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

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Abstract
Throughout the COVID-19 pandemic nasopharyngeal or nose/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 healthcare 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 titres collected in samples were analysed 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.

Introduction
The World Health Organisation declared COVID-19 a global pandemic on March 11th 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 omicron that 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 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/ 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 programmes. In particular this is likely to have a significant impact on asymptomatic testing. NTS sampling for PCR is also resource and labour 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 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 trialled 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 – one 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[21–23].

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 impacted test results.

Results

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

NTS testing is considered the gold-standard for SARS-CoV-2 diagnosis. However this sampling method is uncomfortable and deters individuals from regular testing. This is particularly challenging for high risk groups such as healthcare workers who are often exposed to patients with COVID-19 and who have to maintain a presence at work. Previously we developed a methodology to screen for SARS-CoV-2 in nasopharyngeal swabs stored in viral transport medium (VTM) collected from symptomatic individuals[24]. To determine whether saliva samples can be used for detecting SARS-CoV-2 RNA and to compare the specificity and sensitivity of NTS and saliva samples, the laboratory methodology was further adapted to facilitate viral RNA extraction from saliva (see methods). 109 healthcare workers provided NTS and saliva samples (Study Phase 1a) with an age range from 17 – 64 years (mean 40.2 (SD 1.2), median 41.0 (IQR 28.5-51.0). 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 (Figure 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 (Figure 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%).

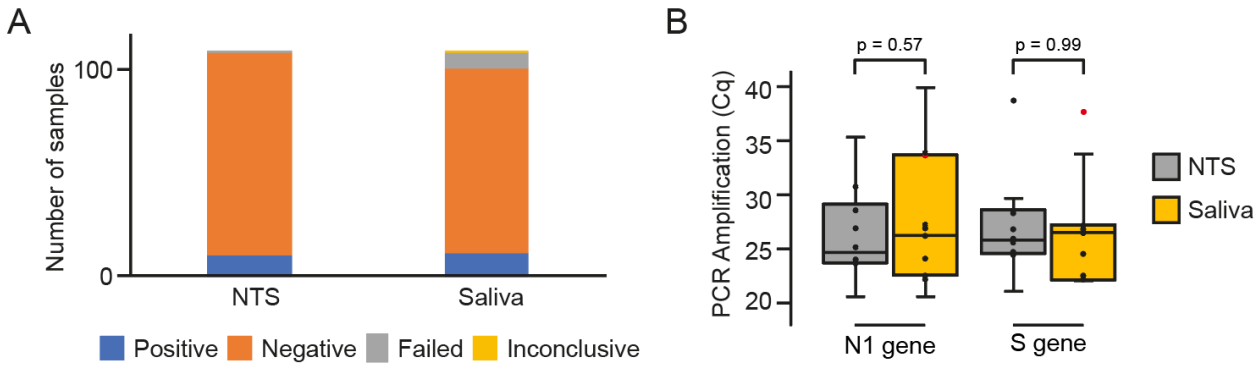

Figure 1 Identification of COVID-19 positive individuals using NTS or saliva samples. (A) Bar chart showing the proportion of positive, negative and failed tests in paired (n=109) NTS and saliva samples. **(B)** Boxplot showing the distribution of N1 gene and S gene Cq values for COVID-positive samples. Red point marks a sample identified as positive in the saliva sample but negative by NTS. P values are for a two tailed Wilcoxon test.

Determination of optimal storage conditions for saliva samples

Often, patient samples are collected some distance from the testing lab necessitating samples to be stored for a period of time. To determine how storage conditions affected the ability to identify SARS-CoV-2 RNA in saliva samples a total of 206 participants each provided two saliva specimens and an NTS sample (Study Phase 1b). Saliva samples were then stored and transported at either ambient or cold (4°C) temperatures. The ages of the participants ranged from 6 – 66 years old (mean 37.7, SD 15.3; median 38.00,IQR 27-51), and included 147 (71.4%) females and 59 (28.6%) males. From these paired samples, 28 NTS specimens were found to be positive for SARS-CoV-2 (14%).

19 positive and 6 negative samples were selected at random and the cognate saliva samples shipped at ambient or cold temperatures were analysed and compared to the corresponding NTS results (Supplementary Table 2). Results were concordant between the two 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 (Figure 2).

