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Saliva versus Upper Respiratory Swabs

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Equivalent for Severe Acute Respiratory Syndrome Coronavirus 2 University Screening while Saliva Positivity Is Prolonged After Symptom Onset in Coronavirus Disease 2019 Hospitalized Patients

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Reopening of schools and workplaces during the ongoing coronavirus disease 2019 (COVID-19) pandemic requires affordable and convenient population-wide screening methods. Although upper respiratory swab is considered the preferable specimen for testing, saliva offers several advantages, such as easier collection and lower cost. In this study, we compared the performance of saliva with upper respiratory swab for severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) detection. Paired saliva and anterior nares specimens were collected from a largely asymptomatic cohort of students, faculty, and staff from the University of Pennsylvania. Paired saliva and combined nasopharyngeal/oropharyngeal (NP/OP) specimens were also collected from hospitalized patients with symptomatic COVID-19 following confirmatory testing. All study samples were tested by real-time PCR in the Hospital of the University of Pennsylvania. In the university cohort, positivity rates were 37 of 2500 for saliva (sensitivity, 86.1%) and 36 of 2500 for anterior nares (sensitivity, 83.7%), with an overall agreement of 99.6%. In the hospital study cohort, positivity rates were 35 of 49 for saliva (sensitivity, 89.3%) and 28 of 49 for NP/OP (sensitivity, 75.8%), with an overall agreement of 75.6%. A larger proportion of saliva than NP/OP samples tested positive after 4 days of symptom onset in hospitalized patients. Our results show that saliva has an acceptable sensitivity and is comparable to upper respiratory swab, supporting the use of saliva for SARS-CoV-2 detection in both symptomatic and asymptomatic populations. *(J Mol Diagn 2022, 24: 727—737; https://doi.org/10.1016/j.jmoldx.2022.03.012)*

In early 2020, as the coronavirus disease 2019 (COVID-19) pandemic spread rampantly across the world, mitigation efforts, such as stay-at-home orders, were put in place to halt the progression of the virus. As schools and workplaces began to reopen in many parts of the world during the summer of 2020, affordable and convenient population-wide screening methods became ever more necessary.[1] University populations were of specific concern, with a high concentration of individuals living and studying in close quarters and being active in the surrounding communities. Successful strategies to prevent infection at universities included a well-designed plan for screening, contract tracing, isolation, and compliance with policies.[2—4] A quick,

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yet robust, method for collection and testing was necessary to meet the optimal screening strategy.

Although upper respiratory swabs [nasopharyngeal (NP), oropharyngeal (OP), midturbinate, and anterior nares (AN)] have been considered the preferable specimens for testing, several factors, such as supply shortage, need for trained personnel, technical difficulties, variability with specimen collection, patient discomfort, and risk of exposure to health care workers, remain a limitation for scalability.[1] Many studies have assessed the performance of saliva as an alternative specimen because of its ease of collection and low cost. Because of the noninvasiveness and simplicity of saliva collection, patients can self-collect at either a collection site or home with minimal risk. Unlike swab specimen collection, saliva can be collected in a sterile, nuclease-free tube and be stable at room temperature for extended periods without stabilizing additives.[5,6] In addition, studies have shown that saliva is a good biological fluid for testing because of contact with the oral mucosa and salivary glands, which have high expression of the angiotensin-converting enzyme 2 receptor that binds severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2).[7]

Although saliva proves to be an acceptable specimen, several studies show conflicting results about its performance in comparison to respiratory swab specimens. The reported clinical sensitivity of saliva compared with NP swab ranges widely, from 60.7% to 100%.[6,8–30] Some studies have reported that saliva has a significantly lower detection rate than swab specimens, whereas others show similar detection rates between the two specimens.[8,10,11,15,19,25,28,31] Saliva specimens could generate a false-negative result in asymptomatic individuals, when collected later in the disease course in symptomatic patients, or when using testing methods that lack an RNA extraction step.[20,31] From the technical side, saliva can be difficult to manipulate because of variable viscosity, which complicates automation of sample manipulation and PCR amplification.[1,15,17,32]

This study investigated the performance of saliva for SARS-CoV-2 detection, with the ultimate goal to use saliva as an alternative specimen for a large-scale student screening program at our university. The sensitivity and specificity of saliva was compared with upper respiratory swab in two settings: screening of a largely asymptomatic university population and monitoring of hospitalized patients with symptomatic COVID-19.

Materials and Methods

Collection

University Samples

Paired AN and saliva specimens were collected from students, faculty, and staff from the University of Pennsylvania under institutional review board protocol number 844126 from October to December 2020. AN specimens were obtained by a health care provider in 0.9% saline or viral transport medium. Saliva was self-collected into a sterile 5-mL tube containing no preservatives with health care worker observation following verbal consent to participate in the study. There were no restrictions with regard to eating, drinking, smoking, or brushing the teeth before testing. Saliva samples were not diluted. Specimens were transported via courier to the testing laboratory at the Hospital of the University of Pennsylvania and tested within 48 hours or frozen at −80°C until tested.

