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Higher SARS-CoV-2 detection of oropharyngeal compared with nasopharyngeal or saliva specimen for molecular testing: a multicentre randomised comparative accuracy study

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ABSTRACT

Background Testing is critical for detecting SARS-CoV-2 infection, but the best sampling method remains unclear.

Objectives To determine whether nasopharyngeal swab (NPS), oropharyngeal swab (OPS) or saliva specimen collection has the highest detection rate for SARS-CoV-2 molecular testing.

Methods We conducted a randomised clinical trial at two COVID-19 outpatient test centres where NPS, OPS and saliva specimens were collected by healthcare workers in different orders for reverse transcriptase PCR testing. The SARS-CoV-2 detection rate was calculated as the number positive by a specific sampling method divided by the number in which any of the three sampling methods was positive. As secondary outcomes, test-related discomfort was measured with an 11-point numeric scale and cost-effectiveness was calculated.

Results Among 23 102 adults completing the trial, 381 (1.65%) were SARS-CoV-2 positive. The SARS-CoV-2 detection rate was higher for OPSs, 78.7% (95% CI 74.3 to 82.7), compared with NPSs, 72.7% (95% CI 67.9 to 77.1) (p=0.049) and compared with saliva sampling, 61.9% (95% CI 56.9 to 66.8) (p<0.001). The discomfort score was highest for NPSs, at 5.76 (SD, 2.52), followed by OPSs, at 3.16 (SD 3.16) and saliva samples, at 1.03 (SD 18.8), p<0.001 between all measurements. Saliva specimens were associated with the lowest cost, and the incremental costs per detected SARS-CoV-2 infection for NPSs and OPSs were US$3258 and US$1832, respectively.

Conclusions OPSs were associated with higher SARS-CoV-2 detection and lower test-related discomfort than NPSs for SARS-CoV-2 testing. Saliva sampling had the lowest SARS-CoV-2 detection but was the least costly strategy for mass testing.

Trial registration number NCT04715607.

WHAT IS ALREADY KNOWN ON THIS TOPIC

⇒ Nasopharyngeal specimens are considered the gold standard respiratory sample for SARS-CoV-2 testing, but the evidence level for the recommendation is low.

WHAT THIS STUDY ADDS

⇒ Oropharyngeal specimens have a higher detection rate for SARS-CoV-2 testing compared with nasopharyngeal specimens, while saliva had the lowest detection rate but was the least costly strategy for mass testing.

HOW THIS STUDY MIGHT AFFECT RESEARCH, PRACTICE OR POLICY

⇒ The SARS-CoV-2 Detection in Saliva, Oropharyngeal and Nasopharyngeal Specimens study provides evidence to include oropharyngeal specimens for higher SARS-CoV-2 detection rate and a better tolerated testing strategy.

Linked

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INTRODUCTION

SARS-CoV-2 testing is critical in diagnosing COVID-19, and billions of molecular diagnostic tests have been performed during the pandemic.[1] Asymptomatic transmission of SARS-CoV-2 is considered the 'Achilles' heel' to control the pandemic spread of COVID-19, and many countries have implemented mass testing strategies to limit transmission.[2–4] The gold standard for detecting SARS-CoV-2 RNA is reverse transcriptase PCR (RT-PCR) testing of an upper respiratory tract specimen.[5] Optimal specimen collection is the most important step in the diagnosis of infectious diseases but there is international disagreement of the preferred type of respiratory specimen for SARS-CoV-2 testing. A nasopharyngeal swab (NPS) specimen has been considered the sampling method with the highest sensitivity for SARS-CoV-2 testing but is associated with considerable test discomfort and cost.[6] Accordingly, an oropharyngeal swab (OPS) specimen is preferred in some countries[7] and is currently used for mass testing during the local COVID-19 outbreaks in China.[8] In contrast, the Infectious Diseases Society of America (IDSA) warns against using OPS instead of NPS specimens,[9] but still only two small retrospective studies have compared them for SARS-CoV-2 testing in a community setting.[10] Instead, IDSA considers self-collected saliva specimens a low-cost alternative with comparable detection rate to NPS specimens

for SARS-CoV-2 testing.[11] However, despite millions of people being tested for SARS-CoV-2 daily, there is still uncertainty about the sensitivity, cost and preferences for respiratory specimens used in public testing.

