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Evaluation of observed and unobserved self-collection of saline gargle samples for the detection of SARS-CoV-2 in outpatients

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ABSTRACT

The diagnostic sensitivity of observed and unobserved self-collected saline gargle samples for the molecular detection of SARS-CoV-2 in adults and school-aged children was evaluated against a reference standard of health care worker collected nasopharyngeal flocked swab.

A total of 46 participants had a positive nasopharyngeal swab sample; of these, 10 were in the observed phase and 36 were in the unobserved phase. Only one matching saline gargle sample tested negative and this was in the unobserved phase, giving an overall sensitivity of 98%. Average viral target Ct values were higher in the saline gargle samples. RNaseP Ct values were lower in unobserved collected samples compared to observed collected samples.

Unobserved self-collection of saline gargle samples is a promising outpatient testing method for COVID-19 diagnosis. The self-collection method has potential to simplify the diagnostic cycle and facilitate implementation of COVID-19 testing, particularly in settings with limited access to health care workers.

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1. Background

The COVID-19 global pandemic has shown the potential to overwhelm the capacity of local and national health care systems (Cesari and Montero-Odasso, 2020) and many areas of the world continue to have very little vaccine coverage. Ongoing Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) testing is needed to identify cases in real time to prevent widespread community transmission, evaluate and monitor vaccine effectiveness, and monitor for new strains. Given the range of symptoms and severity of illness, clinical recognition of cases can be very difficult, and therefore laboratory detection has been the cornerstone of surveillance and containment. However, rapid implementation of laboratory testing for SARS-CoV-2 on such a large scale has presented numerous challenges including deployment of assays with poor performance, lack of availability of high throughput testing platforms, and bottle-necks in procurement of preanalytical materials

and testing reagents (Callahan et al., 2020). One of the key weaknesses in the diagnostic cycle has been the challenges related to sample acquisition. The generally preferred (Canadian Public Health Laboratory Network Best Practices for COVID-19, 2020) specimen type, flocked nasopharyngeal (NP) swabs, had not been able to meet the dramatic increases in global demand and are often difficult to procure particularly in resource limited settings. Additionally, collection of NP swab samples requires trained healthcare workers (HCWs), which constrains human resources where HCWs are needed in other areas, such as in the clinical care of COVID-19 cases, other clinical programs and vaccine roll out.

Saline or water mouth rinse gargle sample collection is a promising noninvasive alternative sample type with high user acceptability and similar diagnostic performance when compared with HCW collected NP swabs (Dumaresq et al., 2021; Goldfarb et al., 2021; Kandel et al., 2021; LeBlanc et al., 2021). Saline gargle (SG) samples are also amenable to self-collection, which reduces the number of trained HCWs needed for collection. The present study aimed to evaluate if unobserved self-collected SG samples perform as well as HCW collected NP swabs for the detection of SARS-CoV-2 in outpatients

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2. Methods

2.1. Setting and study participants

This prospective study was carried out with outpatients presenting to the BC Children's and Women's Hospital Campus COVID -19 Collection Centre in Vancouver, Canada. Participants were eligible for the study if they were 4 years of age and older. Individuals with a contraindication to NP swab collection or with tracheostomies were excluded.

Eligible participants were invited to participate in the investigation by providing a SG sample in addition to a HCW collected NP swab. After obtaining verbal consent, their agreement and demographic information were recorded. This project was reviewed by the BC Children's and Women's Research Ethics Board and deemed a quality improvement/quality assurance (QI/QA) activity.

2.2. Specimen collection and processing

From September 4th to September 29th, 2020 SG specimens were self-collected with collection observed by nursing staff, and from September 30th to October 29, 2020 SG sample collection was performed in a separate room with no observation and no guidance provided by HCWs.

Participants in the unobserved phase were directed to a clinic room where they were provided with a SG collection kit. Participants were eligible if they had not eaten, drank, or brushed their teeth within the hour prior to sample collection. Written and visual collection instructions were displayed in the room (supplemental material), instructing users to open 5 mL vials of sterile 0.9% saline (Addipak®, Teleflex Medical, Research Triangle Park, NC, USA), squeeze the contents into their or their child's open mouth, swish the contents for 5 seconds followed by tilting their heads back and gargling for 5 seconds. Instructions indicated that users should repeat this swish/gargle cycle 2 more times and then expel the saline into a funnel mouthed sterile empty polypropylene container (Access Pacific Collection tube Fayege Inc, China). For unobserved collection, a video was also made available that patients and/or parents could watch (<https://youtu.be/ZvqjkbD-moA>). A nurse came to the room afterwards to collect the NP swab (flexible mini-tip flocced swabs with 3 mL universal viral transport system media, Beckon Dickinson, Sparks, MD) via the left naris unless participant stated preference for the right naris. NP swabs were inserted the distance from the naris to the external ear canal, then rotated five times and left in place for five seconds prior to being removed.

