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Major Article

Evaluation of RT-qPCR of mouthwash and buccal swabs for detection of SARS-CoV-2 in children and adults



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Key Words:

SARS-CoV-2
Mouth wash
Buccal swab
COVID-19

A B S T R A C T

Background: The use of nasopharyngeal (NP) swabs as a specimen collection method to diagnose SARS-CoV-2 infection is frequently perceived as uncomfortable by patients and requires trained personnel. In this study, detection rate of SARS-CoV-2 in mouthwash samples and buccal swabs were compared in both children and adults.

Material and methods: In patients admitted to hospital with confirmed COVID-19 within the previous 72 hours, NP and buccal swabs as well as mouthwash samples were collected. RT-qPCR was performed on all samples.

Results: In total, 170 samples were collected from 155 patients (137 adults and 18 children). Approximately 91.7% of the collected NP swabs were positive in RT-PCR compared to 63.1% of mouthwash samples and 42.4% of buccal swabs. Compared to NP swabs, the sensitivity of using mouthwash was 96.3% and 65.4% for buccal swabs in NP swab samples with a CT value <25. With increasing CT values, sensitivity decreased in both mouthwash and buccal swabs. The virus load was highest during the first week of infection, with a continuous decline observed in all three collection methods over time.

Discussion: Mouthwash presents an alternative collection method for detecting SARS-CoV-2 in the case of unfeasible NP swab sampling. Buccal swabs should not be used due to their low sensitivity.

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INTRODUCTION

Infection rates with *severe acute respiratory syndrome coronavirus type 2* (SARS-CoV-2) and subsequently morbidity and mortality remain high. Rapid identification and isolation of infected individuals are crucial for targeted infection control and mitigation measures. The current gold standard to diagnose early infection is the nucleic acid-based polymerase chain reaction (PCR) performed on naso-oro-pharyngeal swabs (NP). However, NP swab sampling is accompanied by several disadvantages. It is frequently perceived as uncomfortable by patients and is therefore not well tolerated particularly by children.^{1–3} Furthermore, trained healthcare personnel with protective equipment are required for sampling.^{4,5} If sampling is not performed adequately its sensitivity declines.⁶ In patients with complex anatomy or cases of improper sampling, adverse events, such as

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Ethics approval: The study was approved by the local ethics committee of the Vienna Health Care Group (EK-20-190-0820).

Consent to participate: Written informed consent was obtained from adults. Written informed consent was obtained by patients (age 14 to 18 years) or by patients and legal guardians (age 7–13 years).

Consent for publication: All authors gave consent for publication.

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cerebrospinal fluid leakage, temporomandibular joint dislocation and swab breakage in the nasopharyngeal area, may occur.^{7,8} Furthermore, especially in children, patients with mental disability or preexisting naso- or pharyngeal disease, NP swabs might not be obtainable. Additionally, sneezing or coughing during NP swab sampling might generate aerosols which again could increase SARS-CoV-2 transmission.

Since viral replication of SARS-CoV-2 has been shown to be highest in the upper respiratory tract tissues,⁹ the collection and evaluation of other respiratory specimens for SARS-CoV-2 detection should be considered. In a recent study, a high viral load of SARS-CoV-2 in sputum and saliva was reported.¹⁰ However, critically ill adults or children are often unable to spontaneously produce sufficient amounts of saliva or sputum.¹⁰ Findings of previous studies concerned with detection of SARS-CoV-2 in saliva specimens were limited by small sample size and diverse methodology.^{11–14} Whether PCR from saliva mouthwash is an accurate method to detect SARS-CoV-2 in symptomatic patients remains, therefore, yet to be determined.

This study aims to compare the sensitivity and specificity of saliva samples collected as mouthwash and buccal swabs (using a standardized collection method) with the gold standard, NP swabs, in adults and children. Mouthwash and buccal swabs facilitate producing and collecting saliva and avoid discomfort for the patient, with the potential for self-collection.

METHODS

The study took place at two different hospitals in Vienna, Austria. Pediatric and adolescent patients were recruited at one site (Clinic Ottakring, Vienna), adult patients at another (Clinic Favoriten, Vienna). The study was approved by the local ethics committee of the Vienna Health Care Group (EK-20-190-0820).

