SARS-CoV-2 detection on self-collected saliva or anterior nasal specimens compared with healthcare personnel-collected nasopharyngeal specimens

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Summary: Self-collected saliva and anterior nasal specimens (ANS) were evaluated for SARS-CoV-2 detection by rRT-PCR against reference nasopharyngeal specimens in 730 participants. Sensitivity in saliva and ANS was high overall (≥80%) and among participants with symptoms (≥87%) or culturable virus (≥94%).

Abstract

Background. Nasopharyngeal specimens (NPS) are commonly used for SARS-CoV-2 testing but can be uncomfortable for patients. Self-collected saliva or anterior nasal specimens (ANS) for SARS-CoV-2 detection are less invasive but the sensitivity of these specimen types has not been thoroughly evaluated.

Methods. During September–November 2020, 730 adults undergoing SARS-CoV-2 testing at community testing events and homeless shelters in Denver provided self-collected saliva and ANS specimens before NPS collection and answered a short survey about symptoms and specimen preference. Specimens were tested for SARS-CoV-2 by rRT-PCR; viral culture was performed on a subset of specimens positive by rRT-PCR. Sensitivity of saliva and ANS for SARS-CoV-2 detection by rRT-PCR was measured against NPS. Subgroup analyses included test outcomes by symptom status and culture results.

Results. Sensitivity for SARS-CoV-2 detection by rRT-PCR appeared higher for saliva than for ANS (85% vs. 80%) and among symptomatic participants than among those without symptoms (94% vs. 29% for saliva; 87% vs. 50% for ANS). Among participants with culture-positive SARS-CoV-2 by any specimen type, sensitivity of saliva and ANS by rRT-PCR was 94% and 100%, respectively. Saliva and ANS were equally preferred by participants; most would undergo NPS again despite being least preferred.

Conclusions. Saliva was slightly more sensitive than ANS for SARS-CoV-2 detection by rRT-PCR. Both saliva and ANS reliably detected SARS-CoV-2 among participants with symptoms. Self-collected saliva and ANS offer practical advantages, are preferred by patients, and might be most useful for testing people with COVID-19 symptoms.

Key words: SARS-CoV-2; COVID-19; nasopharyngeal; anterior nasal; saliva

BACKGROUND

Testing for SARS-CoV-2, the virus that causes COVID-19, was first authorized to be performed on multiple specimen types as part of the CDC 2019-Novel Coronavirus (2019-nCoV) Real-Time Reverse Transcription Polymerase Chain Reaction (rRT-PCR) Diagnostic Panel [1], with nasopharyngeal specimens (NPS) being the most widely used specimen type [2]. NPS can be uncomfortable for the patient and should be performed by a trained healthcare professional in appropriate personal protective equipment (PPE) [3], as the procedure can generate infectious aerosols from sneezing and coughing caused by irritation of the nasopharynx [4]. Self-collected anterior nasal specimens (ANS) or saliva for SARS-CoV-2 detection could decrease patient discomfort during specimen collection, decrease the need for trained healthcare professionals to perform specimen collection, conserve PPE, reduce testing-associated transmission risk, and improve testing uptake [4-6].

Prior studies have suggested that rRT-PCR testing of ANS and saliva can reliably detect SARS-CoV-2 in patients with COVID-19 [7-9] though these specimen types have not been systematically evaluated as screening tools in high-volume testing events or in congregate settings such as homeless shelters. To evaluate if testing of self-collected ANS or saliva specimens might accurately and reliably detect SARS-CoV-2 in real-life settings, participants were enrolled during testing events in communities in Denver, Colorado that are disproportionately affected by the COVID-19 pandemic. Participants answered questions about symptoms and specimen preference after providing ANS, saliva, and NPS. Test performances for SARS-CoV-2 detection by rRT-PCR for self-collected ANS and saliva were compared to the standard healthcare personnel-obtained NPS. To understand the relationship between test sensitivity by specimen type and presence of culturable virus, a

subset of paired NPS and ANS specimens from participants who tested positive for SARS-CoV-2 by rRT-PCR was sent for viral culture. This multilayered evaluation was designed to inform programmatic decisions about whether the less invasive specimens of saliva and ANS might be suitable alternatives to the traditional NPS for testing and screening programs in community and congregate living settings.

METHODS

Project Design, Setting, and Population

We performed a cross-sectional evaluation of adults seeking SARS-CoV-2 testing at testing events held by Denver Public Health in Denver, Colorado during September–November 2020. Testing events took place at walk-up or drive-up sites in communities disproportionately affected by the pandemic and at shelters for people experiencing homelessness. We enrolled both symptomatic and asymptomatic participants. Participant inclusion criteria were (1) attending a SARS-CoV-2 testing event; (2) ≥18 years of age at date of testing; (3) able and willing to provide informed consent; and (4) willing to comply with study procedures. We excluded participants who reported receiving a previous positive SARS-CoV-2 test. This project was determined to be not human subject research by the Colorado Multiple Institutional Review Board (IRB) and by the Colorado Department of Public Health and Environment (CDPHE) IRB as a public health surveillance activity. This activity was reviewed by CDC and was conducted consistent with applicable federal law and CDC policy.[§] All participants provided verbal informed consent prior to enrollment.

Specimen Collection and Transport

Trained healthcare personnel observed and coached participants to self-collect an ANS (inserting a polyester foam swab 1.0–1.5 cm and rotating it for 10–15 seconds in each nostril) and to provide a saliva specimen (1–5 mL) by spitting several times into a sterile container [3]. Healthcare personnel then collected NPS (mini-tip flocked polyester swab inserted through one naris to the nasopharynx and rotated for 5 seconds) [3]. ANS were immediately placed into a sterile transport tube containing 2–3 mL of sterile saline; NPS were immediately placed into a sterile transport tube containing 2–3 mL of viral transport medium (AccuViral Collection Kit™). All specimens were placed immediately in a cooler with ice packs. On the same day of collection, after each testing event, coolers were transported to the CDPHE laboratory and moved to 4°C refrigerators until processing within 72 hours of collection [3].

