Molecular detection of SARS-CoV-2 from oropharyngeal swabs performed with or without specimen collection from the palatine tonsils – a research proposal for a multicenter randomized controlled trial

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Introduction

Background and rationale

Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) testing strategy is essential for the diagnostic work up and screening during the coronavirus disease 19 (COVID-19) pandemic. Proper upper respiratory specimen collection is the most critical step in the diagnosis of SARS-CoV-2 virus in public settings and standard sample guidelines are recommended[1]. An oropharyngeal swab (OPS) specimen is the preferred sample in many countries, either alone or combined with nasal swab[2, 3]. However, there is a great variability in the diagnostic accuracy for OPS with a 95% confidence interval from 52-100% reported in systematic reviews[4, 5] and The Infectious Diseases Society of America therefore do not recommend OPS for SARS-CoV-2 testing[6]. Many of the previous studies performing OPS also lack detailed description of the sample technique, which makes it difficult to compare the different diagnostic accuracy results. Currently, there are no standardized oropharyngeal sample guidelines, and some only collect specimen from the posterior oropharyngeal wall while other recommend also to include a swab of the palatine tonsils for SARS-CoV-2 testing (Figure A). The Danish Health Authority advised to avoid the palatine tonsils in the oropharyngeal swab in the beginning of the COVID-19 pandemic, but later changed their recommendation so it was up to the individual testing center to decide[7]. However, studies suggest that the palatine tonsils could have a tissue tropism for SARS-CoV-2 which may improve the SARS-CoV-2 detection during sampling[8]. This may explain the variation of sensitivity reported, but no clinical studies have explored the differences between the two sample techniques. We therefore plan to conduct a randomized clinical trial to compare an oropharyngeal swab with or without the palatine tonsils.

