Use of Self-Collected Saliva Samples for the Detection of SARS-CoV-2

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Abbreviations: SARS-CoV-2, SARS coronavirus-2; FDA, US Food and Drug Administration; EUA, emergency use authorization; RT-PCR, reverse transcription polymerase chain reaction; NP, nasopharyngeal; CDC, Centers for Disease Control and Prevention; Ct, cycle threshold; E, envelope; NUC, nucleocapsid; ORF, open reading frame; E, envelope.

ABSTRACT

Objective: Using a US Food and Drug Administration (FDA) emergency use authorization (EUA) reverse transcription polymerase chain reaction (RT-PCR) method, we examined the analytic performance accuracy of saliva specimens as compared to nasopharyngeal (NP) specimens in symptomatic patients. Correlation between test results and symptoms was also evaluated.

Methods: Over a 5-week period in 2020, 89 matched saliva and nasopharyngeal swabs were collected from individuals exhibiting symptoms consistent with SARS-CoV-2. Specimens were tested with an FDA EUA-approved RT-PCR method, and performance characteristics were compared.

Results: The concordance rate between saliva and nasopharyngeal testing was 93.26%. The mean cycle threshold value of saliva when compared to the NP specimen was 3.56 cycles higher. As compared to NP swab, saliva testing demonstrates acceptable agreement but lower sensitivity.

Conclusion: When compared to a reference method using NP swabs, the use of saliva testing proved to be a reliable method. Self-collected saliva testing for SARS-CoV-2 allows for a viable option when trained staff or collection materials are in short supply.

As part of the management of the COVID-19 pandemic, accurate detection of infected individuals lies at the forefront of health care. The current criterion standard specimen type, nasopharyngeal (NP) swabs, must be collected by health care professionals and later analyzed via reverse transcription polymerase chain reaction (RT-PCR). The lack of sufficient supplies (collection swabs, viral transport medias, collection tubes) and availability of health care professionals to facilitate viral collection poses a persistent challenge in the detection of SARS coronavirus-2 (SARS-CoV-2). Discomfort reported by patients along with possible transmission associated with the NP collection method highlights the importance of pursuing alternative sampling methods for SARS-CoV-2. Self-collected saliva is a noninvasive sample type that may improve detection of SARS-CoV-2 as compared to NP swabs.

The consideration of saliva as a sample type centers around differences in sensitivities to NP swabs and the potential of increasing access to COVID-19 diagnostic tests. As new strains of SARS-CoV-2 continue to emerge and spread, the urgent need for efficient collection and testing methods remains pivotal. Utilization of self-collected saliva may diminish the possibility of SARS-CoV-2 transmission as compared to assisted NP swab testing. Other studies note test results from self-collected saliva specimens demonstrate similar analytical performance to NP samples collected by trained health care professionals. In the summer of 2020, the pandemic greatly affected minorities and disenfranchised communities due to preexisting conditions, crowded living spaces, and lack of access to COVID-19 testing. Newer variants of the virus may affect such communities yet again, further highlighting the importance of providing access to reliable testing with readily collectable specimens. Self-collected saliva samples for detection of the virus could potentially increase access to testing, thus reducing the negative effects on underserved communities.

Our study objective was to assess the viability of saliva as a sample type in detection of SARS-CoV-2, as compared to the standard NP swab, using a high-throughput US Food and Drug Administration (FDA) emergency use authorization (EUA) method. We analyzed cycle threshold (Ct) values to discern sensitivity differences between NP swabs and saliva samples to illustrate the potential utility of saliva as a sample type.

Materials and Methods

The study was approved by the Mayo Clinic Florida institutional review board (IRB# 20-004895). In recruiting study participants,
clinical researchers invited individuals via telephone to participate in the study. Informed consent was digitally obtained via remote technology (iPad) by an email containing a link to the study consent form. In addition to completing the digital consent form, written consent was received from each participant on the date of specimen collection.

In the study, 89 participants between the ages of 20 and 83 were sampled from August 25, 2020, to October 2, 2020. Participants were identified as symptomatic through self-reporting the following symptoms or occurrences through the nurse triage call line: loss of sense of taste and/or smell, fever greater than 37°C, cough, shortness of breath, headache, sore throat, nasal congestion, diarrhea, vomiting, or nausea. The collection from study participants was held in conjunction with community testing at a COVID drive-through facility.

