Saliva Is a Promising Alternative Specimen for the Detection of SARS-CoV-2 in Children and Adults

Rebecca Yee,a Thao T. Truong,a Pia S. Pannaraj,b,c Natalie Eubanks,a Emily Gai,a Jaycee Jumarang,b Lauren Turner,b Ariana Peralta,b Yesun Lee,b Jennifer Dien Bard,a,b

aDepartment of Pathology and Laboratory Medicine, Children’s Hospital Los Angeles, Los Angeles, California, USA
bDepartment of Pediatrics, Division of Infectious Diseases, Children’s Hospital Los Angeles, Los Angeles, California, USA
cKeck School of Medicine, University of Southern California, Los Angeles, California, USA

Rebecca Yee and Thao T. Truong contributed equally; author order was determined based on reversed alphabetical order.

ABSTRACT Testing efforts for severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) have been burdened by the scarcity of testing materials and personal protective equipment for health care workers. The simple and painless process of saliva collection allows for widespread testing, but enthusiasm is hampered by variable performance compared to that of nasopharyngeal swab (NPS) samples. We prospectively collected paired NPS and saliva samples from a total of 300 unique adult and pediatric patients. SARS-CoV-2 RNA was detected in 32.2% (97/300) of the individuals using the TaqPath COVID-19 Combo kit (Thermo Fisher). Performance of saliva and NPS was compared against the total number of positives regardless of specimen type. The overall concordances for saliva and NPS were 91.0% (273/300) and 94.7% (284/300), respectively. The values for positive percent agreement (PPA) for saliva and NPS were 81.4% (79/97) and 89.7% (87/97), respectively. Saliva yielded detection of 10 positive cases that were negative by NPS. For symptomatic and asymptomatic pediatric patients not previously diagnosed with COVID-19, the performances of saliva and NPS were comparable (PPA, 82.4% versus 85.3%). The overall values for PPA for adults were 83.3% and 90.7% for saliva and NPS, respectively, with saliva yielding detection of 4 fewer cases than NPS. However, saliva performance for symptomatic adults was identical to NPS performance (PPA of 93.8%). With lower cost and self-collection capabilities, saliva can be an appropriate sample choice alternative to NPS for detection of SARS-CoV-2 in children and adults.

KEYWORDS COVID-19, SARS-CoV-2, nasopharyngeal swab, pediatric, saliva

Accurate and timely molecular testing for severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), the causative agent of the ongoing coronavirus disease 2019 (COVID-19) pandemic, has been crucial for informing patient management, public health decision making, contact tracing, and infection control. The Infectious Diseases Society of America (IDSA) guidelines recommend testing for SARS-CoV-2 by reverse transcriptase PCR (RT-PCR) on specimen samples which include nasopharyngeal swabs (NPS), mid-turbinate swabs, or nasal swabs rather than oropharyngeal swabs (OPS) or saliva alone (1). However, testing efforts have been hampered by supply chain shortages due to an unprecedented demand for testing materials such as swabs, universal transport media, and personal protective equipment for health care workers (2). The simplicity of saliva collection has certainly increased its interest as an alternative specimen for detection of SARS-CoV-2.

Compared to NP specimen collection, saliva is less invasive, circumvents the need for swabs, and requires minimal supervision with the option for self-collection.
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Previous studies have indicated that saliva is a promising specimen for detection of other respiratory viruses by RT-PCR, including influenza virus and common non-SARS human coronaviruses (3–5). To date, the U.S. Food and Drug Administration has issued several emergency use authorizations for laboratory-developed diagnostic tests using saliva. More recent studies have shown that use of saliva has moderate to high sensitivity and specificity compared to NPS for detection of SARS-CoV-2 in sample-to-answer as well as traditional assays that require extraction prior to PCR (6–14). These studies vary widely in sample collection method and testing platforms, and more data are needed to standardize best collection and processing practices.

There is tremendous motivation to pursue saliva collection from children, not only because of the simplicity in specimen collection but to also avoid the unnecessary discomfort of nasopharyngeal swab collection. There is also huge interest in saliva as a primary specimen type to detect SARS-CoV-2 during the school year. Hence, it is important to understand the dynamics of viral detection in children, which has implications for their contribution to transmission of SARS-CoV-2. Unfortunately, data on the use of saliva to detect SARS-CoV-2 in pediatric patients are sparse. The few reports available on the performance of saliva specimens for children showed poor detection of SARS-CoV-2, with sensitivities of 53 to 73%; however, such studies suffer from small sample sizes (15–17). In this study, we evaluated and compared prospectively collected paired saliva and NP specimens from both pediatric and adult patients for detection of SARS-CoV-2. We also compared the differences in viral load in asymptomatic and symptomatic COVID-19 patients.

