ORIGINAL ARTICLE

Self-collected unstimulated saliva, oral swab, and nasopharyngeal swab specimens in the detection of SARS-CoV-2

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Abstract

Objectives The presence of SARS-CoV-2 virus in the saliva of patients infected with COVID-19 has been confirmed by several studies. However, the use of saliva for the diagnosis of COVID-19 remains limited, because of the discrepancies in the results, which might be due to using different saliva sampling methods. The purpose of this study was to compare the consistency of SARS-CoV-2 detection using two different saliva sampling methods (oral swab and unstimulated saliva) to that of the standard nasopharyngeal swab.

Methods Fifty-five subjects were recruited from a pool of COVID-19 inpatient at the Hospital Israelita Albert Einstein (HIAE), Brazil. Nasopharyngeal swab, oral swab, and self-collected unstimulated saliva samples were examined for SARS-CoV-2 using RT-PCR.

Results Self-collected unstimulated saliva demonstrated 87.3% agreement in the detection of SARS-CoV-2 virus as compared with the nasopharyngeal swab, while oral swab displayed 65.9% agreement when compared to nasopharyngeal swab and 73% when compared to self-collected unstimulated saliva.

Conclusion Unstimulated self-collected saliva samples have shown a higher agreement with the nasopharyngeal swab samples for SARS-COV-2 detection than that obtained when using oral swab samples.

Clinical relevance This study compares the accuracy of COVID-19 test using different saliva sampling methods to that of nasopharyngeal swab. Given the need for a simple self-applied test that can be performed at home, our findings support the efficacy of self-collected unstimulated saliva samples in the diagnosis of SARS-CoV-2 infection, alleviating the demands for swab supplies, personal protective equipment, and healthcare personnel.

Keywords Coronavirus · Coronavirus infections · SARS-CoV-2 · COVID-19 · Saliva · Biomarkers

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Introduction

Population-level employable rapid testing is part of a strategy, where testing should be widely available, preferably outside of healthcare or hospital settings, avoiding the overload of resources and the risk of transmission to other patients and healthcare professionals [1]. To date, the gold standard for the diagnosis of SARS-CoV-2 infection is reverse transcription-polymerase chain reaction (RT-PCR) analysis of respiratory tract swabs [2]. However, oropharyngeal and nasopharyngeal collection requires trained professionals to be performed and is associated with discomfort and inconvenience to the patients, in addition to increasing risk of virus transmission, thereby halting the possibility of employing this test in a large population setting [3–5]. Therefore, it is rather imperative to design a convenient and easily accessible test to control the spread of a highly infectious disease such as COVID-19 before vaccines or anti-viral treatment become widely available.

Saliva specimen collection is one of the simplest ways to collect body fluids and represents an important source for the diagnosis and monitoring of coronavirus infection [6]. Saliva sampling has the advantage of being safe, easy to perform, and noninvasive, and may be self-collected repeatedly without discomfort to the patient. Several studies have confirmed a reliable detection of SARS-CoV-2 in the saliva of patients with COVID-19. However, the application of saliva testing for COVID-19 has been hampered due to discrepancies in the reported reliability of the test results. It is highly possible that the method of saliva sample collection could potentially be the source of the observed discrepancies in the reliability of the test results. Regarding the sampling methods, saliva samples used for COVID-19 testing have been collected in different ways, oral swab and unstimulated saliva [6]. To the best of our knowledge, there is no study that directly compared the accuracy of these two methods of sampling to that of the standard nasopharyngeal swab.

Therefore, the aim of this study was to compare the consistency of SARS-CoV-2 detection using saliva samples (oral swab and self-collected unstimulated saliva) to that of standard nasopharyngeal swab.

Methods

Ethics

The study protocol was approved by the National Ethics in Human Research Committee of Brazil (CONEP, protocol #4172–20). All patients were individually informed about the nature of the proposed study and its risks and benefits, and signed informed consent forms.

Subject population and selection criteria

COVID-19 subjects aged 18–90 years were recruited from May 2020 to June 2020 from a pool of COVID-19 inpatients at the Hospital Israelita Albert Einstein (HIAE), Brazil. Inclusion criteria were patients with positive SARS-CoV-2 determined by the nasopharyngeal/oropharyngeal swab RT-PCR method and recorded in the medical record, within 3 days of hospitalization. Excluded were pediatric patients, patients with a negative RT-PCR result for SARS-CoV-2 at the time of recruitment, and patients who had other clinical illness that in the opinion of the investigators could interfere with compliance to sample collection.