Participant Survey

After specimen collection, trained interviewers asked structured survey questions from participants in English or Spanish. Questions included demographic characteristics, detailed assessment for COVID-19 symptoms, whether participants had close contact with a known case of COVID-19 in the past 2 weeks, which specimen type they preferred, and whether they would agree to be tested again by specimen type (Supplement Figure 1). COVID-19 symptoms in this investigation were defined as reports of new or worsening fever (measured or subjective) or chills, cough, shortness of breath or difficulty breathing, fatigue, myalgia, headache, anosmia or ageusia, sore throat, congestion or nasal discharge, nausea or vomiting, or diarrhea [10]. Data were entered into Research Electronic Data Capture (REDCap) software (Vanderbilt).

Testing for SARS-CoV-2 by rRT-PCR

All specimens were processed and tested for SARS-CoV-2 at the CDPHE laboratory using the CDC 2019-Novel Coronavirus (2019-nCoV) Real-Time RT-PCR Diagnostic Panel assay protocol [1]. Nucleic acid extraction was performed according to the instructions for use authorized by the U.S. Food and Drug Administration for the assay. Saliva specimens were vortexed for one minute, then incubated at room temperature for 30 seconds before removing the slightly separated supernatant from a viscous bottom layer; nucleic acid extraction was performed on the supernatant. Results were considered positive for SARS-CoV-2 when cycle threshold (Ct) values for the viral nucleocapsid protein genes N1 and N2 were <40.00 [11]. Ct values from rRT-PCR tests are inversely correlated to the amount of viral genetic material present in the specimen [12].

Testing for SARS-CoV-2 by Viral Culture

A subset of paired ANS and NPS from participants who tested positive for SARS-CoV-2 by rRT-PCR by one or more specimen type was placed in cryovial boxes on ice packs and shipped to CDC for viral culture. Given limited viral culture testing capacity (maximum 100 specimens) and because of the practical challenges of processing saliva specimens for culture [13], only ANS and NPS were submitted for viral culture. Specimens prioritized for this subset included 1) participants with discrepant results by rRT-PCR (e.g., a participant who tested positive by NPS but negative by ANS or saliva, or a participant who tested negative by NPS but positive by ANS or saliva) and 2) specimens with the lowest Ct values. These criteria were systematically applied until the allowed quantity of 100 specimens was identified. Viral culture was performed using Vero-CCL-81 cells as previously described [14]. rRT-PCR was performed on specimens that developed cytopathic effect to confirm isolation

of infectious virus in culture. Recovery of infectious virus was confirmed if the Ct of the recovered isolate was at least 2 Ct lower than the clinical specimen [15].

Statistical Analyses

Analyses were performed to evaluate and compare test performance of self-collected ANS and saliva specimens with NPS. Sensitivity, specificity, positive and negative predictive values (PPV and NPV, respectively), and likelihood ratios positive and negative (LRP and LRN, respectively), were computed for ANS and saliva using NPS as the reference standard; corresponding 95% score confidence intervals (CI) were calculated for each measure. Sensitivity and 95% CI of ANS or saliva for detection of SARS-CoV-2 by rRT-PCR were also computed, using culturable virus on any specimen as the reference standard. Not considering NPS as the reference standard, Cohen's kappa statistics (95% bootstrap CIs) were computed as measures of agreement between ANS and NPS, and between saliva and NPS. Pearson's correlations and 95% CI were calculated between rRT-PCR genetic targets N1 and N2 on ANS and NPS. Receiver-operating-characteristic (ROC) analysis of rRT-PCR Ct values was used to identify the cutoff Ct value which yielded maximum equal sensitivity and specificity. All statistical analyses were conducted using R software (version 4.0.2; Vienna, Austria).

RESULTS

Of 730 total participants enrolled, 452 (61.9%) were enrolled at 10 community testing events and 278 (38.0%) at seven homeless shelter testing events. Age, gender, and race and ethnicity distributions differed among participants enrolled at community sites and homeless shelters (Table 1). Participants enrolled at community sites tended to be younger (54.2% vs 30.6% aged ≤40 years) and more often female (57.3% vs 16.2%) compared to

participants enrolled at homeless shelters. Hispanic ethnicity was more commonly reported by participants at community sites (44.0%) compared to participants at homeless shelters (20.9%); White, non-Hispanic race/ethnicity were more commonly reported by participants at homeless shelters (51.8%), compared to participants at community sites (39.2%). Overall, 36.8% of participants reported one or more symptom(s) consistent with COVID-19 [10] at the time of testing, with a much higher proportion of symptomatic participants enrolled at community sites (47.8%) than at homeless shelters (19.1%). Close contact with a person with confirmed COVID-19 was reported by 33.0% of participants at community sites compared to 3.6% of participants at homeless shelters. In total, 84 (11.5%) participants were positive for SARS-CoV-2 by rRT-PCR by at least one specimen type; 82 (97.6%) were enrolled at community sites.

Specimen Type Preference and Willingness to be Tested Again by Specimen Type

Saliva was the most preferred specimen type overall (45.9%), followed by ANS (37.3%), and NPS (13.4%). Specimen type preference did not differ by testing site type. The majority of participants from both sites indicated they would be willing to be tested again by ANS (98.5% at community sites and 93.2% at homeless shelters), saliva (98.2% at community sites and 94.6% at homeless shelters), and NPS (89.2% at community sites and 85.6% at homeless shelters) if each were the only specimen type being collected for testing.

