Clinical Infectious Diseases

MAJOR ARTICLE

Mass Screening of Asymptomatic Persons for Severe Acute Respiratory Syndrome Coronavirus 2 Using Saliva

Isao Yokota,[1,a] Peter Y. Shane,[2,a] Kazufumi Okada,[1] Yoko Unoki,[1] Yichi Yang,[1] Tasuku Inao,[1] Kentaro Sakamaki,[3] Sumio Iwasaki,[4] Kasumi Hayasaka,[4] Junichi Sugita,[4] Mutsumi Nishida,[4] Shinichi Fujisawa,[4] and Takanori Teshima[2,4,5]

[1]Department of Biostatistics, Hokkaido University Graduate School of Medicine, Sapporo, Japan, [2]International Medical Department, Hokkaido University Hospital, Sapporo, Japan, [3]Center for Data Science, Yokohama City University, Yokohama, Japan, [4]Division of Laboratory and Transfusion Medicine, Hokkaido University Hospital, Sapporo, Japan, and [5]Department of Hematology, Hokkaido University Graduate School of Medicine, Sapporo, Japan

(See the Editorial Commentary by Azzi on pages e566–8.)

Background. Coronavirus disease 2019 (COVID-19) has rapidly evolved to become a global pandemic, largely owing to the transmission of its causative virus through asymptomatic carriers. Detection of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) in asymptomatic people is an urgent priority for the prevention and containment of disease outbreaks in communities. However, few data are available in asymptomatic persons regarding the accuracy of polymerase chain reaction testing. In addition, although self-collected saliva samples have significant logistical advantages in mass screening, their utility as an alternative specimen in asymptomatic persons is yet to be determined.

Methods. We conducted a mass screening study to compare the utility of nucleic acid amplification, such as reverse-transcription polymerase chain reaction testing, using nasopharyngeal swab (NPS) and saliva samples from each individual in 2 cohorts of asymptomatic persons: the contact-tracing cohort and the airport quarantine cohort.

Results. In this mass screening study including 1924 individuals, the sensitivities of nucleic acid amplification testing with NPS and saliva specimens were 86% (90% credible interval, 77%–93%) and 92% (83%–97%), respectively, with specificities >99.9%. The true concordance probability between the NPS and saliva tests was estimated at 0.998 (90% credible interval, .996–.999) given the recent airport prevalence of 0.3%. In individuals testing positive, viral load was highly correlated between NPS and saliva specimens.

Conclusion. Both NPS and saliva specimens had high sensitivity and specificity. Self-collected saliva specimens are valuable for detecting SARS-CoV-2 in mass screening of asymptomatic persons.

Keywords. SARS-CoV-2; COVID-19; saliva; PCR; LAMP.

Since its discovery in Wuhan, China, in late 2019, severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) has rapidly created a global pandemic of coronavirus disease 2019 (COVID-19). The fast evolution of this pandemic has been attributed to the fact that most transmissions occur through people who are presymptomatic or asymptomatic [1–3]. Accordingly, detection of the virus in asymptomatic people is a problem that requires urgent attention for the prevention and containment of the outbreak of COVID-19 in communities [4]. Currently, COVID-19 is diagnosed by detecting the nucleic acids of SARS-CoV-2, typically through real-time quantitative reverse-transcription polymerase chain reaction (qRT-PCR) testing of nasopharyngeal swab (NPS) specimens [5, 6]. However, few data are available regarding the accuracy of qRT-PCR testing in asymptomatic persons, on which the implications of the current testing strategy depend. The sensitivity and specificity of PCR testing need to be elucidated to save unnecessary quarantine and contact tracing, while minimizing new infections from presymptomatic persons.

Recently, NPS specimen collection has been under scrutiny, as this method requires specialized healthcare workers and the use of personal protective equipment (PPE) to mitigate the risk of viral exposure. Consequently, self-collected saliva has been reported to have several advantages over NPS. As the name implies, self-collection of saliva eliminates the close contact in sampling, obviating the need for PPE. In addition, providing saliva is painless and minimizes discomfort for the test subject. However, although we and others have shown the value of saliva as a diagnostic specimen in symptomatic patients [7–12], the utility of saliva in detecting the virus in asymptomatic persons remains to be elucidated.

METHODS

We conducted a mass screening study to determine and compare the sensitivity and specificity of nucleic acid amplification using paired samples (NPS and self-collected saliva) for the detection of SARS-CoV-2 in 2 cohorts of asymptomatic individuals. All distributable data are provided in Supplement 2.

Received 13 August 2020; editorial decision 2 September 2020; published online 25 September 2020.

[a]I. Y. and P. Y. S. contributed equally to this work.

Correspondence: T. Teshima, Department of Hematology, Hokkaido University Graduate School of Medicine, N15, W7, Kita-ku, Sapporo 060-8638, Japan (teshima@med.hokudai.ac.jp).

