


Evaluation of water gargle samples for SARS-CoV-2 detection using Abbott ID NOW COVID-19 assay

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Abstract

The Abbott ID NOW™ COVID-19 assay has been shown as a reliable and sensitive alternative to reverse transcription-polymerase chain reaction (RT-PCR) testing from nasopharyngeal or nasal samples in symptomatic patients. Water gargle is an acceptable noninvasive alternative specimen for severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2) detection by RT-PCR. The objective of this study was to evaluate the performance of water gargle samples for the detection of SARS-CoV-2 using the ID NOW. Residual gargle samples were randomly selected among positive standard of care (SOC)-nucleic acid amplification test (NAAT) samples. For testing on ID NOW, the manufacturer's instructions were followed, except for the specimen addition step: 500 µl of the gargle specimen was added to the blue sample receiver with a pipette and gently mixed. Among the 202 positive samples by SOC-NAAT, 185 were positive by ID NOW (positive percent agreement [PPA]) = 91.6% (95% confidence interval [CI]: 86.9–95.0). For the 17 discordant samples, cycle threshold (C_t) values were all ≥ 31.0 . The PPA was significantly lower among asymptomatic patients (84.4%; 95% CI: 73.2–92.3) versus symptomatic patients (95.2%; 95% CI: 89.8–98.2). The performance of the ID NOW for the detection of SARS-CoV-2 infection on gargle samples is excellent when C_t values are < 31.0 and for patients that have COVID-19 compatible symptoms.

KEYWORDS

Abbott ID NOW, COVID-19, gargle, SARS-CoV-2

1 | INTRODUCTION

Since the beginning of the COVID-19 pandemic, detection of severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2) infected individuals is used by clinicians and public health authorities for case management, infection control and contact tracing. Reverse transcription-polymerase chain reaction (RT-PCR) testing on nasopharyngeal swabs (NPS) is the most commonly used sample type for SARS-CoV-2 detection.¹ However, many countries have faced shortages of NPS and viral transport medium, leading investigators to develop alternative sampling methods.² In addition, NPS requires trained healthcare workers and is uncomfortable or painful, which can hamper repeated screening.³ Nasal swab, saliva, or water mouth rinse/gargle have been the most studied alternative specimens for SARS-CoV-2 detection by RT-PCR.^{4–13} In the province of Québec, natural spring water gargle has been extensively used for SARS-CoV-2 detection following the G-SPIT study that showed acceptable performance compared to oronasopharyngeal swab, particularly for symptomatic patients.^{6,14,15}

To improve turnaround time, point-of-care (POC) tests have been developed, allowing for rapid testing at or near the specimen collection site. POC tests have the potential to allow earlier detection and isolation of confirmed SARS-CoV-2 infections, thereby reducing household and community transmission.¹⁶ Compared to POC antigenic tests, POC molecular tests have better clinical sensitivity and a lower detection limit for SARS-CoV-2.¹⁷

The Abbott ID NOW™ COVID-19 assay is an isothermal nucleic acid amplification test (NAAT) targeting the RdRp segment of the SARS-CoV-2 genome. It is authorized as a lab-based and POC diagnostic assay for the qualitative detection of nucleic acids from direct anterior nasal, nasopharyngeal, or throat swabs from individuals suspected of having COVID-19, within the first 7 days of symptom onset.¹⁸ ID NOW has been shown as a reliable and sensitive alternative to RT-PCR testing from NPS or nasal samples in symptomatic patients, without the need for subsequent confirmation of negative results.^{19,20}

The main objective of this study was to evaluate the performance of natural spring water gargle samples for the detection of SARS-CoV-2 using the ID NOW, compared to standard of care (SOC) NAAT assay.

