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Diagnostic Microbiology and Infectious Disease 101 (2021) 115450

Contents lists available at ScienceDirect

Diagnostic Microbiology and Infectious Disease

journal homepage: www.elsevier.com/locate/diagmicrobio

The reliability of saliva for the detection of SARS-CoV-2 in symptomatic and asymptomatic patients: Insights on the diagnostic performance and utility for COVID-19 screening

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ARTICLE INFO

Article history:
Received 24 February 2021
Revised in revised form 1 June 2021
Accepted 1 June 2021
Available online 6 June 2021

Keywords:
COVID-19
SARS-CoV-2
Saliva
Nasopharyngeal swab
Screening
Asymptomatic

ABSTRACT

Current literature has focused on testing saliva in symptomatic patients, and little information is available regarding saliva performance in asymptomatic severe acute respiratory syndrome-coronavirus-2 (SARS-CoV-2) infection. We compared paired saliva and nasopharyngeal swabs (NPS) collected from 33 symptomatic and 12 asymptomatic known SARS-CoV-2-positive patients. Saliva had an overall sensitivity of 59%, a specificity of 95%, and a negative predictive value of 98%. Saliva demonstrated higher sensitivity in symptomatic (80%) vs. asymptomatic individuals (36%) ($P = 0.006$), and in high-risk (symptomatic, febrile and/or with comorbidities) (82%) vs. low-risk (asymptomatic, afebrile, and no comorbidities) (22%) patients ($P = 0.0002$). Cycle threshold (Ct) values in NPS specimens were higher in saliva-negative vs. saliva-positive cases ($P = 0.02$ and <0.001). Overall, these findings show that despite saliva's low sensitivity in asymptomatic SARS-CoV-2 infections, it can detect infections with lower Ct values and a potentially higher chance of viral transmission. Additional studies are warranted to fully evaluate saliva as a screening test for coronavirus disease-2019.

1. Introduction

Severe acute respiratory syndrome-coronavirus-2 (SARS-CoV-2), the causative agent of coronavirus disease-2019 (COVID-19), continues to infect thousands of people and claim thousands of lives globally on a daily basis (WHO). With case numbers continuing to rise in several parts of the world, there continues to be shortages in medical resources, including specimen collection supplies (e.g., nasopharyngeal swabs, transport media) and personal protective equipment (PPE) (Moreno-Contreras et al., 2020; Nikhil et al., 2020). The World Health Organization (WHO) recommends the use of respiratory secretions, specifically nasopharyngeal swabs (NPS), as the specimen of choice for the detection of SARS-CoV-2 detection (Corman et al., 2020; World Health Organization, 2020), using real-time reverse transcriptase-polymerase chain reaction (RT-PCR) (Wang et al., 2020a, 2020b). However, beside requiring uninterrupted supplies of swabs and transport media, NPS is an invasive and uncomfortable method requiring a trained professional for sample collection, with an increased risk of viral transmission between the patient and the individual collecting the specimen.

Saliva has been the focus of recent investigations for its utility in the diagnosis of SARS-CoV-2 infection. Testing saliva allows for self-collection in a form that has been shown to be accepted by the public (Valentine-Graves et al., 2020), limiting the risks of interactions between the public and health care workers who are frequently exposed to SARS-CoV-2, and potentially reducing the need for PPE. Saliva does not require specific collection supplies; thus, it is not affected by the frequent supply-demand imbalance. Salivary specimens have been proven to be stable in variable conditions and for a prolonged period (Berenger et al., 2020; Matic et al., 2020; Ott et al., 2020). Additionally, as an inexpensive and easy-to-collect specimen type, it represents a promising candidate for point-of-care testing and mass screening of asymptomatic individuals.

Chen *et al* found that salivary glands express angiotensin-converting enzyme II (ACE2), the likely cell receptor for SARS-CoV-2, and identified the virus in saliva samples from patients with COVID-19 (Chen et al., 2020). Early reports have revealed that saliva can harbor SARS-CoV-2 and can be used to monitor viral loads (To et al., 2020).

