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User acceptability of saliva and gargle samples for identifying COVID-19 positive high-risk workers and household contacts

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ABSTRACT

Throughout the COVID-19 pandemic nasopharyngeal or nose and/or throat swabs (NTS) have been the primary approach for collecting patient samples for the subsequent detection of viral RNA. However, this procedure, if undertaken correctly, can be unpleasant and therefore deters individuals from providing high quality samples. To overcome these limitations other modes of sample collection have been explored. In a cohort of frontline health care workers we have compared saliva and gargle samples to gold-standard NTS. 93% of individuals preferred providing saliva or gargle samples, with little sex-dependent variation. Viral titers collected in samples were analyzed using standard methods and showed that gargle and saliva were similarly comparable for identifying COVID-19 positive individuals compared to NTS (92% sensitivity; 98% specificity). We suggest that gargle and saliva collection are viable alternatives to NTS swabs and may encourage testing to provide better disease diagnosis and population surveillance.

1. Introduction

The World Health Organization declared COVID-19 a global pandemic on March 11, 2020 and called on all countries to ramp up their testing strategies. Unfortunately, the COVID-19 virus remains a significant threat to public health as it continues to evolve, as has been seen for the emergence of the alpha (January, 2021), delta (June, 2021) and omicron (November, 2021) variants. Recent evidence suggests that omicron has reduced virulence compared to alpha and delta variants but that omicron has an increased transmission rate [1]. More variants are likely to arise, particularly in parts of the world that do not have good access to vaccines and large numbers of immunocompromised individuals. Testing therefore remains critical as part of a risk stratified approach to detect, isolate, and contain the virus, and will be key in facilitating the sustained reopening of society [2].

The recommended initial diagnostic sampling route for symptomatic individuals is combined nose throat swab (NTS) specimens tested using nucleic acid amplification tests (NAATs) such as quantitative reverse transcription PCR (RT-qPCR) [3]. However, in the UK and many other countries, individuals are recommended to use formal NTS testing in conjunction with lateral flow devices to facilitate rapid at-home testing. In PCR testing swab specimens are obtained from the nasopharynx and posterior pharynx and/or tonsillar areas [4], whilst lateral flow devices use NTS or just nose swabs.

Many find the procedure to collect NTSs uncomfortable or unpleasant which could impact uptake of, or compliance with testing and screening programs. In particular this is likely to have a significant impact on asymptomatic testing. During the COVID-19 pandemic frontline health workers have been regularly tested, who are often exposed to patients with COVID-19 and who have to maintain a presence at work. Although these individuals know the benefits of testing there is a risk that due to the unpleasant nature of taking nasopharyngeal swabs thoroughly, as well as testing fatigue, that over time adherence or sample quality might decrease. NTS sampling for PCR is also resource and labor intensive and testing capacity has been limiting in light of increased demand for tests and mass screening proposals. Furthermore, travel to a testing facility is often required to obtain a formal NTS and there is a risk of nosocomial transmission to the individual performing or facilitating the test due to the close contact required as well as the potential to induce

Abbreviations: NTS, nose throat swab; NAAT, nucleic acid amplification test; VTM, viral transport medium

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involuntary coughing or sneezing. In order to overcome these barriers various alternative testing modalities have been explored.

Saliva has emerged as a promising alternative to nasopharyngeal swab testing as it is convenient, non-invasive, less resource intensive, and can be reliably self-administered. Saliva sampling is already an established practice in genetics to obtain nucleic acid samples, and has been used in the diagnosis of a number of respiratory viral infections prior to the COVID-19 pandemic, including other coronaviruses [5–7]. It has now been trialed in various healthcare settings internationally as an alternative diagnostic method in the detection of SARS-CoV-2 [8–14]. Studies examining concordance rates of saliva with NTS testing have reported varying results—1 study demonstrated increased sensitivity of saliva compared with NTS [15], while another reported that in a community setting saliva testing was less sensitive than NTS [16]. However, a recent meta-analysis of the available evidence concluded that saliva NAAT diagnostic accuracy is similar to that of NTS NAAT [17].

Pharyngeal gargle specimens have also been shown to be a useful sample type for detection of respiratory viruses including coronaviruses [7,18-20] and have shown comparability with NTS in the detection of SARS-CoV-2, although the available literature is more limited [21–26].