We conducted a multicentre randomised clinical study to compare head-to-head SARS-CoV-2 detection rate, test-related discomfort and cost-effectiveness between NPSs, OPSs and saliva sampling for molecular testing.

METHODS

Study design

The SARS-CoV-2 Detection in Saliva, Oropharyngeal and Nasopharyngeal Specimens (SAMPLE) trial[12] was an investigator-initiated clinical trial comparing three diagnostic tests with test sequence randomised at two public COVID-19 test centres in Copenhagen, Denmark. The SAMPLE protocol was registered with the Danish Data Protection Agency (Protocol No. P-2021-34) and ClinicalTrials.gov database (number NCT04715607) and has been published.[12] We followed the Consolidated Standards of Reporting Trials reporting guidelines (see online supplemental appendix A) and no deviations from the SAMPLE protocol[12] were made.

Patient involvement

Patients who had performed different swabs for SARS-CoV-2 testing—as a part of a previous study—were interviewed about their testing preferences. Based on this feedback, we included saliva as a self-sampled specimen in the study design and added participant test preferences as an essential outcome measure. The participants in the study will be informed of the results through a dedicated website (https://www.urt-sample.com/) once the trial is published.

Participants

Citizens (16 years or older) who requested a free-of-charge SARS-CoV-2 RT-PCR test at the Valby or Taastrup COVID-19 outpatient test centres, Copenhagen, Denmark, were eligible for enrolment. Participants with medical conditions that did not allow regular swab sampling (eg, tracheostomy or laryngectomy performed) or who could not understand written and spoken Danish was excluded from trial enrolment. The Danish health authorities recommended that anyone with COVID-19 symptoms or close contact with someone with SARS-CoV-2 infection should be isolated and RT-PCR tested. As a part of the national mass testing strategy, asymptomatic citizens were also encouraged to undergo testing weekly.[13] Government-financed RT-PCR testing was therefore available to the public without the need for referral. Citizens received oral and written study information on arrival at the test centre. Citizens who signed an informed consent form were enrolled as participants and provided with a link to an online Research Electronic Data Capture (Vanderbilt, USA) questionnaire (see online supplemental appendix A).

Randomisation and masking

To avoid bias due to the sampling sequence, we randomly allocated the participants in a 1:1:1 ratio to three groups with different orders for saliva, OPS and NPS specimen collection; NPS→OPS→saliva, OPS→saliva→NPS or saliva→NPS→OPS (see figure 1, online supplemental appendix A). The randomisation was performed for each test case without blinding from the