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https://doi.org/10.1016/j.diagmicrobio.2021.115450
0732-8893/

Since then, several studies comparing saliva to NPS have been published, with the majority of results suggesting comparable saliva sensitivity to that of NPS (Azzi et al., 2020; Becker et al., 2020; Caulley et al., 2020; Hanson et al., 2020; Jamal et al., 2020; Manabe et al., 2020; Pasomsub et al., 2020; Riccò et al., 2020; Sakanashi et al., 2021; Skolimowska et al., 2020; Sutjipto et al., 2020; Vaz et al., 2020). In some studies, saliva outperformed NPS in terms of sensitivity, highlighting a limitation of the latter method to be considered as the standard of care (Kojima et al., 2020; Moreno-Contreras et al., 2020; Wyllie et al., 2020). Such findings led the FDA to issue emergency use authorizations to Yale and Rutgers laboratories for the use of saliva as a sample for SARS-CoV-2 testing in symptomatic individuals suspected of COVID-19 (Rutgers Clinical Genomics Laboratory; Yale School of Public Health, Department of Epidemiology of Microbial Diseases).

With the majority of studies on saliva being performed on confirmed infections or symptomatic patients, limited literature exists on the assessment and reporting of saliva performance in asymptomatic individuals (Vogels et al., 2021; Wyllie et al., 2020; Yokota et al., 2020). In this study, we evaluated the diagnostic value of saliva compared to NPS in patients presenting with symptoms suggestive of COVID-19, as well as asymptomatic SARS-CoV-2 positive patients identified as part of the institutional universal admission screening protocol.

2. Materials and methods

2.1. Ethics

The study was approved by the University of Louisville Institutional Review Board. All study aspects were carried out in accordance with the approved Institutional Review Board protocol (IRB# 20.0374). All enrolled participants acknowledged understanding the aims of the study and signed a consent form prior to collection of demographics, clinical data, and samples.

2.2. Specimen collection

An infectious diseases physician selected inpatients previously determined to be positive for the virus, while eligible patients presenting to the emergency room with symptoms suggestive of COVID-19 were selected by a physician on duty. Each patient was asked if they would voluntarily provide a saliva specimen for comparative purposes following the collection of a NPS specimen which served as the comparator or "gold standard." A Patient Information Form, approved by the IRB, describing the aim of the study and the purpose of acquiring an additional specimen was given to the patient along with a collection container (sterile urine cup or a 50 conical tube) and instructions to expectorate into the collection device. We recommended 3 mL of saliva but accepted a minimum of 1 mL. Following the acquisition of a NPS, obtained by an experienced, trained health care provider, the swab was placed and transported in 3 mL of sterile Universal Transport Medium (UTM) (M4, Becton Dickinson, Sparks, MD; REMEL, Lenexa, KS) for SARS-CoV-2 testing, and 1 to 3 mL of saliva was collected. Both specimens were tested on-demand; in the event of a delay in testing, the saliva specimens were stored at 2°C to 8°C and tested within 12 hours of receipt.

2.3. Definitions

Symptomatic patients were those who presented to our emergency room with symptoms consistent with COVID-19, including cough, shortness of breath, dyspnea, fever, abdominal pain, diarrhea, and loss of taste or smell sensation. Patients with early-onset symptomatic infections were tested and confirmed within ≤4 days from onset of symptoms, while late-onset symptomatic infections were tested and confirmed >4 days from symptom onset. Several patients had underlying comorbidities, including hypertension, cardiovascular disease, diabetes mellitus, malignancy, asthma, COPD, HIV infection, hepatic cirrhosis and ethanol/drug abuse. A high-risk individual is someone who was either symptomatic, febrile at time of testing or had an underlying comorbidity. A low-risk individual showed no symptoms, was afebrile, and had no underlying comorbidities. Fever was chosen as a criterion separate from other symptoms because it can represent either a symptom or a sign.