MATERIAL COLLECTION AND PROCESSING

Mouthwash

The Saliva Collection System from Greiner Bio-One¹⁵ was used to collect saliva samples. Collection occurred through thorough rinsing of the oral cavity for 2 minutes using the saliva extraction solution Balanced-Salt-Solution buffer. Subsequently, the solution was expelled into the small empty collection beaker of the Greiner Bio-One system.

Buccal and NP swabs

Buccal swab sampling was performed on both buccal sides. NP swab sampling was performed on both nostrils and oropharynx sampling using a single swab. FLOQSwab (Modell) swab preserved in universal transport medium (Copan UTM system, both, CA) was used.

Study participants

Adult patients admitted to the Department of Infectious Diseases and Tropical Medicine, Clinic Favoriten due to COVID-19 (confirmed by RT-qPCR within the previous 72 hours) were asked to participate in the study. After obtaining written consent, mouthwash samples were collected by the patient under supervision by a healthcare personnel. NP and buccal swab sampling were performed by a trained health care staff member.

At the second site, the Department of Pediatrics and Adolescent Medicine, Clinic Ottakring, patients above the age of 7 years were asked to participate in the trial. Written informed consent

was obtained by patients (age 14–18 years) or by patients and legal guardians (age 7–13 years). Due to small number of eligible pediatric inpatients with COVID-19, specimens were obtained either from pediatric in- and outpatient to facilitate recruitment. All inpatients were previously tested positive for SARS-CoV-2 by NP swab within 72 hours. Outpatients eligible for study entry either had to have contact with a known COVID-19 case and symptoms of any kind or signs and symptoms highly suspicious of SARS-CoV-2 infection (such as anosmia or atypical pneumonia) without known contact. Only outpatients who were tested positive for SARS-CoV-2 by in-house PCR at study entry, were included in data evaluation.

Processing

The NP and buccal swabs were stored in virus transport medium. All sample types were immediately sent to the laboratory, where RT-qPCR was performed within 24 hours after specimen collection. The reference method for comparing cycle threshold (CT values) comprised a diagnostic SARS-CoV-2 RT-qPCR test. RNA was extracted from 200 μ l of NP or throat wash supernatants using the MagnaPure 24 platform with MagNA Pure 24 Total NA Isolation Kit (Roche Diagnostics GmbH, Austria), according to a standard protocol, and was eluted in 50 μ l. Detection of SARS-CoV-2 RNA was performed using a commercial primer/probe mix (LightMix ModularDx Kit: SARS and Wuhan CoV E-gene, TIB Molbiol, Germany) and LC Multiplex RNA Virus Master (Roche Diagnostics GmbH, Austria) on a z480 real-time PCR instrument (Roche Diagnostics GmbH, Austria). Nuclease-free water and a synthetic RNA control provided with the primer/probe mix were included as respective no-template control and positive control. Ten μ l of the probe and master mix were pooled, respectively, and RT-qPCR was performed according to the manufacturer's instructions.

Statistics

Continuous variables are presented as means with standard deviations, and categorical variables as counts and percentages. The McNemar test was used to calculate differences in dichotomous dependent samples and the Chi-Square test independent variables. For small sample numbers ($n < 5$), Fisher's exact test was used. The sensitivity and specificity were calculated using a 2×2 table. Pearson's correlation calculation was used to evaluate the correlation between cycle threshold (CT value) and time since symptom onset or outcome (death in hospital vs discharge). A P -value $< .05$ was considered statistically significant. IBM SPSS Statistics 26 was used for statistical analysis.

RESULTS

Basic demographics and outcome

At total of 170 samples were collected in 155 patients (137 adult and 18 pediatric patients). One patient refused a NP swab, in two patients mouthwash samples were not collected, two pediatric patients only tolerated oropharyngeal swab. In general, the sample collection method mouthwash and buccal swab was tolerated well from all participants.

The basic demographics and outcome parameter are listed in Table 1.