Hospitalized Patient Samples

Following informed consent under institutional review board protocol number 823392, approved by the University of Pennsylvania institutional review board, paired saliva and combined NP/OP specimens (paired study test) were collected from 37 SARS-CoV-2−infected patients admitted to Hospital of the University of Pennsylvania's inpatient unit from July 2020 to January 2021. All patients were symptomatic and had the diagnosis of COVID-19 confirmed on or before admission with nucleic acid amplification by PCR of an NP swab specimen; the date of this first positive test was considered as the date of diagnosis. The following data were collected for each patient: age, sex, race (self-reported), date of symptom onset, date of diagnosis (first positive test), date of paired study test, date of admission, date of discharge, and survival status at discharge. Enrollment was limited to patients who could spontaneously produce saliva (eg, hospitalized but nonintubated; score of 4 to 6 on the World Health Organization scale).[33] Saliva was self-collected into a sterile specimen container, containing no preservatives, with health care worker observation. Viscous saliva samples were diluted 1:1 with lavage saline before testing. Contemporaneous NP/OP swabs were collected using flocked swabs (Copan) by a health care professional following CDC guidelines (*https://www.cdc.gov/coronavirus/2019-nCoV/lab/guidelines-clinical-specimens.html*, updated October 25, 2021, last accessed January 17, 2021). NP and OP specimens were combined in a single tube with viral transport media. Twenty-five subjects provided one set of paired saliva and NP/OP swab samples, and 12 subjects provided two sets of paired samples several days apart (49 sets total). Saliva and NP/OP viral transport medium samples were frozen within 2 hours of collection and stored at −80°C until tested.

Testing of Paired Specimens

AN specimens from University of Pennsylvania were run on Emergency Use Authorization (EUA) PCR-based methods at Hospital of the University of Pennsylvania for screening purposes. A variety of PCR-based assays were acquired at Hospital of the University of Pennsylvania at the beginning of the pandemic in an effort to increase capacity for SARS-CoV-2 testing. Therefore, AN samples were tested by different methods, depending on their availability during routine testing in the clinical laboratory. The following EUA

Table 1 Summary of Gene Detection and Ct Cutoff for Testing Methods Used

Gene	Abbott m2000	Roche cobas 6800	Thermo TaqPath	DiaSorin simplexa	GenMark ePlex	Fluidigm AdvantaDx*
N	++		+		+	
N1						+
N2						+
S			+	+		
E		+				
ORF1ab		+	+	+		
LOD	28	35	37	32	N/A	26
Ct cutoff	31.5	42	40	40	N/A	32
Sample types tested	AN	AN	AN, NP/OP	AN	AN	Saliva

*All saliva samples were tested with this method.

+, gene targets detectable; AN, anterior nares; LOD, limit of detection; N/A, this information is not publicly available; NP, nasopharyngeal; OP, oropharyngeal.

PCR-based assays were used for testing AN specimens, according to the instructions for use: ePlex SARS-CoV-2 Test (GenMark Diagnostics, Inc., Carlsbad, CA), TaqPath COVID-19 Combo Kit (Life Technologies Corp., Pleasanton, CA), cobas SARS-CoV-2 on the 6800 (Roche Diagnostics, Indianapolis, IN), Simplexa COVID-19 Direct (DiaSorin Molecular LLC, Cypress, CA), or Abbott RealTime SARS-CoV-2 (Abbott Molecular Inc., Des Plainer, IL). All the EUA platforms qualitatively detect either multiple targets on a single gene or one or more of the following genes in SARS-CoV-2 genome: *N* gene, *S* gene, *ORF1ab*, and/or *E* gene (Table 1). Detection of SARS-CoV-2 was determined by each manufacturer's algorithms.

NP/OP hospitalized patient specimens were tested using the EUA-approved TaqPath COVID-19 Combo Kit. The NP/OP specimens were extracted with the KingFisher Flex Magnetic Particle Processor with 96 Deep-Well Head (Life Technologies Corp.) using the protocol of MagMAX Viral/Pathogen II Nucleic Acid Isolation Kit (Life Technologies Corp.) with a sample input volume of 200 μL. After extraction, the TaqPath COVID-19 Combo Kit assay was prepared manually and loaded onto either an Applied Biosystems 7500, 7500 Fast, or 7500 FastDx Real-Time PCR Systems (Applied Biosystems, Waltham, MA) for amplification. Positivity of SARS-CoV-2 was based on the detection of three genes: *N* gene, *S* gene, and *ORF1ab*. The EUA COVID-19 Interpretive Software (v1.3 or v1.5; Applied Biosystems) was used to analyze the raw data and produce an overall interpretative result.

