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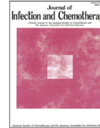

Original Article

Evaluation of clinical utility of novel coronavirus antigen detection reagent, Espline® SARS-CoV-2

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ABSTRACT

Background: To prevent the novel coronavirus disease 2019 (COVID-19) pandemic caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), it is necessary to perform early identification and isolation of people shedding the infectious virus in biological materials with high viral loads several days prior to symptom onset. Rapid antigen tests for infectious diseases are useful to prevent the pandemic spread in clinical settings.
Methods: We evaluated a SARS-CoV-2 antigen test, Espline® SARS-CoV-2 reagent, with reverse transcription polymerase chain reaction (RT-PCR) as reference test, using 129 nasopharyngeal swab specimens collected from COVID-19 hospitalized patients or from patients suspected having COVID-19-like symptoms. Out of these, 63 RT-PCR positive and 66 RT-PCR negative specimens were identified.
Results: Among 63 RT-PCR positive specimens, 25 were positive in the Espline test. Test sensitivity was estimated based on the 532.4 copies/reaction of SARS-CoV-2 RNA obtained through receiver operating characteristic analysis. When the specimens were classified based on time since symptom onset, Espline test sensitivity were 73.3% and 29.2% in specimens collected before day 9 and after day 10, respectively.
Conclusion: Although the overall sensitivity of the Espline® SARS-CoV-2 reagent compared with RT-PCR is less, this antigen test can be useful in identifying people with high risk of virus transmission with high viral loads in order to prevent the pandemic and is useful for diagnosing COVID-19 within 30 min

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1. Introduction

Coronavirus disease (COVID-19) is a respiratory disease caused by the novel coronavirus, severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) [1–3]. It became a global pandemic within a short period since the outbreak began in China in early 2020 [4]. It is reported that more than 17 million people have been infected with this virus and that more than 600,000 people had died till end of July 2020 [5,6]. To prevent viral spread, early detection and isolation of infected people who may be asymptomatic or presymptomatic is necessary [7]. Reverse transcription polymerase chain reaction (RT-PCR) for SARS-CoV-2 genomic RNA is the main procedure used in viral identification [8–10].

Though RT-PCR is a highly sensitive and specific assay, highly skilled personnel and special devices are required because it is prone to analyze contamination and degradation. In addition, the total cost and time required by RT-PCR are also issues. Rapid antigen tests developed on the POCT device have been widely used in diagnosing infectious diseases such as influenza and has been effective in preventing pandemics despite the fact that these tests have less sensitivity compared to PCR [11–14].

SARS-CoV-2 infectious viruses shed from patients have been isolated from respiratory specimens collected from patients with high viral loads, as RT-PCR (RT-PCR) Ct values of these samples were below 25 within several days since symptom onset [15–20]. It is possible that rapid antigen tests would be convenient in clinical settings, similar to their use in other infectious diseases.

Recently, rapid and easy SARS-CoV-2 antigen tests have been developed on an immunochromatography device [21–25]. Several reagents have been evaluated, and evaluation data have been

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published by the Foundation for Innovative New Diagnostics [26]. However, it is difficult to compare the performance of each since there are no standard materials for evaluation.

Espline® SARS-CoV-2 reagent was recently developed and approved as an in-vitro diagnostic in Japan [23,27]. Here we report the performance of this reagent using nasopharyngeal swab samples collected from in-patients, and evaluated this reagent in comparison with RT-PCR and in relation to the number of days since symptom onset.

2. Methods

2.1. Specimens and treatment

Informed consent was obtained from all participants of this study. The study protocol was approved by the Ethics Committee of Faculty of Medicine, Toho University (No. A20028_A20020_A20014_A19099). Nasopharyngeal swab specimens were collected according to the manufacturer swab specimens for Espline® SARS-CoV-2 or through standard procedures for RT-PCR, and these were treated with Espline specimen treatment solution (ETS) or suspended in universal virus transfer medium (UVT), respectively. Because of the limitation in number of specimens collected from COVID-19 patients by ETS, we used previously collected specimens stored in UVT. These specimens in UVT had been stored at −80 °C after RT-PCR testing. For specimens directly treated with ETS, RNA for RT-PCR use was immediately extracted from an aliquot of the treated samples using the QIAamp Viral RNA mini kit (QIAGEN, Germany) to minimize the effect of dilution for sample volume requirement of BD MAX™ ExK TNA-3 kit (Becton Dickinson, U.S.A.), and 20 μL aliquots of the remaining treated specimens were used for the Espline test.

