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Investigation of saliva, tongue swabs and buccal swabs as alternative specimen types to nasopharyngeal swabs for SARS-CoV-2 testing

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ABSTRACT

Throughout the ongoing SARS-CoV-2 pandemic, the recommended sample type for initial diagnostic testing for SARS-CoV-2 infection has been a nasopharyngeal swab. Shortages in swabs and difficulties in obtaining nasopharyngeal swabs in certain patient groups has prompted research into alternative specimen types for the diagnosis of COVID-19. The aim of this study was to assess how 'simply collected' saliva along with tongue swabs and buccal swabs preformed as an alternative specimen type for SARS-CoV-2 detection. It was observed that saliva samples allowed for the detection of 85.3% of positive patients, tongue swabs allowed for the detection of 67.6% of positive patients and buccal swabs allowed for detection of 20.8% of positive patients, when compared to nasopharyngeal swabs. From this data, it could be concluded that using simple saliva collection can provide a less invasive and reliable alternative method for the detection of SARS-CoV2 particularly in those patients where invasive sampling is difficult and where regular repeat testing is required.

1. Background

Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2), the causative agent of COVID-19, has been responsible for significant morbidity and mortality worldwide and is still currently having large impact on health care systems globally. The importance of fast turnaround of accurate and reliable results has been paramount for management of patients and implicating effective infection control measures, both in and out of the healthcare setting.

The sample type which is currently recommended for initial diagnostic testing for current SARS-CoV-2 infections is a nasopharyngeal swab (NPS) [1]. NPS collection and testing remains the recommended testing standard as this sample type has been highlighted as providing the highest diagnostic sensitivity in early infection [2, 3]. The collection of NPS requires trained staff as incorrect sample collection will lead to inconsistent results [3]. Furthermore, collection of correctly taken NPS can be difficult to collect from certain patient groups, for example paediatric patients and patients with learning difficulties or dementia.

Shortages in swabs has prompted research into alternative specimen types for the diagnosis of COVID-19. Studies have reported mixed results on the usage of saliva as an appropriate sample for SARS-CoV-2 detection [4–6]. However, previous studies suggest simply collecting saliva in a sterile container allows for successful detection of other respiratory viruses [7, 8]. The aim of this study was to assess how 'simply collected' saliva along with tongue swabs (TS) and buccal swabs (BS) performed as an alternative specimen type for SARS-CoV-2 detection. These sample types were selected for investigation due to the large number of angiotensin converting enzyme 2 (ACE-2) receptors found on the tongue and oral mucosa [9].

2. Objective

Investigate saliva, TS and BS as an alternative specimen type for SARS-CoV-2 detection in comparison to the recommended sample type NPS.

3. Study design

NPS, saliva and TS were collected from 260 patients. Samples were collected from consenting patients who presented to the Emergency Department.

Subsequently, NPS, saliva, TS and BS were collected from patients of the previous cohort with known SARS-CoV-2 infection confirmed through RT-PCR. All patients had capacity and provided verbal consent

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to the collection of additional samples.

Samples were then processed and tested using the Allplex™ SARS-CoV-2 RT-PCR assay (Seegene, South Korea) to allow for comparison of Ct values. Specimens that returned a Ct 40> were regarded as negative.

4. Results

NPS, saliva and TS from 260 patients were tested through PCR for the detection of SARS-CoV-2. Of these patients, 226 tested negative for SARS-CoV-2 and 34 patients tested positive. A comparison of the results retrieved across this different sample types from positive patients is displayed in Table 1.

NPS are the recommended sample type for SARS-CoV-2 testing, therefore saliva and TS were compared to NPS. NPS from the 34 positive patients detected SARS-CoV-2 whereas saliva enabled detection of 29 positives, 85.3%, and TS lead to detection of 22 positives, 67.6% (Table 1). Indeterminate results were obtained from both saliva ($n = 1$) and TS ($n = 2$), meaning a positive or negative result could not be assigned with confidence to these specimens. This may have been due to insufficient viral RNA in the sample or possibly indicates inconsistency in sampling. Any specimen reporting as indeterminate was documented but excluded from further analysis. Results received from SARS-CoV-2 negative patient's saliva and TS were in 100% concordance with NPS (Table 1).

Interestingly, the NPS taken from Patients 5, Patient 8 and Patient 12 was positive but the corresponding saliva and TS were negative. The Ct values for the NPS were mid-30 range and may suggest individuals with weak Ct values in NPS may not have detectable virus in saliva or TS. It may be hypothesised that lingering viral RNA remains for a longer time in NPS, this is speculative and would require further investigation. Again, variables such as sample collection, viral load and stage of infection are likely to affect results [10].

Out of the positive patient cohort, 24 patients agreed to supply a buccal swab for comparison to other sample types. All sample types were taken at the same time to avoid variability. BS proved to be an inferior sample type detecting only 20.8% of positive patients (Table 1).

For buccal specimens obtained from known positive patients ($n = 24$), only 5 were reported as positive and the Ct values obtained were consistently higher, by at least 3 Ct values, than that of other specimen types. Due to the small sample number, buccal samples were not included in the Ct value comparison.

