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EDITOR'S CHOICE

Sample collection and transport strategies to enhance yield, accessibility, and biosafety of COVID-19 RT-PCR testing

Padmapriya Banada[1], David Elson[1]†, Naranjargal Daivaa[1]†, Claire Park[1], Samuel Desind[1], Ibsen Montalvan[2], Robert Kwiatkowski[3], Soumitesh Chakravorty[1,3], David Alland[1,*] and Yingda L. Xie[1,*]

Abstract

Introduction. Non-invasive sample collection and viral sterilizing buffers have independently enabled workflows for more widespread COVID-19 testing by reverse-transcriptase polymerase chain reaction (RT-PCR).

Gap statement. The combined use of sterilizing buffers across non-invasive sample types to optimize sensitive, accessible, and biosafe sampling methods has not been directly and systematically compared.

Aim. We aimed to evaluate diagnostic yield across different non-invasive samples with standard viral transport media (VTM) versus a sterilizing buffer eNAT- (Copan diagnostics Murrieta, CA) in a point-of-care diagnostic assay system.

Methods. We prospectively collected 84 sets of nasal swabs, oral swabs, and saliva, from 52 COVID-19 RT-PCR-confirmed patients, and nasopharyngeal (NP) swabs from 37 patients. Nasal swabs, oral swabs, and saliva were placed in either VTM or eNAT, prior to testing with the Xpert Xpress SARS-CoV-2 (Xpert). The sensitivity of each sampling strategy was compared using a composite positive standard.

Results. Swab specimens collected in eNAT showed an overall superior sensitivity compared to swabs in VTM (70% vs 57%, P=0.0022). Direct saliva 90.5%, (95% CI: 82%, 95%), followed by NP swabs in VTM and saliva in eNAT, was significantly more sensitive than nasal swabs in VTM (50%, P<0.001) or eNAT (67.8%, P=0.0012) and oral swabs in VTM (50%, P<0.0001) or eNAT (58%, P<0.0001). Saliva and use of eNAT buffer each increased detection of SARS-CoV-2 with the Xpert; however, no single sample matrix identified all positive cases.

Conclusion. Saliva and eNAT sterilizing buffer can enhance safe and sensitive detection of COVID-19 using point-of-care GeneXpert instruments.

INTRODUCTION

Accurate, efficient, and biosafe detection of SARS-CoV-2 in both symptomatic and asymptomatic individuals with active COVID-19 infection is an essential public health strategy for preventing transmission and controlling the COVID-19 pandemic. Although nasopharyngeal (NP) swabs are a preferred specimen type, the invasiveness of this procedure, potential for variable collection quality, and need for supervised collection with biohazard risks have hindered the scalability of this testing method [1–4]. Non-invasive sampling methods such as saliva [5–9] or self-collected nasal or oral swabs [10, 11] combined with rapid, CLIA-waived COVID-19 assays [12, 13] have shown promise to enable broader testing of at-risk populations and increase public access to COVID-19 testing. Sterilizing buffers such as the guanidine-thiocyanate transport

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Author affiliations: [1]The Public Health Research Institute and Department of Medicine, Rutgers New Jersey Medical School, Newark, NJ 07103, USA; [2]University Hospital, Newark, NJ 07103, USA; [3]Cepheid, Sunnyvale, CA, USA.
*Correspondence: Yingda L. Xie, ylx1@njms.rutgers.edu; David Alland, allandda@njms.rutgers.edu
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Abbreviations: CI, confidence interval; CLIA, Clinical Laboratory improvement Amendment; CT, cycle threshold; eNAT, eNAT™ commercial transport medium; EUA, Emergency use authorization; FDA, United States Food and Drug Administration; NP, Nasopharyngeal; RT-PCR, real-time polymerase chain reaction; SD, standard deviation; VTM, universal viral transport medium; Xpert, Xpert Xpress SARS-CoV-2 test.
†These authors contributed equally to this work
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buffer eNAT (Copan diagnostics Murrieta, CA) have also been found to enable more biosafe NP swab transport and testing by reverse-transcriptase polymerase chain reaction (RT-PCR) platforms outside of carefully controlled environments [14; 15]. As a combined strategy, non-invasive sampling and sterilizing buffers such as eNAT have the potential to further enhance yield, biosafety, and accessibility of COVID-19 RT-PCR testing in broad settings. However, to date, this has not been systematically evaluated.

We recently demonstrated that in saliva samples, eNAT buffer leads to viral inactivation, with at least 5-log reduction in viable SARS-COV-2, and stabilization of viral RNA [16]. With the premise that eNAT could optimize yield and simplify transport and handling of saliva and other non-invasive samples, we evaluated and compared the yield of eNAT versus VTM across different non-invasive samples using the Cepheid Xpert Xpress SARS-COV-2 test ('Xpert'). Xpert is an FDA-EUA approved rapid, integrated, cartridge-based RT-PCR test that can be run on widely existing GeneXpert instruments used in over 130 countries. To our knowledge, this is the first study to demonstrate the use of a viral inactivating buffer across various non-invasive samples in a point-of-care test to deliver a complete and scalable workflow for biosafe handling and testing of COVID-19 samples.

