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Validation of self-collected buccal swab and saliva as a diagnostic tool for COVID-19

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Background: Effective management of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) requires large-scale testing to identify and isolate infectious carriers. Self-administered buccal swab and saliva collection are convenient, painless, and safe alternatives to the current healthcare worker (HCW)-collected nasopharyngeal swab (NPS).

Methods: A cross-sectional single-centre study was conducted on 42 participants who had tested positive for SARS-CoV-2 via an NPS within the past 7 days. Real-time polymerase chain reaction (RT-PCR) was performed and cycle threshold (Ct) values were obtained for each test. The positive percent agreement (PPA), negative percent agreement (NPA), and overall agreement (OA) were calculated for the saliva samples and buccal swabs, and compared with NPS.

Results: Among the 42 participants, 73.8% (31/42) tested positive by any one of the three tests. With reference to NPS, the saliva test had PPA 66.7%, NPA 91.7%, and OA 69.0%; the buccal swab had PPA 56.7%, NPA 100%, and OA 73.8%.

Conclusion: Self-collected saliva tests and buccal swabs showed only moderate agreement with HCW-collected NPS. Primary screening for SARS-CoV-2 may be performed with a saliva test or buccal swab, with a negative test warranting a confirmatory NPS to avoid false-negatives, minimize discomfort, and reduce the risk of spread to the community and HCWs.

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Introduction

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), the causative agent of coronavirus disease 2019 (COVID-19), was first identified in December 2019 (Liu et al., 2020a). In March 2020, COVID-19 was declared a pandemic by the World Health Organization.

One of the main reasons for the high rate of transmission of SARS-CoV-2 is the significant proportion of asymptomatic but infective carriers. Hence safe and effective detection of symptomatic COVID-19 patients through appropriate large-scale testing is of paramount importance.
Table 1
Baseline demographic and clinical characteristics of the patients, the results of paired nasopharyngeal, buccal, and saliva tests, and results of the patient experience survey (n = 42).

| Demographics | Nasopharyngeal swab | Buccal swab | Saliva test |
|--------------|---------------------|-------------|-------------|
|               | Positive (n = 30)   | Negative (n = 12) | p-Value | Positive (n = 17) | Negative (n = 25) | p-Value | Positive (n = 21) | Negative (n = 21) | p-Value |
| Age (years)   | 43.3 ± 8.5          | 48.2 ± 2.9   | 0.009     | >0.950 | 42.1 ± 8.1 | 46.5 ± 6.9 | 0.066     | >0.950 | 42.5 ± 8.9 | 46.9 ± 5.5 | 0.062 |
| Sex           |                      |              |           |         |            |            |           |         |            |            |        |
| Male          | 28 (93.3)           | 12 (100)    |           |         | 16 (94.1) | 24 (96.0) |           |         | 20 (95.2) | 20 (95.2) |         |
| Female        | 2 (6.7)             | 0           |           |         | 1 (5.9)   | 1 (4.0)   |           |         | 1 (4.8)   | 1 (4.8)   |         |
| Race          |                      |              |           |         |            |            |           |         |            |            |        |
| Indian        | 13 (41.3)           | 5 (41.7)    |           |         | 3 (17.6)  | 15 (60.0) |           |         | 6 (28.6)  | 12 (57.1) | 0.138 |
| Chinese       | 6 (20.0)            | 2 (16.7)    |           |         | 5 (29.4)  | 3 (12.0)  |           |         | 6 (28.6)  | 2 (9.5)   |         |
| Others        | 11 (36.6)           | 5 (41.7)    |           |         | 9 (52.9)  | 7 (28.8)  |           |         | 9 (42.9)  | 7 (33.3)  |         |

Table 2
Comparison between nasopharyngeal and buccal swabs (a), and between nasopharyngeal swab and saliva test (b), with positive percent agreement, negative percent agreement, positive predictive value, and negative predictive value.

| Nasopharyngeal swab | Positive | Negative |
|---------------------|----------|----------|
| (a) Buccal swab     |          |          |
| Positive            | 17       | 0        |
| Negative            | 13       | 12       |
| PPV = 95.2%         | 52.4%    | Overall agreement = 69.6% |
| NPA = 91.7%         |         |          |
| (b) Saliva test     |          |          |
| Positive            | 20       | 1        |
| Negative            | 10       | 11       |
| PPV = 95.2%         | 52.4%    | Overall agreement = 73.8% |
| NPA = 91.7%         |         |          |

SD, standard deviation. The p-value was based on the independent t-test or Mann–Whitney test for continuous variables, as appropriate, and Fisher’s exact test for categorical variables.