NP Swab Collection
Trained health care professionals collected NP swab specimens from all study participants while abiding by standard precautionary techniques. Swabs placed in viral transport media (Remel) were then sent to the testing laboratory every 60 minutes (+20 minutes transport; total time range, 20–80 minutes) on wet ice after collection to reduce potential degradation of the patient samples. NP swabs were tested in the laboratory within 24 hours of receipt.

Saliva Collection
Supervised by a health care provider, participants were provided a self-collection kit for collection of saliva, which featured kit instructions in the form of a video tutorial and paper handout. Using an FDA EUA-approved self-collection device (Spectrum DNA), participants were to spit approximately 2.0 mL into the device. The instructions then required participants to add 1.50 mL of an RNA stabilization/lysing agent to their sample. Once completed, the samples were placed on wet ice and sent every 60 minutes (+20 minutes transport; total time range, 20–80 minutes) to the laboratory for testing.

Nucleic Acid Extraction and SARS-CoV-2 Detection
The two matched sample specimens (saliva and NP) were transported to the testing laboratory and tested with the cobas SARS-CoV-2 test using the cobas 6800 system (Roche Molecular Diagnostics). NP specimens were processed according to the manufacturer’s recommendations under the FDA EUA. Saliva specimens were considered an alternative specimen type and processed as a laboratory-developed test. Saliva specimens were checked to meet the 2.0 mL collection volume by visual inspection before testing. NP specimens were stored at 4°C–8°C and tested within 24 hours of collection at the clinical laboratory, and saliva specimens were stored at 4°C–8°C and tested within 1 to 20 days of collection.

The targets detected in the cobas assay consisted of the ORF1ab and SARS-CoV-2 envelope (E) genes. In the interpretation of results from the instrument’s software, specimens positive for ORF1ab and E targets or ORF1ab alone were classified as presumptive positive. When positive for E alone, results were reported as indeterminate, and both targets resulting as negative were reported as SARS-CoV-2 undetected. Ct values for each target were collected and later analyzed to assess sensitivity.

Statistical Analyses
The study compared qualitative results between NP specimens and the participant’s matched self-collected saliva samples. Diagnostic accuracy was analyzed with a 2-by-2 table in detecting SARS-CoV-2 between the two sample types, and a Cohen’s coefficient agreement was analyzed. The symptoms were tabulated in an agreement table to view the most prevalent symptoms among the participants. Pearson coefficient was generated through a regression analysis equation.

Results
The 89 participants consisted of 38 men (42.7%) and 51 women (57.3%); the participant mean age was 48 years old (range, 21–84 years). Among the participants, the self-reported onset of infection had a median of 3 days with an upper limit of 7 days. The most common symptoms from all symptomatic participants consisted of muscle or body aches (34.8%), headache (33.7%), cough (32.6%), and fever or chills (30.3%). The remaining symptoms included difficulty breathing, fatigue, loss of taste or smell, sore throat, congestion or runny nose, nausea, and diarrhea (TABLE 1). Among symptomatic participants with a positive SARS-CoV-2 result in NP, the most common symptom consisted of loss of smell or taste (50%), nasal congestion (31.2%), diarrhea (25.0%), and difficulty breathing (25.0%) (FIGURE 1).

Eighty-nine matched specimens for both NP and saliva were found to be of sufficient volume and acceptable for testing. Saliva samples tested positive for SARS-CoV-2 among 17 participants (19.1%), and NP specimens tested positive among 19 participants (21.3%) demonstrating a statistically significant difference ($P < .0001$). Both specimen types demonstrated positive agreement for detection of SARS-CoV-2 in 15 patients. Saliva samples detected 2 positive specimens that remained undetected (negative) in the matched NP specimen. Conversely, NP specimens detected 4 positive samples that were undetected in the paired saliva samples. Sensitivity and specificity of saliva samples was calculated at 78.95% (15/19), and 97.14% (68/70), respectively. The overall agreement between the 2 tests was 93.26%, positive agreement was 89.5% (17/19), with a substantial kappa agreement of 79.1% (TABLE 2).