METHODS

Study population and sample collection

To collect SARS-CoV-2 positive samples from COVID-19 PCR confirmed participants, we conducted a sub-study to an observational cohort study of hospitalized and emergency room COVID-19 patients at University Hospital (UH) affiliated with the Rutgers New Jersey Medical School in Newark, NJ, USA. All patients presenting to UH were routinely screened by a SARS-CoV-2 RT-PCR test. This study was approved by the Rutgers Institutional Review Board for human subject research (Rutgers IRB # Pro2020001138). Eligible patients included adults (age ≥18) who tested SARS-CoV-2 PCR-positive by the in-hospital NP swab PCR tests (most commonly Simplexa COVID-19 Direct EUA (Diasorin Molecular LLC, Cypress, CA). Patients that could not or did not consent, were pregnant or breastfeeding, prisoners, or who were unable to provide any respiratory specimens were excluded. Trained study personnel collected one NP swab (baseline only), two oral swabs, two nasal swabs, and a saliva sample from all participants who consented to all sample types. A subset of participants being evaluated for hospital outcomes in the parent study continued to be sampled longitudinally by oral swabs, nasal swabs, and saliva every 2–3 days until discharge. All swab types were immediately placed into 3 ml of sterile Universal Viral Transport Medium (VTM; Labscoop, Little Rock, AR) whereas a second nasal and oral swab was collected and immediately placed into 3 ml of eNAT (COPAN Diagnostics, Murrieta, CA, USA). A thinner nylon tip swab designed for nasopharyngeal (NP) sampling was used to obtain the NP swab (baseline only), and a thicker nylon tip swab designed for oral and nasal samples (Copan diagnostic, Murrieta, CA) was used for these sample types. NP swab collection was performed in accordance with CDC guidelines [17]; oral swab collection was performed by swabbing both buccal surfaces and tongue with an alternating order of collection for each media; nasal (anterior nares) swab collection was performed by rotating the swab 1 cm inside the nostril for 10–15 s, alternating nostrils for each media. Additionally, participants were instructed to self-collect a posterior saliva sample by clearing the back of their throat, then collecting 4 ml of saliva into a marked, empty, sterile wide-mouth cup (though any volume over 0.5 ml was accepted). All specimens were transported at room temperature and stored in a 2–4 °C prior to testing, which occurred within 48 h of sample collection.

Testing by xpert xpress SARS-Cov-2 ('Xpert')

NP, nasal, and oral swabs were tested by adding 300 µl of the sample (either in VTM or eNAT) directly to the Xpert SARS-Cov-2 test cartridges and the test was run in the GeneXpert system as per the manufacturer's instructions. The saliva sample was tested using three different methods. First, 300 µl of the saliva sample was directly added into the Xpert test cartridge ('saliva direct' sample). Additionally, the same saliva sample was swabbed with two separate swabs (thicker nylon tip swabs) for ten twirls followed by incubating each swab in the saliva for ~10–20 s ('saliva swab' sample). Each saliva swab sample was then transferred into test tubes containing 3 ml of either VTM or 2 ml of eNAT buffer and mixed well. From each of these mixtures, 300 µl were added directly to the sample chamber of Xpert cartridges. Saliva samples <300 µl were tested only by swabbing in eNAT and VTM. We also compared saliva samples directly diluted in 1:1, 1:2 and 1:4 ratios of saliva to eNAT. A minimum volume of 700 µl of saliva was needed to test all saliva processing methods: 'saliva direct', saliva swab in eNAT and all three dilutions. For saliva samples with volumes less than 700 µl, we prioritized saliva direct and saliva swab testing. Out of the 44 saliva direct positive samples tested with eNAT ratios, 1:1 dilution was not performed for one saliva sample due to insufficient volume. One each of the sample types had an error either due to pressure aborts (Error 2008) or probe check error (Error 5017) or instrument hardware error (Error 2025) and were not repeat tested. The saliva:eNAT mixtures were then tested using the GeneXpert system by adding 300 µl of the mixture to the Xpert SARS-CoV-2 test cartridge. The effect on the assay inhibition, N2 gene cycle threshold (Ct), percent positive rate and cartridge pressure values were evaluated.