2.4. SARs-CoV-2 testing

If the saliva specimen was viscous, an equal volume of UTM was added to liquefy the specimen before testing. RT-PCR testing was performed on the DiaSorin Simplexa Direct amplification system (DiaSorin Molecular LLC, Cypress, CA) according to the manufacturer's directions. 50 μL of Simplexa COVID-19 Direct Kit Reaction Mix (MOL4150) was added to the "R" well of the 8-well Direct Amplification Disc (DAD) followed by adding 50 μL of nonextracted NPS or saliva samples to the "SAMPLE" well. Data collection and analysis were performed with LIAISON® MDX Studio software. The system's components include, the Simplexa™ COVID-19 Direct EUA Assay, the LIAISON® MDX (with LIAISON® Studio Software), the Direct Amplification Disc, and associated accessories. The assay targets 2 regions of the SARS-CoV-2 genome: ORF1ab (open reading frame 1ab) and S (spike glycoprotein) genes, differentiated with FAM and JOE fluorescent probes. An RNA internal control is used to detect RT-PCR failure and/or inhibition. Results were reported as Positive, Negative, or Invalid. A result was considered positive if one or both targets (ORF1ab or S gene) were detected. Invalid results were retested with a new Reaction Mix vial from the same kit or a new kit.

2.5. Statistical analysis

Patient characteristics were calculated for all cases. Statistical analysis was performed using R Studio Version 3.6.1. Continuous variables were reported as means with standard deviations, and categorical variables were reported as frequencies and percentages. Assessment of difference in significance for the duration of symptom onset to specimen collection between the saliva-positive and saliva-negative COVID-19 patient groups was conducted using a χ^2 test of independence. Sensitivity, specificity, negative and positive predicative values, and accuracy were calculated for saliva tests. Positive and negative predictive value calculations were based on a 5% disease prevalence. Fischer exact tests and tests of equal proportions were used to identify significant differences in saliva test sensitivity between 4 groups (symptomatic vs. asymptomatic; high risk vs. low risk; asymptomatic high risk vs. asymptomatic low risk; early-onset symptomatic vs. late-onset symptomatic). Significant differences in mean Ct values between concordant NPS and saliva tests for the S and ORF1ab genes were assessed using paired t-tests for 7 groups (all concordant cases, symptomatic, asymptomatic, early symptom onset, and late symptom onset). Lastly, Welch two sample t-tests were utilized to assess significant differences in mean Ct values between NPS and saliva tests for the S and ORF1ab genes between asymptomatic and symptomatic groups and early and late symptom onset groups, respectively. P values <0.05 were used to denote significance for all tests conducted.

3. Results

A total of 61 patients were screened for inclusion between the period from Apr 30, 2020 to Jun 15, 2020, of whom 48 were included (26 males and 22 females). The 13 patients excluded from the study included 1 patient who drank water immediately before saliva collection, 3 who had invalid saliva specimens on repeat testing following

Table 1
Patients' demographics.

Age (years ± SD)	39.9 (±15.5)	-
Sex		
Male	26	
Female	22	
Patients with COVID19	58% (28/48)	
Proportion of patients with comorbidities	47% (21/45)	
COVID19+	38% (8/21)	
Proportion of symptomatic patients	73% (33/45)	
COVID19+	45% (15/33)	
Average symptom onset to test	6.3 (± 8.5)	
Average symptom onset to test in saliva-positive patients (n = 16)	5.2 (± 5.8)	$P = 0.44$
Average symptom onset to test in saliva-negative patients (n = 11)	3.7 (± 3.1)	

redilution in UTM and 9 patients with specimens tested on a platform other than DiaSorin. The average patient age was 39.9 years (±15.5). Clinical data were available in 45 patients. Thirty-three patients presented to our emergency room with symptoms consistent with COVID-19, and 12 were asymptomatic SARS-CoV-2 positive patients identified following universal admission screening. Patients with asymptomatic infection were admitted due to medical conditions unrelated to COVID-19 (labor and delivery, stroke, cholecystitis, psychiatric illness, syncope, and trauma). None of the asymptomatic individuals disclosed having any prior COVID-19 symptoms, recent exposure or contact with COVID-19 patients, and none of them developed symptoms during the course of their hospital stays. Comorbidities were present in 47% (21/45) of patients. Symptomatic COVID-19 cases were predominantly mild and did not require hospital admission, except one severe case requiring intubation and mechanical ventilation. Patient demographics are highlighted in Table 1.