To analyze the correlation between paired PCR Ct values among NP specimens vs saliva samples, 2 Pearson correlation coefficients were generated for the 2 targets, ORF1ab and E. The correlation (R value) was 0.739 for ORF; the saliva deviation remained above the line of equivalence (FIGURE 2). In addition, the correlation (R value) was 0.729 for E and illustrated a saliva deviation above the line of equivalence (FIGURE 3).

The Ct values between the saliva samples and NP specimen demonstrated different values for both ORF1ab and E. Positive saliva samples targeting ORF1ab had a mean of 27.17 cycles (SD, ±4.28; range, 17.87–33.40). The positive NP specimens targeting ORF demonstrated a mean of 24.85 cycles (SD, ±5.95; range, 16.68–34.24) A statistically significant difference between the means were noted (95% CI, 0.33–4.41; $P = .026$) (FIGURE 4). In targeting the E region amongst positive saliva samples, the mean was 28.32 cycles (SD, ±4.77; range, 18.52–35.63). NP specimens targeting E had a mean of 24.76 cycles (SD, ±6.46; range, 16.56–36.85) A statistically significant difference between the means was noted (95% CI, 0.06–7.03; $P = .046$) (FIGURE 5). The paired saliva samples demonstrated a narrower
TABLE 1. Clinical Symptoms Detected in all Participants (n = 69)

| Characteristic               | Overall (%) |
|------------------------------|-------------|
| Muscle or body aches         | 31/69 (45.4) |
| Headache                     | 30/69 (34.8) |
| Cough                        | 29/69 (32.6) |
| Fever or chills              | 27/69 (31.2) |
| Sore throat                  | 27/69 (31.2) |
| Fatigue                      | 26/69 (29.2) |
| Congestion or runny nose     | 16/69 (23.1) |
| Diarrhea                     | 8/69 (9.0)   |
| Nausea or vomiting           | 6/69 (6.7)   |
| Loss of smell/taste          | 6/69 (6.7)   |
| Difficulty breathing         | 4/69 (4.5)   |

TABLE 2. Comparison of Cobas Testing Results: Nasopharyngeal vs Saliva

| Saliva specimen | Detected | Not Detected | Total |
|-----------------|----------|--------------|-------|
| Detected        | 15       | 2            | 17    |
| Not detected    | 19       | 70           | 89    |

Discussion

The study assessed the differences in test accuracy between NP specimens and self-collected saliva samples in symptomatic individuals using an FDA EUA method for detection of SARS-CoV-2. The majority of participants demonstrated symptoms, with the most commonly reported being muscle/body aches, headache, cough, and fever/chills. Saliva is shown to be an acceptable collection specimen, as patients reported no dissatisfaction or adverse events from use of the self-collection device. Overall, detection of SARS-CoV-2 in saliva was 19.1% and 21.3% in NP specimens, with overall 93.26% concordance of positive/negative results between the NP and saliva specimens. The saliva test sensitivity was calculated at 78.95%; thus, the potential for false-negative results compared to NP swabs does exist. This observation may be less relevant when the actual prevalence of SARS-CoV-2 is low; however, it does not lessen the potential adverse downstream effects of false-negative results for patient misdiagnosis, need for additional testing, and possible delays in infection control. No PCR inhibition was noted in either specimen type, PCR inhibitors present in saliva were most likely inactivated when SpectrumDNA lysis buffer was added to the sample specimen.

In this study, we sought to identify the differences in SARS-CoV-2 detection between NP swabs and saliva to prove the viability of saliva as a comparable sample. As a rule, use of Ct values for quantitative comparison of different PCR assays has been strongly discouraged due to myriad factors other than viral load that affect Ct values. However, as true SARS-CoV-2 quantitation was not available, Ct values were considered a reasonable surrogate metric for comparison as the same tests, test platforms, and testing laboratory were used in this study. The Ct values were used to compare and assess test sensitivities, based on results from positive SARS-CoV-2 RT-PCR (FIGURES 4 and 5). The higher the Ct value, the less sensitive the test, due to low viral load in saliva. Among the positive samples, saliva samples generally demonstrated a higher Ct value, indicating lower levels of SARS-CoV-2 detection in saliva specimens than NP specimens. In agreement with previous studies, we note that saliva specimens tended to have higher Ct values, whereas NP specimens demonstrated lower Ct values. A study by Hitzienbichler et al suggests that close resemblances in Ct values reflect slight analogous sensitivities. In their study, these authors were able to draw such conclusions through confirming similar median viral loads in different specimen types.
In our study, the discordant rate was 6.7% (6/89), highlighting the differences in detection based on sample type. Due to inadequate sample volumes and severe constraints on reagent availability, additional testing of discrepant saliva specimens could not be performed. Results of 4 positive NP swab specimens were considered true (standard of care testing). In 2 saliva specimens that were SARS-CoV-2 positive but had negative matched NP specimens, 1 patient was SARS-CoV-2 nucleocapsid antibody positive 13 days after PCR collection, indicating exposure had occurred at some point. In the other case, the patient was negative for influenza A/B but no other viral testing was performed, and the true cause of respiratory disease was not determined. The discordant results are likely a consequence of the medium to low viral load demonstrated in saliva specimens in this study. Our study had an overall concordance of 93.26%, thus supporting saliva as a viable specimen type for SARS-CoV-2 detection.