If practical to implement locally the use of saliva or gargle could be an alternative diagnostic modality for clinical staff and community testing, and be a means of increasing testing capacity and versatility. This mode of testing may also be well suited for the collection of samples from children, for example in a school setting, and for asymptomatic testing, for example those being routinely tested in the health and social care sector. We therefore set out to investigate the feasibility and utility of both saliva and pharyngeal gargle sampling methods, their relative acceptability, and their validity in the detection of the SARS-CoV-2 virus compared with nasopharyngeal testing. As samples are often stored before analysis, we extended the study by exploring how sample storage conditions impact the ability to identify SARS-CoV-2 RNA in saliva samples.

2. Materials and methods

2.1. Patient population and study design

Symptomatic NHS Lothian Health Care Workers (HCWs) or their symptomatic household contacts attending the drive through NHS Lothian Staff COVID-19 testing Centre were invited to participate during the study periods (Phase 1a, 1b and 2). The initial phase of the study (Phase 1a) included symptomatic HCWs only with ages ranging from 17 to 64 years (mean 40.2 (SD 1.2), median 41.0 (IQR 28.5–51.0). Phase 1b and 2 included symptomatic HCWs and their symptomatic household contacts reflecting the guidance for testing at that time. In Phase 1b ages ranged from 6 to 66 years (mean 37.7, SD 15.3; median 38.00, IQR 27–51), and in Phase 2 ages ranged from 8 to 67 years (mean 38, SD 14; median 37, IQR 22). Children aged 5 years or younger were excluded as were individuals who had eaten, had a drink, smoked, chewed gum, or brushed their teeth within 30 minutes prior to the test.

Participants received an information sheet and were consented at the time of sample collection. They received normal clinical care with the samples analyzed anonymously but linked to provide results. The study was a 'Quality Improvement Project' registered with the NHS Clinical Governance Support Team.

2.2. Sample collection

2.2.1. Study Phase 1a and 1b: Saliva sampling

Symptomatic HCWs, or their symptomatic household contacts (Phase 1b) were offered a saliva test in addition to their routine NTS. Phase 1a took place between 20 and 22 May, 2020 and Phase 1b took place between 5 and 16 October, 2020. Paired nasopharyngeal and oropharyngeal specimens were obtained by trained testing center staff prior to saliva testing. Those who agreed to take part were asked to produce a saliva sample by repeatedly pooling saliva in their mouth and spitting into a universal specimen container. In Phase 1a participants were asked to provide one 5 ml saliva sample; these specimens were transported to the lab by cold chain in cool boxes with ice packs. During Phase 1b participants were asked to produce 2 saliva samples at the same time (2 ml saliva per container), 1 stored and transported in a 4°C refrigerator, and the other at ambient temperature (average 15°C).

2.2.2. Study Phase 2: Saliva and Gargle sampling

The second phase of the study took place between 2 and 13 November, 2020. HCWs or their symptomatic household contacts were offered saliva and pharyngeal gargle tests in addition to routine upper respiratory swab testing. NTS specimens were obtained by testing center staff prior to saliva and gargle specimens. Saliva was obtained as per phase 1 but only one 2 ml sample was required. For gargle specimens, participants were asked to gargle 10 ml of 0.9% saline for 20 seconds then deposit the gargle liquid into a universal specimen container. Included participants provided all 3 specimen types in a specified order (NTS, saliva, gargle). Participants were then asked to select their preferred testing modality and to provide reasons for their choice.

3. Laboratory processing

Previously we developed a methodology to screen for SARS-CoV-2 in nasopharyngeal swabs stored in viral transport medium (VTM) collected from symptomatic individuals [27]. The laboratory methodology was further adapted to facilitate viral RNA extraction from saliva and gargle specimens.

3.1. Phase 1a: Saliva sampling (cold storage and transport of saliva specimens)

Saliva and corresponding NTS specimens were processed at the Institute of Genetics and Cancer (IGC) Laboratories on the Western General Hospital Campus, Edinburgh. Existing equipment and reagents were used as per previously validated protocol for COVID-19 RT-qPCR using Thermofisher TaqPath CE-IVD kits [27]. 200 μl saliva or NTS specimen was lysed with 250 μl TNA lysis buffer (Omega Biotek) containing carrier and control RNA. The saliva samples were treated with proteinase K, then each sample extracted using the Omega Biotek MAG-BIND VIRAL DNA/RNA kit on a Thermofisher Kingfisher Flex according to the supplier's Supplementary Protocol for NP Swabs (April 2020 version). Testing was performed using a ABI TaqPath COVID-19 Multiplex Assay for the N, ORF and S genes on a ABI 7500 Fast Real-Time PCR machines [27].