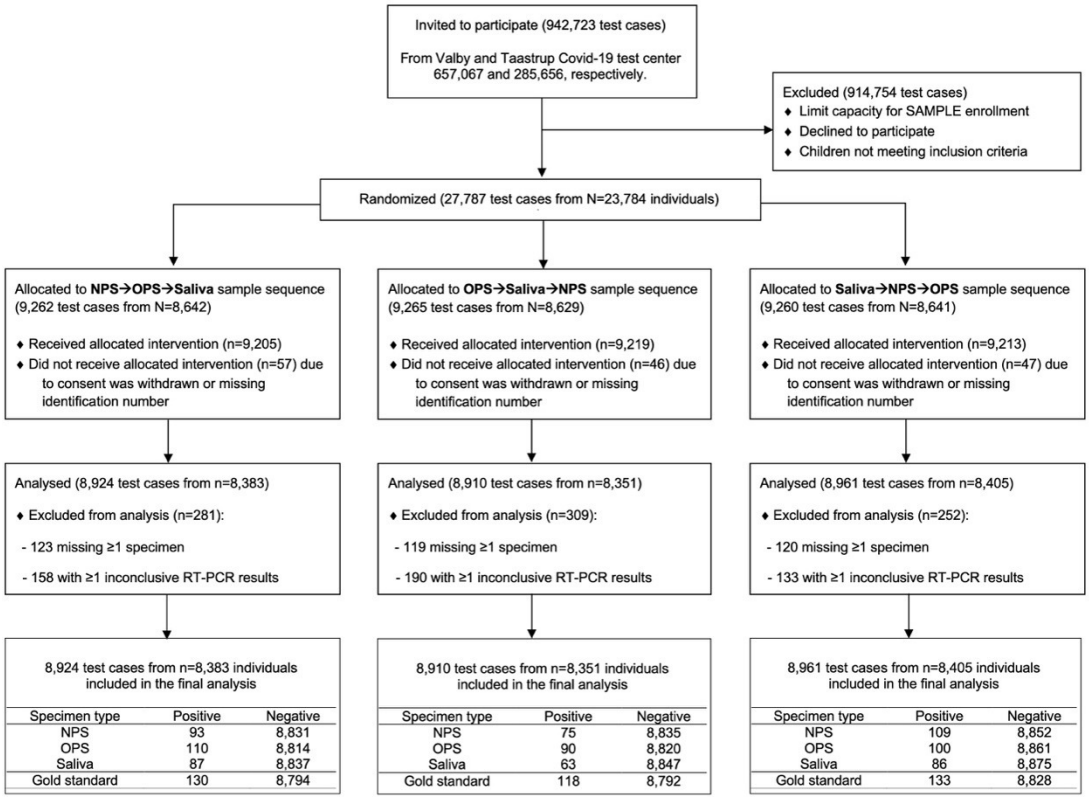

Figure 1 Flow diagram illustrating the tests included in the study. NPS, nasopharyngeal swab; OPS, oropharyngeal swab; RT-PCR reverse transcriptase PCR; Saliva, saliva sampling; SAMPLE, SARS-CoV-2 Detection in Saliva, Oropharyngeal and Nasopharyngeal Specimens. Gold standard is defined as test cases with one or more specimen types with detection of SARS-CoV-2.

interventions and if a participant was enrolled more than once, a unique randomisation was performed each time.

Procedures

Upper respiratory specimens were collected by healthcare workers using nylon-flocked OPSs and NPSs (Wuxi NEST Biotechnology, Jiangsu, China). The NPS was inserted into the nasal cavity until resistance was met by the posterior nasopharyngeal wall[14] while the OPS collected specimens from both palatine tonsils and the posterior oropharyngeal wall as recommended by WHO (see online supplemental appendix A for detailed sampling descriptions). Saliva was collected with the drooling technique[11] into a 50 mL skirted tube (AHN Biotechnologie, Nordhausen, Germany). To ensure standardised collection methods, respiratory specimens were only obtained by healthcare workers who had received additional competency-based training (see online supplemental appendix A for detailed training material).[15 16] All specimens were placed into separate sterile tubes with 2 mL of inactivation transport medium (Wuxi NEST Biotechnology).

Laboratory methods

All samples were stored at 4°C (range 2°C–6°C) at the test site before transport to the Technical University of Denmark, Lyngby, Denmark, for SARS-CoV-2 RT-PCR testing. A Beckman i7 robotic platform extracted nucleic acids from 200 µL of medium using an in-house silica-based procedure (see online supplemental appendix A for details). SARS-CoV-2 RNA was detected on a Rotor-Gene Q PCR system (QIAGEN, Düsseldorf, Germany) using a multiplexed version of the Centers for Disease Control N-gene one-step RT-PCR targeting two N-gene segments. Samples with a cycle threshold (Ct) <34 for at least one N-gene segment target were defined as positive for SARS-CoV-2 RNA. The RNase P ribozyme (RNase P) was used to assess the presence of human genetic material, and tests were considered inconclusive if RNase P Ct values were >23.00 for NPS specimens, >27.43 for OPS specimens and >28.36 for saliva specimens (see online supplemental appendix A).

Outcomes

The main objective of the SAMPLE trial was to compare the detection of SARS-CoV-2 infection in NPS, OPS and saliva specimens collected during mass testing. As secondary outcomes, we aimed to compare the test discomfort and incremental costs per SARS-CoV-2 infection detected using the different sample methods. We used the WHO classification of SARS-CoV-2 infection as a study participant having a positive RT-PCR for SARS-CoV-2 RNA in one or more respiratory specimens (reference standard).[17] The reference standard was used to calculate and compare the detection rate for saliva, OPS and NPS (index tests).[18] The SARS-CoV-2 detection rate of the combined OPS/NPS specimen was estimated by adding the individual molecular test result from NPS together with OPS, meaning the OPS/NPS was considered positive if either the NPS, OPS or both specimens were positive. This means that the combined OPS/NPS detection rate could only be falsely negative if both OPS and NPS were negative while saliva was positive. After all three samples were collected, the participants were asked to rate their discomfort on an 11-point numeric scale (NRS) ranging from 0 (no discomfort) to 10 (worst possible discomfort) and the likelihood to get retested for each sample type[19] (see online supplemental appendix A).