Both SG and NP samples were brought to the laboratory within 2 hours of collection.

2.3. Laboratory testing

NP swab samples were first extracted with the QiaSymphony automated extractor using the DSP virus/pathogen minikit (Qiagen, Germantown, MD) and subsequently tested with a laboratory developed test (LDT) within 24 hours of collection on the Applied Biosystems 7500 Fast Real-Time PCR System (Life Technologies, Carlsbad, CA). This previously described assay (5) detects 2 viral targets (E gene and RdRP gene) and also the human RNaseP gene, which is a marker of amount of human nucleic acid present in the sample. If NP swab was positive, SG samples were extracted and tested on the same platform. The SG sample was stored at room temperature until initial testing. Samples were considered positive if they tested positive for both targets (E gene and RdRP gene) with a threshold cycle (Ct) value of less than 40.

2.4. Statistical analysis

The McNemar exact test was used to determine the comparability of SG sample testing compared to NP swabs sample testing. The design of the study did not allow for assessment of specificity of sample types. The difference in Ct values for the RdRP gene detected from the HCW collected NP swab and the concordant self-collected saline gargle sample were compared using 2-sided paired *t* test. The difference in Ct values for RNaseP (as a surrogate marker of sample quality) were compared using an unpaired 2-sample *t* test. Statistical significance was set as $P < 0.05$ and all testing was done using GraphPad Prism ver 9.0.2 for Windows (GraphPad Software, San Diego, CA).

3. Results

In the initial observed phase (September 4th–29th, 2020) there were 758 participants who provided both sample types, of which 10 had a positive NP swab sample (1.3%). In the unobserved phase (September 30th–October 29th, 2020), there were a total of 1987 participants who provided both sample types, of which 36 had a positive

Table 1

PCR testing results for SARS-CoV-2 positive participants with matching saline gargle samples.

Sample ID	NP swab		Saline gargle	
	E Gene	RdRP	E Gene	RdRP
OB10	16.19	15.77	20.3	20.6
OB8	25.9	25.34	31.58	31.28
OB6	22.27	21.17	28.89	28.22
OB7	27.91	26.32	32.67	32.21
OB1	17.41	16.99	27.68	27.63
OB2	18.6	18.2	26.6	26.6
OB3	17.6	17.3	23.6	23.4
OB4	19.1	19	30.8	30.6
OB5	22.8	22	29	29.4
OB9	22.4	21.7	31.7	31.6
UNOB1	33.43	33.69	33.3	33.3
UNOB2	25.28	24.93	22.2	21.7
UNOB3	15.92	15.87	27.6	27.8
UNOB4	18.87	18.64	19.39	18.85
UNOB5	36.42	37.3	33.61	32.67
UNOB6	19.51	19.32	34.21	33.49
UNOB7	19.34	19.12	25.54	25.04
UNOB9	26.99	28.01	32.41	32.54
UNOB10	34.92	34.51	Negative	Negative
UNOB11	20.7	20.3	28.22	28.19
UNOB12	18.6	18.2	28.81	28.33
UNOB13	37.2	35.3	31.53	30.95
UNOB14	29.3	29	30.17	29.95
UNOB15	19	18.2	23.89	23.54
UNOB16	17.7	18.6	25.51	25.65
UNOB17	28.66	28.89	30.59	31.51
UNOB18	28.99	29.11	27.93	28.74
UNOB19	20.98	22.31	32.4	32.8
UNOB20	19.31	19.52	25.53	25.98
UNOB21	19.42	20.1	18.035	18.49
UNOB22	27.47	27.95	25.66	26.68
UNOB23	19	19.24	26.72	30.27
UNOB24	20.34	20.28	25.45	25.87
UNOB25	27.5	27.72	27.62	28.15
UNOB26	20.2	20.48	27.06	27.83
UNOB27	18.53	18.63	33.54	34.15
UNOB28	22.25	22.29	25.92	26.48
UNOB29	16.98	17.44	21.69	22.33
UNOB30	17.43	17.9	20.48	21.28
UNOB31	29.28	29.59	35.42	36.9
UNOB32	20.3	20.8	28.6	29.3
UNOB33	17.3	17.9	24.3	25.05
UNOB34	25.4	25.8	35.39	35.05
UNOB35	21.9	22.4	24.94	25.34
UNOB36	32.3	33	34.91	36.07
UNOB37	20	20.6	27.67	28.37