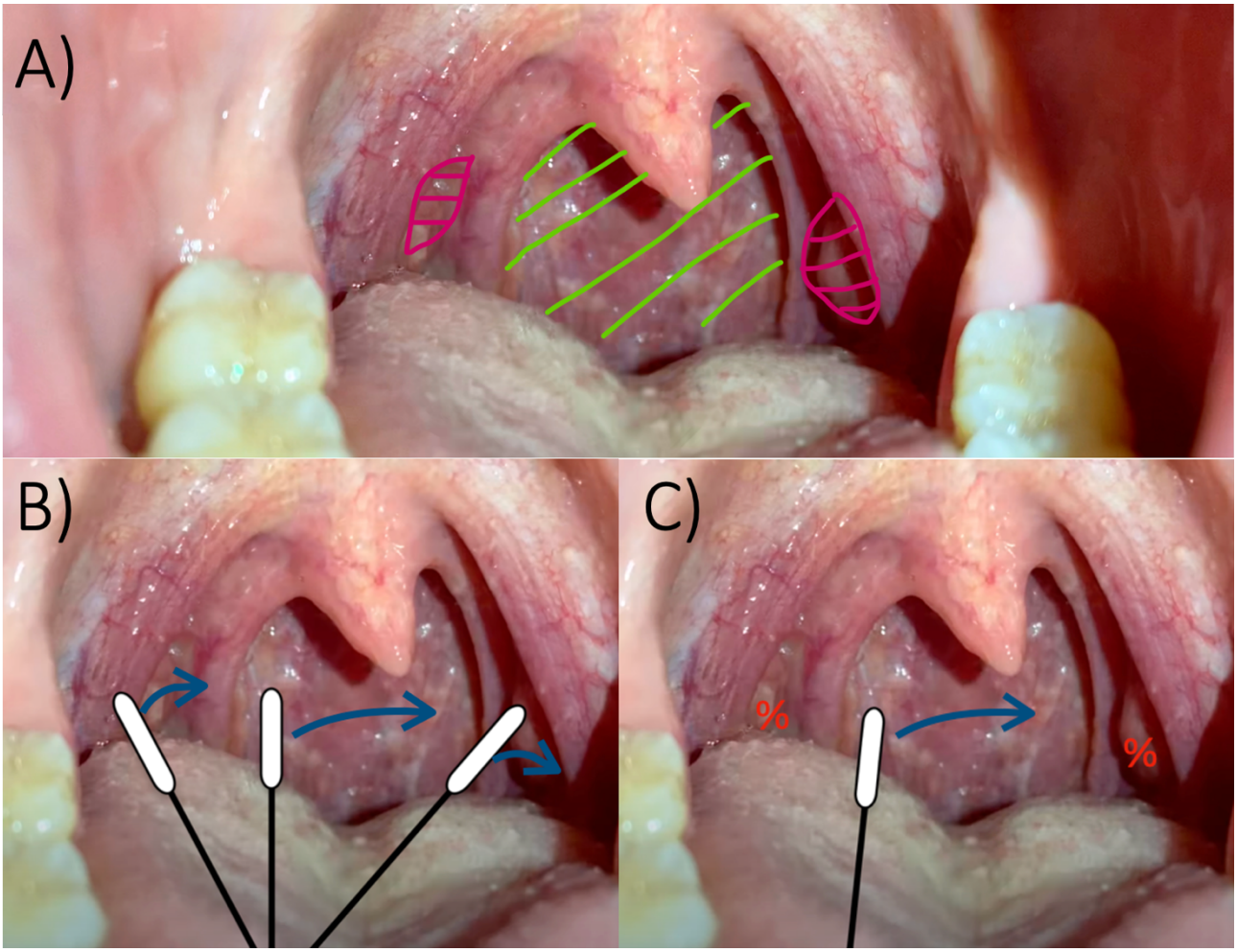


FIGURE A. *Image A. Anatomic visualization of the posterior oropharyngeal wall (green) and palatine tonsils (pink). Image B. Oropharyngeal swab including the posterior oropharyngeal wall and palatine tonsils. Image C. Oropharyngeal swab including the posterior oropharyngeal wall, but avoiding the palatine tonsils.*

Research question

In a cohort of individuals tested for COVID-19 at a public test center, what is the sensitivity of an OPS sample, including the palatine tonsils, compared to only swabbing the posterior oropharyngeal wall in molecular detection of SARS-CoV-2?

Methods

We will conduct a randomized, controlled study to compare the molecular detection rate of SARS-CoV-2 by an OPS performed from the posterior oropharyngeal wall and the palatine tonsils (intervention group) **or** the posterior oropharyngeal wall only (control group). This trial is approved by the Danish Data Protection Agency (Protocol No. P-2022-803) and reported to the Regional Ethics Committee of the Capital Region of Denmark who considered it exempted from further processing (Protocol No. H-22022937). The protocol was registered with the ClinicalTrials.gov database (NCT05611203). A grant was received from the Novo Nordisk Foundation (Grant number NNF21SA0069151) without influence on study design, data collection, data analysis, writing of the report, and the decision to submit the results for publication.

Study setting

Participants will be enrolled in a free public Covid-19 Testcenter in Valby and Hillerød, Capital Region, Denmark starting from November 2022. All samples will be sent for molecular testing at Statens Serum Institut and data analysis will be performed at Rigshospitalet.
Only special trained healthcare workers at Valby and Hillerød Testcenter will collect specimens from the participants enrolled in the study. Before enrollment, all healthcare workers will receive training and a competency-based assessment of their skills (appendix 1).

Eligibility criteria

Individuals with or without symptoms of upper respiratory tract infection age 18 or above who visit Valby or Hillerød Testcenter for RT-PCR-testing for SARS-CoV-2 will be offered participation in the study. Participation is voluntary, and participants are required to provide oral and written consent to participate prior to entering the study (appendix 2).
The exclusion criteria are individuals with a tracheostomy, laryngectomy, or prior oropharyngeal cancer surgery that would make the OPS difficult. Further, individuals without a Danish civil

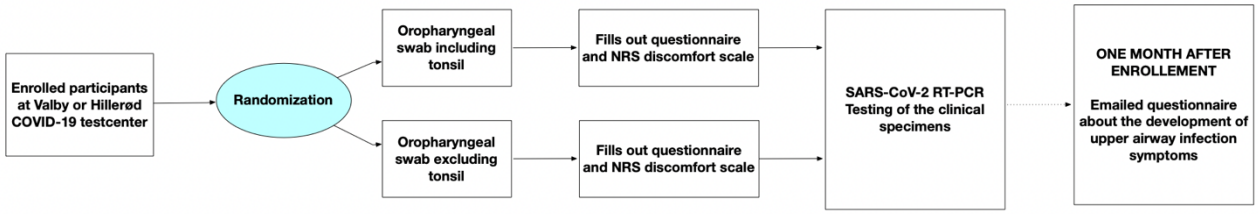
registration number (CPR) will be excluded to participate. Individuals who are not included in the study will have the standard oropharyngeal swab performed in the test center.

The same individual will only be allowed to participate in the study once. If a participant is enrolled in the study more than once, only the first enrollment (or the first positive test case) will be included, while the following test results will be excluded from the statistical analysis.

