CORRESPONDENCE

Saliva or Nasopharyngeal Swab Specimens for Detection of SARS-CoV-2

TO THE EDITOR: Rapid and accurate diagnostic tests are essential for controlling the ongoing Covid-19 pandemic. Although the current standard involves testing of nasopharyngeal swab specimens by quantitative reverse-transcriptase polymerase chain reaction (RT-qPCR) to detect SARS-CoV-2, saliva specimens may be an alternative diagnostic sample.[1-4] Rigorous evaluation is needed to determine how saliva specimens compare with nasopharyngeal swab specimens with respect to sensitivity in detection of SARS-CoV-2 during the course of infection.

A total of 70 inpatients with Covid-19 provided written informed consent to participate in our study (see the Methods section in Supplementary Appendix 1, available with the full text of this letter at NEJM.org). After Covid-19 was confirmed with a positive nasopharyngeal swab specimen at hospital admission, we obtained additional samples from the patients during hospitalization. We tested saliva specimens collected by the patients themselves and nasopharyngeal swabs collected from the patients at the same time point by health care workers.

Using primer sequences from the Centers for Disease Control and Prevention, we detected more SARS-CoV-2 RNA copies in the saliva specimens (mean log copies per milliliter, 5.58; 95% confidence interval [CI], 5.09 to 6.07) than in the nasopharyngeal swab specimens (mean log copies per milliliter, 4.93; 95% CI, 4.53 to 5.33) (Fig. 1A, and Fig. S1 in Supplementary Appendix 1). In addition, a higher percentage of saliva samples than nasopharyngeal swab samples were positive up to 10 days after the Covid-19 diagnosis (Fig. 1B). At 1 to 5 days after diagnosis, 81% (95% CI, 71 to 96) of the saliva samples were positive, as compared with 71% (95% CI, 67 to 94) of the nasopharyngeal swab specimens. These findings suggest that saliva specimens and nasopharyngeal swab specimens have at least similar sensitivity in the detection of SARS-CoV-2 during the course of hospitalization.

Because the results of testing of nasopharyngeal swab specimens to detect SARS-CoV-2 may vary with repeated sampling in individual patients,[5] we evaluated viral detection in matched samples over time. The level of SARS-CoV-2 RNA decreased after symptom onset in both saliva specimens (estimated slope, −0.11; 95% credible interval, −0.15 to −0.06) (Fig. 1C) and nasopharyngeal swab specimens (estimated slope, −0.09; 95% credible interval, −0.13 to −0.05) (Fig. 1D). In three instances, a negative nasopharyngeal swab specimen was followed by a positive swab at the next collection of a specimen (Fig. 1D); this phenomenon occurred only once with the saliva specimens (Fig. 1C). During the clinical course, we observed less variation in levels of SARS-CoV-2 RNA in the saliva specimens (standard deviation, 0.98 virus RNA copies per milliliter; 95% credible interval, 0.08 to 1.98) than in the nasopharyngeal swab specimens (standard deviation, 2.01 virus RNA copies per milliliter; 95% credible interval, 1.29 to 2.70) (see Supplementary Appendix 1).

Recent studies have shown that SARS-CoV-2 can be detected in the saliva of asymptomatic persons and outpatients.[1-3] We therefore screened 495 asymptomatic health care workers who provided written informed consent to participate in our prospective study, and we used RT-qPCR to test both saliva and nasopharyngeal samples obtained from these persons. We detected SARS-CoV-2 RNA in saliva specimens obtained from 13 persons who did not report any symptoms at or before the time of sample collection. Of these 13 health care workers, 9 had collected matched nasopharyngeal swab specimens by themselves on the same day, and 7 of these specimens tested negative (Fig. S2). The diagnosis in the 13 health care workers with positive saliva specimens was later confirmed in diagnostic testing of additional nasopharyngeal samples by a CLIA (Clinical Laboratory Improvement Amendments of 1988)–certified laboratory.