2.2. Quantitative RT-PCR (RT-PCR)

RT-PCR analysis was performed according to the “Pathogen Detection Manual 2019-nCoV Ver.2.6.1” provided by the National Institute of Infectious Diseases [28]. Total RNA was extracted from nasopharyngeal swabs using QIAamp Viral RNA mini kit (QIAGEN, Hiden, Germany), or with BD MAX™ ExK TNA-3 (Swab) (Becton Dickinson, U.S.A.). Then, one-step RT-qPCR was performed using QuantStudio® 5 (Applied Biosystems™, U.S.A.) or BD MAX™ open system, respectively. TaqMan Fast Virus 1-Step Master Mix (Thermo Fisher Scientific, U.S.A.) and BD MAX™ TNA MMK (SPC) were used as one-step RT-qPCR master mixes. The primer set (NIID_2019-nCOV_N_F2 and NIID_2019-nCOV_N_R2) and FAM labeled probe (NIID_2019-nCOV_N_P2) were used as these were previously reported as targeting gene N [10,28]. The detection limit value of the measurement system was set at 5 copies/reaction. Using AccuPlex™ SARS-CoV-2 Reference Material Kit (Seracare, U.S.A.) with known RNA copies, we examined the effect of using ETS on RNA extraction, and we estimated that the recovery rate of RNA from the specimen treated by ETS is approximately 30% by comparison with that obtained using the reference material diluted in UVT (data not shown). The RNA copies by RT-PCR were normalized with the reference materials.

2.3. Espline SARS-CoV-2 rapid antigen test

Espline® SARS-CoV-2 (Fujirebio Inc., Japan) is an immunochromatography assay based on sandwich enzyme immunoassay methods and makes use of monoclonal antibodies that recognize SARS-CoV-2 N antigen (N−Ag) [27,29]. For UVT specimens, 20 μL of specimen was treated with 10 μL of the concentrated treatment solution (Fujirebio Inc., Japan), and 20 μL of the treated specimen was applied onto the cassette after 5 min of incubation. SARS-CoV-2 N−Ag in a specimen was captured as an immune-complex with alkaline phosphatase (ALP)-conjugated antibodies by other antibodies immobilized at the test-line position on the membrane. The free ALP-conjugated monoclonal antibodies were captured with the anti-ALP monoclonal antibody immobilized at the reference-line position. These captured ALP-conjugates developed blue lines due to the immersion with the chromogenic substrate in the container in the cassette. Line judgment is carried out 30 min after specimen application, and it is judged as positive when both the reference line and the judgment line can be visually confirmed, negative when only the reference line can be confirmed; otherwise, no judgment can be made.

3. Results

Ninety-six nasopharyngeal swab specimens treated with ETS, of which 9 are non-COVID-19 patient specimens and 33 were nasopharyngeal swab samples, which were collected from COVID-19 patients and were suspended in UVT, were subjected to RT-PCR. The number of SARS-CoV-2 RNA copies in the reactions was calculated from Ct values using the formula based on values obtained with reference material and standard RNA. Among 129 specimens, 63 tested positive through RT-PCR.

The Espline reagent detected the antigen in 25 specimens and in 2 specimens among 63 RT-PCR-positive specimens (sensitivity: 39.7% (25/63)) and in 66 RT-PCR negative specimens (specificity: 97.0% 64/66)), respectively (Table 1). The SARS-CoV-2 RNA copy number of the antigen-positive specimens were significantly higher than those in the antigen-negative specimens as the median of the antigen-positive specimens and antigen-negative specimens were 1,702 copies/reaction and 81 copies/reaction, respectively, of which the corresponding Ct values were 28.0 and 32.7, respectively. The lowest RNA copy number of the antigen-positive specimen was 31. These results indicate that the viral loads of the specimens would affect antigen test sensitivity (Fig. 1-A).