3.2. Phase 1b: Saliva sampling (ambient and/or cold storage and transport of saliva specimens)

Saliva and corresponding NTS specimens were processed at the Royal Infirmary of Edinburgh. Two shipping conditions were used to evaluate the stability of SARS-CoV-2 in saliva samples between collection and receipt in the laboratory for testing. Total nucleic acid extraction was conducted on the bioMerieux easyMAG or EMAG (bioMerieux Inc, Durham, NC); briefly, for all individual specimens tested, 200 μl of the sample was added to 2 ml NucliSENS Lysis Buffer (bioMerieux) and extracted into 110 μl of eluate. Testing was performed for the E and S genes on ABI 7500FAST Dx instruments using the RealStar SARS-CoV-2 RT-PCR Kit (Altona-Diagnostics) according to the manufacturer's instructions. Saliva samples were pre-treated with proteinase K whereby 200 μl of sample was mixed with 25 μl of molecular grade proteinase K (NEB) and then inactivated by heating at 95°C for 10 minutes prior to extraction.

3.3. Phase 2: Saliva, and Gargle sampling

Saliva samples were processed as per Phase 1b. Gargle samples (1 ml) were mixed with 1 ml VPSS (Viral PCR Sample Solution, E&O Laboratories; 53% guanidine thiocyanate, 44 mM Tris-HCl pH 6.4, 20 mM EDTA, 25 TX-100) and incubated for 10 minutes to ensure inactivation of virus [18] before proceeding to extraction as described above. Pre-treatment with proteinase K was not required for gargle specimens.

Discrepant samples were tested for the *RPP30* gene, which encodes the human RNase P protein subunit P30 [28].

4. Statistical analysis

The diagnostic accuracy of saliva and gargle samples was determined by estimating sensitivity and specificity with exact binomial 95% confidence intervals (CIs) using detection rate in NTS as the gold standard. The significance of sample type and/or shipping conditions on Cq values was determined using the Wilcoxon Test for paired samples and the results plotted using the ggpubr package (v.0.4.0) for R. All analyses were performed using R software (ver. 4.0.3). The effect of gender and age on sample collection method choice was assessed using Fisher's Exact Test.

5. Results

5.1. Detection of SARS-CoV-2 RNA in saliva and NTS samples

A total of 109 health care workers provided NTS and saliva samples (Study Phase 1a). 79 were female (72.5%), and 29 were male (26.6%). Of the 109 paired samples there was a 0.9% (n = 1) and 7.3% (n = 8) amplification failure rate for NTS and saliva respectively, which may be due to high sample viscosity (Fig. 1A). 10 NTS samples were found to be positive for SARS-CoV-2 RNA (Supplementary Table 1), of these all-paired saliva samples were also identified as positive, whilst a further positive sample was identified, resulting in a total of 11 positive saliva specimens. This specimen had a relatively high Cq value in the TaqPath assays compared to other positive samples (33.5, 34.5, and 37.6 for the N, ORF and S genes respectively) (Supplementary Table 1), however the distributions of Cq values for this small sample sets were similar (Fig. 1B) indicating that saliva can be used for identifying COVID-19 positive individuals. Compared to NTS testing, sensitivity for saliva testing was 100% (95% CI, 69.1%–100.0%) and specificity was observed to be 98.9% (95% CI, 94.0%–99.97%).

5.2. Determination of optimal storage conditions for saliva samples

In this phase of the study a total of 206 participants including 147 (71.4%) females and 59 (28.6%) males each provided 2 saliva specimens and an NTS sample (Study Phase 1b). Saliva samples were then stored and transported at either ambient or cold (4°C) temperatures. Samples were shipped the same day (within 8 hours of specimen collection). From these paired samples, 28 NTS specimens were found to be positive for SARS-CoV-2 (14%).

