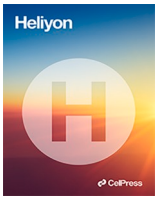
Heliyon 10 (2024) e27188

Contents lists available at ScienceDirect

Heliyon

journal homepage: www.cell.com/heliyon



Research article

Comparison of RT-PCR and antigen test sensitivity across nasopharyngeal, nares, and oropharyngeal swab, and saliva sample types during the SARS-CoV-2 omicron variant

Gregory L. Damhorst [a,b,1], Jessica Lin [a,c,1], Jennifer K. Frediani [a,d,1], Julie A. Sullivan [a,e], Adrianna Westbrook [a,f], Kaleb McLendon [a,g], Tyler J. Baugh [a,g], William H. O'Sick [a,g], John D. Roback [a,g], Anne L. Piantadosi [a,b,g], Jesse J. Waggoner [a,b], Leda Bassit [a,h], Anuradha Rao [a,e], Morgan Greenleaf [a], Jared W. O'Neal [a,i], Seegar Swanson [a], Nira R. Pollock [a,j], Greg S. Martin [a,i,**], Wilbur A. Lam [a,c,e,k,***], Joshua M. Levy [a,l,m,*]

[a] *Atlanta Center for Microsystems-Engineered Point-of-Care Technologies, Emory University, USA*
[b] *Division of Infectious Diseases, Emory University School of Medicine, USA*
[c] *Wallace H. Coulter Department of Biomedical Engineering, Emory University and Georgia Institute of Technology, USA*
[d] *Nell Hodgson Woodruff School of Nursing, Emory University, USA*
[e] *Department of Pediatrics, Emory University School of Medicine, USA*
[f] *Pediatric Biostatistics Core, Department of Pediatrics, Emory University School of Medicine, USA*
[g] *Department of Pathology and Laboratory Medicine, Emory University School of Medicine, USA*
[h] *Laboratory of Biochemical Pharmacology, Department of Pediatrics, Emory University School of Medicine, USA*
[i] *Emory University School of Medicine, Division of Pulmonary, Allergy, Critical Care and Sleep Medicine, USA*
[j] *Department of Laboratory Medicine, Boston Children's Hospital, USA*
[k] *Aflac Cancer and Blood Disorders Center of Children's Healthcare of Atlanta, USA*
[l] *Emory University School of Medicine, Department of Otolaryngology-Head and Neck Surgery, USA*
[m] *Sinonasal and Olfaction Program, National Institute on Deafness and Other Communication Disorders, NIDCD/NIH*

ARTICLE INFO

Keywords:
SARS-CoV-2
COVID-19 testing
SARS-CoV-2 variants
Point of care testing

ABSTRACT

Limited data highlight the need to understand differences in SARS-CoV-2 omicron (B.1.1.529) variant viral load between the gold standard nasopharyngeal (NP) swab, mid-turbinate (MT)/anterior nasal swabs, oropharyngeal (OP) swabs, and saliva. MT, OP, and saliva samples from symptomatic individuals in Atlanta, GA, in January 2022 and longitudinal samples from a small familial cohort were tested by both RT-PCR and ultrasensitive antigen assays. Higher concentrations in the nares were observed in the familial cohort, but a dominant sample type was not found among 39 cases in the cross-sectional cohort. The composite of positive MT or OP assay for both RT-PCR and antigen assay trended toward higher diagnostic yield but did not achieve significant difference. Our data did not identify a singular preferred sample type for SARS-CoV-2 testing, but higher levels of saliva nucleocapsid, a trend toward higher yield of composite OP/

* Corresponding author. Atlanta Center for Microsystems-Engineered Point-of-Care Technologies, Emory University, USA.
** Corresponding author. Atlanta Center for Microsystems-Engineered Point-of-Care Technologies, Emory University, USA.
*** Corresponding author. Atlanta Center for Microsystems-Engineered Point-of-Care Technologies, Emory University, USA.
E-mail addresses: greg.martin@emory.edu (G.S. Martin), wilbur.lam@emory.edu (W.A. Lam), joshua.levy2@emory.edu (J.M. Levy).
[1] Gregory Damhorst, Jessica Lin and Jennifer K Frediani contributed equally to this work.

https://doi.org/10.1016/j.heliyon.2024.e27188
Received 4 April 2023; Received in revised form 29 November 2023; Accepted 26 February 2024
Available online 29 February 2024

MT result, and association of apparent MT or OP predominance with symptoms warrant further study.

1. Introduction

Nasopharyngeal (NP) sampling by a trained healthcare professional is the gold standard for the diagnosis of SARS-CoV-2 infection [1]. Due to the complexity and discomfort of NP sampling, anterior nares (AN), nasal mid-turbinate (MT), and oropharyngeal (OP) swabs and expectorated saliva have garnered interest as alternative sample types in SARS-CoV-2 assays [2–8]. AN and MT sampling are employed in at-home rapid antigen tests with US Food and Drug Administration emergency use authorization (EUA) [9]. The emergence of the omicron (B.1.1.529) variant, regional differences in sampling practices, and social media trends reignited debate over the most effective method for sample collection in persons suspected of having COVID-19 [10–13].

Early meta-analyses of data corresponding to the alpha (B.1.1.7) variant demonstrated the highest sensitivity in NP swabs or combined nasal-OP swabs [4,14–16]. Following the emergence of delta (B.1.617.2) and omicron (B.1.1.529) variants, there are minimal data addressing the relative clinical sensitivity of sample types. Meanwhile, there are few systematic comparisons of the impact of sample type on rapid antigen test sensitivity and specificity, limiting understanding of optimal sampling techniques [17,18].

The omicron variant has over 50 mutations relative to the wild-type virus, which has been postulated to affect tropism, the anatomic distribution of virus in the upper respiratory tract, and the prevalence of symptoms such as odynophagia [19]. Some evidence has suggested omicron replicates more easily in the bronchial compared to lung parenchymal tissues, which contrasts with measurements of delta and wild-type viruses [20]. This highlights potential differences in tissue-dependent replication profiles that may be present in the upper airways, thus impacting diagnostic test performance. Although it has been nearly two years since emergence of the omicron variant, few studies have examined this question. One published study concluded combined nasal-OP swabs produced a similar sensitivity to NP swabs for nucleic acid amplification testing, and another concluded that combined nasal-OP swabs increased the sensitivity of rapid antigen tests [12,13]. One preprint study shows that the omicron variant may be more readily detected in saliva compared to MT samples by RT-PCR [21], while another showed lower sensitivity of OP compared to AN swab [22].

