Supplementary Appendix A
SAMPLE trial

Todsen T, *et al. Thorax* 2023; 78:1028–1034. doi: 10.1136/thorax-2022-219599

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A. National test recommendations and sample selection

We performed the SAMPLE trial at Valby and Taastrup COVID-19 test centers in the urban area of Copenhagen during the second lockdown period in Denmark. Individuals who arrived at the Valby COVID-19 test center from 12th January – 20th May and Taastrup COVID-19 test center from 8th March - 20th May were invited to participate in the SAMPLE trial. During this period, a mass testing strategy was pursued in Denmark with up to 34 SARS-CoV-2 test per thousand people in Denmark (see Figure 1A). (1) Every citizen could have a free COVID-19 test without prior medical evaluation during this period. Danish citizens were encouraged to take free biweekly COVID-19 tests and have documentation for negative tests to access restaurants, shops and educational institutions. In addition to the public test centers offering free RT–PCR tests oropharyngeal swab (OPS) specimens, rapid antigen tests of nasopharyngeal swab (NPS) specimens were also freely available at many test centers as part of the national mass testing strategy. (2) Due to the risk of false-positive rapid antigen tests, it was recommended to perform confirmatory RT–PCR immediately after a positive rapid antigen test. During the SAMPLE trial period, 942,723 tests were performed at Valby and Taastrup COVID-19 test centers in total, with 7,078 (0.78%) positive for SARS-CoV-2 from RT–PCR of OPS specimens (see Table 1A). Out of the 942,723 SARS-CoV-2 tests performed, 27,787 participants (or 2.9%) were included in the SAMPLE trial.

Table 1A. Total number of daily tests and positive rates from Valby and Taastrup COVID-19 test centers.

	Valby Testcenter			Taastrup Testcenter		
	Weekly tests, n	Positive, n	Positive, %	Weekly tests, n	Positive, n	Positive, %
	Start up Valby January 10th,2021					
Week 2	20058	343	1.71			
Week 3	23902	297	1.24			
Week 4	22774	204	0.90			
Week 5	25196	185	0.73			
Week 6	25428	152	0.60			
Week 7	29074	176	0.61			
Week 8	33258	209	0.63			
Week 9	34823	207	0.59	Start-up Taastrup March 8th, 2021		
Week 10	36969	189	0.51	27596	258	0.93
Week 11	29196	163	0.56	26361	205	0.78

Week 12	29410	209	0.71		24845	195	0.78
Week 13	36760	252	0.69		25165	186	0.74
Week 14	38081	264	0.69		33240	263	0.79
Week 15	41074	313	0.76		24968	198	0.79
Week 16	43882	218	0.50		27196	211	0.78
Week 17	42427	317	0.75		23847	172	0.72
Week 18	50343	410	0.81		26381	203	0.77
Week 19	48990	480	0.98		24520	238	0.97
Week 20	45422	439	0.97		21537	190	0.88
	Last Day May 20th, 2021						

Figure 1A. Daily SARS-CoV-2 tests per thousand people in Denmark (blue) compared to the United States (red) during the SAMPLE trial

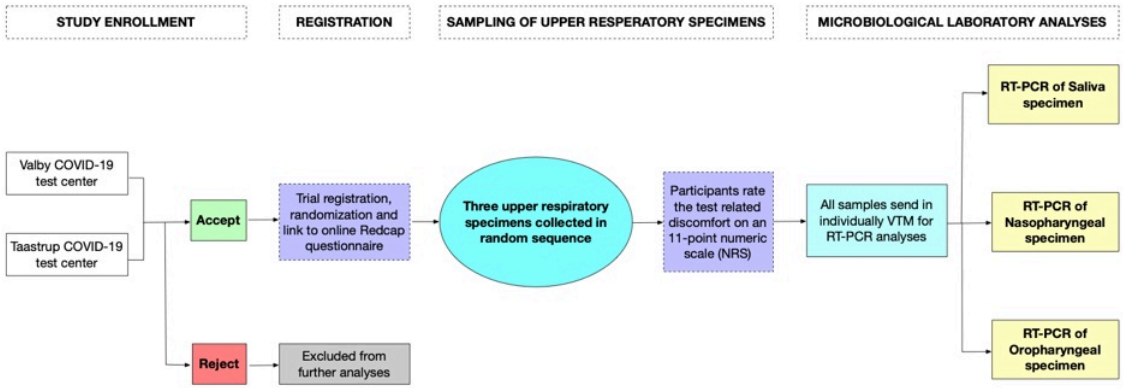
Chart and data from: Ritchie H, Mathieu E, Rodés-Guirao L, et al. Coronavirus pandemic (COVID-19). Our World in Data (https://ourworldindata.org/coronavirus).

B. Randomization and sample size calculations

Trial design

We conducted a randomized crossover prospective trial with participants enrolled from Valby and Taastrup COVID-19 test centers (see Figure 1B for the study flow chart).

Figure 1B



Randomization

The participants were randomized in a 1:1:1 ratio to three groups with different orders of saliva, OPS, and NPS samples (A: NPS→OPS→Saliva, B: OPS→Saliva→NPS, C: Saliva→NPS→OPS); see Figure 2B. A randomization list was made with block sizes of 60 participants for each test center before initiating the study. The randomization list was incorporated in the REDCap Randomization Module so that participants were randomized during enrollment and registration at the test centers.

Figure 2B

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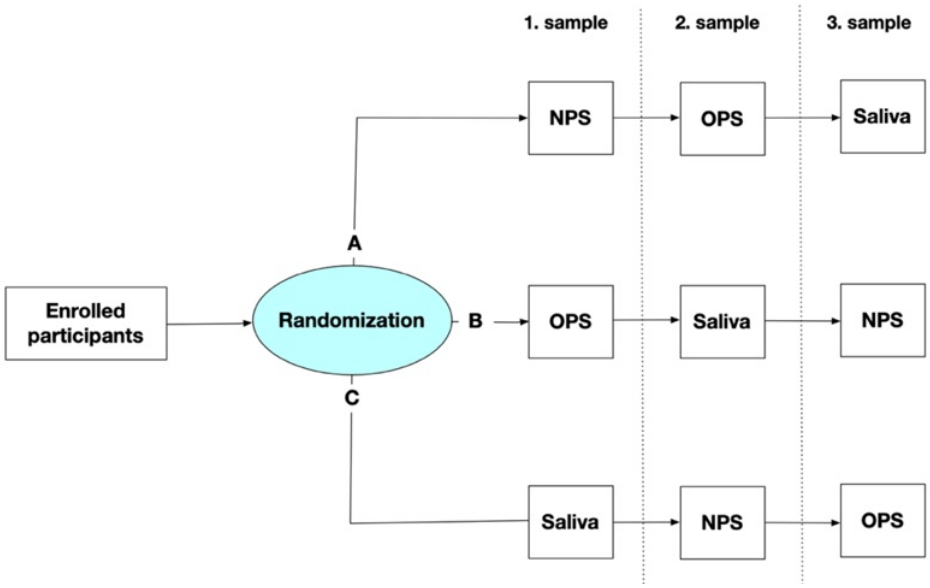

Sample size

According to our power analysis (with a power of 80% and a significance level of 0.05), a minimum of 273 SARS-CoV-2-positive OPS specimens were included in the trial. The power analysis was based on an expected 25.6% difference in detection rate between OPS and NPS specimens. (3) Anticipating that approximately 5% of the included participants will be excluded from final analyses due to missing data, we increased the minimum number of SARS-CoV-2-positive OPS specimens included in the study to 287 participants. As we expect the rate of test positivity to be between 0.5-4% during the inclusion period, the total number of included participants would be between 6,700-55,200.

Framework

Our hypothesis in the trial protocol is that the NPS specimens would have a 25.6% better detection rate than the OPS for SARS-CoV-2 detection during mass testing. (3) This

assumption was used for the power calculation for the superiority hypothesis testing framework.

Statistical interim analysis and stopping guidance

We predefined to stop the study when 287 SARS-CoV-2 OPS-positive participants were included in the study. The number of OPS-positive participants included in the study was monitored on a weekly basis by Nikolai Kirkby, and the study ended when the minimum number of positive participants was reached. Only the number of positive OPS test results were counted and no interim analyses were performed during the study period. No adjustment of the significance level or analyses to stop the trial early were planned.

Changes in Protocol During Study

Originally, the RT–qPCR testing was defined as inconclusive if the cycle threshold (Ct) was > 34 for the N-gene segment targets and an RNase P Ct > 23. However, due to different distribution values of RNase P Ct between sample methods, OPS specimens' cutoff was changed to RNase P Ct > 27.4, and the cutoff for saliva specimens was changed to RNase P Ct > 28.4 (see section G for Microbiology laboratory testing procedures). No change was made for the pre-defined definition of SARS-CoV-2 OPS-positive test results.

In the SAMPLE questionnaire, the participants were asked about any previous positive tests before the study enrollment. However, they were not required to define whether they had a positive rapid antigen test or RT–qPCR test. To validate the information about previous positive SARS-CoV-2 infections, all the participants with a positive test were also search for after the trial ended in The Danish Microbiology Database (MiBa) for previous test results (see section D - SAMPLE questionnaire and prior positive tests).

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C. Quality control

Quality control at the test centers

An SOP (standard operating procedure) was provided for the local test centers to ensure that the trial was conducted and data were generated in compliance with the research protocol. The SOP included detailed descriptions of all the work processes at the test centers and was updated throughout the study to correct for missing descriptions and changes in the work processes.

An audit was conducted on a weekly basis by a research staff member who supervised the overall conduct of the trial and monitored the data documentation at the COVID-19 test centers. The audit was followed up by a weekly meeting with the responsible research staff at the COVID-19 test centers and Primary Investigators. At these meetings, a follow-up on the quality data was presented (e.g., rate of missing questionaries and registration of participants/test), and possible problems were addressed.

To ensure correct and standardized collection of upper respiratory specimens by the staff at the test centers, all staff involved in the SAMPLE trial completed a competence-based training session (see full description of this training in Appendix 2). Furthermore, frequent audits of the collection of upper respiratory specimens (NPS, OPS and saliva collection) were conducted by both internal and external health care professionals (experience nurses and otolaryngology residents). Direct formative assessment during the collection of upper respiratory specimens from participants was performed with the SAMPLE checklist.

Quality assurance and control were also addressed after the collection of data was completed, as data cleaning was conducted on the datasets to ensure that the data were correct, consistent and usable. The data cleaning included standardizing the datasets, identifying duplicate records, fixing spelling and formatting errors and correcting mistakes such as missing data. Another data cleaning was conducted after the merging of the datasets to ensure a high quality of the merged and final dataset. Furthermore, data verification was performed for all the participants with one or more positive SARS-CoV-2 test results. The data verification included a check of the consistency between the data in

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the merged dataset and the original datasets, and it was found that all data matched between the datasets.

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D. SAMPLE questionnaire and prior positive tests

An online link (or QR code with link) to a questionnaire was provided to the participants after enrollment in the SAMPLE study at either the Valby or Taastrup Test center. The participants were requested to answer the online questionnaire before they had the saliva, OPS and NPS specimens collected. They used their mobile phones to fill in their unique CPR number and answered the questions from the *pretest questionary* (see Figure 1D). If they did not have a phone, they were assisted by an employee at the test center to fill out the Redcap questions on a computer at the test site. After the participants had completed all the specimen collections for the SAMPLE trial, they filled in a *posttest questionary* (see Figure 2D) in paper form with a pen and put in a collection box on the way out of the room. The *posttest questionary* also included an 11-point numeric scale (NRS) ranging from 0 (no discomfort) to 10 (worst possible discomfort) for each sample type. (4) As we experienced some participants who did not fill in the online *pretest questionary,* we also added questions regarding the intake of food and beverages to the *posttest questionary* during the initial phase of the study. The results regarding food and beverage intake of the participants primarily came from the *posttest questionary,* and if missing here, it was filled with answers to the pretest questionary.

To validate the information about previous positive SARS-CoV-2 infections prior to the SAMPLE trial, all the participants with a positive test were also found in The Danish Microbiology Database (MiBa). (5) Information on test bookings and results from all individuals in Denmark who had a prior PCR or antigen test for SARS-CoV-2 was captured in MiBa as part of the national surveillance system. (6) Here, a search for the date of a previous positive SARS-CoV-2 test from RT–PCR or antigen testing results was performed to define whether the current positive results were due to new, current or reinfection with SARS-CoV-2. As some of the participants arrived for confirmatory RT–PCR immediately after they received positive antigen testing as part of the national screening, we defined these tested positive the same day or day before as a new infection. Participants with a positive test 2-60 days before a positive SARS-CoV-2 test in the SAMPLE trial were defined as having a positive test late during a current SARS-CoV-2 infection. If participants had a positive test > 60 days ago, we defined it as a reinfection in accordance with the definition by the

European Centre for Disease Prevention and Control. (7) Detailed information on prior positive test results in MiBa from all participants with positive test results in the SAMPLE trial is shown in Table S7.

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Figure 1D: Pretest questionary (Redcap online link)

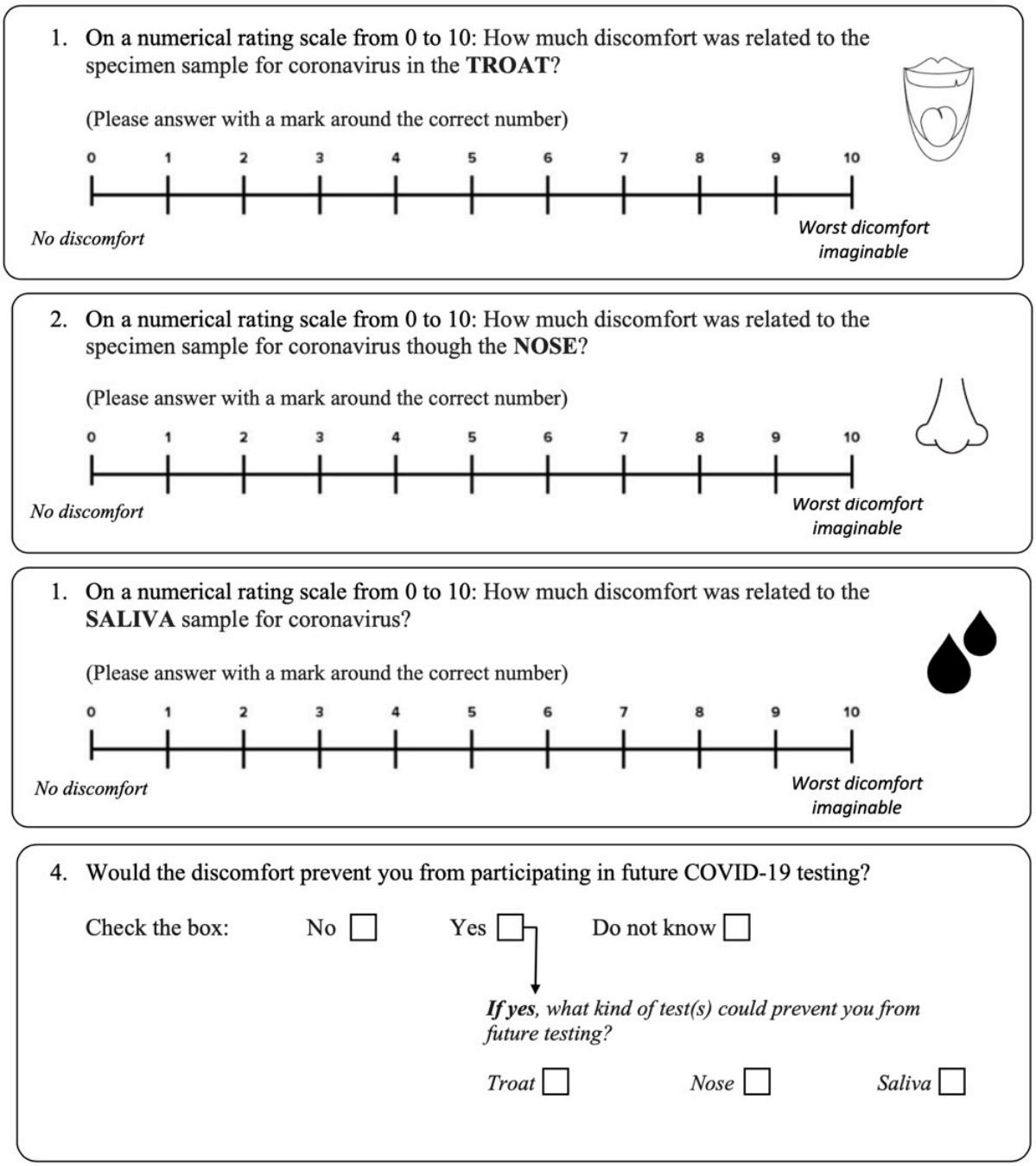
General data	
CPR number	_____ (DDMMYY-XXXX)
Today´s date	_____
Where are you about to be/just been tested?	○ Valby ○ Tåstrup
Questionnaire regarding COVID-19	
Have you previously been tested for COVID-19?	○ Yes ○ No
Have you previously been tested in the nose or mouth?	○ Mouth ○ Nose ○ Both
Have you previously tested positive for COVID-19?	○ Yes ○ No
Date of positive COVID test	_____
Have you been vaccinated against COVID-19?	○ Yes ○ No
Date of the first vaccination	_____
Have you received your second vaccine?	○ Yes ○ No
Date of the second vaccination	_____
Are you in quarantine until a negative COVID-19 response?	○ Yes ○ No
Why have you booked an appointment for a COVID-19 test?	○ I have COVID-19-like symptoms (e.g., fever, general tenderness, sore throat, cough, fatigue, diarrhea, headache, impaired sense of taste or smell, skin rash, eye cataracts, shortness of breath) ○ I have to be treated at a hospital or another facility and take a test

	beforehand (e.g., planned surgery, dentist). o I have been in contact with an infected person o My profession (also covers business travel) o I need to visit a vulnerable person o I have been or are going abroad (not business travel). o I wish for a test before an event that I have to attend (e.g., wedding, sporting event, etc.). o I have previously been sick with a corona-like disease. o I have a suspicion that I may have been infected with COVID-19 (e.g., by my participation in a major event, concert, sporting event). o Other/I participate in a population survey o I follow the recommendations of regular testing.
How long have you had symptoms?	o One day o Two days o Three days o Four days o Five days o Six days or more
What symptoms do you have? (Select one or more)	o Fever o General tenderness o Sore throat o Cough o Fatigue o Diarrhea o Headache o Impaired sense of taste or smell o Skin rash o Eye cataracts o Shortness of breath
What relationship do you have/had to the infected person? (Select one or more)	o I have been warned via the Smittestop app.