A total of 28 patients (58%) tested positive for the SARS-CoV-2 virus by NPS and/or saliva. NPS was positive in 27 patients vs. saliva in 17 patients. The overall positive percent agreement was 96% for NPS and 61% for saliva. One patient tested positive for SARS-CoV-2 only on a saliva sample (Table 2). Among the SARS-CoV-2 positive symptomatic patients, the average onset of symptoms to specimen collection time was 6.3 (±8.5) days. There was no significant difference between the time from symptom onset to specimen collection between the saliva-positive and saliva-negative COVID-19 patient groups (Table 1).

Assuming NPS as the "gold standard," saliva showed an overall sensitivity of 59% and specificity of 95%, with a negative predictive value of 98% (calculated at a prevalence of 5%). Interestingly, saliva

Table 2
Results of parallel testing of NPS and saliva specimens across the entire cohort.

Saliva	Nasopharyngeal swab			Total
		+	–	
	+	16	1	17
	–	11	20	31
Total		27	21	48

sensitivity was 80% in the symptomatic group compared to only 36% in the asymptomatic group ($P = 0.006$). We then sought to stratify patients into high-risk and low-risk categories for harboring the SARS-CoV-2 virus as previously described in the methods section. Saliva showed a significantly higher sensitivity in the high-risk group (82%) compared to the low-risk group (22%) ($P = 0.0002$). No difference was found comparing saliva in early-onset symptomatic vs. late-onset symptomatic patients (Table 3).

Ct values were recorded on all SARS-CoV-2 positive samples. This included paired samples from 28 SARS-CoV-2 positive patients. Four NPS specimens were positive for the ORF1ab gene only (all were saliva-negative). One saliva specimen was positive only for ORF1ab (NPS-negative patient). The average Ct values in NPS samples were 21.8 ± 6.2 (range: 11.5–33.3) for the S gene and 22.1 ± 5.9 (13.6 –32.9) for the ORF1ab gene. In saliva, the average Ct values were 24.8 ± 4.8 (16.4–32.6) for the S gene and 25.3 ± 4.8 (16.5–32.6) for the ORF1ab gene. RNA internal control values in both NPS and saliva specimens were comparable (32.3 ± 1.6 vs. 32.6 ± 1.4). On average, the Ct value in saliva was 3.0 cycles higher for the S gene ($P = 0.04$) and 3.2 cycles higher for the ORF1ab gene ($P = 0.03$) compared to NPS. Similar differences were found when comparing Ct values between NPS and saliva within the asymptomatic group as well as within the early-onset symptomatic patient group, with the difference being less evident within the late-symptomatic patient group (Figs. 1 and 2).

To assess the possible role of viral load, as reflected by the Ct value, on the sensitivity of saliva in the detection of SARS-CoV-2, we compared NPS Ct values in patients with saliva-negative vs. saliva-positive samples. NPS-positive, saliva-negative cases showed significantly higher Ct values on NPS for both S and ORF1ab genes than NPS-positive, saliva-positive cases, with P values of 0.02 and <0.001, respectively (Fig. 3).

4. Discussion

In our study, saliva showed a relatively low overall sensitivity (59%) compared to NPS. Interestingly, however, the sensitivity was

Table 3
Diagnostic test evaluation for saliva.

	Sensitivity (95% CI)	Specificity (95% CI)	PPV* (95% CI)	NPV* (95% CI)	Accuracy* (95% CI)	P value (for sensitivity)
Saliva (overall)	59% (39%–78%)	95% (76%–100%)	40% (9%–82%)	98% (97%–99%)	93% (82%–99%)	
Saliva (symptomatic) (n = 33)	80% (52%–96%)	100% (81%–100%)	100%	99% (97%–100%)	99% (88%–100%)	0.006
Saliva (asymptomatic) (n = 12)	36% (11%–69%)	100% (3%–100%)	100%	97% (95%–98%)	97% (69%–100%)	
Saliva (high risk) (n = 33)	82% (57%–96%)	100% (79%–100%)	100%	99% (97%–100%)	99% (88%–100%)	0.0002
Saliva (low risk) (n = 12)	22% (3%–60%)	100% (29%–100%)	100%	96% (95%–97%)	96% (68%–100%)	
Early symptomatic (≤4 days) (n = 21)	80% (44%–97%)	100% (72%–100%)	100%	99% (96%–100%)	99% (82%–100%)	>0.999
Late symptomatic (>4 days) (n = 12)	80% (28%–99%)	100% (59%–100%)	100%	99% (94%–100%)	99% (72%–100%)	

PPV = positive predictive value; NPV = negative predictive value; CI = confidence interval.
* PPV, NPV, and accuracy were calculated based on an estimated prevalence of 5%.