**MATERIALS AND METHODS**

**Study design.** A total of 300 unique patients (inpatients, outpatients, and household members of diagnosed COVID-19 patients) were enrolled in this study between 8 June and 28 August 2020. Demographic data, including age, gender, and symptoms, were collected. Participants were asked if they had previously tested positive for COVID-19. Paired samples were collected from individuals with unknown COVID-19 status as well as from patients previously positive for SARS-CoV-2. Both symptomatic and asymptomatic patients were enrolled in the study. Study design conducted at Children’s Hospital Los Angeles was approved by the Institutional Review Board (IRB) under IRB numbers CHLA-20-00124 and CHLA-18-00098.

**Sample collection.** At least 3 ml of saliva was self-collected under the observation of a health care worker who subsequently collected an NPS sample for parallel testing. Patients were instructed to avoid eating, drinking, smoking, chewing gum, and chewing tobacco 30 min prior to collection. They were asked to work up saliva by gently rubbing the outside of their cheeks and gently spitting without coughing or clearing their throats. Saliva was collected in a sterile cup and NPS were immediately placed in viral transport medium (Becton Dickinson, Franklin Lakes, NJ). Samples were either sent to the clinical laboratory within 1 h from collection or stored at 4°C and sent to the clinical laboratory within 4 h from collection. Samples were stored at 4°C and tested within 48 h from collection or stored at −80°C prior to testing.

**qRT-PCR assay for SARS-CoV-2 RNA.** Paired nasopharyngeal swabs and saliva samples were sent to the Clinical Virology Laboratory at Children’s Hospital Los Angeles. Total nucleic acid was extracted from 250 μl of undiluted saliva samples using the Thermo Fisher KingFisher Flex specimen processing system with the Applied Biosystems MagMAX viral/pathogen nucleic acid isolation kit (Thermo Fisher, Waltham, MA) and eluted to 50 μl of total nucleic acid. Real-time quantitative reverse transcriptase PCR (qRT-PCR) was performed using the TaqPath COVID-19 Combo kit (Thermo Fisher). A positive result for SARS-CoV-2 detection was determined by amplification of at least one of the three genes targeted (N gene, S gene, or ORF1ab gene) using a cutoff threshold cycle (C_T) value of <40. When multiple targets were detected in a sample, the C_T values for those targets were averaged (18). When a single target was positive, the exact C_T value was used. A valid negative result for SARS-CoV-2 detection was determined by amplification of MS2 internal control using a cutoff C_T value of <32.

**Data and statistical analysis.** A composite gold standard approach was used to determine a true positive case. Any positive detected from either NPS or saliva was considered a true positive, and positive percent agreement (PPA) and negative percent agreement (NPA) were calculated based on this. Statistical analyses comparing different C_T values and days between onset of symptoms and test date were performed using a Mann-Whitney test.

**RESULTS**

During an 11-week period (8 June to 28 August 2020), SARS-CoV-2 RNA was detected in a total of 97 out of 300 individuals, of which 43 (44.3%) were <19 years of age. The median ages were 37.5 years (range, 19 to 58) and 12 years (range, 4 to 18) in
our adult and pediatric COVID-19 positive cohorts, respectively. A female predominance was noted (61/97 [62.9%]). Of the 97 COVID-19-positive patients, 55 (56.7%) were symptomatic at the time of collection, with a median of 10 days between symptom onset and time of collection. Twenty-seven (27.8%) patients were known to be positive for SARS-CoV-2 prior to enrollment. Since individuals in entire households were enrolled, it was not surprising that an overwhelming proportion of our cohort (73/97 [75.3%]) reported exposure to a COVID-19-positive individual.

The overall concordances of saliva and NPS were 91.0% (273/300) and 94.7% (284/300), respectively. When analyzing all 97 positive patients, it was determined that SARS-CoV-2 RNA was detected from both NPS and saliva for 69 patients, from saliva only for 10 patients, and from NPS only for 18 patients. The overall values for PPA for saliva and NPS were 81.4% (79/97) and 89.7% (87/97), respectively, compared to a total number of positive cases identified by RT-PCR (Table 1). The NPA was 100% for both specimen types.