To inform diagnostic sampling practices in both home and point of care settings, we sought to quantify antigen and molecular test performance across three sample types among patients infected with SARS-CoV-2 during the recent omicron surge. This was done in two complementary cohort studies: First, findings from a familial cohort were examined in a longitudinal qualitative analysis. Second, a cross-sectional cohort was analyzed to determine (1) the relative diagnostic performance of MT, OP, and saliva samples, (2) differences in cycle threshold (Ct) value – the number of cycles it takes for the PCR signal to reach a predefined threshold for positivity, which is inversely related to RNA copy number – and quantitative antigen measurements, and (3) the impact of symptoms.

2. Methods

This study was approved by the Emory University Institutional Review Board under STUDY00001082. All experiments were performed in accordance with relevant guidelines and regulations. Recruitment was completed during the height of the omicron surge in the metro Atlanta, GA area between January 10, 2022 and February 1, 2022. Written or verbal informed consent was obtained from all participants prior to providing specimens and questionnaire data.

2.1. Longitudinal sampling of a familial cohort

Following exposure of two related individuals to a known case of COVID-19, both individuals and a third family member provided verbal consent over the phone and were provided with self-collection kits and collection instructions for AN swab, OP swab, and passive expectorated saliva samples (supplementary information). Each participant provided almost daily specimens, which were refrigerated immediately and transported on ice to the Emory/Children's Laboratory for Innovative Assay Development (ELIAD) for analysis within 72 h of collection.

2.2. Testing site sample collection

MT swab, OP swab, and passive expectorated saliva samples were collected consecutively from individuals at community and hospital-based sites affiliated with Emory University Hospital, Grady Memorial Hospital, and Children's Healthcare of Atlanta as part of the RADx program [23]. Participants were enrolled if they indicated COVID-19 symptom onset within the prior 7 days on a questionnaire and consented to sample collections. Asymptomatic patients, those with symptoms associated with COVID-19 for greater than 7 days, and those unable to provide informed consent were excluded. All specimens were collected by trained research staff wearing appropriate personal protective equipment (face mask, face shield or goggles, gown and gloves) [24]. Specimens were collected according to standard protocols (see supplementary methods) and transported on ice to ELIAD for analysis within 72 h of sample collection. Clinical and demographic variables were collected in a centralized, web-based database (REDcap, Nashville, TN).

2.3. RT-PCR testing

Reverse transcriptase polymerase chain reaction (RT-PCR) testing for MT and OP samples was performed using the Cepheid GeneXpert Dx Instrument System according to the manufacturer's instructions for MT swab testing. Assay result as positive or negative was reported by the GeneXpert according to the manufacturer's threshold. The corresponding Ct value was retrieved from GeneXpert System Software. Saliva was processed in accordance with the SalivaDirect dual-plexed RT-PCR protocol and assayed on the ABI QuantStudio 5 Real-Time PCR system [25–27].

2.4. Antigen concentration

Nucleocapsid antigen was quantified using the SARS-CoV-2 N Protein Advantage assay on the Quanterix Simoa HD-X platform (Billerica, MA) according to protocols for research use [28,29]. Samples above the clinical cutoff of 3.00 pg/mL were considered positive (supplementary methods).

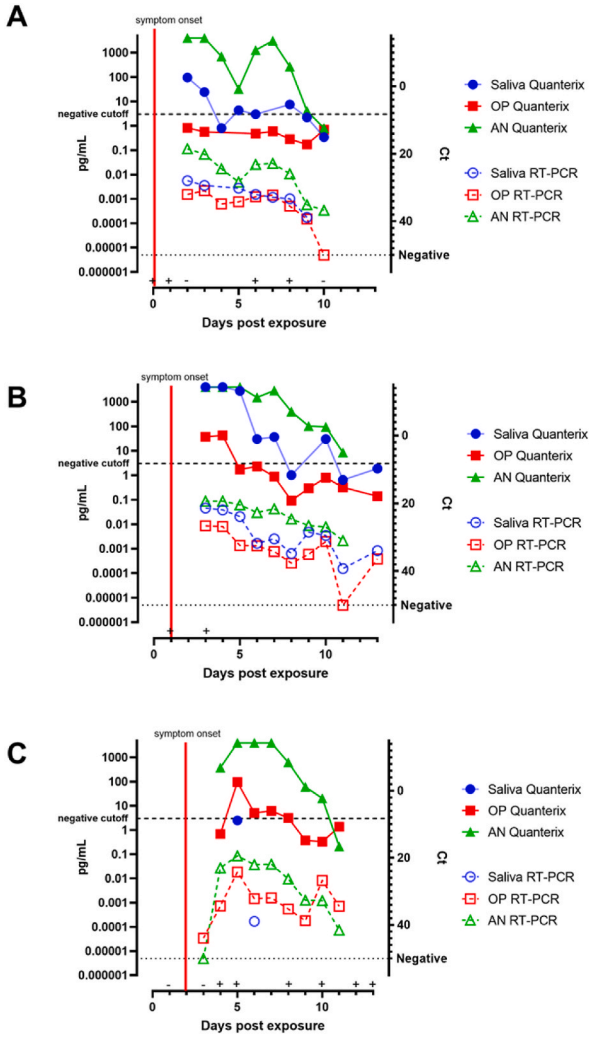


Fig. 1. Comparison of antigen concentration and Ct value of different sample types over the course of disease in a familial cohort. A-C) Three COVID-19 positive patients from the same family cohort were tracked from the time of exposure over the course of disease. Each family member (A, B, or C) self-collected AN, OP, and saliva samples, which were assayed by RT-PCR and the Quanterix Simoa HD-X. The time of symptom onset is indicated by a red line for each patient. Results of self-administered point of care tests are indicated as at least one positive result (+) or all negative results (−) on the x-axis (Table S1). Some antigen measurements were too high to be quantified and are assigned a value of 4000 pg/mL in this figure, which is above the highest quantified antigen level.