An advantage of our study lies in the use of the automated high-throughput cobas 6800 instrument for testing both the saliva sample and the NP specimen. Previous studies highlight the accurate detection of SARS-CoV-2 in NP swabs using the cobas method. It should be noted that saliva is not a specimen type approved under EUA for the cobas SARS-CoV-2 test, and that NP swabs are considered the specimen source for the most accurate results. Early and accurate detection of COVID-19 serves to disrupt the transmission of the virus and better aid in contact tracing. Importantly, supply chain challenges resulting in the lack of reagents and NP swabs may enhance consideration of saliva collection as a viable specimen alternative for SARS-CoV-2 detection. The noninvasive nature of saliva testing coupled with patient self-collection increases access to SARS-CoV-2 testing and limits potential SARS-CoV-2 exposure to health care workers.

This study suggests increased access and availability to reliable SARS-CoV-2 detection with diminished patient discomfort by using self-collected saliva samples. The ongoing pandemic has disproportionately affected underserved Black and Hispanic communities due to some preexisting conditions, such as crowded housing conditions and inconsistent access to health care. In the peak of the pandemic, supply shortages and limited access to COVID-19 testing contributed to inadequate testing coverage overall among minority communities. Our study demonstrates that the application of saliva testing can provide efficient and reliable results using an easily collected specimen type. Implementation of saliva testing in minority communities could potentially increase detection and contribute to contact tracing efforts and decrease the disproportionate rate of testing. This study does not assess the exact applicability of such innovation; rather, it serves to show how our findings could be beneficial to disenfranchised and minority groups. Previous studies have shown adoption of mobile testing units for HIV in underserved communities increased the access to testing and early detection for minorities and disenfranchised individuals. Therefore, mobile testing units for detecting SARS-CoV-2 by collecting saliva samples may serve to further diminish the disparate rate.

Our study has several limitations. The sample size (n = 89) was limited by the availability of viral transport media, saliva collection devices, and reagents for molecular testing in mid-2020. This affected our ability to perform testing on known SARS-CoV-2 negative individuals and additional testing on discrepant specimens. The symptoms reported, disease severity, and date of symptom onset relied on self-reporting bias, which may have affected accuracy in detection of SARS-CoV-2 symptoms. Participants in this study were limited to symptomatic individuals presenting to a drive-through COVID testing site. Although participants were instructed not to eat or drink for 30 minutes prior to saliva collection, they were not directly observed by collection staff.

**Conclusion**

In this proof-of-concept study the utility and diagnostic accuracy of self-collected saliva specimens for detection of SARS-CoV-2 among symptomatic patients compared favorably to NP specimens. Self-collection of saliva for SARS-CoV-2 testing is a viable option when...
trained health care providers are unavailable or when a lack of NP swabs exist. Furthermore, Ct values in saliva specimens are comparable to NP specimens for detection of SARS-CoV-2. Clearly, additional studies are needed to fully characterize and validate assay analytical performance with a saliva sample matrix. Future studies should aim to explore the preferred quantity of saliva needed to detect viral SARS-CoV-2 particles, which could contribute to greater concordance in crossing point thresholds between NP specimens and saliva samples. In addition, given the rapid emergence of multiple genetic variants of SARS-CoV-2, saliva testing may allow for further expansion of testing options. Adoption of saliva sampling may alleviate challenges associated with increased need for testing due to supply chain challenges noted during the ongoing COVID-19 pandemic.

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