All saliva specimens were tested using the EUA-approved Advanta Dx SARS-CoV-2 RT-PCR Assay (Fluidigm Corp., South San Francisco, CA). After inactivation, the specimens were processed through a pre-amplification of the N1 and N2 targeted genes and *RNase P* reference gene. Of note, this assay does not have a nucleic extraction or a reverse transcription step. Following pre-amplification, the specimens were diluted and mixed with master mix. The specimen mix and primer mix of each N1, N2, and *RNase P* were loaded into individual inlets on an integrated fluid circuit (IFC) chip. The IFC chip was loaded onto Juno or IFC Controller RX and then placed onto the Biomark HD (Fluidigm Corp.), where the assay detects SARS-CoV-2 by

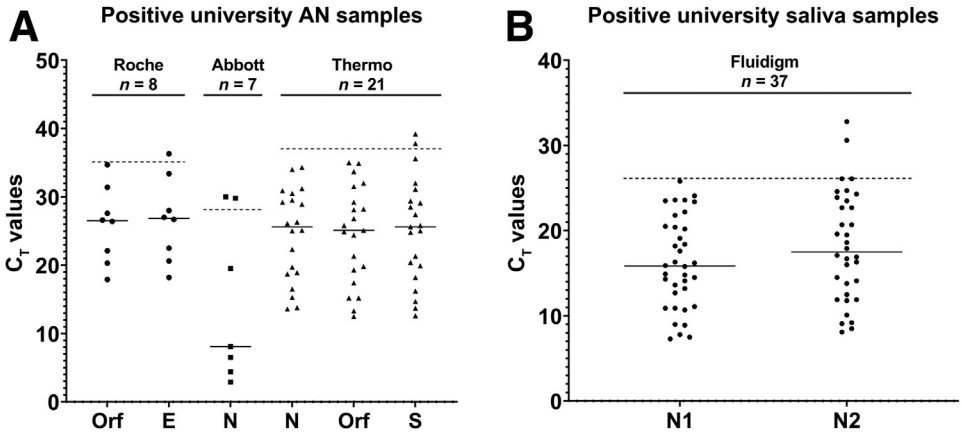

Figure 1 Positive university sample C_T ranges. C_T values for positive anterior nares (AN; **A**) and saliva (**B**) samples are shown, with the median represented by a **solid line**. C_T values are presented by gene targets on each assay. The limit of detection of each assay is represented by a **dashed line**.

RT-PCR amplification. Advanta Dx SARS-CoV-2 EUA Interpretive Software (v4.5.2 or v4.7.1; Fluidigm Corp.) was used to analyze and to produce an overall interpretative result based on cycle threshold (C_T) values and reproducible replicates. Inconclusive results were excluded from the study as each inconclusive result is defined differently by each method and not directly comparable (ie, loading error versus 1 of 3 targets detected). Samples were run according to the Fluidigm EUA, except for the use of liquid handlers to automate technically challenging and time-consuming steps. Liquid handlers (Perkin-Elmer, Waltham, MA) were utilized for specimen aliquoting, pre-amplification plate setup, diluting pre-amplified material, and IFC loading. Protocols for the liquid handlers were customized by PerkinElmer following the Advanta Dx SARS-CoV-2 RT-PCR Assay EUA instructions for use. Assay volumes were modified to accommodate dead volume for the liquid handlers, but ratios were not changed.

C_T Values

C_T values were obtainable from all testing platforms, except the ePlex SARS-CoV-2 Test. C_T values between the assays are not directly comparable because of inherent differences in the assay designs. The Abbott RealTi*m*e SARS-CoV-2 assay detects two targets on the *N* gene with the same fluorophore, thus inflating the single C_T value reported by this assay. The remaining assays result in a single C_T value for each target. A pre-amplification in the Fluidigm Advanta Dx SARS-CoV-2 RT-PCR Assay results in lower C_T values than standard RT-PCR without pre-amplification. Therefore, C_T values were used to identify samples near an assay's limit of detection (LOD) or cutoff, but not for direct comparison between assays. The LOD and cutoff for each assay was either stated in the EUA or an approximate number based on correspondence with vendor of assay, as these exact values are often obtained through proprietary interpretive algorithms (Table 1).

Statistical Analysis

Characteristics of hospitalized patients were analyzed for descriptive statistics and displayed as number and percentage for categorical variables and mean ± SD or median (range) for continuous variables. The McNemar test was used to compare the detection rate for NP/OP and saliva in hospitalized patients. The clinical sensitivity and specificity with 95% CIs were calculated for the detection of SARS-CoV-2 in swab and saliva using the opposite specimen type as reference method.[34] Following discordant sample resolution, the following formulas were employed: sensitivity = true positives ÷ (true positives + false negatives); and specificity = true negatives ÷ (true negatives + false positives). Exact Cochran-Armitage Test for Trend was used to analyze positivity rates over time for nonrepeated measures in the hospitalized cohort (using only the first test results per subject). Statistical analysis was performed using Prism 9 (GraphPad Software, San Diego, CA) and SAS software 9.4 (SAS Institute Inc., Cary, NC).