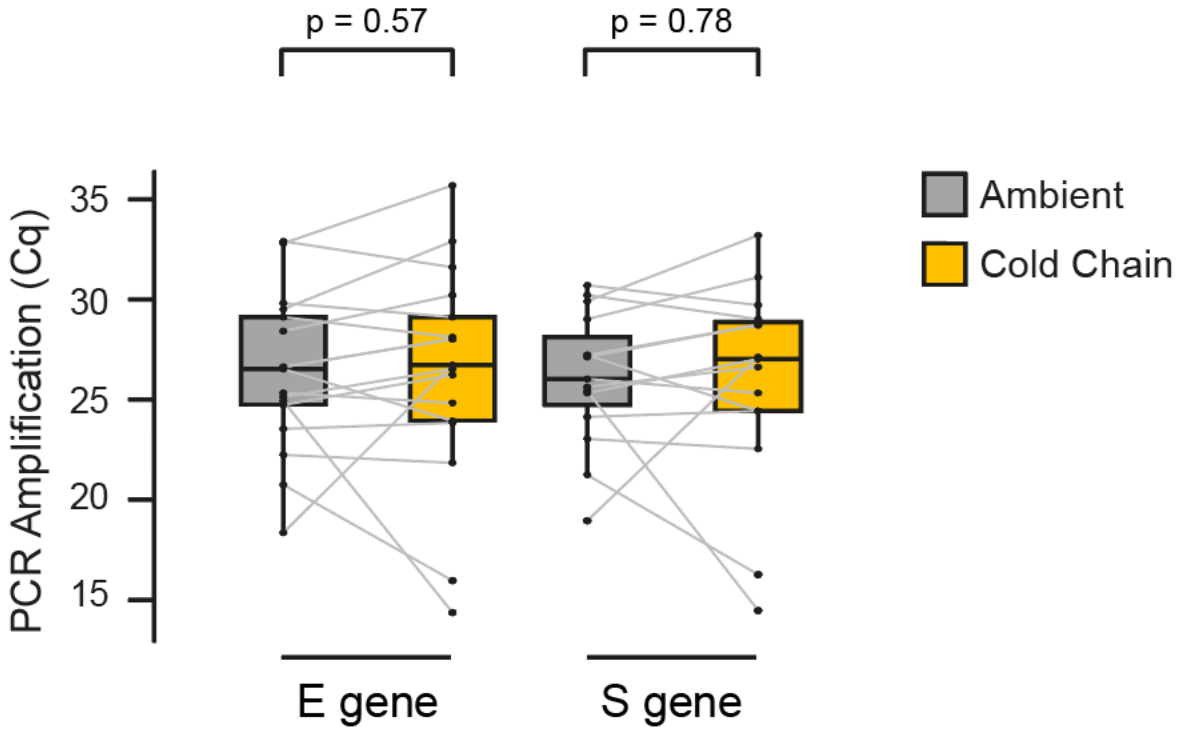

Figure 2 Effect of shipping conditions on SARS-CoV-2 RNA stability in saliva samples. Comparison of Cq values (E gene and S gene) in paired saliva/NTS samples following shipment to the laboratory under room temperature (RT) or cold chain (CC) conditions. P values are for a two tailed Wilcoxon test.

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

During the COVID-19 pandemic frontline health workers have been regularly tested, predominantly by NTS. 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. We therefore initiated a large testing programme (Study Phase 2) to explore alternative COVID-19 testing modalities by comparing the user acceptability and results of dependent NTS, gargle and saliva samples. Samples were collected from 261 individuals with ages ranging from 8 – 67 years old (mean 38, SD 14; median 37, IQR 22) and 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. 37 positive and 30 negative NTS specimens were selected at random and the cognate saliva and gargle specimens were analysed 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[25].

After discounting inhibited samples there were 65 NTS/gargle pairs (Supplementary data 3). 62 of the 65 NTS/ 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/ gargle pairs (Table 1). Of the 61 remaining NTS/ saliva pairs after discounting inhibited samples (6/67 for saliva), 57 of the 61 NTS/ saliva pairs were concordant (32 positive and 25 negative pairs). SARS-CoV-2 RNA was detected only in the NTS specimen in 3 of the NTS/ saliva pairs. Notably there was one 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).

