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Note

Does the timing of saliva collection affect the diagnosis of SARS-CoV-2 infection?

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ABSTRACT

We evaluated the optimal timing of saliva sample collection to diagnose the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infection. We obtained 150 saliva samples at four specific time points from 13 patients with confirmed SARS-CoV-2 infection. The time points were (1) early morning (immediately after waking), (2) immediately after breakfast before tooth brushing, (3) 2 h after breakfast, and (4) before lunch. On the 2nd hospital day, patients collected saliva at the four time points by themselves. We collected samples at two time points, (1) and (3), from the 3rd hospital day to day 9 following symptom onset. In 52 samples collected at the four time points, there was no significant difference. Meanwhile, there was no significant difference in the positive proportion or the viral load between the two time points in both analyses by the day from symptom onset and by all samples. In this study, there was no difference in the positive proportions in saliva collected at various time points within 9 days after symptom onset. The timing of saliva collection was not affected by the diagnosis of SARS-CoV-2 infection.

The coronavirus disease 2019 (COVID-19) caused by severe acute respiratory coronavirus 2 (SARS-CoV-2), and it occurred in December 2019 in Wuhan, China [1]. SARS-CoV-2 is highly contagious and continues to spread worldwide. *Nasopharyngeal swabs* are the primary sampling methods used to detect SARS-CoV-2. However, swab sampling is invasive and can pose a risk of infection for healthcare workers. Researchers suggest that saliva collected within 9 days after symptom onset is a useful sample for the molecular diagnosis of COVID-19 [2]. The Ministry of Health, Labor and Welfare in Japan has allowed "PCR assay by saliva collected within 9 days after symptom onset [3]." Several studies have reported that the detection sensitivity of the test using saliva is comparable to that of nasopharyngeal swab specimen [2,4]. The saliva collection procedure is non-invasive, easy to collect, and can reduce the risk of virus transmission to healthcare workers. Recently, some studies reported that the SARS-CoV-2 molecular test using the posterior oropharynx samples collected in the early morning showed high sensitivity [5]. Moreover, in SARS-CoV-2 molecular tests, one case report stated that saliva collected in the early morning is desirable in terms of detection capability [6]. Therefore, the timing of saliva collection may also affect the results of the SARS-CoV-2 molecular test. In this study, we evaluated the optimal timing of saliva collection for SARS-CoV-2 molecular tests.

We conducted an observational study of patients with COVID-19 admitted to Sapporo Medical University Hospital between August 2020 and March 2021. On admission, nasopharyngeal swabs were collected and tested using the SARS-CoV-2 molecular test to confirm the infection. The specific time points were defined as the time point 1: early morning (immediately after waking, before teeth brushing, mouth rinsing, and eating breakfast), time point 2: right after breakfast before tooth brushing, time point 3: 2 h after breakfast, and time point 4: just before lunch. On the 2nd hospital day, patients collected saliva at four specific time points by themselves. From the 3rd hospital day to day 9

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from symptom onset, they were collected at two specific time points, the time points 1 and 3. After breakfast, the patients rinsed their mouths with water to exclude residues in the oral cavity. We asked patients to pool saliva in their mouth for 5–10 min. We then asked them to expectorate 1–2 ml of saliva into a sterile PP Screw Cup 50 (Asia Kizai Co., Ltd. Tokyo, Japan). The saliva specimens were frozen at −80 °C as soon as possible, and preserved until measurement. The median on the storage period was 17 days (range 0–74 days). All patients had general cognitive skills, and understood and implemented the saliva specimen collection method. Additionally, the researchers monitored them the first few times and checked the volume of saliva in all timing of saliva collection. If the volume is not enough, we asked the patients to excrete at the least 1 ml of saliva. All patients ate breakfast every day; however, we do not know if they ate the snacking. Because we asked patients to rinse their mouths with water after eating something, it is unlikely effects of food residues for PCR assays. The cycle threshold (Ct) values and viral loads of saliva specimens collected at the four time points were obtained and analyzed. The SARS-CoV-2 molecular tests were performed on a LightCycler480 System (Roche, Basel, Switzerland) using the Ampdirect™ 2019-nCoV Detection Kit (Shimadzu Corporation, Kyoto, Japan). The researcher analyzed the samples according to the manufacturer's protocol [7]. The samples were judged as positive or negative based on the threshold cycle (Ct) value. For example, when the Ct value of the sample was 45 or less, it was considered positive. Our laboratory used Standard RNA for nCoV (Shimadzu Corporation) to calculate the viral load. We evaluated the positive proportion and viral load for the optimal timing of sample collection. First, we compared the positive proportion of samples collected at the above four specific time points on the 2nd hospital day. Next, we compared the positive proportion and viral load of samples collected at two specific time points from hospital day 2 to day 9 after symptom onset. Statistical analyses were performed using the Statistical Package for the Social Sciences (SPSS version 24.0) (SPSS Inc, Chicago, IL, USA). As appropriate, we compared the positive proportion using the chi-square test or Cochran's Q-test. We compared the viral load using the Mann-Whitney *U* test. Statistical significance was set at $p < 0.05$. This study is a prospective study and has been approved by the Sapporo Medical University Hospital Institutional Review Board, and informed consent was obtained from all patients (Ethics number 322-113).