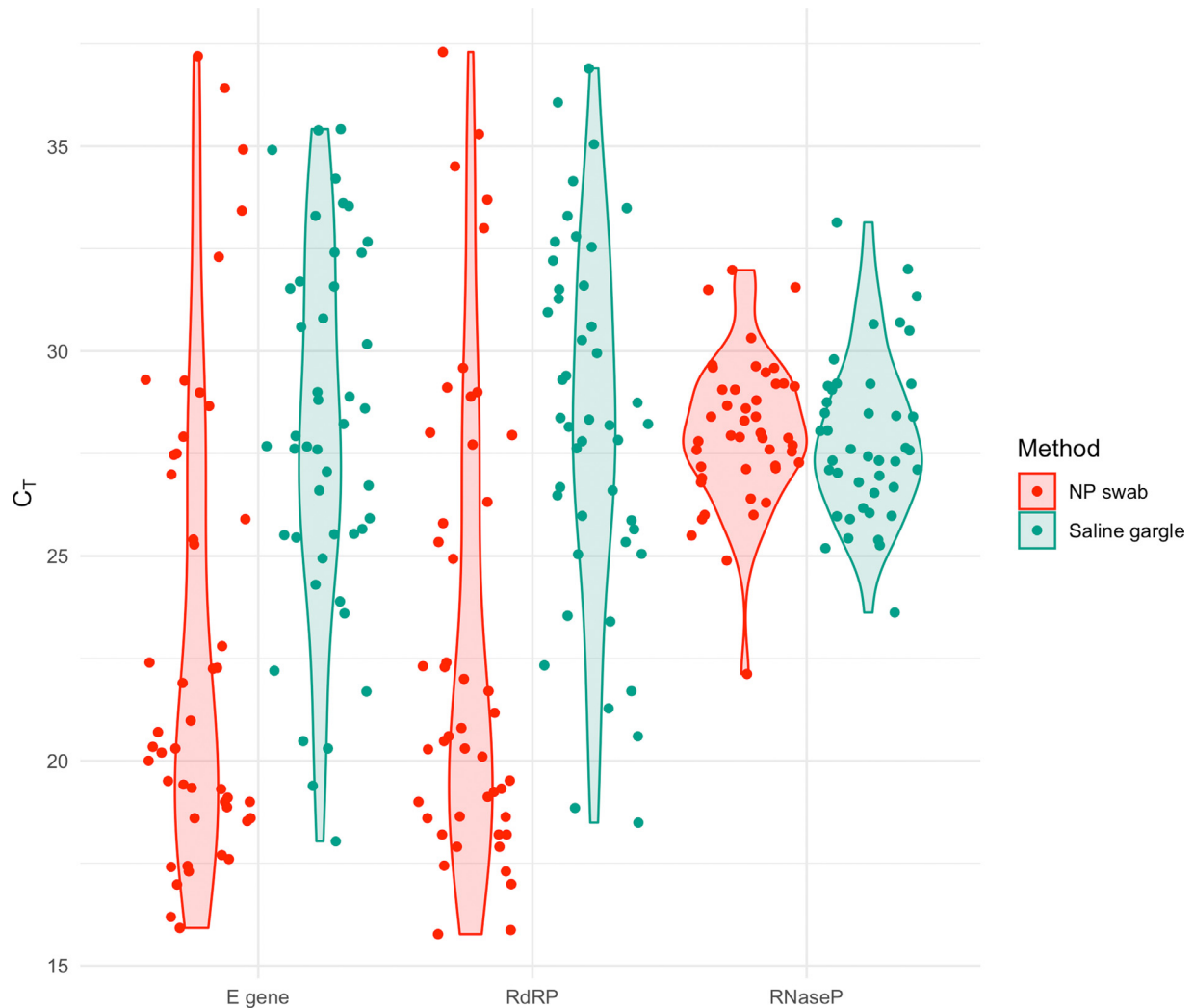


Fig. 1. Cycle threshold (Ct) values for E gene, RdRP and RNaseP targets in NP swab and matching saline gargle samples.

sample (2.6%). The median age for all positive participants was 29.5 years (interquartile range 14–41 years) and 14 (30.4%) were \leq 18 years of age.

Results of molecular testing are shown in [Table 1](#). One participant with a positive HCW collected NP swab had a negative matching saline gargle sample, giving an overall sensitivity of 97.8% (95% confidence interval 88.7%–99.6%). The one false negative saline gargle sample was in the unobserved group (with mean Ct value for viral targets in the matching NP swab being 34.7), giving a sensitivity for the unobserved method of 97.2% (95% confidence interval of 85.8%–99.5%). All saline gargle samples had both viral genes detected and there were no invalid results. The McNemar test revealed no significant difference in detection between the HCW collected NP swab and overall saline gargle sample (2 tailed P value = 1.0). All pediatric samples ($n = 14$) had matching NP and saline gargle testing results.