As the clinical symptoms that COVID-19 patients experience are often non-specific, the current method of detection relies heavily on molecular techniques. It is recommended that samples for testing are obtained from the upper respiratory tract rather than the lower respiratory tract (Diseases CIPoC, 2020). These samples include nasopharyngeal swabs (NPS), the current standard test, as well as oropharyngeal swabs, saliva specimens, and nasal aspirates.

The accuracy of COVID-19 detection varies according to the viral load in the different respiratory tract samples. In the first 14 days after the onset of illness, SARS-CoV-2 has most reliably been detected in sputum samples, which have been shown to contain the highest viral load, followed by nasal swabs (Pan et al., 2020; Yoon et al., 2020; Mohammadi et al., 2020). There are several disadvantages of the NPS, which is currently the most widely used test for diagnosis. Firstly, the NPS can only be performed by trained healthcare workers (HCWs). The patient needs to travel to the swabbing facility, which increases the risk of community spread. Secondly, to perform the swab, the medical staff must be in close contact with the patient. Coughing or retching by the patient could produce a large number of aerosolized droplets, increasing the risk of transmission to HCWs (Qian et al., 2020). Thirdly, this increases the burden on the currently heavily strained healthcare system by diverting a lot of resources to the diagnosis of SARS-CoV-2. Furthermore, a significant proportion of suspected cases who reside in the community are asymptomatic and are only called up for testing as a result of contact-tracing from a confirmed COVID-19 case and hence are unlikely to present for testing.

To counter these disadvantages, our group seeks to validate diagnostic tests that can be performed by the patient at home, and if validated, may have comparable concordance to NPS. A meta-analysis by Mohammadi et al. showed that alternatives, such as the sputum test, are more accurate in diagnosing SARS-CoV-2 compared
to the NPS, despite the NPS being the most widely used test for diagnosis (Mohammadi et al., 2020). Sputum tests are limited in their applicability due to the high prevalence of infected patients who are either asymptomatic or do not have a productive cough and thus are unable to produce sputum. However, this finding highlights the potential and need to continue searching for better alternatives.

In instances where an NPS is not possible, the US Centers for Disease Control and Prevention (CDC) and the US Food and Drug Administration (FDA) recommend a HCW-collected oropharyngeal specimen as an alternative. Indeed, active viral replication in the upper respiratory tract of young to middle-aged patients with mild cases of COVID-19 has been demonstrated previously, with peak viral shedding during the first week of symptoms (Wölfel et al., 2020). There is also emerging evidence to suggest that supervised self-collected oral fluid specimens perform similarly to HCW-collected NPS specimens for the detection of SARS-CoV-2 infection (Kojima et al., 2020). Hence, we decided to evaluate the effectiveness of these two options as alternative diagnostic tests.

In order to validate the use of buccal swabs and saliva specimens as alternative diagnostic tests for SARS-CoV-2, our group performed a cross-sectional study of NPS, self-collected buccal swabs, and self-collected saliva specimens obtained concurrently in order to determine the positive percent agreement (PPA), negative percent agreement (NPA), overall agreement (OA), positive predictive value (PPV), and negative predictive value (NPV). The advantage of self-collected buccal swabs or saliva specimens is two-fold: (1) it facilitates specimen collection without patients leaving their home, thus improving compliance and ease of collection, and (2) it reduces the risk of community spread and transmission to HCWs. This would revolutionize the management of SARS-CoV-2, where suspect cases can send in a specimen for testing without breaching quarantine notice, and thus increase detection rates without compromising the safety of others.

### Methods

**Setting and participants**

A cross-sectional single-centre study was conducted on 42 individuals who had previously tested positive for SARS-CoV-2 via an NPS within the past 7 days and who were isolated at the Singapore General Hospital (SGH). SGH is the largest tertiary hospital in Singapore and is one of the main referral hospitals for treating COVID-19 patients.

The inclusion criteria were participants diagnosed with SARS-CoV-2 infection by NPS, between the ages of 21 and 80 years. Patients who were unable to produce oral secretions for self-collection were excluded from the study. Written informed consent was obtained. This study was approved by the institutional ethics review board (CIRB Ref. No. 2020/2655).