Ct values obtained for each sample type were widely distributed, however the Ct values obtained for NPS (range: Ct 17 – Ct 36.06) were consistently lower than that of their comparable saliva (range: Ct 20.57 – Ct 36.40) and TS (Ct 25.40 – Ct 36.84), suggesting a greater concentration of virus collected through NPS. The data reported no significant difference in Ct values obtained for both NPS and saliva (Fig. 1). However, the Ct values obtained from TS were significantly higher than those obtained for NPS ($p \leq 0.01$; Fig. 1). This further suggested TS are not an appropriate sample type for detecting SARS-CoV-2.

Table 1
Summary of results reported across each different sample type (**-**: Negative PCR result; +: Positive PCR result; **IND**: Indeterminate PCR result; N/A: Not applicable).

PATIENT	NPS (n = 34)	SALIVA (n = 34)	TS (n = 34)	BS (n = 24)
1	+	+	+	N/A
2	+	+	–	N/A
3	+	+	+	N/A
4	+	+	+	N/A
5	+	–	–	N/A
6	+	+	+	N/A
7	+	+	–	N/A
8	+	+	+	N/A
9	+	+	+	N/A
10	+	+	+	N/A
11	+	–	–	–
12	+	+	+	–
13	+	+	+	+
14	+	+	+	–
15	+	+	+	IND
16	+	+	+	+
17	+	–	–	IND
18	+	+	+	–
19	+	+	+	IND
20	+	+	+	IND
21	+	IND	–	–
22	+	+	+	+
23	+	+	+	–
24	+	+	+	–
25	+	+	+	–
26	+	+	+	+
27	+	+	–	–
28	+	+	–	–
29	+	+	IND	–
30	+	+	+	IND
31	+	+	+	IND
32	+	+	–	–
33	+	–	–	–
34	+	+	+	+
Total positive	34	29	23	5
Total negative	0	4	10	13
Total indeterminate	0	1	1	6
Accuracy	**100.0%**	**85.3%**	**67.6%**	**20.8%**

5. Discussion

The aim of this study was to assess how saliva, TS and BS preformed as an alternative specimen type for SARS-CoV-2 detection in comparison to the recommended sample type. NPS was taken as the Gold Standard to which other sample types were compared. Saliva samples allowed for the detection of 85.3% of positive patients, TS allowed for the detection of 67.6% of positive patients and BS allowed for detection of 20.8% of positive patients, when compared to NPS.

Saliva, TS and BS are less invasive and are therefore attractive in different patient groups, particularly in children individuals with ENT issues and individuals with learning difficulties, who cannot comfortably give a NPS. TS and BS may be taken from children or other patients who may not be able too easily offer an NPS. In light of this data, it would be recommended to avoid using TS or BS.

TS and BS were selected as viable candidates for investigation due to the abundance ACE-2 receptors found on the tongue, the SARS-CoV2

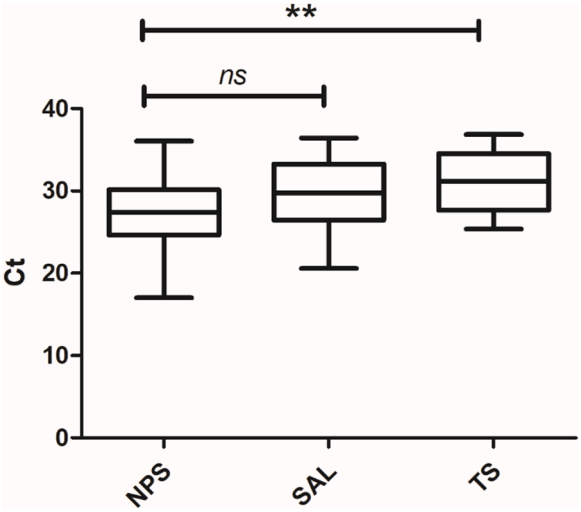

Fig. 1. Comparison of Ct values obtained across sample types (*ns:* $p > 0.05$; **: $p \leq 0.01$).

binding receptor on human cells [9]. Paired TS and BS collected alongside NPS highlighted that this sample types are sub-optimal compared to NPS and saliva, only detecting 67.6% and 20.8% of positive patients, respectively.

It is important to note that another aim of this study was to test saliva as a suitable sample type, but to do so in a way that supply chain restrictions would not hinder the ability to collect samples. Therefore saliva samples were collected simply into sterile universal containers. This was important with the aim of avoiding supply chain restrictions that were observed with swabs during the 1st wave of the pandemic. From this data, saliva represents a reliable and useful alternative sample for SAR-CoV2 testing particularly in the difficult patient groups outlined above. Saliva was in 100% concordance with NPS when calling negative results.

There are many variables affecting test results, for example, quality of samples in terms of timing and sample taking technique along with previous positivity of a patient and background prevalence. This provides a challenge to uniformity across studies evaluating different sample types. However from this data, using simple saliva collection can provide a less invasive and reliable alternative method for the detection of SARS-CoV2 particularly in those patients where invasive sampling is difficult and where regular repeat testing is required.

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Declaration of Competing Interest

The authors have no conflicts to declare

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