Clinical Infectious Diseases® 2021;73(3):e559–65

DOI: 10.1093/cid/ciaa1388

Design and Population

The contact-tracing (CT) cohort included asymptomatic persons who had been in close contact with patients with clinically confirmed COVID-19 and a positive qRT-PCR result with a an NPS sample. Close contact was defined as being within approximately 2 meters of an infected person. Contact tracing was implemented by tracing the links for each infected person, identified by 2 public health centers between 12 June and 7 July 2020. A separate cohort, the airport quarantine (AQ) cohort, enrolled asymptomatic travelers arriving at Tokyo and Kansai international airports between 12 and 23 June 2020. In both cohorts, participants were requested by the medical officers to provide saliva samples in addition to mandatory NPS samples. Saliva samples were self-collected in a sterilized 15-mL polystyrene sputum collection tube (Toyo Kizai) in a partitioned booth. Multiple partitioned booths enabled parallel sample collection with expeditious flow of test subjects, with high feasibility of saliva testing, especially in the context of mass screening. All specimens were transported at 4°C and analyzed within 48 hours at the central laboratory (SRL).

All NPS samples in the CT cohort were tested with qRT-PCR. The NPS samples in the AQ cohort were tested with either qRT-PCR or reverse-transcription loop-mediated isothermal amplification (RT-LAMP) [13, 14] at the discretion of the airport quarantine medical staff. All saliva samples in both cohorts were analyzed with both qRT-PCR and RT-LAMP. This study was approved by the Institutional Ethics Board of Hokkaido University Hospital (Division of Clinical Research Administration no. 020-0116), and informed consent was obtained from all individuals.

Diagnostic Tests

The method of collection for both saliva and NPS specimens was the same across all participants at all sites. All saliva specimens were self-collected in sterilized 15-mL polystyrene sputum collection tubes (Toyo Kizai) and transported at 4°C without transport medium. The NPS samples were collected using FLOQSwabs (COPAN), placed in transport medium, and transported at 4°C. Samples were analyzed within 48 hours at the central laboratory (SRL). The saliva samples were was diluted 4-fold with phosphate-buffered saline and centrifuged at 2000*g* for 5 minutes to remove cells and debris. RNA was extracted from 200 µL of the supernatant or NPS samples using the QIAsymphony DSP Virus/Pathogen kit and QIAamp Viral RNA Mini Kit (Qiagen). Nucleic acids of SARS-CoV-2 were detected using qRT-PCR or RT-LAMP. The RT-LAMP assay for NPS samples was performed only at the Tokyo airport quarantine station, while the RT-LAMP assay for saliva samples was performed at a central laboratory SRL, using the same system and methods.

Regardless of the test site, all qRT-PCR tests for both NPS and saliva samples were performed using the same methods, according to the National Institute of Infectious Diseases manual [15]. Briefly, 5 µL of the extracted RNA was used as a template. One-step qRT-PCR was performed using the THUNDERBIRD Probe One-step qRT-PCR Kit (Toyobo) and 7500 Real-time PCR Systems (Thermo Fisher Scientific). The cycle threshold (Ct) values were obtained using N2 primers (NIID_2019-nCOV_N_F2 and NIID_2019-nCOV_N_R2) and a probe (NIID_2019-nCOV_N_P2). RT-LAMP was performed to detect SARS-CoV-2 RNA, using Loopamp 2019-SARS-CoV-2 Detection Reagent Kit (Eiken Chemical). The final reaction volume, containing 10 µL of viral RNA extract and 15 µL of primer mix with SARS-CoV-2–specific primers, was dispensed into a reaction tube with dried amplification reagents, including Bst DNA polymerase and avian myeloblastosis virus (AMV) reverse-transcriptase. This tube was incubated at 62.5°C with turbidity readings (optical density, 650 nm) and monitored for 35 minutes using the Loopamp Realtime Turbidimeter (Eiken Chemical).

Statistical Analysis

Test values for qRT-PCR and RT-LAMP methods were illustrated using scatterplots and Kendall coefficient of concordance W as nonparametric intraclass correlation coefficient, taking nonlinearity and censored value into consideration. The performance of diagnostic tests was evaluated by calculating sensitivity Se_{NPS}/Se_{saliva} and Sp_{NPS}/Sp_{saliva}, where Se indicates sensitivity and Sp, specificity. Sensitivity was the positive probability in the infected population, and specificity the negative probability in the noninfected population. To evaluate the concordance between NPS and saliva tests, true concordance probability was defined as follows: $p(Se_{NPS})(Se_{saliva}) + (1 - p)(Sp_{NPS})(Sp_{saliva})$, where p is the prevalence of SARS-CoV-2.

Although qRT-PCR using NPS may be the best-performing test available, it is not a "gold (reference) standard" without known clinical outcomes. Therefore, Se_{NPS}, Se_{saliva}, Sp_{NPS}, Sp_{saliva}, and p were jointly estimated using a bayesian latent class model [16–18], because this method can estimate these parameters without a reference standard and also account for change of plans and rare positive cases. The prior distribution of specificity Sp_{NPS}, Sp_{saliva} were $\beta(201, 1)$, reflecting the results of the in-hospital screening, all negative in >200 consecutive individuals, with none subsequently developing COVID-19 (data not shown). The prior distribution of Se_{NPS}, Se_{saliva}, and p were $\beta(1, 1)$. The corresponding true concordance probability was estimated under varying prevalence values. Sensitivity analysis estimated the true concordance probability when the sensitivities of saliva and NPS were equal, and when the sensitivity of saliva was 10% lower than that of NPS.