Focusing on pediatric patients only, the overall values for PPA were 79.1% for saliva and 88.4% for NPS collected. Performance of saliva (PPA, 82.4%) and NPS (PPA, 85.3%) were comparable when only first-time-positive pediatric patients were analyzed for both symptomatic and asymptomatic patients. Specifically, testing using saliva detected the same number of COVID-19 cases as NPS (both at 78.6%) in the asymptomatic pediatric cohort and missed only one positive case (85% versus 90%) in the symptomatic cohort (Table 2). The performance of saliva remained high for both young and older children. In children ages 4 to 10 years, saliva and NPS achieved PPA of 83.3%. Additionally, saliva was able to capture all 6/6 (100%) symptomatic patients in this age group, as opposed to the 5/6 (83.3%) for NPS. In patients between 11 and 18 years old, one positive case was missed by saliva (PPA, 81.8% versus 86.4%), but the performance was superior when testing only asymptomatic patients (PPA, 87.5% versus 75.0%), with detection of an additional case (Table 2).

For adult patients, the overall values for PPA were 83.3% and 90.7% for saliva and NPS, respectively. In contrast to the pediatric data, saliva performed better for symptomatic patients, with identical PPA to NPS (93.8%), but poorly for asymptomatic adults

| Sample group and type | First-time positives | All positives |
|-----------------------|----------------------|--------------|
|                        | n                    |             |
| All samples            |                      |             |
| Saliva [no. (%)]       | 70                   | 97           |
| NP [no. (%)]           | 62 (88.6)            | 87 (89.7)    |
| Pediatric (all ages)   |                      |             |
| n                      | 34                   | 43           |
| Saliva [no. (%)]       | 28 (82.4)            | 34 (79.1)    |
| NP [no. (%)]           | 29 (85.3)            | 38 (88.4)    |
| <10 yrs                |                      |             |
| n                      | 12                   | 15           |
| Saliva [no. (%)]       | 10 (83.3)            | 12 (80.0)    |
| NP [no. (%)]           | 10 (83.3)            | 13 (86.7)    |
| 11–18 yrs              |                      |             |
| n                      | 22                   | 28           |
| Saliva [no. (%)]       | 18 (81.8)            | 22 (78.6)    |
| NP [no. (%)]           | 19 (86.4)            | 25 (89.3)    |
| Adult                  |                      |             |
| n                      | 36                   | 54           |
| Saliva [no. (%)]       | 29 (80.6)            | 45 (83.3)    |
| NP [no. (%)]           | 33 (91.7)            | 49 (90.7)    |
(PPA, 68.2% versus 86.4). Findings were comparable even when only first-time-positive patients were analyzed (Tables 1 and 2).

The average differences in Ct values between saliva and NPS samples were not statistically different (Ct, 28.7 versus 29.1) (Fig. 1A and B). Based on linear regression analy-
sis in which \( y \) values of saliva (y axis) are plotted against the \( x \) values of NPS (x axis) from the paired sample, the equation \( y = 0.9994x \) suggests that \( y \) values from the two sample types are approximately equivalent to one another (Fig. 1C). In addition, the \( y \) values of saliva and NPS samples remain comparable regardless of age and disease status (symptomatic versus asymptomatic) (Fig. 2).

Importantly, SARS-CoV-2 RNA was detected for 28 (28.9%) patients in only one sample type (10 saliva samples and 18 NPS). Most of these patients were older than 10 years (25/28 [89.3%]) (see Table S1 in the supplemental material). Saliva-only positive patients were tested ranging from 3 to 43 days post-symptom onset, compared to 7 to 31 days post-symptom onset for NPS-only positive patients. The overall \( y \) values between saliva-only and NPS-only positives were comparable (\( y \)s of 32.4 versus 32.5), with 88.8% (NPS-positive only) and 80% (saliva-positive only) of the samples having a \( y \) of over 30 (Fig. 3).

The average \( y \) value derived from cases detected by both saliva and NPS was lower than when only one sample type was positive \( (y, 28.9 \text{ versus } 32.4; P < 0.001) \). Symptomatic patients were more likely to have SARS-CoV-2 RNA detected from both sample types \((\text{OR} = 3.37; P = 0.01)\).

**DISCUSSION**

Testing saliva specimens can circumvent the shortage of collection supplies and may be a sufficient noninvasive and more cost-effective alternative for SARS-CoV-2 testing (4). The sensitivity of saliva for detection of SARS-CoV-2 has been shown to be less than that of NPS in other studies, ranging from 72% to 86% (19, 20). We demonstrated an overall PPA of 81.4% in saliva versus 89.7% in NPS in our entire cohort. Comparable performance of saliva to NPS was shown for children who were previously unknown positive patients (both symptomatic and asymptomatic patients) and also for symptomatic adults only. To our knowledge, this is the first and largest study dem-

**FIG 2** \( C_T \) values from saliva and nasopharyngeal swab specimens collected from our SARS-CoV-2-positive asymptomatic (open circles) and symptomatic (filled circles) patients in our adult (A) populations and pediatric cohort (B).