Results

University Samples

A total of 2500 paired samples (AN and saliva) were collected from participants from October to December 2020. Of the 2500 pairs, approximately 150 pairs were from participants who were symptomatic (approximately 6%). The remaining pairs were from asymptomatic participants. In this study population, the SARS-CoV-2 detection rate was approximately 1% for both AN and saliva (36/2500 and 37/2500, respectively). Figure 1 shows the range of C_T values for each gene target from positive samples by AN and saliva; C_T values ranged from 2.9 to 39.2 for AN and from 7.3 to 32.8 for saliva across all genes.

An overall agreement of 99.6% (2489/2500) was observed between AN and saliva specimens (Table 2). Using AN as the reference standard, the sensitivity and specificity of saliva were 86.1% (95% CI, 73.9%−98.3%) and 99.8% (95% CI, 99.6%−100%), respectively. Alternatively, using saliva as the reference standard, the sensitivity and specificity of AN were 83.7% (95% CI, 70.8%−96.8%) and 99.8% (95% CI, 99.6%−100%), respectively. The calculated sensitivity and specificity of AN and saliva show that both specimen types perform similarly in detecting SARS-CoV-2.

Most AN samples (2497/2500) were run on either the Thermo TaqPath (n = 1570), Abbott m2000 (n = 414), or

Table 2 Concordance of SARS-CoV-2 Results on Paired Anterior Nares and Saliva in the University Population

	Anterior nares		
Saliva	Positive	Negative	Total
Positive	31	6	37
Negative	5	2458	2463
Total	36	2464	2500
Overall concordance, %	99.6		
Saliva sensitivity, %	86.1 (95% CI, 73.9−98.3)		
Saliva specificity, %	99.8 (95% CI, 99.6−100)		
Anterior nares sensitivity, %	83.7 (95% CI, 70.8−96.8)		
Anterior nares specificity, %	99.8 (95% CI, 99.6−100)		

Table 3 Concordance of SARS-CoV-2 Results by Individualized Anterior Nares Assays

	Anterior nares assays					
	Thermo TaqPath		Abbott m2000		Roche cobas 6800	
Saliva	Positive	Negative	Positive	Negative	Positive	Negative
Positive	18	5	5	1	8	0
Negative	3	1544	2	406	0	505
Overall concordance, %	99.5		99.3		100	
Saliva sensitivity, %	85.7 (95% CI, 69.6–101)		71.4 (95% CI, 31.8–111)		100	
Saliva specificity, %	99.7 (95% CI, 99.5–99.9)		99.8 (95% CI, 99.6–100)		100	

Roche cobas for the 6800 of 8800 ($n = 513$). Concordance, sensitivity, and specificity were calculated individually to compare saliva with each assay (Table 3). Although the overall concordance and specificity were similar compared with all assays, the sensitivity of saliva varied (71.4% to 100%) depending on the comparator assay.

The discordant samples are outlined in Table 4. There were 11 total discordant samples, five of which were positive by AN and negative by saliva (samples 1 to 5) and six of which were negative by AN and positive by saliva (samples 6 to 11). Two of the five positive AN samples that resulted negative by saliva had C_T values near the LOD of the AN assay (samples 1 and 4). The remaining three positive AN samples that resulted negative by saliva were repeated again, with negative saliva results. This suggests five false negatives by the saliva assay (samples 1 to 5). Two discordant samples (samples 6 and 9) were negative by AN and classified as false positives by saliva. Three discordant samples (samples 7, 10, and 11) were negative by AN but positive by saliva and were all classified as saliva true positives; these saliva samples were repeated multiple times, with reproducible positive results. Participant 11 had a positive AN 1 and 2 days following the saliva-positive–AN-negative pair, indicating the saliva sample was a true positive. Sample 8 resulted negative by AN but had C_T values near the LOD of the saliva assay, which was interpreted as a saliva true positive.

To attempt a direct comparison between specimens, a portion of saliva and swab samples was quantified in copies/μL against a standard curve of the ATCC (Manassas, VA) control (VR-3280SD) on the Fluidigm and TaqPath assays. Twenty positive saliva and five positive swab samples were used; because of sample limitations, not all positive samples were able to be quantified. Quantified samples ranged from 1 to approximately 131,000 copies/μL. Of the four sample pairs, two samples had comparable viral loads, one sample had a lower viral load in saliva, and one sample had a lower viral load in the

Table 4 Description of Discordant Results between Anterior Nares and Saliva

	Nares		Saliva				
Sample	Result	Positive replicates	Result	Positive replicates	Food/drink	Notes about discordance	Discordant classification
1	Positive	1/1	Negative	0/6	No	AN: positive with high Ct	Saliva false negative
2	Positive	1/1	Negative	0/2	No	AN: positive with low Ct	Saliva false negative
3	Positive	2/2	Negative	0/1	UKN	AN: positive with low Ct in both replicates	Saliva false negative
4	Positive	1/1	Negative	0/5	Yes	AN: positive with high Ct	Saliva false negative
5	Positive	2/2	Negative	0/3	UKN	AN: participant tested 2 days later with positive result	Saliva false negative
6	Negative	0/1	Positive	1/3	Yes	Saliva: positive with detection in 1 of 2 *N* gene targets	Saliva false positive
7	Negative	0/1	Positive	3/4	No	Saliva: positive with low Ct in 3 of 4 replicates	Saliva true positive
8	Negative	0/1	Positive	2/2	UKN	Saliva: positive with high Ct	Saliva true positive
9	Negative	0/2	Positive	1/1	UKN	AN: participant tested 6 days later with negative result	Saliva false positive
10	Negative	0/2	Positive	4/4	UKN	Saliva: positive with low Ct in all replicates	Saliva true positive
11	Negative	0/1	Positive	4/4	Yes	AN: participant tested 1 and 2 days later with positive results Saliva: positive with high Ct	Saliva true positive

AN, anterior nares; UKN, unknown.