	○ I have been contacted by Coronaopsporing/Corona-hotline with the message that I have been in close contact with an infected person. ○ Someone from my household. ○ A close relative (nonhousehold member). ○ A good friend/acquaintance. ○ Someone from my work (e.g., a colleague or customer). ○ An event or major event. ○ Other
When were you sick?	○ < 7 days ago ○ Between 7–14 days ago ○ Between 2 and 4 weeks ago ○ More than 4 weeks ago ○ Unknown
Which profession do you have?	○ I work in the municipal health, social and elderly sector. ○ I work in the regional health sector (hospital system). ○ I work in the private health and elderly sector. ○ I work with children and adolescents. ○ I work at an institution (e.g., residence, prison, etc.). ○ I am in touch with many people (e.g., the transport sector, sales, restaurant industry). ○ I have to make a business trip. ○ I returned from a business trip and thus want a test. ○ Other
Comment	_____
Who are you going to visit?	○ I need to visit an elderly person in a nursing home. ○ I have to visit an elderly person in their own home. ○ I have to visit a person at the hospital. ○ I need to visit a vulnerable nonhospitalized person in their own home.

	○ I have to visit a vulnerable person at an institution or similar. ○ I have to visit a person at an institution or similar. ○ Other
Why have you been or are going abroad?	○ I am going on holiday and want a test before leaving. ○ I have returned from vacation and want a test in connection with the homecoming. ○ I have to visit or have visited family abroad. ○ I have to travel abroad for reasons other than vacation. ○ Other
Do you have problems with dry mouth on a daily basis?	○ Yes ○ No ○ Unknown
Comment	_____
Have you eaten within the last 30 minutes?	○ Yes ○ No
Have you been drinking within the last 30 minutes?	○ Yes ○ No
Further comments	_____

Figure 2D: Posttest questionnaire about test-related discomfort using the Numerical Rating Scale (NRS)



E. Training curriculum for health care workers involved in SAMPLE trial

All health care workers selected to collect upper respiratory specimens for the SAMPLE trial received additional competency-based training in NPS and OPS specimen collection. The technique to collect upper respiratory specimens followed the recommendations of the World Health Organization (WHO) to ensure a correct and standardized sample method (see details in the "*Guidelines for collecting and handling the upper respiratory specimens*" section). (8) Furthermore, they also received training on how to instruct the participants in collection of saliva by the drooling technique. (9) We devolved teaching material for the SAMPLE trial, including instructional videos and visual guides about how to perform NPS, OPS and saliva specimen collection (see www.urt-sample.com). The training was composed of a didactic teaching session about the upper-airway anatomy and video demonstration of the NPS and OPS collection techniques in real life. Afterward, the health care workers received hands-on training with OPS sampling practiced on a life-sized airway mannequin (Airsim Advance Crico, Trucorp, Belfast, N. Ireland), and the NPS practiced on a free 3D-printed nose model (10) we modified for the teaching session (see Figure 1E). (11)

Figure 1E: Example of the 3D-printed nose model and the mannequin used for upper respiratory specimen collection training

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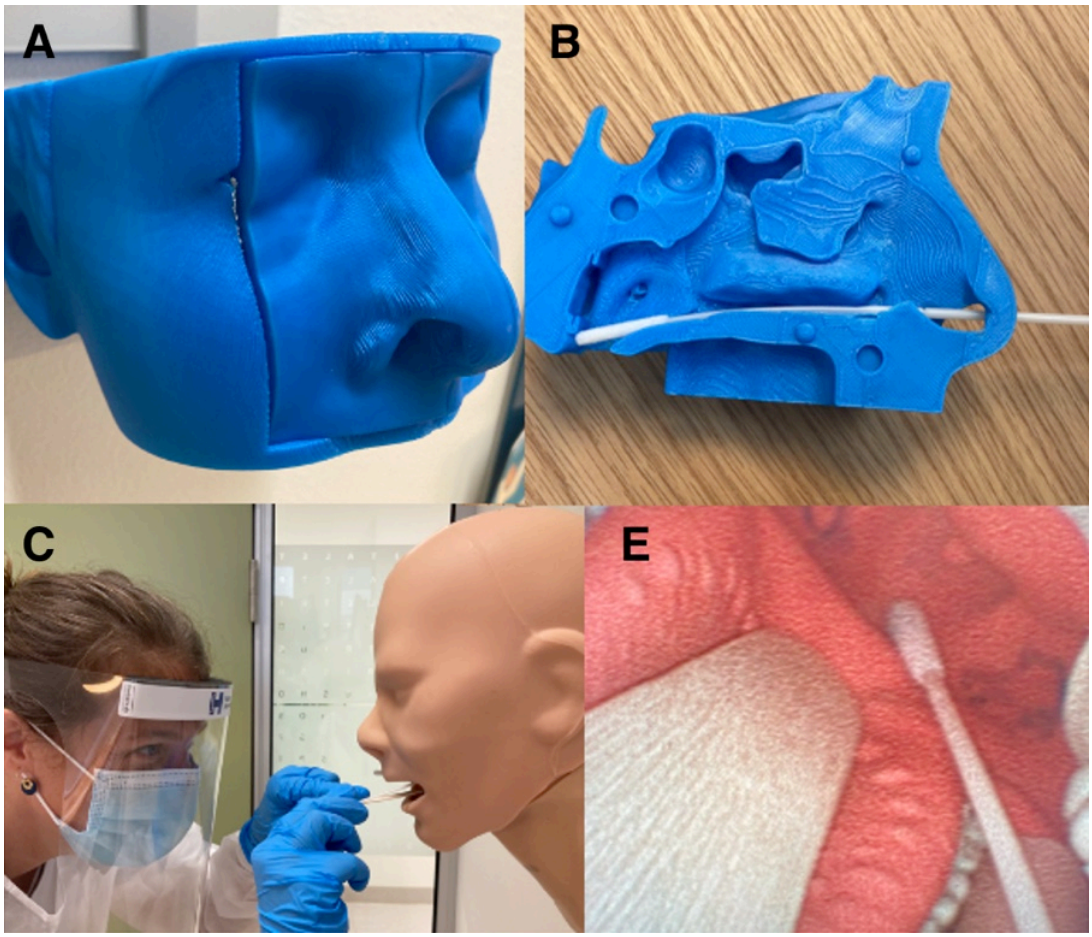

A and B: 3D-printed nose model used for NPS specimen collection training.

C and D: Airway mannequin used for OPS specimen collection training.

Performance-based skills assessment was performed with procedure-specific checklists (see Figure 1E-3E), and theoretical knowledge was assessed with multiple-choice questions (see Figure 4E) validated in a previous study. (11) The teaching session was approximately one hour and was provided by an otorhinolaryngologist or an experienced nurse who completed a train-the-trainer. After the health care workers completed the simulation-based training, they were assessed during the performance of OPS and NPS specimen collection of participants with procedure-specific checklists. Only selected staff who completed the competency-based training were allowed to be involved in specimen collection of the participants enrolled in the SAMPLE study.

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Figure 1E. Checklist to assess the performance of nasopharyngeal swab specimen collection

	NAME _____	Correctly performed	Not correctly performed	*Not relevant*
1	The swab is performed with the correct use of personal protective equipment.	☐	☐	☐
2	The swab is performed in compliance with infection hygiene	☐	☐	☐
3	The patient is instructed to lean the head slightly back throughout the procedure.	☐	☐	☐
4	The swab tip is inserted in the nasal cavity in an upgoing direction (depending on how much the head is leaned back).	☐	☐	☐
5	The swab is inserted correctly in the midline and angled downwards for a direction following the floor of the nose.	☐	☐	☐
6	The swab is inserted until resistance is met by the posterior wall of the nasopharynx.	☐	☐	☐
7	The swab should sit in the nasopharynx in a couple of seconds and be rotated three times around.	☐	☐	☐
8	The swab is withdrawn slowly while being rotated.	☐	☐	☐
9	The swab is inserted into the vial following applicable guidelines.	☐	☐	☐
	Number correct	____		

	Bad	Unacceptable	Acceptable	Good	Excellent
GENERAL ASSESMENT	☐	☐	☐	☐	☐

Figure 2E. Checklist to assess the performance of oropharyngeal swab specimen collection

	NAME _____	Correctly performed	Not correctly performed	*Not relevant*
1	The swab is performed with the correct use of personal protective equipment.	☐	☐	☐
2	The swab is performed in compliance with infection hygiene.	☐	☐	☐
3	The swab is performed in alignment with the height of the patient in order to get a good visualization of the oropharyngeal wall.	☐	☐	☐
4	The swab is held correctly between the thumb and the first and second finger.	☐	☐	☐
5	The patient is instructed in saying "ahhh" so the soft palate will rise and improve the visualization. If relevant, a tongue depressor is used.	☐	☐	☐
6	The swab is inserted and withdrawn without touching the tongue or the cheeks.	☐	☐	☐
7	The swab collects sufficient material from the oropharyngeal wall and both tonsils with a rotating or in a painting movement (Alternatively, just swab the posterior oropharyngeal wall if a bad cough reflex is stimulated).	☐	☐	☐
8	The swab is inserted into the vial following applicable guidelines.	☐	☐	☐
	Number correct	____		

	Bad	Unacceptable	Acceptable	Good	Excellent
GENERAL ASSESSMENT	☐	☐	☐	☐	☐

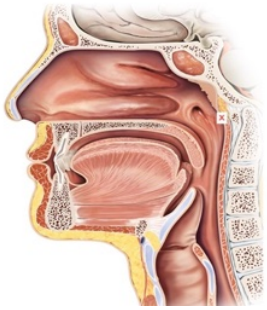
Figure 3E. Checklist to assess the performance of collection of saliva specimens

	NAME _____	Correctly performed	Not correctly performed	*Not relevant*
1	The saliva collection is performed in compliance with infection hygiene.	☐	☐	☐
2	Chewing gum is handed out in order to stimulate saliva production (if relevant).	☐	☐	☐
3	The patient is instructed correctly to lean the head slightly forward and let the saliva collect in the front of the mouth without swallowing.	☐	☐	☐
4	The patient is instructed to collect saliva and not try to hack up secretions while collecting the saliva.	☐	☐	☐
5	The saliva collection is finished by collecting 2 ml saliva except for foam.	☐	☐	☐
6	1 ml saliva is pipetted safely from the collection tube to the vial.	☐	☐	☐
	Number correct answers	____		

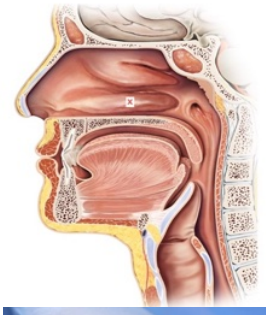
	Bad	Unacceptable	Acceptable	Good	Excellent
GENERAL ASSESSMENT	☐	☐	☐	☐	☐

Figure 4E. MCQ to assess general knowledge about the collection of nasopharyngeal swab, oropharyngeal swab and saliva specimens

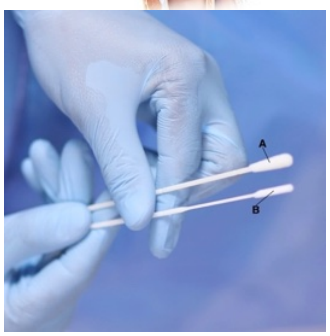
NASOPHARYNGEAL SWAB



1. **What anatomical structure is seen by the cross-mark on the picture**
   - **a.** The posterior oropharyngeal wall
   - **b.** **The posterior nasopharyngeal wall (x)**
   - c. The inferior turbinate



2. **What anatomical structure is seen by the cross-mark on the picture**
   - **a.** The posterior oropharyngeal wall
   - **b.** The posterior nasopharyngeal wall
   - **c.** **The inferior turbinate (x)**



3. **What swab is used for the nasopharynx**
   - **a.** The thick and rigid one
   - **b.** **The flexible and fine shafted one (x)**
   - **c.** Both of the above can be used

4. **When the tip of the swab has entered the nasal cavity, the direction of the tip changes, so it is pointing**
   - **a.** **Down toward the earlobe (x)**
   - **b.** Up toward the scalp
   - **c.** To the side toward the ear

5. **How far is the swab inserted**
   - **a.** 6-9 cm
   - **b.** 9-12 cm
   - **c.** **Until resistance is met by the posterior nasopharyngeal wall (x)**

6. **When the swab has met the posterior nasopharyngeal wall do the following**
   - **a.** **Let the swab sit for a couple of seconds and rotate it three times around (x)**
   - **b.** Let the swab sit for 10 seconds
   - **c.** Rotate the swab around while counting to 10

7. **The swab is subsequently withdrawn from the nose**

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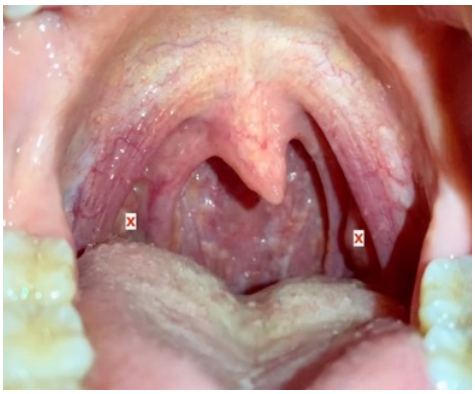


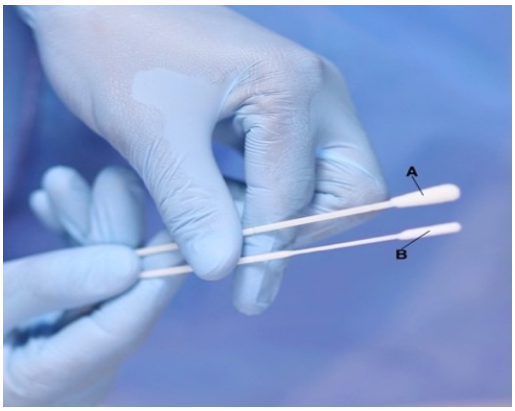
a. In a fast movement
b. Slowly with a painting-like-movement
c. **Slowly with a rotating motion (x)**

8. **What is a frequent error that leads to resistance or pain while inserting the swab**
   a. **The swab is directed upward (x)**
   b. The swab is directed downward
   c. The swab is held between the first and the second finger

9. **What do you do if there is resistance or pain before the swab is inserted into the nasopharynx?**
   a. **Withdraw the swab and change the direction of the tip of the swab more downward before it is carefully inserted again (x)**
   b. Push through until the swap has passed the resistance
   c. Withdraw the swab slightly and change the direction of the swab while aiming higher up into the nose

10. **What do you do if there is still resistance after the second attempt in the same nostril?**
    a. The swab is sent as it is
    b. The swab is thrown away, and the specimen is discarded
    c. **A new attempt is made in the opposite nostril instead (x)**

OROPHARYNGEAL SWAB



11. **What swab is used for the oropharynx**

    a. **The thick and rigid one (x)**
    b. The flexible and fine shafted one
    c. Both of the above can be used

12. **What anatomical structure is seen by the cross-marks on the pictures**

    a. The posterior wall of the oropharynx
    b. **The tonsils (x)**
    c. The anterior pillars of the fauces

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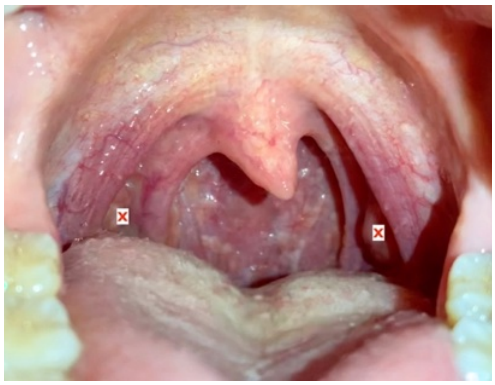
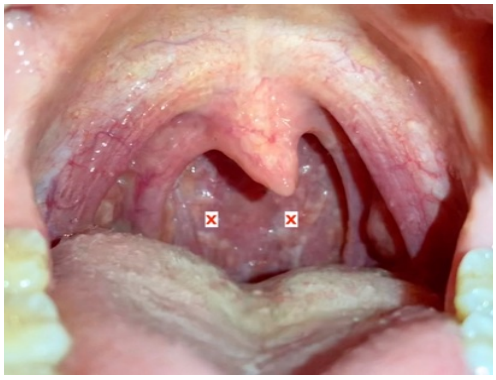
13. **What anatomical structure is seen by the cross-marks on the pictures**



    a. The posterior wall of the oropharynx
    b. **The tonsils (x)**
    c. The anterior pillars of the fauces

14. **What anatomical structure is seen by the cross-mark on the picture**



    a. **The posterior wall of the oropharynx (x)**
    b. The tonsils
    c. The anterior pillars of the fauces

15. **If the visualization of the posterior wall of the oropharynx is obstructed by the tongue**

    a. Swab the tongue
    b. Push the tongue down with the swab to be able to swab the oropharynx
    c. **Use a tongue depressor to provide a better view (x)**

SALIVA COLLECTION

1. **Explain to the patient that during the saliva sampling they should be**
    a. Leaning the head back and letting the saliva be collected in the front in the mouth without swallowing
    b. **Leaning the head slightly forward and letting the saliva be collected in front of the mouth without swallowing (x)**
    c. They should hack up secretions to collect material for the specimen.