To estimate the viral loads required for antigen detection, receiver operating characteristic (ROC) analysis was done with SARS-CoV-2 RNA titers and based on the concordance of the antigen test and RT-PCR test results. The value obtained using the minimum distance method was 532.4 copies/mL (Fig. 1-B). Specimens with higher RNA titers than this number were collected from days earlier than symptom onset compared to those specimens with lower RNA copies. The median duration since symptom onset were 9.5 d, 16 d, and 19 d for specimens with both antigen and RT-PCR positive, those with antigen negative/RT-PCR positive, and those with both antigen and RT-PCR negative, respectively.

We examined the cumulative sensitivities and agreement rates of the antigen test using RT-PCR as the reference along with the collection days since the symptom onset. Both rates are peaking at 8–9 d (Fig. 2-A); therefore, we compared the cumulative sensitivities in specimens collected before 9 d and after 10 d since symptom onset. These rates were 73.3% (11/15) and 29.2% (14/48) in specimens collected before 9 d and after 10 d, respectively (Table 1). RNA titers were also higher in specimens collected within 9 d since symptom onset compared to those collected after 10 d (Fig. 2-B). The maximum and minimum SARS-CoV-2 RNA titers of four RT-PCR positive/antigen-negative specimens collected within the 9 d were 1,320 and 7, respectively (Fig. 2-C). Antigen test sensitivities of 77.3% (17/22) and 90.9% (10/11) were observed when specimens were classified by their RNA titers at 523.4 copies/mL and when they were classified by both RNA titer and the days of specimen collection before 9 d, respectively (Fig. 2-C). Espline® SARS-CoV-2 reagent could be useful for detecting SARS-CoV-2 infected persons efficiently in specimens collected within 9 d since symptom

Table 1
Comparison between SARS-CoV-2 Antigen and RNA detection among specimens classified by days since symptom onset.

days since symptom onset	RT-PCR	All			≦9			≧10		
		Positive	Negative	Total	Positive	Negative	Total	Positive	Negative	Total
Antigen	Positive	25	2	27	11	0	11	14	2	16
	Negative	38	64[a]	102	4	12[a]	16	34	52	86
	Total	63	66	129	15	12	27	48	54	102

[a] The number includes 9 specimens collected from non-COVID-19 patients.

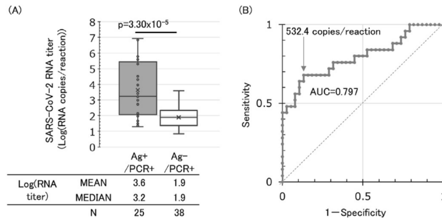

Fig. 1. Characterization of SARS-CoV-2 antigen-positive and negative specimens. (A) Box-plot of SARS-CoV-2 viral loads in nasopharyngeal swabs: the specimens of Espline positive (Ag+)/RT-PCR positive (PCR+) and Espline negative (Ag-)/RT-PCR positive (PCR+) were plotted. Mean and median values of the RNA titers are indicated at the bottom of the panel. The p-value of the t-test between RNA titers of the specimens is indicated at the top of the panel. (B) ROC curve of the concordance of SARS-CoV-2 antigen-positive result with the SARS-CoV-2 RNA positive specimens.

onset. Viral loads of these specimens were higher than 500 copies/reaction of the SARS-CoV-2 RNA (corresponding to Ct values less than 30).

4. Discussion

Espline® SARS-CoV-2 reagent exhibited approximately 40% overall sensitivity, which was lower than those previously reported [21,22]. We assume that differences in sensitivity reflect the differences in specimen sets between the clinical studies. Median Ct values of antigen-positive specimens, antigen-positive/RT-PCR positive specimens, and specimen collection days since symptom onset used in this study were quite different from those in a previous study: most Ct values of the specimens were less than 25, and the previously used specimen collection day was 5 d after symptom onset. As such, it was estimated that the Espline test could give high sensitivity when it was evaluated using the same sample set used in the previous study.