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

	NTS		
Gargle	**Positive**	**Negative**	**Total**
Positive	34	0	34
Negative	3	28	31
Total	37	28	65
Sensitivity	91.9% (95% CI,78.1% - 98.3%)		
Specificity	100.0 % (95% CI,87.7 % - 100.0%)		

	Saliva	**NTS**	
	Positive	**Negative**	**Total**
Positive	32	1	33
Negative	3	25	28
Total	35	26	61
Sensitivity	91.4% (95% CI, 76.9% - 98.2%)		
Specificity	96.2% (95% CI, 80.4% - 99.9%)		

No significant differences were observed in the Cq values between corresponding saliva and gargle specimens (Figure 3). However, there were five discrepant saliva/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 one saliva sample that tested positive for SARS-CoV-2 RNA while the corresponding NTS and gargle were negative.

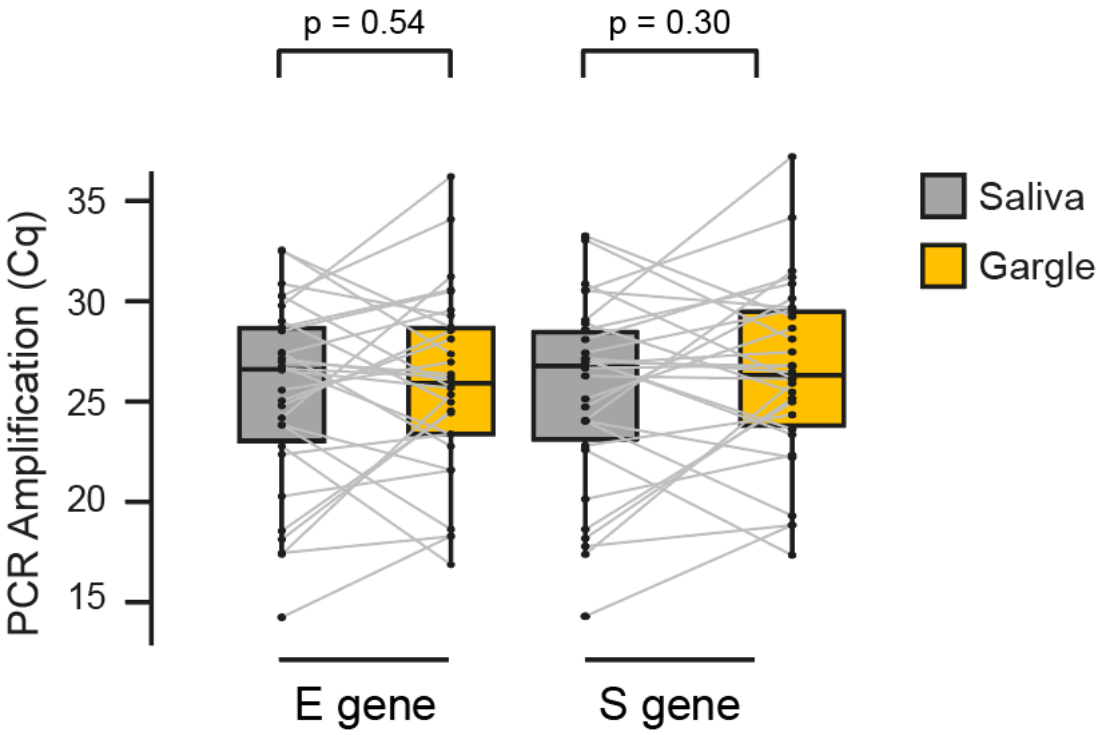

Figure 3. SARS-CoV-2 RNA amplification in saliva and gargle samples. Comparison between Cq values (E gene and S gene) in paired saliva and gargle samples.

Of the 261 patients who participated in Phase 2, 133 (51%) preferred the gargle method, 109 (41.7%) 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).

Table 2. Preferential testing method stratified by gender and age.

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

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 three 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 vs 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 healthcare professional and reduces the use of PPE and other resources at testing centres 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 programmes, 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.

There is limited available literature comparing the validity and acceptability of both saliva and gargle specimens with NTS. One study by Goldfarb et al[20] carried out in children aged 4-12 years found that gargle was significantly more sensitive than saliva when compared to NTS. However, the order of sample collection was alternated 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.

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.