Table 5 Characteristics of 37 Hospitalized COVID-19 Patients

Characteristic	Value
Age, median (range), years	57.9 (28 to 82)
Sex, *n* (%)	
Male	22 (59)
Female	15 (41)
Race, *n* (%)	
Asian	3 (8)
Black	21 (57)
White	13 (35)
Time from diagnosis to paired study test, mean ± SD (range), days	4.2 ± 3.1 (0 to 11)*
Time from symptom onset to paired study test, mean ± SD (range), days	9.2 ± 3.6 (1 to 17)*
Time from symptom onset to admission, mean ± SD (range), days	6.3 ± 3.4 (0 to 14)
Length of hospital stay, mean ± SD (range), days	6.5 ± 4.1 (1 to 21)

*Calculation based on 49 samples.

swab sample. The number of quantified paired samples ($n = 4$) was too low to draw a true correlation conclusion. Any variability could be due to poor sample collection or biological difference.

Discordant samples 7 and 10 had low viral loads (1 and 20 copies/μL) in the saliva sample, confirming our classification of true saliva positives. These low viral loads are close to the LOD and, given biological differences, could explain the negative result in the paired swab.

Overall, five samples were most likely saliva false negatives, two samples were most likely saliva false positives, and four samples were most likely saliva true positives (AN false negatives). Both specimen types produced a similar small number of likely false negatives, suggesting neither specimen type to be overtly better than the other. After determining the most likely resolution of these discordant samples, the resolved sensitivity and specificity of saliva were 87.5% (95% CI, 76.5%−98.5%) and 99.9% (95% CI, 99.8%−100%), respectively, and the resolved sensitivity and specificity of AN were 90.0% (95% CI, 80.2%−99.8%) and 100%, respectively. These differences in sensitivity were not statistically significant.

Hospitalized Patient Samples

At least one paired NP/OP-saliva sample was obtained from 37 hospitalized patients. A second paired sample was obtained from 12 patients later during their admission, resulting in a total of 49 paired NP/OP-saliva samples (25 patients with one sample and 12 patients with two samples). The mean time from symptom onset to paired NP/OP-saliva testing was 9.2 days (range, 1 to 17 days) and from diagnosis (first positive NP test) to paired NP/OP-saliva testing was 4.2 days (range, 0 to 11 days). The mean length of stay in the hospital was 6.5 days (range, 1 to 21 days), and all the patients were discharged alive (Table 5).

Positivity for SARS-CoV-2 was seen in 57.1% (28/49) of all NP/OP samples and 71.4% (35/49) of all saliva samples. Testing results were inconclusive in 8.2% (4/49) of NP/OP samples. Figure 2 shows the range of C_T values for each gene target from positive samples by NP/OP and saliva; the range of C_T values was 16 to 40 for NP/OP samples and 5.9 to 27.8 for saliva samples. When excluding samples with inconclusive results, the overall concordance between NP/OP and saliva was 75.6% (95% CI, 61.3%−85.8%). Using NP/OP as reference standard, sensitivity of saliva was 89.3% (95% CI, 77.2%−100.0%) and specificity of saliva was 52.9% (95% CI, 20.3%−85.6%) (Table 6). Alternatively, using saliva as the reference standard, the sensitivity and specificity of NP/OP were 75.8% (95% CI, 58.9%−92.6%) and 75.0% (95% CI, 46.7%−100.0%), respectively, when excluding samples with inconclusive results. Although the proportion of positive results was the same in NP/OP and saliva within the first 4 days of symptom onset (100%), there was a larger proportion of saliva samples testing positive at 5 to 8, 9 to 12, and after 13 days of symptom onset (Figure 3); these differences, however, were not statistically significant. Although positivity rates of NP/OP and saliva decreased with longer symptom onset (Figure 3), this trend was not statistically significant ($P = 0.20$ for saliva, and $P = 0.12$ for NP/OP).