Definitions

We compared samples that were collected contemporaneously (sample comparison set) and applied a composite SARS-CoV-2 positive reference standard, defined as at least one sample type being positive in the sample comparison set. We did not compare sample sets in which no samples were positive, as we reasoned that the PCR-negative samples

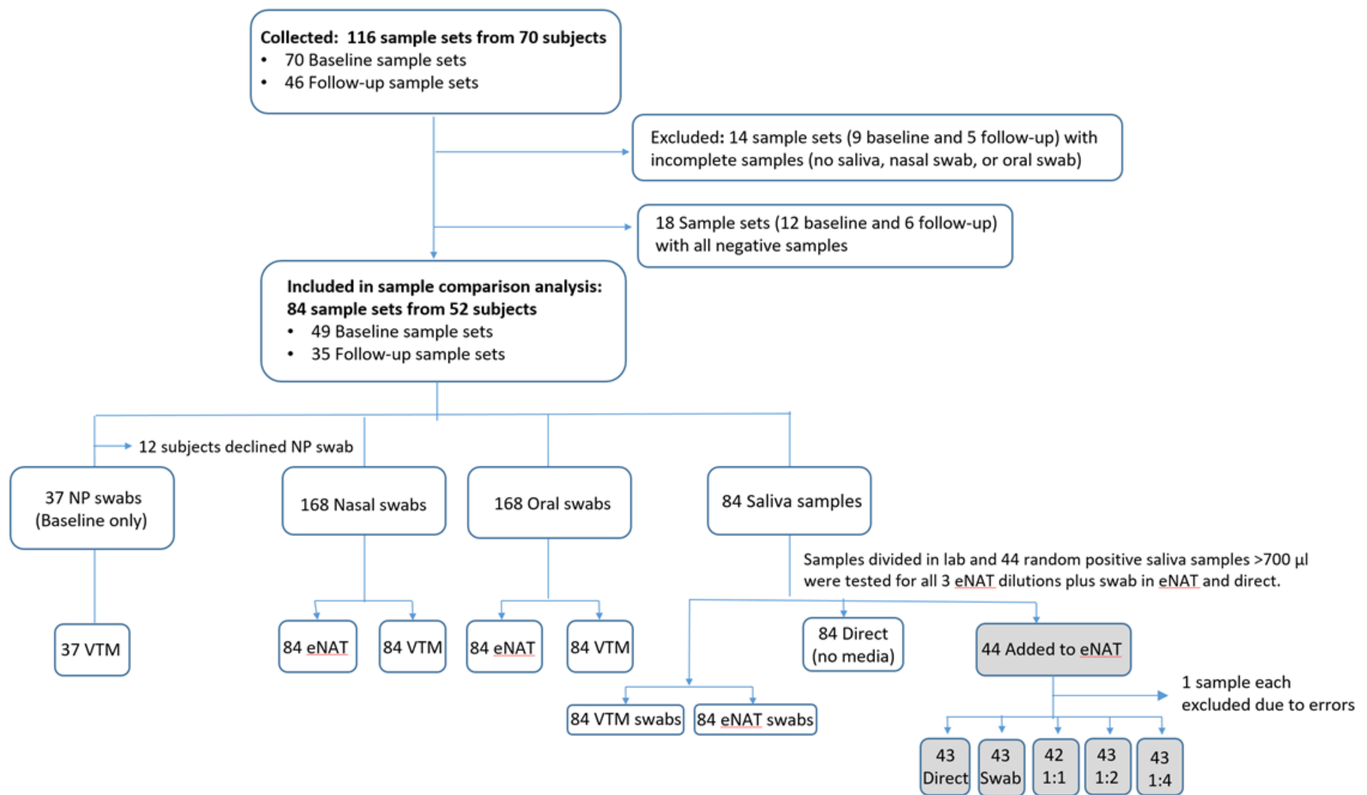

Fig. 1. Study flowchart.

from these individuals could not be considered false negative due to biological variability in sampling over time, but they were also not suitable as true negative comparators due to known COVID-19 status of these individuals. To confirm the discordancy that negative comparison sets was not due to difference in the in-hospital versus Xpert PCR tests, we obtained leftover media from positive NP swabs of a random subset of six participants. We then tested this archived sample as validation samples on Xpert. Xpert correctly detected SARS-CoV-2 in all six of these archived samples.

Statistical analyses

Standard statistical analyses (average, standard deviation, and *t*-test) and proportion of positive tests by each sampling method were compared by Chi-square, *t*-test or z-test as appropriate, using Microsoft Excel 365 for Windows, GraphPad Prism version eight or online software (http://vassarstats.net/propdiff_ind.html). Scatter plots for Ct values showing the mean and SD were included for the positive samples.