2. **During saliva collection, it is important that the patient**
    a. **Is relaxed and takes time to collect the saliva, since stress can induce dry mouth (x)**
    b. Massages the cheeks during the sample collection
    c. Rinses the mouth with water

3. **When is the sample collection with saliva finished**
    a. When 4 ml of saliva is collected except the foam
    b. When there is 2 ml of saliva collected inclusive the foam
    c. **When there is 2 ml of saliva collected except the foam (x)**

F. Guidelines for collecting and handling upper respiratory specimens

Upper respiratory specimens were only collected by selected and specially trained health care workers at the COVID-19 test centers, who followed the SAMPLE Standard Operating Procedure (SOP) and completed a competency-based training program; see further information "*SAMPLE SOP*" and "*Training curriculum for health-care workers involved in SAMPLE specimen collection*". OPS and NPS were performed according to the guidelines (see Figures 1F and 2F) with nylon-flocked swabs (Wuxi NEST Biotechnology Co., Jiangsu, China) and transported in tubes with 2 mL inactivation transport medium (ITM) manufactured by Wuxi NEST Biotechnology Co., Jiangsu, China.

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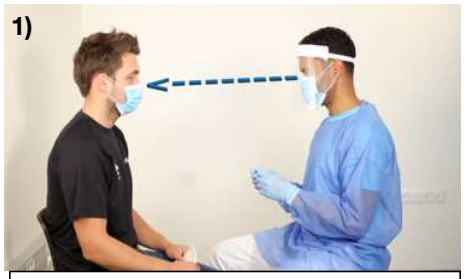
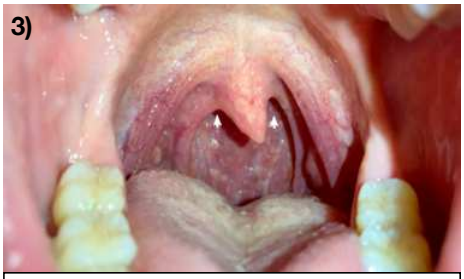
Figure 1F. Clinical guidelines for collection of oropharyngeal swab specimens

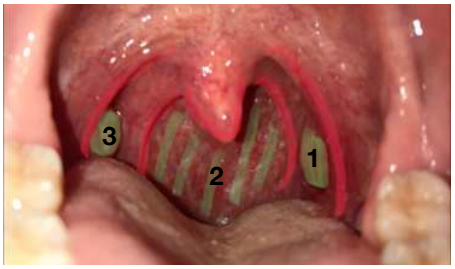


Ensure you are in the same level as the patient in order to have a good view to the oropharynx. Ask the patient to take off the mask during the procedure.

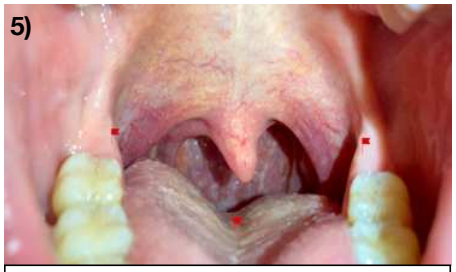
Hold the swab between the thumb and the first and second finger like a pencil.



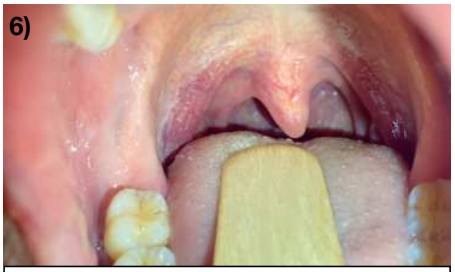
Ask the patient to open the mouth and say "aaah", so the soft palate will rise.



Insert the swab and swipe with rotating movements over one tonsil (1), the posterior wall of the oropharynx (2), and finish by swiping the swab over the other tonsil (3).

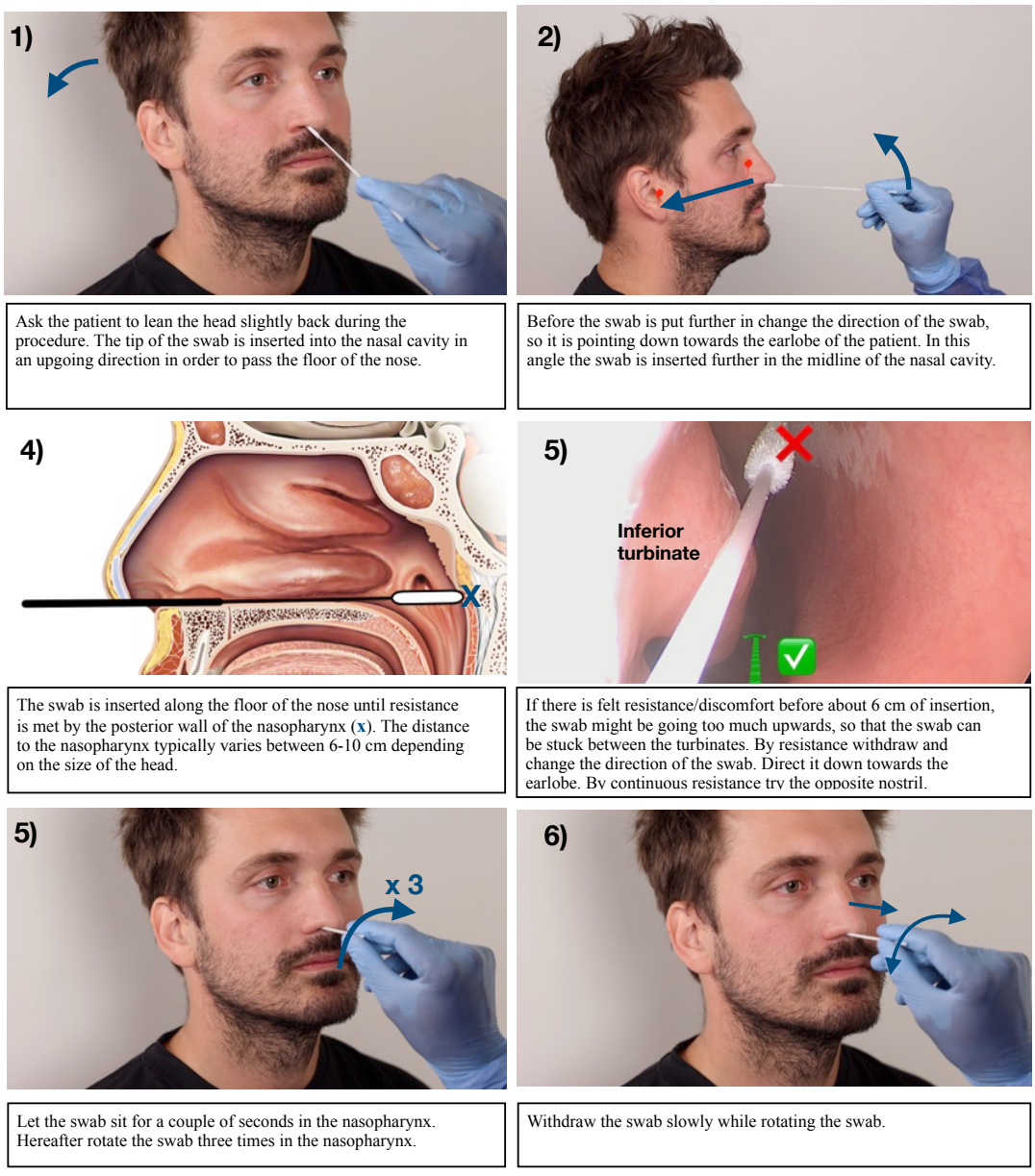


Withdraw the swab without touching the cheeks, the teeths, or the gums.



If the visualization of the posterior wall of the oropharynx is obstructed by the tongue, use a tongue depressor to get a better view.

Figure 2F. Clinical guidelines for collection of nasopharyngeal swab specimens



Saliva was collected with the drooling technique (6), and the participants were instructed by the health care workers to lean their head slightly forward and to passively pool saliva in the mouth (see Figure 3F). When a pool of saliva accumulated in the mouth of the participants, they were instructed to gently spit the saliva in a 50 ml collection tube without coughing or clearing their throat during the procedure. If they had problems with producing at least 2 mL saliva, they were offered a neutral paraffin gum (MORSA GmbH, Krumbach, Germany) to stimulate saliva production (see Figure 4F).

Figure 4F. Example of neutral paraffin gum used in the study

After completing the sample, 1 mL of the saliva was pipetted into an identical ITM tube as the swabs (Wuxi NEST Biotechnology Co., Jiangsu, China) by a health care worker.
The ITM tube with the respiratory specimens was stored at a refrigerator at the test center until the end of the day where they were transported to the laboratory at Technical University of Denmark, Lyngby, Denmark, for SARS-CoV-2 RT–PCR testing.

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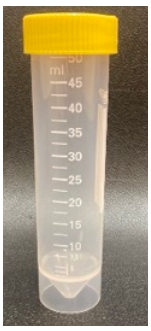
Figure 3F. Clinical guidelines for collection of saliva specimens with the drooling technique

1)



Hand out a saliva collection tube for and a small piece of neutral chewing gum (only if needed) to help stimulation of saliva.

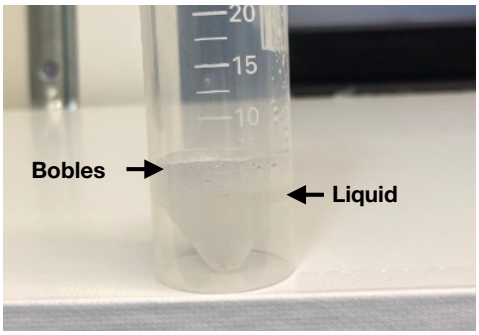
2)

Explain to the citizen that they should lean their head slightly forward and let the saliva passive collect in their mouth without swallowing.

3)

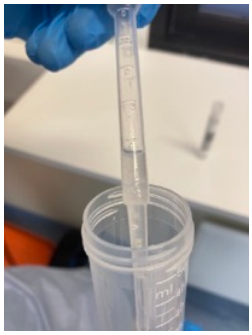
Tell the participant to relax and let the saliva collect in front of the mouth. When a pool has been collected, it should be spitting into the collection tube. The participant should avoid coughing or clearing the throat during sampling.

4)



Repeat the process until about 2ml of saliva is collected (filled with liquid for the first line on the tube. If needed, chewing gum can be handed out to stimulate saliva production.

5)



Use a pipette to suck up 1 ml of saliva from the collection tube.

6)

Transfer the saliva from the pipette into the tube with virus transport medium for saliva collection (marked with number-S).

G. Microbiology laboratory testing procedures

All samples were stored in tubes with 2 mL of inactivation transport medium (Wuxi NEST Biotechnology Co., Jiangsu, China) containing buffered chaotropic salt solution (3 molar guanidine iso-thiocyanate). The tubes with Saliva specimens had the final concentration of guanidine iso-thiocyanate reduced to 2 molar as the 2 mL of inactivation transport medium was diluted by 1 mL of saliva. The tubes were transferred daily to the Danish Technology University Lyngby (DTU), Denmark, for RT–PCR testing. The SARS-CoV-2 RNA was detected based on the CoviDetect – COVID-19 Multiplex RT-qPCR assay from PentaBase (PentaBase APS, Odense, Denmark). The PentaBase assay is a modified version of the Centers for Disease Control (CDC) 2019-nCoV RT-PCR diagnostic panel. (12) The RNA was extracted from 200 µL of the sample material using the para-magnetic particle-based RNAadvance Viral XP kit (Beckman Coulter, Indianapolis, NV, USA) processed on the Biomek i7 platform (Beckman Coulter, Indianapolis, NV, USA). RNA was eluted in a volume of 30 µL, of which 5 µL was transferred to the PCR-master mix. The master mix was prepared by combining 10 µL 2x Mastermix One Step PrimeScript III, RT-qPCR mix (cat.no. RR600, TaKaRa Bio Europe AB, Sweden), and 5 µL 4x primer/probe mix. RT-PCR was performed to detect two Nucleocapsid protein gene (N-gene) targets of SARS-CoV-2 and one human RNase P ribozyme (RNase P) gene target (See Table 1G). (13) The RNase P was used to confirm the presence of human DNA in the sample and assessed if the PCR amplification was adequately performed (the amplification of RNase P-gene segment indicated removal of PCR inhibiting substances). Gene amplification was performed on a Rotor-Gene Q PCR system (QIAGEN, Düsseldorf, Germany) using the thermal profile: reverse transcription at 52° C for 5 minutes, initial polymerase activation at 95° C for 10 seconds, 7 cycles of denaturation at 95° C for 5

seconds with annealing/elongation at 66° C for 30 seconds, and 38 cycles of denaturation at 95 ° C for 5 seconds and 60° C for 30 seconds.

Table 1G. Sequences of primers/probes used

Primer/probe	Sequence (5'-3')
N1 Forward primer	GACCCCAAAATCAGCGAAAT
N1 Reverse primer	CGCAGTATTATTGGGTAAACC
N1 Probe (5'-FAM/3'-Unknown)	ACCCCGCATTACGTTTGGTGGACC
N2 Forward primer	AGGAACTGATTACAAACATTGGC
N2 Reverse primer	TGTAGGTCAACCACGTTCCC
N2 Probe (5'-HEX/3'-Unknown)	TGCACAATTTGCCCCAGCG

RT–qPCR testing was defined as positive for SARS-CoV-2 RNA if the cycle threshold (Ct) was < 34 for at least one N-gene segment target (see Table 2G). To ensure the specimen samples contained representative human cellular material, the amplification of RNase P was measured. The NPS specimen was used as the reference specimen, and the test was categorized as inconclusive with RNase P Ct > 23 (see Figure 1 for a histogram of distribution values). The consequence was that 0.28% of the NPS samples was defined as inconclusive (see Figure 1). Using the same principles, a cutoff value of RNase P Ct > 27.4 was set for OPS specimens, and RNase P Ct > 28.4 was set for saliva specimens (see Figures 2G and 3G).