Table 1
Basic demographics and outcome parameter of adult and minor participants

Characteristic	All	Adult	Pediatric	P-value
Number of patients (%)	155	137 (88.39)	18 (11.61)	
Age (years ± SD)	51.46 (±23.39)	60.98 (±17.67)	13.55 (±3.01)	<.0001
Female sex (%)	65 (41.94)	53 (38.69)	12 (66.67)	.024
Time since symptom onset (days ± SD)	7.80 (±4.60)	8.18 (± 4.33)	2.80 (± 1.72)	<.0001
Length of hospital stay (days) (min-max) *	9 (1-58)	10 (2-58)	5 (1-13)	.006
Asymptomatic at sampling (%)	10 (6.45)	2 (1.46)	8 (44.44)	<.0001
Disease severity				
Mild (%)	61 (39.35)	43 (31.39)	18 (100)	<.0001
Pneumonia, no supplemental oxygen (%)	34 (21.94)	34 (24.82)	0 (0%)	.0135
Pneumonia, requiring supplemental oxygen (%)	55 (35.48)	55 (40.15)	0 (0%)	.0003
ARDS/ MOF (%)	5 (3.23)	5 (3.65)	0 (0%)	1.000
Course & outcomes				
Discharge	146 (94.19)	128 (93.43)	18 (100)	.5995
ICU admission	9 (5.81)	9 (6.57)		.600
Death (%)	9 (5.81)	9 (6.57)	0 (0%)	1.000
Samples				
Number (%)	170	152 (89.41)	18 (10.59)	
NP swab (CT ± SD)	30.15 (±7.75)	30.31 (±7.72)	28.72 (±8.19)	.362
Mouthwash (CT ± SD)	35.44 (±6.21)	35.40 (±6.36)	35.81 (±4.76)	.931
Buccal swab (CT ± SD)	36.95 (±10.21)	37.17 (±10.61)	34.58 (±3.47)	.108

ARDS, acute respiratory distress syndrome; CT, cycle threshold; min, minimum; max, maximum; MOF, multi organ failure; NP, nasopharyngeal swab SD, standard deviation.

*Three outpatients in the pediatric group without hospital admission.

Performance of mouthwash and buccal swab samples

Of the collected NP swabs, 91.7% (155 of 170) were positive in RT-qPCR compared to 63.1% (106 of 168) of mouthwash samples and 42.4% (72 of 170) of buccal swab samples. In three patients, RT-qPCR results of mouthwash samples were positive, while the NP swab was negative, with a mean CT value of 40.89. One patient showed a positive result in the buccal swab (CT value of 40.99) and was negative in the NP swab sample.

Compared to the gold standard, the NP swab, the overall sensitivity of mouthwash samples was 67.3% compared to 45.8% in buccal swabs. Among pediatric patients, 83.3% of NP swabs were positive, compared to 55.5% of mouthwash samples, and 33.3% of buccal swabs, respectively. CT values were lowest in NP swabs (mean 28.7, SD 8.2) compared to mouthwash samples (mean 35.1, SD 4.8), and buccal swabs (mean 34.6, SD3.5).

The overall sensitivity with different CT values is shown in Table 2.

Mouthwash was shown to be significantly more sensitive compared to buccal swabs regarding the overall sensitivity ($P < .001$) and in NP swab samples with a CT value less than 30 ($P = .021$). As seen in Figure 1, the mean CT value in NP positive swab samples was 30.16 (SD 7.05, range 16.34-40.99), in mouthwash 35.4 (SD 5.2, range 22.89-40.99), and in buccal swabs 37.05 (SD 5.2, range 22.89-40.99).

The difference of the mean CT value was demonstrated to be significant between NP swab and mouthwash samples ($P < .001$), NP and buccal swab samples ($P < .001$), and mouthwash and buccal swab samples ($P < .001$).

A significant correlation between time from symptom onset and CT value was calculated using NP swabs ($r = 0.420$, $P < .001$), mouthwash ($r = 0.442$, $P < .001$), and buccal swabs ($r = 0.315$, $P = .009$). As depicted in Figure 2, there is a peak at the start of symptoms, followed by a continuous decline.