The pre-analytical costs for the different sampling methods were calculated using a microcosting approach. We measured the time for registration and collection of the different specimen types at the Valby COVID-19 test centre, Copenhagen, Denmark, and calculated the salary and material cost per test (see online supplemental appendix A for further details).

We calculated the mean Ct values for the N1-gene segment from SARS-CoV-2 RNA RT-PCR for the different specimens, and the distributions of SARS-CoV-2 variants were analysed by Sanger sequencing or whole genome sequencing (see online supplemental appendix A).

Statistical analysis

According to the power analysis,[12] with 273 SARS-CoV-2-positive OPS specimens, the trial had 80% power to detect a 26% difference in detection rate between OPS and NPS sampling given an inconclusive rate of 5% and an alpha of 0.05 (see online supplemental appendix A for details). Anticipating that approximately 5% of the included participants will be excluded from final analyses due to missing data, we predefined to stop the study when 287 SARS-CoV-2 OPS-positive participants were enrolled.

Data are correlated due to repeated observations within participants and test dates. To account for the hierarchical data structure, differences in primary and secondary outcomes were examined using generalised estimating equations (GEEs). By using this approach, a comparison between specimen types is performed within participant and test date. A logistic regression analysis was performed using GEEs to compare the detection rate of SARS-CoV-2 among the saliva, OPS, NPS and combined samples. The ORs and 95% CIs were calculated with adjustments for the effect of the test centre and the order of tests performed (randomisation). An exchangeable working correlation matrix was applied with each test date for each participant as a cluster (subject). The Ct values from positive RT-PCR samples and the NRS discomfort scores were compared using a general linear model with mixed effects (Ct) and GEE models (NRS discomfort). A 5% significance level was applied. Statistical Analysis Software V.9.4 (SAS Institute, North Carolina, USA) was used for the statistical analyses.

Post hoc analysis

We performed some supplementary analyses to examine the robustness of the diagnostic findings. The definition of RT-PCR-positive test for SARS-CoV-2 infection was changed to Ct <25 for both one and two N-gene segments to explore the consequences of a higher test specificity for the SARS-CoV-2 detection rate between specimen types. To explore a potential bias from the distribution of the inconclusive test results, we included the inconclusive results as negative test results in a subgroup analysis. Furthermore, we evaluated the diagnostic agreement (positive and negative agreement) between the respiratory specimens. Post hoc subgroup analyses were also performed on symptomatic and asymptomatic participants and among participants who had eaten half an hour before testing or not. We also analysed the distribution of positive test results for specimens stratified by randomisation and the previous COVID-19 infection.

Health economic analysis

We performed a cost-effectiveness analysis using Monte Carlo second-order simulation and fitted the test sensitivity parameters to beta distributions, whereas the cost parameters were applied to gamma distributions (see online supplemental appendix A for further details). The mean pre-analytical cost for SARS-CoV-2 infection detected per 100 000 persons for each specimen type

was calculated. The costs were used to estimate incremental costs per SARS-CoV-2 infection detected versus the most inexpensive sample method. All economic analyses were conducted in Excel (Excel 2019 V.16.0, Microsoft, Washington, USA) (see online supplemental appendix B for the health economic simulation model in Excel).

Role of the funding source

The funder of the study had no role in study design, data collection, data analysis, data interpretation or writing of the report.

RESULTS

From 22 January 2021 to 20 May 2021, a total of 23 784 adults were randomised at the Valby and Taastrup COVID-19 test centres. A total of 27 787 test cases were performed as some participants were tested more than once during the trial period. Only test cases with valid molecular results from all three specimens were included in the final analyses and in total 992 test cases (3.6%) were excluded due to missing identifiers/withdrawn consent (total n=150), missing test results (total n=362) and inconclusive results (total n=481) (figure 1). In the final analyses, 26 795 test cases from 23 102 individual participants with RT-PCR results for all three respiratory specimens (80 385 RT-PCR test results in total) were included (see demographics and clinical characteristics in table 1).