Table 2G. Interpretation of laboratory results used in all three upper respiratory specimens

2019 nCoV_N1	2019 nCoV_N2	RNase P	Result Interpretation	Diagnose
+	+	±	SARS-CoV-2 detected	Positive
+	-	±	SARS-CoV-2 detected	Positive
-	+	±	SARS-CoV-2 detected	Positive
-	-	+	SARS-CoV-2 not detected	Negative
-	-	-	Invalid result	Inconclusive

Figure 1G. Histogram of the Ct values of human genetic material from nasopharyngeal swab specimens

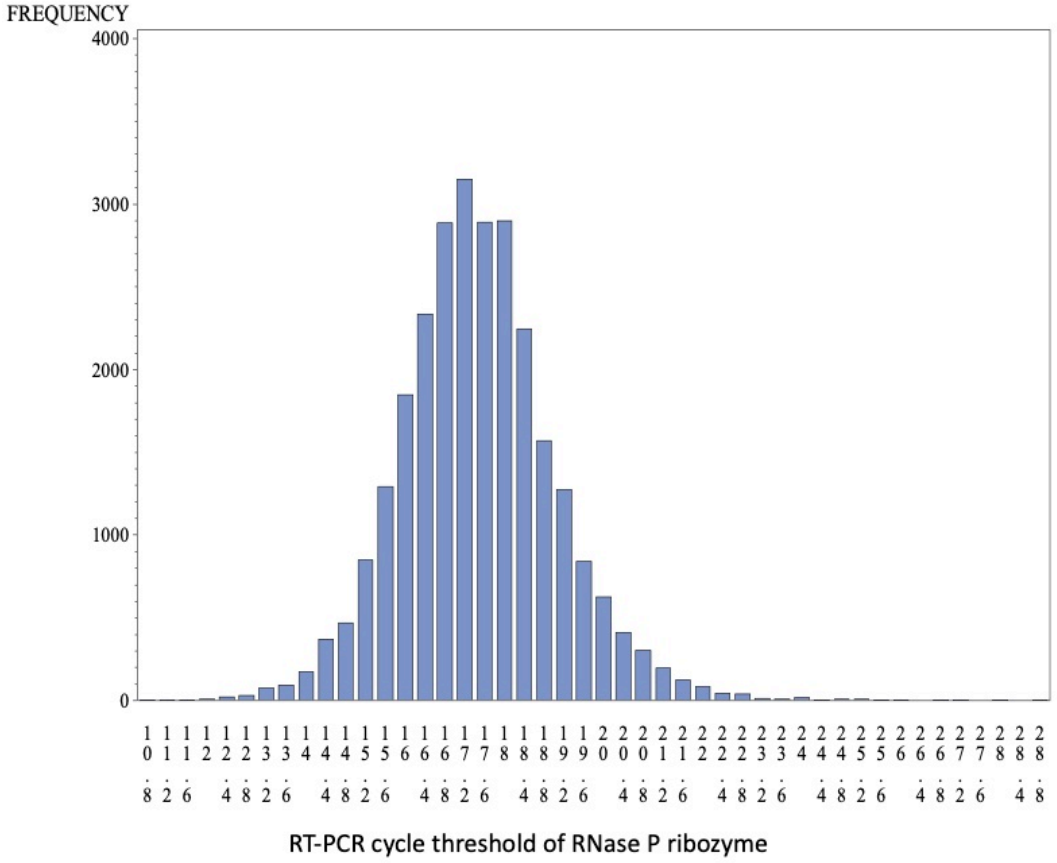


Figure 2G. Histogram of the Ct values of human genetic material from oropharyngeal swab specimens

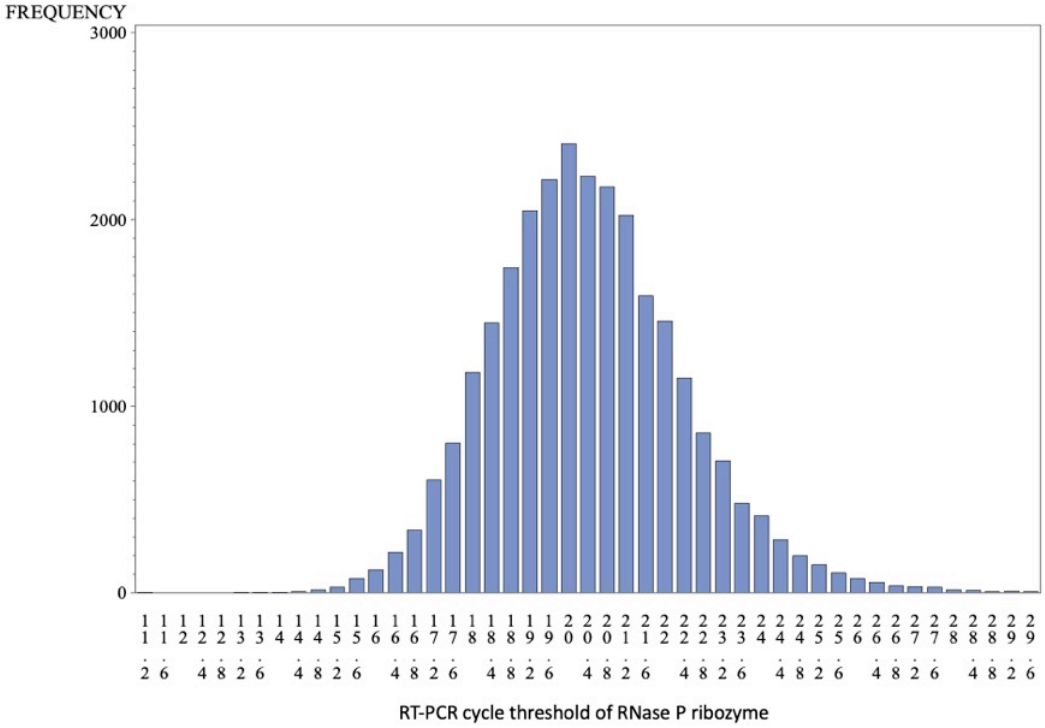
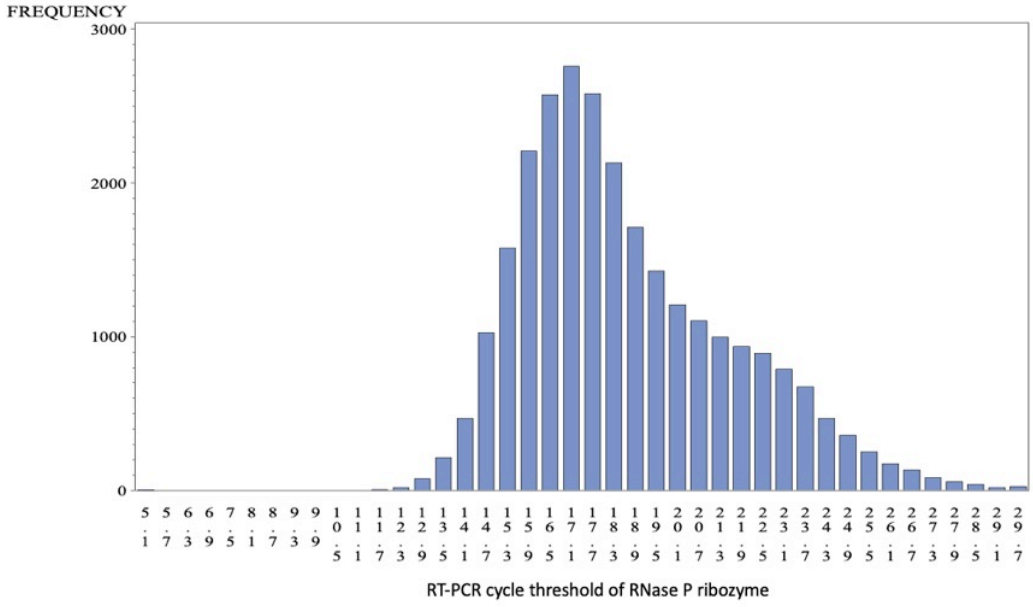


Figure 3G. Histogram of the Ct values of human genetic material from saliva specimens



Mutation analyses were performed for all specimens (NPS, OPS or Saliva) with detected SARS-CoV-2 RNA. The virus variant of concern was classified using the WHO nomenclature

(Table 3G). (14) Sanger sequencing of a Spike-gene segment was used to identify signature mutations of actively circulating variants seen during the study period in Denmark. Total nucleic acids were extracted, and the gene segment was amplified by RT-PCR. The remaining PCR reagents and small DNA fragments were removed to yield a highly pure amplicon. The amplicons were mixed with sequencing primer and shipped by currier transport to Eurofins Genomics (Eurofins, Luxembourg, Luxembourg) for Sanger sequencing. (15). Data from the sample material (NPS, OPS or Saliva) yielding the best quality of sequence data was used for the identification of mutations.

In the case of low-quality sequencing results, aberrant or inconsistent mutations patterns within sample sets (OPS, NPS or Saliva), the virus variant was investigated by Whole Genome Sequencing (WGS). WGS was performed on the Oxford Nanopore Technologies MinION or Mk1C devices (Oxford Nanopore Technologies, United Kingdom) using the R9.4.1 512-pore flow cell. The Artic Network 1200 bp. Amplicon protocol https://artic.network/ was used for Tiled PCR covering the full length of the SARS-CoV-2 genome and for library preparation. The assembled sequence output was analyzed with respect to Pango liange (https://www.pango.network/) and allocated to the best fitting WHO variant of interest. Samples were classified as "Unknown" if insufficient sequence data for variant classification was found from both Sanger sequencing and WGS.

Table 3G. SARS-CoV-2 Variants of concern (VOC) analyzed in the SAMPLE trial

WHO classification	**Contry of first identification**	**Mutation gene**								
		69_70del	L452R	T478K	E484K	E484Q	N501Y	Q677H	P681R	P681H
Alpha	England	+	-	-	-	-	+	-	-	+
Alpha		+	-	-	+	-	+	-	-	+
Beta (B.1.351) Gamma (P1)	South Africa (B.1.351) Brazil (P1) Colombia (B.1.621)	-	-	-	+	-	+	-	-	+/-
Eta	Several countries	+	-	-	+	-	-	+	-	-
Kappa (B.1.617.1) Delta (B.1.617.2) Epsilon (B.1..427/B.1.429)*	India (B.1.617) USA (B.1.427/B.1.429)	-	+	+/-	-	+/-	-	-	+/-	-

* As the this group was dominated by the Delta variant during the SAMPLE trial, all these mutations patterns were classified as "Delta". Delta variants were confirmed by Whole Genome Seqeuncing for specimins with sufficient viral RNA contents.

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H. Details for the health economic analysis

The pre-analytical costs for the different sampling methods were calculated using the salary and material cost at the Covid-19 test centers using a microcosting approach. We anticipated that the saliva specimen could be self-collected and send in the collection tube without need for viral transport medium. We performed a cost-effectiveness analysis using a second-order Monto Carlo simulation model taking account of parameter uncertainty. (16) This multivariate sensitivity analysis examines the effect of simultaneous changes in different variables on the outcomes. (17) (18) Using second-order Monte Carlo simulations allowed us to incorporate the real distributions of all input variables. Each simulation was based on different values drawn randomly from the distribution of each variable, yielding simulated population outcomes for costs and utilities/health outcomes. The Monte Carlo 2nd order simulation was performed based on detected incidences per 100,000 persons for each specimen type (see Supplementary Appendix B). For the overall population analysis with a SARS-CoV-2 prevalence of 1%, it was estimated that testing using saliva specimen would identify 619.73 (95% CI, 569.05 to 667.34) cases of SARS-CoV-2 infection per 100,000 persons, which was ~108 cases less compared with NPS, ~168 less compared with OPS, and ~318 less compared with combined NPS-OPS. The cost-effectiveness results for local settings with other prevalences of SARS-CoV-2, salary and material costs can be estimated using our Monte Carlo 2nd simulation model in Excel (see *Supplementary Appendix B*). We did not include transportation and laboratory costs in our economic analysis as we assume it to be identical. All cost estimates were converted from DKK to U.S. dollars using current exchange rates.

Data input collection

The material costs were collected from a testcenter in the Capital Region, Denmark (Valby Testcenter). The material cost for saliva specimen included a sterile collection tube, while the cost for OPS and NPS included tubes with 2 mL of inactivation transport medium (ITM) (Table H2). All material cost parameters are presented in Table H1 with cost and standard error. The relevant material costs per test for each type of SARS-CoV-2-test are illustrated in Table H2.

Table H1: All relevant equipment cost parameters collected in Danish Kroners and converted into USD ($) reported with the standard error.

Parameter description	Cost ($)	Standard error ($)
Medical mask	0.11	0.01
Eye protection	3.09	0.31
Gown	2.39	0.24
Gloves	0.27	0.03
Tongue spatula	0.01	0.00
Disinfection wipe	0.10	0.01
Nylon-flocked oropharyngeal swabs	0.32	0.03
Tubes with inactivation transport medium	1.58	0.16
Nylon-flocked nasopharyngeal swabs	0.32	0.03
Tube for saliva collection	0.22	0.02
Rack for storage of sample tubes	0.07	0.01
1L hand sanitizer	2.52	0.25
1 garbage bag	0.08	0.01

Table H2: Relevant equipment cost parameters per test for each type of sampling method

Parameter description	Cost ($)			
	OPS	NPS	Saliva	NPS+OPS
Medical mask	0.00	0.00	-	0.00
Eye protection	0.07	0.09	-	0.11
Gown	0.06	0.07	-	0.09
Gloves	0.27	0.27	-	0.27
Tongue spatula	0.01	-	-	0.01
Disinfection wipe	0.01	0.01	0.01	0.01
Nylon-flocked oropharyngeal swabs	0.32	-	-	0.32
Tubes with inactivation transport medium	1.58	1.58		1.58
Nylon-flocked nasopharyngeal swabs	-	0.32	-	0.32
Tube for saliva collection	-	-	0.22	-
Rack for storage of sample tubes	0.07	0.07	0.07	0.07
1L hand sanitizer	0.01	0.01	0.01	0.01
1 garbage bag	0.00	0.00	0.00	0.00

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We estimated the staff cost for each sampling method and, therefore, measured the time for registration of the participants and collection of the different specimen types (including disinfection and change of gloves) at the Valby COVID-19 test center, Copenhagen, Denmark. We measured the staff time used to register the participants' identification numbers and labelling of the test tubes to take 24 seconds based on 50 measurements of time used for registration of participants. We assume the registration time would be the same for NPS, OPS and saliva specimens for the cost-effectiveness analysis. The time it took the health care staff to perform OPS, and NPS specimens were measured while the self-collected saliva specimen was taken without staff time. We estimated the time for collecting each specimen by performing 106 measurements of time usage per sampling method from 12 different workers at Valby Testcenter. The average time use per test type for each of the 12 workers is reported in Table H3. We used the staff time to collect OPS and NPS specimens in the cost analyses and estimate the time for a change of gloves and disinfection. However, we anticipated that one healthcare worker would be used to observe ten people performing a saliva self-sample with the drooling technique, and the staff time used for sample collection was, therefore, 1/10 of the calculated sample time for saliva (see Table H4). As the staff did not need to change gloves during the saliva sample, only the time used to disinfect the surface of saliva tubes was estimated to take 2 seconds in the cost evaluation (See Table H4).

Table H3: The average time for collection of each type of specimen type for each of the 12 workers measured from 106 samples at Valby Testcenter

Time (seconds) for collection of each specimen type			
	NPS	OPS	Saliva*
Test center worker A	33.5	21.0	80.2
Test center worker B	39.9	21.9	105.0

Test center worker C	53.3	24.1	95.6
Test center worker D	51.1	37.9	111.3
Test center worker E	57.3	30.1	103.7
Test center worker F	84.4	30.9	78.3
Test center worker G	60.7	44.6	120.8
Test center worker H	46.1	18.9	104.2
Test center worker I	50.2	35.0	75.4
Test center worker J	60.4	35.3	130.9
Test center worker K	50.3	35.9	102.0
Test center worker L	40.0	27.0	95.6
Average time	**52.3**	**30.2**	**100.3**

* Time for participants to collect a saliva specimen.

The total time usage was multiplied by 1.5 to account for overhead time usage. It was estimated that a worker would be able to do test sampling for 5.625 hours per work shift, based on experience from the test center. The parameters were used to calculate the staff cost per test based on the time needed to collect the specimen, and the staff salary including pension, annual leave, and labor market contribution of DKK 220 per hour[1], which converts into $33.61.

Table H4: Time usage for each SARS-CoV-2-test specimen collection method used to calculate the staff cost per test

Parameter	OPS		NPS		Saliva		NPS+OPS	
	Sec.	Std. error	Sec.	Std. error	Sec.	Std. error	Sec.	Std. error
Registration	24.00	2.40	24.00	2.40	24.00	2.40	24.00	2.40
Disinfect and gloves	35.00	3.50	35.00	3.50	2.00*	0.20	35.00	3.50
Time for sample collection	30.20	N/A	52.28	N/A	10.00**	N/A	82.48	N/A
Total time usage	89.20	-	111.28	-	36.00	-	141.48	-
Including overhead time usage	133.80	-	166.92	-	54.00	-	212.22	-
No. of tests per worker per day	151.35	-	121.32	-	375.00	-	95.42	-

[1] Apacta: https://apacta.com/tools-medarbejder-kostpris/?gclid=EAIaIQobChMI3rO0udL98AIVARd7Ch2T_gT_EAAYAiAAEgKcKPD_BwE

	Cost ($)			
Staff cost per test	1.67	2.08	0.67	2.64

Sec.=seconds, NPS = nasopharyngeal swab, OPS = oropharyngeal swab and Saliva = saliva sampling.

** Estimated time for disinfection for saliva tubes (no need for use of staff gloves due to saliva self-sampling)*

*** Estimated time based on an assumption that the staff time used to handle saliva samples was 1/10 of average time it took participants to collect the samples.*

Table H5: The estimated cost per test for the different sample type

Sample	Cost, $
Saliva	0.98
OPS	4.06
NPS	4.49
NPS/OPS	5.43

NPS = nasopharyngeal swab, OPS = oropharyngeal swab and Saliva = saliva sampling.