Methods

Study Phase 1a and 1b: Saliva sampling

NHS Lothian Health Care Workers (HCWs) or their symptomatic household contacts attending the drive through NHS Lothian COVID-19 testing Centre were offered a saliva test in addition to their routine NTS. Phase 1a took place between 20th – 22nd May 2020 at the Chalmers Hospital, Edinburgh, and Phase 1b took place between 5th – 16th October 2020 at the Western General Hospital, Edinburgh. In May the predominant variant was clade 20A whilst in October the predominant variant was clade 20E (EU1). These individuals had been referred for testing via the NHS Lothian Occupational Health Department. Children under the age of 5 years were excluded due to the level of compliance required to produce the specimen. Individuals were also excluded if they had eaten, had a drink, smoked, chewed gum, or brushed their teeth within the 30 minute period preceding the test. A written information leaflet was provided to each eligible attendee and verbal consent for involvement was obtained prior to participation in the study. Paired nasopharyngeal and oropharyngeal specimens (referred to as nose/ throat swabs (NTS) for the purposes of this paper) were obtained by trained testing centre 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 coolboxes with ice packs. During Phase 1b participants were asked to produce 2 saliva samples (2 ml saliva per container), one stored and transported in a 4°C refrigerator, and the other at ambient temperature.

Study Phase 2: Saliva and Gargle sampling

The second phase of the study took place between the 2nd – 13th November 2020. NHS Lothian HCWs or their symptomatic household contacts attending the drive through COVID testing centre at the Western General Hospital, Edinburgh were offered both saliva and pharyngeal gargle tests in addition to routine upper respiratory swab testing. Children aged 5 years or less were once again excluded along with individuals who had had eaten, had a drink, smoked, chewed gum, or brushed their teeth within the 30 minute period preceding the test. A written information leaflet was provided to each eligible attendee and verbal consent was obtained as per phase 1. NTS specimens were obtained by testing centre staff prior to saliva and gargle specimens. Participants were asked to provide one saliva sample and one gargle sample. Saliva was obtained as per phase 1 but only one 2 ml sample was required in phase 2. 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. The saliva and gargle specimens were transported to the lab at ambient temperature.

User acceptability of sampling

Participants who provided all 3 specimen types in Phase 2 were asked to select their preferred testing modality and to provide reasons for their choice.

Laboratory processing

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[25]. 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[25].

Phase 1b: Saliva sampling (ambient/ 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 min prior to extraction.

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 min to ensure inactivation of virus before proceeding to extraction as described above.

Discrepant samples were tested for the *RPP30* gene, which encodes the human RNase P protein subunit P30[25].

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/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.

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Supplementary Table 1. Full results for Phase 1a NTS and Saliva samples showing amplification (Cq). U = Undetermined. Green, positive NTS samples; beige, failed samples; blue, inconclusive samples