2.5. Lateral flow assay testing

In a subset of participants, a second OP swab was collected simultaneously and one AN swab was collected by trained research staff for use with the Quickvue OTC home test (Quidel, San Diego, CA). The test was performed according to the manufacturer's instructions by study staff within 4 min of sample collection. The familial cohort also provided AN and off-label OP results on a subset of days from Quickvue which was performed at home by the participant according to the manufacturer's instructions for AN sampling.

2.6. Statistical analysis

Participants who tested positive for SARS-CoV-2 RNA or nucleocapsid protein in at least two of six possible test combinations (RT-PCR or antigen testing of MT, OP or saliva) (n = 39) were included in the analysis. Not all participants were able to contribute all three sample types. Positive percent agreement (PPA) for a sample type was defined as the number of positive results for that sample type divided by the total number of the sample type tested from included individuals. 95% confidence intervals were calculated using the formula for standard error.

Comparisons of Ct values and quantitative antigen measures were performed with a rank-sum test using 0 pg/mL for a negative antigen test. A Ct value of 50 was used for negative RT-PCR, which exceeds the highest Ct value in specimens determined to be positive for SARS-CoV-2 by the GeneXpert or SalivaDirect assays. Absolute agreement of each assay across sample types was calculated via an intraclass correlation coefficient (ICC) using a two-way mixed effects model in IBM SPSS Statistics for Windows, version 28 (IBM Corp., Armonk, N.Y., USA). To evaluate impact of sample type and duration of symptoms, Ct value and antigen concentrations were log transformed for normality and then analyzed in a mixed effects model accounting for repeated measures from the same participant using SAS 9.4 (Cary, NC). Subjects were treated as a random effect while time and location were considered fixed effects. In the mixed effects models, antigen negative samples were assigned a value of 2.99 pg/mL and negative RT-PCR results were again assigned a Ct

Table 1
Characteristics of study population and sample collection.

Variable	Level	Overall N = 39
Age	Median (IQR)	29.03 (22.99, 40.61)
Sex	Female	19 (48.72)
	Male	20 (51.28)
Race	White	21 (53.85)
	Black/African American	15 (38.46)
	Asian	3 (7.69)
Ethnicity	Hispanic	4 (10.26)
	Non-Hispanic	35 (89.74)
Received COVID-19 Vaccine	Yes	32 (82.05)
	No	7 (17.95)
Type of Vaccine Received		
	Boosted (1 J&J or 2 mRNA and booster)	14 (35.90)
	Primary Series (1 J&J or 2 mRNA only)	16 (41.03)
	Incomplete Series (1 mRNA only)	2 (5.13)
	Unvaccinated	7 (17.95)
Previous COVID-19 Diagnosis	Yes	8 (20.51)
	No	31 (79.49)
Days Since Symptom Onset*	Median (IQR)	2.00 (1.00, 4.00)
Symptoms in the Preceding 14 Days	Congestion/rhinorrhea	29 (74.36)
	Cough	29 (74.36)
	Headache	29 (74.36)
	Sore throat	23 (58.97)
	Fatigue	21 (53.58)
	Myalgias	18 (46.15)
	Chills	13 (33.33)
	Arthralgias	7 (17.95)
	Fever	6 (15.38)
	Nausea	6 (15.38)
	Diarrhea	5 (12.82)
	Photophobia	4 (10.26)
	Shortness of breath	4 (10.26)
Symptoms At Time of Test	Congestion/rhinorrhea	26 (66.67)
	Cough	25 (64.10)
	Headache	20 (51.28)
	Fatigue	19 (48.72)
	Sore throat	16 (41.03)
	Myalgias	15 (38.46)
	Chills	8 (20.51)
	Nausea	5 (12.82)
	Diarrhea	4 (10.26)

Symptoms with less than 10% prevalence are omitted.

value of 50.

3. Results

3.1. Familial cohort

In longitudinal self-collection by a 3-participant familial cohort aged 30–39 (n = 2) and 60–69 (n = 1) each of the participants provided almost daily samples for RT-PCR and ultrasensitive antigen assay over a period of 8–11 days (Fig. 1A–C). Participants also provided the results of point-of-care (POC) diagnostic tests when available (Table S1). All three participants were infected with the Omicron variant (Table S2).We observed consistently higher antigen concentrations and lower Ct values suggesting higher viral loads in AN samples compared to paired OP and saliva samples (Fig. 1).

3.2. Cross-sectional cohort participants and characteristics

MT, OP, and saliva specimens were collected from 121 individuals in the cross-sectional cohort of whom 39 were included in the analysis. 19 (48.7%) were female and 32 (82.1%) had received at least one dose of a COVID-19 vaccine (Table 1). Median days since symptom onset was 2.0 (IQR 1.0–4.0). 26 (66.7%) reported congestion or rhinorrhea and 16 (41.0%) reported sore throat (Table 1) at time of evaluation. Spike single-nucleotide polymorphism (SNP) or full viral genome sequencing were able to be performed for 30 of 39 participants, all of which identified the omicron variant (Table S2) [30–33]. National surveillance data report greater than 98% circulating B.1.1.529 during the study period (Table S3) [34].