We excluded five patients after 9 days following symptom onset on admission. Thirteen patients were included in this study, seven women (54%), with a median age of 55 years (range 19–82 years). The median on the first sampling day was day 4 after symptom onset (range 3–7 days). Of the 13 patients, 10 were mild disease, three were moderate disease on admission and did not require intensive care. Eight patients had fever or chills, six had new loss of taste or smell, five had fatigue, four had coughs, three had headache, two had nausea or vomiting, one had muscle or body ache, sore throat, nasal congestion, or runny nose on admission. In the medication, 4 patients were treated with favipiravir and 2 patients were treated with dexamethasone. The remaining seven patients received only symptomatic treatment without specific therapy. All patients have cured COVID-19. We collected 150 saliva samples from patients within 9 days following symptom onset at four specific time points. Comparing positive proportions for each time point on the 2nd hospital day, there was no significant difference ($p = 0.19$) (Table 1). Comparing positive proportions at time points 1 and 3 in all samples obtained from the 2nd hospital day to day 9 from symptom onset, there was no difference ($p = 1.00$). In the same way, comparing positive proportions of SARS-CoV-2 disease severity, there were no significant differences between mild ($p = 0.83$) and moderate disease ($p = 0.31$). In the analysis of positive proportions for each day from symptom onset, there were no significant differences between samples in the early morning and ones at 2 h after breakfast, and the positive proportions reduced day by day (Fig. 1). In addition, viral loads of the SARS-CoV-2 for each day from symptom onset at the two time points showed no significant difference (Fig. 2).

We evaluated the optimal timing of saliva sample collection for SARS-CoV-2 molecular tests. There were no significant differences in the positive proportions and viral loads according to the timing of saliva collection in this study. Although a study recommended a posterior oropharynx sample collected in the early morning at the point of detection sensitivity [5], it would be difficult to practice in outpatient care. This study indicated that saliva could be collected regardless of the timing. There is a possibility of implicating the specimen type as the cause of this difference. This study used saliva from the oral cavity, whereas another study used samples expectorated from the posterior oropharynx [5]. It has been reported that SARS-CoV-2 is present at high concentrations in the upper respiratory tract [8]. The oral cavity secretes saliva from various glands, such as the parotid, submandibular, sublingual, and minor salivary glands [9]. In contrast, samples from the posterior oropharynx were collected by the patients themselves in the study. Because it is derived from the upper respiratory tract and includes the nasopharynx component, it is more like sputum than saliva. Moreover, sputum collected in the early morning includes a higher proportion of bacteria, than that collected at other points in the tuberculosis test. This is because the body accumulates respiratory secretions during sleep [10]. Therefore, using samples from the posterior oropharynx collected early in the morning may also be recognized in the test for SARS-CoV-2. In addition, a previous case report found that the positive proportions of saliva samples collected in the early morning were more likely to be higher than those of saliva samples collected during the day [6]. However, it would be beneficial if we compared the timing of samples collected on the same day. SARS-CoV-2 screening tests using saliva have been practiced at the airport for quarantine in Japan, and the virus needs to be detected in the saliva of asymptomatic individuals [4]. It has been reported that asymptomatic individuals have lower viral loads of SARS-CoV-2 than symptomatic individuals, and thus it is difficult to detect it [11]. One study demonstrated that the asymptomatic infection proportion may be as high as 40–45% [12]. Furthermore, another study acknowledged that silent disease transmission during the pre-symptomatic and asymptomatic stages was responsible for more than 50% of the overall attack proportion in COVID-19 outbreaks [13]. Therefore, appropriate sample collection for the SARS-CoV-2 testing is needed to detect the virus and prevent its spread. This study indicated

Table 1
Positive proportions of the SARS-CoV-2 molecular test in saliva collected at four specific time points.