**Demographic and clinical data collection, and survey on patient experience**

Sociodemographic data and symptoms at the time of sampling were obtained via a questionnaire administered by a study team member, and through a review of the medical records. After sample collection had been completed, the patient’s experience was surveyed. They were asked to select the test that best fit each of the following qualities: (1) comfort, (2) convenience, and (3) personal preference.

**Sample collection and processing**

Saliva, buccal, and nasopharyngeal samples were obtained from each participant in that order. For the saliva specimen, the participant was asked to cough deeply five times and pool saliva in
their mouth for 1–2 min prior to collection, and then gently spit 1–2 ml of saliva into a 60-ml sterile closed-top plastic collection container (BMH 921406, Biomedia, Singapore). Subsequently, for the buccal sample, the participant was asked to pool any phlegm or secretions in their mouth, rub the swab (sterile swab in a round tube; 300264, Deltalab, Spain) on the internal surfaces of both cheeks, above and below the tongue, both gums, and on the hard palate for a total of 20 s to ensure that the swab was saturated with oral fluid. The swab was then placed in the tube with the lid secured. Then, the NPS (MSC-96000-5T, Miraclean, China) was collected by a trained HCW for all patients, as per the standard hospital protocol (SingHealth, 2021). All swabs were processed in 1 ml of lysis buffer (Cobas Omni Lysis Reagent, P/N 06997538190) and an in-house RT-PCR was performed on all specimens based on the protocol of Corman et al. (Corman et al., 2020). The results for SARS-CoV-2, including the E-gene cycle threshold (Ct) values, were correlated to those of the NPS. All tests with signals that crossed the detection threshold were considered positive.

**Statistical analysis**

The independent t-test or Mann–Whitney test was applied to continuous variables, as appropriate, and Fisher’s exact test was applied to categorical variables. The results of the saliva and buccal swab tests were individually compared to the results of the NPS and the PPA, NPA, OA, PPV and NPV were calculated. Comparisons between the saliva sample and NPS Ct values and between the buccal swab and NPS Ct values were performed using the paired t-test. The statistical analyses were conducted using IBM SPSS Statistics version 20.0 (IBM Corp, Armonk, NY, USA).

**Results**

All of the patients who met the inclusion criteria were recruited and no patients were excluded from this study. Among the 42 participants who had previously tested positive for SARS-CoV-2 via an NPS, 73.8% (31/42) tested positive by any one of the three tests (RT-PCR on saliva sample, buccal swab, NPS). Table 1 reports the baseline demographics of those who tested positive and negative, for the three diagnostic tests for SARS-CoV-2 (Table 1). Participants who remained NPS-positive at the time of study recruitment were younger and more likely to be symptomatic (Table 1). The survey of participant experience showed that 59.5% (25/42) of participants ranked the buccal swab as the most preferred, most convenient, and most comfortable to collect. Overall, 40.5% (17/42) of participants chose the saliva test as the most convenient and most comfortable means for first-line SARS-CoV-2 testing. 38.1% chose the saliva test as the most preferred means of first-line SARS-CoV-2 testing. Only one participant ranked the NPS as the most preferable (1/42), while none of them felt that it was convenient or comfortable.

The incidence of SARS-CoV-2 in this cohort of patients, who had previously tested COVID-19-positive as diagnosed using the NPS (the current standard of care test), was 71.4% (n = 30/42). With reference to the NPS, the buccal swab had a PPA of 56.7%, NPA of 100%, OA of 73.8%, PPV of 100%, and NPV of 48% (Table 2A). The viral load was lower in the buccal specimen, with the mean Ct value for the buccal swab being higher than that of the NPS (27.19 ± 2.48 vs 21.66 ± 5.60, p < 0.001) (Figure 1A). With reference to the NPS, the saliva sample had a PPA of 66.7%, NPA of 91.7%, OA of 69.0%, PPV 95.2%, and NPV of 52.4% (Table 2B). There was no difference in Ct values between the saliva and NPS specimens (25.77 ± 5.60 vs 22.95 ± 6.03, p = 0.057), suggesting a similar viral load in the two samples (Figure 1B).

The presence of symptoms at the time of swab collection was associated with better diagnostic accuracy. Fifty-three percent of symptomatic participants tested positive by both NPS and buccal swab (p = 0.017), while 63.3% were positive by both NPS and saliva sample (p = 0.004) (Table 3). There was no statistically significant association between Ct values and the time since symptom onset, for all three diagnostic modalities (Figure 2). Comparison of Ct values for the paired NPS, saliva samples, and buccal swabs are shown in Figure 3.