RESULTS

Participant enrollment and characteristics

Between 12 June 2020 and 23 October 2020, 70 subjects were enrolled into the study (Fig. 1). From these 70 enrollees, a total of 116 sample comparison sets were collected - 70 at baseline and 46 at follow-up time-points. Of note, some participants consented to all sample types except for NP swabs. Of the 116 comparison sets, 84 sample sets from 52 participants were complete with all specimen types and had at least one sample positive (by the composite reference standard) and were thus included in the sample comparison analysis (Fig. 1). Characteristics of the 52 participants in the analysis population (participants with at least one study sample positive for SARS-CoV-2) are shown in Table 1 and characteristics of the 13 participants with all negative samples are shown in Table S1 (available in the online version of this article). Among the 52 participants in the analysis population, 41 (79%) had symptoms potentially consistent with COVID-19 whereas 11 (21%) of these participants presented to the hospital for non-COVID indications, had no respiratory symptoms (asymptomatic), and were incidentally found to be COVID-19 positive by screening. Among the 41 symptomatic COVID-19 patients, nine (22%) did not require oxygen and had mild-moderate infection. Average participant age was 55, 37% were female, and the most common comorbidities were hypertension and diabetes.

On average, the baseline collection took place 2 days after the last positive in-hospital NP swab PCR test for participants in the analysis group, and 3 days for participants

Table 1. Characteristics of participants in the analysis population (participants with at least one study sample positive for SARS-CoV-2)

	Analysis population(N=52)
Mean Age in years (SD)	55 (15.1)
# of Men (%)	33 (63%)
# of Women (%)	19 (37%)
Ethnicity (%)	
Hispanic	35 (67%)
Black	15 (29%)
White	2 (4%)
Comorbidities	
Hypertension	27 (52%)
Diabetes mellitus	16 (31%)
Coronary artery disease	7 (13%)
Chronic kidney disease	4 (8%)
Lung disease (e.g. COPD)	8 (15%)
No chronic disease	19 (36%)
COVID symptoms (%)	
Cough	33 (64%)
Shortness of breath	32 (62%)
Fever	31 (60%)
Diarrhoea	13 (25%)
Chest pain	10 (19%)
No COVID symptoms	11 (21%)
Oxygen Support Required (%)	
None	20 (38%)
Nasal canula	29 (56%)
Non-invasive mechanical ventilation	2 (4%)
Intubation	1 (2%)
Symptom duration prior to baseline collection: **mean (range)**	7 days (1–23 days)
Days between in-hospital NP swab PCR and baseline collection: **mean (range)**	2 days (0–10 days)
Number of follow-up time-points per participant: **mean (range)**	1.5 (0–10)
Number of follow-up time-points per participant: **mean (range)**	1.5 (0–10)
Participants with negative NP swab PCR collected in routine clinical follow-up during hospitalization	19 (38%)

with no positive samples. The biological variability of PCR positivity from samples collected several days apart was evident in the discordancy of longitudinal in-hospital NP swab PCR testing results even when the same test was used. Nineteen (38%) of the 52 participants in the analysis group had at least one subsequent negative in-hospital NP swab PCR test during their hospital admission (Table 1). Additionally, we validated 100% agreement of the in-house test with Xpert (all the original samples were Xpert positive) from the original left-over positive NP swab specimen of six participants. These observations support that positive-negative discordancy across time was likely biological or sampling variability and unlikely due to discordancy between the in-hospital and Cepheid tests, and is consistent with previous comparative performance of Xpert Xpress SARS-CoV-2 with other SARS-CoV-2 RT-PCR platforms [12, 18, 19].

Comparative testing of different respiratory specimens in Xpert Xpress SARS-COV-2

A total of 84 sample comparison sets from 52 patients were included in the sample comparison analysis based on the composite reference, where at least one specimen in the comparison set was positive. Seventeen of these patients had follow-up samples collected on alternative days during their hospital stay. Thus, a total of 84 sets (49 baseline and 35 follow-up sets) of all specimen types were included in the analysis. Of the 49 completed baseline collections, 12 participants declined NP swab, leaving a total of 37 sample sets that could be analysed with NP swab.