Randomization to intervention and control group

After enrollment, the participants will be randomized in a 1:1 ratio having either oropharyngeal swab performed including the posterior oropharyngeal wall and both palatine tonsils (intervention group) or only including the posterior oropharyngeal wall and avoiding the palatine tonsils (control group), see Figure B.
A block randomization list was generated by T.T. using an online randomization software[7] afterward uploaded to REDCap. The randomization group will be disclosed in connection with trial registration in REDCap using the REDCap "randomize" function. The oropharyngeal sample will afterward be performed by the trained healthcare workers with or without the palatine tonsils depending on the randomization to either the intervention or control group.

Figure B. Study flowchart.



The swabs will be placed in separate tubes dry and sent to the clinical microbiology laboratory for detection of SARS-CoV-2 by single-target RT-PCR at Statens Serum Institute. Samples with viral cycle threshold (Ct) values below 38 are considered positive, 38-40 inconclusive and above 40 negative[8].

All participants are required to fill out a survey on-site about their reason for being tested, prior Covid-19 infection(s), vaccinations status, symptoms, and if any prior tonsillectomy has been performed (appendix 2).
All healthcare workers participating in the data collection have been given a unique ID which will be registered for each OPS performed in the study. Further, the healthcare worker will also be asked to rate the Mallampati score of the participants including in the study (appendix 5).
All data will be documented on-site in a secure web database (REDCap, appendix 3).

Outcomes

The primary outcome will be reported as:

- SARS-CoV-2 RNA by RT-PCR test result (positive, negative, inconclusive)

The secondary outcome will be reported as:

- SARS-CoV-2 RT-PCR cycle threshold (Ct) value
- Test discomfort on a 11-point NRS-scale
- Development of COVID-19 disease after testing
- SARS-CoV-2 detection rate for each healthcare worker
- Mallampati Score of participants being tested

Sample size

Based on a SARS-CoV-2 test positive proportion on 13% in the Danish capital region the last week of October 2022, we expected proportion of positive to increase to about 20% during the following study period[9]. The power calculation was based on an expected improvement in diagnostic accuracy of 25% by including the palatine tonsils in the oropharyngeal specimen collection compared to a sample without the tonsils. We therefore estimated that a sample of 2,188 participants (with about 438 with SARS-CoV-2 infection) would provide the trial with 80% power at a 5% significance level with an expected test positive proportion of 20% for SARS-CoV-2

among the participants tested [10]. As we expect about 10% missing due to dropout and missing data, we aim to include 2.407 participants in the study. All individuals entering the testing facility who meet the eligibility criteria are offered to participate in the study. We expect about 300-700 tests performed at both COVID-19 test centers each day and an assumed participation at 10-30%. We therefore expect to complete the study within two-three months.

Statistical analysis

A study participant is considered to have a SARS-CoV-2 infection if the oropharyngeal sample is RT–PCR positive (reference standard). Participants with an inconclusive RT-PCR test result will be included in the analyses as a negative test result following an intention-to-diagnose approach[11]. Differences in the proportion of SARS-CoV-2 positive tests between the intervention and the control group will be compared using binary logistic regression using test center as fixed effect and a generalized estimating equation to adjust for clustering of data within the healthcare workers performing the sample. The difference in SARS-CoV-2 detection rate between healthcare workers will also be reported separately to estimate the inter-person variance. The Ct values from positive RT–PCR samples and the NRS discomfort scores will be compared using a general linear model with mixed effects (Ct) and GEE models (NRS discomfort). The 95% confidence intervals (CI) will be presented. Categorical data will be summarized with number and percentage while continuous data will be summarized by mean and standard deviation. We will not perform tests of statistical significance for baseline characteristics. 5% significance level was applied. Statistical Analysis Software ver. 9.4 (SAS Institute, North Carolina, U.S.) was used for the statistical analyses.

Planned subgroup analyses

We planned to do a sensitivity analysis using a lower cycle threshold (Ct) < 25 for positive SARS-CoV-2 definition to explore the consequences of a higher test specificity for the SARS-CoV-2 detection rate between specimen types. We also plan to estimate the sensitivity and specificity using Bayesian latent class analysis for accounting for an imperfect reference standard. Further, we planned to do subgroup analyses exploring the distribution of positive test results for participants stratified by symptoms, previous COVID-19 infection, vaccinations status, prior

tonsillectomy and Mallampati score. To explore a potential bias from the distribution of the inconclusive test results, we excluded the inconclusive results in a subgroup analyses.