Testing Performance of ANS and Saliva by rRT-PCR

Conclusive (positive or negative) results were available for 544 saliva, 609 ANS, and 665 NPS specimens; 467 (64.0%) participants had conclusive results for all 3 specimen types (Figure 1). Compared to NPS, overall sensitivity for detection of SARS-CoV-2 by rRT-PCR on saliva specimens was 85.2% (95% CI 73.4 – 92.3%) and for ANS was 80.0% (95% CI 68.7 – 87.9%) (Table 2). Sensitivity was higher among participants reporting at least one COVID-19

symptom at the time of specimen collection (93.6% [95% CI 82.8 – 97.8%] for saliva; 86.8% [95% CI 75.1 – 93.5%] for ANS) and also when results from saliva and ANS were combined (87.5% [95% CI 77.2 – 93.5%]). Among asymptomatic participants, sensitivity was low (28.5% for saliva; 50.0% for ANS). Compared to NPS, specificity and NPV were ≥96% for both saliva and ANS, including among asymptomatic participants. Analysis of test performance by testing location type was limited by low numbers of positive tests among participants at homeless shelters during the study period (Supplement Table). Using Cohen's kappa coefficient, agreement between saliva and NPS was 0.85 (95% CI 0.77 – 0.93) and between ANS and NPS was 0.83 (0.76 – 0.91).

Comparison of Mean Ct Values by Specimen Type

For all participants, Ct values for the N1 and N2 rRT-PCR targets were strongly correlated (r = 0.994 [95% CI 0.990 – 0.996]) (Supplement Figure 2); thus, only N1 Ct values are reported. Among participants with a valid result by all three specimen types and with SARS-CoV-2 detected in the NPS, mean Ct values of NPS were lower (indicating a higher concentration of target nucleic acid in the specimen) among participants positive by all three specimen types (18.1 [95% CI 16.9–19.3]) than among participants positive only by NPS (32.4 [95% CI 25.4–39.4]). The difference of means of Ct values among participants positive by only NPS compared to the Ct values among participants positive by all three specimen types was 14.3 (95% CI 5.8–22.8).

SARS-CoV-2 Detection by Viral Culture

A total of 48 ANS and 51 NPS specimens from 51 participants were submitted for viral culture (Figure 1); of these, conclusive (positive or negative) culture results were available for 50 NPS and 46 ANS from 50 participants (Table 3). Culturable virus was detected in specimens from 19 (38%) participants, 6 in both NPS and ANS specimens, 12 in NPS only, and 1 in ANS only. Using culturable virus in any specimen type as the reference, sensitivity for SARS-CoV-2 detection by rRT-PCR was 100% (95% CI 83.2%–100%) for both NPS and ANS and 93.8% (95% CI 71.7%–99.7%) for saliva. In addition, among the 19 participants with culturable virus, one rRT-PCR result for saliva was inconclusive and one was invalid. Ct values for the N1 target were associated with overall culture result. ROC analysis showed that a Ct value of 18.7 was the optimal cutoff for detection of culturable SARS-CoV-2 (Supplement Figure 3); among specimens positive for SARS-CoV-2 by rRT-PCR, those with lower Ct values (<19) were more often positive by culture, while specimens with higher Ct values (≥19) were more often negative by culture (Figure 2).

DISCUSSION

In this investigation, most participants positive for SARS-CoV-2 by rRT-PCR on NPS also tested positive by self-collected saliva and ANS, especially participants reporting current symptoms. Agreement of results between specimen types (saliva and NPS; ANS and NPS) was high for participants reporting current symptoms. Adopting less invasive specimen collection could improve testing uptake, but the benefits of saliva and ANS for testing should be weighed carefully against the loss of sensitivity in asymptomatic people.

These findings suggest that several strategies might be applied to optimize SARS-CoV-2 detection in self-collected saliva or ANS. When patients are unable or unwilling to undergo NPS collection, or when PPE or trained healthcare personnel are limited, self-collected saliva or ANS could be offered, acknowledging that some infections might be missed that would have been detected by NPS. Notably, most participants with discordant results between the three specimens had NPS Ct values >30, consistent with decreased genetic material in the sample and potentially nonviable virus, which is also consistent with prior studies [16-18]. Additionally, when evaluating the limited number of specimens that were tested for SARS-CoV-2 by viral culture and found to have culturable virus present, sensitivity by rRT-PCR of both saliva and ANS was high (94% and 100%, respectively), suggesting that the lower sensitivity of saliva and ANS may not be clinically relevant.

Given the low sensitivity among asymptomatic individuals, self-collected saliva or ANS specimens are likely to be most useful to test people reporting current COVID-19 symptoms. Testing both saliva and ANS in parallel for individual participants appeared to increase sensitivity, but this strategy may be impractical given the increased burden on laboratory resources. Pooled saliva/ANS specimens could be a subject of future research.

While saliva specimens had a modestly higher overall sensitivity than ANS for SARS-CoV-2 detection in this investigation, the sensitivity of saliva among asymptomatic participants was notably lower than among symptomatic participants and lower than has been previously reported [19]. Some participants were unable to produce saliva, while others were able to produce saliva but in volumes insufficient for testing (Figure 1). Laboratory personnel reported that saliva was difficult to process and often required additional processing to yield valid results, consistent with prior reports [13, 20]. The usefulness of saliva as a specimen

might be limited if participants are unable to produce adequate specimens or if the laboratory has limited resources to resolve saliva processing issues. One approach that has shown promise to standardize sampling of the oral cavity for SARS-CoV-2 testing is the saline mouth rinse/gargle method [21], which might overcome challenges when saliva production is limited. Because our study objective was to evaluate test performance during real-life testing events, we did not exclude participants who ate, drank, brushed their teeth, or smoked immediately prior to sampling, which might have affected test performance of saliva specimens [22]. Future studies that optimize sampling of the oral cavity are particularly relevant in light of recent data supporting that the oral cavity might have a direct role in SARS-CoV-2 transmission [23].