Discordant samples were further investigated to determine potential causes for disagreement (Table 7). There were 15 total discordant samples, three of which were positive in NP/OP and negative in saliva (samples 1 to 3) and eight of which were negative in NP/OP and positive in saliva (samples 8 to 15). Four inconclusive samples in NP/OP corresponded with two positives and two negatives in saliva (samples 4 to 7). Inconclusive results on the NP/OP samples represent a sample with low positivity (1 of 3 SARS-CoV-2 gene targets detected); variability in detection is expected in these low positive specimens. The three positive NP/OP results were negative in saliva because of dilution of the saliva (1:1 dilution with saline). Six of the negative NP/OP samples were collected 8 to 15 days after symptom onset. The other two negative NP/OP samples were positive in saliva, but the

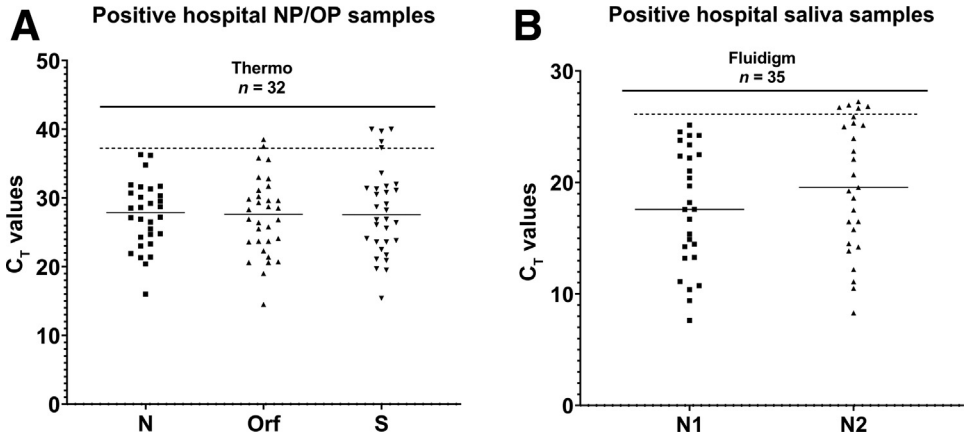

Figure 2 Positive hospital sample C_T ranges. C_T values for positive nasopharyngeal (NP)/oropharyngeal (OP; **A**) and saliva (**B**) samples are shown, with the median represented by a **solid line**. C_T values are presented by gene targets on each assay. The limit of detection of each assay is represented by a **dashed line**.

C_T value was near the LOD of the saliva assay, which is an indication of low viral copy. The four inconclusive NP/OP samples had C_T values close to the LOD of the NP/OP assay.

Overall, six samples were reclassified as low positives for which inconsistent results are not unexpected, three samples were saliva false negatives, possibly because of dilution of saliva, and six samples were NP/OP false negatives occurring during the second week after symptom onset. NP/OP specimens had more false-negative results than saliva, suggesting that saliva may be better at detecting SARS-CoV-2 infection later in the disease course.

Discussion

Substantiating saliva as a reliable specimen for screening SARS-CoV-2 could assist in reducing cost and risk of implementing screening protocols for schools and workplaces.[1] Many private companies and federal workplaces are requiring rigorous testing for their unvaccinated workforce and others to continue operations while maintaining a safe environment.[35,36] This manuscript presents the comparison data of swab and saliva from screening of a large mostly asymptomatic university population and from monitoring in hospitalized patients with confirmed COVID-19.

Saliva had good sensitivity (86.1%), high specificity (99.8%), and excellent overall percentage agreement (99.6%) when compared with AN in the university cohort. Following these favorable results, a screening program was put in place whereby all students returning to the University of Pennsylvania for the spring semester of 2021 were tested at least twice a week, with a test turnaround time of 24 hours. Approximately 13,000 tests were performed weekly with this high-throughput system, and positivity rates remained low (0.07% to 0.79%). This quick and affordable screening strategy allowed for a safe and successful campus reopening. Similar screening programs using saliva have also been implemented in other universities and school systems (*https://www.voanews.com/a/student-union_us-universities-roll-out-covid-spit-tests/6194786.html*, last accessed August 31, 2021;

Table 6 Concordance of SARS-CoV-2 Results in Paired NP/OP-Saliva Samples of Hospitalized COVID-19 Patients

	NP/OP			
Saliva	Positive	Negative	Inconclusive*	Total
Positive	25	8	2	35
Negative	3	9	2	14
Inconclusive	0	0	0	0
Total	28	17	4	49
Overall concordance, %		75.6		
Saliva sensitivity, %		89.3 (95% CI, 77.2—100.0)		
Saliva specificity, %		52.9 (95% CI, 20.3—85.6)		
NP/OP sensitivity, %		75.8 (95% CI, 58.9—92.6)		
NP/OP specificity, %		75.0 (95% CI, 46.7—100.0)		

*Inconclusive NP/OP samples were due to detection of only one of three genes. Overall concordance, sensitivity, and specificity calculations excluded inconclusive results.

NP, nasopharyngeal; OP, oropharyngeal.