Quality assurance

All the healthcare workers who will be including participants for this study will be trained and have at least three months prior experience in oropharyngeal swabs. Further, they will complete a special standardized training- and certification program taught by a board-certified otorhinolaryngologist to ensure all healthcare workers perform the same technique on the participants in the intervention and control group. A two-hour long training session will cover the theoretical and practical part of oropharyngeal sample techniques and the study design with following elements:

- A walk-through of the practical study setup and how participants are enrolled, and diagnostic interventions are performed
- Theory on oropharyngeal swabbing including upper airway anatomy and anatomical variations/Mallampati Score.
- Practical exercises in oropharyngeal swab technique.

Checklists outlining the two different sampling techniques for oropharyngeal swabs with and without palatine tonsils, respectively, will be used to assess the swab performance of all the healthcare workers by a board-certified otolaryngologist or special trained nurse before study enrollment (see appendix 1).
An on-site healthcare professional is in charge of internal daily quality assurance (N.T.G.).

Follow-up

The participants enrolled in the study will be asked to agree to be contacted in case of a positive test result. About one month after a positive test results the participants will receive an online questionnaire or phone call to follow up. The follow-up questions can be found in appendix 3.

Ethics and safety considerations

The purpose of the study is to compare the detection rate of the two different sampling techniques. Both techniques used in this study are implemented in the Danish healthcare system and are thus considered reliable methods for detecting and diagnosing infection with SARS-CoV-2. The study protocol is approved by the local ethical committee and the participants of the study are not subject to any adverse intervention and are put at no greater risk than the risk associated with a regular SARS-CoV-2 test.

Data plan

Data will be collected on-site in the secure database REDCap. Identifiers such as CPR-number, healthcare worker-ID and sample-ID are registered directly in the database for each new participant. The survey will be marked with a REDCap-ID allowing the research group to later match records with the physical surveys. Surveys are filled out on site by the participants and healthcare workers and stored in accordance with GDPR-legislation until data entry. Data entry will be done either by on-site healthcare workers continuously throughout the data collection period or by the research group once data collection is completed. No data analysis will be conducted before data collection is completed.

Declaration of interests

The authors have no competing interests to declare.

Access to data

The anonymized data will be made publicly available through an open access database after publication.

Appendices

Appendix 1: Quality assurance checklists

Checklist for oropharyngeal swab (OPS) including palatine tonsils						
Name:		Correct	Incorrect	Not relevant		
1	OPS performed with proper use of protection equipment					
2	OPS performed in compliance with infection prevention guidelines					
3	OPS performed in alignment with the participant ensuring proper visualization of the back wall of the oropharynx					
4	The swab is placed correctly between thumb, index and middle finger					
5	Participant is instructed to say “aaaah” making the soft palate rises and a spatula is used					
6	The swab is inserted and retracted without making contact with the mucous membrane of the oral cavity or the tongue					
7	The swab gathers sufficient material from the back wall of the oropharynx including both palatine tonsils in a rotating or brushing movement					
8	The swab is placed in the sample tube in accordance with local guidelines					
Total score						
		Poor	Unacceptable	Good	Acceptable	Excellent
General assessment						

Checklist for oropharyngeal swab (OPS) excluding palatine tonsils

Name:		Correct	Incorrect	Not relevant
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8	The swab is placed in the sample tube in accordance with local guidelines			
Total score				

	Poor	Unacceptable	Good	Acceptable	Excellent
General assessment					

Appendix 2: Survey for participants

Survey to be filled out by participant

1. **Are you vaccinated against COVID-19?**

☐YES ☐NO

2. **Have you previously been infected with COVID-19?**

☐YES ☐NO

3. **Please indicate your reason for getting tested today:**

☐Symptoms ☐Close contact ☐Positive quick test

☐Screening ☐Test ahead of social gathering or nursing home visit ☐Other

If 'yes' to symptoms, which symptoms do you have?

☐Sore throat ☐Headache ☐Cough ☐Muscle and joint pain

☐Fever ☐Fatigue ☐Reduced sense of smell or taste

If "yes" to symptoms, for how many days have you had symptoms?