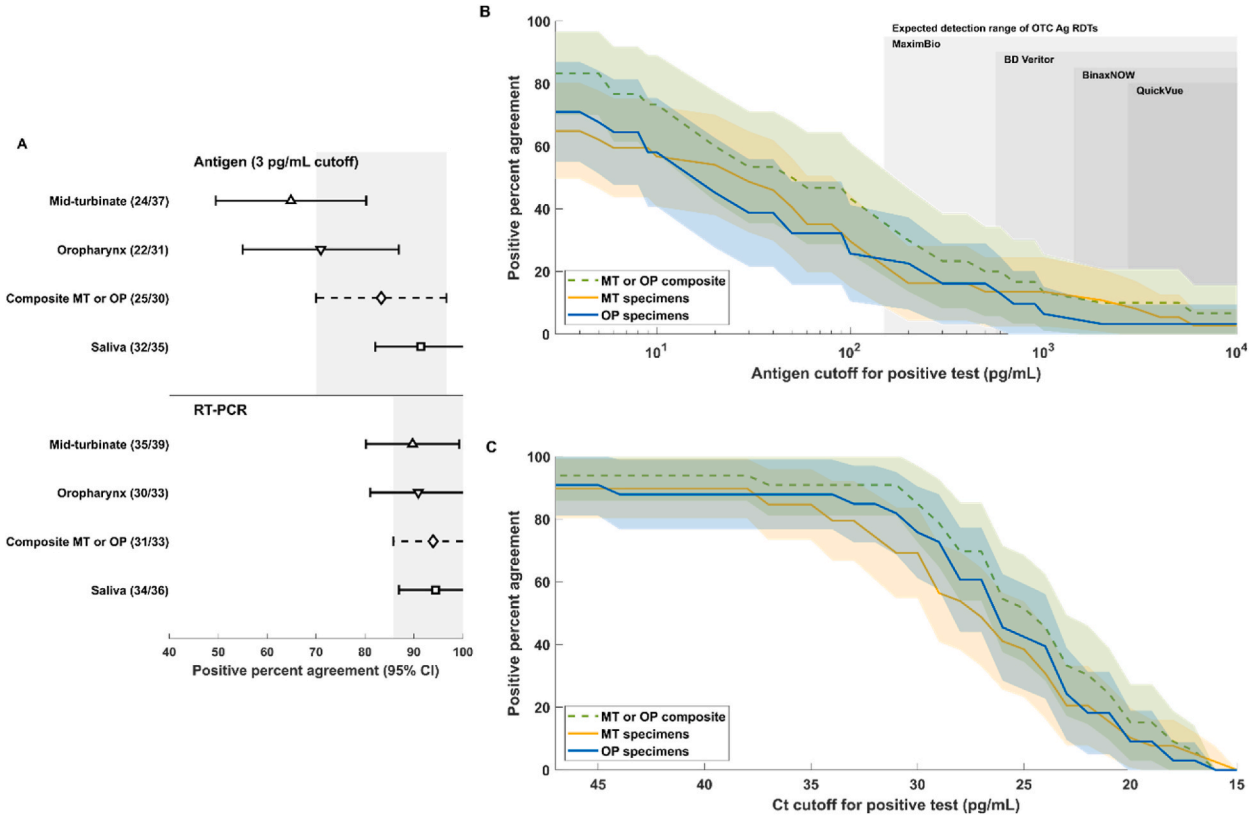


Fig. 2. Positive percent agreement (PPA) of RT-PCR and antigen measurements of different specimen types. All included specimens came from participants who had two or more of the three specimens positive for SARS-CoV-2 by RT-PCR or antigen detection. PPA is calculated as the total number of positive results of that sample type divided by the total number of specimens of that sample type tested. Results are included in the composite MT or OP only if the participant had both MT and OP samples tested. (A) Antigen PPA determined by a cutoff of 3 pg/mL and RT-PCR (threshold cycle cutoff based on respective assay). (B) Antigen PPA for MT, OP and MT or OP composite for a range of antigen cutoffs. Shaded regions denote 95% CI calculated using the formula for standard error. Expected ranges for commercially available EUA rapid diagnostic tests based on local testing in comparison with the Quanterix assay are shown in gray (see Table S4). (C) RT-PCR PPA for MT, OP and MT or OP composite versus threshold cycle cutoff. Shaded regions denote 95% CI calculated using the formula for standard error.

3.3. Diagnostic performance

Among the 39 included participants, RT-PCR was performed on 39 MT, 33 OP, and 36 saliva specimens. Nucleocapsid was measured in 37 MT, 31 OP and 35 saliva specimens (Table 1). PPA [95% CI] for RT-PCR was 89.7 [80.2–99.3], 90.9 [81.1–100] and 94.4 [87–100], in MT, OP and saliva specimens, respectively, while antigen detection exhibited 64.9 [49.5–80.2], 71 [55–86.9], and 91.4 [82.2–100] PPA at a cutoff of 3 pg/mL (Fig. 2A). A composite result was also considered among individuals who had both MT and OP specimens analyzed to understand dual sampling strategies, where only one positive result on the MT or the OP specimens was required to consider the composite result positive. , PPA of the composite result was 93.9 [85.8–100] for RT-PCR (n = 33) and 83.3 [70–96.7] for antigen detection (n = 30). McNemar test did not show significant difference between the diagnostic performance of MT only and the composite result for either antigen (p = 0.07) or RT-PCR (p = 0.48).

The diagnostic performance of antigen detection at varying cutoffs was examined, revealing a higher PPA for the composite result of MT or OP but with significant overlap of the 95% CI for the individual sample types. This was observed throughout the entire range of cutoffs (Fig. 2B) including the reference ranges of common over the counter (OTC) rapid diagnostic tests (RDTs; Table S4). PPA for RT-PCR over the range of Ct values is shown in Fig. 2C. This analysis produced similar results in a supplemental analysis where an alternate reference standard only requiring one of six sample type-assay combinations to be positive was used (Figs. S1 and S2).

Sub-analysis comparing diagnostic performance of OP antigen testing in participants with presence and absence of sore throat at the time of sampling showed PPA [95% CI] of 92.3 [77.8–100] and 55.6 [32.6–78.5], respectively (Fig. S3). OP antigen concentration between participants with (median [IQR] = 22.9 [11.9–206.8]) and without (6.8 [0–90.5]) sore throat did not achieve statistical significance (p = 0.09). Stratification by presence or absence of rhinorrhea showed overlapping PPA for MT sample antigen testing (68 [49.7–86.3] with rhinorrhea, 58.3 [30.4–86.2] without) but stark difference was observed in performance at thresholds above 20 pg/mL including regions without overlap of the 95% CI (Fig. S4). MT antigen concentrations for participants with and without rhinorrhea were (median [IQR]) 33.1 [8.6–200.4] and 4.6 [0–18.9], respectively (p = 0.07).