This comparison provides important information: First, the duration since symptom onset greatly affects sensitivity of antigen testing. SARS-CoV-2 viral load is highest several days after symptom onset and decreases rapidly [15,17,30]. The cumulative sensitivity and agreement of the Espline test using RT-PCR as reference test were better before 9 d than after 10 d (Fig. 2-A). It also reported that infectious virus could be isolated from specimens with high viral loads collected 8–9 d since symptom onset [16,20,31,32]. In addition, asymptomatic carriers with high viral loads would act as the epicenter for viral spread [30]. These findings suggest that the antigen test would be very effective in the rapid identification of virus carriers to prevent future epidemics.

We observed four RT-PCR-positive/antigen-negative cases in patients exhibiting COVID-19 symptoms 9 d since symptom onset (Table 1). Viral loads of three of these cases were very low, with less than 100 copies/reaction in the SARS-CoV-2 RNA titers, suggesting that transmission risk from these patients is low; however, using the rapid antigen test should not exclude the use of nucleic acid testing for COVID-19 diagnosis. Although two discrepancies between antigen-positive/RT-PCR negative specimens might be caused by false-positive reaction of the Espline test or by false-negative reaction of RT-PCR, it should be noted that these two discrepancies were collected from COVID-19 patient specimens, of which specimens obtained at previous observation points were RT-PCR positive, indicating that antigen testing gave the correct COVID-19 diagnosis in these cases.

Our study has some important limitations, including limited number of specimens collected before 5 days since symptom onset and bias might have existed in in-house developed RT-PCR test based on the standard protocol established in NIID, which would affect sensitivity and cutoff analysis in this study. We think that the international standards for antigen and RNA, and the panels of specimens would be required for proper evaluation of COVID-19 tests.

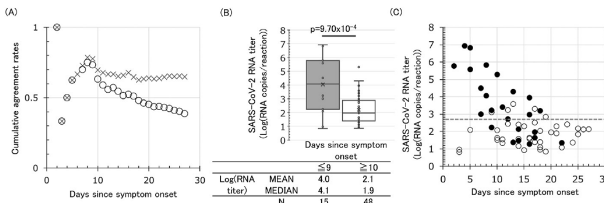

Fig. 2. Characterization of SARS-CoV-2 RNA positive specimens. (A) The cumulative agreement rates for positive, and positive or negative judgment between the antigen test and RT-PCR test along days since symptom onset were plotted as open circles and crosses, respectively. (B) Box-plot of SARS-CoV-2 RNA titers in Ag+/RNA+ and Ag−/RNA+ specimens. Mean and median values of RNA titers are indicated at the bottom of the panel. The p-value of the t-test between the RNA titers in Ag+/RNA+ and Ag−/RNA+ specimens is indicated at the top of the panel. (C) SARS-CoV-2 viral loads in nasopharyngeal swabs were plotted against the days of collection after symptom onset. Specimens of Ag+/RNA+ and Ag−/RNA+ were indicated by closed circles and open circles, respectively. The vertical dash-line indicates the viral load of 532 copies/reaction.

In conclusion, the sensitivity of Espline® SARS-CoV-2 reagent is limited in comparison with RT-PCR. This rapid antigen test is useful as it does not require a special device, it can diagnose COVID-19 within 30 min, and it can identify people with a high risk of virus transmission in order to prevent pandemics.

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ICMJE statement

Contributors YI was responsible for the organization and coordination of the study. KA, TN and SY were responsible for the data analysis. YI, KA, SY and KT prepared this study design. KK and TM contributed to take informed consent and collection of clinical specimens. All authors contributed to the writing of the manuscript.

Declaration of competing interest

SY is employees of Fujirebio, Inc. The other authors have no conflict of interest to declare.