2 | MATERIALS AND METHODS

This study was conducted in three large, multisite, university-affiliated laboratory networks in Montréal, Sherbrooke and Quebec City, located in the province of Québec, Canada, during four distinct periods: December 2020, July–August 2021, November 2021, and December 2021–January 2022. In the three institutions, water gargle samples are acceptable specimens for SARS-CoV-2 detection by NAAT for routine COVID-19 testing. Gargle samples were collected as described before.^{5,6,14} Briefly, patients were asked not to eat, drink, or smoke for 15 min before sampling. They were provided with a cup containing 5 ml of natural spring water and were told to gargle with the water for 5 s in the mouth, 5 s in the throat, then to repeat

this process once and spit out as much as possible in the original cup. They were then asked to transfer their gargle specimen to a tube, which was subsequently sent to a laboratory for SOC-NAAT testing. Gargle samples used in this study were collected as part of routine COVID-19 screening activities in all three institutions. A convenience sample set of residual gargle samples were randomly selected among the SARS-CoV-2 positive samples to be analyzed using the ID NOW COVID-19 assay in all three institutions. Selection of positive SOC-NAAT samples was blinded for cycle threshold (C_t) values. Some negative samples were also included randomly to explore the risk of false-positive as we (data not shown) and others had observed this problem in the past with Abbott Panbio POC rapid antigen test.²¹ No personal data were collected outside of the information available on the standard COVID-19 laboratory form (date of collection and if the patient had symptoms or a close contact, according to public health criteria).

Multiple SOC-NAAT assays for SARS-CoV-2 detection are used in participating laboratories. Commercial assays are: Simplexa™ COVID-19 Direct Kit (DiaSorin Molecular LLC), cobas® SARS-CoV-2 performed with the cobas®6800/8800 systems (Roche), and Allplex™ 2019-nCoV assay with thermal lysis (Seegene). Laboratory developed tests (LDTs) targeting the structural protein envelope E gene were performed on LightCycler® 480 II Instrument (Roche) and CFX96 Touch Real-Time PCR System (BioRad) after thermal lysis or RNA extraction using NucliSens easyMAG (bioMérieux) as previously described.^{4,6} Samples were considered positive if they met the interpretation criteria of the manufacturer or if C_t values were ≤ 40 for LDTs.

After SOC-NAAT was performed on gargle samples, residuals were kept at 4°C for a maximum of 48 h until ID NOW testing or kept at -80°C if testing was performed ≥ 48 h following sampling. At the Quebec City laboratory, an inactivation step (70° for 15 min) is performed on gargle samples before testing on all instruments including ID NOW. For gargle samples tested on ID NOW, the manufacturer instructions were followed, except for the specimen addition step. At this step, 500 μ l of the specimen was added to the blue sample receiver with a pipette and gently mixed to avoid bubble formation. In addition, for a subset of the tested samples, an additional ID NOW test was done by soaking the ID NOW swab in the gargle specimen for 10 s before processing according to the manufacturer instructions.

Stata 16 (StataCorp LLC) was used to calculate positive percent agreement (PPA) and confidence intervals (CI). The validation study and publication of results was permitted without obtaining formal written consent from patients as part of l'Urgence sanitaire de la Santé Publique du Québec, since results are published without identification of participants and for diagnostic purposes and assay validation.

3 | RESULTS

Overall, 213 gargle samples were analyzed using ID NOW (202 positives and 11 negatives by SOC-NAAT for SARS-CoV-2). Each gargle sample represents a single patient, among which 128 were symptomatic, 56 were close contacts of a positive case or within an outbreak

investigation (data on the presence of symptoms not available), 14 were asymptomatic and information was missing for 15 individuals. The 11 negative SOC-NAAT samples were negative on ID NOW.

Among the 202 positive samples by SOC-NAAT, 185 were positive by ID NOW (Table 1), which corresponds to a PPA of 91.6% (95% CI: 86.9–95.0). Although the PPA was lower in the last period of the study (88.6% vs. 93.2%), corresponding to the Omicron emergence (fifth wave of the pandemic in the province of Québec), the difference was not statistically significant. The PPA was significantly lower at the Quebec city laboratory (80.0%; 95% CI: 64.4–90.9), where heat inactivation was performed; excluding these 40 samples, PPA raise to 94.4% (95% CI: 89.7–97.4). Freezing and thawing before performing ID NOW did not affect performance (Table 1). The PPA was significantly lower among asymptomatic patients (84.4%; 95% CI: 73.2–92.3) versus symptomatic patients (95.2%; 95% CI: 89.8–98.2).