Compared to NP swabs, the sensitivity of mouthwash and buccal swab samples is highest during the first week since symptom onset. Subsequently, a strong decline in the sensitivity is observed for buccal swabs. The difference is demonstrated to be significant on days 7-12 ($P = .057$) and day >13 ($P = .039$) since symptom onset. The difference at up to day six since symptom onset was not observed to be significant ($P = .057$); however, a tendency was found.

Outcome

The CT value in NP swab ($r = -0.288$, $P = .004$) and mouthwash samples ($r = -0.249$, $P = .010$) were demonstrated to correlate negatively with hospital mortality. There was no correlation found using buccal swabs ($r = -0.128$, $P = .284$).

DISCUSSION

Our data demonstrate that sample collection via mouthwash is a potential method that can be used if NP swabbing is not possible. Although CT values were shown to be higher in mouthwash samples and the overall sensitivity was significantly lower than in NP swab samples, the sensitivity in patients with high virus load (CT value

Table 2
Sensitivity of qRT-PCR mouthwash and buccal swab compared to qRT-PCR of NP swab depending on the CT value

	Overall	NP swab			
		CT <30	CT <22	CT 22-25	CT 25-30
Mouthwash	67.3 % (103 of 153)	90.3 % (65 of 72)	100.0 % (23 of 23)	90.9 % (20 of 22)	81.5 % (22 of 27)
Buccal swab	45.8 % (71 of 155)	76.7 % (56 of 73)	82.6 % (19 of 23)	77.3 % (17 of 22)	71.4 % (20 of 28)

CT, cycle threshold; min, minimum; max, maximum; NP, nasopharyngeal swab.