A total of 19 positive and 6 negative samples were selected at random and the cognate saliva samples shipped at ambient or cold temperatures were analyzed and compared to the corresponding NTS results (Supplementary Table 2). Results were concordant between the 2 saliva samples stored under different conditions, but compared to the NTS samples only 17 samples were identified as being COVID-positive giving a sensitivity of 89.5% (95% CI, 66.9%–98.7%) and a specificity of 100% (95% CI, 54.1%–100.0%).

The objective of this phase was to compare how different shipping conditions might influence the ability of the laboratory to detect SARS-CoV-2 RNA, which presumably will be a reflection of viral RNA in the saliva samples. As noted, samples were concordantly called irrespective of shipping method, but it might be anticipated that due to RNA degradation at room temperature there would be a concomitant increase in Cq values. However, statistically there was no difference in Cq values between saliva samples stored at 4°C (Cold Chain) or at ambient temperature for the E gene (P = 0.57) or S gene target (P = 0.78) and the data distributions were similar (Fig. 2).

5.3. User acceptability of saliva and gargle sample for SARS-CoV-2 RNA detection

Samples were collected from 261 individuals with a gender breakdown of 22.2% male and 77.8% female. Out of 261 individuals 46 (18%) were found to have NTS specimens positive for SARS-CoV-2 RNA. A total of 37 positive and 30 negative NTS specimens were selected at random and the cognate saliva and gargle specimens were analyzed by RT-qPCR. Internal control amplification failed in 3% of gargle samples and 9% of saliva samples, despite all being positive for the human RPP30 housekeeping gene [27].

After discounting inhibited samples there were 65 NTS/gargle pairs (Supplementary data 3). 62 of the 65 NTS and/or gargle pairs were concordant (34 positive and 28 negative pairs) whilst SARS-CoV-2 RNA was detected only in the NTS specimen and not in the gargle specimen in 3 of the NTS and/or gargle pairs (Table 1). Of the 61 remaining NTS and/or saliva pairs after discounting inhibited samples (6/67 for saliva), 57 of the 61 NTS and/or saliva pairs were concordant (32 positive and 25 negative pairs). SARS-CoV-2 RNA was

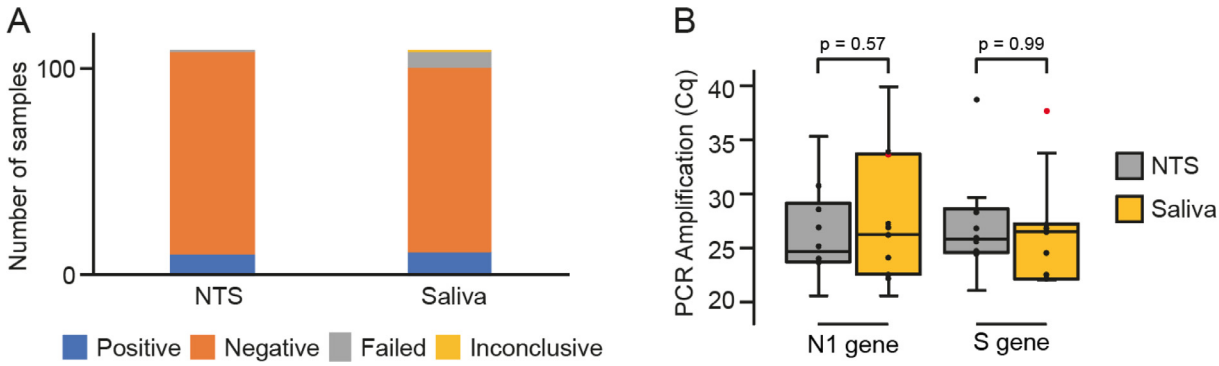

Fig. 1. Identification of COVID-19 positive individuals using NTS or saliva samples. (A) Bar chart showing the proportion of positive, negative and failed tests in paired (n = 109) NTS and saliva samples. (B) Boxplot showing the distribution of N1 gene and S gene Cq values for COVID-positive samples. Red point marks a sample identified as positive in the saliva sample but negative by NTS. P values are for a 2 tailed Wilcoxon test. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