The sample size in the CT cohort was calculated as 250, based on the prevalence of 0.1, and 25 positive specimens were needed to keep the width of the 90% credible interval (CI) of sensitivity within .3 with a sensitivity of 0.7. The

sample size in the AQ cohort was calculated as 1818, based on the probability that the 90% CI of specificity >99.0% would be 0.8 when the specificity is 99.5%.

The point estimate and 90% CI were used for the median and 5th to 95th percentiles, respectively. All statistical analyses were conducted using SAS software, version 9.4. SAS codes for the bayesian latent class model are provided in the Supplementary Material.

RESULTS

Demographics

Of the 2558 persons screened, consent was obtained from 1940 persons (75.8%) and 1924 persons were included for analysis (Figure 1). The most common reason for exclusion was the presence of symptoms (n = 95 [33%]) and declined consent (n = 493 [22%]) in the CT and AQ cohorts, respectively. Only 16 persons (0.82%) among those who agreed to participate were excluded owing to insufficient saliva volume, confirming the feasibility of self-collection. Background characteristics of the 161 and 1763 persons in the CT and AQ cohorts, respectively, are shown in Table 1.

In the CT cohort, age and sex data were not made available from many participants for procedural reasons. This population mainly consisted of relatively young people between 20 and 50 years of age. In the AQ cohort, the number of participants by the last point of embarkation was 467 (26%) from Europe (Amsterdam, Frankfurt, and London), 583 (33%) from Asia and Oceania (Bangkok, Jakarta, Manila, Seoul, Shanghai, Sydney, and Taipei), and 713 (40%) from North America (Chicago, Los Angeles, Seattle, and Vancouver). Because of the reduced number of international flights during this period, passengers from Central and South Americas, Africa, and the Middle East may have arrived via transit through any of the regions.

Table 1. Background Characteristics of Study Participants

Characteristic	Participants, No. (%)[a]	
	CT Cohort	AQ Cohort
Sex		
Female	26 (16.1)	832 (47.2)
Male	44 (27.3)	927 (52.6)
Unknown	91 (56.5)	4 (0.2)
Age, median (IQR), y	44.9 (29.8–66.4)	33.5 (22.6–47.4)
Age, y		
≤19	2 (1.2)	299 (17.0)
20–29	16 (9.9)	433 (24.6)
30–39	13 (8.1)	344 (19.5)
40–49	9 (5.6)	324 (18.4)
50–59	8 (5.0)	230 (13.0)
60–69	9 (5.6)	97 (5.5)
≥70	13 (8.1)	34 (1.9)
Unknown	91 (56.5)	2 (0.1)
Last point of embarkation		
North America	…	713 (40.4)
Asia and Oceania	…	583 (33.1)
Europe	…	467 (26.5)

Abbreviations: AQ, airport quarantine; CT, contact-tracing; IQR, interquartile range.
[a]Data represent no. (%) of participants unless otherwise specified.

Sensitivity, Specificity and True Concordance

In the CT cohort, SARS-CoV-2 was detected in 41 NPS and 44 saliva samples; in 38 individuals, both samples tested positive (Table 2). In 114 persons, both tests were negative, which resulted in 152 of 161 matches. In the AQ cohort, viral RNA was detected in 5 NPS and 4 saliva samples, among 1763 individuals (Table 2).

The sensitivities of NPS and saliva tests were 86% (90% CI, 77%–93%) and 92% (83%–97%), respectively (Figure 2A), and the respective specificities were 99.93% (99.77%–99.99%) and 99.96% (99.85%–100.00%) (Figure 2B). The estimated prevalences in the CT and AQ cohorts were 29.6% (90% CI, 23.8%–35.8%) and 0.3% (.1%–.6%), respectively. The true

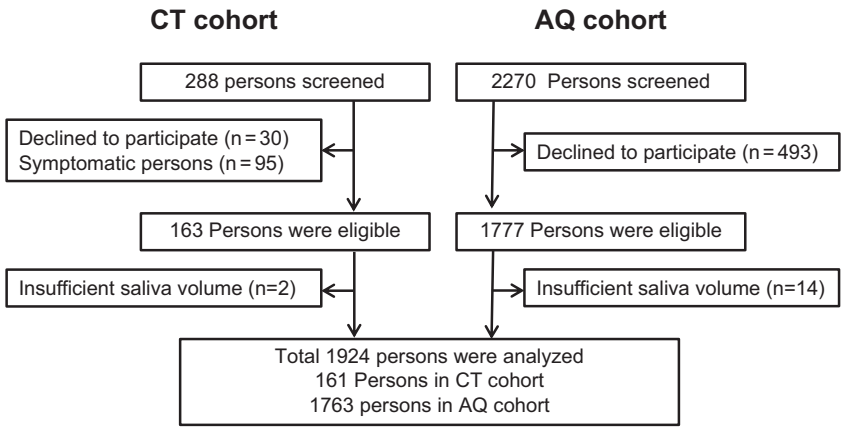

Figure 1. Flow diagram of participants. Abbreviations: AQ, airport quarantine; CT, contact-tracing.

