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SHORT COMMUNICATION

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Comparison of SARS-CoV-2 detection with the Cobas® 6800/8800 system on gargle samples using two sample processing methods with combined oropharyngeal/nasopharyngeal swab

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Abstract

Background: Gargle samples have been proposed as a noninvasive method for detection of SARS-CoV-2 RNA. The clinical performance of gargle specimens diluted in Cobas® PCR Media and in Cobas® Omni Lysis Reagent was compared to oropharyngeal/nasopharyngeal swab (ONPS) for the detection of SARS-CoV-2 RNA.

Study Design: Participants were recruited prospectively in two COVID-19 screening clinics. In addition to the ONPS, participants gargled with 5 ml of natural spring water split in the laboratory as follows: 1 ml was added to 4.3 ml of polymerase chain reaction (PCR) media and 400 µl was added to 200 µl of lysis buffer. Testing was performed with the Cobas® SARS-CoV-2 test on the Cobas® 6800 or 8800 platforms.

Results: Overall, 134/647 (20.7%) participants were considered infected because the ONPS or at least one gargle test was positive. ONPS had, respectively, a sensitivity of 96.3% (95% confidence interval [CI]: 91.3–98.5); both gargle processing methods were slightly less but equally sensitive (90.3% [95% CI: 83.9–94.3]). When ONPS and gargle specimens were both positive, the mean cycle threshold (C_t) was significantly higher for gargles, suggesting lower viral loads.

Conclusion: Gargle specimens directly added in PCR Media provide a similar clinical sensitivity to chemical lysis, both having a slightly, not significantly, lower sensitivity to ONPS.

KEYWORDS
inactivation methods, molecular methods, SARS-CoV-2, specimen collection, transport medium

1 | INTRODUCTION

Accurate and rapid diagnosis of SARS-CoV-2 infection represents the cornerstone of public health interventions. Since the onset of the pandemic, the gold standard for the diagnosis of SARS-CoV-2 infection has been a reverse-transcription polymerase chain reaction (RT-PCR) test applied on a combined oropharyngeal and nasopharyngeal swab (ONPS). However, limitations such as the need for substantial human and material resources for ONPS collection and the discomfort associated with this invasive sample

have led to the evaluation of alternative specimen collection methods.[1]

Gargle samples have been previously reported with promising results as an autonomous, convenient, and noninvasive specimen collection method for detection of SARS-CoV-2 by a nucleic acid amplification test (NAAT).[2–5] However, processing of specimens potentially infected with SARS-CoV-2 still requires manipulation in a biosafety level 2 laboratory and specimen inactivation.[6,7] The Cobas® SARS-CoV-2 test performed in Cobas® 6800 and 8800 is fully automated and requires a lysis step of samples before testing in the instrument.[8] Considering the important number of specimens sent to the laboratory for screening purposes, the addition of a chemical lysis step for inactivation before detection of SARS-CoV-2 RNA, as suggested by the manufacturer, hampers the efficiency of testing and the workflow in the laboratory.

The Cobas® PCR Media, a transport medium that contains guanidine hydrochloride in Tris-HCL buffer, was found to significantly reduce SARS-CoV-2 plaque formation units (PFU) and median tissue culture infectious doses.[6,9] Adding directly gargle samples to polymerase chain reaction (PCR) media instead of using additional lysis protocols for specimen inactivation, could simplify sample processing for SARS-CoV-2 detection. However, the impact on the clinical sensitivity of SARS-CoV-2 RNA detection after diluting gargle samples in PCR media compared to chemical lysis, as well as its performance compared to ONPS, is unknown.

The main objective of this study was to evaluate the concordance in samples obtained from symptomatic individuals of SARS-CoV-2 detection with the two-target Cobas® SARS-CoV-2 test (Roche Molecular Diagnostics) performed in Cobas® 6800 and 8800 on ONPS, gargle specimens diluted in Cobas® PCR Media and in Cobas® Omni Lysis Reagent.

