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Protocol Article

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SARS-CoV-2 in saliva, oropharyngeal and nasopharyngeal specimens

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ABSTRACT

INTRODUCTION: The reference test to evaluate patients with suspected respiratory virus infection is a real-time reverse transcription-polymerase chain reaction (RT-PCR) from a nasopharyngeal swab (NPS). However, other specimen collection methods such as an oropharyngeal swab (OPS) or saliva specimen are also used for SARS-CoV-2 testing during the ongoing COVID-19 pandemic. However, it remains unclear if rates of SARS-CoV-2 detection differ between sampling methods. This study will compare the rates of SARS-CoV-2 detection by saliva, OPS, and NPS sampling in a public setting.

METHODS: Individuals referred for outpatient SARS-CoV-2 testing will be invited to participate in a prospective clinical study. They will have saliva, OPS and NPS specimens collected that will be analysed separately for SARS-CoV-2 RNA by RT-PCR. The rate of SARS-CoV-2 detection in saliva, OPS and NPS will be compared using a logistic regression mixed-effect model analysis. A sample of 19,110 participants is required at an expected 1.5% test-positive rate in order to detect a 25.6% difference. The total sample size will be adjusted as the test-positive rate changes.

CONCLUSIONS: This study will provide evidence for the optimal site of specimen collection to detect SARS-CoV-2. The results may help guide the health authorities.

FUNDING: This is an investigator-initiated trial based on an unrestricted grant from the Novo Nordisk Foundation and the Aage og Johanne Louis-Hansens Fond. The foundations have had no say in the decisions on study design or reporting.

TRIAL REGISTRATION: ClinicalTrials.gov (ID: NCT04715607).

A comprehensive testing strategy is recommended during the current COVID-19 pandemic. The gold standard for detection of SARS-CoV-2 in an outpatient test setting is to apply a real-time reverse transcription-polymerase chain reaction (RT-PCR) of SARS-CoV-2 to an upper respiratory tract specimen. A nasopharyngeal swab (NPS) collected by a healthcare worker is the conventional method for obtaining a clinical specimen for viral testing [1, 2], whereas oropharyngeal swabs (OPS) are recommended in some countries [3, 4]. A drawback of the NPS and OPS is that the swabs may be technically challenging, may cause patient discomfort and trigger sneezing. Alternatively, the Centers for Disease Control and Prevention consider that other non-invasive upper respiratory specimen collection methods are acceptable during the COVID-19 pandemic, e.g. a saliva specimen [5]. In

contrast, the World Health Organization (WHO) recommends performing both OPS and NPS at the same time (OPS/NPS) and does not recommend using saliva as a single specimen for testing [6]. However, it remains unclear how the rate of detection may differ when using different sampling methods as the studies performed during the COVID-19 pandemic are small, have mainly enrolled hospitalised patients and have excluded individuals without symptoms or with mild symptoms in ambulatory test settings [7, 8]. The aim of the trial of SARS-CoV-2 detection in saliva, oropharyngeal and nasopharyngeal specimens (SAMPLE) is to compare the diagnostic sensitivity of RT-PCR, patient discomfort and costs when using the different specimen collection methods for COVID-19 testing.

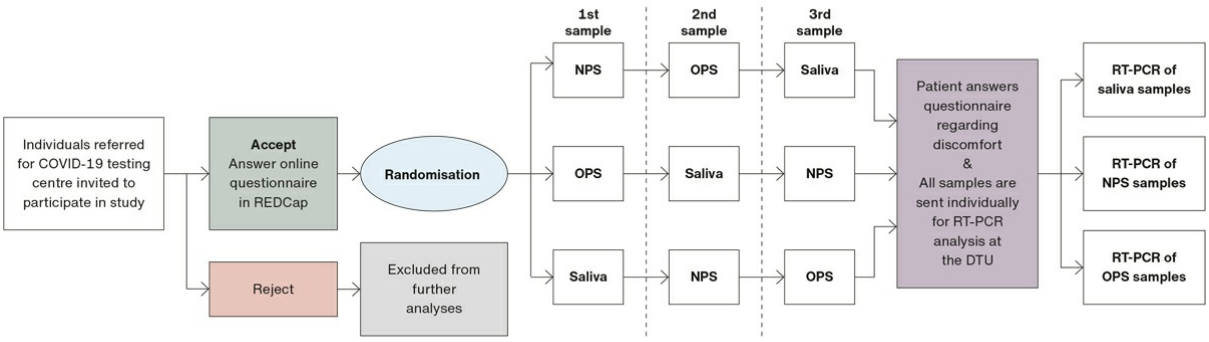
Research question: What is the detection rate of SARS-CoV-2 in saliva, OPS or NPS specimens tested by RT-PCR in individuals tested for COVID-19 at a public test centre?

