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Short communication

A combined oropharyngeal/nares swab is a suitable alternative to nasopharyngeal swabs for the detection of SARS-CoV-2

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ABSTRACT

Given the global shortage of nasopharyngeal (NP) swabs typically used for respiratory virus detection, alternative collection methods were evaluated during the COVID-19 pandemic. This study showed that a combined oropharyngeal/nares swab is a suitable alternative to NP swabs for the detection of SARS-CoV-2, with sensitivities of 91.7% and 94.4%, respectively.

1. Introduction

The first reports of 2019 novel coronavirus disease (COVID-19) caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) emerged from China in December 2019, but quickly spread as a pandemic. [1–3] Laboratory testing for SARS-CoV-2 plays an essential role in infection control and public health mitigation strategies; however, testing has been hampered by global supply chain shortage nasopharyngeal (NP) swabs and universal transport medium (UTM). As such, alternative collection methods were rapidly evaluated, including nasal swabs, oropharyngeal (OP) swabs, throat washings, and saliva [4–8]. While NP swabs in UTM are the specimen of choice for respiratory virus testing, a recent study demonstrated the feasibility of COVID-19 testing from nasal sample collected with a swab typically used for chlamydia and gonorrhea testing: the Aptima Multitest swab (Hologic, Inc.) and its accompanying specimen transport medium (STM). [9] This study sought to further validate the Aptima swab/STM collection kit for the detection of SARS-CoV-2 using a single swab approach to sample the oropharynx and anterior nares (OP/Na).

2. Methods

In assessment centers prioritizing areas with suspected community spread of SARS-CoV-2, specimens were collected for COVID-19 testing from 190 individuals using two different collection devices: a flocked NP swab in 3 mL UTM (Copan Diagnostics Inc., Murrieta, CA) and combined OP/Na sampling using the Aptima Multitest swab in 2.9 mL of STM (Hologic, Inc., San Diego CA), according to an accompanying instructional video (https://vimeo.com/397169241). Each specimen was stored at 4 °C until testing, and an aliquot was stored at −80 °C. Both swabs were run in parallel within 12 h of collection using two molecular methods. First, the SARS-CoV-2 assay, was performed on a Cobas 6800 system (Roche Diagnostics). For UTM (NP swab material), 600 µL was processed directly, as recommended by the manufacturer, whereas for the OP/Na, 200 µL of STM was diluted into 1 mL of Cobas omni Specimen Diluent prior to use due to the presence of high concentrations of detergents. [9] Second, a Total Nucleic Acid (TNA) extraction on a MagNApure LC 2.0 instrument (Roche Diagnostics) was performed, followed by real-time RT-PCR [i.e. laboratory-developed test (LDT) designed at the British Columbia Centre for Disease Control (BCCDC) (Vancouver, BC)]. Briefly, TNA was extracted from 200 µL of specimen (NP or OP/Na), eluted into 50 µL of elution buffer, and 5 µL was used as template in a triplex real-time RT-PCR, with primers and probes targeting the SARS-CoV-2 envelope (E) [10] and RNA-dependent RNA polymerase (RdRp), as well as those targeting an endogenous internal control, ribonuclease P (RNaseP). Amplification was performed on an Applied BioSystems 7500 Fast system (Thermo Fisher Scientific), and threshold cycles (Ct) values were determined by the manufacturer

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software. Results for each instrument were classified as positive or negative, and specimens yielding discrepant results were subjected to testing using the Xpert Xpress SARS-CoV-2 assay (Cepheid). Each test was compared to a modified reference standard defined as concordant results from at least two methods with different genetic targets. Sensitivity and specificity were calculated from 2 × 2 contingency tables with 95% confidence intervals (CI) for each collection (NP or OP/Na swabs) and instrument (LDT and commercial assay) using online software (https://www.medcalc.org/calc/diagnostic_test.php). A Fisher exact test was used to assess differences and $P \leq 0.05$ was considered statistically significant.

3. Results and discussion

The limited and unpredictable supply of NP swabs during the COVID-19 pandemic prompted the evaluation of swabs that were readily available and commonly used for sexually transmitted infections. Of 190 paired NP and OP/Na specimens collected, 154 negative results were obtained and 36 patients tested positive by at least one molecular method (18.9% positivity rate). Regardless of the swab (NP or OP/Na) or methods used (LDT or commercial), the specificity was 100.0% (155/155) [95% CI: 97.7–100.0%]. Using the LDT, the sensitivity of NP swabs for the detection of SARS-CoV-2 was 94.4% (34/36) [95% CI: 81.3%–99.3%] compared to 91.7% (33/36) [95% CI: 77.5–98.3%] for the OP/Na swabs. Using the commercial assay, the sensitivity for NP swabs was 100.0% (36/36) [95% CI: 90.3%–100.0%] compared to 88.9% (32/36) [95% CI: 73.9–96.9%] for the OP/Na specimens. While the sensitivity of OP/Na was lower than NP swabs using the LDT or commercial assays, no significant differences were observed ($P = 0.679$ and 0.115, respectively).