As shown in Fig. 2a, undiluted saliva added directly to the cartridge ('direct saliva') gave the highest detection rate at 90.5% (76/84), followed by NP-VTM (86.5%, 32/37) and saliva in eNAT buffer (84.5%; 71/84), which were significantly higher compared to nasal or oral swabs (P<0.0001). Saliva in VTM (71.4%; 60/84) also performed better than oral swabs in VTM (50%; 42/84) or eNAT (58%; 49/84), as well as nasal swabs in VTM (50%; 42/84) or eNAT (67.8%; 57/84). We further analysed N2-gene cycle threshold (Ct) values for all positive samples as shown in Fig. 2b. Average N2 gene Ct values were the earliest for NP-VTM (32±5.4) and saliva direct (Ct=34.2±5.8) and most delayed for oral-VTM (37.5±4.9). The Ct range difference was statistically significant between saliva direct and oral-VTM (P<0.0001), oral-eNAT (P=0.0003) and saliva-VTM (P=0.0026). However, there was no significant difference of N2-Ct range for NP-VTM (P=0.28), nasal-VTM (P=0.09), nasal-eNAT (P=0.82) and saliva-eNAT (P=0.26) compared to saliva direct (Fig. 2b). There were three negative NP specimens that were detected in saliva, which we observed to have N2 Ct values of 39.4, 40.3 and 36.1 (Fig. S1c), indicating below LOD level viral loads [20] possibly contributing to the discrepancy. Only one of the sample sets was positive by NP swab (Ct=35.4) but negative in saliva direct and both saliva swabs (VTM and eNAT). Overall, we found that saliva performed better or equal to NP swabs in detecting COVID-19 positive patients. Similarly, the samples that were negative by other respiratory specimens (nasal or oral swab) but detected by saliva swab in VTM or eNAT had an overall delayed N2-Ct values of >37, indicating better performance in saliva for samples with low viral load (sub-LoD) or less variability in saliva collection.

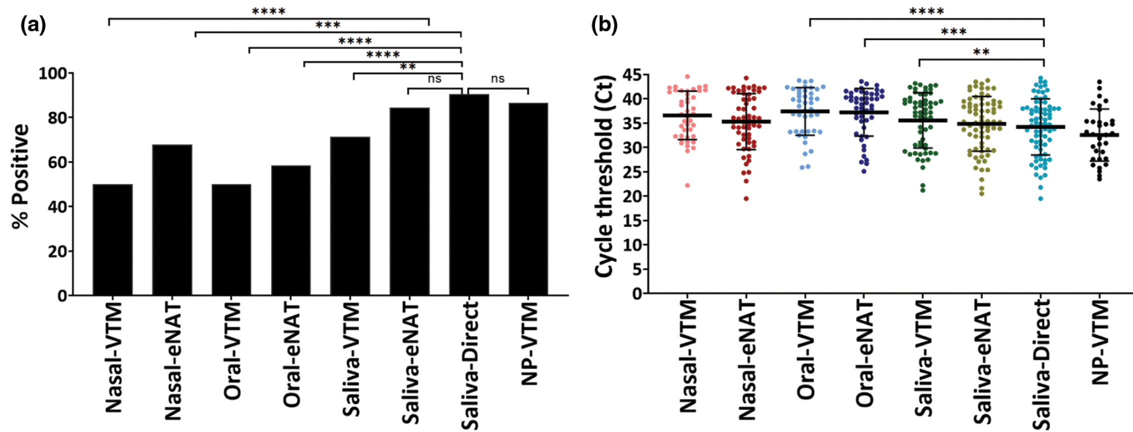

Fig. 2. Comparative testing of different respiratory specimens using the Xpert Xpress SARS-CoV-2 test. (A) Percent positive rate and (B) N2 gene cycle threshold (Ct) values of samples from all participants with at least one SARS-CoV-2 positive sample (*N*=84 for all samples and *N*=37 for NP swab). NP=Nasopharyngeal: VTM=Viral transport medium; eNAT=eNAT transport media, Copan diagnostics. ns=not statistically different. ****P<0.0001; ***P<0.001, **P=0.02.

Influence of transport media on detection across all sample types

We also evaluated if the composition of different transport media, specifically VTM and eNAT, had any influence on the detection sensitivity. As described, nasal and oral swabs were collected in both VTM and eNAT whereas saliva was collected from patients in an empty sterile cup, then subsequently swabbed and stored in VTM and eNAT. As shown before in Fig. 2A, compared to VTM, eNAT increased the positivity rate by about 20% (40/84 vs 57/84) for nasal swabs (P=0.008), followed by 12% for saliva (60/84 vs 70/84, P=0.065) and 6% for oral swabs (42/84 vs 47/84, P=0.43). When data from all sample types were combined to compare the two media, eNAT offered over 12% advantage (142 vs 174 out of 252 samples) in overall detection rate compared to VTM (P=0.003).

Optimizing the use of eNAT buffer for saliva

Compared to saliva swabbed into eNAT, direct saliva yielded an overall delayed SPC-Ct values in the Xpert test, indicating possible PCR inhibition and increased (>60 PSI) in-cartridge pressure values (Fig. S2b). Saliva diluted into eNAT at a ratio of 1:2 (N=43) yielded the second highest PCR positive rate (97.7%, 42/43) after saliva direct (100%, 43/43) (Fig. 3). Dilutions of 1:1 and 1:4 yielded 95% (40/42) and 93% (40/43) positive rate, respectively. Saliva swabs in eNAT showed the lowest sensitivity at 86% (37/43, P>0.05; Fig. 3a). A sample missed by 1:2 and 1:4 dilutions and another by 1:1 and 1:4 dilutions, had delayed N2-Ct values of 44.3 and 41.7 with saliva direct, respectively, indicating the influence of Poisson distribution for viral loads considerably below the limit of detection. Whereas the average N2-Ct values were similar (ca. 33–34) for all saliva