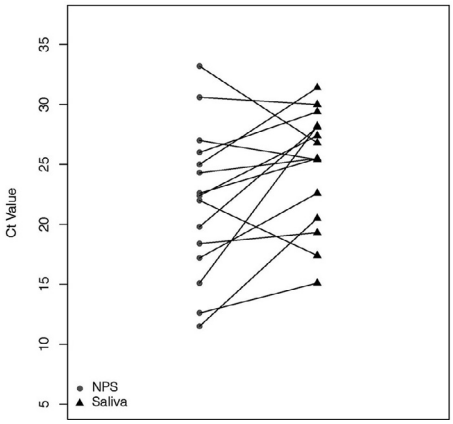

Fig. 1. Ct values in paired NPS and saliva samples.

significantly higher in patients who were symptomatic (80%) and those who we considered being high-risk individuals (82%). These results suggest that saliva has comparable sensitivity to NPS especially in individuals with high clinical suspicion for COVID-19 and is significantly less sensitive than NPS in detecting SARS-CoV-2 in asymptomatic individuals with no known recent prior exposure and significantly higher Ct values (i.e., lower viral loads).

Viral load characteristics in SARS-CoV-2 infection may offer an explanation of our findings. Evidence shows that the viral load peaks early in the course of infection (first week), and the duration of viral shedding can be variable, with a median of 12 to 20 days and lasting up to 63 days postsymptom onset (Widders et al., 2020). Higher estimated viral loads and prolonged viral shedding were found to positively correlate with disease severity and immune status (Liu et al., 2020; Widders et al., 2020). It was also found that asymptomatic infections and milder cases of COVID-19 have faster viral clearance than severe cases (Chau et al., 2020; Liu et al., 2020). These findings suggest that the course of illness, symptom development and duration of viral shedding may depend on the acquired viral inoculum and the immune status of individuals. Thus, asymptomatic infections would be suspected to generally have lower viral loads and enhanced viral clearance compared to the more severe COVID-19 cases. Furthermore, a prior study showed that saliva had lower positivity rates in asymptomatic individuals than NPS (although not statistically significant) (Chau et al., 2020), and another study showed a higher proportion of saliva-positive tests in severe COVID-19 cases compared to mild infections (Nagura-Ikeda et al., 2020). Our results show a significantly lower sensitivity for saliva in asymptomatic compared to symptomatic individuals. Additionally, Ct values in saliva-negative, NPS-positive COVID-19 cases were significantly higher than those in saliva-positive, NPS-positive cases, suggesting higher viral loads in the latter. In general, we found that saliva showed higher mean Ct values than NPS in paired specimens, suggesting that the viral load in saliva is generally lower than in NPS. This difference was less evident in samples collected later in the course of infection (>4 days from symptom onset) when NPS showed the highest mean Ct values, suggesting that the viral load peaks early during infection and perhaps falls more rapidly in NPS than saliva.

Although saliva showed lower sensitivity in asymptomatic patients, our findings suggest that it can play a vital role in mass-screening for COVID-19. It is imperative to differentiate between the identification of viral RNA by RT-PCR tests and the ability to shed and transmit viable viruses. It has been shown that later in the infection (>21 days) NPS detects a significantly larger number of positive