Table 2. Diagnostic Results of Nasopharyngeal Swab and Saliva Sample Testing

	Saliva Sample Result			
	CT Cohort (n = 161)		AQ Cohort (n = 1763)	
NPS Sample Result	Positive	Negative	Positive	Negative
Positive	38	3	4	1
Negative	6	114	0	1758

Abbreviations: AQ, airport quarantine; CT, contact-tracing; NPS, nasopharyngeal swab.

concordance probability was estimated at 0.998 (90% CI, .996–.999) in the AQ cohort. As shown in Figure 3, when the prevalence was varied from 0% to 30%, the point estimate for the true concordance probability ranged from 0.934 to 0.999, and the lower limit of the 90% CI was never below .9. True concordance probability with varying estimation constraints of sensitivity is shown to be very high (Supplementary Material), and therefore the qRT-PCR results from saliva and NPS appeared to be sufficiently consistent.

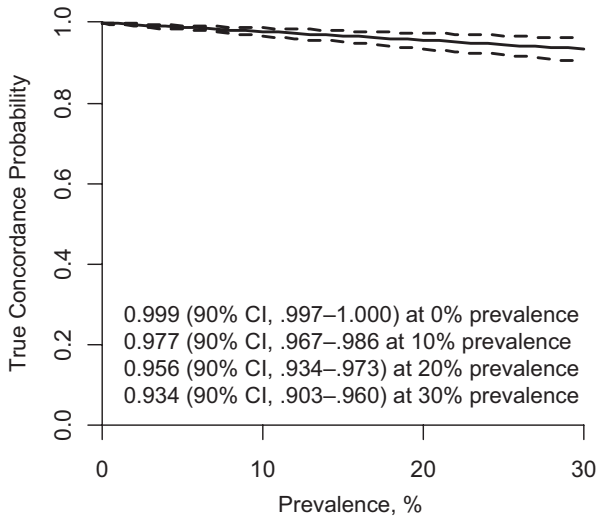

Figure 3. True concordance probability of diagnosis, comparing nasopharyngeal swab and saliva sample tests in populations with varying rates of prevalence. Solid line indicates point estimates, and dashed lines, 90% credible interval (CI).