METHODS

This is a comparative prospective diagnostic accuracy study reported according to the STARD guidelines [9] and registered with ClinicalTrials.gov (ID: NCT04715607). The study was approved by the Danish Data Protection Agency (Protocol No. P-2021-34) and the Copenhagen Regional Ethical Committee (classified as a quality improvement study, Protocol No. H-21003614). We will invite individuals referred for outpatient SARS-CoV-2 testing in Copenhagen, Denmark, at Testcenter Danmark in Valby and Taastrup to participate in the study on a volunteer basis. The enrolled patients will have an OPS and an NPS performed by a trained healthcare worker, who will also instruct and supervise the collection of saliva.

OPS is the standard collection method for SARS-CoV-2 testing specimens at public test centres in Denmark [4]. The saliva, OPS and NPS samples will be placed in separate tubes with universal transport media and sent to the laboratory of clinical microbiology at the Technical University of Denmark (DTU) for SARS-CoV-2 RT-PCR testing. The criteria for a positive RT-PCR test result will be a cycle threshold (Ct) value below 34. To minimise any bias in the sampling method, the order of the sample methods will be randomised by REDCap Software during participant enrolment (see **Figure 1**). The participants will be invited to complete a questionnaire to assess their pain or discomfort during the procedures. Furthermore, they will complete a questionnaire about their symptoms and the number of sick days. All data will be documented on-site in a secure web database (REDCap).

FIGURE 1 Flow chart of study design.



DTU = Technical University of Denmark; NPS = nasopharyngeal swab; OPS = oropharyngeal swab; RT-PCR = real-time reverse transcription-polymerase chain reaction.

Specimen collection techniques

NPS

trained healthcare worker will insert a fine-shafted flexible nylon-flocked swab (nasopharyngeal swab, NEST Biotechnology, Jiangsu, China) following the floor of the nose until resistance is met at the posterior pharynx

(equivalent to the distance from the opening of the nostril to the earlobe). Here, the swab is left in place for one second and rotated three times before being withdrawn slowly by a rotating motion. The swab is placed into a vial with 2 ml of viral transport medium (IMT DNA/RNA preservation media) and stored at room temperature until transportation to the laboratory at the DTU for same-day RT-PCR.

OPS

trained healthcare worker will perform the OPS with a rigid-shaft nylon-flocked swab rubbed over a tonsil and the posterior oropharynx with a rotating or painting movement. The swab is inserted without touching the tongue and gums. If visualisation of the posterior oropharyngeal wall is obstructed by the tongue, a tongue depressor is used to improve visibility. The swab is placed into a vial identical to the NPS swab and handled in the same manner.

Saliva

Saliva is collected by the drooling technique. The participants are given paraffin gum to stimulate saliva production and instructed to tilt the head forward to allow the saliva to pool in the mouth without swallowing. Next, they will guide the collected pool of saliva through the mouth and into a 50 ml skirted tube. Participants are asked to avoid spitting or clearing their throat during the collection. They are allowed a maximum of two minutes to produce the saliva specimen. Saliva is collected without restriction on intake of food or beverage prior to enrolment. One ml of the saliva will be pipetted into a vial identical to the NPS and OPS swabs and handled in the same manner.

Training of healthcare workers

Healthcare workers who participate in the collection of upper-respiratory samples are experienced staff who have performed > 1,000 OPS. Staff are trained in proper infection prevention and control precautions. All staff receive additional training prior to the initiation of SAMPLE including handpost and video instructions on how to perform saliva, OPS and NPS collection. After reading the handouts and watching the videos, a short didactic lesson about the upper-airway anatomy and the steps in the techniques for collecting upper respiratory samples will be given by an experienced nurse who has received formal training by a specialist in otorhinolaryngology. Subsequently, the OPS technique will be demonstrated on a life-sized airway demonstration model, and NPS will be demonstrated on a 3D-printed simulator for nasopharyngeal swab collection [10]. All the participants will then perform the OPS and NPS on the demonstration model and receive feedback on their swabbing technique. Finally, at the end of the session, they will need to pass a multiple-choice questionnaire with theoretical questions and skills assessment using a checklist to evaluate the OPS and NPS performance on the demonstration model [11].

SARS-CoV-2 real-time reverse transcription-polymerase chain reaction testing of the clinical specimens

All swabs are processed as routine samples by laboratory technicians at the DTU who are blinded to the sampling method. Nucleic acid is extracted from 200 µl of medium using an in-house silica-based procedure on a Beckman i7 robotic platform. SARS-CoV-2 RNA is detected using a multiplexed version of the CDC N-gene one-step RT-PCR, targeting two N-gene segments and the RNase P ribozyme as inhibition control and for the assessment of the presence of human genetic material [7]. Samples with Ct < 34 for at least one target are considered positive. Negative samples with RNase P Ct > 23 are considered inconclusive (RNase P median Ct = 19.5).

Clinical outcome measures

Due to the high sensitivity of RT-PCR, we will define a participant with an RT-PCR-positive result from either saliva, OPS or NPS as being SARS-CoV-2 infected. The saliva/OPS/NPS results will be used as the diagnostic reference to calculate the sensitivity for the saliva, OPS, NPS and combined tests.

