

Correspondence

Accuracy and stability of saliva as a sample for reverse transcription PCR detection of SARS-CoV-2

COVID-19 prevalence has increased worldwide. Reverse transcription (RT)-PCR-based SARS-CoV-2 detection has majorly contributed to COVID-19 diagnosis. Although nasopharyngeal swab samples are commonly used for RT-PCR, infection risk is high among the healthcare personnel during sample collection. Saliva, which can be self-collected by patients even at home, has been proposed as a sample for RT-PCR-based SARS-CoV-2 detection, thus potentially reducing the infection risk among healthcare personnel.^{1,2} However, few studies have assessed the accuracy of RT-PCR analysis using multiple saliva samples. Furthermore, salivary ribonuclease is speculated to affect the analysis of stored samples.³

From 15 May to 16 July 2020, we obtained nasopharyngeal swabs and saliva samples simultaneously, from patients admitted to Keio University Hospital (Tokyo, Japan) for COVID-19 treatment and from the university staff presenting symptoms suggesting acute viral infections, including fever, upper or lower respiratory symptoms, or diarrhoea. Nasopharyngeal swab samples were collected by trained medical staff using a FLOQ SWAB and a

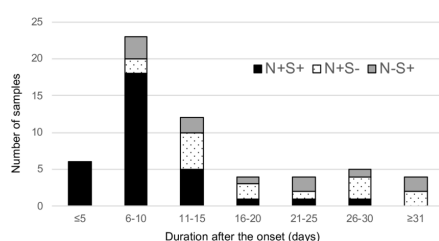


Figure 1 Association between days from onset and reverse transcription (RT)-PCR analysis of SARS-CoV-2-positive samples. Samples obtained up to 10 days after COVID-19 onset showed positive results with both nasopharyngeal swabs and saliva samples on RT-PCR (N+S+) except for only five samples with discordant results. However, samples obtained after 11 days from COVID-19 onset were likely to display discordance between the two samples; 13 samples were positive for nasopharyngeal swabs and negative for saliva on RT-PCR (N+S-), and eight samples were negative for nasopharyngeal swabs and positive for saliva on RT-PCR (N-S+).

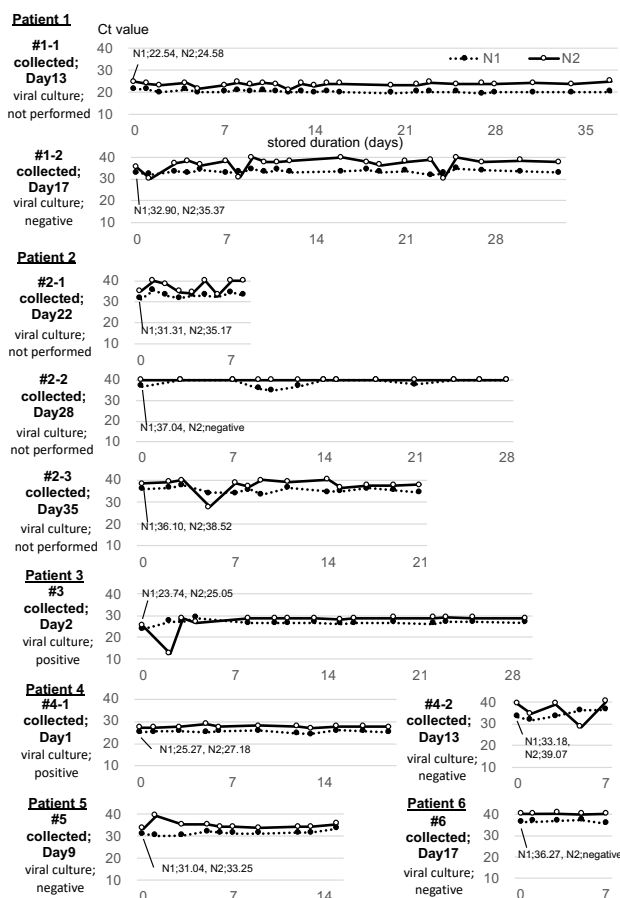


Figure 2 Storage of saliva samples at 25°C and changes in CT values. Ten reverse transcription (RT)-PCR positive saliva samples were transferred to ribonuclease-free microtubes and stored at 25°C, and the RT-PCR assay was repeated every 1 - 3 days until the sample was exhausted. Dotted lines indicate CT values of N1 primers, and solid lines indicate those of N2 primers. CT values of >40 or no elevation of the amplification curve are indicated as CT values of 40. Initial CT values varied among samples according to the duration after symptom onset (shown as day X), except for one sample (#2-2) with a high initial CT value presenting varied results, RT-PCR results remained positive. An occasional temporal dip was observed in the CT values of N2 primers.

