

Clinical Feasibility of a Saliva-Based Antigen Qualitative Test for Severe Acute Respiratory Syndrome Coronavirus 2

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Background: Nasopharyngeal swabs (NPS) are generally used as specimen samples for antigen qualitative tests for severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2). The principle of the reaction to the antigen protein is the same when saliva is used, and saliva samples were reported to be as accurate as NPS for real-time reverse transcription quantitative polymerase chain reaction (RT-qPCR) testing to identify SARS-CoV-2. Unlike NPS collection, self-collected saliva does not expose healthcare workers to the risk of infection. In this study, we evaluated the feasibility of using saliva samples for a SARS-CoV-2 antigen qualitative test (TA2107SA) under development.

Methods: Saliva samples were collected from patients with confirmed or suspected COVID-19 infection and analyzed. The sensitivity, specificity, and concordance index of the antigen qualitative test were calculated using an RT-qPCR test as reference.

Results: Saliva samples were collected from 105 patients. The mean interval from onset to specimen collection was 5.7 days. The mean cycle threshold (Ct) value of RT-qPCR was 31.3. The sensitivity, specificity, and concordance index were 70.7%, 100%, and 0.85, respectively. In 33 patients with Ct values <30, the results of both the RT-qPCR and antigen tests were positive. The sensitivity of the saliva-based TA 2107SA SARS-CoV-2 antigen qualitative test was slightly lower than that of the conventional antigen qualitative test using NPS samples from the same patient.

Conclusion: Saliva-based antigen qualitative tests for SARS-CoV-2 are an alternative option during a pandemic.

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Key words: SARS-CoV-2, COVID-19, rapid antigen test, point of care test, saliva

Introduction

Since December 2019, the pandemic caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) pandemic has had a substantial impact worldwide. Although highly effective vaccines have been developed, the emergence of new variants has further complicated this public health problem¹. SARS-CoV-2 can be transmitted even in the asymptomatic period before onset and clinical presentation of coronavirus disease 2019 (COVID-19). After onset, symptoms resemble those of the common cold, or an upper/lower respiratory tract infection^{2,3}.

Thus, a simple, rapid test is required for diagnosing viral infections, such as COVID-19, that have nonspecific initial symptom. Early detection of SARS-CoV-2 is crucial for preventing subsequent transmission of the infection.

The standard laboratory test for symptomatic COVID-19 patients is the real-time reverse transcription quantitative polymerase chain reaction (RT-qPCR) method, which uses nasopharyngeal swabs (NPS). However, RT-qPCR testing is not suitable for rapid screening because it requires expensive equipment and several hours to complete. Recently, antigen qualitative testing has become

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popular as the first point-of-care test (POCT) for patients with suspected COVID-19 infection. It is suitable for primary screening because the results can be determined within 30 min with a commercially available test^{4,6}. However, collection of NPS is problematic because of the potential for healthcare workers to be exposed to droplets, whereas saliva can be easily collected. In previous reports, RT-qPCR of saliva specimens and NPS had comparable detectability, especially within 7 days of onset^{7,8}. Although there is less RNA in saliva samples than in NPS⁹, an automated quantitative chemiluminescence enzyme immunoassay using saliva specimens can accurately measure the amount of viral antigen with a sensitivity comparable to that of RT-qPCR tests¹⁰.

Unfortunately, the performance of saliva-based antigen qualitative tests has been unreliable, and they have not been used clinically¹¹. However, recent technological advances in technology have increased the sensitivity of antigen qualitative tests⁶, which now have the potential for clinical use through the use of saliva specimens.

In this study, we evaluated the feasibility of a saliva-based SARS-CoV-2 antigen qualitative test (TA2107SA, TAUNS Laboratories, Inc.).

Materials and Methods

Ethics Approval

This study was approved by the Ethics Committee of Nippon Medical School Chiba Hokusoh Hospital (No. 904).

Patients

We analyzed data from 368 patients with confirmed or suspected COVID-19 infection who visited the COVID-19 outpatient clinic at Nippon Medical School Hokusoh Hospital for treatment during the period from July 21 through September 2, 2021. Saliva samples were collected from 105 patients. Data on age, sex, days from onset to specimen collection, presence of pneumonia, and serum makers of severity were recorded¹². Pneumonia was diagnosed by reviewing a chest computed tomography scan.