The primary outcome will be reported as

The detection rate of SARS-CoV-2 RNA for saliva, OPS, NPS, OPS/NPS or combined OPS/NPS/saliva

Diagnostic sensitivity of detecting SARS-CoV-2 RNA for saliva, OPS, NPS or OPS/NPS.

Secondary outcome

SARS-CoV-2 RT-PCR Ct values

OPS, NPS and saliva test discomfort and likelihood to get retested

Frequency of mutations in SARS-CoV-2

Costs for each of the three tests (OPS, NPS and saliva) and incremental costs per additional infection detected using different testing strategies.

Statistics

The rate of SARS-CoV-2 detection in saliva, OPS, and NPS will be compared using logistic regression mixed-effect analysis. We will adjust for the effect of the test centre and the order of tests performed. Odds ratios and 95% confidence intervals (CI) for comparison of saliva, ONS, and NPS will be calculated. In case the number of observations for some of the combinations is too small, McNemar's test will be performed instead. The Ct values and visual analogue scale (VAS) will be compared using paired sample t-test and mixed-effect general linear model. To evaluate if the detection rate of OPS/NPS is significantly higher than the detection rate using a single test (saliva, OPS or NPS), we will test if the difference in detection rates is different from zero. The level of statistical significance is $p < 0.05$.

Sample size calculations

The study will be conducted during the second period of lockdown in Denmark with a positive rate of the RT-PCR test at the COVID-19 Test Centres in Copenhagen of 2.6% in the week before the study starts. Due to the lockdown, we estimate that the positive rate will decrease by approx. 40-50% weekly with an average of 1.5% during a three-week study period. Therefore, the sample size was determined to provide adequate power for assessment of the primary outcome estimating an incidence of SARS-CoV-2 infection at 1.5% of all the test results during the study period. Assuming that NPS would have a 25.6% higher sensitivity than OPS [7], we estimated that a sample of 18,200 participants would provide the trial with 80% power at a 5% significance level for a low correlation between the OPS and NPS test. This corresponds to including a total of 273 individuals with a positive OPS test. Anticipating that approx. 5% of the participants will be lost to follow-up or fail to complete all tests, at least 19,110 participants are required. However, if the positive rate changes in other ways than expected, this will change the sample size needed to provide adequate power for assessment of the primary outcome (**Table 1**). Test-positive rates will therefore be monitored during the study period to ensure that our sample size calculation assumptions remain correct. No data analyses will be conducted before data collection is completed.

TABLE 1 Sample size calculation. Different incidence rates of SARS-CoV-2 infection and corresponding sample sizes needed for the trial with 80% power at a 5% significance level.

	SARS-CoV-2 test-positive results using the OPS test, %								
	0.5	0.75	1.0	1.5	2.0	2.5	3.0	3.5	4.0
Paired test: individuals, n	55,200	36,800	25,300	18,200	13,600	10,800	9,000	7,700	6,700

OPS = oropharyngeal swab.

Participants

Participants are all volunteers who are attending the test facilities to obtain a PCR test. Participants are required to provide oral and written informed consent for participation before entering the study. Subsequently, they will be asked to answer an online questionnaire through a link to a secure web database (REDCap) on their smartphone. Individuals who do not carry a smartphone will be given the possibility to answer the questionnaire on a site computer assisted by study staff. The specimen collection will then be performed in a random order and the participants will be asked to assess their discomfort on a VAS during each procedure. The randomisation will be registered in Redcap along with the discomfort score and will then be merged together with the participant's questionnaire answers.

The inclusion criterion is 16 years or more of age. The exclusion criteria are neck breathers (tracheostomy/laryngectomy patients) or other nasopharyngeal or oropharyngeal anomalies that do not allow for sampling using swabs. The participants include symptomatic persons and asymptomatic persons who have or have not come into close contact with an infected individual.

Economic analyses

The costs of each test are calculated using the ingredients method, including equipment costs and personnel costs. We will calculate the incremental costs per additional infection detected and estimate 95% CIs using the Monte Carlo simulation.

Data-sharing statement

Following de-identification, data will be shared upon request from researchers with an interest in the research field who have a sound proposal. This includes data sharing to methodological and meta-analysis studies and proposals should be directed to the corresponding author.

Trial registration: ClinicalTrials.gov (ID: NCT04715607).

DISCUSSION

The results of this study will provide evidence for the most sensitive specimen collecting method for SARS-CoV-2 RNA by RT-PCR. Furthermore, the cost-effectiveness and patient discomfort between the specimen collecting methods will also be explored. We hypothesise that NPS will be the more accurate single specimen with which to detect SARS-CoV-2, but also that it will be associated with more discomfort during sampling which may decrease the likelihood of future testing. Each test method has advantages and drawbacks that we aim to explore in this study. Our findings will provide evidence for the testing recommendations by the international and national health authorities.

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Conflicts of interest Potential conflicts of interest have been declared. Disclosure forms provided by the authors are available with the article at ugeskriftet.dk/dmj

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