As shown in Table 2, for the 17 discordant samples, C_t values were all ≥ 31.0 , likely due to lower viral loads in these samples. Among the 174 samples with C_t values < 31.0 by SOC-NAAT, the PPA was 100% (95% CI: 97.9–100); among the 28 samples with C_t values ≥ 31 by SOC-NAAT, only 11 were positive with ID NOW (PPA: 39.3%; 95% CI: 11.5–59.4).

We also analyzed a subset of 93 samples (including the 11 negative SOC-NAAT samples) using the ID NOW swab soaked in the gargle specimen (Table 3). Among the 82 SOC-NAAT positive samples, one was missed by ID NOW only when using 500 μ l of gargle specimen (SOC-NAAT C_t value of 31.2) and four were missed only when using the swab soaked in gargle specimen (SOC-NAAT C_t values of 27.5, 28.1, 31.2, and 31.2).

4 | DISCUSSION

This study is the first to evaluate water gargle samples using the ID NOW platform. It demonstrates an excellent performance of water gargle for the detection of SARS-CoV-2 using the ID NOW COVID-19 assay when C_t values are < 31.0 and among symptomatic patients. The method of directly using 500 μ l of the gargle specimen for the assay showed better performance compared to soaking ID NOW swabs in the specimen. This is probably explained by a lower quantity of viral particles being transferred to the blue sample receiver using the soaked swab. Sample addition using the pipette also had the advantage of reducing bubble formation (by removing them easily), thus possibly reducing the number of invalid results. There is also no

	ID NOW		PPA % (95% CI)
	+	-	
Study period			
December 2020 to November 2021 (n = 132) ^a	123	9	93.2% (87.5–96.8)
December 2021 to January 2022 (n = 70) ^b	62	8	88.6% (78.7–94.9)
Laboratory			
Montréal (n = 86)	85	1	98.8% (93.7–100)
Sherbrooke (n = 76)	68	8	89.5% (80.3–95.3)
Quebec city (n = 40)	32	8	80.0% (64.4–90.9)
Sample treatment or conservation			
Heat inactivation before SOC-NAAT (n = 40)	32	8	80.0% (64.4–90.9)
Conservation at 4°C for < 24 h after SOC-NAAT (n = 135)	126	9	93.3% (87.7–96.9)
Freezing after SOC-NAAT (n = 27)	27	27	100% (87.2–100)
Presence of symptoms			
Yes (n = 124)	118	6	95.2% (89.8–98.2)
No (n = 64) ^c	54	10	84.4% (73.2–92.3)
Unknown (n = 14)	13	1	92.9% (66.2–99.8)
Total	185	17	91.6% (86.9–95.0)

TABLE 1 ID NOW results according to study period, laboratory, sample treatment, and presence of symptoms (n = 202 samples positive by SOC-NAAT).

Abbreviations: CI, confidence interval; PPA, positive percent agreement; SOC-NAAT, standard of care-nucleic acid amplification testing.

^aThe first three “pre-Omicron” study periods were pooled together: December 2020 (n = 5), July–August 2021 (n = 58), and November 2021 (n = 69).

^bAmong the 70 samples, 40 were tested at the laboratory where heat inactivation was performed before SOC-NAAT and ID NOW.

^cAmong the 64 samples, 52 were close contacts of a positive case or within an outbreak.

TABLE 2 ID NOW results for the 202 positive SOC-NAAT gargle samples.

Comparator SOC-NAAT	ID NOW		SOC-NAAT C _t values, mean (range)	
	+	-	Concordant samples	Discordant samples
cobas [®] SARS-CoV-2 test (n = 126)	111	15	ORF1ab gene: 26.5 (17.7–32.9) E gene: 27.3 (18.1–35.2)	ORF1ab gene (n = 10 ^a): 32.5 (31.2–33.5) E gene: 35.4 (32.7–37.5)
Allplex [™] 2019-nCoV assay (n = 38)	38	0	E gene: 24.6 (14.7–32.8) N gene: 29.6 (21.0–37.1) RdRp gene: 27.3 (17.3–32.8)	N/A
Simplexa [™] COVID-19 Direct Kit (n = 5)	5	0	S gene: 22.8 (17.9–26.2) ORF1ab gene: 23.7 (19.1–26.8)	N/A
LDT/RNA extraction or thermal lysis (n = 33)	31	2	E gene: 27.6 (20.2–33.9)	E gene: 32.8 (31.2–34.5)
Total	185	17		

Abbreviations: C_t, cycle threshold; LDT, laboratory developed test; N/A, not applicable; SARS-CoV-2, severe acute respiratory syndrome coronavirus-2; SOC-NAAT, standard of care-nucleic acid amplification testing.