Patients with discrepant NP and OP/Na results are summarized in (Table 1). With the exception of patient 4, the other five patients with discrepant NP and OP/Na results had specimens with low viral loads (Table 1). Low viral loads are known to occur in the early and late stages of COVID-19 illness [4–6,11–19], and false negative results can arise from differences in analytical sensitivity between methods (Table S1) [20,21], the variability in specimen collection, or factors influencing specimen stability or recovery of SARS-CoV-2 RNA during specimen transport, storage or processing [4,13]. For example, three different SARS-CoV-2 targets were detected between the various PCR methods used for testing of specimens from patient 1, yet high Ct values were observed for these targets (Table 1). High Ct values are suggestive of low viral loads, and it is known that detection of PCR targets near the limit of detection lacks reproducibility. [20,21] Therefore, low viral loads and differences in analytical sensitivity of the various molecular methods could explain differences in SARS-CoV-2 detection between the NP and OP/Na collections (Table S1). Similar arguments could be made for patients 2–4, who were either asymptomatic or in the pre-symptomatic stage of infection where low viral loads can occur [4–6,11–19]. Discrepant results for patients 5 and 6 were in the setting of known positive cases, with symptoms predating their sample collection by 14 and 18 days, respectively. Waning viral loads over time in the upper respiratory tract are well documented for SARS-CoV-2; however, discrepant NP and OP/Na results from sampling in the later stages of illness may be of little clinical significance, as detection of SARS-CoV-2 RNA does not imply infectivity [4,6,11,19]. Further analyses are underway to correlate SARS-CoV-2 detection, and better understand viral shedding from various anatomical sites in patients stratified by disease onset, clinical presentation, and outcomes.

Interestingly, patient 4 had a positive NP swab with low Ct values (i.e. high viral load) by three different methods, but the OP/Na on the same patient was negative. The exogenous internal control in the commercial assay was amplified from the OP/Na specimen (arguing against the presence of PCR inhibitors); however, the LDT endogenous control in the OP/Na reaction was near the cutoff (Ct value of 34.9). While an unlikely alternative explanation could be a false-positive

Table 1
Relevant characteristics among ambulatory patients in whom results of paired nasopharyngeal and oropharyngeal/nares swabs were discrepant for the detection of SARS-CoV-2.

Patient	LDT					6800					Xpert		Symptoms	Comments
	NP		OP/Na			NP			OP/Na		NP			
	Ct (RdRp)	Ct (E)	Result	Ct (RdRp)	Ct (E)	Result	Ct (Orf1ab)	Ct (E)	Result	Ct (Orf1ab)	Ct (E)	Result	Ct (E)	Ct (N2)	Result		
1	ND	ND	NEG	ND	35.3	POS	ND	38.6	POS	35.8	37.6	POS	ND	40.0	POS	Yes, but onset not recorded	High Ct values (low viral load)
2	ND	ND	NEG	36.2	34.7	POS	ND	38.3	POS	33.9	36.2	POS	41.6	41.2	POS	No	High Ct values (low viral load)
3	37.8	35.7	POS	ND	37.6	POS	33.6	35.9	POS	ND	ND	NEG	35.2	37.5	POS	No	High Ct values (low viral load)
4	27.3	26.8	POS	ND	ND	NEG	27.5	28.2	POS	ND	ND	NEG	25.4	28.2	POS	No	High Ct value (35.9) observed for RNaseP with the OP/Na during testing with the LDT suggests issue in collection or transport
5	33.7	33.1	POS	ND	ND	NEG	30.7	33.1	POS	ND	ND	NEG	30.5	33.5	POS	Yes, with onset 18 days prior	High Ct values (low viral load)
6	33.6	33.4	POS	ND	ND	NEG	32.1	34.1	POS	ND	ND	NEG	34.2	37.4	POS	Yes, with onset 14 day prior	High Ct values (low viral load)

*Discrepant analysis using Xpert testing was only performed on nasopharyngeal swabs in UTM, as the OP/Na showed reduced sensitivity for this assay (Table S1). Abbreviations: Threshold cycle (Ct), envelope (E); laboratory-developed test (LDT); nucleoprotein (N2); not available (N/A); not detected (ND); negative (NEG); nasopharyngeal (NP); oropharyngeal/nares (OP/Na); positive (POS); RNA-dependent RNA polymerase (RdRp); ribonuclease P (RNaseP).

result for the NP swab, it is more likely that there were collection or transport deficiencies for the OP/Na specimen.

The data obtained from this study represents a relatively short time period in a community setting with a mixed population of asymptomatic and mildly-symptomatic patients. While OP/Na swabs collection showed excellent performance for the detection of SARS-CoV-2, as previously shown for nasal sampling [9], one should exercise caution in applying these findings to other patient populations, collection devices, or laboratory methods [4,22]. For example, upper respiratory specimens like NP or OP/Na might have poor performance in hospitalized adults with progression of COVID-19 to lower tract disease [4].

Overall, this study demonstrated that OP/Na sampling is a suitable alternative to NP swabs for the detection of SARS-CoV-2 in ambulatory patients, especially when symptomatic. To our knowledge, this is the largest head-to-head comparison of NP and OP/Na swabs for the detection of SARS-CoV-2, and the first study to evaluate the performance of the OP/Na collection with an Aptima Multitest swab for SARS-CoV-2 detection.

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Ethics

This project was a quality assurance initiative and did not require research ethics board review.

Declaration of Competing Interest

The authors have no conflicts to declare.

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Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.jcv.2020.104442.

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