Self-Collected Oral Fluid and Nasal Swab Specimens Demonstrate Comparable Sensitivity to Clinician-Collected Nasopharyngeal Swab Specimens for the Detection of SARS-CoV-2

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Abstract

We compared self-collected oral fluid swab specimens with and without clinician supervision, clinician-supervised self-collected mid-turbinate (nasal) swab specimens, and clinician-collected nasopharyngeal swab specimens for the detection of SARS-CoV-2. Supervised oral fluid and nasal swab specimens performed similarly to clinician-collected nasopharyngeal swab specimens. No sample type could detect SARS-CoV-2 infections amongst all positive participants.

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MAIN TEXT

The 2019 severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), which causes COVID-19, was first detected in Wuhan, China in late 2019 [1]. On 20 January 2020, the first case of COVID-19 was reported in the United States [2]. After more than 118,000 cases were detected in 114 countries with over 4,000 deaths, the World Health Organization declared COVID-19 pandemic [3].

The ideal specimen for the detection of SARS-CoV-2 is unknown. Currently, trained health care professionals and specialized collection devices are recommended for the collection of nasopharyngeal swab specimens [4]. This requires staffing of health care workers, who could be performing other duties, and the use of personal protective equipment (PPE), during a severe shortage. Additionally, patients report discomfort during nasopharyngeal swab specimen collection, which may deter patients from being tested [5]. The use of mid-turbinate (nasal) swab and oral fluid specimens could potentially greatly increase health worker safety and the number of persons tested. We recruited participants recently tested for SARS-CoV-2 to assess differences in specimen types and collection methods for SARS-CoV-2 testing.

Methods

We recruited participants that recently tested for SARS-CoV-2 at a CLIA-certified, high-complexity laboratory. The patient population and recruitment methods are described below.

Testing population

We recruited non-hospitalized persons tested for SARS-CoV-2 in Los Angeles County, California, that included symptomatic adults older than age 65, those with a chronic disease, first responders, and law enforcement officers that may have been exposed to SARS-CoV-2. We aimed to recruit 30 persons that tested negative for SARS-CoV-2 and 30 persons that

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tested positive. Participants were contacted via telephone or email and provided with details of the study. Participants were given a study information sheet and gave verbal informed consent.

Specimen collection methods

We obtained unsupervised self-collected oral fluid swab specimens, clinician-supervised self-collected oral fluid swab specimens, clinician-supervised self-collected mid-turbinate (nasal) swab specimens, and clinician-collected posterior nasopharyngeal swab specimens.

For the unsupervised self-collected oral fluid swab specimens, we provided written instructions with the testing kit, which included a sterile swab and a tube with an RNA preservative media (DNA/RNA Shield™ solution, Zymo Research Corp., Irvine, CA, USA). Participants were instructed to cough deeply three to five times collecting any phlegm or secretions in their mouth, rub the swab on both cheeks, above and below the tongue, both gums, and on the hard palate for a total of 20 seconds to ensure the swab was saturated with oral fluid. Following that, participants were instructed to place the swab into the tube, secure the lid, invert the tube three to five times, and place the capped tube into a collection bag. Unsupervised specimen collection was observed by a clinician but from a greater distance than the supervised collections, and the clinician did not provide any feedback to the participant. For the clinician-supervised self-collected oral fluid swab specimens, the same instructions were provided and a clinician provided real time feedback. Without clinician feedback, some unsupervised patients did not cough before self-collecting their sample.

For the clinician-supervised self-collected nasal swab specimen, a kit was provided that included a flocked swab (Copan Diagnostics, Murrieta, CA, USA) and the same collection

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media as described above The participant was verbally instructed to insert the swab into one nostril to the depth of three to four cm, rotate the swab for five to ten seconds, place the swab into the collection tube, invert the tube three to five times, and place the capped tube into a collection bag. Posterior nasopharyngeal swab specimens were collected by a clinician with the recommended medical technique using nasopharyngeal swabs (Becton Dickinson and Company, Franklin Lakes, NJ, USA) [6].