There were 26 795 test cases in which all 3 specimens had a valid result, and of these, 381 (1.41%) had at least one specimen positive for SARS-CoV-2 (reference standard). Of the 381 participants with SARS-CoV-2 infection (none were included more than once in the study), 277, 300 and 236 had positive molecular tests from NPSs, OPSs and saliva specimens, respectively. The detection rate for SARS-CoV-2 was 72.7% (95% CI 67.9 to 77.1) for NPSs; 78.7% (95% CI 74.3 to 82.7) for OPSs and 61.9% (95% CI 56.9 to 66.8) for saliva sampling (figure 2). The detection rate was 6.0% points higher for OPSs than for NPSs (p=0.049) and 10.8% points higher for NPSs than for saliva sampling (p<0.001) (table 2). The number of positive RT-PCR test results for the combination of NPSs/OPSs was 357. The detection rate for NPSs/OPSs was 93.7% (95% CI 90.8 to 95.9) and 21% points higher compared with NPS specimens (p<0.001) and 15% points higher than OPS specimens (p<0.001). Of 381 cases in which any of the three tests were positive, NPS was positive in 277 (72.7%), OPS was positive in 300 (78.7%) and both were positive in 220 (57.7%). In the 137 cases where they disagreed, OPS was more likely to be positive (80) than NPS (57) (see online supplemental appendix A, table S1,S2).

Of the 26 795 test cases, 22 118 (82.5%) answered the online questionnaire (see table 1 for responses and number of missing for each question). Most of the participants, 99.6%, were tested as part of the national screening strategy, that is, the majority were either asymptomatic or did not have a history of close contact with a SARS-CoV-2-positive individual. Among the SARS-CoV-2-positive participants, 26.6% reported symptoms, and of these, 87.7% were tested within 3 days of the onset of symptoms (see table 1 and online supplemental appendix A table S3). The OR of SARS-CoV-2 detection for OPS versus NPS specimens was 1.19 when focusing on asymptomatic participants, while the OR was reduced to 1.01 when focusing on symptomatic participants (see online supplemental appendix A table S4). The SARS-CoV-2 detection rate of saliva specimens was 64.9% (95% CI 57.2% to 72.1%) and 59.7% (95% CI 52.4% to 66.7%) among participants who had eaten half an hour before testing or not, respectively (see online supplemental appendix A table S5). The number of inconclusive results was 115 (0.4%) for NPS, 107 (0.4%) for OPS and 265 (1%) for saliva specimens and the diagnostic results stratified by inclusion or exclusion of inconclusive test results are reported in online supplemental appendix A

Table 1 Baseline demographics and clinical characteristics of the participants overall and stratified by detection of SARS-CoV-2 infection (reference standard)

		Reference standard*	
	Overall	Positive n=381	Negative n=26 414
Age, mean (SD)	42.2 (15.2)	39.5 (15.5)	42.3 (15.2)
Female sex	13 549 (50.6%)	164 (43.0%)	13 385 (50.7%)
Vaccination status			
Vaccinated	1777 (8.1%)	16 (5.3%)	1761 (8.1%)
Not vaccinated	20 285 (91.9%)	286 (94.7%)	19 999 (91.9%)
Missing	4733	79	4654
Test centre			
Valby	19 050 (71.1%)	287 (75.3%)	18 763 (71.0%)
Taastrup	7745 (28.9%)	94 (24.7%)	7651 (29.0%)
Randomisation order			
1 (NPS→OPS→saliva)	8924 (33.3%)	130 (34.1%)	8794 (33.3%)
2 (OPS→saliva→NPS)	8910 (33.3%)	118 (31.0%)	8792 (33.3%)
3 (Saliva→NPS→OPS)	8961 (33.4%)	133 (34.9%)	8828 (33.4%)
Prior positive test			
Yes	1181 (5.5%)	79 (27.2%)	1102 (5.2%)
No	20 193 (94.5%)	212 (72.8%)	19 981 (94.8%)
Missing	5421	90	5331
Days since positive test†			
0–1 days (part of initial testing)	63 (5.6%)	36 (46.2%)	27 (2.6%)
2–60 days (late infection stage)	87 (7.6%)	23 (29.5%)	64 (6.0%)
>60 days (reinfection)	989 (86.8%)	19 (24.4%)	970 (91.4%)
Test reason			
Symptoms			
Yes	1149 (5.2%)	81 (26.6%)	1068 (4.9%)
Missing	4649	77	4572
Exposure to COVID-19	1430 (6.4%)	67 (22.0%)	1363 (6.2%)
Screening			
Yes	19 486 (88.0%)	156 (51.4%)	19 330 (88.5%)
No	81 (0.4%)	0 (0.0%)	81 (0.4%)
Days since first symptom‡			
1 day	411 (36.3%)	34 (42.0%)	377 (35.8%)
2–3 days	538 (47.4%)	37 (45.7%)	501 (47.6%)
4–6 days	185 (16.3%)	10 (12.3%)	175 (16.6%)
Missing	15	0	15