[Fig. 1](#) shows a violin plot of the Ct values for NP swab samples and matching saline gargle samples. There was a higher average Ct value for the viral RdRP target (+5.5, 95% confidence interval 4.09–6.93) and E gene (+5.26 95% confidence interval 3.86, 6.67) but no difference in the RNaseP target Ct values (-0.19, 95% confidence interval -1.11 to 0.72). Unobserved saline gargle samples had a slightly lower Ct value (-2.434, 95% confidence interval -3.795 to -1.072) suggesting a higher concentration of human cellular material in the unobserved collected samples.

4. Discussion

We found that unobserved self-collected SG samples perform as well as HCW collected NP swabs for the detection of SARS-CoV-2 in outpatients. There appeared to be a higher amount of human nucleic acid in the unobserved self-collected samples, which may reflect more aggressive mouth rinsing when unobserved, although the clinical relevance of this is unclear. Others have compared unobserved to HCW observed self-collected saliva samples and found that there was a difference in the performance ([Fernández-González et al., 2021](#)), with the observed saliva samples appearing to have better performance. One potential explanation for the preserved performance of unobserved saline gargle samples is the relatively high user acceptability for this sample collection method (5). A prior evaluation of user acceptability across a number of self-collected sample types including throat swabs, nasal swabs and saliva samples found that saline gargle samples were the most preferred by patients ([Finlayson-Trick et al., 2021](#)).

This study builds on previous research that demonstrated the noninferiority of this self-collection method when compared to HCW-collected NP swabs for the detection of SARS-CoV-2 among outpatients (5). In particular, the current study found that unobserved self-collected SG samples across a wide range of ages had high sensitivity, which has implications for implementation in community

settings. The finding of higher Ct values (i.e., lower viral loads) in SG samples when compared with NP swab samples is not unexpected given that it is a diluted sample type that uses lavage to better sample the entire oropharynx. This is similar to the higher Ct values but still improved sensitivity of bronchioalveolar lavage versus sputum samples for the molecular diagnosis of pulmonary tuberculosis (Theron et al., 2014). Unobserved self-collected SG sampling has the potential to alleviate human resource constraints, and given the high user acceptability may help address “testing fatigue” within communities where continued surveillance is needed (Street et al., 2020). Additionally, there is the potential to expand the scope of testing within community settings where human resource inputs and the logistics required for HCW collected NP swabs were insurmountable barriers, such as within schools, at border crossings or remote locations. It is recognized that promptly detecting people with COVID-19 is critical to pandemic containment efforts (Lyng et al., 2021). As schools and businesses attempt to re-open and stay open, contact networks increase in size and complexity, which reinforces the need to strengthen and expand capacity of laboratory diagnostic processes. However, test sensitivity and specificity of various methods, testing frequency, and cost are critical variables in the feasibility of scaling up viable practical and sustainable testing strategies within large cohort settings (Lyng et al., 2021). The high sensitivity of unobserved self-collected SG sampling method, alongside high acceptability and cost savings potential due to the lack of need for personal protective equipment or trained HCWs for sample acquisition, makes this a promising potential solution.

While our study design had several strengths, including participants across the pediatric and adult age ranges, comparison between observed and unobserved self-collection methods and enrolling outpatient individuals where largest burden of testing is performed, our study also had a number of limitations. The primary limitation of our study was the relatively small number of participants and the fact that we did not also test patients who had a negative NP swab sample. Therefore it is possible that saline gargle samples actually have a higher sensitivity than NP swabs. We also did not assess the performance in asymptomatic cases. Further research is recommended among asymptomatic cases as well as within large cohort community settings.

In conclusion, unobserved self-collection of SG samples is a promising outpatient collection method for COVID-19 diagnosis. The self-collection method is noninvasive and avoids the need for personal protective equipment, health worker collection and expensive swabs, which has potential to address a key bottle neck in the diagnostic cycle to facilitate more timely and equitable access to SARS-CoV-2 testing in many regions.

Declaration of competing interest

The authors report no conflicts of interest relevant to this article

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Author contributions

DMG, LMNH, PT, JAS, and GNA-R conceptualized the study and the methodology of the study with input from MM. IK conducted the investigation and MD was involved in study management. DMG, VJG and JNB conducted the statistical analyses and MWK wrote the first draft of the manuscript. All authors contributed to the interpretation and have read and approved the final manuscript.

Supplementary materials

Supplementary material associated with this article can be found in the online version at doi:[10.1016/j.diagmicrobio.2021.115566](https://doi.org/10.1016/j.diagmicrobio.2021.115566).

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