Surveying and sampling

We collected samples in private areas of participant homes. We collected symptom data immediately prior to sampling. Sampling methods are detailed above. For each patient, all samples were collected within a 30-minute window. Samples were transported to the laboratory at ambient temperature for testing on the day of collection.

Specimen extraction and testing

We processed samples from the specimen collection tubes. We lysed and extracted RNA from samples (RNA purification kit, Norgen Biotek Corp., Thorold, ON, Canada) using an automated instrument (Resolvex A200, Tecan Group Ltd., Zürich, Switzerland) on a 96-well plate. We used a reverse transcription-quantitative polymerase chain reaction (RT-qPCR) assay that utilized a single color TaqMan probe with a modified version of the qualitative detection of SARS-CoV-2 (N1, N2 primer/probe assay) designed and validated by the Centers for Disease Control and Prevention (CDC) (Integrated DNA Technologies, Coralville, IA, USA) [7]. We recorded cycle threshold values for tests. We detected human Ribonuclease P RNA with an additional single color TaqMan assay, in a parallel reaction using an aliquot of the extracted participant specimen to serve as a control for specimen extraction, specimen adequacy, and RT-PCR inhibition. We ran samples on an RT-qPCR

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System (CFX 96™ Touch RT-PCR Detection System or CFX 96™ Connect RT-PCR Detection System, Bio-Rad, Hercules, CA, USA).

Ethics statement

The Institutional Review Board of the University of California Los Angeles reviewed and approved the study (reference number 20-000545).

Results

We recruited 45 participants. The median age of study participants was 42 years (Interquartile range [IQR], 31 to 52 years). Of the participants, 29 tested positive for SARS-CoV-2 viral RNA in at least one specimen. All 29 participants that tested positive for COVID-19 in at least one specimen had prior symptoms. Of the participants, 23 (51%) of 45 participants reported active symptoms; 21 of those 23 had COVID-19. Symptoms and likely transmission source are documented in the Supplemental Table.

Overall, we collected 180 specimens from 45 participants. Of those specimens, one specimen was lost and two specimens had insufficient sample for laboratory analysis. Therefore, 177 specimens yielded results (Figure). Clinician-supervised oral fluid swab specimens detected 26 (90%) of 29 infected individuals, clinician-supervised nasal swab specimens detected 23 (85%) of 27, clinician-collected posterior nasopharyngeal swab specimens detected 23 (79%) of 29, and unsupervised self-collected oral fluid swab specimens detected 19 (66%) of 29. There was no difference in testing performance when comparing those with and without active symptoms.

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When comparing cycle threshold values, clinician-collected posterior nasopharyngeal swab specimens had an average cycle threshold value of 25.88 (standard deviation (SD): 5.90; Supplemental Figure), clinician-supervised self-collected nasal swab specimens had an average cycle threshold value of 30.49 (SD: 5.59), clinician-supervised self-collected oral fluid swab specimens had an average cycle threshold value of 34.13 (SD: 3.63), and unsupervised self-collected oral fluid swab specimens had an average cycle threshold value of 33.48 (SD: 3.26).

Discussion

We found that clinician-supervised self-collected specimens for SARS-CoV-2 detection were feasible. No single specimen type identified all participants with COVID-19. The performance of clinician-supervised self-collected oral fluid and nasal swab specimens was similar to clinician-collected nasopharyngeal swab specimens. Unsupervised self-collected oral fluid swab specimens performed worse in this study sample.

The CDC currently recommends the use of nasopharyngeal or oropharyngeal swab specimens either collected by a health care worker or self-collected mid-turbinate or anterior nares samples in symptomatic patients in a health care setting, including a supervised drive-through setting, if nasopharyngeal swab specimens are not available [4]. Prior studies reported that SARS-CoV-2 detection was similar among oral fluid and mid-turbinate specimens when compared to nasopharyngeal swabs specimens [8, 9]. It was found in one of those studies that multiple anatomic site testing may improve the sensitivity and reduce false-negative test results.