Values are numbers and percentages (n, %) unless stated otherwise.
*Reference standard: one or more specimen types with positive RT-PCR test for SARS-CoV-2 RNA.
†Among individuals with a prior positive test.
‡Among individuals with symptoms.
NPS, nasopharyngeal swab; OPS, oropharyngeal swab; RT-PCR, reverse transcriptase PR; Saliva, saliva sampling.

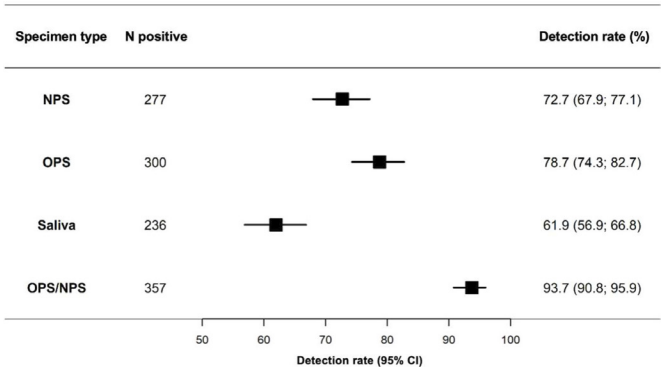

Figure 2 Detection rate for RT-PCR testing of NPS, OPS, saliva and OPS/NPS sampling based on participants (n=381) with confirmed SARS-CoV-2 infection. NPS, nasopharyngeal swab; OPS, oropharyngeal swab; RT-PCR reverse transcriptase PCR; Saliva, saliva sampling.

table S6–S8. The full anonymised dataset can be seen as online supplemental appendix C.

The mean Ct value was lower for NPSs, at 17.56 (8.17 SD), than for OPSs, at 18.95 (6.48 SD), and saliva sampling, at 22.64 (5.73 SD) (p<0.001 between all measurements) (online supplemental appendix A figure S1). If we changed the definition of PCR positive test to include cases with one or two N-targets with Ct <25, then the detection rate was 79.9% or 79.8% for NPSs; 92.9% or 93.8% for OPSs; 66.1% or 57.4% for saliva sampling and 97.6% or 97.9% for NPSs/OPSs, respectively (see further details in online supplemental appendix A figure S2-S3, and Table S9-11). Subgroup analyses explored the detection rate for each specimen stratified for prior SARS-CoV-2 infection and randomisation sequence (see online supplemental appendix A table S12–S15). The distributions of SARS-CoV-2 variants were as follows: 252 (66%) were B.1.1.7 (alpha), 27 (7%) were B.1.617.2 (delta), 2 (0.5%) were B.1.525 (eta) and 100 (26%) were unknown variants (see online supplemental appendix A table S16).

Of the 26795 test cases, 26258 (98%) reported discomfort on the 11-point numeric scale (NRS). The mean NRS score was highest for NPSs, at 5.76 (SD 2.52), followed by OPSs, at 3.16 (SD 2.38) and saliva sampling, at 1.03 (SD 1.83), p<0.001 between all measurements (see online supplemental appendix A figure S4). The number of participants who responded (26.813 in total) that they might refrain from future testing because of test-related discomfort was 1967 (7.34%) for NPSs, 232 (0.87%) for OPSs and 165 (0.62%) for saliva sampling (p<0.001 between all pairwise comparisons).