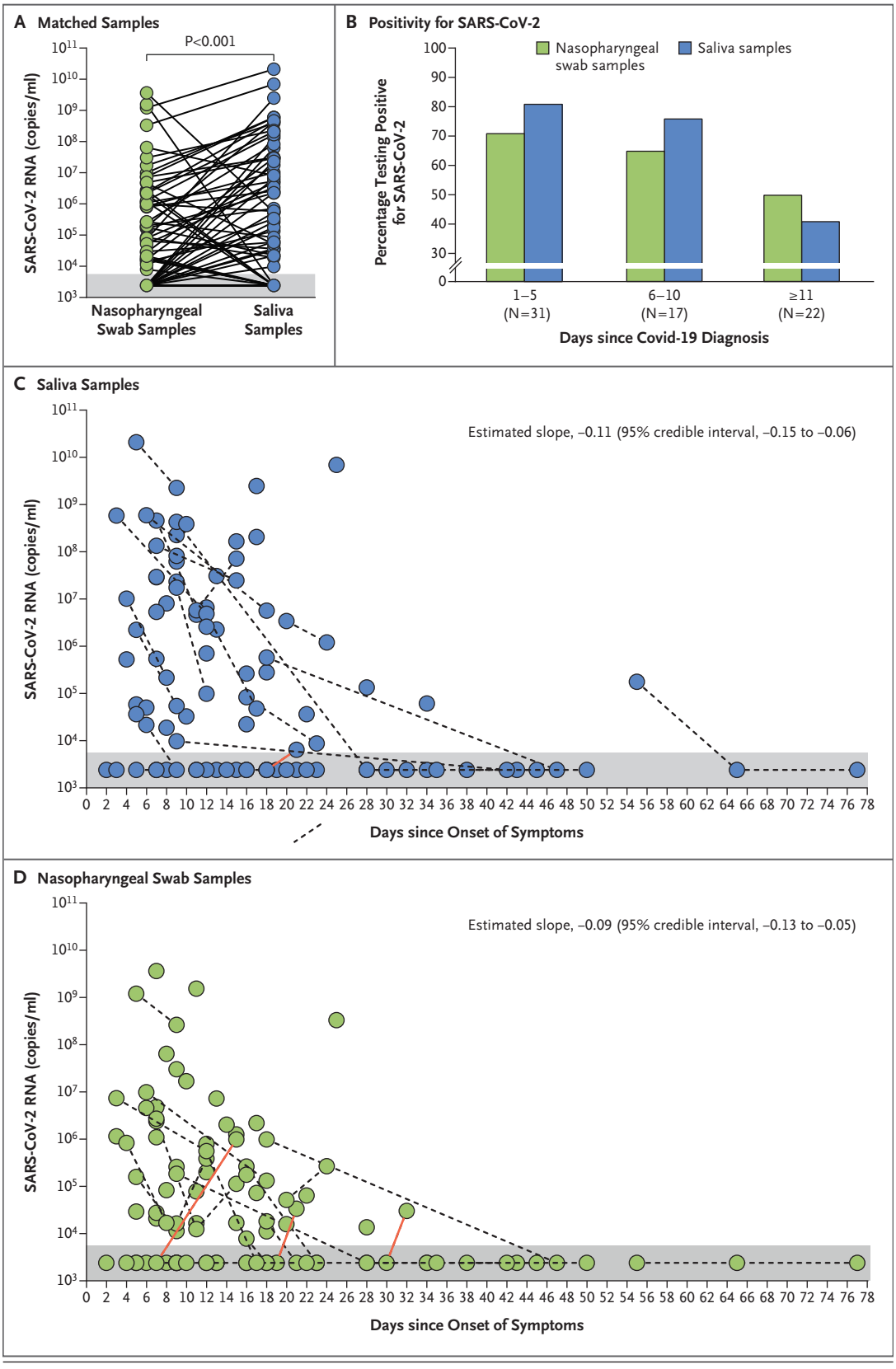

Figure 1 (facing page). SARS-CoV-2 RNA Titers in Saliva Specimens and Nasopharyngeal Swab Specimens.

Samples were obtained from 70 hospital inpatients who had a diagnosis of Covid-19. Panel A shows SARS-CoV-2 RNA titers in the first available nasopharyngeal and saliva samples. The lines indicate samples from the same patient. Results were compared with the use of a Wilcoxon signed-rank test ($P<0.001$). Panel B shows percentages of positivity for SARS-CoV-2 in tests of the first matched nasopharyngeal and saliva samples at 1 to 5 days, 6 to 10 days, and 11 or more days (maximum, 53 days) after the diagnosis of Covid-19. Panel C shows longitudinal SARS-CoV-2 RNA copies per milliliter in 97 saliva samples, according to days since symptom onset. Each circle represents a separate sample. Dashed lines indicate additional samples from the same patient. The red line indicates a negative saliva sample that was followed by a positive sample at the next collection of a specimen. Panel D shows longitudinal SARS-CoV-2 RNA copies per milliliter in 97 nasopharyngeal swab specimens, according to days since symptom onset. The red lines indicate negative nasopharyngeal swab specimens there were followed by a positive swab at the next collection of a specimen. The gray area in Panels C and D indicates samples that were below the lower limit of detection of 5610 virus RNA copies per milliliter of sample, which is at cycle threshold 38 of our quantitative reverse-transcriptase polymerase chain reaction assay targeting the SARS-CoV-2 N1 sequence recommended by the Centers for Disease Control and Prevention. To analyze these data, we used a linear mixed-effects regression model (see Supplementary Appendix 1) that accounts for the correlation between samples collected from the same person at a single time point (i.e., multivariate response) and the correlation between samples collected across time from the same patient (i.e., repeated measures). All the data used to generate this figure, including the raw cycle thresholds, are provided in Supplementary Data 1 in Supplementary Appendix 2.

Variation in nasopharyngeal sampling may be an explanation for false negative results, so monitoring an internal control for proper sample collection may provide an alternative evaluation technique. In specimens collected from inpatients by health care workers, we found greater variation in human RNase P cycle threshold (Ct) values in nasopharyngeal swab specimens (standard deviation, 2.89 Ct; 95% CI, 26.53 to 27.69) than in saliva specimens (standard deviation, 2.49 Ct; 95% CI, 23.35 to 24.35). When health care workers collected their own specimens, we also found greater variation in RNase P Ct values in nasopharyngeal swab specimens (standard deviation, 2.26 Ct; 95% CI, 28.39 to 28.56) than in saliva specimens (standard deviation, 1.65 Ct; 95% CI, 24.14 to 24.26) (Fig. S3).

Collection of saliva samples by patients themselves negates the need for direct interaction between health care workers and patients. This interaction is a source of major testing bottlenecks and presents a risk of nosocomial infection. Collection of saliva samples by patients themselves also alleviates demands for supplies of swabs and personal protective equipment. Given the growing need for testing, our findings provide support for the potential of saliva specimens in the diagnosis of SARS-CoV-2 infection.

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