Indicate number of days: _____day(s)

4. **Have you had your tonsils removed??**

☐YES ☐NO

5. **On a scale from 0-10, how uncomfortable was today's test for COVID-19?**

No discomfort Worst possible discomfort

To be filled out by personnel:

REDCap ID:_____

Personnel ID:_____

Participant's Mallampati score:

☐**1** ☐**2** ☐**3** ☐**4**

Appendix 3: REDCap database

REDCap-ID	
Date	DD-MM-YYYY
Randomization	Oropharyngeal swab including palatine tonsils Oropharyngeal swab excluding palatine tonsils
CPR-number	
Sample ID	
Are you vaccinated against COVID-19?	YES NO
Have you previously been infected with COVID-19?	YES NO
Please indicate your reason your reason for getting tested today	Symptoms Close contact Positive quick test Screening Test ahead of social gathering / visit to a nursing home Other
If "YES" to symptoms, what symptoms do you have?	Sore throat Headache Cough Muscle and joint pain Fever Fatigue Reduced sense of smell or taste
If "YES" to symptoms, for how many days have you had symptoms?	
Have you had your tonsils removed?	YES NO
On a scale from 0-10, how uncomfortable was today's test for COVID-19?	0 1

	2 3 4 5 6 7 8 9 10
Personnel ID	
Participant's Mallampati score	1 2 3 4
FOLLOW UP	
Have you since the test and until four weeks later had symptoms of COVID-19?	YES NO
If 'yes' to symptoms, when did you develop symptoms?	I had symptoms when I got tested I developed symptoms 1-3 days after the test I developed symptoms 3-5 days after the test I developed symptoms 5-7 days after the test I developed symptoms 1-2 weeks after the test I developed symptoms 2-3 weeks after the test I developed symptoms 3-4 weeks after the test I do not know / I do not wish to disclose
If 'yes' to symptoms, which symptoms did you develop?	Sore throat Headache Cough Muscle- / joint pain Fever Fatigue Reduced sense of smell / taste Other
If 'yes' to fever, what was you highest temperature?	

If 'yes' to fever, how many days did you have a fever for?	
If 'yes' to symptoms, how many days did you have symptoms in total?	
Think about period from one week before your test until four weeks after. Which of the following statements describes best how you felt at your worst?	I had no symptoms I was sick and stayed home I was admitted to the hospital due to COVID-19
Have you taken days off work / from school during your infection with COVID-19?	No, I have not taken any days off Yes, I have taken days off between one week before the test until four weeks after Yes, I have taken days off later than four weeks after Not relevant
If 'yes' to having taken days off, how many days have you taken off in total?	
Do you smoke?	Yes No I used to smoke, but I do not smoke anymore Not relevant / I do not wish to disclose
If 'yes' to smoking, which of the following statements describes you best?	I smoke sometimes, e.g. at parties I smoke daily (less than 10 cigarettes or equivalent to that) I smoke daily (10 or more cigarettes or equivalent to that) I smoke e-cigarettes I do not know / I do not wish to disclose
If 'yes' to having smoked previously, which statement describes you best?	I have not smoked for more than 5 years I have smoked within the last 5 years, but I do not smoke anymore
Think about the past half year before the test. Which of the following statements describes your level of physical activity best?	I do hard exercise / elite level sports regularly and several times per week I exercise or perform heavy gardening tasks or similar at least four times per week I walk, bike or do other lighter exercise at least four times per week (incl. walking/biking to work and lighter gardening) I read, watch TV or do other still activities I do not know / I do not wish to disclose

Think about the past half year before the test. How would you describe you physical condition?	Very good Good Alright Below average Bad I do not know / I do not wish to disclose
Have you been diagnosed with a chronic illness by a doctor previous to your test for COVID-19?	No, I have not been diagnosed with a chronic illness Yes, diabetes Yes, asthma Yes, COPD or chronic pulmonary disease Yes, high blood pressure Yes, chronic of frequent headache, including migraine Other chronic illnesses I do not know / I do not with to disclose

Appendix 4: Participant information

Deltagerinformation om deltagelse i videnskabeligt forsøg

Forsøgets titel: Sammenligning af COVID-19 test ved podning i mundsvælget med eller uden mandler.

Vi vil spørge, om du vil deltage i et videnskabeligt forsøg, der udføres af Rigshospitalet i samarbejde med akutberedskabet i Region Hovedstaden og Hvidovre Hospital.

Før du beslutter, om du vil deltage i forsøget, skal du fuldt ud forstå, hvad forsøget går ud på, og hvorfor vi gennemfører forsøget. Vi vil derfor bede dig om at læse denne deltagerinformation. Du vil også modtage mundtlig deltagerinformation, hvor du kan stille de spørgsmål, du eventuelt har til forsøget.

Hvis du beslutter dig for at deltage i forsøget, vil vi bede dig om at underskrive en samtykkeerklæring. Det er frivilligt at deltage i forsøget, og du kan når som helst og uden at give en grund trække dit samtykke tilbage.

Nytte ved forsøget

Ved at indgå i forsøget hjælper du med at skaffe værdifuld viden om, hvordan man bedst foretager COVID-19 test. Det er med til at sikre, at vi kan bruge de testmetoder, der er bedst til at finde de smittede og samtidigt er mindst mulig ubehagelig for borgerne.