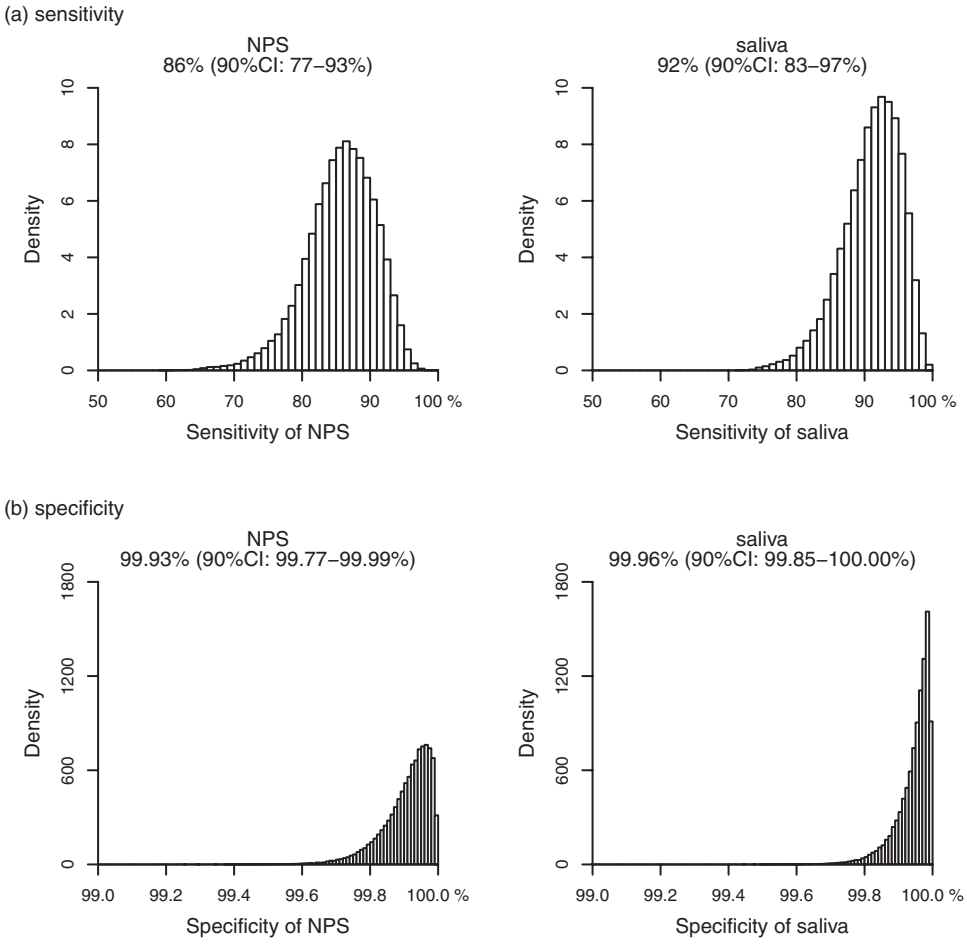

Figure 2. Sensitivity (*A*) and specificity (*B*) of nasopharyngeal swab (NPS) and saliva sample testing, shown as histograms of posterior distributions. Point estimates are shown with 90% credible intervals (CIs), representing the 5th to 95th percentiles.

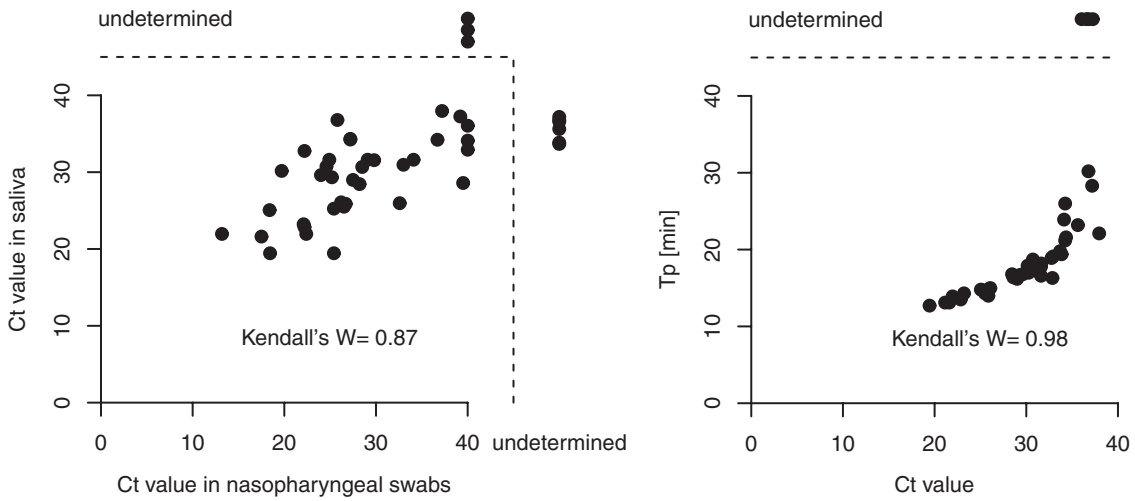

Figure 4. Comparison of viral load between nasopharyngeal swab (NPS) and saliva samples. *A,* Cycle threshold (Ct) values determined with quantitative reverse-transcriptase polymerase chain reaction (qRT-PCR) test of NPS and saliva samples. *B,* Time to detection of positive results with reverse-transcriptase loop-mediated isothermal amplification (RT-LAMP) testing of saliva samples plotted against Ct values determined by qRT-PCR of saliva samples. Kendall *W* represents Kendall coefficient of concordance. Data from samples without a positive test are not shown.

Comparison of Viral Load Between NPS and Saliva Samples

A scatterplot of the Ct values for qRT-PCR from the 45 positive samples (either NPS or saliva) is shown in Figure 4A. All 3 samples that were negative by saliva and positive by NPS had Ct values of 40 with the NPS qRT-PCR test. On the other hand, 6 who had negative NPS and positive saliva samples had Ct values between 33.7 and 37.2 with saliva qRT-PCR. The Kendall coefficient of concordance was 0.87, indicating that the viral load was equivalent between NPS and saliva samples.

Test Values of RT-PCR and RT-LAMP Methods

To confirm the equivalence of the qRT-PCR and RT-LAMP methods, we plotted the time to detection of positive results with RT-LAMP against the Ct values determined with qRT-PCR testing of 44 saliva samples (Figure 4B). Four samples that were negative by RT-LAMP and positive by qRT-PCR had Ct values ranging from 36.0 to 37.3, indicating very low viral loads (Kendall coefficient of concordance, 0.98). Excluding these 4 samples, concordance between qRT-PCR and RT-LAMP was demonstrated in 87 saliva specimens (36 positive and 51 negative) in the CT cohort. In the AQ cohort, all 1763 samples (4 positive and 1759 negative) were concordant.

DISCUSSION

This study examined the accuracy of detecting SARS-CoV-2 with qRT-PCR of NPS and saliva samples in a significant number (n = 1924) of asymptomatic individuals. Our results showed that qRT-PCR in both specimens had specificity >99.9% and sensitivity of approximately 90%, validating the current practice of detecting SARS-CoV-2 infection by means of nucleic acid amplification.