This evaluation had several limitations. First, participants were enrolled in a single large urban area and might not be representative of other communities. Second, the changing dynamics of SARS-CoV-2 test-seeking behavior and SARS-CoV-2 incidence in Denver during the enrollment period [24, 25] limited ability to compare test performance by testing location type. Overall positivity rates in Denver during the period of study enrollment ranged from 2% in early September, 2020 to 12% in November, 2020. More specifically, at Denver Public Health testing events during this period, the positivity rates ranged from 2 – 33% at community sites and 0 – 8% in homeless shelters. In addition, survey responses were self-reported; therefore, responses might be subject to social desirability or recall biases. Finally, because only a nonrandom subset of samples was evaluated by viral culture, inferential statements based on the findings reported here should be evaluated in a separate study to ensure generalizability.

Strengths of this analysis include its design using specimens collected from actual community events and inclusion of a diverse group of participants from events that frequently had relatively high positivity rates. The addition of symptom status, Ct values, and viral culture data to the qualitative rRT-PCR results supports a more complete understanding of the usefulness of ANS and saliva specimens to detect clinically meaningful SARS-CoV-2 infection.

While use of self-collected saliva and ANS specimens offers practical advantages, challenges to collect and process saliva might be a limitation [13]. Understanding the benefits and limitations of less-invasive specimen collection procedures for SARS-CoV-2 testing should inform public health efforts to design testing programs most appropriate to the local context and population and could possibly improve test uptake. Development of SARS-CoV-2 testing programs should consider differences in test sensitivity by specimen type, logistical and practical factors of offering testing in different settings, and specimen preferences by those seeking testing. For high volume testing events, self-collected ANS specimens are a preferred alternative to more invasive NPS by patients, are easier to collect and process than saliva, and reliably detect SARS-CoV-2 among people who are symptomatic.

Notes

§: See e.g., 45 C.F.R. part 46, 21 C.F.R. part 56; 42 U.S.C. §241(d); 5 U.S.C. §552a; 44 U.S.C. §3501 et seq.

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FIGURE LEGENDS

Figure 1. Flowchart of the participants enrolled and the specimens submitted for SARS-CoV-2 rRT-PCR
and viral culture. Abbreviations: NPS, nasopharyngeal specimen; ANS, anterior nasal specimen

Figure 2. Correlation of Ct values for the SARS-CoV-2 genetic target N1 by rRT-PCR on nasopharyngeal specimen (NPS) and anterior nasal specimen (ANS), by culture result. *A*, Viral culture
results from NPS. *B*, Viral culture results from ANS.
Abbreviations: Ct, cycle threshold; NPS, nasopharyngeal specimen; ANS, anterior nasal specimen.

Table 1. Self-reported demographics, clinical and epidemiologic characteristics, specimen preference, and overall SARS-CoV-2 positivity by rRT-PCR by testing location type.

Participant Characteristic	All Testing Sites N = 730 No. (%)	Community Testing Sites n = 452 No. (%)	Homeless Service Sites n = 278 No. (%)	Difference of Proportions	95% CI	*P*-value
Age group, yrs						
18–40	330 (45.2)	245 (54.2)	85 (30.6)	0.24	0.16, 0.31	
41–65	334 (45.8)	167 (36.9)	167 (60.1)	−0.23	−0.30, −0.16	<0.001
>65	66 (9.0)	40 (8.8)	26 (9.4)	−0.01	−0.05, 0.04	
Gender						
Male	420 (57.5)	190 (42.0)	230 (82.7)	−0.41	−0.47, −0.34	
Female	304 (41.6)	259 (57.3)	45 (16.2)	0.41	0.35, 0.47	<0.001
Other	5 (0.7)	3 (0.7)	2 (0.7)	−0.0006	−0.02, 0.01	
Race/Ethnicity						
Black, non-Hispanic	85 (11.6)	44 (9.7)	41 (14.7)	−0.05	−0.103, −0.002	
White, non-Hispanic	321 (44.0)	177 (39.2)	144 (51.8)	0.13	−0.20, −0.05	
Asian, non-Hispanic	17 (2.3)	12 (2.7)	5 (1.8)	0.01	−0.02, 0.03	
Hispanic	257 (35.2)	199 (44.0)	58 (20.9)	0.23	0.16, 0.30	<0.001
Native American/Alaska Native, non-Hispanic	14 (1.9)	4 (0.9)	10 (3.6)	−0.03	−0.06, −0.01	
Other, non-Hispanic	33 (4.5)	15 (3.3)	18 (6.5)	-0.03	−0.07, −0.0005	
New or worsening symptom(s)[a] reported at time of testing						
Any symptom	269 (36.8)	216 (47.8)	53 (19.1)	0.29	0.22, 0.35	<0.001
Any one of cough, shortness of breath or difficulty breathing, anosmia or ageusia	168 (23.0)	139 (30.8)	29 (10.4)	0.20	0.15, 0.26	<0.001