Formål med forsøget

Formålet med forsøget er at sammenligne den diagnostiske sikkerhed for to forskellige måder at foretage COVID-19 test på.

Normalt bliver man i Danmark podet med vatpind gennem munden for at samle materiale til PCR-test for COVID-19. Det varierer fra region til region hvorvidt podningen indbefatter mandlerne. Der mangler undersøgelser, der giver os viden om podning af mandlerne øger sikkerheden af testen eller ej. Vi vil derfor i dette lodtrækningsforsøg foretage podningen i svælget med eller

uden samtidig podning af mandlerne for at undersøge, hvilken metode er den bedste. For at få viden om borger præferencer vil du også blive spurgt ind til testubehag ved undersøgelsen. Når studiet er overstået, vil vi således være klogere på hvilken metode der bør anbefales i danske test sammenhæng.

Plan for forsøget

Alle undersøgelser vil blive udført under samme besøg, og du kan forvente at dette kan tage omkring 5 minutter længere. Du vil få taget podning i munden samt besvare et kort spørgeskema.

Podningen i mundsvælget foregår ved, at man fører en vatpind ind bag ganen (standardpraksis). Bliver du randomiseret til at skulle have podet mandlerne også, foregår det ved samme podning. Prøven vil blive sendt til PCR-analyse som vanligt og svartiden vil ikke blive forlænget.

For at undersøge om testen også giver et resultatet som er i overensstemmelse med efterfølgende sygdomsudviklingen, vil vi eventuelt efter podningen også lave et opslag i din elektroniske patientjournal for at hente information om behov for indlæggelse, behov for medicinsk behandling, samt tidligere vaccinationsstatus og COVID-19 infektion. Ved positiv test vil du også blive kontaktet nogle uger efter afslutning af forsøget (e-boks eller telefon) for opfølgning på udviklingen af mulige alvorlige symptomer på COVID-19.

Bivirkninger, risici, og ulemper

Du kan muligvis forvente lidt ekstra ubehag ved podning af mandlerne, men ellers udføres podningen ligesom den aktuelle standard praksis.

Etisk og databehandling (fortrolighed)

Forsøget varetages på initiativ af Rigshospitalet, Region Hovedstadens Akutberedskab, og Hvidovre Hospital med uafhængig økonomisk støtte af Novo Nordisk Fonden. Projektet er godkendt af Videnskabsetisk Komite og Videnscenter for Dataanmeldelser, og håndtering af persondata vil blive håndteret med fuld fortrolighed efter gældende retningslinjer.

Adgang til forsøgsresultater

Forsøgets resultater vil blive offentliggjort umiddelbart efter forsøgets afslutning i et fagfællebedømt videnskabeligt tidsskrift.

Vi håber, at du med denne information har fået tilstrækkeligt indblik i, hvad det vil sige at deltage i forsøget, og at du føler dig rustet til at tage beslutningen om din eventuelle deltagelse. Hvis du har spørgsmål eller vil vide mere om forsøget, er du meget velkommen til at kontakte forsøgsansvarlig medicinstuderende Benedikte Hartvigsen (benedikte.hartvigsen@regionh.dk).

Vi håber du vil have mulighed for at bruge 5 minutter ekstra på at indgå i projektet i forbindelse med, at du skal testes for COVID-19.

På forhånd tak for din interesse i at hjælpe med vores forskningsprojekt!