We report for the first time the accuracy of viral detection using natural clinical specimens from asymptomatic persons [19]; the sensitivity was higher than the 52%–71% reported in symptomatic patients [5, 20–23]. The COVID-19 literature to date has been consistent in identifying the peak viral load at symptom onset, with subsequent decline [7, 20, 24–27], suggesting the possibility of higher presymptomatic viral load. More recent studies have also shown that infectiousness peaks on or before symptom onset [28] and that live virus can be isolated from asymptomatic individuals [29]. Concomitantly, there have been reports of discrepancy between viral load as detected by qRT-PCR and contagiousness [29–31], which may be of utmost importance in controlling outbreaks, as the potential to infect close contacts lends credibility to the current strategy of self-quarantine. Although the relationship between contagiousness and viral load needs further investigation, abrogation of early infectiousness may also be an effective drug development target.

The current findings further suggest that saliva may be a beneficial alternative to nasopharyngeal fluid in detecting SARS-CoV-2 in asymptomatic carriers. Comparisons between paired samples have shown equivalent utility, with similar sensitivities and specificities. However, self-collected saliva samples have significant advantages over NPS samples, especially in the setting of mass screening. Saliva collection is noninvasive and does not require specialized personnel or the use of PPE, which saves time and costs In addition, providing saliva is painless and minimizes discomfort for the patient. These significant advantages became immediately apparent during our sample collection at the airport quarantine, where the queue of international arrivals filtered smoothly through multiple collection booths. Self-collection of saliva enables parallel sample collection, which

is simply more conducive to simultaneous mass screening of a large number of individuals, in settings such as social and sporting events.

Previous studies comparing viral loads between NPS and saliva samples have found conflicting results. Wyllie et al [27] showed that the viral load was higher in saliva than in NPS samples , while others have reported results to the contrary [9, 26]. Our results clearly showed the viral loads to be equivalent between NPS and saliva specimens in asymptomatic individuals, and both may be useful in detecting viral RNA.

Some NPS samples at Tokyo's international airport and all saliva samples were analyzed by means of RT-LAMP, an isothermal nucleic acid amplification technique. RT-LAMP has several advantages over standard RT-PCR, including rapid turn-around time, ease of implementation, and potential utility at point of care using a simple device. RT-PCR of NPS samples was conditionally approved in Japan on 31 March 2020 and is increasingly being used as an alternative to RT-PCR, specifically for mass screening at point of care, including sites such as quarantine stations. The development of a novel portable viral detection system based on RT-LAMP has recently been reported by a group in Illinois and others, showing RT-LAMP to be highly sensitive and specific with equivalent accuracy when directly compared with RT-PCR [12, 32–37].

In the current study, we confirmed the accuracy of RT-LAMP in a large population of asymptomatic persons, using saliva samples; no individual was RT-LAMP negative with NPS samples and positive with saliva samples. It is unlikely that the sensitivity of RT-LAMP is significantly less than that of qRT-PCR, and that testing by RT-LAMP instead of qRT-PCR in some instances had minimal impact on our conclusions. Our study suggests that RT-LAMP may be a useful alternative to RT-PCR for the diagnosis of SARS-CoV-2, especially when diagnosis is required at the point of sample collection.

Among the limitations of any diagnostic modality is the possibility of obtaining false results with serious consequences. Persons infected with SARS-CoV-2 who have false-negative test results may be left in society without the necessary precautions to keep them from transmitting the virus, and noninfected persons with false-positive results may undergo unnecessary quarantine and labor-intensive contact-tracing measures. Although the high specificity of qRT-PCR reported herein may be reassuring in individual cases, the implications of mass testing depend on the prevalence of disease in the subject population.

However, point prevalence is unknowable a priori and extremely difficult to assess in rapidly evolving outbreaks from carriers with relatively long presymptomatic periods. Rather, insights on mass testing may be gained by carefully monitoring test positivity in relation to the total number of tests performed. For example, with >99.9% specificity, a positive result in 5% of all tests would indicate that >4.9% (of the 5%) are true-positives, with a positive predictive value of ≥98%. On the other hand, if only 0.3% of all tests return positive results (eg, in isolated localities with very little disease), the positive predictive would be (0.3% − 0.1%)/0.3% = 0.67, erroneously labeling one-third of all positive tests. Because this value is dependent on the prevalence of disease, mass testing using a highly specific test will remain effective as long as test positivity remains relatively high.

The current study lacks longitudinal data and clinical confirmation of positive cases, without which the 2 sample sets are critical to comparison. In the absence of a true diagnostic reference standard, however, we used the most appropriate statistical model available, as described in Methods. Nonetheless, this is the first study in asymptomatic individuals comparing paired of NPS and saliva samples for the detection of COVID-19. Rapid detection of infection in asymptomatic patients is critical for the prevention of COVID-19 outbreaks in communities and hospitals. Mass screening of the virus using self-collected saliva samples can be performed easily, noninvasively, and with minimal risk of viral transmission to healthcare workers.

Supplementary Data

Supplementary materials are available at *Clinical Infectious Diseases* online. Consisting of data provided by the authors to benefit the reader, the posted materials are not copyedited and are the sole responsibility of the authors, so questions or comments should be addressed to the corresponding author.