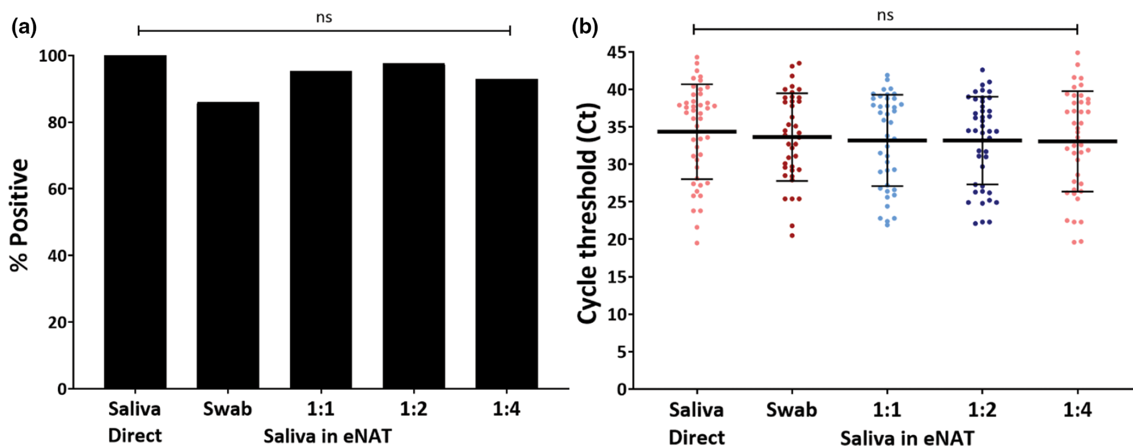

Fig. 3. eNAT as a transport media for saliva. Saliva diluted with eNAT at 1:1, 1:2, and 1:4 ratio showing (A) Percent positive rate and (B) N2-Ct values from patient saliva samples tested directly (*N*=44), as a swab in eNAT (*N*=44), diluted 1:1 (*N*=44), 1:2 (*N*=42), and 1:4 (*N*=42) in eNAT transport media. ns=not statistically significant.

conditions tested (P>0.05), the SPC-Ct values were earlier with saliva in eNAT compared to saliva direct (31.25±1.74, P<0.001, Fig. S2c), suggesting that PCR inhibition was mitigated by the addition of eNAT to an appreciable extent.

Operational characteristics of processing saliva in GeneXpert cartridges

To evaluate saliva processing profiles in the GeneXpert cartridges, we analysed the sample processing control (SPC) Ct values and in-cartridge pressure values. All respiratory samples collected in either VTM or eNAT did not have any significant difference either with SPC Ct or the max pressure values (Fig. S3A and B). Saliva direct, the only sample type analysed as is without dilution, yielded slightly delayed SPC Ct and higher cartridge pressure values with an average of 58±13.48, with one sample aborting the run due to pressure exceeding 100 psi (vs NP-VTM, P<0.0001). However, when the saliva was swabbed and transferred to VTM or eNAT, average pressure values fell to 53.2±6.06 (P<0.0001) and 52.2±5.4 (P<0.0001), respectively. Dilution with eNAT at 1:1, 1:2 and 1:4 ratio reduced the inhibition from saliva direct (P<0.0001) by lowering the average SPC-Ct values by ~2 Ct values (Ct 29.1 in 1:2 vs 31.2 in saliva direct). There was no significant difference in maximum in-cartridge pressure values with saliva dilution in eNAT (P>0.05), except for swab in eNAT (P=0.02). These results suggest that particles or mucus present in direct saliva samples can occasionally interfere with assay function, and that swab testing may be considered when these situations occur.

DISCUSSION

We found that saliva is an excellent test matrix for the Xpert Xpress SARS-CoV-2 test, providing a sensitivity (90.5% in VTM, 84.5% in eNAT) that is comparable to that of NP swabs (86.5% in VTM) and better than nasal (50% in VTM, 67.8% in eNAT) and oral swabs (50% in VTM, 58% in eNAT). This finding is consistent with previously published studies using other RT-qPCR modalities [21–26]. Although a handful of previous studies have looked at saliva tested in the Xpert SARS-CoV-2 [12, 27, 28], to our knowledge this study is the first study to comprehensively test multiple non-invasive sampling methods, in the setting of both symptomatic and asymptomatic SARS-CoV-2 infection, with and without the use of a sterilizing sample/transport buffer. By applying a composite reference standard for a positive sample, we observed that saliva enhanced the detection of SARS-CoV-2 compared all other sampling types, consistent with similar observations from other studies [1, 5–7]. It is worth noting that no sample matrix was 100% sensitive compared to the composite reference standard. Discordancy between sample matrices was most pronounced in samples that had a delayed cycle threshold indicating low viral load. This suggests that for patients with high-risk or severe disease, testing with multiple samples and perhaps multiple sample types when clinical suspicion is high may provide the highest sensitivity and negative predictive value for SARS-CoV-2 to guide treatment decisions.