Health economic results

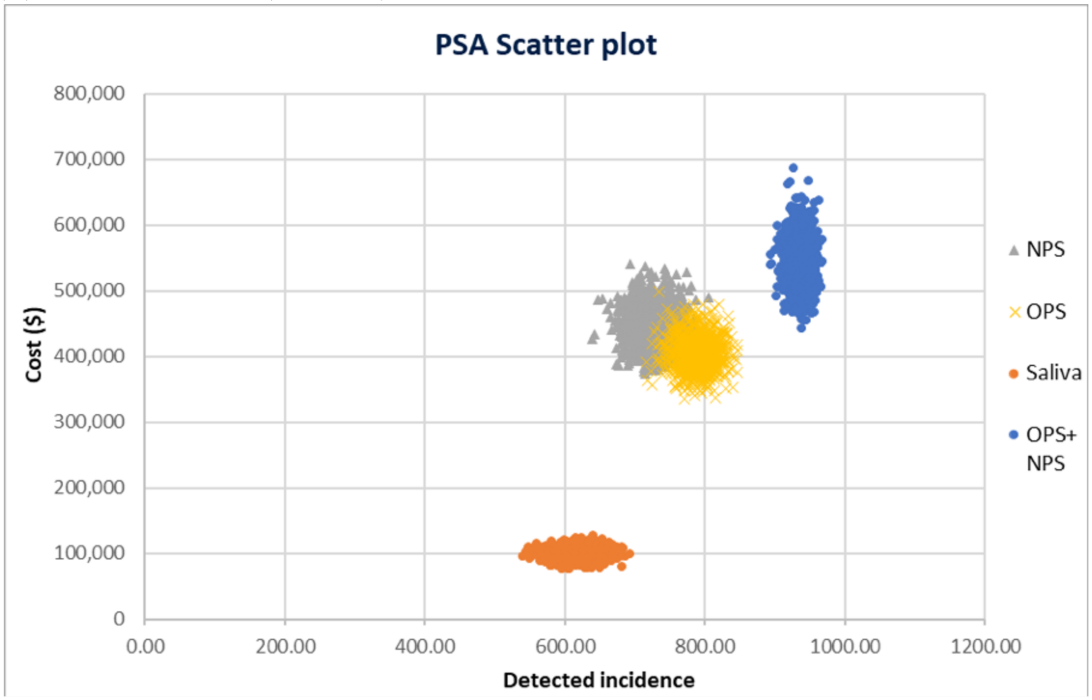
The mean results from the 2nd order Monte Carlo simulation in a population with a SARS-CoV-2 prevalence of 1% were presented in

Table H6. The results are also illustrated in a cost-effectiveness scatterplot in Figure H1. The comparisons of OPS, NPS, and NPS+OPS versus the saliva specimen collection method are presented in the incremental cost-effectiveness scatterplots in Figure H2, Figure H3, and Figure H4 The results from the 2nd order Monte Carlo simulation indicated that the saliva test was the cheapest but least effective specimen collection method in all simulation iterations.

Table H6: Results from the 2nd-order Monte Carlo simulation presented as the mean and 95% uncertainty interval for the overall population (1.0% prevalence of SARS-CoV-2)

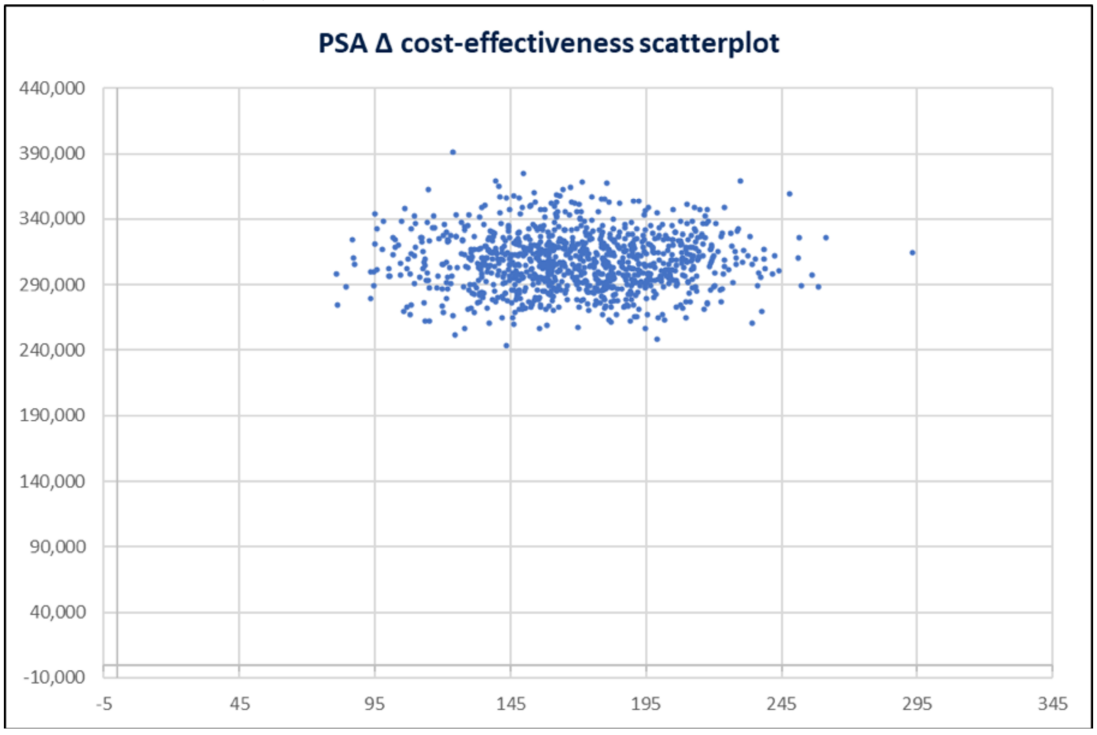
Test	Cost ($)	Detected incidence	Cost ($) per detected incidence	Δ cost ($) per detected incidence vs. saliva
Saliva	98,162.80 (82,372.24 - 116,514.76)	619.73 (569.05-667.34)	158.91	-
OPS	405,521.50 (359,343.62-458,301.88)	788.18 (744.51-827.36)	515.68	1,831.62 (1,281.90-2,987.95)
NPS	448,739.02 (394,941.25-507,981.74)	727.69 (681.31-771.21)	618.19	3,257.85 (1,952.95-8,451.86)
NPS+ OPS	542,480.23 (479,601.21-614,552.92)	937.55 (910.78-959.22)	579.83	1,401.46 (1,139.01-1,754.65)

Figure H1: Cost-effectiveness scatterplot illustrating the iterations from the 2nd order Monte Carlo simulation in a population with a SARS-CoV-2 prevalence of 1.0%



Similar to the initial Monte Carlo simulation, the NPS and OPS-tests are overlapping in multiple of the iterations in both cost and effectiveness, while the saliva specimen sampling method and NPS also overlap concerning effectiveness.

Figure H2: Incremental cost-effectiveness scatterplot comparing OPS and saliva test illustrating the 2nd order Monte Carlo simulation (1.0% SARS-CoV-2 prevalence)



Y-axis = cost (dollars), X-axis = detected SARS-CoV-2 incidence

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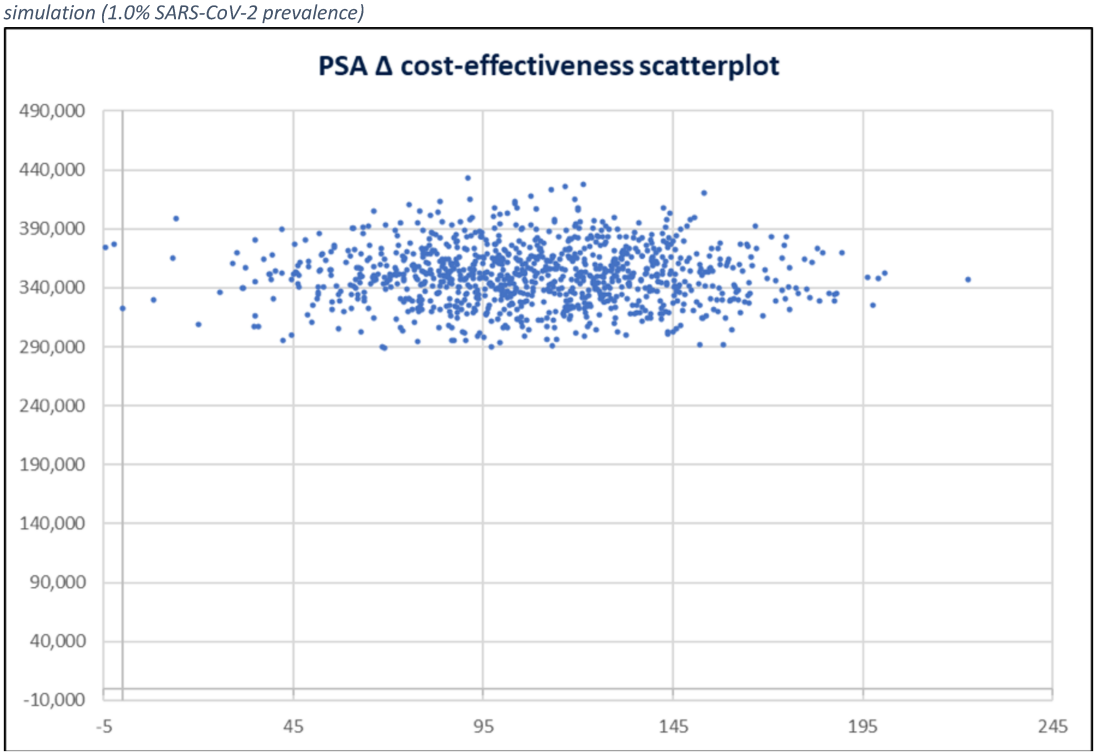
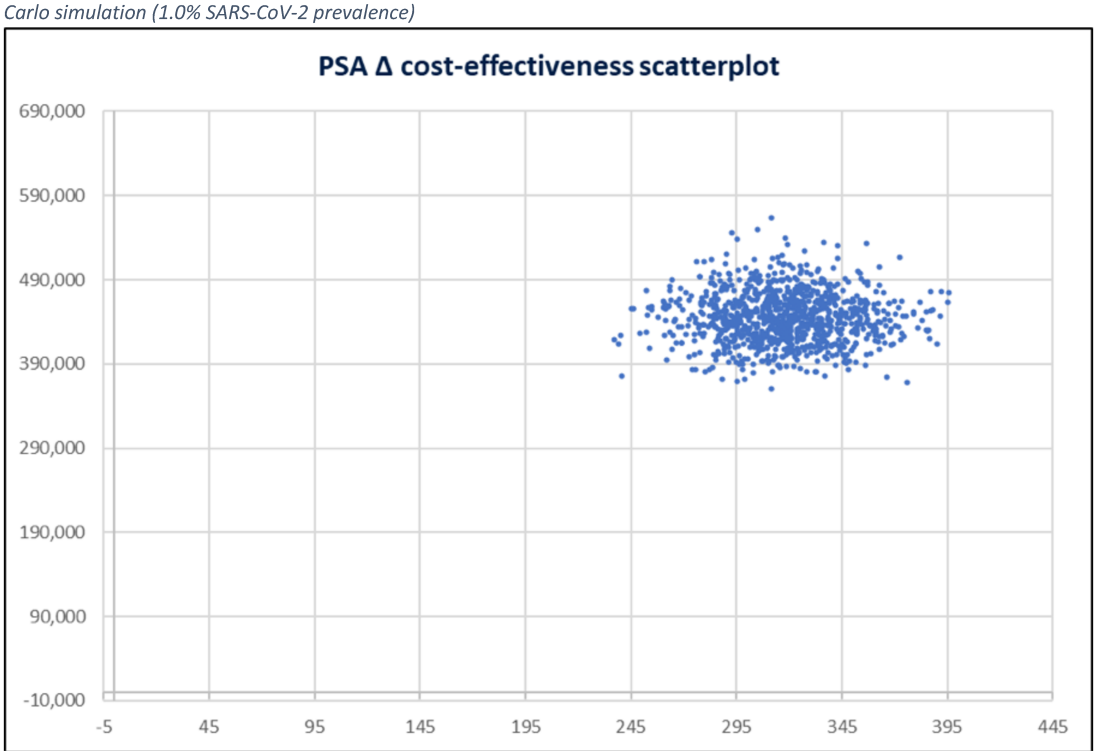
Figure H3: Incremental cost-effectiveness scatterplot comparing NPS and saliva test illustrating the 2nd order Monte Carlo simulation (1.0% SARS-CoV-2 prevalence)



Y-axis = cost (dollars), X-axis = detected SARS-CoV-2 incidence

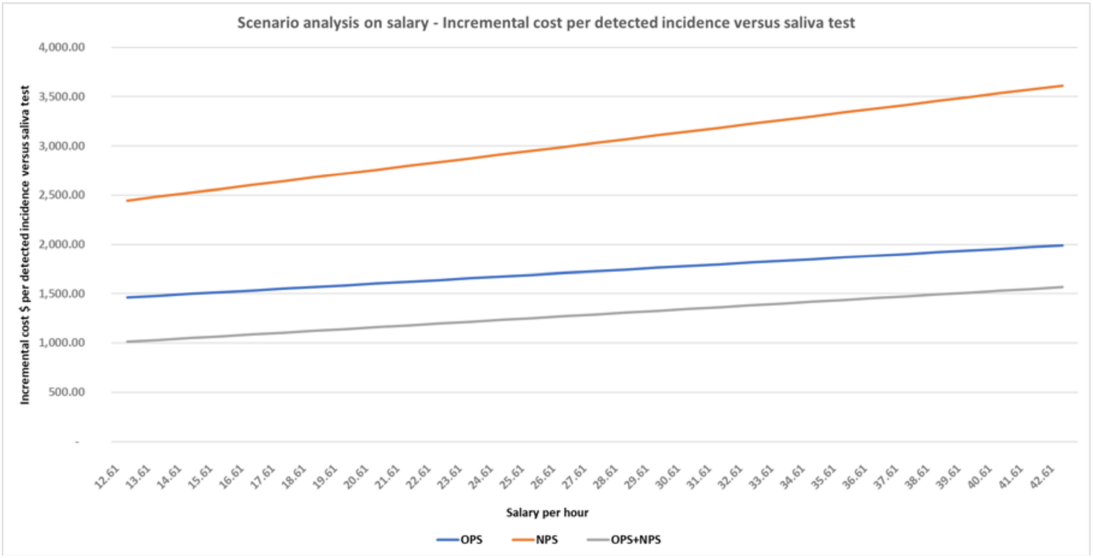
Figure H4: Incremental cost-effectiveness scatterplot comparing NPS+OPS and saliva test illustrating the 2nd order Monte Carlo simulation (1.0% SARS-CoV-2 prevalence)



Y-axis = cost (dollars), X-axis = detected SARS-CoV-2 incidence

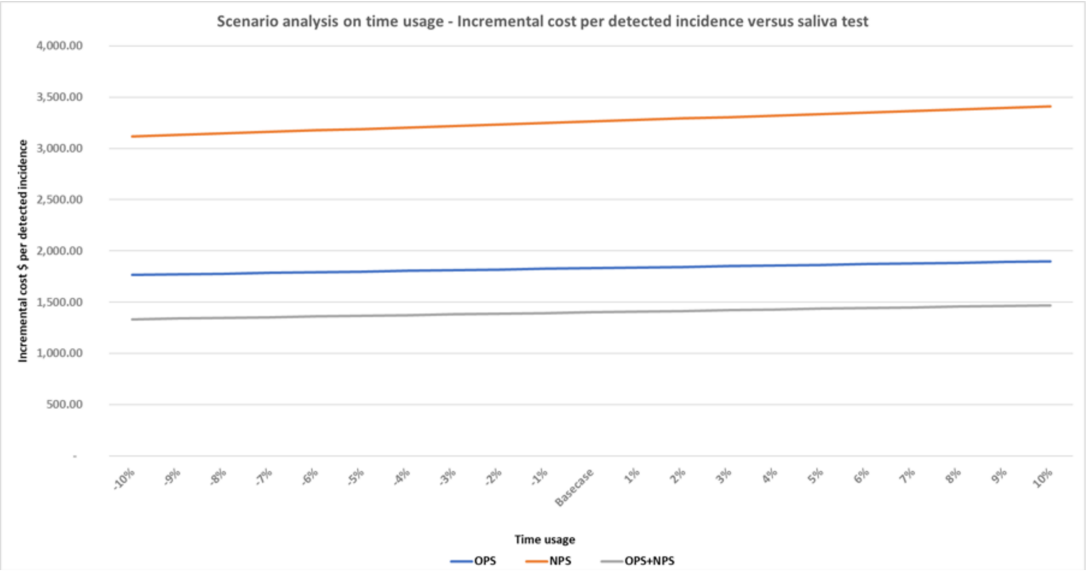
Post-hoc secondary parameter scenario analyses were conducted to analyze the impact of alteration in parameter inputs. The analyzed parameters were staff salary, time usage, and prevalence level. The results of the scenario analyses were presented as incremental cost per detected incidence versus testing using saliva specimen in a graph format. These results are presented in Figure H5, Figure H6, and Figure H7, and illustrate that the prevalence of SARS-CoV-2 is the most impactful parameter in relation to the incremental cost per detected incidence, as a low prevalence of SARS-CoV-2 results in a higher incremental cost per detected incidence in the comparison with the saliva test.