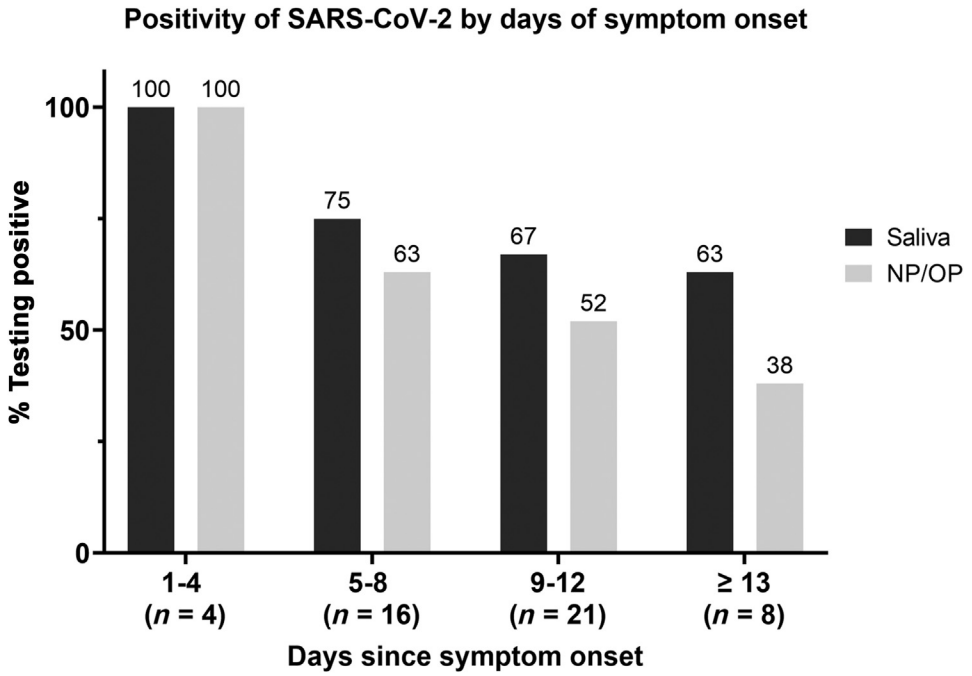

Figure 3 Percentages of positivity for SARS-CoV-2 in paired nasopharyngeal (NP)/oropharyngeal (OP) and saliva samples over time in hospitalized COVID-19 patients. Data are shown for 1 to 4, 5 to 8, 9 to 12, and ≥13 days after symptom onset. Differences between NP/OP and saliva samples are not statistically significant.

https://coloradosun.com/2021/01/18/colorado-schools-education-students-teachers-covid-19-testing, last accessed August 31, 2021; *https://www.gmu.edu/news/2021-01/george-mason-university-expands-testing-and-tracking-behind-faculty-research*, last accessed August 31, 2021; *https://news.illinois.edu/view/6367/1795135071*, last accessed August 31, 2021).[1,37]

A similar sensitivity (89.3%) was seen in saliva samples of hospitalized symptomatic patients with confirmed SARS-CoV-2 infection. These results are consistent with most studies, where the sensitivity of saliva using PCR-based methods is estimated to be in the 80% to 90% range.[10,12–14,17,20,22–24,27,29] Accordingly, three meta-analyses comparing the performance of saliva with upper respiratory swabs (NP, OP, AN, or NP/OP) reported a sensitivity ranging from 85% to 91% in saliva.[38–40]

Traditionally, upper respiratory swabs have been considered a better specimen for SARS-CoV-2 detection.[8,10,11,15,19,25,28] Meta-analyses have shown that the sensitivity of NP swab is approximately 98%, higher

Table 7 Description of Discordant Results between NP/OP and Saliva Results in Samples of Hospitalized Patients

	NP/OP		Saliva		
Sample no.	Result	Positive replicates	Result	Positive replicates	Reason for discordance
1	Positive	1/1	Negative	0/4	Saliva was diluted
2	Positive	1/1	Negative	0/3	Saliva was diluted
3	Positive	1/1	Negative	0/1	Saliva was diluted
4	Inconclusive	0/1	Negative	0/1	NP/OP sample near LOD of the swab assay
5	Inconclusive	0/1	Negative	0/1	NP/OP sample near LOD of the swab assay
6	Inconclusive	0/1	Positive	3/3	NP/OP sample near LOD of the swab assay
7	Inconclusive	0/1	Positive	1/3	NP/OP sample near LOD of the swab assay
8	Negative	0/1	Positive	4/4	8 Days after symptom onset
9	Negative	0/1	Positive	1/1	10 Days after symptom onset
10	Negative	0/1	Positive	4/4	10 Days after symptom onset
11	Negative	0/1	Positive	2/2	15 Days after symptom onset
12	Negative	0/1	Positive	2/2	9 Days after symptom onset
13	Negative	0/1	Positive	2/2	Near the LOD of the saliva assay
14	Negative	0/1	Positive	1/1	10 Days after symptom onset
15	Negative	0/1	Positive	1/1	Near the LOD of the saliva assay

LOD, limit of detection; NP, nasopharyngeal; OP, oropharyngeal.

than the sensitivity of saliva.[38,40] In our study, however, the sensitivity of upper respiratory swab was 83.7% for AN in the university cohort and 75.8% for NP/OP in hospitalized patients, lower than the sensitivity of saliva in both populations. After retesting the discordant samples and reclassifying some of the saliva samples as false positives, the sensitivity of AN in the university cohort increased to 90%. Despite NP swab being considered the gold standard for SARS-CoV-2 detection,[41] the use of AN swab in this study was justified by its easier and faster collection, patient comfort, and acceptable sensitivity for screening purposes.