Notes

Author contributions. I. Y., K. S., J. S., M. N., and TT determined the study design. I. Y., P. Y. S., Y. U., S. I., K. H., M. N., S. F, and T. T. collected the data. I. Y., K. O., Y. U., Y. Y., T. I., and K. S. performed statistical analysis. I. Y., P. Y. S, and T. T. drafted the manuscript, and all authors critically reviewed and approved the final manuscript.

Acknowledgments. The authors thank Tokyo and Kansai airport quarantine stations for their cooperation; we thank Megumi Aoki, Miwa Aoki, Nana Arai, Satomi Araki, Cao Cuicui, Kazumi Hasegawa, Masato Horiuchi, Nao Kurita, Dr Aki Nakamura, Chiho Okabe, Mana Okamura, Yusuke Sakai, Dr Akahito Sako, Natsumi Satake, Maki Shimatani, Kaki Tanaka, Maina Toguri, Sachiko Tominaga, and Hana Wakasa for assistance in collecting saliva samples.

Financial support. This work was supported by Health, Labour and Welfare Policy Research (grant 20HA2002).

Potential conflicts of interest. I. Y. reports speaker fees from Chugai Pharmaceutical, Japan Tabacco, Pharmaceutical Division, and Nippon Shinyaku, outside the submitted work. P.Y. S. reports speaker fees from AYUMI Pharmaceutical, Japan Pharmaceutical Manufacturers Association, Alexion Pharmaceuticals, and Kyowa Kirin, outside the submitted work. T. T. reports honoraria from Merck Sharp & Dohme, Takeda Pharmaceutical, Pfizer Japan, Bristol-Myers Squibb, and Kyowa Hakko Kirin; advisory board fees from Merck Sharp & Dohme, Takeda Pharmaceutical, and Novartis Pharma; research funding from Kyowa Hakko Kirin, Novartis Pharma, Chugai Pharmaceutical, Sanofi, Astellas Pharma, Teijin Pharma, Fuji Pharma, and Nippon Shinyaku; manuscript preparation from Novartis Pharma and Janssen Pharmaceutical; and grants from Japan Society for the Promotion of Science Kakenhi (grant 17H04206) and the Center of Innovation Program from Japan Science and Technology Agency, all outside the submitted work. All other authors report no potential conflicts. All authors have submitted the ICMJE Form for Disclosure of Potential Conflicts of Interest. Conflicts that the editors consider relevant to the content of the manuscript have been disclosed. K. S. reports personal fees from Taiho Pharmaceutical Co., Ltd., Chugai Pharmaceutical Co., Ltd. and Novartis Pharma K. K.