**Discussion**

In this study, it was found that saliva tests and buccal swabs were comparable to each other and were in moderate agreement with NPS for the detection of SARS-CoV-2, with PPA between 56%
and 66% and PPV between 95% and 100%. On the other hand, both saliva tests and buccal swabs performed comparably to NPS in detecting negative cases, with an NPA of 90% to 100%. Overall, they were moderately comparable to NPS with an OA of 69% to 74%. This paves the way for larger validation studies to support the use of self-collected saliva samples or buccal swabs, without the risk of community spread or spread to HCWs.

Most coronaviruses are known to replicate in the epithelial cells of the respiratory tract. The viral shedding pattern in SARS-CoV-2 has been shown to be similar to that of the influenza virus, with peak viral shedding in the first week of illness (Wölfel et al., 2020; Zou et al., 2020). NPS RT-PCR remains the reference test for the diagnosis of COVID-19 in most parts of the world, despite results showing that sample positivity is highest for alternative tests like those on sputum specimens (Mohammadi et al., 2020). Moreover, there is emerging evidence alluding to similar detection rates with alternative sample collection methods that are comparable, feasible, and safe, including saliva, posterior oropharyngeal saliva, and throat washing.

Self-collected saliva offers a promising prospect for sample collection. This is due to its relative convenience, comfort, and subjective participant preference compared to the NPS. It is also reported to have a reduced time and cost (To et al., 2019), and decreased risk of transmission in the community and to HCWs, making it a convenient and safe means of mass testing.

There is increasing evidence that the viral load in saliva is comparable to or higher than that in the nasopharynx (Yoon et al., 2020; Mohan Rao et al., 2020; Iwasaki et al., 2020; Liu et al., 2020b). The present study showed that the Ct values were comparable for saliva and NPS, representing a similar viral load in the two samples. A longitudinal study in Korea showed that the highest viral load was in the nasopharynx, but that the viral load was also remarkably high in the saliva, and the virus was detected in the saliva up to day 6 of hospitalization and day 9 of illness (Yoon et al., 2020).

Azzi et al. showed a high overall concordance rate of 97.4% between NPS and saliva, and also showed that there was no statistically significant difference in viral load between the two types of sample (Iwasaki et al., 2020; Azzi et al., 2020). The present study results also showed a high PPV of 95.2% compared to NPS, the current standard diagnostic test for SARS-CoV-2. However, the low PPA of 66.7% may limit its suitability to replace NPS as the gold standard diagnostic test. Existing studies have also shown varying PPA or concordance rates when comparing saliva detection rates to those of NPS, with reported positive concordance rates ranging from 45.6% (Mohan Rao et al., 2020) to 94.8% (Chen et al., 2020).

The great differences in concordance rates may be accounted for by the limitations in sample collection. The lower PPA might also be limited by the amount and sample collection technique used for the saliva test. Moreover, considering the similar viral load in saliva and NPS, this further supports that the technique might be the reason leading to some saliva tests being completely negative. Unfortunately, our study did not investigate the quality of the saliva produced. Kojima et al. observed that there were differences in the positive detection rates between clinician supervised versus unsupervised self-collected saliva tests and NPS, which further supports that the collection technique plays a big role in the positive detection rates (Kojima et al., 2020).

Another possible explanation is the duration from diagnosis to sample collection. Wyllie et al. showed that a higher percentage of saliva samples remained positive up to 10 days after the COVID-19 diagnosis compared to NPS (81% vs 71%) (Wyllie et al., 2020), which is a result supported by other studies (Mohammadi et al., 2020; Becker et al., 2020; Williams et al., 2020). Although the present study results showed a non-significant relationship between the interval after symptom onset and sample collection, other studies have reported the possibility that NPS and saliva samples are most equivalent early in the illness when compared to samples collected beyond the first week (Mohammadi et al., 2020; Mwaddah et al., 2020).

Not surprisingly, the presence of symptoms increased the PPA for saliva compared to NPS (79.2%, 19/24).

Overall, these findings provide supporting evidence for recommending saliva as an alternative modality that is safer, more comfortable and convenient to collect, and sufficiently accurate for making a clinical diagnosis.

Very few studies have evaluated the use of buccal swabs, with conflicting results: two studies showed that a self-collected buccal swab performed less accurately than the NPS (Mwaddah et al., 2020; Kam et al., 2020), while another showed that the results were comparable (Kojima et al., 2020). Our results showed that the buccal swab performed less accurately than the NPS due to its lower PPA rate of 56.7%. It also had a lower viral load, as reflected by the significantly higher Ct value obtained. However, the buccal swab was found to be the most convenient, comfortable, and preferred test according to the study participants. Thus, buccal swabs may have a role in specific populations who might not be able to spontaneously produce saliva, such as in young children and older patients (Kam et al., 2020).