Sample collection

The collection of nasopharyngeal and saliva samples was performed within 3 days of hospitalization. Senior nurse staff took nasopharyngeal samples using a flexible, mini-tip swab, which was passed through the patient's nostril until the posterior nasopharynx was reached, left in place for several seconds to absorb secretions then slowly removed while rotating. The swab was placed in a sterile viral transport medium (total volume 3 mL) and sealed securely. Unstimulated whole saliva was self-collected by the patients in a sterile 50-mL tube as previously described [7]. Oral swabs were taken from the back of the tongue, deep groove and floor of the mouth by a trained dentist using a flexible mini-tip swab and placed in the sterile viral transport medium (total volume 3 mL). All samples were immediately transported on ice to the laboratory at the Hospital Israelita Albert Einstein and tested within 48 h of collection [8, 9].

Detection and quantification of viral RNA

Viral RNA was extracted from 200 µL of viral transport medium from the nasopharyngeal swab or from the oral swab or from unstimulated saliva specimens using a QIAsymphony™ RNA Kit (QIAGEN, Hilden, Germany) according to the manufacturer's instructions. Following extraction, RNA was eluted with 60 µL of elution buffer. For SARS-CoV-2 RNA detection in saliva (unstimulated saliva and oral swab), RT-qPCR amplification of the Nucleocapsid (N) and Open reading frame (ORF) 1ab genes was performed using the XGen Master COVID-19 RT-PCR Kit (Mobius Life Science Comércio de Produto para Laboratórios Ltda, Pinhais, Brazil). The sensitivity of the reaction in routine diagnosis varies according to the gene analyzed, as follows: SARS-CoV-2 gene ORF1ab gene—10 copies/reaction; SARS-CoV-2 N gene—50 copies/reaction. Saliva samples were classified as positive for SARS-CoV-2 when the cycle threshold (CT) for both N and ORF1ab genes was

lower than 38. For SARS-CoV-2 RNA detection in nasopharyngeal swab specimen (NPS), RT-qPCR amplification of the Envelope (E) and N genes was performed as previously described [10]. NPS samples were classified as positive for SARS-CoV-2 when the CT for both E and N genes was lower than 38 (Fig. 1).

Statistical analysis

The results were presented by estimated mean values, 95% confidence intervals (95% CI) and p-values. The CT measurements obtained in saliva collection were compared to the CT measurements in nasopharyngeal swab collection using models of generalized estimation equations (GEE) with gamma distribution, considering the relationship between the different collections performed in the same patient. The same calculation was performed to compare the CT measurements obtained from unstimulated saliva, oral swab, and nasopharyngeal swab collections. The sensitivity of each test (self-collected unstimulated saliva and oral swab) was determined by the proportion of positive results to that of the gold standard (nasopharyngeal swab). All analyses were performed with the SPSS program (IBM Corp, IBM SPSS Statistics for Windows, Version 24.0, Armonk, NY) considering a 5% significance level.

Results

A total of 55 patients with positive COVID-19 RT-PCR using nasopharyngeal swab were included in the study. Of the 55 patients, 48 (87.3%) were also positive with self-collected unstimulated saliva. Forty-one patients also provided

oral swab samples, and of those, 27 patients (65.9%) were positive for SARS-CoV-2 RNA detection (Table 1).

Unstimulated self-collected saliva and nasopharyngeal swab specimens in the detection of SARS-CoV-2

In the RT-qPCR of saliva samples, two genes from SARS-CoV-2 were amplified, ORF1ab and N genes. Amplification of N gene was observed in all saliva samples while amplification of ORF1ab, only in 72.8%. Table 2 shows the distribution of CT by the N and ORF genes in unstimulated saliva compared to the E and N genes in nasopharyngeal swab.

We observed differences between the CT for N gene in saliva samples (CT N_S) and the CT for gene E (CT E) in nasopharyngeal swab specimens, where the mean CT E was -4.1 (95%CI: -6.8 to -1.5; $p = 0.001$). However, there was no statistical difference between CT for N gene (CT N) from self-collected unstimulated saliva specimens and nasopharyngeal swab ($p = 0.183$). For the nasopharyngeal swab, there was no statistical difference between the CT E ($p = 0.080$) and CT N ($p = 0.496$) values (Table 3).