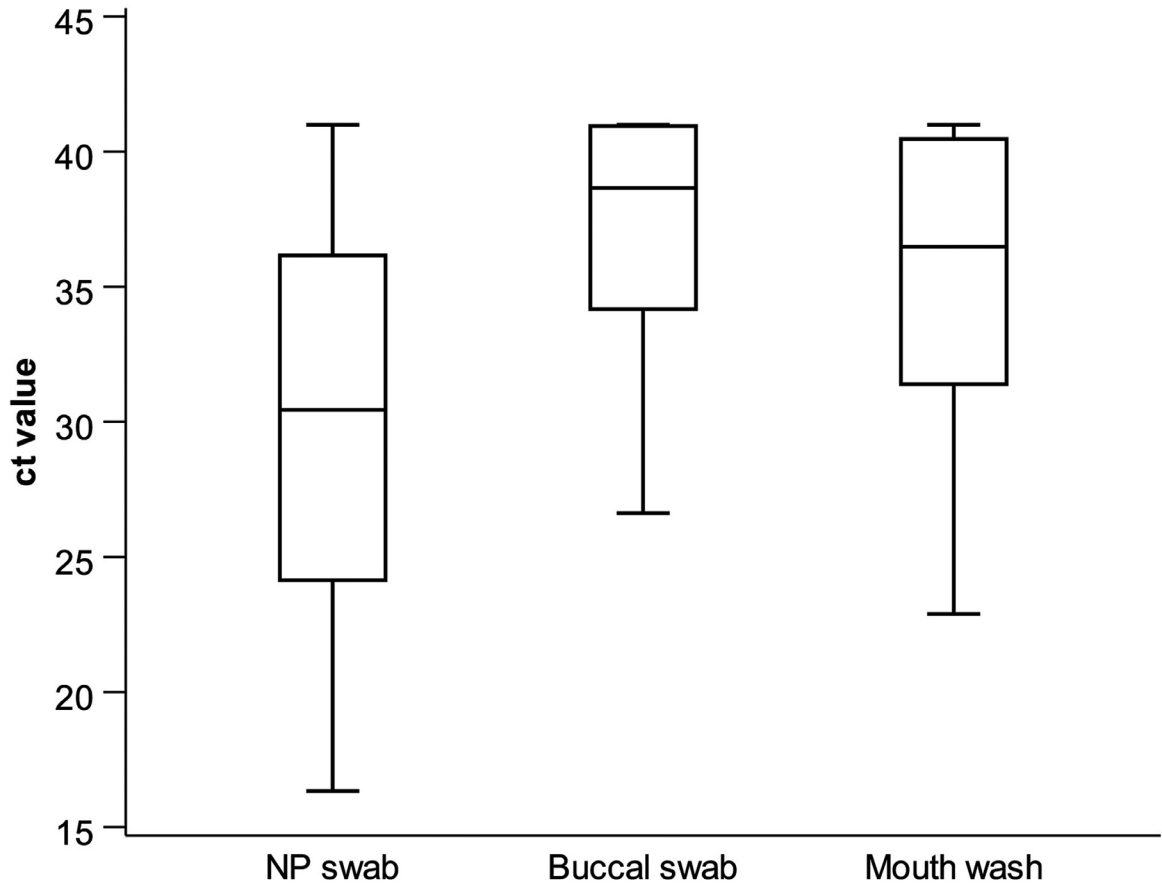


Fig 1. Median with interquartile range of CT value in qRT-PCR of NP swab, buccal swab and mouthwash.

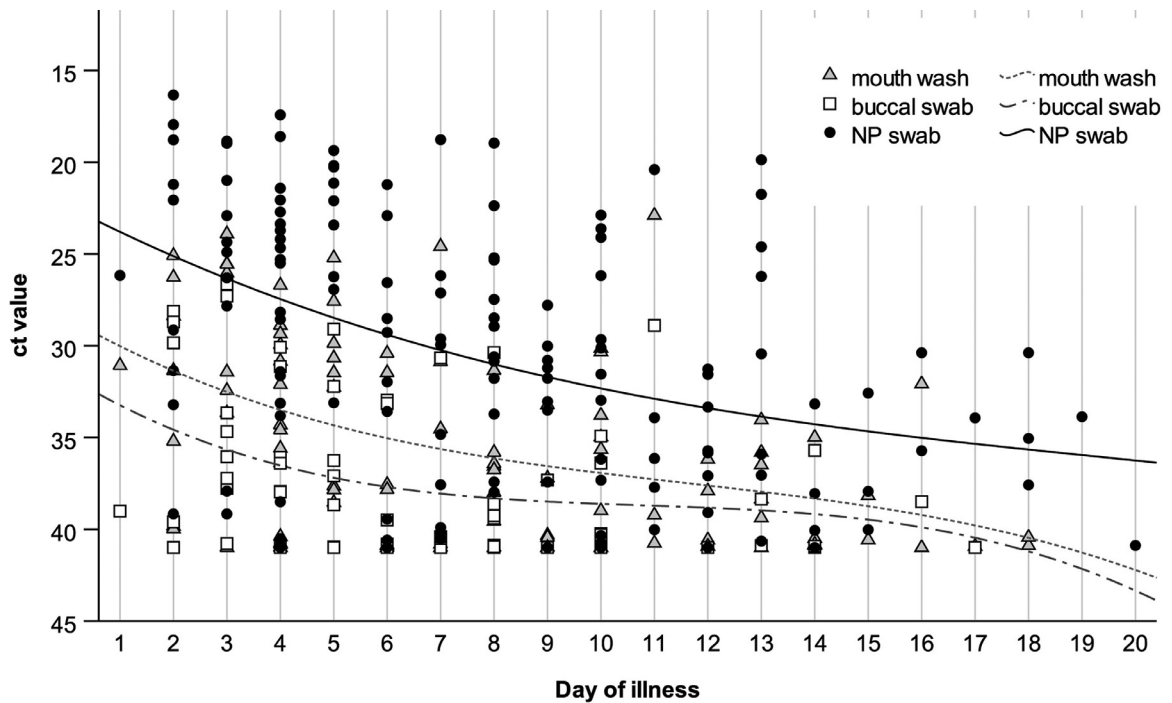


Fig 2. Trend of CT value in the course of time since symptom onset.

≤25) is 95.5% compared to NP swabs. In three patients, mouthwash samples were demonstrated to have a higher sensitivity than NP swabs.