3.4. Viral load distribution and agreement between sample types

Distribution of Ct value, a surrogate marker for RNA copy number or viral load, did not show significant differences in comparison

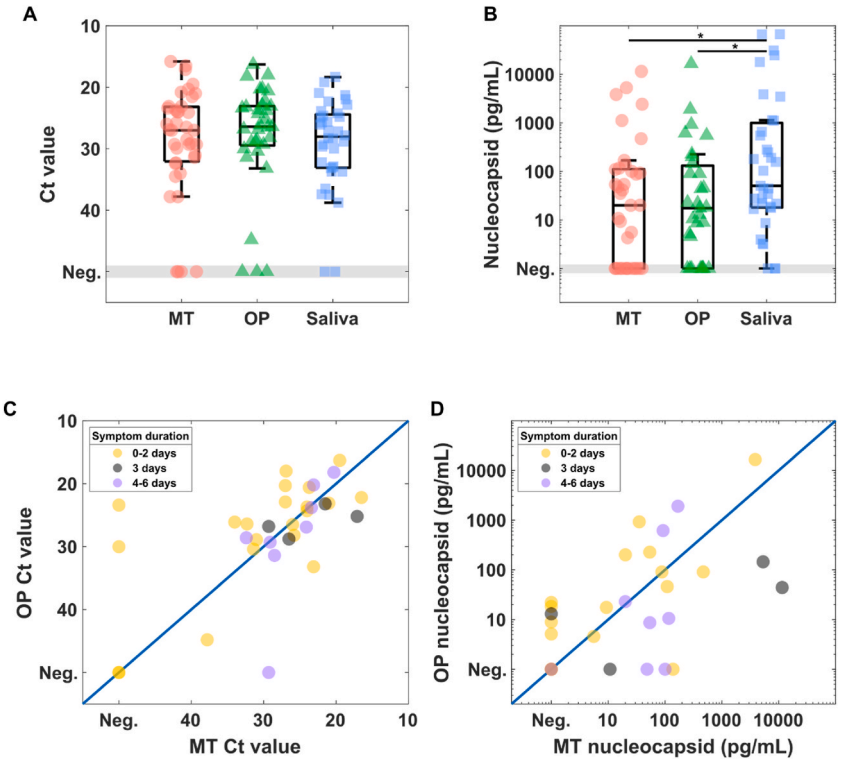


Fig. 3. Time-independent comparison of (A) Ct value and (B) nucleocapsid antigen concentration distributions for the three sample types. Significant differences in distribution of values based on rank-sum test were only observed for the comparisons of antigen concentration between MT and Saliva (p = 0.02) as well as OP and Saliva (p = 0.02). Boxplots display median values (center line), IQR (box) and range (whiskers) excluding outliers (data points more than 1.5*IQR above the upper limit of the IQR). OP vs MT plots demonstrate distribution of (C) Ct values and (D) antigen concentration for paired specimens in which both OP and nares predominant phenotypes are observed among individuals tested early (<3 days) and late (>3 days) after symptom onset.

of any two sample types across the cohort (MT vs OP: p = 0.47, saliva vs OP: p = 0.21, MT vs saliva: p = 0.56; Fig. 3A). Meanwhile, nucleocapsid antigen measurements were significantly higher in saliva compared to MT or OP samples (p = 0.02 for both comparisons; Fig. 3B), but not in the comparison of MT to OP samples (p = 0.99).

A model assuming independence and models assuming correlation between observations were applied to each assay result and a likelihood ratio test between nested models was employed to determine the model of best fit which would be used for the final analysis. Three possible correlation structures were considered: autoregression, Toeplitz, and unstructured correlation. The highest-level correlation structure that returned a significantly better fit than the previous structure was used for analysis. A Toeplitz covariance structure and an unstructured covariance matrix allowed the best fit mixed effects model for the antigen concentrations and Ct values, respectively. Sample type was significantly associated with mean antigen concentrations (p = 0.05), but not with Ct values (Table 2).

Paired MT and OP samples showed poor absolute agreement (interclass correlation [95% CI] 0.03 [−0.03, 0.09] for Ct values and 0.06 [−0.04, 0.16] for antigen concentration). Ct values for OP and MT samples were directly compared as these have identical collection methods except for the anatomic site being swabbed. Ct value and antigen measurements both revealed a mix of paired samples with greater concentration in the OP sample (OP-predominant phenotype) and greater concentration in the MT sample (MT-predominant phenotype) and do not demonstrate clear trends if sampled early (<3 days) or late (>3 days) after symptom onset (Fig. 3C–D, Fig. S5).

3.5. Rapid diagnostic testing

Testing of 17 of the 124 individuals in this study (Table S5) with separate AN and OP specimens on a common OTC RDT (Quickvue OTC home test) showed 4 true positives and 15 true negatives for AN sampling and 2 false negatives and 14 true negatives for OP sampling. There were no false positives for any sample type.

3.6. Investigation of possible confounding factors

Ct value and antigen concentrations were examined separately based on duration of symptoms at time of sampling. Comparisons of Ct or antigen distribution did not differ significantly in pairwise comparison of sample types on any day of symptoms (all p values > 0.08; Fig. S6). Results from the mixed effects model found no evidence that duration of symptoms nor vaccination status were significantly associated with Ct value (p = 0.05 and p = 0.76, respectively) or antigen concentrations (p = 0.78 and p = 0.94, respectively; Table 2). Sensitivity analysis excluding negative RT-PCR (i.e. Ct values that were imputed as 50) in the main analysis

Table 2
Impact of sample type and duration of symptoms on antigen concentrations and Ct values using a mixed effect linear model (n = 49).