Speciment Number	TaqPath NTS	Amplification (Cq)				TaqPath Saliva	Amplification (Cq)			
	Result	**MS2**	**Ngene**	**ORFgene**	**Sgene**	**Result**	**MS2**	**Ngene**	**ORF**	**Sgene**
1	Negative	23.64	U	U	U	Negative	24.606	U	U	U
2	Negative	24.474	U	U	U	Fail	U	U	U	U
3	Negative	24.34	U	U	U	Negative	24.948	U	U	U
4	Negative	24.149	U	U	U	Negative	24.534	U	U	U
5	Positive	24.855	24.145	23.366	24.422	Positive	25.025	26.834	26.242	26.67
6	Negative	25.099	U	U	U	Negative	24.215	U	U	U
7	Negative	25.015	U	U	U	Fail	U	U	U	U
8	Negative	24.745	U	U	U	Negative	24.023	U	U	U
9	Negative	25.485	U	U	U	Negative	25.103	U	U	U
10	Negative	24.606	U	U	U	Negative	25.859	U	U	U
11	Negative	25.376	U	U	U	Negative	23.628	U	U	U
12	Positive	24.715	24.023	24.951	25.551	Positive	24.569	20.562	20.922	21.985
13	Negative	24.298	U	U	U	Negative	24.077	U	U	U
14	Negative	24.612	U	U	U	Negative	24.975	U	U	U
15	Positive	24.471	28.526	27.939	28.234	Positive	25.527	33.859	32.922	33.688
16	Negative	24.253	U	U	U	Negative	25.801	U	U	U
17	Negative	24.774	U	U	U	Negative	26.002	U	U	U
18	Negative	24.514	U	U	U	Negative	25.181	U	U	U
19	Negative	25.001	U	U	U	Negative	25.388	U	U	U
20	Negative	24.535	U	U	U	Negative	23.448	U	U	U
21	Negative	24.201	U	U	U	Negative	25.874	U	U	U
22	Negative	24.368	U	U	U	Negative	27.561	U	U	U
23	Negative	24.196	U	U	U	Negative	24.444	U	U	U
24	Negative	25.153	U	U	U	Negative	25.565	U	U	U
25	Negative	24.52	U	U	U	Negative	24.933	U	U	U
26	Negative	25.344	U	U	U	Negative	24.808	U	U	U
27	Negative	24.628	U	U	U	Negative	26.142	U	U	U
28	Negative	23.868	U	U	U	Negative	26.017	U	U	U
29	Negative	23.806	U	U	U	Negative	25.125	U	U	U
30	Negative	23.946	U	U	U	Negative	24.39	U	U	U
31	Negative	24.269	U	U	U	Negative	24.372	U	U	U
32	Negative	24.53	U	U	U	Negative	24.197	U	U	U
33	Negative	24.852	U	U	U	Negative	24.54	U	U	U
34	Negative	25.703	U	U	U	Negative	25.323	U	U	U
35	Negative	26.76	U	U	U	Fail	35.686	U	U	U
36	Negative	26.005	U	U	U	Negative	24.164	U	U	U
37	Negative	25.478	U	U	U	Negative	24.6	U	U	U
38	Negative	25.569	U	U	U	Negative	23.101	U	U	U
39	Negative	25.791	U	U	U	Negative	24.134	U	U	U
40	Negative	24.741	U	U	U	Negative	24.142	U	U	U
41	Negative	25.468	U	U	U	Negative	24.935	U	U	U
42	Negative	24.729	U	U	U	Inconclusive	22.603	35.974	U	U
43	Negative	25.233	U	U	U	Negative	25.125	U	U	U
44	Negative	26.097	U	U	U	Negative	25.231	U	U	U
45	Negative	24.328	U	U	U	Negative	25.942	U	U	U
46	Negative	25.652	U	U	U	Negative	24.277	U	U	U
47	Negative	27.159	U	U	U	Negative	24.433	U	U	U
48	Negative	25.397	U	U	U	Negative	25.033	U	U	U
49	Negative	25.334	U	U	U	Negative	24.843	U	U	U
50	Negative	24.726	U	U	U	Negative	24.731	U	U	U
51	Negative	24.387	U	U	U	Negative	25.505	U	U	U
52	Negative	24.402	U	U	U	Negative	25.129	U	U	U
53	Negative	24.729	U	U	U	Negative	23.928	U	U	U
54	Negative	25	U	U	U	Negative	25.269	U	U	U
55	Negative	24.445	U	U	U	Negative	23.924	U	U	U
56	Negative	24.699	U	U	U	Negative	24.324	U	U	U
57	Negative	25.35	U	U	U	Negative	25.254	U	U	U
58	Positive	25.007	25.095	25.764	25.924	Positive	23.96	26.168	26.156	27.145
59	Negative	24.899	U	U	U	Negative	23.146	U	U	U
60	Negative	25.357	U	U	U	Negative	24.243	U	U	U
61	Negative	26.072	U	U	U	Fail	34.529	U	U	U
62	Fail	31.002	U	U	U	Negative	24.667	U	U	U
63	Negative	24.707	U	U	U	Negative	23.902	U	U	U
64	Negative	24.565	U	U	U	Negative	24.889	U	U	U
65	Positive	24.289	20.528	20.928	21.099	Positive	23.775	27.193	26.336	26.803
66	Negative	26.113	U	U	U	Negative	25.473	U	U	U