På vegne af forskningsgruppen

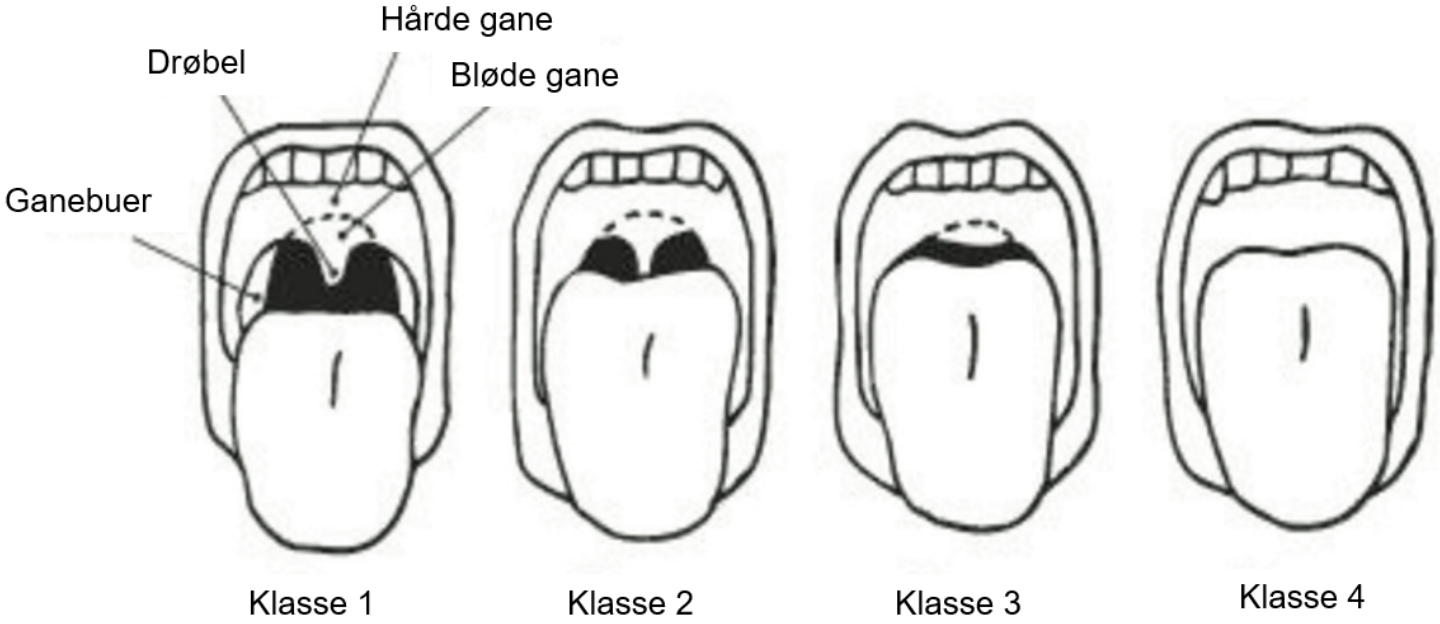
Benedikte Hartvigsen, medicinstuderende, Københavns Universitet

Thomas Benfield, overlæge, professor, Infektionsmedicinsk Afdeling – Hvidovre Hospital

Annette Kjær Ersbøll, Professor, Region Hovedstadens Akutberedskab

Nina Steenhard, Ph.d., Testcenter Danmark – Statens Serum Institut

Tobias Todsen, speciallæge, lektor, Afdeling for Øre-Næse-Halskirurgi og Audiologi – Rigshospitalet



Klasse 1: Udsyn til den bløde gane, drøblen, mandler og ganebuer,

Klasse 2: Udsyn til den bløde gane og drøblen samt delvist til mandlerne.

Klasse 3: Udsyn til den bløde gane og den øverste del af drøblen.

Appendix 6: Statistical Analysis Plan (SAP)

Title: Molecular detection of SARS-CoV-2 from oropharyngeal swabs performed with or without specimen collection from the palatine tonsils – a multicenter randomized controlled trial

SAP version 1, 11122022

Senior statistician: Professor Annette Kjær Ersbøll, PhD

Chief investigator: Associate Professor Tobias Todsen, MD, PhD

1. Statistical principles

1.1 Confidence intervals and *P* values

The level of statistical significance will be $p < 0.05$ and 95% confidence interval will be reported.

1.2 Adherence and Protocol deviations

Definition of adherence to the intervention

Adherence is defined as participants who has a full registration of identification number (CPR number), test center site for collection of specimen and randomization to control or intervention group. Further, the participants need to complete the OPS specimen collection send for molecular testing.

Compliance is assessed based on the number and percent of subjects who have correct registration information and representative samples for RT-PCR.

Description of adherence

The adherence to the intervention will be summarized in the study flowchart and number and "% compliance" will be summarized.

Definition of protocol deviations for the trial

The participants will be excluded from final analysis if one or more of the following deviations from the testing protocol was fund:

Missing identification number (CPR number), no registered test center sites or no randomization (intervention or control) registered.
Missing RT-PCR result

Description of which protocol deviations will be summarized
The number and type of protocol deviation will be registered, and number of participants removed will be summarized in figure with study flow.

2. Trial Population

2.1 Screening data
We aim to invite individuals from Valby and Hillerød Covid-19 test centers to represent citizens from two urban areas in Copenhagen, Denmark to participate in the study.

2.2 Eligibility
All individuals being 18 years or older will be invited to participate in the SAMPLE trial. The same individual will only be allowed to participate in the study once.