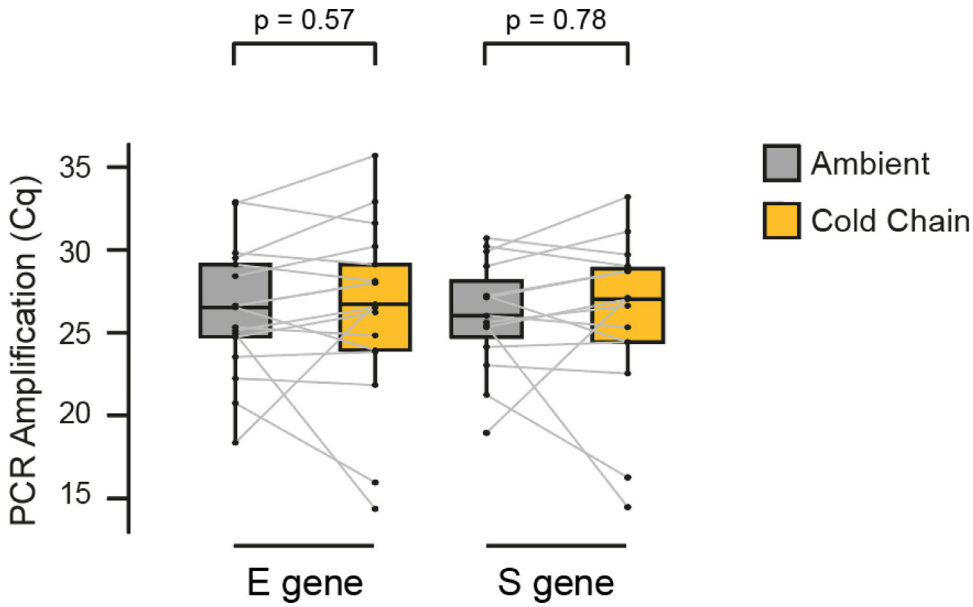

Fig. 2. Effect of shipping conditions on SARS-CoV-2 RNA stability in saliva samples. Comparison of Cq values (E gene and S gene) in paired saliva and/or NTS samples following shipment to the laboratory under room temperature (RT) or cold chain (CC) conditions. *P* values are for a 2 tailed Wilcoxon test.

Table 1
Comparison of gargle versus paired NTS and saliva versus paired NTS for the detection of SARS-CoV-2 RNA.

Gargle	NTS			Saliva	NTS		
	Positive	Negative	Total		Positive	Negative	Total
Positive	34	0	34	Positive	32	1	33
Negative	3	28	31	Negative	3	25	28
Total	37	28	65	Total	35	26	61
Sensitivity	91.9% (95% CI,78.1%–98.3%)			Sensitivity	91.4% (95% CI, 76.9%–98.2%)		
Specificity	100.0 % (95% CI,87.7 %–100.0%)			Specificity	96.2% (95% CI, 80.4%–99.9%)		

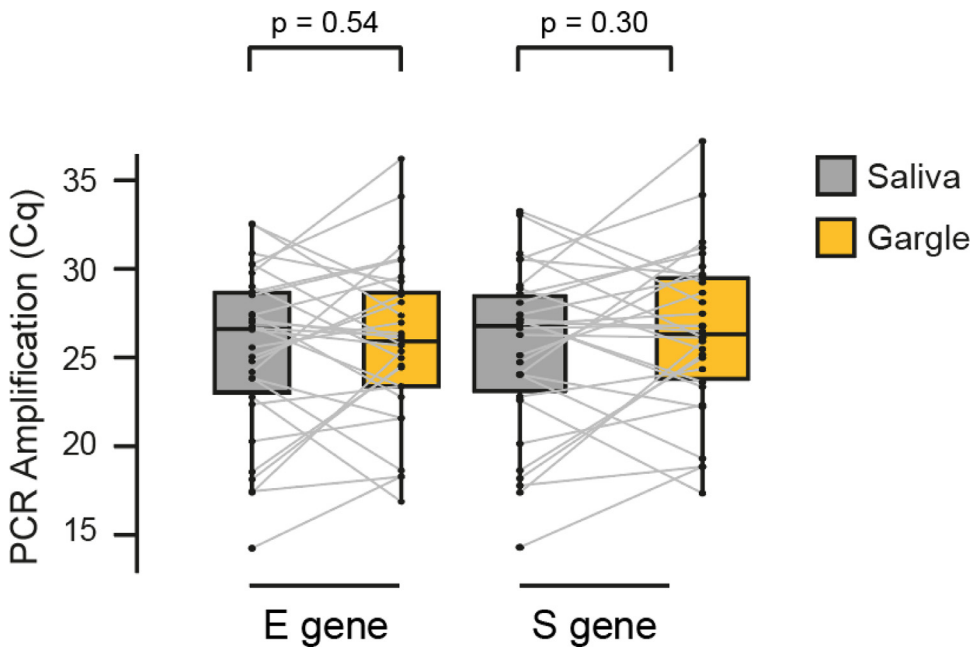

Fig. 3. SARS-CoV-2 RNA amplification in saliva and gargle samples. Comparison between Cq values (E gene and S gene) in paired saliva and gargle samples.