References

[1] Wu F, Zhao S, Yu B, Chen Y-M, Wang W, Song Z-G, et al. A new coronavirus associated with human respiratory disease in China. Nature 2020;1–8. https://doi.org/10.1038/s41586-020-2008-3.
[2] Zhu N, Zhang D, Wang W, Li X, Yang B, Song J, et al. A Novel coronavirus from patients with pneumonia in China. N Engl J Med 2019;2020. https://doi.org/10.1056/nejmoa2001017.
[3] Chen N, Zhou M, Dong X, Qu J, Gong F, Han Y, et al. Epidemiological and clinical characteristics of 99 cases of 2019 novel coronavirus pneumonia in Wuhan, China: a descriptive study. Lancet 2020. https://doi.org/10.1016/s0140-6736(20)30211-7.
[4] WHO Director-General's opening remarks at the media briefing on COVID-19 - 11 March 2020. https://www.who.int/dg/speeches/detail/who-director-general-s-opening-remarks-at-the-media-briefing-on-covid-19—11-march-2020; 2020.
[5] Dong E, Du H, Gardner L. An interactive web-based dashboard to track COVID-19 in real time. Lancet Infect Dis 2020;20:533–4. https://doi.org/10.1016/s1473-3099(20)30120-1.
[6] COVID-19 dashboard by the center for systems science and engineering (CSSE) at Johns Hopkins University (JHU). https://gisanddata.maps.arcgis.com/apps/opsdashboard/index.html#/bda7594740fd40299423467b48e9ecf6; 2020.
[7] Kucharski AJ, Klepac P, Conlan AJK, Kissler SM, Tang ML, Fry H, et al. Effectiveness of isolation, testing, contact tracing, and physical distancing on reducing transmission of SARS-CoV-2 in different settings: a mathematical modelling study. Lancet Infect Dis 2020. https://doi.org/10.1016/s1473-3099(20)30457-6.
[8] Individual EUAs for molecular diagnostic tests for SARS-CoV-2. n.d, https://www.fda.gov/medical-devices/coronavirus-disease-2019-covid-19-emergency-use-authorizations-medical-devices/vitro-diagnostics-euas#individual-molecular. [Accessed 1 August 2020].
[9] CDC's diagnostic test for COVID-19 only and supplies. n.d, https://www.cdc.gov/coronavirus/2019-ncov/lab/virus-requests.html. [Accessed 1 August 2020].
[10] Shirato K, Nao N, Katano H, Takayama I, Saito S, Kato F, et al. Development of genetic diagnostic methods for novel coronavirus 2019 (nCoV-2019) in Japan. Jpn J Infect Dis 2020;73. https://doi.org/10.7883/yoken.jjid.2020.061. JJID.2020.061.
[11] Sasaki T, Kubota-Koketsu R, Takei M, Hagihara T, Iwamoto S, Murao T, et al. Reliability of a newly-developed immunochromatography diagnostic kit for pandemic influenza A/H1N1pdm virus: implications for drug administration. PloS One 2012;7:e50670. https://doi.org/10.1371/journal.pone.0050670.
[12] Mitamura K, Sugaya N. Diagnosis and treatment of influenza. Uirusu 2006;56:109–16. https://doi.org/10.2222/jsv.56.109.
[13] Zazueta-García R, Canizalez-Roman A, Flores-Villaseñor H, Martínez-Garcia J, Llausas-Vargas A, León-Sicairos N. Effectiveness of two rapid influenza tests in comparison to reverse transcription-PCR for influenza A diagnosis. J Infect Dev Ctries 2014;8:1–8. https://doi.org/10.3855/jidc.3726.
[14] Green DA, StGeorge K. Rapid antigen tests for influenza: rationale and significance of the FDA reclassification. J Clin Microbiol 2018;56:e00711–8. https://doi.org/10.1128/jcm.00711-18.
[15] Walsh KA, Jordan K, Clyne B, Rohde D, Drummond L, Byrne P, et al. SARS-CoV-2 detection, viral load and infectivity over the course of an infection. J Infect 2020. https://doi.org/10.1016/j.jinf.2020.06.067.
[16] Scola BL, Bideau ML, Andreani J, Hoang VT, Grimaldier C, Colson P, et al. Viral RNA load as determined by cell culture as a management tool for discharge of SARS-CoV-2 patients from infectious disease wards. Eur J Clin Microbiol 2020;39:1059–61. https://doi.org/10.1007/s10096-020-03913-9.