Figure H5: Scenario analysis on the hourly salary for the testcenter staff's impact on the incremental cost per detected incidence. The incremental cost per detected incidence is presented versus saliva test



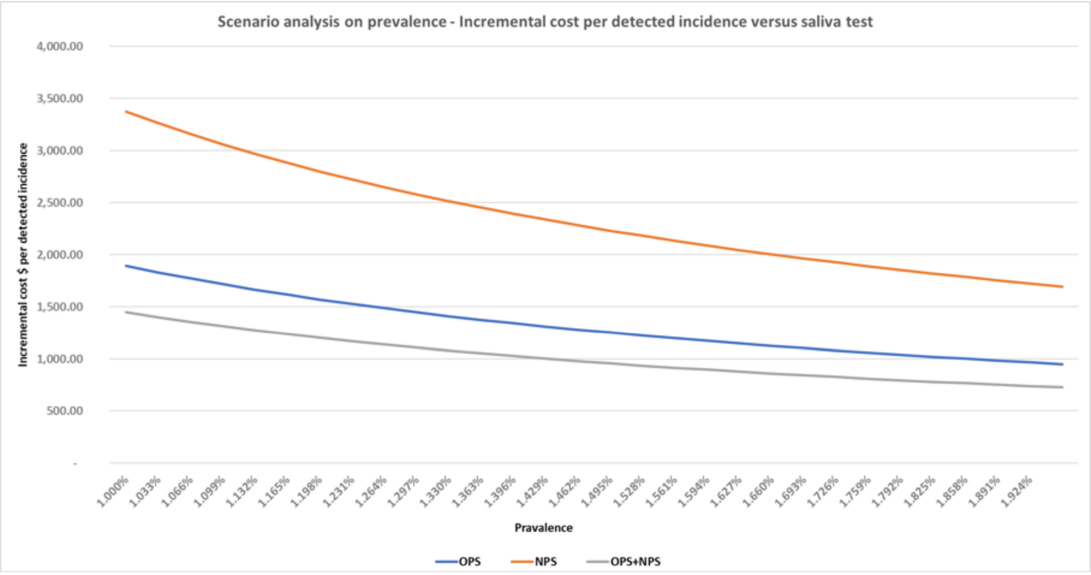
The scenario analysis indicates that the analysis is robust to alterations in the salary level

Figure H6: Scenario analysis on the time-usage per SARS-CoV-2-test sampling's impact on the incremental cost per detected incidence. The incremental cost per detected incidence is presented versus saliva test



The scenario analysis indicates that the analysis is robust to alterations in the use of time per test

Figure H7: Scenario analysis on the prevalence of SARS-CoV-2's impact on the incremental cost per detected incidence. The incremental cost per detected incidence is presented versus saliva test



The scenario analysis indicates that the differences in the incremental cost per detected incidence are higher with a lower prevalence of SARS-CoV-2.

Table H7: Scenario analysis on the prevalence of SARS-CoV-2's impact on the incremental cost per detected incidence. The incremental cost per detected incidence is presented versus saliva test. The analysis is calculated based on mean cost and prevalence; thus, this analysis did not utilize inputs from the Monte Carlo simulation.

Δ cost ($) per detected case for different incidences vs. saliva				
Test	**SARS-CoV-2 prevalence 0.01%**	**SARS-CoV-2 prevalence 0.1%**	**SARS-CoV-2 prevalence 1%**	**SARS-CoV-2 prevalence 10%**
OPS	183,106.32	18,310.63	1,831.06	183.11
NPS	326,199.63	32,619.96	3,262.00	326.20
NPS+ OPS	140,072.40	14,007.24	1,400.72	140.07

The scenario analysis indicates that the differences in the incremental cost per detected incidence are higher with a lower prevalence of SARS-CoV-2.

Table H8: Scenario analysis when the laboratory cost for RT-PCR testing is included

	Mouth	Nose	Saliva	OPS+NPS
Cost of material use per sample	$ 2.39	$ 2.42	$ 0.31	$ 2.79
Cost of staff per sample	$ 1.67	$ 2.08	$ 0.67	$ 2.64
Cost for laboratory test	$ 5.00	$ 5.00	$ 5.00	$ 10.00
Total cost per sample	$ 9.06	$ 9.49	$ 5.98	$ 15.43
Prevalence in %	0.01			
Sensitivity of test	0.7874	0.727	0.6194	0.937
No. of prevalent incidences detected per 100,000 samples	787.4	727	619.4	937

	Mouth	Nose	Saliva	OPS+NPS
Cost per 100,000 samples	$ 906,049.55	$ 949,421.74	$ 598,430.94	$ 1,543,300.86
Cost per detected incidence	$ 1,150.69	$ 1,305.94	$ 966.15	$ 1,647.07
Incremental cost per detected incidence vs. cheapest	$ 1,831.06	$ 3,262.00	-	$ 2,975.03

Including laboratory costs for RT-PCR testing (conservative estimate of 5$ per RT-PCR test) will increase the cost for OPS+NPS testing as the results combine two molecular test results from both NPS and OPS. However, sending both specimens for the same molecular testing would reduce the laboratory cost for OPS+NPS to the same level as a single specimen.

I. Effect on RT–PCR testing results when changing the definition of positive to Ct <25

Of the 26,794 test cases, 254 (0.95%) were positive for SARS-CoV-2 in one or more clinical specimens when using the definition of Ct<25 to define SARS-CoV-2 infection (reference standard). The number of positive RT-PCR test results and the SARS-CoV-2 detection rate for the different sample methods were then 203, 236, 168 and 248 for NPSs, OPSs, saliva, and NPSs/OPSs specimens, respectively. The detection rate for SARS-CoV-2 was 79.9% (95% CI, 74.5 to 84.7) for NPSs; 92.9% (95% CI, 89.0 to 95.8) for OPSs; 66.1% (95% CI, 60.0 to 71.9) for saliva sampling; and 97.6% (95% CI, 94.9 to 99.1) for NPSs/OPSs (See Figure 2). The detection rate was 13.0% points higher for OPSs than for NPSs (95% CI, 9.1 to 17.8) and 13.8% points higher for NPSs than for saliva sampling (95% CI, 9.8 to 18.6), see Table 2. Combined OPS/NPS specimens had a 17.7% points higher detection rate than NPS specimens (95% CI, 13.2 to 23.0) and a 4.7% points higher detection rate than OPS specimens (95% CI, 2.5 to 8.1).

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J. STARD checklist

Section & Topic	No	Item	Reported on page #
TITLE OR ABSTRACT			
	1	Identification as a study of diagnostic accuracy using at least one measure of accuracy (such as sensitivity, specificity, predictive values, or AUC)	1
ABSTRACT			
	2	Structured summary of study design, methods, results, and conclusions (for specific guidance, see STARD for Abstracts)	2-3
INTRODUCTION			
	3	Scientific and clinical background, including the intended use and clinical role of the index test	4
	4	Study objectives and hypotheses	4
METHODS			
Study design	**5**	Whether data collection was planned before the index test and reference standard were performed (prospective study) or after (retrospective study)	5
Participants	**6**	Eligibility criteria	5-6
	7	On what basis potentially eligible participants were identified (such as symptoms, results from previous tests, inclusion in registry)	5-6
	8	Where and when potentially eligible participants were identified (setting, location and dates)	5-6
	9	Whether participants formed a consecutive, random or convenience series	5-6
Test methods	**10a**	Index test, in sufficient detail to allow replication	6-7
	10b	Reference standard, in sufficient detail to allow replication	6-7
	11	Rationale for choosing the reference standard (if alternatives exist)	4
	12a	Definition of and rationale for test positivity cut-offs or result categories of the index test, distinguishing pre-specified from exploratory	7
	12b	Definition of and rationale for test positivity cut-offs or result categories of the reference standard, distinguishing pre-specified from exploratory	7
	13a	Whether clinical information and reference standard results were available to the performers/readers of the index test	-
	13b	Whether clinical information and index test results were available to the assessors of the reference standard	-
Analysis	**14**	Methods for estimating or comparing measures of diagnostic accuracy	8-9
	15	How indeterminate index test or reference standard results were handled	8-9, Appendix A
	16	How missing data on the index test and reference standard were handled	8-9, Appendix A
	17	Any analyses of variability in diagnostic accuracy, distinguishing pre-specified from exploratory	9
	18	Intended sample size and how it was determined	Appendix A
RESULTS			
Participants	**19**	Flow of participants, using a diagram	Figure 1
	20	Baseline demographic and clinical characteristics of participants	Table 1
	21a	Distribution of severity of disease in those with the target condition	12
	21b	Distribution of alternative diagnoses in those without the target condition	12
	22	Time interval and any clinical interventions between index test and reference standard	6

Test results	**23**	Cross tabulation of the index test results (or their distribution) by the results of the reference standard	Figure 1, Appendix A
	24	Estimates of diagnostic accuracy and their precision (such as 95% confidence intervals)	11
	25	Any adverse events from performing the index test or the reference standard	12
DISCUSSION			
	26	Study limitations, including sources of potential bias, statistical uncertainty, and generalisability	15
	27	Implications for practice, including the intended use and clinical role of the index test	16-18
OTHER INFORMATION			
	28	Registration number and name of registry	5
	29	Where the full study protocol can be accessed	5
	30	Sources of funding and other support; role of funders	5

K. CONSORT checklist

CONSORT 2010 checklist of information to include when reporting a randomised trial*

Section/Topic	Item No	Checklist item	Reported on page No
Title and abstract			
	1a	Identification as a randomised trial in the title	1
	1b	Structured summary of trial design, methods, results, and conclusions (for specific guidance see CONSORT for abstracts)	2-3
Introduction			
Background and objectives	2a	Scientific background and explanation of rationale	4
	2b	Specific objectives or hypotheses	4
Methods			
Trial design	3a	Description of trial design (such as parallel, factorial) including allocation ratio	5-6
	3b	Important changes to methods after trial commencement (such as eligibility criteria), with reasons	Appendix, page 8
Participants	4a	Eligibility criteria for participants	5-6
	4b	Settings and locations where the data were collected	5-6
Interventions	5	The interventions for each group with sufficient details to allow replication, including how and when they were actually administered	6-7
Outcomes	6a	Completely defined pre-specified primary and secondary outcome measures, including how and when they were assessed	7-8
	6b	Any changes to trial outcomes after the trial commenced, with reasons	Appendix, page 8
Sample size	7a	How sample size was determined	8
	7b	When applicable, explanation of any interim analyses and stopping guidelines	8
Randomisation: Sequence generation	8a	Method used to generate the random allocation sequence	Appendix, page 6-7
	8b	Type of randomisation; details of any restriction (such as blocking and block size)	Appendix, page 6-7

Allocation concealment mechanism	9	Mechanism used to implement the random allocation sequence (such as sequentially numbered containers), describing any steps taken to conceal the sequence until interventions were assigned	Appendix, page 6-7
Implementation	10	Who generated the random allocation sequence, who enrolled participants, and who assigned participants to interventions	Appendix, page 6-7
Blinding	11a	If done, who was blinded after assignment to interventions (for example, participants, care providers, those assessing outcomes) and how	6
	11b	If relevant, description of the similarity of interventions	6-7
Statistical methods	12a	Statistical methods used to compare groups for primary and secondary outcomes	8-9
	12b	Methods for additional analyses, such as subgroup analyses and adjusted analyses	9-10
Results			
Participant flow (a diagram is strongly recommended)	13a	For each group, the numbers of participants who were randomly assigned, received intended treatment, and were analysed for the primary outcome	11
	13b	For each group, losses and exclusions after randomisation, together with reasons	p11, figure 2 and Appendix, Table S9
Recruitment	14a	Dates defining the periods of recruitment and follow-up	11
	14b	Why the trial ended or was stopped	11
Baseline data	15	A table showing baseline demographic and clinical characteristics for each group	Figure 1
Numbers analysed	16	For each group, number of participants (denominator) included in each analysis and whether the analysis was by original assigned groups	Figure 2
Outcomes and estimation	17a	For each primary and secondary outcome, results for each group, and the estimated effect size and its precision (such as 95% confidence interval)	11-12
	17b	For binary outcomes, presentation of both absolute and relative effect sizes is recommended	11-12
Ancillary analyses	18	Results of any other analyses performed, including subgroup analyses and adjusted analyses, distinguishing pre-specified from exploratory	12-13 and Appendix Table S3, S5, S8, S10, S13-S19 and Figure S4.
Harms	19	All important harms or unintended effects in each group (for specific guidance see CONSORT for harms)	No harms observed

Discussion			
Limitations	20	Trial limitations, addressing sources of potential bias, imprecision, and, if relevant, multiplicity of analyses	16-17
Generalisability	21	Generalisability (external validity, applicability) of the trial findings	15-16
Interpretation	22	Interpretation consistent with results, balancing benefits and harms, and considering other relevant evidence	17-19
Other information			
Registration	23	Registration number and name of trial registry	5
Protocol	24	Where the full trial protocol can be accessed, if available	5
Funding	25	Sources of funding and other support (such as supply of drugs), role of funders	5

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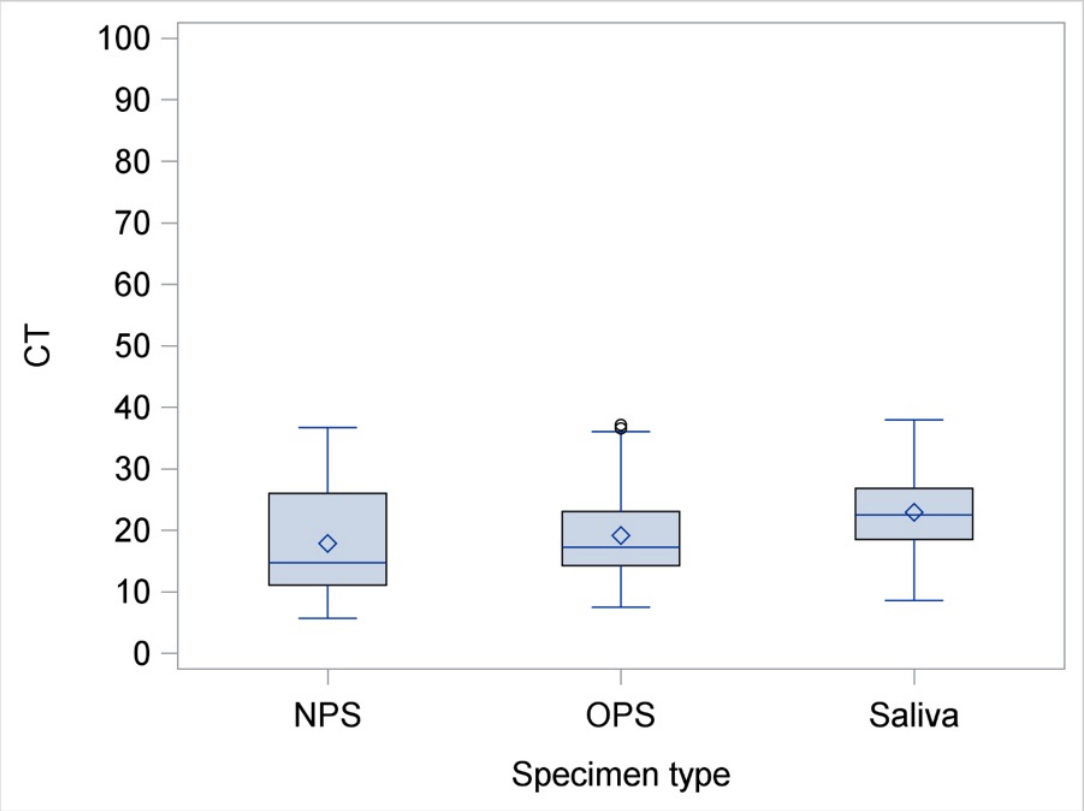
Supplementary Figures

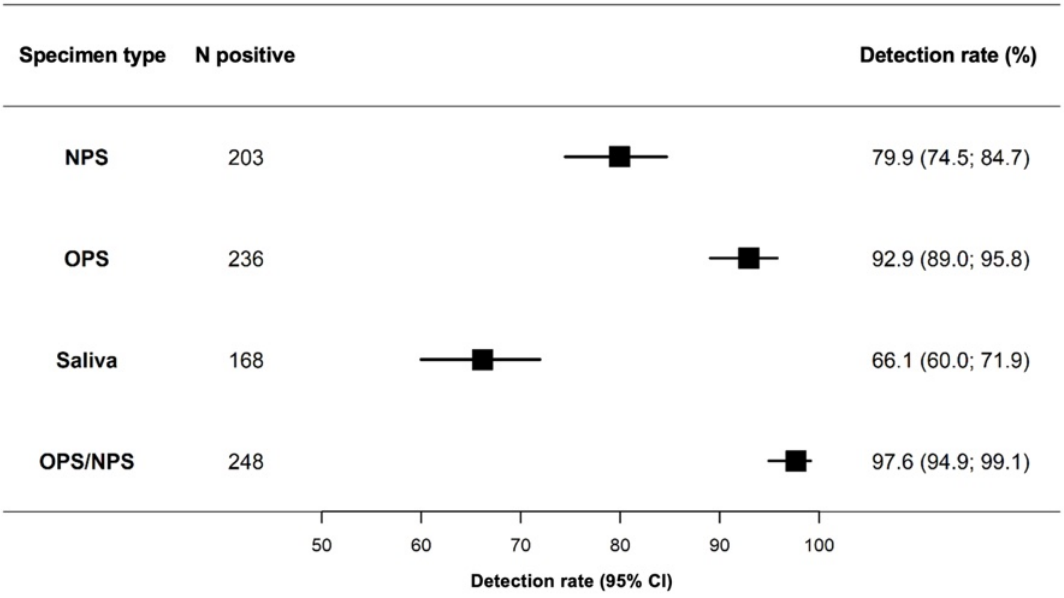
Figure S1. Cycle threshold (Ct) for NPS, OPS and saliva