At least two of fever (measured or subjective) or chills, rigors, myalgia, headache, sore throat, nausea or vomiting, diarrhea, fatigue, congestion/nasal discharge	187 (25.6)	172 (38.1)	15 (5.4)	0.33	0.27, 0.38	<0.001
Flu-like symptoms (Fever AND either cough or sore throat)	66 (9.0)	62 (13.7)	4 (1.4)	0.12	0.09, 0.16	<0.001
Fever (measured or subjective) or chills	90 (12.3)	84 (18.6)	6 (2.2)	0.16	0.12, 0.21	<0.001
Cough	79 (10.8)	61 (13.5)	18 (6.5)	0.07	0.03, 0.11	0.004
Shortness of breath or difficulty breathing	86 (11.8)	69 (15.3)	17 (6.1)	0.09	0.05, 0.13	<0.001
Fatigue	87 (11.9)	76 (16.8)	11 (4.0)	0.13	0.09, 0.17	<0.001
Myalgia	108 (14.8)	97 (21.5)	11 (4.0)	0.18	0.13, 0.22	<0.001
Headache	66 (9.0)	54 (11.9)	12 (4.3)	0.08	0.04, 0.11	0.001
Anosmia or ageusia	76 (10.4)	73 (16.2)	3 (1.1)	0.15	0.12, 0.19	<0.001
Sore throat	116 (15.9)	109 (24.1)	7 (2.5)	0.22	0.39, 0.49	<0.001
Congestion or nasal discharge	66 (9.0)	42 (9.3)	24 (8.6)	0.01	−0.04, 0.05	0.87
Nausea or vomiting	30 (4.1)	21 (4.6)	9 (3.2)	0.01	−0.02, 0.04	0.46
Diarrhea	165 (22.6)	156 (34.5)	9 (3.2)	0.31	0.26, 0.36	<0.001
Close contact with known COVID-19 case within prior 2 weeks	159 (21.8)	149 (33.0)	10 (3.6)	0.29	0.24, 0.34	<0.001
Preferred specimen type						
Self-collected ANS	272 (37.3)	174 (38.5)	98 (35.3)	0.03	−0.04, 0.10	0.67
Self-collected saliva	335 (45.9)	205 (45.4)	130 (46.8)	−0.01	−0.09, 0.06	
Healthcare provider-collected NPS	98 (13.4)	64 (14.2)	34 (12.2)	0.02	−0.03, 0.07	
Willingness to be tested again by that specimen type, if it were the only specimen being collected for testing						

Self-collected ANS	704 (96.4)	445 (98.5)	259 (93.2)	0.05	0.02, 0.09	<0.001
Self-collected saliva	707 (96.8)	444 (98.2)	263 (94.6)	0.04	0.01, 0.07	0.07
Healthcare provider-collected NPS	641 (87.8)	403 (89.2)	238 (85.6)	0.04	−0.01, 0.09	0.44
Positive for SARS-COV-2 by at least one specimen type[b]	84 (11.5)	82 (18.1)	2 (0.7)	0.17	0.14, 0.21	<0.001

Abbreviations: ANS, anterior nasal specimen; NPS, nasopharyngeal specimen

[a] Symptoms defined as new or worsening fever (subjective or objective) or chills, cough, shortness of breath or difficulty breathing, fatigue, muscle or body aches, headache, new anosmia or ageusia, sore throat, congestion or nasal discharge, nausea or vomiting, diarrhea.

[b] Including participants who had a missing, invalid, or inconclusive test result for any specimen type of anterior nasal specimen, saliva, or nasopharyngeal specimen.

Table 2. Test performance of self-collected anterior nasal specimen (ANS) and saliva for the detection of SARS-CoV-2 by rRT-PCR, using healthcare provider-performed nasopharyngeal specimen as the reference.

	Proportion (% [95% CI])					
			Asymptomatic at testing		Any symptoms[a] at testing	
Test Characteristic	**ANS[b] n = 563**	**Saliva[c] n = 497**	**ANS[b] n = 332**	**Saliva[c] n = 299**	**ANS[b] n = 230**	**Saliva[c] n =197**
Sensitivity	52/65 (80.0 [68.7, 87.9])	46/54 (85.2 [73.4, 92.3])	6/12 (50 [25.3, 74.6])	2/7 (28.5 [8.2, 64.1])	46/53 (86.8 [75.1, 93.5])	44/47 (93.6 [82.8, 97.8])
Specificity	492/497 (99.0 [97.6, 99.6])	436/442 (98.6 [97.1, 99.4])	317/320 (99.1 [97.2, 99.7])	289/292 (99.0 [97.0, 99.7])	175/177 (98.9 [96.0, 99.7])	147/150 (98.0 [94.3, 99.3])
LRP	79.7 [34.1, 187.6]	62.9 [28.9, 137.7]	53.3 [15.7, 173.0]	27.8 [5.8, 114.4]	76.8 [21.5, 280.5]	46.8 [16.3, 137.5]
LRN	0.20 [0.12, 0.31]	0.15 [0.08, 0.27]	0.50 [0.26, 0.75]	0.72 [0.36, 0.93]	0.13 [0.07, 0.25]	0.065 [0.02, 0.18]
PPV	52/57 (91.2 [81.0, 96.2])	46/52 (88.5 [77.0, 94.6])	6/9 (66.7 [35.4, 87.9])	2/5 (40 [11.8, 76.9])	46/48 (95.8 [86.0, 98.8])	44/47 (93.6 [82.8, 97.8])
NPV	492/505 (97.4 [95.6, 98.5])	436/444 (98.2 [96.5, 99.1])	317/323 (98.1 [96.0, 99.2])	289/294 (98.3 [96.1, 99.3])	175/182 (96.2 [92.2, 98.1])	147/150 (98.0 [94.3, 99.3])
Agreement[d]	0.83 [0.76, 0.91]	0.85 [0.77, 0.93]	0.56 [0.27, 0.84]	0.32 [0, 0.79]	0.89 [0.81, 0.96]	0.92 [0.85, 0.98]

Abbreviations: ANS, anterior nasal specimen; CI, confidence interval; LRP, likelihood ratio positive; LRN, likelihood ratio negative; PPV, positive predictive value; NPV, negative predictive value.