Regarding buccal swabs, our findings are consistent with other studies^{3,16} that demonstrate the detection of SARS-CoV-2 via RT-qPCR in buccal specimens and a high viral load during the first week of disease or diagnosis. Similar to our findings, the authors report significantly higher CT values in buccal swab and lower sensitivity compared to NP swab.

Other reports on the use of saliva for SARS-CoV-2 detection emerged in the last months.^{11,14} However, these were criticized for the small sample size and that, unlike sampling with nasopharyngeal swabs, collection and processing methods for saliva specimens differed broadly among studies.

Mouthwash, that we used in our study, using the Saliva Collection System from Greiner Bio-One and following the given construction, enables standardized collection.

Interestingly, not only does the viral load influences the sensitivity (the higher the CT value, the less sensitivity), but the time from symptom onset was also found to play a role. The highest sensitivity was observed during the first week, correlating with the highest detected viral load in mouthwash samples.

Up to now, especially data from pediatric patients on SARS-CoV-2-detection in saliva specimens are scarce.¹⁷ In recent trial, authors report a sensitivity of 87.7% when compared to NP swabs in children presenting for COVID-19 screening.¹⁸ Guzmán-Ortiz et al.¹⁹ report a sensitivity of 82.3% in saliva specimens compared to NP swabs from pediatric patients with COVID-19. Sensitivity was lower in this trial, which might be attributable to different methodology and a high-rate of asymptomatic patients in our pediatric population. In contrast to our study, in these studies saliva was collected by spitting in a collection tube with and without gargeling first.

Caulley et al. demonstrated that in paired NP and saliva samples taken from adults, in 20% of patients SARS-CoV-2-PCR was detected only in saliva specimens.²⁰ Cases of children that test positive for SARS-CoV-2 by PCR only in saliva samples have been reported in other trials.^{18,19} Similarly, among our pediatric patient population, one patient only tested positive by mouthwash while remaining negative in NP and buccal swab. In our adult population three patients only tested positive by mouthwash. Testing saliva samples additionally to NP swabs in symptomatic patients could potentially increase diagnostic yield while minimizing discomfort. We postulate that collecting saliva samples via mouthwash might increase sensitivity compared to spitting saliva in a collection tube.

Mouthwash collection was well tolerated by all participants in the present study, even by young children. Collection could be self-performed, minimizing the transmission risk for healthcare personnel. In our protocol, patients only had to rinse the fluid in their mouths and not gargle the solution, therefore no aerosol droplets were produced. Furthermore, samples by mouthwash can be obtained at home, enabling broad application.

No adverse events (such as aspiration, nausea, or vomiting) or abortion of mouth rinsing was observed during the study period. This study demonstrates that using mouth washes to detect SARS-CoV-2 could be both save and feasible, even in patients with dyspnea or children and adolescents with limited compliance.

Limitations and strengths

Although several studies have evaluated saliva or sputum for SARS-CoV-2 detection, this is the first known trial utilizing

mouthwash samples without gargling. The strength of the present study is the inclusion of both minors and adults, as well as symptomatic and asymptomatic individuals. However, the rate of asymptomatic patients was only 5.8%, therefore, data validity in this group is limited. Further studies are required to evaluate the sensitivity of mouthwash samples in individuals with asymptomatic or mild COVID-19. We included a high number of adults, but only 18 pediatric patients participated in the trial. Many children and/or legal guardians denied study participation due to fear of additional NP sampling. Another strength of the study is the standardized sample collection using a collection system.

CONCLUSION

Similar to previous studies, our data shows that detection of SARS-CoV-2 in saliva specimens is possible. However, due to the low sensitivity and higher CT values compared to the NP swabs, buccal swabbing does not represent a suitable screening modality for COVID-19.

Though, RT-qPCR of mouthwash samples appears to be a reliable, easy-to-use collection method for the detection of SARS-CoV-2. Despite a lower sensitivity compared to NP swabs overall, especially in the first week of symptom onset, in low resource settings (ie, few NP sampling equipment, shortage of trained personnel), and patients, where NP sampling is not feasible (ie, children, reduced collaboration, underlying medical conditions) detection of SARS-CoV-2 in mouthwash samples is a suitable alternative.

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