**FIG 3** \( C_T \) values of adult (A) and pediatric (B) patients tested positive by both nasopharyngeal swab and saliva, nasopharyngeal swab only, and saliva only are depicted in reference to when they were tested since symptom onset (days).
onstrating support for utilization of saliva in the pediatric age group and comparison of performance of saliva between pediatric and adult cohorts.

It is important to note that testing of saliva caught 10 additional COVID-19 cases that were negative by NPS. Our findings are consistent with results from other studies demonstrating how saliva specimens can identify otherwise missed cases of not only COVID-19 but also influenza and respiratory syncytial virus (RSV) (4, 6, 20). In this study, of the 18 cases that were detected by NPS only, 7 (38.9%) were in asymptomatic adults, a subpopulation that performed poorly with detection of SARS-CoV-2 in saliva. Additionally, over 80% of NPS-positive-only patients exhibited Ct values past 30.0, suggesting that false negatives are attributable to lower viral loads. Additionally, our study showed that the performance of saliva is not dependent on age, which is corroborated by recent studies which also reported that age had no impact on viral load and detection of SARS-CoV-2 (17, 21), including in pediatric populations.

While some studies argue that viral load is highest in saliva within the first week of symptom onset, others have shown that saliva can be more sensitive than NPS throughout the course of infection or sometimes produces intermittent positive results over the course of a few weeks (22). A small, longitudinal pediatric study from South Korea found that SARS-CoV-2 RNA was more readily detected from saliva within the first few days of symptom onset, followed by a drastic decline in viral load compared to that in NPS (16). In contrast, we report the detection of SARS-CoV-2 in saliva for up to 43 days, compared to 32 days for NPS.

While several studies have shown that NPS yield lower Ct values than saliva for symptomatic adult patients (8, 10, 11), we report no significant difference in Ct values between saliva and NPS for either our adult or pediatric patients. Our findings corroborate a recent study of 19 adults that reported no significant differences (7). Interestingly, a recent study demonstrated that for adult populations, performance of saliva was better than that of NPS in detecting SARS-CoV-2 in asymptomatic individuals, but our results suggest that saliva was a poor alternative to NPS for asymptomatic adults, missing 4 cases that were NPS positive (23). However, it must be noted that in our cohort of older children (11 to 18 years old), the performance of saliva was superior to that of NPS for detection in first-time-positive asymptomatic individuals. The conflicting findings between studies may be due to differences in saliva collection protocol, collection device, and age of patient (13). Inadequate specimen collection of either NPS or saliva may also contribute to false-negative results. We were able to demonstrate comparable detection of a human gene internal control (RNase P) in 127 paired NPS (mean C<sub>t</sub>, 23.8; range, 21.3 to 29.0) and saliva (mean C<sub>t</sub>, 21.5; range, 17.9 to 26.3) samples using an alternate SARS-CoV-2 RT-PCR assay (data not shown), suggesting adequate specimen collection in our cohort. Additionally, there are inherent difficulties in working with a more viscous sample that may be more prone to more sampling variabilities. In our hands, the number of invalid results were minimal and did not require dilution of saliva samples with a buffer, as has been reported in other studies (9, 10, 13). Such differences in methodology may account for the variability in the performance of saliva reported in other studies. A more thorough comparison and standardization of saliva collection and processing needs to be evaluated.

Limitations of this study include the small sample size of both children, particularly younger children, and adults from a single medical institution. Also, this study included only outpatients, patients admitted to the emergency department, and family members who volunteered to enroll in the study, which can bias our findings regarding the role of COVID-19 exposure in specimen performance. Since viral load may or may not be correlated with clinical manifestations, further studies should be conducted in inpatient or intensive care unit (ICU) settings, as the spectrum of disease ranges from asymptomatic to causing severe illness (24–26). Finally, despite a standardized protocol utilized during the collection of the saliva samples, it can be challenging for children to properly salivate into a collection device. The volume of saliva obtained may also vary
among patients due to excessive bubbles and other factors despite the same amount of saliva being processed for testing.

Conclusions. Our study reveals that saliva is a reliable diagnostic specimen for the detection of SARS-CoV-2 RNA by RT-PCR, particularly for both symptomatic and asymptomatic children and symptomatic adults. Moreover, saliva was able to identify additional COVID-19 cases that were otherwise missed by NPS. With saliva collection being more cost-effective and noninvasive, it offers a feasible approach for widespread testing of SARS-CoV-2 in inpatient settings and in the community.

SUPPLEMENTAL MATERIAL
Supplemental material is available online only.

SUPPLEMENTAL FILE 1, PDF file, 0.1 MB

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All authors declare no conflict of interest.

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