	Ct value			Antigen concentration		
	Mean	95% CI	P-value	Mean	95% CI	P-value
Sample Type			0.19			0.045
Mid-turbinate	28.38	(24.72, 32.57)		22.76	(5.73, 90.44)	
Oropharyngeal	27.58	(23.85, 31.90)		19.29	(4.68, 79.44)	
Saliva	29.68	(25.94, 33.97)		93.65	(23.04, 380.66)	
Days of symptoms			0.05			0.78
0	41.12	(29.10, 58.11)		7.96	(0.28, 229.27)	
1	25.36	(22.09, 29.10)		60.43	(15.58, 234.44)	
2	32.01	(26.97, 37.98)		26.71	(5.01, 142.51)	
3	23.75	(19.02, 29.65)		125.61	(14.94, 1055.95)	
4	27.82	(23.79, 32.54)		58.76	(12.74, 270.91)	
5	29.14	(18.71, 45.37)		32.90	(0.47, 2310.46)	
6	23.98	(15.40, 37.33)		18.70	(0.27, 1312.91)	
Vaccination Status			0.76			0.94
Received at least 1 dose	28.96	(25.83, 32.46)		33.39	(11.16, 99.89)	
Never vaccinated	28.11	(23.04, 34.31)		35.68	(5.10, 249.51)	
Sample Type			0.23			0.045
Mid-turbinate	28.05	(24.74, 31.80)		22.76	(5.73, 90.44)	
Oropharyngeal	27.35	(23.95, 31.24)		19.29	(4.68, 79.44)	
Saliva	29.28	(25.94, 33.06)		93.65	(23.04, 380.66)	
Days of symptoms			0.06			0.78
0	39.41	(28.75, 54.02)		7.96	(0.28, 229.27)	
1	25.38	(22.41, 28.75)		60.43	(15.58, 234.44)	
2	30.92	(26.49, 36.08)		26.71	(5.01, 142.51)	
3	23.90	(19.57, 29.20)		125.61	(14.94, 1055.95)	
4	27.46	(23.83, 31.63)		58.76	(12.74, 270.91)	
5	29.14	(19.56, 43.40)		32.90	(0.47, 2310.46)	
6	24.08	(16.17, 35.87)		18.70	(0.27, 1312.91)	
Vaccination Status			0.74			0.94
Received at least 1 dose	28.61	(25.81, 31.73)		33.39	(11.16, 99.89)	
Never vaccinated	27.82	(23.25, 33.29)		35.68	(5.10, 249.51)	

showed similar results (Table S6).

4. Discussion

Trends in self-testing for COVID-19 have raised interest in understanding diagnostic performance of specimens obtained from distinct anatomic sites during the omicron era. We collected specimens from a familial cohort of 3 individuals following exposure to SARS-CoV-2 and 121 individuals presenting for testing, of whom 39 were included because they had at least two positive test results between RT-PCR and antigen testing of MT, OP and saliva specimens.

Longitudinal samples from the familial cohort showed highest viral load in self-collected AN specimens compared to self-collected OP and saliva specimens for all three individuals. Plotting these measurements suggested similar viral kinetics in all three sample types and that the AN consistently remained the sample type of highest viral content. As such, this cohort appeared to maintain a nasal-predominant phenotype among samples collected by non-healthcare trained participants.

In contrast, our cross-sectional cohort data did not provide clear evidence that one sample type was more reliable for clinical testing. The cross-sectional cohort demonstrated a mix of MT-predominant and OP-predominant phenotypes and there was not a sample type with consistently higher viral RNA or nucleocapsid antigen across all participants. Trends in absolute PPA of simultaneous nares and OP testing favor further study. A recent pre-print describes early false-negative nares rapid antigen testing in a small cohort despite positive RT-PCR in saliva in the same period [35]. Our data did not show a clear trend toward nares or OP-predominant phenotypes for those testing early or late after symptom onset (Fig. 3C–D). Although there were 4 individuals with detectable OP antigen and undetectable MT antigen less than 3 days after symptom onset, another individual had detectable MT antigen and undetectable OP antigen in this same window. Finally, although our data show apparently higher antigen yield of saliva, there are no validated OTC RDTs that utilize saliva and, notably, we did not see this relative advantage with molecular testing.

Limitations of our study include use of self-collected swabs in the familial cohort, which are generally not considered as reliable as those collected by trained research staff. Though some evidence supports the adequacy of self-collected AN and MT swabs, OP sampling is an inherently more challenging self-collection procedure [36,37]. There were likely differences in duration of sample storage for the familial cohort compared to the cross-sectional cohort prior to analysis which are not expected to contribute significantly to our results, though may have more substantial effects on antigen measurements than molecular testing [38]. In the cross-sectional cohort, we cannot exclude the effect of sampling variability in producing the observed phenotypes. Further investigation with repeat sampling of participants is needed. Our study also excludes asymptomatic individuals, which limits generalization of our findings.

Consistencies in testing methodology across sample types is also am important consideration. RT-PCR assays were performed with a common assay (Cepheid GeneXpert) for MT and OP samples. Saliva RT-PCR was performed using the SalivaDirect protocol. Because of these differences, and because neither assay has EUA as a quantitative test, conclusions are limited from comparisons of Ct values between saliva and the other specimens.

Comparisons across all three sample types in antigen testing are addressed by our study format. While the quantity of cellular material and mucous may be inherently different between sample types with different collection methodologies, the pragmatic question of viral antigen yield can be examined in our study because a consistent sample volume and a common assay was used for nucleocapsid measurements across all sample types. Thus, our data suggest that higher antigen levels may be found in saliva specimens, but calculation of PPA failed to show clear benefit in diagnostic yield. Conclusions as to the utility of saliva for antigen testing are limited by missing specimens from some participants and a small sample size.

The observation of apparent OP- and MT-predominant phenotypes is a key finding in our data and a strength of our study that should motivate further investigation. Isolated reports of OP-positive, nares-negative tests early during symptomatic COVID-19 have motivated practices of combined oropharyngeal and nasal sampling. Yet testing with OTC tests in a subset of our population did not produce any positive OP specimens. Our data is compatible with the hypothesis that viral loads in individuals with COVID-19 during the omicron surge manifest distinctly as OP- or MT-predominant phenotypes. Our sub-analyses (Figs. S3–S5) also suggest symptoms could be a clue to phenotyping. Examination of this phenomenon in larger sample sizes is warranted and should include longitudinal re-sampling of participants and association of symptoms with test performance of each sample type.

In total, our data do not support a preferred singular anatomic location for sampling with SARS-CoV-2 diagnostics during the omicron surge. However, trends toward increasing sensitivity when both MT and OP results are considered may suggest a benefit can be achieved by testing multiple sample types concurrently. Further rigorous study should investigate the benefit and safety of combined sampling methods.

Funding source

This work was supported by the National Institute of Biomedical Imaging and Bioengineering at the National Institutes of Health under award Numbers U54 EB027690-03S1 and U54 EB027690-03S2 and the National Center for Advancing Translational Sciences of the National Institutes of Health under Award Number UL1TR002378. The content is solely the responsibility of the authors and does not necessarily represent the official views of the National Institutes of Health.

IRB approval

This study was approved by the Emory University Institutional Review Board under STUDY00001082.