The correlation of detection rates and symptoms still remains controversial. Zou et al. showed that the viral load in asymptomatic patients was similar to that in symptomatic patients with NPS and throat swabs (Zou et al., 2020), while Chau et al. showed that viral loads were equivalent in symptomatic patients, but lower in asymptomatic patients in saliva (Chau et al., 2020). The current study found that none of the tests showed an evident association with the time since symptom onset. However, symptomatic patients were more likely than asymptomatic patients to be true-positive with both the buccal swab and saliva test.

Taken together, buccal and saliva samples show a moderate agreement with the NPS, and are reasonable alternatives to the current gold standard NPS for the diagnosis of COVID-19. In view of the comparable viral load, high PPV and OA, moderate PPA, and greater patient comfort and convenience, we

![Figure 4. Recommended clinical workflow for population SARS-CoV-2 screening.](image-url)
recommend that the initial screening NPS be replaced with a saliva test or buccal swab for community testing. If the result is positive, the individual can be managed as COVID-19-positive, due to the high PPV when compared to the NPS. If the result is negative, a confirmatory NPS should be performed before discharging the patient, to mitigate the moderate PPA value and ensure that they are truly COVID-negative (Figure 4). This workflow is timely, especially in the context of mass screening strategies where resources, in particular HCWs, are scarce and saliva tests or buccal swabs can easily increase testing rates due to their ease of collection without a further strain on the healthcare system. The requirement for a confirmatory NPS for negative cases combines the sensitivity of NPS with the high NPA of buccal swab and saliva samples, while saving costs, minimizing discomfort, and reducing the risk of spread to the community and to HCWs.

This study has several strengths. Firstly, it was possible to conduct self-collection of buccal swab and saliva sample specimens, testing the feasibility of widespread application in the community with an assessment of participant acceptability of the various test modalities, which showed saliva tests and buccal swabs to be superior to the NPS. Secondly, we were also able to recruit a variety of symptomatic and asymptomatic patients with various stages of their disease from symptom onset.

This study also has several limitations. Firstly, the study was conducted on only a small number of adult patients. Furthermore, only patients who had previously tested SARS-CoV-2-positive were included, limiting the generalizability of results to population screening; in population screening, the individuals are predominately asymptomatic and the incidence is lower, thus reducing the PPV. The participants were also recruited within 7 days of testing positive by NPS RT-PCR instead of immediately after diagnosis, thus resulting in 26.2% of participants who tested negative in at least one of the three diagnostic tests in this study. The timing of test assessments may also have resulted in the lack of association between viral load and the timing of symptom onset. We also note that the NPS is not the ‘gold standard’ test due to its high false-negative rate. Other studies have shown that alternatives like sputum and bronchoalveolar lavage have a higher sensitivity in diagnosing SARS-CoV-2 (Mohammadi et al., 2020; Wang et al., 2020). However, this was the most widely used test at the time of writing. This study was also limited to the adult population. Chong et al. showed that saliva tests might not be useful in the paediatric population (Chong et al., 2020), which might limit their use as a population screening test. Even though the samples were self-collected, they were still conducted in the context of a healthcare setting and under the supervision of a HCW. Thus, the performance of the tests may be overestimated, with a better quality of sample collection as compared to the actual quality in the general community. Hence, larger validation studies need to be performed in both symptomatic and asymptomatic patients before buccal and saliva samples can be routinely recommended in the clinical setting.

In conclusion, saliva samples and buccal swabs were comparable to each other and were in moderate agreement with NPS for the detection of SARS-CoV-2. Patient preference was greatest for both saliva samples and buccal swabs when compared to NPS, in terms of comfort and convenience. Primary screening for SARS-CoV-2 may be performed with a saliva or buccal test. A negative test warrants a confirmatory NPS to avoid false-negatives. Buccal swabs might be considered in the context of specific cohorts where spontaneous saliva production might be difficult. These self-collection methods represent feasible alternatives that could help reduce the discomfort experienced by patients, save costs, and reduce the risk of community spread and spread to HCWs. Larger trials should be conducted to determine the generalizability of these tests in both the symptomatic and asymptomatic population before they can be used for large-scale community testing.

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**Declaration of Competing Interest**

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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