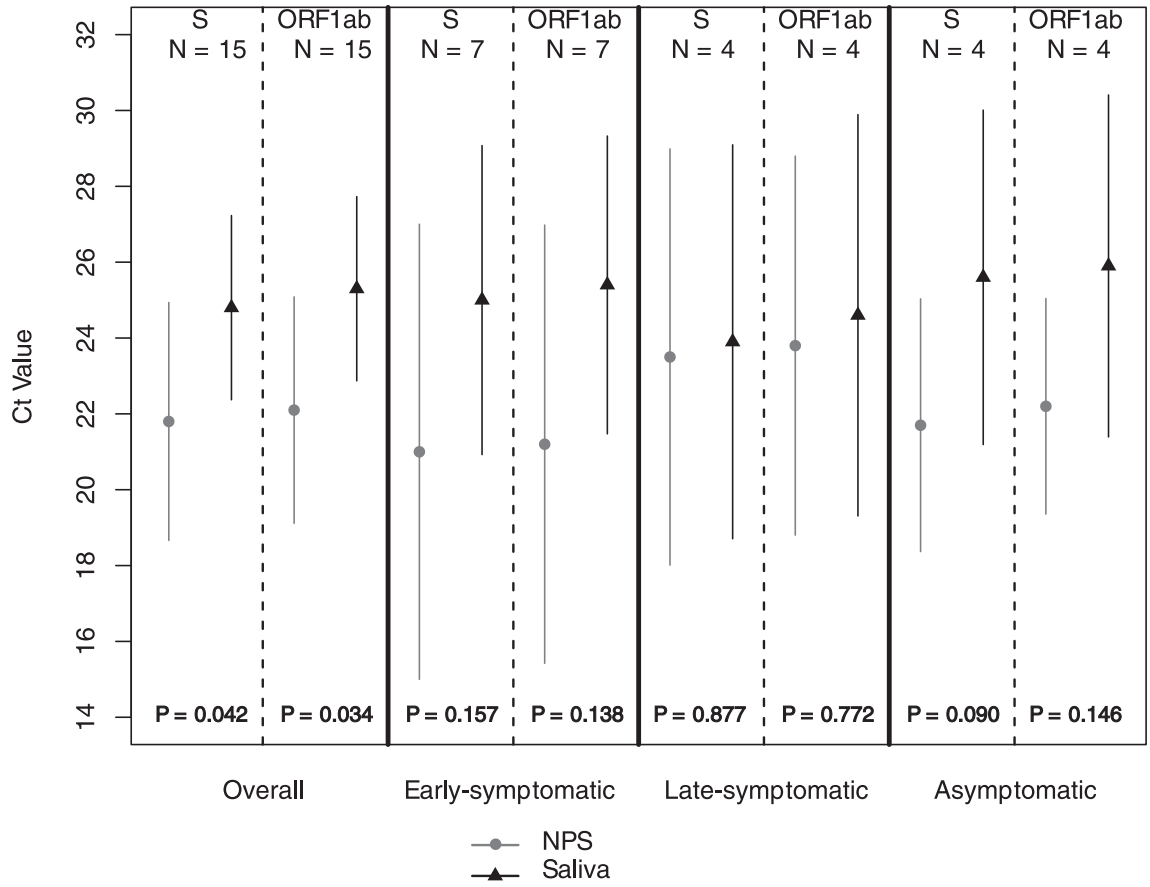

Fig. 2. Mean Ct value by test type (NPS vs. saliva) across different patient categories (Welch two sample t-tests).