The exclusion criteria were individuals with
a tracheostomy, laryngectomy, or prior oropharyngeal cancer surgery
without a Danish civil registration number (CPR)

2.3 Recruitment
A trial flow diagram will be used to summarize the number of included participants with information about:

- Total number of Covid-19 tested individuals during the study period
- Number of participants lost to identify / registrar
- Number of participants excluded from final analyses due missing test results

2.4 Withdrawal/Follow-up

The level of withdrawal and the missing final RT-PCR test results during the study will be tabulated. As they only had a single OPS performed, no dropouts will be expected after the intervention – beside missing data.

2.5 Baseline patient characteristics

List of baseline characteristics for participants:

Measure	Outcome	Description
Demographic data	Age and gender	Data from the Danish civil registration number
Questionnaire	Test reason, symptom description, vaccination status, prior SARS-CoV-2 infection, prior tonsillectomy	Questionnaire registered in RedCap
NRS-scale	Discomfort score for OPS specimen collections	11-item measure of test-related discomfort answered immediately after testing
Mallampati score	A visual assessment of the distance from the tongue base to the roof of the mouth of the participants	4-item measure of amount of space in the mouth to reach the oropharynx assessed by the healthcare workers

Categorical data will be summarized with number and percentage while continuous data will be summarized by mean and standard deviation. We will not perform tests of statistical significance for baseline characteristics.

3. Analysis

3.1 Outcome definitions

The primary outcome:

- The proportion of positive SARS-CoV-2 RNA samples by RT-PCR for intervention and control group

The secondary outcome:

- SARS-CoV-2 RT-PCR cycle threshold (Ct) value
- Test discomfort on a 11-point NRS-scale
- Development of COVID-19 disease after testing
- SARS-CoV-2 detection rate for each healthcare worker
- Mallampati Score of participants being tested

3.1 Analysis methods

Analysis method and treatment effects

Differences in the proportion of SARS-CoV-2 positive tests between the intervention and the control group will be compared using binary logistic regression using test center as fixed effect and a generalized estimating equation to adjust for clustering of data within the healthcare workers performing the sample. The difference in SARS-CoV-2 detection rate between healthcare workers will also be reported separately to estimate the inter-person variance. The Ct values from positive RT–PCR samples and the NRS discomfort scores will be compared using a general linear model with mixed effects (Ct) and GEE models (NRS discomfort).
The 95% confidence intervals (CI) will be presented. The level of statistical significance will be defined as $p < 0.05$.

Adjustment for covariates

The regression analyses will be adjusted for the effect of the test centers and the individual health-care worker performing the sample.

Methods used for assumptions to be checked for statistical methods

Assumptions for the logistic regression analysis included a binary outcome, independent observations and linearity in logit for continuous variables. To account for lack of independent observations, a generalized estimating equation approach will be applied. No continuous variables will be included.

Assumptions for the linear regression analysis included a normal distribution, independent observations, equal variation (homoscedasticity) and linearity in logit for continuous variables. Normally distributed outcomes and homoscedasticity will be evaluated visually by plots of the residuals. To account for lack of independent observations, a generalized estimating equation approach and mixed effect models will be applied. No continuous variables will be included.

Details of alternative methods to be used if distributional assumptions do not hold, e.g., normality, proportional hazards, etc.

If the assumption of a normal distribution of the outcome in the linear regression model is not fulfilled, a transformation of the outcome will be applied (e.g., logarithmic and rank transformations).

Planned subgroup analyses

We planned to do a sensitivity analysis using a lower cycle threshold (Ct) < 25 for positive SARS-CoV-2 definition to explore the consequences of a higher test specificity for the SARS-CoV-2 detection rate between specimen types. We also plan to estimate the sensitivity and specificity using Bayesian latent class analysis for accounting for an imperfect reference standard. Further, we planned to do subgroup analyses exploring the distribution of positive test results for participants stratified by symptoms, previous COVID-19 infection, vaccinations status, prior tonsillectomy and Mallampati score. To explore a potential bias from the distribution of the inconclusive test results, we excluded the inconclusive results in a subgroup analyses.

3.3 Missing data

Participants who will not adhere to the intervention definition (see SAP 1.2) will be reported as missing data and excluded from final analysis. Participants with missing data about their baseline characteristics from the questionnaire will still be included in the statistical analysis of primary outcome and secondary outcome. A table with baseline characteristics will be presented as raw data without the participants with missing data from the questionnaire.

3.4 Harms

Any adverse events during or after the collection of respiratory specimens for the trial will be noted and categorized into acute bleeding or foreign body in upper airway.

3.5 Statistical software

SAS statistical software suite ver. 9.4 (SAS Institute, North Carolina, U.S.)

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