	Days after symptom onset (day)	Timing of saliva collection			
		1	2	3	4
Positive proportion (positive samples/total samples)	4	88% (7/8)	63% (5/8)	88% (7/8)	75% (6/8)
	5	100% (1/1)	100% (1/1)	100% (1/1)	100% (1/1)
	6	100% (1/1)	100% (1/1)	100% (1/1)	100% (1/1)
	7	100% (3/3)	100% (3/3)	67% (2/3)	67% (2/3)
	Total	92% (12/13)	77% (10/13)	85% (11/13)	77% (10/13)

Saliva specimens were collected at four specific time points. Positive proportions of the SARS-CoV-2 molecular test on the 2nd hospital day. Time point 1: early morning (immediately after waking, before tooth brushing, mouth rinsing, and eating breakfast), time point 2: immediately after breakfast (before teeth brushing), time point 3: 2 h after breakfast, and time point 4: before lunch.

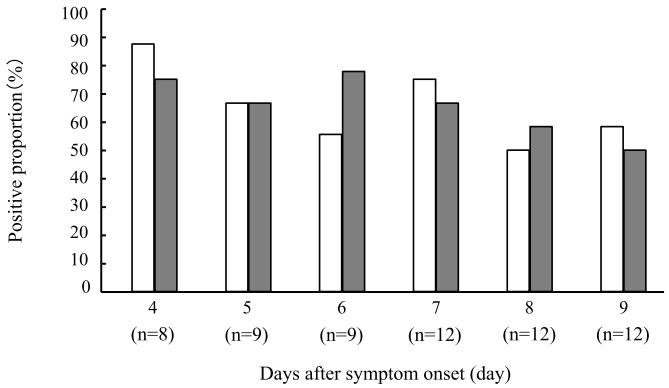

Fig. 1. Transition of positive proportions in the SARS-CoV-2 molecular test for each day after symptom onset.
White bar (□) shows positive proportion of SARS-CoV-2 molecular test in the early morning, gray bar (■) shows one at 2 h after breakfast.

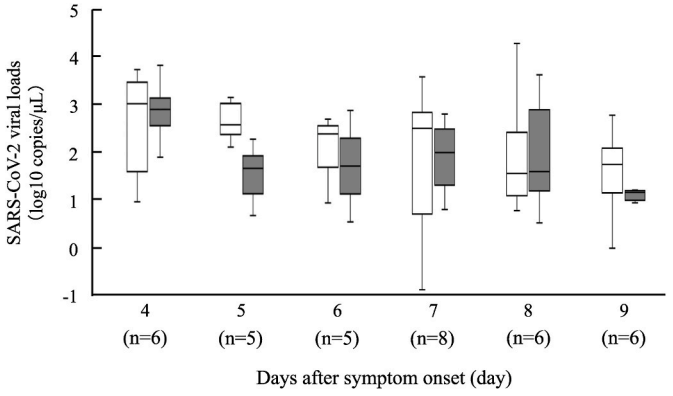

Fig. 2. SARS-CoV-2 viral loads at two time points.
White bar (□) shows positive proportion of SARS-CoV-2 molecular test in the early morning, gray bar (■) shows one at 2 h after breakfast.

that saliva could be obtained at any time in the morning for a diagnosis of the SARS-CoV-2 infection. Our study has several limitations. Firstly, we compared four specific time points including early morning, before breakfast, 2 h after breakfast, and before lunch as the timing of saliva collection. In this study, we could not compare saliva samples collected at the time points of the afternoon. Secondly, we did not consider sample properties. One study reported that a high viscosity could make nucleic acid extraction difficult. Under these circumstances, this could lead to a reduction in the diagnostic accuracy of the SARS-CoV-2 molecular test [14]. In our study, there were differences in viscosity according to collection time in the same patients. We acknowledge that saliva may be collected by patients who are dehydrated and symptomatic. This occurs in about 10% of asymptomatic volunteers [15]. Therefore, the viscosity of saliva may affect the results of the SARS-CoV-2 molecular test. Therefore, appropriate sample collection for SARS-CoV-2 testing is needed to detect the virus and prevent its spread.

To conclude, there was no difference in the positive readings of the SARS-CoV-2 molecular test with reference to the timing of saliva collection, that is within 9 days after symptom onset.

Article type

Note.

Authorship statement

All authors meet the ICMJE authorship criteria. Y.K., K.A., Y.F., and S.T. contributed to the organization and coordination of the trial. S.T. was the chief investigator responsible for the data analysis. Y.K. and R. M. developed the trial design and conducted the investigation. All authors contributed to writing the final manuscript.