Laboratory Testing

RT-qPCR testing was performed in accordance with the protocol of the National Institute of Infectious Diseases in Japan. The cutoff for cycle threshold (Ct) values of PCR test was 37, and a value of 37 or less was defined as positive.

TAUNS Laboratories Inc. developed an officially approved SARS-CoV-2 antigen qualitative test for use with NPS (immunoace). The reported accuracy of immunoace is based on a comparison with RT-qPCR using domestic

clinically preserved NPS samples. The positive concordance rate was 61.7%, the negative concordance rate was 100%, and the overall concordance rate was 71.7%¹³.

The newly developed SARS-CoV-2 antigen qualitative test differ from immunoace, as it is a membrane-based immunochromatography assay that detects SARS-CoV-2 nucleocapsid protein in saliva. The swab is dipped into the collected saliva and mixed with a buffer solution in a tube. Three drops are added to the appropriate well, and positive lines are visually confirmed after 20 minutes. The clinical performance of the immunoace kit was found to be comparable to that of previously approved commercially available antigen qualitative test kit. In this study, we used the Immunoace antigen qualitative testing kit, with NPS samples from the same patient, as a comparison to the TA2107SA salivary antigen qualitative testing kit.

Specimen Collection

We obtained a saliva sample of at least 2 mL from patients after we confirmed that they had not consumed any food or liquid during the 30 min before collection. The collected saliva was immediately frozen and then transported to the laboratory of TAUNS Inc. for testing. NPS samples were collected by 2 skilled physicians at the same time as saliva collection. Antigen testing and Rt-qPCR testing of the NPS samples were immediately performed in the hospital.

Data Analysis and Statistics

Population characteristics are presented as number of persons (%) for categorical variables and as mean, SD, and range for continuous variables. The results were stratified according to whether the Ct value of RT-qPCR was ≤ 30 , whether it was within 7 days of onset, and whether there was pneumonia present. In addition, the association between the Ct value of the Rt-qPCR and the TA2107SA SARS-CoV-2 antigen qualitative test using saliva sample was visualized in a scatterplot, values of the positive group and negative group were compared with the Mann-Whitney U test.

The sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV) with 95% confidence intervals (CIs) of the antigen qualitative test were calculated with RT-qPCR testing as the reference method, as follows:

Sensitivity: True positive (TP)/(TP + False negative (FN))

Specificity: True negative (TN)/(TN + False positive (FP))

PPV: TP/(TP + FP)

Table 1 Performance of the saliva-based antigen qualitative test, with real-time reverse transcription quantitative polymerase chain reaction as reference

	Sensitivity % (95%CI)	Specificity % (95%CI)	PPV % (95%CI)	NPV % (95%CI)	Concordance index (95%CI)
All patients, n=105	70.7 (60.6–79.0)	100 (73.4–100)	100 (93.3–100)	32.5 (20.0–48.0)	0.85 (0.78–0.92)
Patient with high viral load (Ct<30), n=33	100 (87.6–100)		100 (87.6–100)		
Patient with low viral load (Ct≥30), n=72	54.2 (41.6–66.3)	100 (73.4–100)	100 (87.2–100)	32.5 (20.0–48.0)	0.77 (0.66–0.88)
Days from symptom onset≤7 days, n=69	66.1 (53.3–76.9)	100 (67.9–100)	100 (89.3–100)	33.3 (19.1–51.3)	0.83 (0.73–0.92)
Days from symptom onset>7 days, n=36	78.8 (61.9–89.6)	100 (38.2–100)	100 (84.7–100)	30.0 (10.3–60.7)	0.89 (0.78–1.0)
Patients with pneumonia, n=61	69.6 (56.6–80.1)	100 (51.0–100)	100 (89.3–100)	22.2 (9.7–43.8)	0.84 (0.73–0.95)
Patients without pneumonia, n=44	72.2 (55.8–84.2)	100 (62.7–100)	100 (84.7–100)	44.4 (24.3–66.3)	0.86 (0.74–0.96)

CI, confidence interval; Ct, cycle threshold; NPV, negative predictive value; PPV, positive predictive value.

NPV: $TN / (TN + FN)$

The concordance index was used for distribution modeling and was equivalent to the non-parametric area under the curve statistic¹⁴. Additionally, we compared the areas under 2 correlated receiver operating characteristic curves with nonparametric methods to evaluate the discriminatory ability of TA2107SA using saliva samples and Immnoace using NPS samples in antigen qualitative testing to RT-qPCR test with NPS samples¹⁵.