Boxplot of cycle threshold (Ct) for the N1-gene segment from SARS-CoV-2 RNA RT–PCR for test cases with Ct < 100

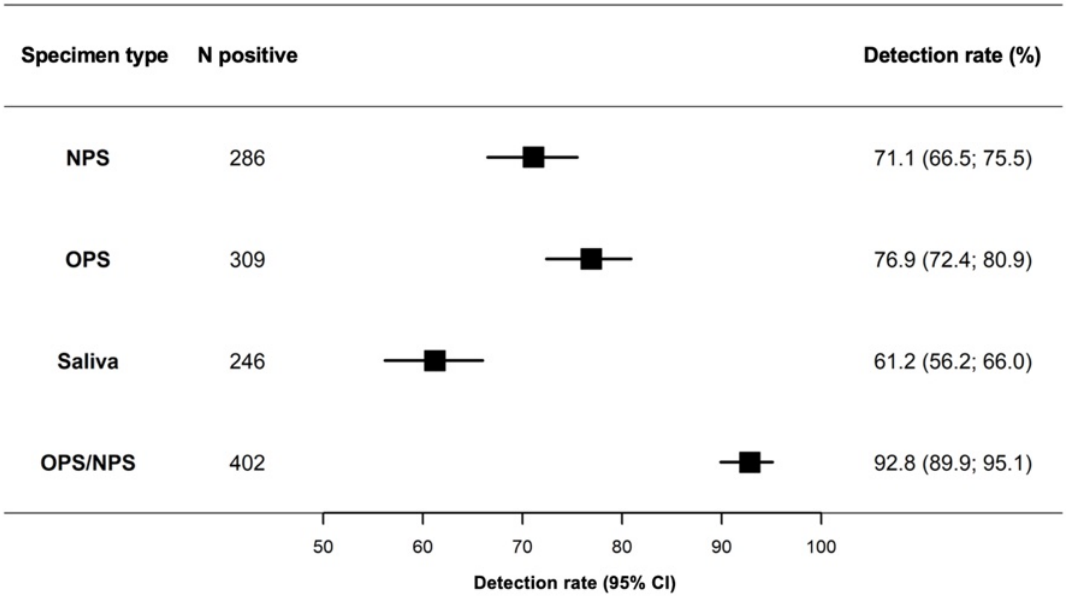
Figure S2. Detection rate for RT–PCR testing (with changed definition of positive to Ct <25 for one N-target)

Detection rate for RT–PCR testing of NPS, OPS, saliva, and OPS/NPS sampling based on participants (n=254) with confirmed SARS-CoV-2 infection.



NPS denotes nasopharyngeal swab, OPS oropharyngeal swab, Saliva saliva sampling, RT–PCR reverse transcriptase polymerase chain reaction, and CI confidence interval.

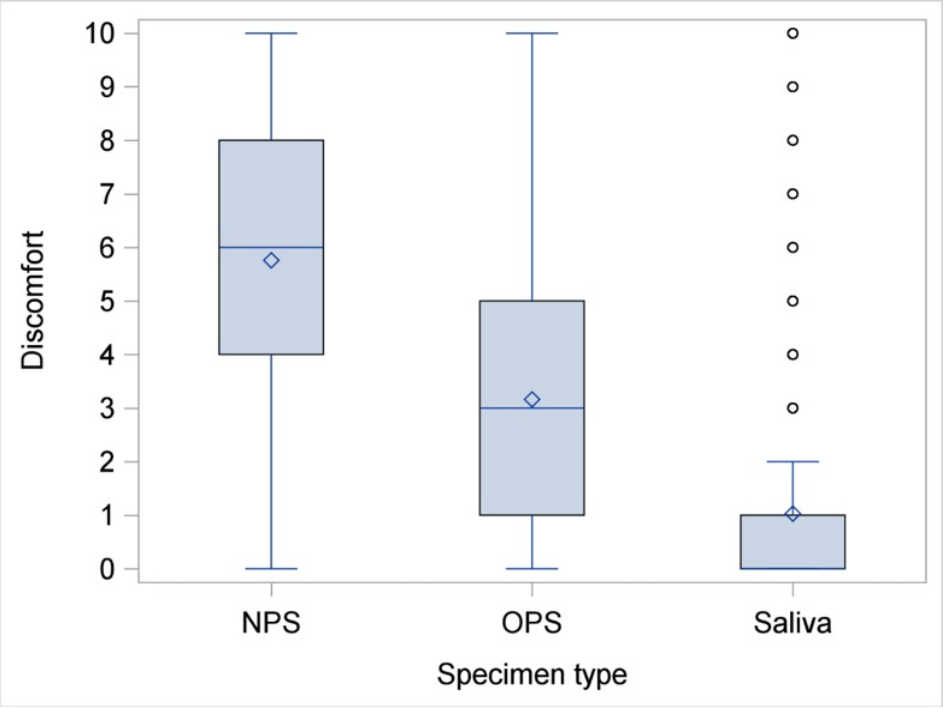
Figure S3. Detection rate for RT–PCR testing (with changed definition of positive to Ct <40 for two N-targets)



NPS denotes nasopharyngeal swab, OPS oropharyngeal swab, Saliva saliva sampling, RT–PCR reverse transcriptase polymerase chain reaction, and CI confidence interval.

Figure S4. Boxplot with NRS ratings of the test-related discomfort

Boxplot with 11-point numeric scale (NRS) ratings of the test-related discomfort from 0 (no discomfort) to 10 (worst possible discomfort) for each sample method, N=26,258 responders out of 26,795 possible test cases (98%).



Supplementary Tables

Table S1: 8-row table with all the possible test-result combinations from different specimen types

Test-result combinations when using a positive definition with **Ct < 34** of one N-gene from ≤ 1 respiratory specimen.

Saliva	NPS	OPS	N
Pos	Pos	Pos	189
Pos	Pos	Neg	0
Pos	Neg	Pos	23
Pos	Neg	Neg	24
Neg	Pos	Pos	31
Neg	Pos	Neg	57
Neg	Neg	Pos	57
Neg	Neg	Neg	26414

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Table S2. Diagnostic agreement between the three specimen types given by positive agreement and negative agreement with corresponding 95% confidence interval

	PA	95% CI	NA	95% CI
NPS vs OPS	76.2%	(72.4%; 80.1%)	99.7%	(99.7%; 99.8%)
NPS vs saliva	73.7%	(69.4%; 78.0%)	99.7%	(99.7%; 99.8%)
OPS vs saliva	79.1%	(75.3%; 82.9%)	99.8%	(99.8%; 99.8%)

Post hoc analysis of the diagnostic agreement. Nasopharyngeal swab (NPS), oropharyngeal swab (OPS), saliva sampling (Saliva), positive agreement (PA), negative agreement (NA) and 95% confidence interval (95% CI).

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Table S3. Additional answers from the SAMPLE pretest questionnaire

Additional baseline demographics and clinical characteristics (from online questionnaire) of the tests overall and stratified by reference standard (defined as RT–PCR-positive results from either NPS, OPS, or saliva specimens). Values are numbers and percentages (N, %) unless stated otherwise.

	Overall	Reference standard[1]	
		Positive	Negative
Are you in quarantine until a negative COVID-19 response? - yes	2,151 (9.8)	153 (50.8)	1,998 (9.2)
Have you previously been tested for COVID-19?	21,443 (97.0)	292 (96.4)	21,151 (97.0)
Have you previously been tested in the nose or mouth?[4]			
Mouth	5,422 (25.3)	50 (17.1)	5,372 (25.4)
Nose	419 (2.0)	13 (4.5)	406 (1.9)
Both	15,565 (72.7)	229 (78.4)	15,336 (72.6)
	1,149 (5.2)	81 (26.6)	1,068 (4.9)
Why have you booked an appointment for a COVID-19 test? (reason for booking a test)			
1: I have COVID-19-like symptoms (e.g. fever, general tenderness, sore throat, cough, fatigue, diarrhea, headache, impaired sense of taste or smell, skin rash, pink eye, shortness of breath)	1,149 (5.2)	81 (26.6)	1,068 (4.9)
2: I have to be treated at a hospital or another facility and take a test beforehand (e.g. planned surgery, dentist)	520 (2.4)	1 (0.3)	519 (2.4)
3: I have been in contact with an infected person	1,475 (6.7)	85 (28.0)	1,390 (6.4)
4: My profession (also covers business travel)	4,438 (20.0)	29 (9.5)	4,409 (20.2)
5: I need to visit a vulnerable person	1,393 (6.3)	8 (2.6)	1,385 (6.3)

6: I have been or are going abroad (not business travel)	568 (2.6)	6 (2.0)	562 (2.6)
7: I wish for a test before an event that I have to attend (e.g. wedding, sporting event, etc.)	3,325 (15.0)	15 (4.9)	3,310 (15.2)
8: I have previously been sick with a corona-like disease	39 (0.2)	5 (1.6)	34 (0.2)
9: I have a suspicion that I may have been infected with COVID-19 (e.g. by my participation in a major event, concert, sporting event)	328 (1.5)	32 (10.5)	296 (1.4)
10: Other/I participate in a population survey	742 (3.4)	8 (2.6)	734 (3.4)
11: I follow the recommendations of regular testing.	9,747 (44.0)	76 (25.0)	9,671 (44.3)
What symptoms do you have?			
Fever[3]	275 (23.9)	40 (49.4)	235 (22.0)
General tenderness[3]	330 (28.7)	41 (50.6)	289 (27.1)
Sore throat[3]	738 (64.2)	44 (54.3)	694 (65.0)
Cough[3]	461 (40.1)	39 (48.2)	422 (39.5)
Fatigue[3]	472 (41.1)	41 (50.6)	431 (40.4)
Diarrhea[3]	50 (4.4)	4 (4.9)	46 (4.3)
Headache[3]	479 (41.7)	54 (66.7)	425 (39.8)
Impaired sense of taste or smell[3]	53 (4.6)	8 (9.9)	45 (4.2)
Skin rash[3]	9 (0.8)	0 (0.0)	9 (0.8)
Eye cataracts[3]	11 (1.0)	4 (4.9)	7 (0.7)
Shortness of breath[3]	44 (3.8)	4 (4.9)	40 (3.8)
Have you been eating or drinking within the last 30 minutes - yes	11,611 (46.3)	168 (46.8)	11,443 (46.3)
What relationship do you have/had to the infected person?			
I have been warned via the Smittestop app[5]	115 (7.8)	3 (3.5)	112 (8.1)
I have been contacted by Coronaopsporing/Corona-hotline with the message that I have been in close contact with an infected person.[5]	61 (4.1)	4 (4.7)	57 (4.1)
Someone from my household[5]	232 (15.7)	31 (36.5)	201 (14.5)

A close relative (non-household member).[5]	217 (14.7)	15 (17.7)	202 (14.5)
A good friend/acquaintance[5]	266 (18.0)	15 (17.7)	251 (18.1)
Someone from my work (e.g. a colle ague or customer)[5]	549 (37.2)	21 (24.7)	528 (38.0)
An event or major event[5]	41 (2.8)	3 (3.5)	38 (2.7)
Other[5]	52 (3.5)	0 (0.0)	52 (3.7)
When were you sick?			
< 7 days ago[6]	14 (35.9)	1 (20.0)	13 (38.2)
Between 7 – 14 days ago[6]	5 (12.8)	3 (60.0)	2 (5.9)
Between 2 and 4 weeks ago[6]	2 (5.1)	1 (20.0)	1 (2.9)
More than 4 weeks ago[6]	14 (35.9)	0 (0.0)	14 (41.2)
Unknown[6]	4 (10.3)	0 (0.0)	4 (11.8)
Which profession do you have?			
I work in the municipal health-, social-, and elderly sector[7]	370 (8.3)	2 (6.9)	368 (8.4)
I work in the regional health sector (hospital system)[7]	120 (2.7)	1 (3.5)	119 (2.7)
I work in the private health and elderly sector [7]	219 (4.9)	0 (0.0)	219 (5.0)
I work with children and adolescents [7]	717 (16.2)	5 (17.2)	712 (16.2)
I work at an institution (e.g. residen-ce, prison, etc.)[7]	164 (3.7)	1 (3.5)	163 (3.9)
I am in touch with many people (e.g. the transport sector, sales, restaurant industry)[7]	992 (22.4)	10 (34.5)	982 (22.3)
I have to make a business trip[7]	211 (4.8)	3 (10.3)	208 (4.7)
I returned from a business trip and thus want a test[7]	69 (1.6)	1 (3.5)	68 (1.5)
Other[7]	1,728 (38.9)	9 (31.0)	1,719 (39.0)

Who are you going to visit?			
I need to visit an elderly person in a nursing home[8]	121 (8.7)	1 (12.5)	120 (8.7)
I have to visit an elderly person in their own home[8]	736 (52.8)	3 (37.5)	733 (52.9)
I have to visit a person at the hospital [8]	51 (3.7)	0 (0.0)	51 (3.7)
I need to visit a vulnerable non-hospitalized person in their own home[8]	356 (25.6)	3 (37.5)	353 (25.5)
I have to visit a vulnerable person at an institution or similar[8]	20 (1.4)	0 (0.0)	20 (1.4)
I have to visit a person at an institution or similar [8]	16 (1.2)	0 (0.0)	15 (1.2)
Other[8]	134 (9.6)	1 (12.5)	133 (9.6)
Why are you going abroad?			
I am going on holiday and want a test before leaving[9]	121 (21.3)	1 (16.7)	120 (21.4)
I have returned from vacation and want a test in connection with the homecoming[9]	77 (13.6)	0 (0.0)	77 (13.7)
I have to visit or have visited family abroad[9]	195 (34.3)	1 (16.7)	194 (34.5)
I have to travel abroad for reasons other than vacation[9]	97 (17.1)	3 (50.0)	94 (16.7)
Other[9]	88 (15.5)	2 (33.3)	86 (15.3)

[1]Reference standard: one or more specimen types are positive
[2]Among individuals with a prior positive test
[3]Among individuals with symptoms
[4]Among individuals tested previously
[5]Among individuals who have been in contact with an infected person (reason 3 for booking a test)

[6]Among individuals who previously have been sick with a corona-like disease (reason 8 for booking a test)
[7]Among individuals who are being tested do to their profession (also covers business travel) (reason 4 for booking a test)
[8]Among individuals who are being tested prior to visiting a vulnerable person (reason 5 for booking a test)
[9]Among individuals who are being tested after or prior to going abroad (not business travel) (reason 6 for booking a test)

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Table S4. Exploratory subgroup analysis of detection rates between specimen types stratified by symptoms

Specimen type	OR (95% CI)*	p-value
Symptoms, N=1,149 tests		
OPS vs. NPS	1.01 (0.97; 1.06)	0.56
NPS vs. saliva	1.07 (1.01; 1.14)	0.025
OPS vs. saliva	1.09 (1.02; 1.16)	0.014
OPS/NPS vs. saliva	1.10 (1.03; 1.18)	0.008
OPS/NPS vs. OPS	1.01 (0.99; 1.04)	0.32
OPS/NPS vs. NPS	1.03 (0.99; 1.07)	0.16
No symptoms, N=20,997 tests		
OPS vs. NPS	1.19 (1.05; 1.36)	0.008
NPS vs. saliva	1.18 (1.01; 1.36)	0.031
OPS vs. saliva	1.40 (1.24; 1.59)	<0.001
OPS/NPS vs. saliva	1.72 (1.50; 1.98)	<0.001
OPS/NPS vs. OPS	1.23 (1.15; 1.31)	<0.001
OPS/NPS vs. NPS	1.47 (1.34; 1.61)	<0.001

NPS denotes nasopharyngeal swab, OPS oropharyngeal swab, Saliva saliva sampling, OR Odds Ratio, and CI confidence interval.

*OR was estimated using generalized estimating equations (GEE) logistic regression.

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Table S5. Exploratory subgroup analysis of the effect on drinking or eating

Subgroup analysis of the effect on drinking or eating before collecting the saliva specimen on detection rate

A total of 11,611 (46.3%) test cases reported eating or drinking 30 minutes before testing in the questionnaire, while 13,481 did not (1703 test cases did not answer the question).

Drinking/eating*	Total number	Positive (reference standard)	Detection rate saliva specimen
Yes	11,611	1.45%	64.9% (95% CI: 57.2%; 72.1%)
No	13,481	1.42%	59.7% (95% CI: 52.4%; 66.7%)

*Participants reported eating or drinking 30 minutes before testing

Reference standard: one or more specimen types are positive, CI: confidence interval

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Table S6. Baseline demographics of included and excluded cases

Baseline demographics and clinical characteristics (from online questionnaire) of the randomized test cases, overall and stratified by being included or excluded from the analysis, N=27,787 tests. Values are numbers and percentages (N, %) unless stated otherwise.

