/10.1016/j.jcv.2020.104500.

[4] Mina MJ, Parker R, Larremore DB. Rethinking COVID-19 test sensitivity – a strategy for containment. N Engl J Med 2020 Nov 26;383(22):e120. https://doi.org/10.1056/NEJMp2025631.

[5] Japanese Ministry of Health, Labour and Welfare. Approval of in vitro diagnostics for the novel coronavirus infection. https://www.pmda.go.jp/files/000235116.pdf. [Accessed 14 January 2021].

[6] Matsuyama S, Nao N, Shirato K, Kawase M, Saito S, Takayama I, et al. Enhanced isolation of SARS-CoV-2 by TMPRSS2-expressing cells. Proc Natl Acad Sci U S A 2020;117:7001–3. https://doi.org/10.1073/pnas.2002589117.

[7] Yamayoshi S, Sakai-Tagawa Y, Koga M, Akasaka O, Nakachi I, Koh H, et al. Comparison of rapid antigen tests for COVID-19. Viruses 2020;12:1420. https://doi.org/10.3390/v12121420.

[8] Rhee C, Kanjilal S, Baker M, Klompas M. Duration of SARS-CoV-2 infectivity: when is it safe to discontinue isolation? Clin Infect Dis 2020:ciaa1249. https://doi.org/10.1093/cid/ciaa1249.