We additionally found that eNAT, a buffer we have previously determined to be effective at inactivating SARS-CoV-2 in-vitro [16], increased the test positivity rates across all non-invasive sample types compared to VTM (P=0.0032), with a saliva to eNAT ratio of 1:2 being optimal in our sample set. We also found that adding eNAT to saliva possibly mitigates the PCR interference from saliva with lower pressure values and recovery of otherwise delayed SPC Ct values seen with direct saliva. These findings suggest that the application of eNAT as a sample buffer may be advantageous not only in safe handling and transport, but also in improving yield and processing capability of non-invasive samples on the Cepheid system.

There were several limitations in this study. First, there were less contemporaneous NP swabs collected with saliva, thereby reducing the number of direct comparisons between these two sample types, although they were found to be comparable. An underlying reason for this – participants declining NP swab collection due to its discomfort – also demonstrates the real-world limitations that would be magnified with larger scale testing such as in schools or the workplace. Secondly, we added eNAT to saliva in the laboratory, whereas the benefit of eNAT would be to sterilize samples immediately after collection and before transport and test set up. However, this allowed us to evaluate the combination of eNAT and saliva under different conditions and inform optimal design of kits to add eNAT immediately to saliva upon collection. Finally, our participants were patients who had either been admitted to the hospital or seen in the emergency department. This population may not be generalizable to ambulatory individuals who would benefit the most from self-collection. However, we captured a diverse patient group in our cohort including those who were never admitted, as well as patients who were detected by universal screening but reported no COVID-19 symptoms.

Altogether, our findings support the use of saliva and eNAT sterilizing buffer with non-invasive samples to enhance effective, safe, and accessible COVID-19 testing and screening in the many health care systems worldwide already using GeneXpert instruments.

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Conflicts of interest
The authors declare that there are no conflicts of interest.

Ethical statement
Written consent was obtained from all study participants under a Rutgers Institutional Review Board for human subject research (Rutgers IRB # Pro2020001138).