Table 2
Preferential testing method stratified by gender and age.

Gender	Male	Female	Total
NTS	4 (6.9%)	15 (7.4%)	19 (7.3%)
Saliva	24 (41.4 %)	85 (41.9%)	109 (41.8%)
Gargle	30 (51.7 %)	103 (50.7 %)	133 (50.9%)
Age (6–67 years)	≤18 years	>18 years	Total
NTS	0 (0%)	19 (7.9%)	19 (7.3%)
Saliva	13 (65%)	96 (39.8%)	109 (41.8%)
Gargle	7 (35%)	126 (52.3%)	133 (50.9%)

detected only in the NTS specimen in 3 of the NTS and/or saliva pairs. Notably there was 1 saliva sample that tested positive for SARS-CoV-2 RNA while the paired NTS and gargle were negative (Table 1). Both E and S genes were detected in this positive saliva specimen (Cq values 31.46 and 31.77 respectively).

No significant differences were observed in the Cq values between corresponding saliva and gargle specimens (Fig. 3). However, there were 5 discrepant saliva and/or gargle pairs (3 positive saliva specimens with a negative corresponding gargle specimen and 2 positive gargle specimens with a corresponding negative saliva specimen). These positive discrepant specimens all had Cq values that were within the interquartile range for positives of that sample type. Notably, there was also 1 saliva sample that tested positive for SARS-CoV-2 RNA while the corresponding NTS and gargle were negative.

Of the 261 patients who participated in Phase 2, 133 (50.9%) preferred the gargle method, 109 (41.8%) preferred the saliva method, and 19 (7.3%) preferred the nasopharyngeal swab method (Table 2) with no apparent gender specific differences (Supplementary Table 4). Similarly, there was no bias in sample test method according to age (Supplementary Table 5).

6. Discussion

Saliva and gargle specimens demonstrated high levels of concordance when compared with NTS specimens which corresponds well with previous studies (saliva sensitivity 93.1% (95% CI, 75.8%–98.8%) phase 1 and 91.4% (95% CI, 76.9%–98.2%) phase 2), gargle sensitivity 91.9% (95% CI,78.1%–98.3%)). This shows both saliva and gargle to be reliable alternative testing modalities to NTS for detection of SARS-CoV-2.

In Phase 1a, a positive saliva specimen was detected where the corresponding paired NTS was negative, and similarly in Phase 2 a positive saliva specimen was detected with corresponding negative NTS and gargle specimens. Both of these positive saliva specimens had relatively high Cq values (>30 for each gene tested). Although these samples were considered as false positives, both saliva specimens could be true positive cases as despite being weakly positive all 3 genes were detected in the positive saliva specimen in Phase 1a, and both E and S gene detected in the positive saliva specimen in Phase 2. The potential for increased sensitivity of saliva compared to NTS has also been described previously [13].

In Phase 1a there was a relatively high level of amplification failure for saliva (7.3%) compared to NTS (0.9%) samples. One possible explanation is the high viscosity of saliva which increases the complexity of specimen handling and requires additional pre-processing steps in the lab to overcome this issue. Since undertaking this study we, and others, have explored alternate methods for reducing saliva sample viscosity including the addition of DTT, proteinase K and sample agitation by vortexing. In contrast to saliva, gargle samples do not have the same challenges but instead produce larger volumes of fluid which could be more difficult for lab handling on automated systems, and may increase the risk of spillage. As there was no significant difference in Cq values detected between saliva samples stored and transported at 4°C versus ambient temperature, cold transport is not required which increases the practicality of these sample types.