67	Negative	24.451	U	U	U	Negative	25.251	U	U	U
68	Negative	25.1	U	U	U	Negative	25.565	U	U	U
69	Negative	24.54	U	U	U	Negative	27.163	U	U	U
70	Negative	25.235	U	U	U	Negative	22.892	U	U	U
71	Negative	24.475	U	U	U	Negative	23.75	U	U	U
72	Negative	24.335	U	U	U	Negative	24.685	U	U	U
73	Negative	24.895	U	U	U	Fail	35.5	U	U	U
74	Negative	24.979	U	U	U	Negative	24.719	U	U	U
75	Negative	25.409	U	U	U	Negative	23.793	U	U	U
76	Negative	25.154	U	U	U	Negative	24.986	U	U	U
77	Negative	25.358	U	U	U	Negative	24.718	U	U	U
78	Negative	24.502	U	U	U	Positive	23.545	33.576	34.473	37.553
79	Negative	24.42	U	U	U	Negative	25.14	U	U	U
80	Negative	23.689	U	U	U	Negative	23.348	U	U	U
81	Negative	25.347	U	U	U	Negative	25.147	U	U	U
82	Negative	24.663	U	U	U	Negative	24.586	U	U	U
83	Negative	25.873	U	U	U	Negative	25.081	U	U	U
84	Negative	24.905	U	U	U	Negative	25.593	U	U	U
85	Positive	24.793	23.678	23.384	24.585	Positive	24.865	22.524	21.686	22.499
86	Negative	23.678	U	U	U	Negative	25.117	U	U	U
87	Negative	24.283	U	U	U	Negative	24.928	U	U	U
88	Negative	24.832	U	U	U	Fail	28.495	U	U	U
89	Negative	24.159	U	U	U	Negative	24.702	U	U	U
90	Negative	24.228	U	U	U	Negative	25.553	U	U	U
91	Negative	24.464	U	U	U	Fail	34.225	U	U	U
92	Positive	24.969	35.225	36.634	38.617	Positive	30.036	39.775	35.627	U
93	Positive	24.643	23.633	24.407	24.711	Positive	25.238	24.068	23.744	24.5
94	Positive	25.635	30.696	29.768	29.619	Positive	23.179	22.117	21.421	22.124
95	Negative	25.605	U	U	U	Negative	26.102	U	U	U
96	Negative	25.337	U	U	U	Negative	23.301	U	U	U
97	Negative	24.281	U	U	U	Negative	26.012	U	U	U
98	Negative	24.932	U	U	U	Negative	24.823	U	U	U
99	Negative	24.67	U	U	U	Negative	24.58	U	U	U
100	Negative	25.797	U	U	U	Fail	27.764	U	U	U
101	Negative	24.457	U	U	U	Negative	24.8	U	U	U
102	Negative	25.254	U	U	U	Negative	26.826	U	U	U
103	Negative	24.284	U	U	U	Negative	24.68	U	U	U
104	Negative	24.581	U	U	U	Negative	24.917	U	U	U
105	Negative	25.411	U	U	U	Negative	26.762	U	U	U
106	Negative	24.642	U	U	U	Negative	25.418	U	U	U
107	Negative	26.032	U	U	U	Negative	24.445	U	U	U
108	Positive	23.944	26.894	26.625	26.808	Positive	23.821	26.122	25.271	26.434
109	Negative	24.718	U	U	U	Negative	25.631	U	U	U
	average	24.925				average	25.297			
	stdev	0.8651				stdev	2.2137			
	average-2SD	23.194				average-2SD	20.87			
	average+2SD	26.655				average+2SD	29.724			
	minimum	23.64				minimum	22.603			

Supplementary Table 2. Sample results from Phase 1b saliva and paired NTS testing (NTS = nose throat swab, IC = internal control). Green, NTS positive; beige, discordant result.