Several studies have reported equivalent or superior sensitivity of saliva compared with upper respiratory swab.[6,9,16,21,26,42–44] For example, Fan et al[44] reported a significantly higher detection rate in saliva than in NP swabs (88.1% versus 76.2%, respectively). Similarly, Wyllie et al[43] detected higher levels of SARS-CoV-2 RNA copies in saliva than in NP samples in hospitalized patients and observed less variation in saliva viral levels during the clinical course. In addition, a higher percentage of saliva samples than NP samples was positive up to 10 days after COVID-19 diagnosis, similar to our results. More recently, preliminary studies have suggested that saliva can be more sensitive than nasal specimens in detecting the Omicron variant, especially during the first few days following infection.[45,46]

One possible explanation for the superior sensitivity of saliva in our study is that the assay used (Advanta Dx SARS-CoV-2 RT-PCR) includes a pre-amplification step. In contrast, the AN and NP/OP samples in our study did not undergo pre-amplification. Other studies have employed heat, mechanical treatments, and chemical treatments to significantly improve the analytic sensitivity of saliva specimens.[32,47–49] Therefore, the combination of saliva and technical aspect, such as heat inactivation or pre-amplification to increase the sensitivity, may enhance the utility of saliva as a specimen. On the other hand, a possible explanation for false-negative results in NP swabs is variation in sampling, resulting from technical difficulties with specimen collection. Using human *RNase P* as an internal control for proper sample collection, Wyllie et al[43] found greater variation in human *RNase P* C_T values in NP swab specimens than in saliva specimens. In addition, variation in the sensitivity (71.4% to 100%) of saliva specimens that was dependent of the comparator AN method was observed. Therefore, the testing methods and controls should be carefully considered when assessing the variability in the performance characteristics.

Higher sensitivity on the order of 94% to 100% has been reported in saliva samples of symptomatic individuals.[6,9,16,18,42] Most of these studies, however, did not assess the time from symptom onset; and it is possible that the subjects were in the first week of the disease when viral titers are maximal.[40] The mean time of symptom onset in the study by Vaz et al[42] was 4 days (interquartile range, 3 to 6 days), which could explain the high sensitivity in that cohort. Although the sensitivity of saliva in our study (89.3%) seems to be lower than in the aforementioned studies, this percentage was calculated longitudinally across a wide range of days since symptom onset (1 to 17 days). In fact, the positivity rate in both saliva and NP/OP was 100% during the first 4 days of symptom onset (Figure 3). Even at 1 to 7 days (data not shown), positivity rates for saliva and NP/OP remained high, at 93% and 86%, respectively. Although there was an apparent trend of decreasing positivity over time, the small sample size was a limiting factor to detect statistically significant differences. Similar to the findings reported by Wyllie et al,[43] there was a higher percentage of saliva samples than NP/OP samples testing positive later in the disease course. Persistence of SARS-CoV-2 in saliva can be clinically useful as patients often seek medical attention if symptoms do not spontaneously resolve, at which time nasal swab testing could be negative.

Although sensitivity and specificity are essential, other factors, such as cost and logistics, also play an important role in choosing a SARS-CoV-2 test. An assessment of the cost, throughput, ease of use, turnaround time, and human resources needed for each of the testing methods employed in this study has been published previously.[3] The use of robotic liquid handlers with the Fluidigm platform shortens the length of this saliva-based assay by approximately 2.5 to 3 hours and reduces technical challenges. This system was used successfully in the current study to yield high-throughput, affordable, and relatively quick results with saliva samples while maintaining a high sensitivity. Therefore, using a saliva-based high-throughput assay for large-scale screening is recommended. Future studies comparing the performance of different saliva-based assays are needed.

The current study has several limitations. In the university cohort, clinical information was not recorded at the time of testing, and therefore a correlation of clinical characteristics with test outcomes was not performed. In addition, it is unknown whether food or fluid intake before testing could have affected the results, because this information was also not consistently documented. In the hospital setting, the sample size was relatively small, reducing statistical power. In both populations, the heterogeneity in testing method for upper respiratory swabs and saliva precluded a direct comparison of C_T values between the assays. Similarly, AN samples were tested with a variety of testing platforms, further complicating this comparison. Moreover, the absence of a true diagnostic gold standard hinders accurate calculation of sensitivity and specificity, as evident by the variation in sensitivity between test methods.

In conclusion, our study provides data to support the use of saliva for SARS-CoV-2 detection in both symptomatic and asymptomatic populations. Our results show that saliva has an acceptable sensitivity and is, at a minimum, comparable to upper respiratory swab. Saliva testing is ideal for large-scale, regular screening programs where sample collection logistics and supply shortages represent a

significant capacity strain and in identifying SARS-CoV-2 infections in patients presenting later in the clinical course.

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