Data availability statement

The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

CRediT authorship contribution statement

Gregory L. Damhorst: Writing – review & editing, Writing – original draft, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Jessica Lin:** Writing – original draft, Data curation. **Jennifer K. Frediani:** Writing – review & editing, Writing – original draft, Project administration, Methodology, Investigation, Data curation, Conceptualization. **Julie A. Sullivan:** Project administration. **Adrianna Westbrook:** Software, Methodology, Formal analysis. **Kaleb McLendon:** Methodology, Investigation. **Tyler J. Baugh:** Methodology, Investigation. **William H. O'Sick:** Methodology, Investigation. **John D. Roback:** Investigation, Data curation, Conceptualization. **Anne L. Piantadosi:** Methodology, Investigation, Formal analysis. **Jesse J. Waggoner:** Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Leda Bassit:** Methodology, Investigation, Conceptualization. **Anuradha Rao:** Methodology, Investigation, Conceptualization. **Morgan Greenleaf:** Project administration, Conceptualization. **Jared W. O'Neal:** Project administration, Methodology. **Seegar Swanson:** Investigation, Formal analysis, Data curation. **Nira R. Pollock:** Methodology, Investigation, Conceptualization. **Greg S. Martin:** Supervision, Project administration, Funding acquisition. **Wilbur A. Lam:** Writing – review & editing, Supervision, Funding acquisition, Conceptualization. **Joshua M. Levy:** Writing – review & editing, Writing – original draft, Supervision, Methodology, Investigation, Conceptualization.

Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests:

Wilbur Lam, Greg Martin reports financial support was provided by National Institute of Biomedical Imaging and Bioengineering. Wilbur Lam, Greg Martin reports administrative support was provided by National Center for Advancing Translational Sciences. This research was supported by funds from the NIDCD Division of Intramural Research to Joshua Levy (DC000097).

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.heliyon.2024.e27188.

References

[1] F.M. Marty, K. Chen, K.A. Verrill, How to Obtain a nasopharyngeal swab specimen, N. Engl. J. Med. 382 (22) (2020) e76.
[2] O. Nikolai, C. Rohardt, F. Tobian, et al., Anterior nasal versus nasal mid-turbinate sampling for a SARS-CoV-2 antigen-detecting rapid test: does localisation or professional collection matter? Inf. Disp. 53 (12) (2021) 947–952.
[3] E.S. Savela, A. Winnett, A.E. Romano, et al., Quantitative SARS-CoV-2 viral-load curves in paired saliva and nasal swabs inform appropriate respiratory sampling site and analytical test sensitivity required for earliest viral detection [Internet], J. Clin. Microbiol. 60 (2) (2021) e0178-521 [cited 2022 Jan 31]; Available from: https://journals.asm.org/doi/abs/10.1128/JCM.01785-21.
[4] S.C.Y. Wong, H. Tse, H.K. Siu, et al., Posterior oropharyngeal saliva for the detection of Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), Clin. Infect. Dis. 71 (11) (2020) 2939–2946.
[5] M. Echavarria, N.S. Reyes, P.E. Rodriguez, et al., Self-collected saliva for SARS-CoV-2 detection: a prospective study in the emergency room, J. Med. Virol. 93 (5) (2021) 3268–3272.
[6] Saegerman C, Donneau A-F, Speybroeck N, et al. Repetitive saliva-based mass screening as a tool for controlling SARS-CoV-2 transmission in nursing homes. Transbound Emerg Dis. 2022 Jul;69(4):e194-e203 [cited 2022 Feb 1];n/a(n/a). Available from: https://onlinelibrary.wiley.com/doi/abs/10.1111/tbed.14280..
[7] S. Ricci, L. Lodi, F. Citera, et al., How home anterior self-collected nasal swab simplifies SARS-CoV-2 testing: new surveillance horizons in public health and beyond, Virol. J. 18 (1) (2021) 59.
[8] M. Valentine-Graves, E. Hall, J.L. Guest, et al., At-home self-collection of saliva, oropharyngeal swabs and dried blood spots for SARS-CoV-2 diagnosis and serology: post-collection acceptability of specimen collection process and patient confidence in specimens, PLoS One 15 (8) (2020) e0236775.
[9] Health C for D and R., In vitro diagnostics EUAs - antigen diagnostic tests for SARS-CoV-2 [Internet], FDA (2022) [cited 2022 Feb 7];Available from: https://www.fda.gov/medical-devices/coronavirus-disease-2019-covid-19-emergency-use-authorizations-medical-devices/in-vitro-diagnostics-euas-antigen-diagnostic-tests-sars-cov-2.
[10] CDC, COVID-19 and your health [Internet], Cent. Dis. Control Prev (2020) [cited 2022 Feb 3];Available from: https://www.cdc.gov/coronavirus/2019-ncov/testing/self-testing.html.
[11] [Internet], How to Do a Rapid Lateral Flow Test for Coronavirus (COVID-19), nhs.uk, 2021 [cited 2022 Jan 30];Available from: https://www.nhs.uk/conditions/coronavirus-covid-19/testing/how-to-do-a-test-at-home-or-at-a-test-site/how-to-do-a-rapid-lateral-flow-test/.
[12] G. Patriquin, J.J. LeBlanc, H.A. Gillis, G.R. McCracken, J.J. Pettipas, T.F. Hatchette, Combined oropharyngeal/nares and nasopharyngeal swab sampling remain effective for molecular detection of SARS-CoV-2 Omicron variant, J. Med. Microbiol. 71 (6) (2022).
[13] B.L. Goodall, J.J. LeBlanc, T.F. Hatchette, L. Barrett, G. Patriquin, Investigating the sensitivity of nasal or throat swabs: combination of both swabs increases the sensitivity of SARS-CoV-2 rapid antigen tests, Microbiol. Spectr. 10 (4) (2022) e0021722.
[14] N.N.Y. Tsang, H.C. So, K.Y. Ng, B.J. Cowling, G.M. Leung, D.K.M. Ip, Diagnostic performance of different sampling approaches for SARS-CoV-2 RT-PCR testing: a systematic review and meta-analysis, Lancet Infect. Dis. 21 (9) (2021) 1233–1245.
[15] R.A. Lee, J.C. Herigon, A. Benedetti, N.R. Pollock, C.M. Denkinger, Performance of saliva, oropharyngeal swabs, and nasal swabs for SARS-CoV-2 molecular detection: a systematic review and meta-analysis [Internet], J. Clin. Microbiol. 59 (5) (2021) e02881-20 [cited 2022 Jan 30];Available from: https://journals.asm.org/doi/abs/10.1128/JCM.02881-20.