Table 1 SARS-CoV-2 virus detection in self-collected unstimulated saliva, oral swab, and nasopharyngeal swab specimens

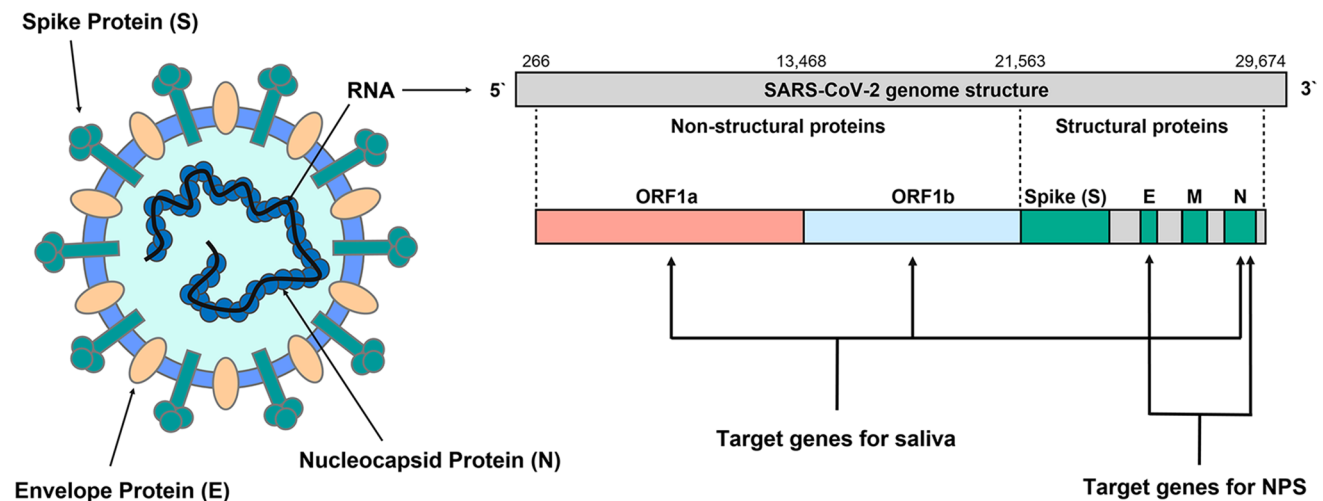| Specimen	Result	
	Negative n (%)	Positive n (%)
Unstimulated saliva ($n = 55$)	7 (12.7)	48 (87.3)
Oral swab ($n = 41$)	14 (34.1)	27 (65.9)
Nasopharyngeal swab ($n = 55$)	0 (0.0)	55 (100.0)

Fig. 1 SARS-COV-2 diagnostic targets: The figure illustrates diagnostic targets for COVID-19 testing. Genes targets used for saliva and for nasopharyngeal swab specimen (NPS) are illustrated

Table 2 Cycle threshold (CT) mean values and the 95% confidence interval (95% CI) comparisons for (A) N gene from self-collected unstimulated saliva and for the E and N genes from nasopharyngeal swab specimens (NPS) ($n = 34$) and (B) ORF gene from self-collected unstimulated saliva and for the E and N genes from NPS ($n = 24$)

Unstimulated saliva		Nasopharyngeal swab	
A	Gene N	Gene E	Gene N
Mean CT	29.9	25.8	28.4
95% CI	28.4; 31.6	23.5; 28.3	26.0; 30.9
B	Gene ORF	Gene E	Gene N
Mean CT	27.5	24.1	26.4
95% CI	25.4; 29.7	21.5; 27.0	23.9; 29.1

Values expressed by estimated means and 95% confidence intervals (95% CI)

Table 3 Differences between cycle thresholds obtained for the N (CT N_S) and ORF (CT ORF) genes in self-collected unstimulated saliva and for the E (CT E) and N (CT N_{NP}) genes in nasopharyngeal swab (NPS) specimens

Sample	CT difference	Estimated mean difference (95% CI)	p-value
Saliva	CT E − CT N_S	−4.1 (−6.8; −1.5)	0.001
	CT N_{NP} − CT N_S	−1.6 (−3.9; 0.7)	0.183
NPS	CT E − CT ORF	−3.3 (−7.0; 0.3)	0.080
	CT N_{NP} − CT ORF	−1.1 (−4.1; 2.0)	0.496

All values are expressed by estimated means, with 95% CIs in parentheses

Self-collected unstimulated saliva, oral swab, and nasopharyngeal swab specimens in the detection of SARS-CoV-2

Of the 55 patients, 21 had concomitantly CT values for E and N genes in nasopharyngeal swab specimens and for N gene in unstimulated saliva and oral swab specimens and 12 had concomitantly CT values for E and N genes in nasopharyngeal swab specimens and for ORF gene in unstimulated saliva and oral swab specimens. Table 4 shows the distribution of CT by the N and ORF genes in unstimulated saliva and oral swab and by the E and N (N_{NP}) genes in nasopharyngeal swab.