SPSS Version 28 (IBM Corp., Armonk, NY, USA) was used for statistical analysis. Statistical significance was set at $P < 0.05$.

Results

The mean (SD) age of all patients was 44.1 (14.2) years (range, 11–80 years). Men accounted for 61 (58.1%) of the patients. The mean period from onset to specimen collection was 5.7 (3.1) days (range, 0–12 days). Sixty-one patients (61.6%) had pneumonia at the time of the visit, of which 44 (44.4%) of whom were hospitalized. The mean Ct value of RT-qPCR testing was 31.3 (3.7) (range, 21.0–36.4).

Table 1 shows the results of the salivary antigen testing. The sensitivity, specificity, PPV, NPV, and concordance index were 70.7%, 100%, 100%, 32.5%, and 0.85, respectively. There were 65 TP results, 27 FN results, 13 TN results, and no FP results.

Among the 33 patients with Ct values <30, both RT-qPCR and antigen tests were positive. The sensitivity of Ct values ≥30 was the lowest. There were no FP results for the saliva samples collected. Patients with a positive

result on the saliva-based TA2107SA SARS-CoV-2 test had significantly lower Ct values on the Rt-qPCR test than did patients with a negative antigen test result (**Fig. 1**).

Table 2 shows the results of the antigen qualitative test and the Rt-PCR test using NPS samples as a control. There were 64 TP results, 31 FN results, 9 TN results, and 1 FP result for the saliva-based TA2107SA.

There was a slight difference between the concordance index of the TA2107SA test using saliva samples and the immunoace test using NPS samples in antigen qualitative testing to RT-qPCR test with NPS samples in the same patient ($P = 0.173$; difference, 0.07; 95%CI, -0.03 to 0.18).

Discussion

This study evaluated the feasibility of a POCT using saliva samples, which was developed to detect SARS-CoV-2 antigens and provide results within 20 min. The diagnostic feasibility of the new antigen test, a saliva-based antigen qualitative test, was compared to salivary RT-qPCR testing. The sensitivity and specificity of the saliva-based antigen test were 70.7% and 100%, respectively, and no FP results were observed. The overall concordance index of the saliva-based antigen qualitative test, with salivary RT-qPCR as control was 0.85 (**Table 1**). Conversely, when the RT-qPCR test with NPS samples was used as the control, the index for the TA2107SA antigen qualitative test using saliva samples was slightly lower, 0.78, and the difference with the immunoace antigen qualitative test using NPS samples was 0.07 (**Table 2**). The positive likelihood ratio for the TA2107SA antigen qualitative test 6.74,

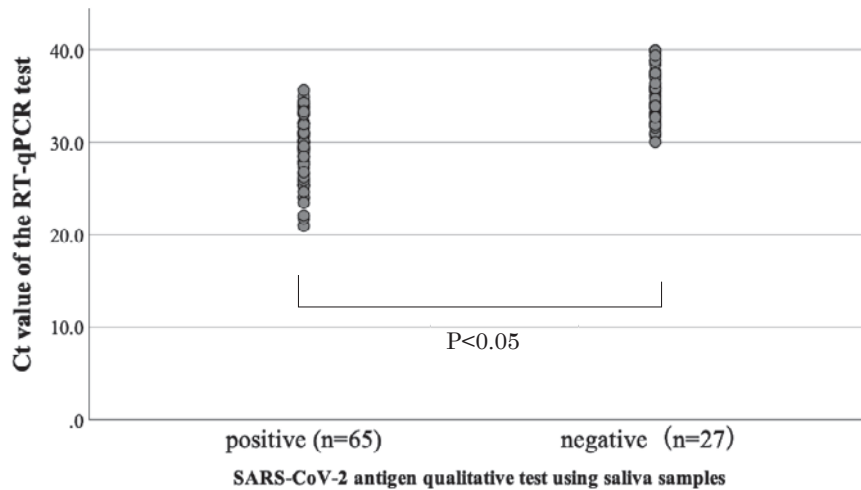


Fig. 1 Scatterplot comparing the cycle threshold (Ct) values of the SARS-CoV-2 reverse-transcription polymerase chain reaction (Rt-qPCR) test and the SARS-CoV-2 antigen qualitative test using saliva samples.