References

1. Qian Y, Zeng T, Wang H, Xu M, Chen J, *et al*. Safety management of nasopharyngeal specimen collection from suspected cases of coronavirus disease 2019. *Int J Nurs Sci* 2020;7:153–156.
2. Jayamohan H, Lambert CJ, Sant HJ, Jafek A, Patel D, *et al*. SARS-CoV-2 pandemic: a review of molecular diagnostic tools including sample collection and commercial response with associated advantages and limitations. *Anal Bioanal Chem* 2020;413:49–71.
3. Kinloch NN, Ritchie G, Brumme CJ, Dong W, Dong W, *et al*. Suboptimal biological sampling as a probable cause of false-negative COVID-19 diagnostic test results. *J Infect Dis* 2020;222:899–902.
4. Surkova E, Nikolayevskyy V, Drobniewski F. False-positive COVID-19 results: hidden problems and costs. *Lancet Respir Med* 2020;8:1167–1168.
5. Pasomsub E, Watcharananan SP, Boonyawat K, Janchompoo P, Wongtabtim G, *et al*. Saliva sample as a non-invasive specimen for the diagnosis of coronavirus disease 2019: A cross-sectional study. *Clin Microbiol Infect* 2021;27:e1–e4:285..
6. Vaz SN, Santana DS, Netto EM, Pedroso C, Wang WK, *et al*. Saliva is a reliable, non-invasive specimen for SARS-CoV-2 detection. *Braz J Infect Dis* 2020;24:422–427.
7. Wyllie AL, Fournier J, Casanovas-Massana A, Campbell M, Tokuyama M, *et al*. Saliva or nasopharyngeal swab specimens for detection of SARS-CoV-2. *N Engl J Med* 2020;383:1283–1286.
8. To KK-W, Tsang OT-Y, Yip CC-Y, Chan K-H, Wu T-C, *et al*. Consistent detection of 2019 novel coronavirus in saliva. *Clin Infect Dis* 2020;71:841–843.
9. Babady NE, McMillen T, Jani K, Viale A, Robilotti EV, *et al*. Performance of severe Acute Respiratory Syndrome Coronavirus 2 real-time RT-PCR tests on oral rinses and saliva samples. *J Mol Diagn* 2021;23:3–9.
10. McCulloch DJ, Kim AE, Wilcox NC, Logue JK, Greninger AL, *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* 2020;3:e2016382.
11. Tu Y-P, Jennings R, Hart B, Cangelosi GA, Wood RC, *et al*. Swabs collected by patients or health care workers for SARS-COV-2 testing. *N Engl J Med* 2020;383:494–496.
12. Chen JH, Yip CC, Poon RW, Chan KH, Cheng VC, *et al*. Evaluating the use of posterior oropharyngeal saliva in a point-of-care assay for the detection of SARS-CoV-2. *Emerg Microbes Infect* 2020;9:1356–1359.
13. Ravi N, Cortade DL, Ng E, Wang SX. Diagnostics for SARS-CoV-2 detection: A comprehensive review of the FDA-EUA COVID-19 testing landscape. *Biosens Bioelectron* 2020;165:112454.
14. Richard-Greenblatt M, Comar CE, Flevaud L, Berti M, Harris RM, *et al*. Copan eNAT Transport System to Address Challenges in COVID-19 Diagnostics in Regions with Limited Testing Access. *J Clin Microbiol* 2021;12:JCM.
15. Mannonen L, Kallio-Kokko H, Loginov R, Jaaskelainen A, Jokela P, *et al*. Comparison of two commercial platforms and a laboratory-developed test for detection of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) RNA. *J Mol Diagn* 2021;S1525-1578:00007-6.
16. Banik S, Saibire K, Suryavanshi S, Johns G, Chakravorty S, *et al*. Inactivation of SARS-CoV-2 virus in saliva using a guanidium based transport medium suitable for RT-PCR diagnostic assays. *PLOS ONE* 2021;16.
17. CDC. Interim guidelines for collecting, handling, and testing clinical specimens for covid-19. 2020. https://www.cdc.gov/coronavirus/2019-ncov/lab/guidelines-clinical-specimens.html
18. Lieberman JA, Pepper G, Naccache SN, Huang ML, Jerome KR, *et al*. Comparison of commercially available and laboratory-developed assays for in vitro detection of SARS-CoV-2 in clinical laboratories. *J Clin Microbiol* 2020;58.
19. Zhen W, Manji R, Smith E, Berry GJ. Comparison of four molecular *in vitro* diagnostic assays for the detection of SARS-CoV-2 in nasopharyngeal specimens. *J Clin Microbiol* 2020;58.
20. Loeffelholz MJ, Alland D, Butler-Wu SM, Pandey U, Perno CF, *et al*. Multicenter evaluation of the cepheid Xpert Xpress SARS-CoV-2 Test. *J Clin Microbiol* 2020;58.
21. Alizargar J, Etemadi Sh M, Aghamohammadi M, Hatefi S. Saliva samples as an alternative for novel coronavirus (COVID-19) diagnosis. *J Formos Med Assoc* 2020;119:1234–1235.
22. Azzi L, Baj A, Alberio T, Lualdi M, Veronesi G, *et al*. Rapid Salivary Test suitable for a mass screening program to detect SARS-CoV-2: A diagnostic accuracy study. *J Infect* 2020;81:e75–e78.
23. Chu AW-H, Chan W-M, Ip JD, Yip CC-Y, Chan JF-W, *et al*. Evaluation of simple nucleic acid extraction methods for the detection of SARS-CoV-2 in nasopharyngeal and saliva specimens during global shortage of extraction kits. *J Clin Virol* 2020;129:104519.
24. Lai CKC, Chen Z, Lui G, Ling L, Li T, *et al*. Prospective study comparing deep throat saliva with other respiratory tract specimens in the diagnosis of novel coronavirus disease 2019. *J Infect Dis* 2020;222:1612–1619.
25. Nagura-Ikeda M, Imai K, Tabata S, Miyoshi K, Murahara N, *et al*. Clinical Evaluation of Self-Collected Saliva by Quantitative Reverse Transcription-PCR (RT-qPCR), Direct RT-qPCR, reverse transcription-loop-mediated isothermal amplification, and a rapid antigen test to diagnose COVID-19. *J Clin Microbiol* 2020;58.
26. Hanson KE, Barker AP, Hillyard DR, Gilmore N, Barrett JW, *et al*. Self-collected anterior nasal and saliva specimens versus health care worker-collected nasopharyngeal swabs for the molecular detection of SARS-CoV-2. *J Clin Microbiol* 2020;58:11.
27. McCormick-Baw C, Morgan K, Gaffney D, Cazares Y, Jaworski K, *et al*. Saliva as an alternate specimen source for detection of SARS-CoV-2 in symptomatic patients using cepheid xpert xpress SARS-CoV-2. *J Clin Microbiol* 2020;58.
28. Wong RC-W, Wong AH, Ho YI-I, Leung EC-M, Lai RW-M. Evaluation on testing of deep throat saliva and lower respiratory tract specimens with Xpert Xpress SARS-CoV-2 assay. *J Clin Virol* 2020;131:104593.

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