When comparing the CT values of the N gene in the oral swab to the CT values in the nasopharyngeal specimens, we observed differences in relation to the CT E ($p < 0.001$) and CT N_{NP}, where the mean CT E was −8.5 (95% CI: −12.2 to −4.7 and the mean CT N_{NP} was −5.6 (95% CI: −9.2 to −1.9; $p = 0.001$) lower than the mean CT N in oral swab.

As for CT values for N gene, the mean CT in unstimulated saliva was −4.4 (95% CI: −6.3 to −2.4; $p < 0.001$) lower than that in the oral swab. Comparing the CTs of the ORF gene in the oral swab to the CTs of the nasopharyngeal specimens, we observed evidence of differences in relation to the CT E and CT N_{NP}, where the mean CT E was −9.3 (95% CI: −14.7 to −3.9; $p < 0.001$) and the mean CT N_{NP} was −6.9 (95% CI: −12.1 to −1.8; $p = 0.004$) less than the mean of the CT ORF values in oral swab. Similarly, the mean CT value of the ORF gene in unstimulated saliva was −8.3 (95% CI: −11.5 to −5.2; $p < 0.001$) less than the average of the value in the oral swab (Table 5).

Types of saliva sampling for SARS-CoV-2 detection

When comparing unstimulated self-collected saliva and oral swab, of the 37 patients with positive results for gene N in self-collected unstimulated saliva, 26 (70.3%) were also positive with the oral swab. Considering the positive results for the ORF gene in unstimulated saliva in 34 patients, 16 (47.1%) were also positive with the oral swab. Regardless of the gene, unstimulated saliva was positive in 37 patients, whereas the oral swab was positive only in 27 of the 37 (73.0%) samples.

Table 4 Mean values and 95% confidence intervals (95% CI) estimated for cycle threshold (CT) measurements for (A) N gene in unstimulated saliva and oral swab and by the E and N genes in nasopharyngeal swab specimens (NPS) ($n = 21$) and (B) ORF gene in unstimulated saliva and oral swab and by the E and N genes in NPS ($n = 12$)

Sample				
Unstimulated saliva		Oral swab	Nasopharyngeal swab	Nasopharyngeal swab
A	Gene N	Gene N	Gene E	Gene N
Mean CT	28.9	33.3	24.8	27.7
95% CI	26.8; 31.1	31.5; 35.1	22.3; 27.5	25.0; 30.7
B	Gene ORF	Gene ORF	Gene E	Gene N
Mean CT	24.3	32.7	23.4	25.7
95% CI	21.8; 27.1	29.4; 36.3	20.3; 26.8	22.7; 29.2

Values expressed by estimated means and 95% confidence intervals (95% CI)

Table 5 Differences between cycle threshold (CT) measurements obtained for the N and ORF genes in self-collected unstimulated saliva and oral swab and for the E and N genes in nasopharyngeal swab specimens ($n = 21$)

CT gene differences (sample source)	Estimated mean difference (95% CI)	p-value
CT E (NPS) − CT N (S)	−4.1 (−7.0; −1.2)	0.003
CT N (NPS) − CT N (S)	−1.2 (−3.8; 1.5)	0.384
CT E (NPS) − CT N (OS)	−8.5 (−12.2; −4.7)	<0.001
CT N (NPS) − CT N (OS)	−5.6 (−9.2; −1.9)	0.001
CT N (S) − CT N (OS)	−4.4 (−6.3; −2.4)	<0.001
CT E (NPS) − CT ORF (S)	−0.9 (−3.7; 1.8)	0.594
CT N (NPS) − CT ORF (S)	1.4 (−1.6; 4.4)	0.594
CT E (NPS) − CT ORF (OS)	−9.3 (−14.7; −3.9)	<0.001
CT N2 (NPS) − CT ORF (OS)	−6.9 (−12.1; −1.8)	0.004
CT ORF (S) − CT ORF (OS)	−8.3 (−11.5; −5.2)	<0.001

All values are expressed by estimated means, with 95% CIs in parentheses. *CT E*, CT value for E gene; *CT N*, CT value for N gene; *CT ORF*, CT value for ORF gene. Sample sources are indicated in parentheses as follows: *(NPS)*, nasopharyngeal swab specimen; *(S)*, saliva; *(OS)*, oral swab