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Declaration of competing interest

The authors declare the following conflict of interests which may be considered as potential competing interests: Satoshi Takahashi received speaker honoraria from MSD K.K. and research grants from Shino-Test Corporation, Roche Diagnostic K. K., Fujirebio Inc., and Abbott Japan Co., Ltd. All other authors declare no conflict of interests.

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References

[1] Chan JF, Kok KH, Zhu Z, Chu H, To KK, Yuan S, et al. Genomic characterization of the 2019 novel human-pathogenic coronavirus isolated from a patient with atypical pneumonia after visiting Wuhan. Emerg Microb Infect 2020;9:221–36. https://doi.org/10.1080/22221751.2020.1719902.
[2] Uwamino Y, Nagata M, Aoki W, Fujimori Y, Nakagawa T, Yokota H, et al. Accuracy and stability of saliva as a sample for reverse transcription PCR detection of SARS-CoV-2. J Clin Pathol 2021;74:67–8. https://doi.org/10.1136/jclinpath-2020-206972.
[3] Ministry of Health labor and Welfare. COVID-19 tests. https://www.mhlw.go.jp/stf/seisakunitsuite/bunya/0000121431_00182.html. [Accessed 13 December 2021].
[4] Yokota I, Shane PY, Okada K, Unoki Y, Yang Y, Inao T, et al. Mass screening of asymptomatic persons for severe acute respiratory syndrome coronavirus 2 using saliva. Clin Infect Dis 2021;73:e559–65. https://doi.org/10.1093/cid/ciaa1388.
[5] Hung DL, Li X, Chiu KH, Yip CC, To KK, Chan JF, et al. Early-morning vs spot posterior oropharyngeal saliva for diagnosis of SARS-CoV-2 infection: implication of timing of specimen collection for community-wide screening. Open Forum Infect Dis 2020;7. https://doi.org/10.1093/ofid/ofaa210. ofaa210.
[6] Tajima Y, Suda Y, Yano K. A case report of SARS-CoV-2 confirmed in saliva specimens up to 37 days after onset: proposal of saliva specimens for COVID-19 diagnosis and virus monitoring. J Infect Chemother 2020;26:1086–9. https://doi.org/10.1016/j.jiac.2020.06.011.
[7] Corporation Shimadzu. manufacturer's protocol of AmpdirectTM 2019-nCoV Detection Kit. https://www.shimadzu.co.jp/reagents/covid-19/pdf/ampncov4.pdf. [Accessed 28 January 2022].
[8] Wölfel R, Corman VM, Guggemos W, Seilmaier M, Zange S, Müller MA, et al. Virological assessment of hospitalized patients with COVID-2019. Nature 2020;581:465–9. https://doi.org/10.1038/s41586-020-2196-x.
[9] Kaufman E, Lamster IB. The diagnostic applications of saliva—a review. Crit Rev Oral Biol Med 2002;13:197–212. https://doi.org/10.1177/154411130201300209.
[10] Ssengooba W, Kateete DP, Wajja A, Bugumirwa E, Mboowa G, Namaganda C, et al. An early morning sputum sample is necessary for the diagnosis of pulmonary tuberculosis, even with more sensitive techniques: a prospective cohort study among adolescent TB-suspects in Uganda. Tuberc Res Treat 2012;2012:970203. https://doi.org/10.1155/2012/970203.
[11] Van Vinh Chau N, Lam VT, Dung NT, Yen LM, Minh NNQ, Hung LM, et al. The natural history and transmission potential of asymptomatic severe acute respiratory syndrome coronavirus 2 infection. Clin Infect Dis 2020;71:2679–87. https://doi.org/10.1093/cid/ciaa711.
[12] Oran DP, Topol EJ. Prevalence of asymptomatic SARS-CoV-2 infection: a narrative review. Ann Intern Med 2020;173:362–7. https://doi.org/10.7326/M20-3012.
[13] Moghadas SM, Fitzpatrick MC, Sah P, Pandey A, Shoukat A, Singer BH, et al. The implications of silent transmission for the control of COVID-19 outbreaks. Proc Natl Acad Sci U S A 2020;117:17513. https://doi.org/10.1073/pnas.2008373117.5.
[14] Jeong JH, Kim KH, Jeong SH, Park JW, Lee SM, Seo YH. Comparison of sputum and nasopharyngeal swabs for detection of respiratory viruses. J Med Virol 2014;86:2122–7. https://doi.org/10.1002/jmv.23937.
[15] Landry ML, Criscuolo J, Peaper DR. Challenges in use of saliva for detection of SARS CoV-2 RNA in symptomatic outpatients. J Clin Virol 2020;130:104567. https://doi.org/10.1016/j.jcv.2020.104567.