[a] Symptoms defined as new or worsening fever (subjective or objective) or chills, cough, shortness of breath or difficulty breathing, fatigue, muscle or body aches, headache, new anosmia or ageusia, sore throat, congestion or nasal discharge, nausea or vomiting, diarrhea.

[b] Analysis restricted to participants with conclusive positive or negative results on both anterior nasal specimens and nasopharyngeal specimens.

[c] Analysis restricted to participants with conclusive positive or negative results on both saliva and nasopharyngeal specimens.

[d] Agreement calculated using Cohen's kappa correlation coefficient; CI computed by bootstrap.

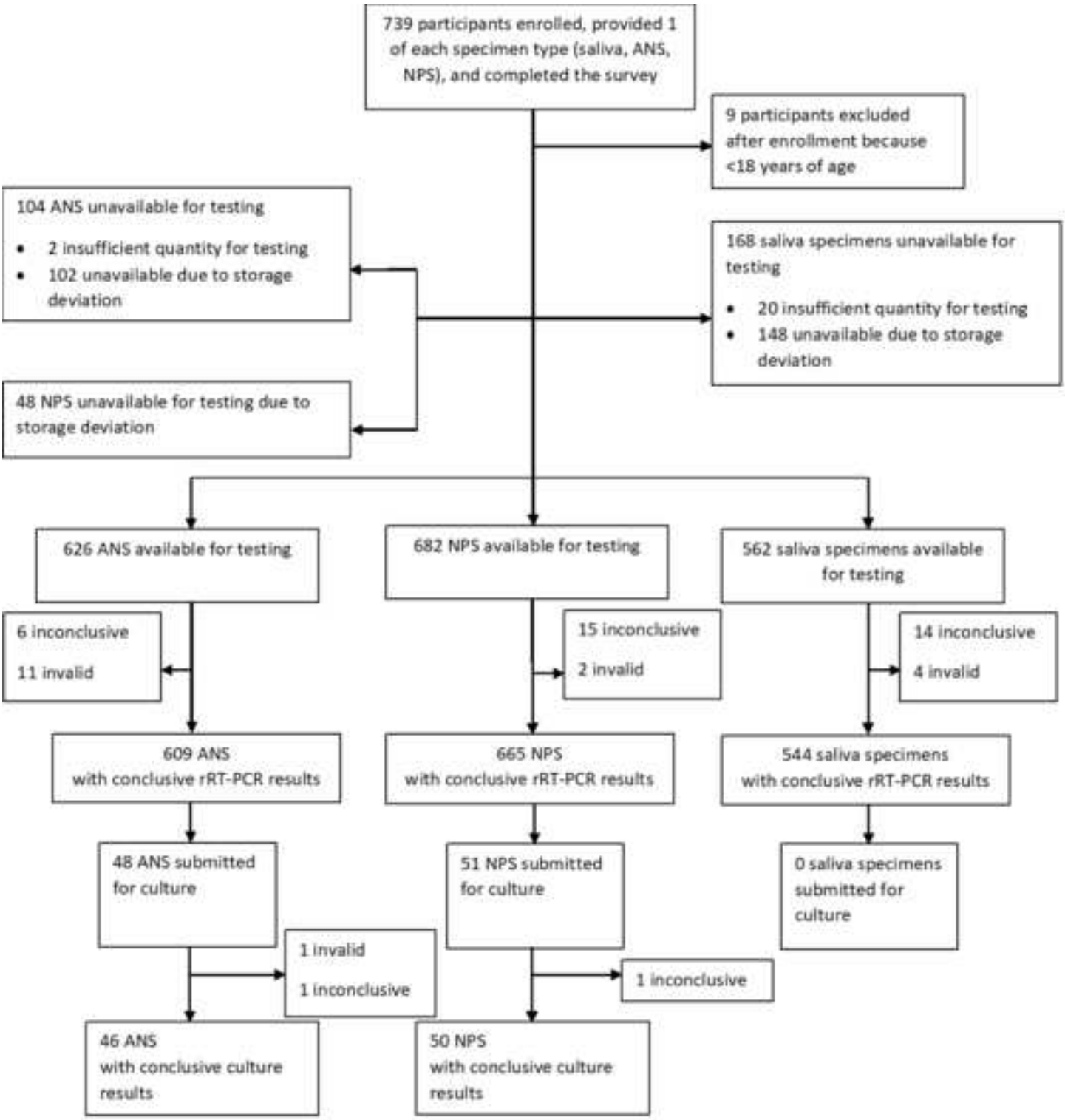
Table 3. SARS-CoV-2 detection by rRT-PCR on nasopharyngeal specimens (NPS), anterior nasal specimens (ANS), and saliva and by culture on NPS and ANS among participants with at least one valid culture result for either NPS or ANS.