	Overall n=27,787	Analysis	
		Included n=26,795	Excluded n=992
Age, mean (SD)	42.3 (15.2)	42.2 (15.2)	43.9 (15.4)
Female sex	14,003 (50.7)	13,549 (50.6)	454 (53.9)
Vaccination status	1,820 (8.1)	1,777 (8.1)	43 (8.0)
Test center, Valby	19,735 (71.0)	19,050 (71.1)	685 (69.1)
Randomization order			
1 (NPS → OPS → Saliva)	9,262 (33.3)	8,924 (33.3)	338 (34.1)
2 (OPS → Saliva → NPS)	9,265 (33.4)	8,910 (33.3)	355 (35.8)
3 (Saliva → NPS → OPS)	9,260 (33.3)	8,961 (33.4)	299 (30.1)
Prior positive test	1,209 (5.5)	1,181 (5.5)	28 (5.5)
Days since positive test[1]			
0-1 days (Part of initial testing)	66 (5.7)	63 (5.6)	3 (10.7)
2-60 days (Late infection stage)	88 (7.5)	87 (7.6)	1 (3.6)
>60 days (Reinfection)	1,013 (86.8)	989 (86.8)	24 (85.7)
Test reason			
Symptoms	1,178 (5.2)	1,149 (5.2)	29 (5.4)
Exposure to Covid-19	1,467 (6.8)	1,430 (6.8)	37 (7.3)
Screening	19,955 (99.6)	19,486 (99.6)	469 (99.8)
Days since first symptom[2]			
1 day	421 (36.2)	411 (36.3)	10 (35.7)
2-3 days	550 (47.3)	538 (47.4)	12 (42.9)
4-6 days	191 (16.5)	185 (16.3)	6 (21.4)
Vaccinated	1,820 (8.1)	1,777 (8.1)	43 (8.0)
Quarantine	2,198 (9.7)	2,151 (9.8)	47 (8.8)

[1]Among individuals with a prior positive test
[2]Among individuals with symptoms

Table S7. Number of specimen types with inconclusive RT-PCR test results

Distribution of inconclusive and conclusive test results stratified on specimen type.

OPS	NPS	SALIVA	Number of participants
+	+	+	26,795
	?	+	109
	+	?	259
	?	?	5
?	+	+	105
	?	+	1
	+	?	1
	?	?	0

+ denotes conclusive RT-PCR result, ? inconclusive RT-PCR results, NPS nasopharyngeal swab, OPS oropharyngeal swab, and Saliva saliva sampling

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Table S8. Exploratory subgroup analysis of detection rate of specimen types stratified by inclusion or exclusion of participants with inconclusive RT-PCR test results

Table S9.A detection ratefor RT–PCR testing of NPS, OPS, saliva, and OPS/NPS sampling based on participants (n=387) with confirmed SARS-CoV-2 infection, where inconclusive test results are considered as negative rather than excluding the test cases with one or more inconclusive test results

Specimen type	N (positive)	Detection rate	95% confidence interval
OPS	304	78.6	74.1; 82.5
NPS	281	72.6	67.9; 77.0
Saliva	237	61.2	56.2; 66.1
OPS/NPS	363	93.8	90.9; 96.0

Table S9.B detection ratefor RT–PCR testing of NPS, OPS, saliva, and OPS/NPS sampling based on participants (n=381) with confirmed SARS-CoV-2 infection, where participants are excluded if one or more of the test results are inconclusive

Specimen type	N (positive)	Detection rate	95% confidence interval
OPS	300	78.7	74.3; 82.7
NPS	277	72.7	67.9; 77.1
Saliva	236	61.9	56.9; 66.8
OPS/NPS	357	93.7	90.8; 95.9

Table S9. Exploratory subgroup analysis of RT-PCR using two N-genes positive definition

Diagnostic results (A) and comparison of detection rates (B) for NPS, OPS, saliva, and OPS/NPS when requiring a cycle threshold (Ct) < 34 of two N-gene (instead of a single N-gene) segments to have a positive test for SARS-CoV-2 infection, N=26,795 test cases.

A)

Specimen type	Positive	Negative	Detection rate (95% CI)
NPS	242	26,553	75.9% (70.8%; 80.5%)
OPS	275	26,520	86.2% (81.9%; 89.8%)
Saliva	203	26,592	63.6% (58.1%; 68.9%)
NPS/OPS	304	26,491	95.3% (92.4%; 97.3%)
Reference standard	319	26,476	

B)

Specimen type	**OR (95% CI)***
Two N-genes, N=26,795 test	
OPS vs. NPS	1.14 (1.06; 1.22)
NPS vs. saliva	1.19 (1.09; 1.31)
OPS vs. saliva	1.36 (1.25; 1.48)
OPS/NPS vs. saliva	1.50 (1.37; 1.65)
One N-gene, N=26,785 tests	
OPS vs. NPS	1.08 (1.00; 1.17)
NPS vs. saliva	1.18 (1.07; 1.29)
OPS vs. saliva	1.27 (1.18; 1.38)
OPS/NPS vs. saliva	1.52 (1.39; 1.66)

NPS: Nasopharyngeal specimen, OPS: Oropharyngeal specimen, saliva: Saliva specimen, Reference standard: one or more specimen types are positive, OR: Odds ratio, CI: confidence interval

*OR was estimated using generalized estimating equations (GEE) logistic regression.

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Table S10. Comparisons of detection rate (primary outcome) between specimen types (with changed definition of positive to Ct <25 for one N-target)

Comparisons of detection rate (primary outcome) between specimen types, N=254 tests cases with a positive gold standard

Specimen type	Δ Detection rate (95% CI)
OPS vs. NPS	13.0% (9.1; 17.8)
NPS vs. saliva	13.8% (9.8; 18.6)
OPS vs. saliva	26.8% (21.4; 32.7)
OPS/NPS vs. saliva	31.5% (25.8; 37.6)
OPS/NPS vs. OPS	4.7% (2.5; 8.1)
OPS/NPS vs. NPS	17.7% (13.2; 23.0)

NPS denotes nasopharyngeal swab, OPS oropharyngeal swab, Saliva saliva sampling, Δ percentage points difference between two detection rates, and CI confidence interval.

Table S11. Comparisons of detection rate (primary outcome) between specimen types (with changed definition of positive to Ct <25 for two N-target)

	Detection rate	95% CI
OPS	93.8%	90.0; 96.5
NPS	79.8%	74.1; 84.6
Saliva	57.4%	50.9; 63.8
OPS/NPS	97.9%	95.2; 99.3

Specimen type	**Δ sensitivity (95% CI)**
OPS vs. NPS	14.1% (9.9; 19.1)
NPS vs. saliva	22.3% (17.2; 28.1)
OPS vs. saliva	36.4% (30.3; 42.8)
OPS/NPS vs. saliva	40.5% (34.3; 47.0)
OPS/NPS vs. OPS	4.1% (2.0; 7.5)
OPS/NPS vs. NPS	18.2% (13.5; 23.6)

NPS denotes nasopharyngeal swab, OPS oropharyngeal swab, Saliva saliva sampling, Δ percentage points difference between two detection rates, and CI confidence interval.

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Table S12. Detection rate stratified by days since positive test

Detection rate (95% CI)	**Days since a positive test**	
	≤60 days (n=59)	**>60 days (n=19)**
OPS	81.4% (69.1; 90.3)	36.8% (16.3; 61.6)
NPS	96.6% (88.3; 99.6)	68.4% (43.5; 87.4)
Saliva	71.2% (57.9; 82.2)	10.5% (1.3; 33.1)
OPS/NPS	100.0% (93.9; 100.0)	100.% (82.4; 100.0)

The SARS-CoV-2 detection rate among the 78 postive participants (Ct<34, one N-target) who answered to have had a prior COVID-19 infection before the enrollment in the SAMPLE trial (59 <= days 60 and 19 > 60 days).

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Table S13. Prior positive registered in the Danish Microbiology Database

SAMPLE participants defined as positive (Ct < 34 of one N-gene from ≤ 1 respiratory specimen) with prior PCR or antigen test for SARS-CoV-2 registered in the Danish Microbiology Database (MiBa)

Days from prior positive test	**Definition**	Test type			**Total**	**Proportion of total number positive (n=381)**
		Antigen test	**RT–PCR**	**RT–PCR + Antigen test**		
0-1	Part of initial testing	47	3	1	51	13.4%
2-60	Retesting during late phase of SARS-CoV-2 infection	7	21	1	29	7.6%
>60	Reinfection	0	19	0	19	5.0%
Total		54	43	2		

Among the SARS-CoV-2 positive SAMPLE participants, 51 (13.4%) had a prior positive test 0-1 day before enrolment in the study. Further, 29 (7.6%) had a prior positive test 2-60 days before enrolment in the study and 19 (5%) had a positive test >60 days before inclusion why these were categorized as having a reinfection.

Table S14: Demographics and baseline characteristics for participants randomised to each sampling arm.

	Randomization		
	1 N=8,924	2 N=8,910	3 N=8,961
Age, mean (SD)	42.3 (15.2)	42.2 (15.2)	42.1 (15.3)
Female sex	4,477 (50.2%)	4,488 (50.4%)	4,584 (51.2%)
Vaccination status			
Vaccinated	581 7.9(%)	604 (8.3%)	592 (8.0%)
Not vaccinated	6,766 (92.1%)	6,720 (91.7%)	6,799 (92.0%)
Missing	1,577	1,586	1,570
Test center			
Valby	6,352 (71.2%)	6,326 (71.0%)	6,372 (71.1%)
Taastrup	2,572 (28.8%)	2,584 (29.0%)	2,589 (28.9%)
Prior positive test			
Yes	386 (5.4%)	379 (5.3%)	416 (5.8%)
No	6,724 (94.6%)	6,737 (94.7%)	6,732 (94.2%)
Missing	1,814	1,794	1,813
Symptoms			
Yes	407 (5.5%)	358 (4.9%)	384 (5.2%)
No	6,974 (94.5%)	6,992 (95.1%)	7,031 (94.8%)
Missing	1,543	1,560	1,546

Table S15: Distribution of test positive results for test specimens stratified by randomization and test center

Distribution of test positive results for test specimens stratified by randomization and test center, N=381

		N	Positive test result, N (%)		
			NPS N=277	OPS N=300	Saliva N=236
Randomization					
	1: NPS → OPS → Saliva	130	93 (71.5%)	110 (84.6%)	87 (66.9%)
	2: OPS → Saliva → NPS	118	75 (63.6%)	90 (76.3%)	63 (53.4%)
	3: Saliva → NPS → OPS	133	109 (82.0%)	100 (75.2%)	86 (64.7%)
Test center					
	1	287	211 (73.5%)	225 (78.4%)	176 (61.3%)
	2	94	66 (70.2%)	75 (79.8%)	60 (63.8%)

Distribution of test positive results for specimens for Testcenter 1 stratified by randomization

TEST CENTER 1		N	Positive test result, N (%)		
			NPS N= 211	OPS N= 225	Saliva N= 176
Randomization					
	1: NPS → OPS → Saliva	102	74 (72.5%)	85 (85%)	68 (68%)
	2: OPS → Saliva → NPS	84	55 (65.4%)	65 (77.3%)	43 (51.1%)
	3: Saliva → NPS → OPS	101	82 (81%)	75 (74.2%)	65 (64.3%)

Distribution of test positive results for specimens for Testcenter 2 stratified by randomization

TEST CENTER 2		N	Positive test result, N (%)		
			NPS N=66	OPS N=75	Saliva N=60
Randomization					
	1: NPS → OPS → Saliva	28	19 (67.9%)	25 (89.2%)	19 (67.9%)
	2: OPS → Saliva → NPS	34	20 (58.8%)	25 (73.5%)	20 (58.8%)
	3: Saliva → NPS → OPS	32	27 (84.3%)	25 (78.1%)	21 (65.6%)

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Table S16. The distributions of SARS-CoV-2 variants of concern

The distributions of SARS-CoV-2 variants and percentage of total (%)

Varians	Total	OPS	NPS	Saliva
Alpha	252 (66%)	234 (93%)	207 (82%)	200 (79%)
Delta	27 (7%)	24 (89%)	22 (81%)	18 (67%)
Eta	2 (1%)	1 (50%)	2 (100%)	1 (50)
Unknown	100 (26%)	41 (41%)	46 (46%)	17 (17%)
Total	381	300	277	236

OPS: Oropharyngeal swab, NPS: Nasopharyngeal swab, Saliva: Saliva sampling

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References

1. Busk PK, Kristiansen TB, Engsig-Karup A. Assessment of the National Test Strategy on the Development of the COVID-19 Pandemic in Denmark. Epidemiologia. 2021;2(4):540-52.
2. The Danish Ministry of Health. Regeringen Tilpasser Teststrategi til Gradvis Genåbning af Samfundet. Available online: https://sum.dk/nyheder/2021/marts/regeringen-tilpasser-teststrategi-til-gradvis-genaabning-af-samfundet (accessed on 02 Januar 2022).
3. Todsen T, Tolsgaard M, Folke F, Jakobsen KK, Ersbøll AK, Benfield T, et al. SARS-CoV-2 in saliva, oropharyngeal and nasopharyngeal specimens. Dan Med J. 2021;68(5).
4. Jensen MP, Karoly P, Braver S. The measurement of clinical pain intensity: a comparison of six methods. Pain. 1986;27(1):117-26.
5. Voldstedlund M, Haarh M, Molbak K, MiBa Board of R. The Danish Microbiology Database (MiBa) 2010 to 2013. Euro Surveill. 2014;19(1).
6. Hansen CH, Michlmayr D, Gubbels SM, Mølbak K, Ethelberg S. Assessment of protection against reinfection with SARS-CoV-2 among 4 million PCR-tested individuals in Denmark in 2020: a population-level observational study. The Lancet. 2021;397(10280):1204-12.
7. European Centre for Disease Prevention and Control. Reinfection with SARS-CoV-2: implementation of a surveillance case definition within the EU/EEA. 8 April 2021. ECDC: Stockholm, 2021.
8. Organization WH. Diagnostic testing for SARS-CoV-2: Interim guidance [Available from: https://apps.who.int/iris/bitstream/handle/10665/334254/WHO-2019-nCoV-laboratory-2020.6-eng.pdf?sequence=1&isAllowed=y.
9. Bastos ML, Perlman-Arrow S, Menzies D, Campbell JR. The Sensitivity and Costs of Testing for SARS-CoV-2 Infection With Saliva Versus Nasopharyngeal Swabs : A Systematic Review and Meta-analysis. Ann Intern Med. 2021.
10. Sananes N, Lodi M, Koch A, Lecointre L, Sananes A, Lefebvre N, et al. 3D-printed simulator for nasopharyngeal swab collection for COVID-19. Eur Arch Otorhinolaryngol. 2020.
11. Todsen T, Bohr A, Hovgaard LH, Eið RC, Benfield T, Svendsen MBS, et al. Valid and Reliable Assessment of Upper Respiratory Tract Specimen Collection Skills during the COVID-19 Pandemic. Diagnostics. 2021;11(11).
12. CDC 2019-Novel Coronavirus (2019-nCoV) Real-Time RT-PCR Diagnostic Panel. Centers for Disease Control and Prevention. (https://www.fda.gov/media/134922/download).
13. Vogels CBF, Brito AF, Wyllie AL, Fauver JR, Ott IM, Kalinich CC, et al. Analytical sensitivity and efficiency comparisons of SARS-CoV-2 RT-qPCR primer-probe sets. Nat Microbiol. 2020;5(10):1299-305.
14. WHO. Tracking SARS-CoV-2 variants [Available from: https://www.who.int/en/activities/tracking-SARS-CoV-2-variants/.
15. Jørgensen TS, Blin K, Kuntke F, Salling HK, Michaelsen TY, Albertsen M, et al. A rapid, cost efficient and simple method to identify current SARS-CoV-2
2 variants of concern by Sanger sequencing part of the spike protein gene. medRxiv preprint. 2021.

16. Groot Koerkamp B, Weinstein MC, Stijnen T, Heijenbrok-Kal MH, Hunink MG. Uncertainty and patient heterogeneity in medical decision models. Med Decis Making. 2010;30(2):194-205.
17. Doubilet P, Begg CB, Weinstein MC, Braun P, McNeil BJ. Probabilistic sensitivity analysis using Monte Carlo simulation. A practical approach. Med Decis Making. 1985;5(2):157-77.
18. O'Hagan A, McCabe C, Akehurst R, Brennan A, Briggs A, Claxton K, et al. Incorporation of uncertainty in health economic modelling studies. PharmacoEconomics. 2005;23(6):529-36.
20. Hui SL, Walter SD. Estimating the error rates of diagnostic tests. Biometrics. 1980;36(1):167-71.
21. Health WOfA. Manual of Diagnostic Tests and Vaccines for Terrestrial Animals. Chapter 1.1.6 – Principles and Methods of Validation of Diagnostic Assays for Infectious Diseases. http://wwwoieint/en/international-standard-setting/terrestrial-manual/access-online/ 2016