[16] Y. Zhou, T.J. O'Leary, Relative sensitivity of anterior nares and nasopharyngeal swabs for initial detection of SARS-CoV-2 in ambulatory patients: rapid review and meta-analysis, PLoS One 16 (7) (2021) e0254559.
[17] M.T.N. Nsoga, I. Kronig, F.J.P. Rodriguez, et al., Diagnostic accuracy of Panbio rapid antigen tests on oropharyngeal swabs for detection of SARS-CoV-2, PLoS One 16 (6) (2021) e0253321.
[18] V. Agulló, M. Fernández-González, VO de la Tabla, et al., Evaluation of the rapid antigen test Panbio COVID-19 in saliva and nasal swabs in a population-based point-of-care study, J. Infect. 82 (5) (2021) 186–230.
[19] SARS-CoV-2 Variants - Stanford Coronavirus Antiviral & Resistance Database (CoVDB) [Internet]. [cited 2021 Dec 19];Available from: https://covdb.stanford.edu/page/mutation-viewer/#omicron.
[20] K.P.Y. Hui, J.C.W. Ho, M. Cheung, et al., SARS-CoV-2 Omicron variant replication in human bronchus and lung ex vivo, Nature (2022) 1–5.
[21] G. Marais, N. Hsiao, A. Iranzadeh, et al., Saliva swabs are the preferred sample for Omicron detection [Internet]. https://www.medrxiv.org/content/10.1101/2021.12.22.21268246v1, 2021.
[22] J. Schrom, C. Marquez, G. Pilarowski, et al., Comparison of SARS-CoV-2 reverse transcriptase polymerase chain reaction and BinaxNOW rapid antigen tests at a community site during an omicron surge : a cross-sectional study, Ann. Intern. Med. 175 (5) (2022) 682–690.
[23] The RADx tech test verification core and the acme POCT in the evaluation of COVID-19 testing devices: a model for progress and change, Ieee Open J Eng Med Biol 2 (2021) 142–151.
[24] Use personal protective equipment (PPE) when caring for patients with confirmed or suspected COVID-19 [Internet]. https://www.cdc.gov/coronavirus/2019-ncov/downloads/A_FS_HCP_COVID19_PPE.pdf, 2020.
[25] C.B.F. Vogels, A.E. Watkins, C.A. Harden, et al., SalivaDirect: a simplified and flexible platform to enhance SARS-CoV-2 testing capacity, Med N Y N 2 (3) (2021) 263–280.e6.
[26] SalivaDirect assay EUA summary [Internet]. https://www.fda.gov/media/141192/download, 2022.
[27] C. Vogels, O.M. Allicock, D.E. Brackney, et al., SalivaDirect™: RNA extraction-free SARS-CoV-2 diagnostics [Internet], protocols.io (2021). https://www.protocols.io/view/salivadirect-rna-extraction-free-sars-cov-2-diagno-btdnni5e.
[28] SARS-CoV-2 N PROTEIN ANTIGEN (RUO) [Internet]. Quanterix. [cited 2022 Feb 14];Available from: https://www.quanterix.com/simoa-assay-kits/sars-cov-2-n-protein-antigen/..
[29] SARS-CoV-2 N Protein Advantage Kit Instructions for HD-X.pdf [Internet]. [cited 2022 Feb 14];Available from: http://portal.quanterix.com.
[30] A. Babiker, K. Immergluck, S.D. Stampfer, et al., Single-amplicon multiplex real-time reverse transcription-PCR with tiled probes to detect SARS-CoV-2 spike mutations associated with variants of concern, J. Clin. Microbiol. 59 (12) (2021) e0144621. https://journals.asm.org/doi/abs/10.1128/JCM.01446-21.
[31] Á. O'Toole, V. Hill, O.G. Pybus, et al., Tracking the international spread of SARS-CoV-2 lineages B.1.1.7 and B.1.351/501Y-V2 with grinch, Wellcome Open Res 6 (2021) 121.
[32] A. Addetia, M.J. Lin, V. Peddu, P. Roychoudhury, K.R. Jerome, A.L. Greninger, Sensitive recovery of complete SARS-CoV-2 genomes from clinical samples by use of swift biosciences' SARS-CoV-2 multiplex amplicon sequencing panel [Internet], J. Clin. Microbiol. 59 (1) (2020) e02226-20 [cited 2022 Feb 11];Available from: https://journals.asm.org/doi/abs/10.1128/JCM.02226-20.
[33] M. Martinez, P.-V. Nguyen, M. Su, et al., SARS-CoV-2 variants in Paraguay: detection and surveillance with an economical and scalable molecular protocol, Viruses 14 (5) (2022) 873.
[34] CDC, COVID data tracker variant proportions [Internet], Cent. Dis. Control Prev (2020) [cited 2022 Apr 21];Available from: https://covid.cdc.gov/covid-data-tracker/#variant-proportions.
[35] B. Adamson, R. Sikka, A.L. Wyllie, P. Premsrirut, Discordant SARS-CoV-2 PCR and rapid antigen test results when infectious: a december 2021 occupational case series [Internet]. https://www.medrxiv.org/content/10.1101/2022.01.04.22268770v1, 2022.
[36] J.J. Waggoner, M.B. Vos, E.A. Tyburski, et al., Concordance of SARS-CoV-2 results in self-collected nasal swabs vs swabs collected by health care workers in children and adolescents, JAMA 328 (10) (2022) 935–940.
[37] D.J. McCulloch, A.E. Kim, N.C. Wilcox, et al., Comparison of unsupervised home self-collected midnasal swabs with clinician-collected nasopharyngeal swabs for detection of SARS-CoV-2 infection, JAMA Netw. Open 3 (7) (2020) e2016382.
[38] G.A. Perchetti, M.-L. Huang, V. Peddu, K.R. Jerome, A.L. Greninger, Stability of SARS-CoV-2 in phosphate-buffered saline for molecular detection [Internet], J. Clin. Microbiol. 58 (8) (2020) e01094-20, https://journals.asm.org/doi/abs/10.1128/JCM.01094-20.