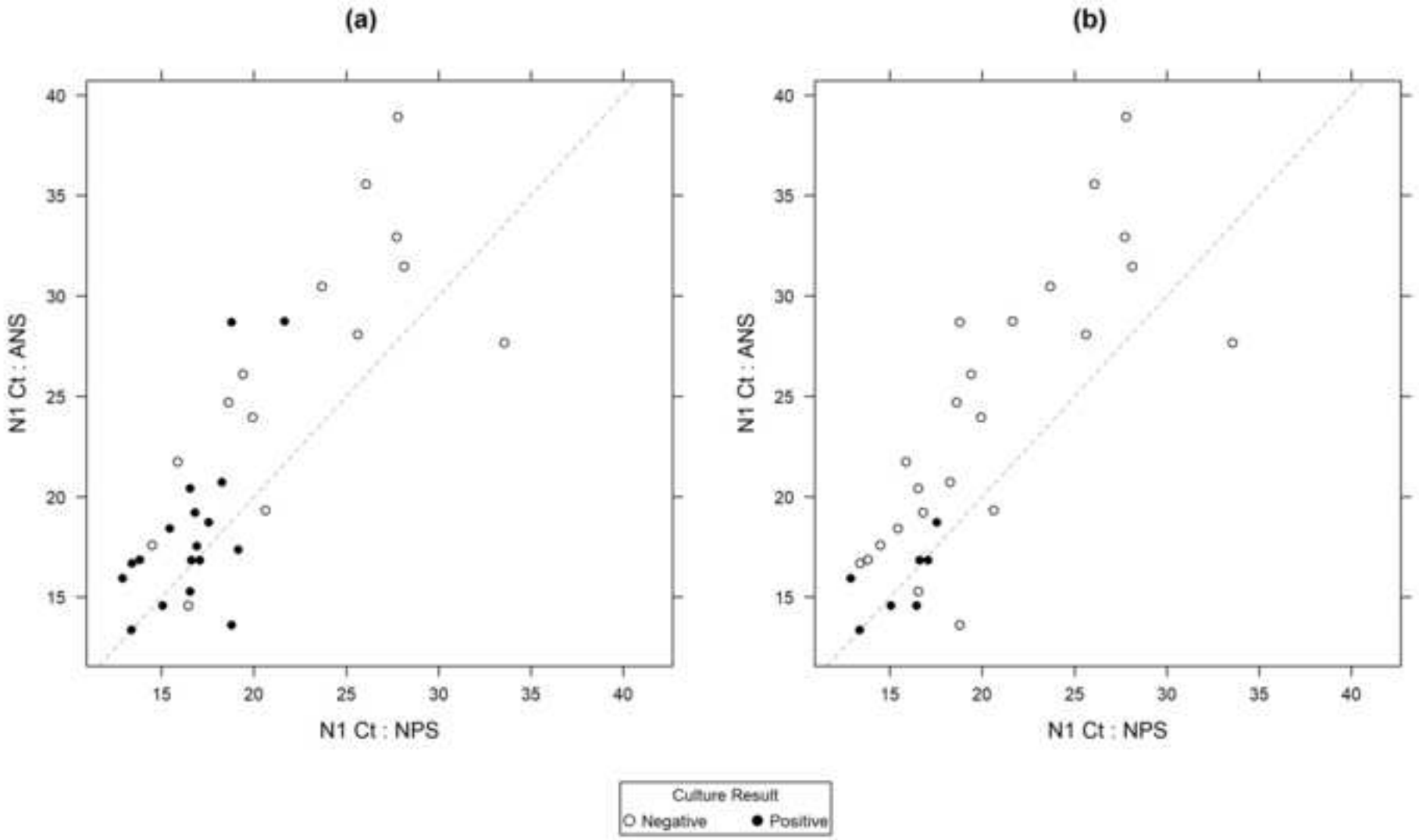
	rRT-PCR Results[a]									Culture Results	
	NPS			ANS			Saliva			NPS	ANS
Participant	Result	Ct (N1)	Ct (N2)	Result	Ct (N1)	Ct (N2)	Result	Ct (N1)	Ct (N2)		
1	Positive	16.4	16.0	Positive	14.6	14.2	Positive	27.5	27.7	Negative	Positive
2	Positive	28.1	28.0	Positive	31.5	31.8	Inconclusive	35.1	>40.0	Negative	Negative
3	Negative	>40.0	>40.0	Positive	31.6	36.2	Negative	>40.0	>40.0	Negative	Negative
4	Positive	27.8	28.1	Inconclusive	38.9	>40.0	Negative	>40.0	>40.0	Negative	Negative
5	Positive	18.8	17.8	Positive	28.7	28.7	Positive	31.6	30.7	Positive	Negative
6	Positive	25.6	25.6	Positive	28.1	28.7	Positive	37.4	35.4	Negative	Negative
7	Positive	17.1	16.1	Positive	16.8	15.6	Negative	>40.0	>40.0	Positive	Positive
8	Positive	32.3	31.6	Negative	>40.0	>40.0	Positive	30.2	28.6	Negative	Negative
9	Positive	25.2	24.9	Negative	>40.0	>40.0	Positive	30.7	31.7	Negative	Negative
10	Positive	18.8	18.0	Positive	13.6	12.2	Positive	12.4	11.0	Positive	Negative
11	Positive	17.5	16.4	Positive	18.7	17.8	Invalid	>40.0	>40.0	Positive	Positive
12	Positive	19.9	18.8	Positive	24.0	22.9	Positive	28.1	27.0	Negative	Negative
13	Positive	27.7	28.0	Positive	32.9	33.0	Positive	24.4	23.9	Negative	Negative
14	Negative	>40.0	>40.0	Negative	>40.0	>40.0	Positive	30.2	29.2	Negative	Negative
15	Positive	36.3	37.4	Negative	>40.0	>40.0	Negative	>40.0	>40.0	Negative	Negative
16	Positive	15.4	14.7	Positive	18.4	18.2	Positive	19.0	19.4	Positive	Negative
17	Positive	33.6	33.9	Positive	27.7	27.7	Inconclusive	>40.0	36.6	Negative	Negative
18	Positive	19.7	18.4	Negative	>40.0	>40.0	Negative	>40.0	>40.0	Negative	Negative
19	Positive	32.7	33.8	Negative	>40.0	>40.0	Negative	>40.0	>40.0	Negative	Negative
20	Positive	21.7	22.9	Positive	28.7	28.6	Positive	24.2	24.2	Positive	Negative
21	Positive	34.6	35.1	Negative	>40.0	>40.0	...	…	…	Negative	Negative
22	Negative	>40.0	>40.0	Negative	>40.0	>40.0	Positive	19.7	22.4	Negative	…