In contrast to NTS, self-collected saliva and gargle samples are easy to obtain, and more acceptable to patients, with the distinct advantage of being a less invasive testing modality. Sampling with these methods also obviates the need for contact with a health care professional and reduces the use of PPE and other resources at testing centers in the face of pervasive testing supply shortages. Home self-sampling using these sample types would avoid the requirement for symptomatic individuals to attend testing facilities and reduce risk of viral transmission to others. This would have particular utility in rural settings where testing facilities are less available. Furthermore, the use of these sample types could increase compliance with testing and screening programs, particularly those who are required to undergo regular asymptomatic screening. Their non-invasive nature may also remove some of the difficulties surrounding consent for and compliance with NTS in populations such as young children and those with cognitive impairment.

Overall gargle specimens were the most acceptable test. This was irrespective of sex with 50.7% of females and 51.7% of males choosing the gargle as their preferred sample method. Saliva was preferred by 41.9% of females and 41.8% of males, while NTS was the most acceptable in only 7.4% of females and 6.9% of males. Of those aged 18 years and under, 65% preferred saliva testing and 35% preferred gargle with none selecting NTS as their preferred testing method. Of those aged >18 years 52.3% preferred gargle testing, 39.8% preferred saliva and 7.9% preferred NTS. Using Fisher's Exact Test, there was no significant association between gender and sample collection method or between age and sample collection method (Supplementary Tables 4 and 5).

Study participants preferring the gargle and saliva samples cited ease of performance and reduced discomfort compared with NTS as reasons for this response. Some individuals chose gargle over saliva as they felt that the saliva sample took longer to produce, whereas the gargle was quicker. Other participants found the saltiness of the saline solution unpleasant and for that reason preferred the saliva test. Those who preferred the NTS offered a variety of explanations including ease, speed, being used to it, the perception of a more accurate result, and being less unpleasant than they had expected. Of important note, the volume of saliva required for this study was greater than that which would be necessary in practice (0.5–1 ml), and reducing the volume required may further increase the acceptability of saliva testing. In addition, the large volumes of saline used in this study are likely unnecessary with most other groups using only 2.5–5mls [24–26].

There is limited available literature comparing the validity and acceptability of both saliva and gargle specimens with NTS. Genelhoud et al. [26] found saliva had a superior sensitivity, specificity, and accuracy than gargle with saliva performing similarly to nasopharyngeal samples. Conversely, Goldfarb et al. [20] found that gargle was significantly more sensitive than saliva when compared to health care worker collected NP swabs. However, in the latter study the order of sample collection was alternated to reduce biasing 1 of these oral sample types which may be a confounding factor as performing mouth rinse prior to saliva sampling is likely to dilute the saliva specimen and thus decrease it's sensitivity. In our study a saliva specimen was obtained prior to saline gargle in all participants. Goldfarb et al. found gargle to be more acceptable than saliva or NTS testing in their study population which is consistent with our findings.

A degree of compliance is required to provide a saliva or gargle sample and further work is required to explore the feasibility of alternative sample collection techniques in individuals unable to comply with the instructions required. Some individuals may also be unable to produce sufficient saliva including those with conditions such as sicca syndrome, or those taking medications that cause xerostomia.

7. Conclusions

Our study confirms that both saliva and gargle sample types are suitable for use as an alternative testing modality to NTS, particularly

in scenarios where the latter cannot be obtained, and for individuals required to undergo repeat asymptomatic screening. These samples are sufficiently stable at room temperature to allow ambient transport to the lab. The option of these alternative sampling techniques increases diagnostic capacity and versatility in the face of ongoing significant testing demands.

Declaration of competing interest

The authors declare that they have no known competing interests.

Authors' contributions

Kirsty McLennan: Conceptualization, Methodology, Investigation, Writing – Original Draft, Writing – Review and Editing. Ellen Barton: Methodology, Investigation, Writing – Original Draft. Christie Lang: Methodology, Investigation, Writing – Original Draft. Ian R. Adams, Conceptualization, Methodology. Gina McAllister: Methodology, Investigation. Martin A. M. Reijns: Conceptualization, Methodology, Writing – Review and Editing. Kate Templeton: Conceptualization, Methodology. Ingólfur Johannessen: Conceptualization, Methodology, Writing – Review and Editing. Alastair Leckie: Conceptualization, Methodology, Writing – Review and Editing. Nick Gilbert: Conceptualization, Writing – Original Draft, Writing – Review and Editing.

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Supplementary materials

Supplementary material associated with this article can be found in the online version at doi:10.1016/j.diagmicrobio.2022.115732.

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