BD UVT container (BD, Franklin Lakes, New Jersey, USA), and saliva samples were collected by patients themselves in sterile containers after 1 min of salivation. Real-time RT-PCR-based SARS-CoV-2 detection was simultaneously performed for both samples, using LightCycler96 (Roche, Basel, Switzerland) using the 2019 Novel Coronavirus Detection Kit (Shimadzu, Kyoto, Japan) in accordance with the manufacturer's instructions using N1 and N2 primers and probes.⁴ Ct values of <40 for either primer were considered as a positive result, and the results were compared between the two samples.

Furthermore, to assess the stability of saliva samples, samples with an adequate residual volume with positive RT-PCR results were selected and transferred to ribonuclease-free microtubes and stored at 25°C, and RT-PCR was repeated every 1-3 days for 7 days and more until the sample

was exhausted. As for the case patients' consent were obtained for sample use, viral culture for detecting infective virus were performed using VeroE6/TMPRSS2 cells and observed for 1 week.⁵

Consequently, 196 saliva and nasopharyngeal swab samples were obtained from 32 hospitalised patients with COVID-19 and 115 symptomatic staff. Thirty-two samples were found positive for both saliva and nasopharyngeal swab samples (N+S+), while 138 were negative for both (N-S-). Fifteen samples were positive for nasopharyngeal swab samples and negative for saliva samples (N+S-), and 11 samples were positive for saliva samples and negative for nasopharyngeal swab samples (N-S+). Overall, saliva and nasopharyngeal swab samples displayed 86.7% concordance with kappa coefficient as 0.625. Although samples collected long after symptom

onset displayed discordant results (figure 1), those obtained within 10 days from symptom onset (n=140) displayed 96.4% concordance between both types of samples (kappa coefficient: 0.883). Only five samples collected from day 6 to day 10 from symptom onset revealed discordant results (two samples collected on day 8 were N+S and one sample collected on day 6 and two samples collected on day 9 were N-S+).

Ten saliva samples at 25°C from six patients with COVID-19 were stored for ≥7 days. Although initial Ct values were varying among samples, repeating the RT-PCR analysis revealed positive results for ≥7 days, with no wide fluctuations in Ct values (figure 2), except for one sample displaying high initial Ct values and inconsistent results. Out of viral cultures of six samples, only two revealed viable virus.

These results indicate that saliva, especially collected within 10 days of symptom onset, can substitute the nasopharyngeal swab samples, concurrent with previous reports.^{6,7} Therefore, saliva samples might be suitable for diagnosis of acute symptomatic patients, and it will decrease the risk for occupational infection of health-care professionals during sample collection without losing accuracy. Additionally, although the sample size was limited, long-term storage of saliva samples herein did not affect the test results even in the presence of ribonuclease in saliva. This suggests that saliva samples collected even at the patients' houses can be transported to distant laboratories without losing sensitivity. Viral culture results imply RNA fragmentation by ribonuclease could result in loss of viability but preserve the detectability by probe without decomposition for days. Therefore, Ct values of salivated samples were not fluctuated over time ex vivo, while initial Ct values were increased over time reflecting decreasing viral burden in vivo. Furthermore, our results about sample stability of saliva demonstrate that contamination of the laboratory environment with SARS-CoV-2-containing saliva might be long lasting and affect the test results for a long period; therefore, caution in handling

saliva samples is critical for a laboratory personnel.

In conclusion, test results of SARS-CoV-2 RT-PCR using saliva collected in an acute phase were as accurate as those using nasopharyngeal swab samples, and saliva sample storage at a room temperature did not affect the test results.

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Handling editor Tahir S Pillay.

Acknowledgements We would like to thank the medical technologists of Keio University Hospital for their great contribution to clinical work on COVID-19. We would also like to thank Ms Yuri Furusawa (University of Tokyo) for her contribution.

Contributors YU and MN equally contributed to this work. YU conceived, designed the study, analysed and interpreted the data, and wrote the manuscript. MN conceived, designed the study, performed the assays and analysed the data. WA, YF, TN, YS-T and KI-H performed the assays. TS, ShoUc, ShuUn, HirokK, SI and HirofK collected the data, and all authors discussed the data and critically reviewed and revised the manuscript. All authors have given final approval for this version of the manuscript to be published.

Funding This study was funded by Keio University Hospital and partly supported by a Research Program on Emerging and Re-emerging Infectious Diseases (JP19fk0108113) from the Japan Agency for Medical

Research and Development and by the National Institutes of Allergy and Infectious Diseases-funded Center for Research on Influenza Pathogenesis (Grant HHSN272201400008C).

Competing interests None declared.

Patient consent for publication Not required.

Ethics approval This study was approved by Ethics Committee of Keio University School of Medicine (20 200 063 and 20190337).

Provenance and peer review Not commissioned; internally peer reviewed.

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To cite Uwamino Y, Nagata M, Aoki W, et al. *J Clin Pathol* 2021;**74**:67–68.

Received 22 July 2020

Accepted 1 September 2020

Published Online First 14 September 2020

J Clin Pathol 2021;**74**:67–68.

doi:10.1136/jclinpath-2020-206972

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