Discussion

Saliva samples can be collected by a variety of methods, such as posterior oropharyngeal saliva, oral swab, and unstimulated saliva. However, the majority of studies used the term saliva failing to describe exactly which sample collection technique was used [6]. In the present study, we directly compared two saliva sampling techniques for SARS-CoV-2 detection. While self-collected unstimulated saliva showed a sensitivity of 87.3% when compared to nasopharyngeal swab, this result was decreased to 65.9% when the saliva samples were collected using oral swab. This finding is relevant in the current pandemic, as the use of a swab for saliva collection does not seem optimal. Using self-collected unstimulated saliva as the sampling method alleviates the need for swabs and healthcare workers. Thus, self-collection of saliva in simple, sterile, nuclease-free tubes, negating the high costs associated with specialized collection devices, could be one of the key obstacles to meet the mass testing demands [11].

Here, we have demonstrated that nasopharyngeal swab exhibits higher sensitivity for COVID-19 testing than unstimulated self-collected saliva. However, unstimulated self-collected saliva showed higher sensitivity than oral swab. While nasopharyngeal swab samples appear to be more suitable for COVID-19 testing, it is important to recognize that saliva-based COVID-19 testing is an important option for frequent/repeated testing [12], as being scalable, and provides a useful sustainable strategy that easily allows periodic testing over time [13].

The sensitivity of self-collected unstimulated saliva was reduced to 78.2% when using only results from ORF gene. Although RT-PCR has been used as the gold standard for diagnosing COVID-19, there are some difficulties involving its application [14]. The first RT-PCR kit made available by China's CDC has as targets the N and ORF1ab genes of SARS-CoV-2 genome, and the infection is confirmed when both genes are amplified. However, there are reports of the amplification of only one of these gene targets [15].

Previous studies have reported a tendency for a higher viral load (or lower CTs) in nasopharyngeal swabs in comparison to saliva samples [16, 17]. However, in the present study, we found no significant differences between CT N in nasopharyngeal swab and in unstimulated saliva specimens ($p = 0.183$). This was not true when comparing CT N of nasopharyngeal swab to oral swab, where the latter presented a higher CT. The sensitivity of different diagnostic methods may vary according to variables such as gene target, sample processing and clinical signs and symptoms at the time of sample collection, as observed in the present study.

Recent studies reported that SARS-CoV-2 could be detected in the saliva of asymptomatic individuals and outpatients [17–20], showing a higher sensitivity than nasopharyngeal swabs for diagnosis of asymptomatic and mild COVID-19 infection [21–23]. In the present study, we have only included inpatients, although we see great potential with saliva's utility outside of the hospital setting.

There are, of course, limitations in the present study. Primarily, only symptomatic inpatients testing positive for COVID-19 [24] were included in the current investigation. Furthermore, as molecular biology supplies were limited, two different COVID-19 RT-PCR kits were used. We also recognize the limited number of study participants who were able to provide all three different samples. Outside of a pandemic setting, where there are no limitations of resources, our future studies will include a larger group of patients, record the operational characteristics (sensitivity, specificity, precision, and so on) of COVID-19 diagnostic test, and comply with Standards for Reporting of Diagnostic Accuracy Studies (STARD) guidelines [25]. Finally, no biological tests have 100% sensitivity and 100% specificity, a fact which needs to be considered when diagnostic results are translated into clinical practice [26].

Future investigations should include a larger sample size and focus on the diagnostic capability of human saliva for identifying COVID-19 with new cost-effective point-of-care technologies, making diagnosis accessible for anyone and anywhere [27]. Self-collected saliva is quite comfortable for patients as well as being easy, low cost [28], and non-invasive with minimal equipment, and does not require a healthcare facility or professionals. It also minimizes the nosocomial transmission of SARS-CoV-2 to healthcare workers. In the current pandemic situation, all research centers, health

agencies, and healthcare providers should explore this diagnostic opportunity and rapidly develop automated molecular point-of-care assays.

Conclusions

Unstimulated self-collected saliva samples have shown a higher agreement with the nasopharyngeal swab samples for SARS-COV-2 detection than that obtain with oral swab samples. Self-collection of unstimulated saliva samples is safe and alleviates demands for supplies of swabs, personal protective equipment, and healthcare personnel. Given the growing need for testing worldwide, our findings support the efficacy of saliva samples in the diagnosis of SARS-CoV-2 infection.

Declarations

Ethics approval All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

Informed consent Informed consent was obtained from all individual participants included in the study.

All authors read and agreed on the final version of the manuscript.

Conflict of interest The authors declare no competing interests.

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