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There is an urgent need to validate reliable specimen collection methods for the detection of SARS-CoV-2 to increase access to safe and easy testing. Our findings support that clinician-collected posterior nasopharyngeal swab specimens has a similar testing sensitivity to clinician-supervised self-collected oral fluid and clinician-supervised self-collected nasal swab specimens for the detection of SARS-CoV-2. Further research on other supervised means of collection, such as video-based instructions or observation and feedback via telehealth, is warranted.

In our sample, there were 6 cases of COVID-19 detected among oral fluid swab specimens, which were not detected in clinician-collected nasopharyngeal swab specimens. It is possible that the detection of SARS-CoV-2 may differ at anatomic sites based on the timing of infection [10, 11]. There were also 3 cases of COVID-19 detected among nasopharyngeal specimens not detected in oral fluid swab specimens. That suggests that testing any single anatomic site may miss some cases of COVID-19, which is consistent with a prior study [12]. We did not find significant differences in cycle threshold values between groups. Prior studies have found that oral fluid provides a similar sensitivity to nasopharyngeal swabs [13-16], particularly when combined with coughing before specimen collection to provide an upper respiratory tract sample.

We found that unsupervised self-collected oral fluid swab specimens detected SARS-CoV-2 in fewer patients than other specimen types, and this discrepancy was unexpected. We observed that without feedback, some unsupervised participants did not cough before self-collecting their sample. A pre-printed study reported that after bronchoalveolar lavage fluid, which is not feasible in the outpatient setting, sputum samples showed the highest positive rate in all stages following a SARS-CoV-2 infection, followed by nasal swabs [17]. Coughing was included as part of this specimen collection protocol and may provide a sputum

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specimen in the oral fluid specimen in addition to saliva. Laboratory studies and a case series have indicated that oral fluid collected after a participant coughs are reliable specimens [13, 15]. This study suggests that coughing may be a critical step when collecting oral fluid swab specimens for the detection of SARS-CoV-2.

Our report has several strengths. We were able to perform self-collected specimen collection for COVID-19 testing. We studied multiple sample types and collection methods, including unsupervised self-collected specimens and clinician-supervised self-collected specimens. Clinician-collected nasopharyngeal specimens were collected in all patients for comparison. All samples were tested at a CLIA-certified, high-complexity laboratory with a validated FDA-authorized COVID-19 assay.

However, our study had a limited sample size due to the current shortage of testing supplies. Our study was not designed to detect statistical differences between specimen types or collection methods. Given the urgency of obtaining results, recruitment took place over a short period.

Conclusions

Supervised self-collected oral fluid and nasal swab specimens performed similarly to clinician-collected nasopharyngeal swab specimens for the detection of SARS-CoV-2. No sample type captured all infections. Supervised self-collected methods were feasible and could enable widespread access to testing by removing the need for a healthcare professional to collect each sample, reducing potential exposure for healthcare professionals and reducing the amount of PPE used for testing.

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Declarations

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Disclaimer

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Potential conflicts:

V.S. is the CSO of Curative Inc. and holds stock in the company. N.K. reports working as a research consultant for Curative Inc. F.T. is the CEO of Curative Inc. and holds stock in the company. All other authors have no potential conflicts.