2 | MATERIALS AND METHODS

This prospective study was conducted in two designated COVID-19 screening clinics in Quebec in November and December 2020. Participants were recruited if they had symptoms of COVID-19 or if they had contact with a positive case. After obtaining informed consent, an ONPS was collected by a trained healthcare professional by swabbing the posterior oropharynx and then inserting the same swab through one nostril and rotating for 5–10 s before removing. A gargle sample was collected after with the following instructions. Participants were asked not to eat, drink, or smoke for 15 min before. They were handled a goblet with 5 ml of natural spring water (ESKA®, St-Mathieu-d'Harricana) and were told to gargle with the water for 5 s in the mouth, 5 s in the throat, to repeat this process once and then to spit as much as possible in the initial goblet. They were then asked to transfer their gargle specimen in a tube that was sent to a designated laboratory for processing and testing.

Gargle specimens were split in the laboratory as follows: 1 ml was added to 4.3 ml of PCR media and 400 µl was added to 200 µl of Cobas® Omni Lysis Reagent for 10 min, as suggested by the manufacturer. ONPS were transported in 4.3 ml of PCR media. Considering that 400 µl of processed sample is actually tested for SARS-CoV-2 RNA in the Cobas instruments, 267 µl of the initial gargle in Omni lysis reagent and 76 µl of the initial gargle in PCR media were tested for SARS-CoV-2 detection with the Cobas® SARS-CoV-2 test authorized by Health Canada, according to the manufacturer's instructions, on the cobas® 6800 in St-Eustache (n = 300) and the Cobas® 8800 in Montreal (n = 347).[8] Samples were considered positive if they met the interpretation criteria of the manufacturer. This assay simultaneously tests both the ORF1 a/b and E-gene viral molecular targets.

In the absence of a gold standard for SARS-CoV-2 RNA detection, data were first analyzed using a contingency table to assess the overall, positive and negative agreement with 95% confidence intervals (CI) calculated. The level of agreement was also assessed using κ statistics. By definition, κ values above 0.75 indicate excellent agreement, values between 0.40 and 0.75 indicate fair to good agreement, and values below 0.40 represent poor agreement beyond chance. The performance of the assay on ONPS and gargle samples was then calculated using a reference standard defined as a positive result from either the ONPS or any of the two processing methods on gargles. This reference standard was chosen because no current gold standard exists and false-negative NPS NAAT results have been reported.[1,10] Sensitivity was calculated using the exact Clopper-Pearson test. Linear regression analysis was performed to compare cycle thresholds (C_t) values between gargle samples. The study received ethical approval from the review boards of each participating institution.

3 | RESULTS

Among 647 participants, three were excluded as reported in Table 1 because an invalid result was obtained for one of their samples because of the presence of a clot interfering with the test. Overall, 134 (20.7%) participants were considered infected. The global agreement, positive agreement, and negative agreement between gargle samples processed in PCR media and ONPS were 97.2% (95% CI: 95.6–98.3; κ value of 0.91 [95% CI: 0.86–0.94]), 89.9% (95% CI: 83.4–94.1) and 99.0% (95% CI: 97.7–99.7), respectively, and between gargle samples processed with lysis buffer and ONPS were 97.4% (95% CI: 95.8–98.4; κ value of 0.92 [95% CI: 0.87–0.95]), 90.7% (95% CI: 84.3–94.7) and 99.2% (95% CI: 97.9–99.8), respectively (Table 1).

Using the reference standard as described in the methods section, NAAT with the ONPS and both gargle processing methods had a sensitivity of 96.3% (95% CI: 91.3–98.5) and 90.3% (95% CI: 83.9–94.3), respectively (Table S2). The negative predictive values were 99.0% (97.7–99.7) for ONPS and 97.5% (95.8–98.7) for gargle samples with each processing protocol.

ONPS positive for SARS-CoV-2 (n = 129) had median C_t values of 22.5 (interquartile range [IQR]: 18.6–25) for the E-gene target and 20.9 for the ORF1 a/b (IQR: 18.5–24.4). When ONPS and gargle

TABLE 1 Detection of SARS-CoV-2 RNA in ONPS, gargle samples in PCR media, and gargle samples in lysis buffer, using the Cobas® 6800/8800 SARS-CoV-2 test ($n = 647$)

Sample		ONPS			Agreement[a] (95% CI)			
		POS	NEG	Invalid	Positive	Negative	Total	κ
Gargle in PCR Media	POS	116	5	2	89.9 (83.4–94.1)	99.0 (97.7–99.7)	97.2% (95.6–98.3)	0.91 (0.86–0.94)
	NEG	13	511	0				
	Invalid	0	0	0				
Gargle in lysis buffer	POS	117	4	2	90.7 (84.3–94.7)	99.2 (97.9–99.8)	97.4% (95.8–98.4)	0.92 (0.87–0.95)
	NEG	12	511	0				
	Invalid	0	1	0				

Abbreviations: CI, confidence interval (calculated using exact Clopper-Pearson test); PCR, polymerase chain reaction; ONPS, oropharyngeal/nasopharyngeal swab.