Ct, cycle threshold; RT-qPCR, real-time reverse transcription quantitative polymerase chain reaction; SARS-CoV-2, severe acute respiratory syndrome coronavirus 2

Table 2 Performance of antigen qualitative tests using saliva samples and nasopharyngeal swab samples, with a real-time reverse transcription quantitative polymerase chain reaction test using nasopharyngeal swab samples as reference

	Sensitivity % (95%CI)	Specificity % (95%CI)	PPV % (95%CI)	NPV % (95%CI)	Concordance index (95%CI)
TA2107SA: Saliva samples, n=105	67.4 (57.4–75.9)	90 (57.4–100)	98.4 (90.9–100)	22.5 (12.1–37.7)	0.78 (0.67–0.89)
Immunoace: NPS samples, n=105	72.6 (62.8–80.6)	100 (67.9–100)	100 (93.6–100)	27.7 (15.7–44.1)	0.86 (0.81–0.90)

CI, confidence interval; Ct, cycle threshold; NPV, negative predictive value; PPV, positive predictive value.

as determined using the sensitivity and specificity values in Table 2.

Several studies have evaluated the diagnostic performance of NPS-based antigen qualitative testing in real clinical samples^{4–6}, but only a few have used saliva samples¹¹. Previous studies reported widely varying sensitivity values (53.2% to 96.7%) for NPS antigen qualitative tests. Some products were unsuitable for clinical use, and false positives were frequent. The sensitivity of 70.7% in our study was comparable to that for an the NPS antigen qualitative test in 2020^{4,5}. In particular, the sensitivity was 100% for samples with Ct values of lower than 30. Because the amount of RNA in saliva is reported to be less than that of NPS, the amount of viral antigen may also be proportionally less. Nevertheless, the sensitivity of 100% at a viral load corresponding to the common onset period suggests that use in clinical situations is feasible. It is advantageous that the test is straightforward, and

dose not expose healthcare workers to highly infectious droplets. However, it does not meet the World Health Organization screening test standard of 80% sensitivity and therefore it may need to be improved. Although there were no false positives, the false omission rate was high (67.5%). There were not many negative samples, so it is unclear if this number is accurate. However, in cases of negative results, additional PCR tests or retests will be required.

Although it has been reported that salivary RT-qPCR tests show a decrease in sensitivity after 7 days⁸, we did not observe such a trend in this study. However, the higher the amount of viral RNA, as indicated by the Ct value of Rt-qPCR in the patient, the more likely the antigen qualitative test would be positive. In the samples collected, there was a weak positive correlation between the number of days from onset to collection and the Ct value (0.184, $P=0.085$), but this was not significant. The

mean Ct value was also higher than that in a previous study, which may have been influenced by the longer storage of saliva samples before testing. In addition, the presence of severe pneumonia did not significantly affect the results. An additional factor that may have contributed to the decreased sensitivity of the saliva-based antigen qualitative test was the inconsistent quality of the samples collected. In this study, patients were instructed not to eat or drink for 1 hour before saliva collection, but dehydrated patients were not completely restricted from drinking.

Our study had limitations. First, it was not a real-world study, as it used saliva samples collected as an adjunct to NPS when patients with confirmed or suspected COVID-19 infection were seen for treatment. Although many patients were already known to be positive for SARS-CoV-2, laboratory personnel were not informed of the results for individual patients. Nevertheless, preconceptions may have influenced the visual confirmation of antigen-positive bands. Second, the date of symptom onset was reported by the patients and may not have been accurate. Six asymptomatic patients were also included, and three of 3 patients had false-negative results. Finally, because the saliva samples were transported to the laboratory and were stored before testing, accuracy might differ from that in actual clinical practice, where the samples are tested immediately. However, the sensitivity of the saliva-based TA2107SA antigen qualitative test was slightly lower than that of the NPS-based immunoassay in the same patients, which were tested in real time. Therefore, there may have been the same impact on inspection accuracy.

The saliva-based TA2107SA antigen qualitative test for SARS-CoV-2 appears to be useful as a POCT, given samples can be collected without exposing of healthcare workers to the risk of infection. The test appears to be an acceptable and realistic options during the current pandemic. However, the present sample was small and additional validation studies are required.

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