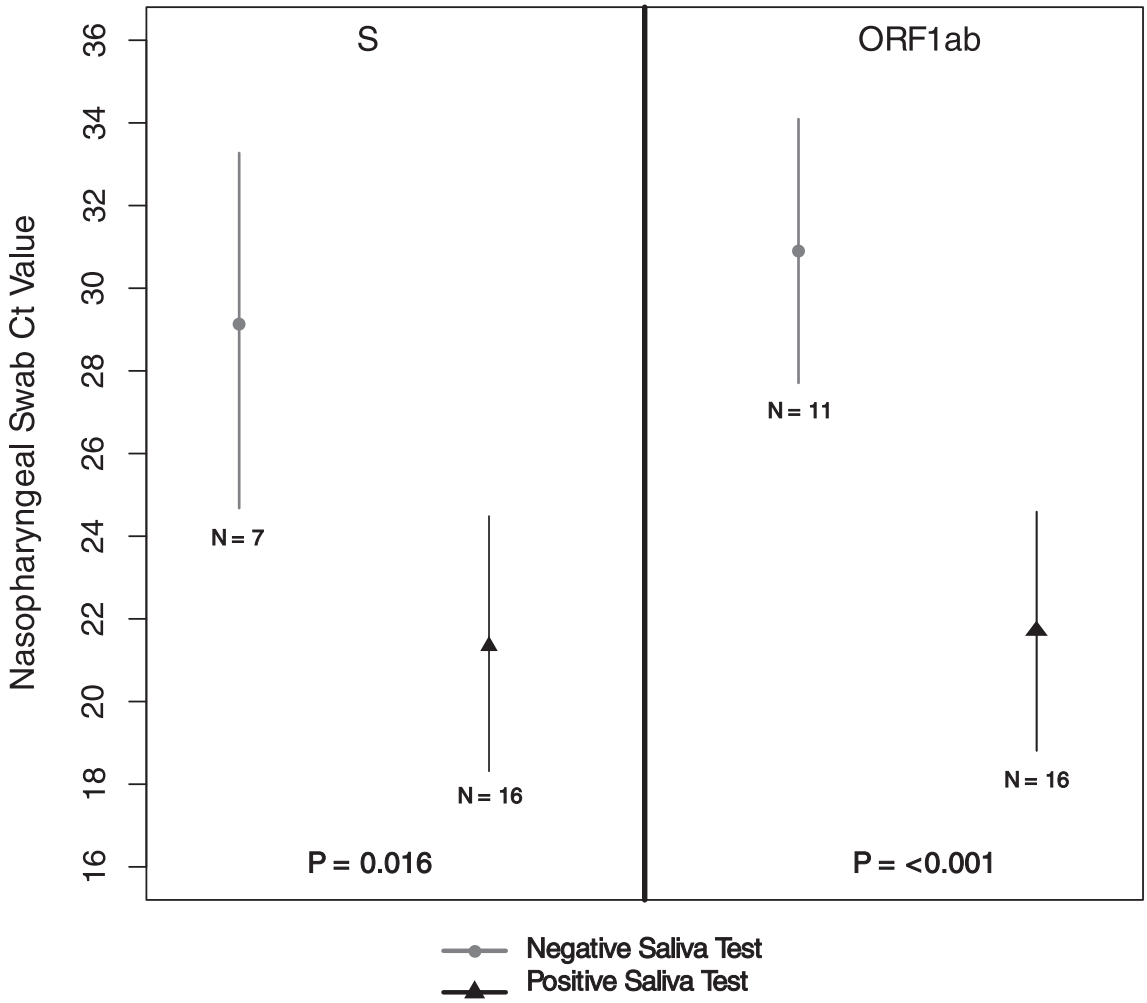

Fig. 3. Difference in mean NPS Ct value between saliva-negative and saliva-positive samples (Welch two sample t-tests).

results compared to oral fluid or anterior nares specimens. At that stage of the disease, those most likely represent inactivated viral RNA shedding (false positive) with no transmission potential. This finding was observed with multiple FDA EUA molecular assays, suggesting that biological variation among specimen types, rather than the assay used for testing, is the main reason for this phenomenon (Turner et al., 2021). Furthermore, viral cultures from patients with prolonged disease onset/ high RT-PCR Ct values failed to identify viable virus (Laferl et al., 2021; Manzulli et al., 2021; van Kampen et al., 2021). In one study comparing saliva to NPS, all viral culture-positive samples had concordantly positive NPS and saliva samples, and no viable virus was isolated from saliva-negative, NPS-positive patients (Manabe et al., 2020). In our study, saliva could detect patients who had lower Ct values (suggestive of higher viral loads) and likely higher risk of viral transmission. In fact, saliva was able to detect all but 2 cases with Ct values below 30, one of which had a minimum Ct value of 29.5 for one of the targets. Our findings, in conjunction with the currently available evidence, strongly suggests that saliva is sensitive enough to detect the majority of "true" active infections with potential viral transmission and have a specificity advantage over NPS by failing to detect the prolonged stage of postdisease viral RNA shedding (false positive). This ultimately gives saliva a high negative predictive value, which warrants its use especially in communities with low infection prevalence (Supplementary Fig. 1).