Table 2 Comparisons of SARS-CoV-2 detection rate (primary outcome) between specimen types, n=381 tests cases with a positive gold standard

Specimen type	Δ detection rate (95% CI)	P value*
OPS versus NPS	6.0% (3.9 to 8.92)	0.049
NPS versus saliva	10.8% (7.8 to 14.3)	<0.001
OPS versus saliva	16.8% (13.2 to 20.9)	<0.001
OPS/NPS versus saliva	31.8% (27.1 to 36.7)	<0.001
OPS/NPS versus OPS	15.0% (11.5 to 18.9)	<0.001
OPS/NPS versus NPS	21.0% (17.0 to 25.4)	<0.001

*Based on calculated OR using generalised estimating equations logistic regression.
Δ, % points difference in detection rate; NPS, nasopharyngeal swab; OPS, oropharyngeal swab; Saliva, saliva sampling.

Table 3 The mean difference in the pre-analytical cost for different sample methods by the second-order Monte Carlo simulation of 100 000 persons with 1.0% prevalence of SARS-CoV-2 infection

Sample	Pre-analytical cost, US$ (95% UI)	Detected infection, n (95% UI)	Pre-analytical cost per detected infection, US$	Δ Pre-analytical cost per detected infection versus saliva, US$ (95% UI)
Saliva	98163 (82 372 to 116 515)	620 (569 to 667)	159	–
OPS	405522 (359 344 to 458 302)	788 (744 to 827)	516	1832 (1282 to 2988)
NPS	448739 (394 941 to 507 982)	728 (681 to 771)	618	3258 (1953 to 8452)
NPS/OPS	542480 (479 601 to 614 552)	938* (911 to 959)	580*	1401* (1139 to 1755)

*The pre-analytical cost per detected infection was estimated based on the combined results from two individual molecular test results for NPS and OPS.
NPS, nasopharyngeal swab; OPS, oropharyngeal swab; Saliva, saliva sampling; UI, uncertainty interval.

The pre-analytical cost for salary and material per test were US$0.98, US$4.06, US$4.49 and US$5.43 for saliva, OPS, NPS and NPS/OPS sampling, respectively (see online supplemental appendix A for further details). The saliva specimen collection method was the most inexpensive, with a sample cost of US$98071 per 100000 persons (table 3). With an estimated SARS-CoV-2 prevalence of 1%, the incremental cost per detected SARS-CoV-2 infection per 100000 persons compared with saliva sampling was US$3258 (95% uncertainty interval (UI) US$1953 to US$8452) for NPSs, US$1832 (95% UI US$1282 to US$2988) for OPSs and US$1401 (95% UI US$1139 to US$1755) for NPSs/OPSs, respectively (see online supplemental appendix A for further details).

DISCUSSION

This study, comparing three specimens collected in randomised sequence, found that OPS specimens had a higher detection rate for molecular SARS-CoV-2 testing than NPS specimens. When they disagreed, the saliva sample was never concordant positive with NPS but sometimes concordant positive with OPS. OPS also appears to be better tolerated and less expensive than NPS. Saliva had a lower detection rate than either OPS or NPS, but was less costly and better tolerated for mass testing.

The SAMPLE trial fully explored the consequences of different SARS-CoV-2 sample strategies by combining molecular test results with data on test discomfort and cost-effectiveness. The randomised trial design allowed us to control for the external confounders affecting the diagnostic results by randomising the order of three paired samples and using the same viral transport media and laboratory equipment for all the RT-PCR analyses. Only healthcare workers who completed a validated competency-based training programme were allowed to collect respiratory specimens for the SAMPLE trial to ensure standardised and high-quality specimen collection.[15] Denmark had one of the highest RT-PCR testing rates for SARS-CoV-2 per capita in the world and the SAMPLE participants were representative of the general urban population tested.[1 20] As 23102 participants were enrolled during mass testing, the SAMPLE trial provides unique insight into the pattern of viral shedding during the initial infection stage of asymptomatic individuals not previously investigated. Very few participants were excluded from the

final analyses, and no difference in clinical characteristics was observed compared with the participants who were included in the analysis (see online supplemental appendix A tables S6–S8). Thus, we believe our findings are valid and generalisable to adults in ambulatory care settings undergoing SARS-CoV-2 molecular testing for both diagnostic and screening purposes.