23	Positive	13.4	12.6	Positive	16.7	14.9	…	…	…	Positive	Negative
24	Positive	16.8	15.8	Positive	19.2	17.9	Positive	25.8	27.0	Positive	Negative
25	Negative	>40.0	>40.0	Positive	19.1	19.3	Inconclusive	>40.0	39.2	Negative	Negative
26	Negative	>40.0	>40.0	Positive	18.8	19.5	…	…	…	Negative	Negative
27	Positive	14.5	13.6	Positive	17.6	16.4	Positive	26.7	25.8	Negative	Negative
28	Positive	16.6	15.6	Positive	16.8	15.8	Positive	23.7	23.0	Positive	Positive
29	Negative	>40.0	>40.0	Negative	>40.0	>40.0	Positive	33.3	32.7	Negative	Negative
30	Positive	29.8	30.7	Negative	>40.0	>40.0	Positive	31.9	32.7	Negative	Negative
31	Positive	16.6	14.5	Positive	15.3	13.3	Positive	33.2	39.2	Positive	Negative
32	Positive	13.8	12.6	Positive	16.9	15.0	Positive	18.0	17.1	Positive	Negative
33	Positive	26.1	26.4	Inconclusive	35.6	>40.0	Inconclusive	>40.0	34.7	Negative	Negative
34	Positive	19.4	19.0	Positive	26.1	25.2	Positive	26.8	26.0	Negative	Negative
35	Negative	>40.0	>40.0	Positive	32.2	30.9	Positive	25.3	23.7	Negative	Negative
36	Negative	>40.0	>40.0	Inconclusive	>40.0	36.0	Positive	31.2	30.5	Negative	Negative
37	Positive	23.7	22.9	Positive	30.5	29.6	Positive	33.2	34.5	Negative	Negative
38	Positive	25.1	25.4	Negative	>40.0	>40.0	Positive	36.5	36.4	Negative	Negative
39	Positive	20.6	19.9	Positive	19.3	18.5	Positive	22.5	21.7	Negative	Negative
40	Positive	31.8	33.6	Negative	>40.0	>40.0	Negative	>40.0	>40.0	Negative	Negative
41	Positive	18.3	16.7	Positive	20.7	18.7	Inconclusive	25.9	>40.0	Positive	Negative
42	Positive	13.4	11.9	Positive	13.4	11.9	Positive	10.3	9.2	Positive	Positive
43	Positive	16.6	15.9	Positive	20.4	19.7	Positive	29.5	28.8	Positive	Negative
44	Positive	15.9	15.1	Positive	21.7	20.7	Positive	26.9	26.5	Negative	Negative
45	Positive	18.6	17.7	Positive	24.7	24.0	Positive	21.0	20.3	Negative	Negative
46	Positive	15.1	14.1	Positive	14.6	13.0	Positive	27.8	27.8	Positive	Positive
47	Positive	12.9	12.2	Positive	15.9	15.2	Positive	22.3	22.2	Positive	Positive
48	Positive	16.9	16.1	Positive	17.5	16.8	Positive	13.5	12.4	Positive	…
49	Positive	35.9	36.2	Negative	>40.0	>40.0	Negative	>40.0	>40.0	Negative	…
50	Positive	19.1	18.2	Positive	17.4	16.5	Positive	28.2	27.7	Positive	…

Abbreviations: NPS, nasopharyngeal specimen, ANS, anterior nasal specimen; Ct, cycle threshold; N1, SARS-CoV-2 nucleocapsid genetic target 1; N2, SARS-CoV-2 nucleocapsid genetic target 2; ..., not tested because of unavailable specimen.

[a] Result outcomes defined per the CDC 2019-Novel Coronavirus (2019-nCoV) Real-Time RT-PCR Diagnostic Panel Emergency Use Authorization [1]: positive, Ct values for N1 and N2 are <40.00; negative, Ct values for N1 and N2 are ≥40.00 and positive control RNase P is <40.00; invalid, Ct values for N1, N2 and positive control RNase P are ≥40.00; inconclusive, Ct values for either N1 or N2 (but not both) are <40.00.


739 participants enrolled, provided 1 of each specimen type (saliva, ANS, NPS), and completed the survey
9 participants excluded after enrollment because <18 years of age
104 ANS unavailable for testing
2 insufficient quantity for testing
102 unavailable due to storage deviation
168 saliva specimens unavailable for testing
20 insufficient quantity for testing
148 unavailable due to storage deviation
48 NPS unavailable for testing due to storage deviation
626 ANS available for testing
682 NPS available for testing
562 saliva specimens available for testing
6 inconclusive
11 invalid
15 inconclusive
2 invalid
14 inconclusive
4 invalid
609 ANS with conclusive rRT-PCR results
665 NPS with conclusive rRT-PCR results
544 saliva specimens with conclusive rRT-PCR results
48 ANS submitted for culture
51 NPS submitted for culture
0 saliva specimens submitted for culture
1 invalid
1 inconclusive
1 inconclusive
46 ANS with conclusive culture results
50 NPS with conclusive culture results


(a)
(b)
N1 Ct : ANS
N1 Ct : NPS
15
20
25
30
35
40
Culture Result
Negative
Positive