Sample Number	Ambient				Cold Chain				NTS Result
	E gene	S gene	IC	Result	E gene	S gene	IC	Result	
MI611007W	26.6	27.3	29.9	Positive	24	24.5	32.9	Positive	Positive
MI611104S	29.2	30.3	31	Positive	28.2	29.1	31.2	Positive	Positive
MI611105G	25.2	25.7	-	Positive	26.6	26.7	-	Positive	Positive
MI611117G	18.4	19	34.2	Positive	26.8	27.2	33.2	Positive	Positive
MI611078S	25	25.5	-	Positive	14.4	14.5	-	Positive	Positive
MI611119N	33	35.9	29.5	Positive	31.7	-	32	Positive[$]	Positive
MI611120R	23.6	24.2	31.5	Positive	23.9	24.5	31.3	Positive	Positive
MI611132X	25.4	26.1	35	Positive	24.9	25.4	-	Positive	Positive
MI611147S	20.8	21.3	31.5	Positive	16	16.3	37.8	Positive	Positive
MI611159S	29.9	30.8	30.3	Positive	29.2	29.8	31.8	Positive	Positive
MI611162K	32.9	-	29.5	Positive[$]	35.8	-	30	Positive[$]	Positive
MI611168X	22.3	23.1	33.7	Positive	21.9	22.6	36.5	Positive	Positive
MI611175M	-	-	30.5	Negative	-	-	31.1	Negative	Positive
MI611185T	24.8	25.4	31.7	Positive	26.3	27.1	30.9	Positive	Positive
MI611189V	26.7	27.3	-	Positive	28.1	28.8	34	Positive	Positive
MI611196C	26.7	27.2	34.5	Positive	28.1	28.8	29.7	Positive	Positive
MI611202Z	28.5	29.1	31.1	Positive	30.3	31.2	30	Positive	Positive
MI611219Y	29.6	30	29.1	Positive	33	33.3	28.6	Positive	Positive
MI611224E	-	-	29.8	Negative	-	-	30.2	Negative	Positive
MI611124G	-	-	29.8	Negative	-	-	30.2	Negative	Negative
MI611123S	-	-	29.1	Negative	-	-	29	Negative	Negative
MI611122L	-	-	29.5	Negative	-	-	29.2	Negative	Negative
MI611121X	-	-	29.8	Negative	-	-	29.4	Negative	Negative
MI611118Z	-	-	30	Negative	-	-	31.4	Negative	Negative
MI611116S	-	-	31.3	Negative	-	-	32.4	Negative	Negative

[$]Confirmed as positive by testing with the CDC N2 gene assay

Supplementary Table 3. Full results from Phase 2 gargle, saliva and dependent NTS testing (NTS = nose throat swab, IC = internal control). Green, NTS positive; beige, discordant result.