References

1. Böhmer MM, Buchholz U, Corman VM, et al. Investigation of a COVID-19 outbreak in Germany resulting from a single travel-associated primary case: a case series. Lancet Infect Dis **2020**; 20:920–8.
2. Bai Y, Yao L, Wei T, et al. Presumed asymptomatic carrier transmission of COVID-19. JAMA **2020**; 323:1406–7.
3. Moghadas SM, Fitzpatrick MC, Sah P, et al. The implications of silent transmission for the control of COVID-19 outbreaks. Proc Natl Acad Sci U S A **2020**; 117:17513–5.
4. Abbasi J. The promise and peril of antibody testing for COVID-19. JAMA **2020**; 323:1881–3.
5. Wang W, Xu Y, Gao R, et al. Detection of SARS-CoV-2 in different types of clinical specimens. JAMA **2020**; 323:1843–4.
6. Wang X, Tan L, Wang X, et al. Comparison of nasopharyngeal and oropharyngeal swabs for SARS-CoV-2 detection in 353 patients received tests with both specimens simultaneously. Int J Infect Dis **2020**; 94:107–9.
7. To KK, Tsang OT, Chik-Yan Yip C, et al. Consistent detection of 2019 novel coronavirus in saliva. Clin Infect Dis **2020**; 71:841–3.
8. Azzi L, Carcano G, Gianfagna F, et al. Saliva is a reliable tool to detect SARS-CoV-2. J Infect **2020**; 81:e45–e50.
9. Williams E, Bond K, Zhang B, Putland M, Williamson DA. Saliva as a noninvasive specimen for detection of SARS-CoV-2. J Clin Microbiol **2020**; 58:e00776-20.
10. Tu YP, Jennings R, Hart B, et al. Swabs collected by patients or health care workers for SARS-CoV-2 testing. N Engl J Med **2020**; 383:494–6.
11. Iwasaki S, Fujisawa S, Nakakubo S, et al. Comparison of SARS-CoV-2 detection in nasopharyngeal swab and saliva. J Infect **2020**; 81:e145–7.
12. Nagura-Ikeda M, Imai K, Tabata S, et al. Clinical evaluation of self-collected saliva by RT-qPCR, direct RT-qPCR, RT-LAMP, and a rapid antigen test to diagnose COVID-19. J Clin Microbiol **2020**; 58:e01438-20.
13. Notomi T, Okayama H, Masubuchi H, et al. Loop-mediated isothermal amplification of DNA. Nucleic Acids Res **2000**; 28:E63.
14. Park GS, Ku K, Baek SH, et al. Development of reverse transcription loop-mediated isothermal amplification assays targeting severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2). J Mol Diagn **2020**; 22:729–35.
15. Manual for the detection of pathogen 2019-nCoV. Version 2.6. Available at: https://www.niid.go.jp/niid/images/epi/corona/2019-nCoVmanual20200217-en.pdf. Accessed 30 June 2020.
16. Joseph L, Gyorkos TW, Coupal L. Bayesian estimation of disease prevalence and the parameters of diagnostic tests in the absence of a gold standard. Am J Epidemiol **1995**; 141:263–72.
17. Johnson WO, Gastwirth JL, Pearson LM. Screening without a "gold standard": the Hui-Walter paradigm revisited. Am J Epidemiol **2001**; 153:921–4.
18. Wang C, Hanson TE. Estimation of sensitivity and specificity of multiple repeated binary tests without a gold standard. Stat Med **2019**; 38:2381–90.
19. Woloshin S, Patel N, Kesselheim AS. False negative tests for SARS-CoV-2 infection - challenges and implications. N Engl J Med **2020**; 383:e38.
20. Zou L, Ruan F, Huang M, et al. SARS-CoV-2 viral load in upper respiratory specimens of infected patients. N Engl J Med **2020**; 382:1177–9.
21. Fang Y, Zhang H, Xie J, et al. Sensitivity of chest CT for COVID-19: comparison to RT-PCR. Radiology **2020**; 296:E115–E117.
22. Ai T, Yang Z, Hou H, et al. Correlation of chest CT and RT-PCR testing in coronavirus disease 2019 (COVID-19) in China: a report of 1014 cases. Radiology **2020**: 200642.
23. Guo L, Ren L, Yang S, et al. Profiling early humoral response to diagnose novel coronavirus disease (COVID-19). Clin Infect Dis **2020**; 71:778–85.
24. To KK, Tsang OT, Leung WS, et al. Temporal profiles of viral load in posterior oropharyngeal saliva samples and serum antibody responses during infection by SARS-CoV-2: an observational cohort study. Lancet Infect Dis **2020**; 20:565–74.
25. Jamal MA, Mohammad M, Coomes E, et al. Sensitivity of nasopharyngeal swabs and saliva for the detection of severe acute respiratory syndrome coronavirus 2. Clin Infect Dis **2021**; 72:1064–6.
26. Becker D, Sandoval E, Amin A, et al. Saliva is less sensitive than nasopharyngeal swabs for COVID-19 detection in the community setting [Preprint]. medRxiv 17 May 2020. Available from: https://www.medrxiv.org/content/10.1101/2020.05.11.20092338v2.
27. Wyllie AL, Fournier J, Casanovas-Massana A, et al. Saliva or nasopharyngeal swab specimens for detection of SARS-CoV-2. N Engl J Med **2020**; 383:1283–6.
28. He X, Lau EHY, Wu P, et al. Temporal dynamics in viral shedding and transmissibility of COVID-19. Nat Med **2020**; 26:672–5.
29. Arons MM, Hatfield KM, Reddy SC, et al; Public Health–Seattle and King County and CDC COVID-19 Investigation Team. Presymptomatic SARS-CoV-2 infections and transmission in a skilled nursing facility. N Engl J Med **2020**; 382:2081–90.
30. La Scola B, Le Bideau M, Andreani J, 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 Infect Dis **2020**; 39:1059–61.
31. Bullard J, Dust K, Funk D, et al. Predicting infectious SARS-CoV-2 from diagnostic samples. Clin Infect Dis **2020**;ciaa638. doi:10.1093/cid/ciaa638.
32. Ganguli A, Mostafa A, Berger J, et al. Rapid isothermal amplification and portable detection system for SARS-CoV-2. Proc Natl Acad Sci U S A **2020**; 117:22727–35.
33. Yan C, Cui J, Huang L, et al. Rapid and visual detection of 2019 novel coronavirus (SARS-CoV-2) by a reverse transcription loop-mediated isothermal amplification assay. Clin Microbiol Infect **2020**; 26: 773–9.
34. Lee JYH, Best N, McAuley J, et al. Validation of a single-step, single-tube reverse transcription loop-mediated isothermal amplification assay for rapid detection of SARS-CoV-2 RNA. J Med Microbiol **2020**; 69:1169–78.
35. Huang WE, Lim B, Hsu CC, et al. RT-LAMP for rapid diagnosis of coronavirus SARS-CoV-2. Microb Biotechnol **2020**; 13:950–61.
36. Chow FW, Chan TT, Tam AR, et al. A rapid, simple, inexpensive, and mobile colorimetric assay COVID-19-LAMP for mass on-site screening of COVID-19. Int J Mol Sci **2020**; 21:5380.
37. Hu X, Deng Q, Li J, et al. Development and clinical application of a rapid and sensitive loop-mediated isothermal amplification test for SARS-CoV-2 infection. mSphere **2020**; 5:e00808-20.