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Figure Legend

Figure 1: COVID-19 detection in self-collected unsupervised and clinician-supervised oral fluid swab specimens, clinician-supervised self-collected nasal swab specimens, clinician-collected posterior nasopharyngeal swab specimens, and pooled results with current symptom status

QNS: Quantity Not Sufficient; +: positive; -: negative

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Symptomatic	Days of symptoms	Unsupervised Self-Collected Oral Fluid	Supervised Self-Collected Oral Fluid	Supervised Self-Collected Nasal Specimen	Clinician-Collected NP	Any Positive
No	18	+	+	QNS	+	+
Yes	7	+	+	QNS	+	+
Yes	21	+	+	+	+	+
Yes	15	+	+	+	+	+
Yes	12	+	+	+	+	+
Yes	10	+	+	+	+	+
Yes	9	+	+	+	+	+
Yes	8	+	+	+	+	+
Yes	8	+	+	+	+	+
Yes	7	+	+	+	+	+
Yes	7	+	+	+	+	+
Yes	6	+	+	+	+	+
Yes	4	+	+	+	+	+

Yes	2	+	+	+	+	+
No	17	-	+	+	+	+
No	14	-	+	+	+	+
Yes	17	-	+	+	+	+
Yes	14	-	+	+	+	+
Yes	5	-	+	+	+	+
Yes	15	+	-	+	+	+
No	7	-	-	+	+	+
Yes	18	-	+	-	+	+
Yes	9	-	-	-	+	+
Yes	17	+	+	+	-	+
No	16	+	+	+	-	+
No	N/A	+	+	+	-	+
No	5	+	+	+	-	+
No	N/A	-	+	-	-	+
Yes	13	-	+	-	-	+
No	N/A	QNS	-	-	-	-
Yes	10	-	-	-	-	-
Yes	7	-	-	-	-	-
No	N/A	-	-	-	-	-

No	N/A	-	-	-	-	-
No	N/A	-	-	-	-	-
No	N/A	-	-	-	-	-
No	N/A	-	-	-	-	-
No	N/A	-	-	-	-	-
No	N/A	-	-	-	-	-
No	N/A	-	-	-	-	-
No	N/A	-	-	-	-	-
No	N/A	-	-	-	-	-
No	N/A	-	-	-	-	-
No	N/A	-	-	-	-	-
No	N/A	-	-	-	-	-
No	N/A	-	-	-	-	-

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Figure 1

Symptomatic	Days of symptoms	Unsupervised Self-Collected Oral Fluid	Supervised Self-Collected Oral Fluid	Supervised Self-Collected Nasal Specimen	Clinician-Collected NP	Any Positive
No	18	+	+	QNS	+	+
Yes	7	+	+	QNS	+	+
Yes	21	+	+	+	+	+
Yes	15	+	+	+	+	+
Yes	12	+	+	+	+	+
Yes	10	+	+	+	+	+
Yes	9	+	+	+	+	+
Yes	8	+	+	+	+	+
Yes	8	+	+	+	+	+
Yes	7	+	+	+	+	+
Yes	7	+	+	+	+	+
Yes	6	+	+	+	+	+
Yes	4	+	+	+	+	+
Yes	2	+	+	+	+	+
No	17	-	+	+	+	+
No	14	-	+	+	+	+
Yes	17	-	+	+	+	+
Yes	14	-	+	+	+	+
Yes	5	-	+	+	+	+
Yes	15	+	-	+	+	+
No	7	-	-	+	+	+
Yes	18	-	+	-	+	+
Yes	9	-	-	-	+	+
Yes	17	+	+	+	-	+
No	16	+	+	+	-	+
No	N/A	+	+	+	-	+
No	5	+	+	+	-	+
No	N/A	-	+	-	-	+
Yes	13	-	+	-	-	+
No	N/A	QNS	-	-	-	-
Yes	10	-	-	-	-	-
Yes	7	-	-	-	-	-
No	N/A	-	-	-	-	-
No	N/A	-	-	-	-	-
No	N/A	-	-	-	-	-
No	N/A	-	-	-	-	-
No	N/A	-	-	-	-	-
No	N/A	-	-	-	-	-
No	N/A	-	-	-	-	-
No	N/A	-	-	-	-	-
No	N/A	-	-	-	-	-
No	N/A	-	-	-	-	-
No	N/A	-	-	-	-	-
No	N/A	-	-	-	-	-
No	N/A	-	-	-	-	-