[a]Invalid results were excluded from calculation of agreement.

TABLE 2 C_t values obtained from ONPS and gargle samples when both results are positive, according to PCR target ($n = 644$[a])

	Positiven (%)	Target[b]	C_t values		
			Median (IQR)	Mean (range)	Mean difference[c]
ONPS	129 (20.0)	E gene ($n = 116$)	22.5 (18.6–25.0)	22.3 (14.8–33.8)	
		ORF1 a/b ($n = 114$)	20.9 (18.5–24.4)	21.7 (14.8–31.4)	
Gargle in PCR media	121 (18.8)	E gene ($n = 116$)	29.9 (26.5–32.6)	29.7 (20.1–37.9)	7.4 (−4.1 to 16.5)
		ORF1 a/b ($n = 114$)	29.1 (25.9–30.9)	28.6 (20.3–34.8)	6.9 (−4.5 to 15.8)
Gargle in lysis buffer	121 (18.8)	E gene ($n = 116$)	29.0 (25.7–31.4)	28.8 (18.6–36.7)	6.3 (−4.2 to 14.9)
		ORF1 a/b ($n=114$)	28.1 (25.0–30.0)	27.6 (18.7–33.3)	5.8 (−4.2 to 14.3)

Abbreviations: IQR, interquartile range; ONPS, oropharyngeal/nasopharyngeal swab; PCR, polymerase chain reaction.

[a]Three of the 647 participants for whom an invalid result was obtained on at least one NAAT were excluded.

[b]When the target was not detected on at least one of the three NAATs, the participant was excluded from this analysis.

[c]Mean C_t difference between the results obtained from the gargle samples and the ONPS.

specimens were both positive, the mean C_t was significantly higher for gargles (Table 2). The magnitude of the increased C_t on gargle samples was similar between both processing methods. As shown in Figure 1, the C_t values were highly correlated between gargle specimens processed in lysis buffer and with PCR media when both tests were positive ($r^2 = 0.85$, $p < 0.0001$ for E-gene target; $r^2 = 0.92$, $p < 0.001$ for ORF1 a/b).

4 | DISCUSSION

Our findings demonstrate a high level of agreement between both gargle sample processing protocols and ONPS for SARS-CoV-2 detection with the Cobas® SARS-CoV-2 test. The lower positive agreement is explained by the slightly lower sensitivity of gargles, although not statistically significant, than ONPS. Both processing methods on gargle samples generated higher C_t values than those obtained with ONPS without having an impact on the clinical sensitivity of these sample processing methods.

Our study is the first to evaluate two processing methods for gargle samples on the Cobas® platform using PCR Media and a lysis buffer. Participants were recruited prospectively, and testing was performed blinded to results obtained with the other samples and processing methods. The number of samples tested and compared to ONPS was substantial. The difference of sensitivity between gargle samples and ONPS was not significant and would require a larger study to reach statistical significance. Our protocol is readily applicable to laboratories that intend to implement gargles and these sample processing protocols for SARS-CoV-2 screening. The addition of gargle specimens directly in PCR media at the screening clinic could facilitate their manipulation and the workflow at the laboratory. Even if our samples were placed in PCR Media at the laboratory in our study, we believe similar results would be obtained if this step was performed at the sample collection site. Moreover, even if gargle samples are more diluted in PCR Media than when processed with a lysis buffer, the positive agreement and sensitivity were not significantly affected in our study. However, this study did not assess the quality of inactivation and did not investigate differences in inactivation efficiency.

Our results suggest that gargle specimens directly added in PCR media could be used for SARS-CoV-2 detection which will simplify the laboratory workflow while preserving a similar clinical sensitivity to a lysis method on the Cobas® platform, even if the C_t values were higher with the use of the PCR media than the standard ONPS sample.

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SUPPORTING INFORMATION

Additional Supporting Information may be found online in the supporting information tab for this article.

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