One larger study by Yokota *et al* assessed saliva in the screening of predominantly asymptomatic contacts of COVID-19 patients and found comparable sensitivity to NPS (Yokota et al., 2020). Since their study predominantly included COVID-19 contacts, it may be assumed that the majority of SARS-CoV-2 positive individuals they identified had been exposed within a short period of time from testing. This was not the case in our study, as asymptomatic patients in our cohort did not disclose any prior exposure, and we simply cannot know or predict when those patients acquired the virus. Assuming faster viral clearance in asymptomatic infections as described in the literature, this explains the low saliva sensitivity in our asymptomatic cohort compared to the aforementioned study. Another recent study tested a saliva-based assay in a large cohort of asymptomatic individuals with promising results. In this study, however, saliva was compared to anterior nares/oropharyngeal swabs, not NPS (Vogels et al., 2021).

Our findings may suggest several clinical applications for saliva in the diagnosis of COVID-19. Saliva demonstrated a high sensitivity for the detection of SARS-CoV-2 in symptomatic individuals, making it a suitable specimen for the primary diagnosis of COVID-19 in that patient group, especially in the setting of swab shortage. Additionally, saliva may play a vital role in COVID-19 screening of asymptomatic individuals in different settings, such as screening individuals who are found to be febrile on temperature measurement, as well as in contact tracing campaigns, nursing home residents, teachers and students returning to schools, and airport departures. In the screening setting, saliva may prove more advantageous than NPS, as the higher sensitivity and lower specificity of the latter, particularly during the prolonged postdisease period of inactive viral RNA shedding, would

lead to more false positive results that would overwhelm contact tracing programs and halt efforts of safe return to school or work.

We acknowledge several limitations to our study. Our patient cohort is small and included some who were seeking health care for medical conditions other than COVID-19; thus, their overall health and immune status may not reflect that of the general population. Preanalytical conditions may have played a role in testing accuracy for saliva. Although instructions were provided to health care workers and patients on optimal methods for sample collection (e.g., no food or drinking 1 hour before sample collection), the laboratory has little or no control over preanalytical factors associated with patient preparation, specimen collection, and timely transport of the specimen to the laboratory which are variables that can impact the quality of laboratory results. Unless we had prior knowledge of such factors, such as drinking within 1 hour before sample collection, as was learned in one case resulting in exclusion from the study, the majority of the saliva specimens that we received were accepted for testing. With the exception of 1 patient, all asymptomatic patients included tested positive for SARS-CoV-2 at least on NPS, limiting the possibility of identifying false positive results. Lastly, several studies reported varying clinical and analytical sensitivities for the DiaSorin Simplexa platform, some of which demonstrating lower sensitivity compared to other assays (Cradic et al., 2020; Lephart et al., 2021; Lieberman et al., 2020; Procop et al., 2020; Tibbetts et al., 2020; Zhen et al., 2020). This may potentially limit the overall reproducibility of our results across diagnostic platforms.

In conclusion, saliva demonstrates comparable sensitivity to NPS in detecting SARS-CoV-2 in symptomatic patients and those with high clinical suspicion for COVID-19. Despite showing lower overall sensitivity in asymptomatic individuals, saliva showed high specificity and negative predictive value and was able to identify asymptomatic individuals with lower Ct values (suggestive of higher viral loads) and potentially higher likelihood of SARS-CoV-2 transmission. More extensive studies conducted on a larger number of asymptomatic SARS-CoV-2-positive individuals are necessary to fully evaluate saliva's diagnostic value as a primary specimen for mass-screening for SARS-CoV-2.

Funding

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

Declaration of competing interest

The authors declare no competing financial/nonfinancial interests.

Authors' contributions

Khaled J. Alkhateeb: Conceptualization, methodology, formal analysis, investigation, writing – original draft, visualization. Meredith N. Cahill: Formal analysis, resources, data curation, writing – review and editing. Adam S. Ross: Resources, writing – review and editing. Forest W. Arnold: Resources, writing – review and editing. James W. Snyder: Conceptualization, methodology, resources, data curation writing – review and editing, visualization, supervision, project administration.

Acknowledgments

We thank the nursing and molecular diagnostics laboratory personnel for their assistance and technical support in collecting some and testing all saliva specimens. We would also like to thank Stephen Furmanek for his assistance and guidance with the statistical analysis.

Supplementary materials

Supplementary material associated with this article can be found in the online version at doi:10.1016/j.diagmicrobio.2021.115450.

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