[17] He X, Lau EHY, Wu P, Deng X, Wang J, Hao X, et al. Temporal dynamics in viral shedding and transmissibility of COVID-19. Nat Med 2020;26:672–5. https://doi.org/10.1038/s41591-020-0869-5.
[18] Calderaro A, Arcangeletti MC, Conto FD, Buttrini M, Montagna P, Montecchini S, et al. SARS-CoV-2 infection diagnosed only by cell culture isolation before the local outbreak in an Italian seven-week-old suckling baby Int J Infect Dis 2020;96:387–9. https://doi.org/10.1016/j.ijid.2020.05.035.
[19] 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 Unit States Am 2020;117. https://doi.org/10.1073/pnas.2002589117. 7001–3.
[20] Wölfel R, Corman VM, Guggemos W, Seilmaier M, Zange S, Müller MA, et al. Virological assessment of hospitalized patients with COVID-2019. Nature 2020;1–5. https://doi.org/10.1038/s41586-020-2196-x.
[21] Porte L, Legarraga P, Vollrath V, Aguilera X, Munita JM, Araos R, et al. Evaluation of novel antigen-based rapid detection test for the diagnosis of SARS-CoV-2 in respiratory samples. Int J Infect Dis 2020. https://doi.org/10.1016/j.ijid.2020.05.098.
[22] Weitzel T, Legarraga P, Iruretagoyena M, Pizarro G, Vollrath V, Araos R, et al. Head-to-head comparison of four antigen-based rapid detection tests for the diagnosis of SARS-CoV-2 in respiratory samples. Biorxiv. 2020. https://doi.org/10.1101/2020.05.27.119255. 2020.05.27.119255.
[23] Omi K, Takeda Y, Mori M. SARS-CoV-2 qRT-PCR Ct value distribution in Japan and possible utility of rapid antigen testing kit. Medrxiv. 2020. https://doi.org/10.1101/2020.06.16.20131243. 2020.06.16.20131243.
[24] Scohy A, Anantharajah A, Bodéus M, Kabamba-Mukadi B, Verroken A, Rodriguez-Villalobos H. Low performance of rapid antigen detection test as front-line testing for COVID-19 diagnosis. J Clin Virol 2020;129:104455. https://doi.org/10.1016/j.jcv.2020.104455.
[25] Diao B, Wen K, Chen J, Liu Y, Yuan Z, Han C, et al. Diagnosis of acute respiratory syndrome coronavirus 2 infection by detection of Nucleocapsid protein. Medrxiv. 2020. p. 2020. https://doi.org/10.1101/2020.03.07.20032524. 03.07.20032524.
[26] Find evaluation update: SARS-COV-2 immunoassays. n.d. https://www.finddx.org/covid-19/sarscov2-eval-immuno/.
[27] Yamakawa K, Fujimoto A, Miyamoto K, Ohshima T, Suzuki T, Nagata N, et al. Development of rapid immunochromatographic enzyme immunoassay for SARS-CoV-2 nucleocapsid protein. Jpn J Med Pharm Sci 2020;77:937–44.
[28] Manual for the detection of pathogen 2019-nCoV Ver.2.6. 2020. https://www.niid.go.jp/niid/images/epi/corona/2019-nCoVmanual20200217-en.pdf.
[29] Ohnishi K, Sakaguchi M, Kaji T, Akagawa K, Taniyama T, Kasai M, et al. Immunological detection of severe acute respiratory syndrome coronavirus by monoclonal antibodies. Jpn J Infect Dis 2005;58:88–94.
[30] Kawasuji H, Takegoshi Y, Kaneda M, Ueno A, Miyajima Y, Kawago K, et al. Viral load dynamics in transmissible symptomatic patients with COVID-19. Medrxiv. 2020. https://doi.org/10.1101/2020.06.02.20120014. 2020.06.02.20120014.
[31] Bullard J, Dust K, Funk D, Strong JE, Alexander D, Garnett L, et al. Predicting infectious SARS-CoV-2 from diagnostic samples. Clin Infect Dis 2020. https://doi.org/10.1093/cid/ciaa638. ciaa638.
[32] Arons MM, Hatfield KM, Reddy SC, Kimball A, James A, Jacobs JR, et al. Presymptomatic SARS-CoV-2 infections and transmission in a skilled nursing facility. N Engl J Med 2020. https://doi.org/10.1056/nejmoa2008457.