Our study has limitations to consider. In our research protocol,[12] we defined a positive reference standard for SARS-CoV-2 infection if one or more of the three index tests were positive for a participant. Our reference standard, therefore, has an incorporation bias, as a positive molecular test from any specimen (index test) would define the reference standard as positive. If all three index tests were negative, we would also define the reference standard as negative, although it might be a false negative.[21] However, as we collected three specimens sent for independent highly sensitive RT-PCR testing, we believe the risk of false positives is very low. All the specimens were also stored in the same VTM and analysed in the same laboratory, and possible false-positive RT-PCR results should be comparable between specimens and will not change the head-to-head comparison of the SARS-CoV-2 detection rate. We also confirmed our conclusions by changing the definition of a positive PCR test to a Ct <25 for two N-gene segments, revealing that the difference in detection rate between OPSs and NPSs increased from 6.0% to 14.1%. All these subgroup analyses, therefore, support the validity of our main results despite not having an independent reference standard for participants with SARS-CoV-2 infection.

NPS are considered the most sensitive specimen for SARS-CoV-2 testing[22] and the IDSA currently advises against using OPS instead of NPS.[9] As such, we were surprised to discover a slightly higher SARS-CoV-2 detection rate among OPS than NPS samples. However, the two retrospective studies exploring OPSs for outpatient SARS-CoV-2 testing included fewer than 500 symptomatic participants,[21 22] and a meta-analysis could not establish any significant difference between OPS and NPS sensitivity.[10] Furthermore, the studies had retrospective designs and lacked precise descriptions of the OPS procedure and staff training. The gag reflex makes OPS a challenging procedure, and there is a risk that the tongue and gums may be swabbed instead of the oropharyngeal cavity (palatine tonsils and the posterior oropharyngeal wall). As all healthcare workers in the SAMPLE trial completed an additional competency-based training programme,[15] we ensured a standardised collection of high-quality respiratory specimens from the different anatomical sites. Another difference compared with previous studies is the enrolment of many asymptomatic participants from mass testing. The difference between the OPS and NPS detection rates was also primarily seen among the asymptomatic compared with the symptomatic participants (19% vs 1%, respectively). A recent study also demonstrated that SARS-CoV-2 was detected in the throat before the nasal cavity in volunteers who had intranasally SARS-CoV-2 inoculation performed in a controlled experimental setup.[23] These findings indicate that the oropharyngeal cavity may play a more significant role than the nasal cavity during the SARS-CoV-2 replication in the initial presymptomatic infection period.

A policy of collecting both OPS and NPS for separate molecular testing (OPS/NPS) would increase the SARS-CoV-2 detection rate by 15% and 21% points compared with single OPS or NPS specimens, respectively. These results are comparable to previous studies[24 25] and support the WHO recommendation to combine OPS and NPS specimens for molecular testing.[16] A limitation of these results is that the diagnostic NPS/OPS results are calculated based on the results from the NPS and OPS specimens sent individually for RT-PCR. As the definition of a SARS-CoV-2 positive participant is any positive molecular test, a combination of test results will, therefore, always result in higher sensitivity. Instead, the two swabs could be sent in the same virus transport medium for one RT-PCR analysis to reduce costs, but we do not know if it could decrease the detection rate compared with our findings.

The detection rate for saliva specimens was 11% points lower than for NPS specimens in our study and thereby a little lower than findings from systematic reviews reporting a 1.6%–7.9% points difference for SARS-CoV-2 testing.[11 26] A reason for the lower detection rate of saliva could be that we included many asymptomatic participants and had a higher number of inconclusive saliva test (1%) compared with NPS and OPS (0.4%). Although saliva specimens had a lower detection rate than NPS and OPS specimens, the pre-analytical cost was one-fourth as expensive due to reduced salary and need for protective equipment than OPS and NPS performed by healthcare workers.

The SAMPLE trial provides evidence for the OPS specimens to have a higher SARS-CoV-2 detection rate, being less costly, and better tolerated than NPS for molecular testing in a public setting. Saliva specimens had a little lower detection rate with a much lower cost per sample, making saliva suitable for mass testing. The combined OPS/NPS specimen achieved the highest SARS-CoV-2 detection rate, and it could be recommended as part of testing during hospital admission, when it is critical to detect and isolate infectious patients. Further research is needed to investigate if our diagnostic results can be generalised to a more heterogeneous population, including children, and if the high sensitivity from OPS molecular testing also is generalisable to rapid antigen tests.

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