Sample Number	Saliva				Gargle				NTS Result
	E gene	S gene	IC	Result	E gene	S gene	IC	Result	
MI611226Y	-	-	29.7	Negative	-	-	29.29	Negative	Negative
MI611228P	-	-	29.22	Negative	-	-	29.06	Negative	Negative
MI611229A	-	-	30.55	Negative	-	-	29.56	Negative	Negative
MI611230D	-	-	29.13	Negative	-	-	29.37	Negative	Negative
MI611231L	-	-	28.94	Negative	-	-	28.51	Negative	Negative
MI611232G	-	-	30.03	Negative	-	-	29.48	Negative	Negative*
MI611233Z	-	-	-	Inhibited	-	-	29.04	Negative	Negative
MI611235Q	-	-	-	Inhibited	-	-	31.48	Negative	Negative
MI611237H	-	-	29.27	Negative	-	-	30.65	Negative	Negative
MI611239W	-	-	-	Inhibited	-	-	30.72	Negative	Negative
MI611240R	-	-	28.05	Negative	-	-	31.03	Negative	Negative
MI611243S	31.46	31.77	28.18	Positive	-	-	30.94	Negative	Negative
MI611244G	-	-	30.38	Negative	-	-	30.53	Negative	Negative
MI611245Z	-	-	-	Inhibited	-	-	31.01	Negative	Negative
MI611421K	-	-	27.96	Negative	-	-	29.13	Negative	Negative
MI611422J	-	-	27.69	Negative	-	-	29.67	Negative	Negative
MI611423V	-	-	29.43	Negative	-	-	30.07	Negative	Negative
MI611424B	-	-	28.34	Negative	-	-	33.03	Negative	Negative
MI611425R	-	-	30.27	Negative	-	-	29.31	Negative	Negative
MI611426X	-	-	27.99	Negative	-	-	-	Inhibited	Negative
MI611427D	-	-	29.15	Negative	-	-	29.38	Negative	Negative
MI611430F	-	-	33.07	Negative	-	-	30.02	Negative	Negative
MI611480G	-	-	28.17	Negative	-	-	29.53	Negative	Negative
MI611481Z	-	-	27.77	Negative	-	-	29.56	Negative	Negative
MI611482Q	-	-	27.96	Negative	-	-	28.86	Negative	Negative
MI611483E	-	-	28.4	Negative	-	-	29.31	Negative	Negative
MI611484H	-	-	27.78	Negative	-	-	33.31	Negative	Negative
MI611486W	-	-	28.68	Negative	-	-	-	Inhibited	Negative
MI611487P	-	-	27.35	Negative	-	-	30.25	Negative	Negative
MI611488A	-	-	29.16	Negative	-	-	29.44	Negative	Negative
MI611227W	28.56	28.62	29.34	Positive	30.53	30.92	28.89	Positive	Positive
MI611234N	32.6	33.1	-	Positive	27.41	28.15	37.67	Positive	Positive
MI611242L	14.26	14.31	-	Positive	18.31	18.86	34.09	Positive	Positive
MI611246N	25.6	26.9	-	Positive	27	27.51	31.1	Positive	Positive
MI611254L	18.15	18.2	-	Positive	24.57	25.19	30.75	Positive	Positive
MI611260M	23.86	24.06	-	Positive	21.62	22.24	33.27	Positive	Positive
MI611261J	17.47	17.8	-	Positive	18.3	18.86	34.99	Positive	Positive
MI611263R	27.04	26.7	-	Positive	26.4	26.84	31.53	Positive	Positive
MI611265D	30.3	30.9	-	Positive	25.75	26.18	31.01	Positive	Positive
MI611267S	26.68	27	-	Positive	26.26	26.79	31.16	Positive	Positive
MI611268G	-	-	30.76	Negative	23.92	24.4	30.56	Positive	Positive
MI611272J	20.3	20.16	31.83	Positive	21.59	22.35	38.44	Positive	Positive
MI611277D	-	-	28.33	Negative	-	-	30.57	Negative	Positive
MI611302X	18.57	18.65	30.12	Positive	24.47	25.01	31.57	Positive	Positive
MI611304L	24.21	24.09	30.95	Positive	31.26	31.54	32.67	Positive	Positive
MI611315D	29.82	29.09	29.4	Positive	36.26	37.26	30.37	Positive	Positive
MI611319Z	30.91	30.56	29.08	Positive	29.32	29.72	30.48	Positive	Positive
MI611328Q	28.74	28.13	29.93	Positive	30.61	31.24	31.23	Positive	Positive
MI611339N	25.07	25.16	29.26	Positive	28.15	28.69	30.36	Positive	Positive
MI611342V	27.42	27.46	34.39	Positive	29.59	29.6	30.59	Positive	Positive
MI611345X	22.39	22.83	30.17	Positive	23.41	24.37	30.95	Positive	Positive
MI611354V	22.8	22.62	29.56	Positive	16.89	17.36	36.02	Positive	Positive
MI611356R	26.6	26.31	30.32	Positive	25.7	26.15	30.63	Positive	Positive
MI611358D	26.79	27.1	28.19	Positive	23.4	23.65	31.85	Positive	Positive
MI611366V	-	-	-	Inhibited	34.12	34.62	30.86	Positive	Positive
MI611367B	27.16	27.18	30.07	Positive	26.13	26.5	30.8	Positive	Positive
MI611380H	23.26	23.28	28.83	Positive	-	-	29.24	Negative	Positive
MI611383A	28.19	28.14	36.79	Positive	-	-	29.38	Negative	Positive
MI611401T	30.3	30.6	-	Positive	34.12	34.21	29.28	Positive	Positive
MI611406R	27.48	27.15	28.8	Positive	22.8	23.38	30.35	Positive	Positive
MI611407X	-	-	-	Inhibited	25.81	26.32	29.31	Positive	Positive
MI611408D	32.54	33.3	28.47	Positive	28.74	29.27	31.32	Positive	Positive
MI611411F	29.04	28.93	29.24	Positive	25	25.5	29.71	Positive	Positive
MI611414M	-	-	30.48	Negative	21.37	22.07	30.69	Positive	Positive
MI611479T	17.4	17.4	34.17	Positive	25.38	25.94	-	Positive	Positive
MI611485Y	24.82	24.76	34.12	Positive	28.57	30.18	-	Positive	Positive
MI611496H	23.86	24.05	30.21	Positive	18.65	19.33	-	Positive	Positive

*Sample Lost - follow-up sample negative

Supplementary Table 4. Association between gender and sample collection preference

	Male	Female	Fishers exact test
NTS	4	15	P = 1
Saliva	24	85	
NTS	4	15	P = 1
Gargle	30	103	
Saliva	24	85	P = 1
Gargle	30	103	

Supplementary Table 5. Association between age and sample collection preference

	18 and under	Over 18	Fishers exact test
NTS	0	19	P = 0.2137
Saliva	13	96	
NTS	0	19	P = 0.597
Gargle	7	126	
Saliva	13	96	P = 0.0984
Gargle	7	126	