^aThe ORF1ab gene was not detected in 5/15 discordant samples.

risk of spillage adding this volume of sample in the blue sample receiver, since there is enough space to add more than 500 µl without interfering with the transfer cartridge process. Water gargle samples have been shown as an adequate alternative to NPS in previous studies.^{5,6,12,14} Even if viral RNA concentrations is lower in gargle samples compared to NPS,^{5,6} the improved patient acceptability and the ease of the sampling protocols are benefits that likely exceed the slight loss of clinical sensitivity of the method.¹³ Notably, in our study, the difference in assay sensitivity was limited to specimens with elevated C_t values by NAAT testing. In addition, thermal lysis seems to have a negative effect on samples with lower viral load, as demonstrated by Pan et al.²² Further studies are needed comparing ID NOW performed immediately after sampling and SOC-NAAT.

One strength of our study is that we used the same sample for both methods, therefore eliminating result variability due to sample quality. We also chose to blind the SOC-NAAT C_t values during sample selection, thus avoiding overestimation of gargle sample performance by selection of only strong positive samples. Of course, if more low viral samples (high C_t values by SOC-NAAT) have been included in our study, the PPA would have been negatively influenced. Conversely, if only symptomatic patients had been included, potentially with a higher viral load, gargle samples performance would have been increased.

Limitations of our study include a possible overestimation of the performance by using gargle as SOC for NAAT, which is associated with lesser sensitivity than using standard NPS.⁶ On the other hand, we may have underestimated the performance of gargle samples on ID NOW by comparing to PCR assays, rather than comparing two different sampling methods directly on ID NOW (side by side study compared with nasal swab obtained at the same time of gargle sample). This should be evaluated in a future study, since it has been demonstrated in a preprint study that the pattern of viral shedding

TABLE 3 ID NOW results for the 93 analyzed gargle samples using 500 µl of the specimen or the swab soaked in the specimen.

	Swab soaked in gargle specimen		Total
	+	-	
500 µl of gargle specimen			
Positive	77 ^a	4 ^a	81
Negative	1 ^a	11 ^b	12
Total	78	15	93

Abbreviation: SOC-NAAT, standard of care-nucleic acid amplification testing.

^aThese 82 samples were positive by SOC-NAAT.

^bThese 11 samples were also negative by SOC-NAAT.

during the course of infection is altered for the Omicron variant with higher viral shedding in saliva relative to nasal samples resulting in improved diagnostic performance of saliva swabs.²³ We could speculate that it will be the same for gargle samples. Our study spanned two different COVID-19 waves in the Quebec province; the first part was caused mostly by Delta variant and the second part by Omicron variant, according to provincial laboratory surveillance (data not shown).

In conclusion, our results show that water gargle samples give an adequate performance on the ID NOW COVID-19 assay and could be used for the diagnosis of COVID-19 or screening for SARS-CoV-2 infection, particularly in patients with COVID-19 compatible symptoms. This sample collection method is often preferred over nasal or NPS and may enable a higher rate of detection from a population-based perspective, in particular when repeat testing is required, due to its better acceptance leading to decreased testing avoidance. If used, we suggest that water gargle specimens be tested without any pretreatment to avoid impact on samples with lower viral load.

AUTHOR CONTRIBUTIONS

Simon Lévesque: Investigation; formal analysis; data curation; data interpretation; statistical analysis; visualization; writing—original draft. **Stéphanie Beauchemin:** Investigation; methodology; writing—review and editing. **Maud Vallée:** Conceptualization; methodology; writing—review and editing. **Jean Longtin:** Data interpretation; writing—review and editing. **Mariève Jacob-Wagner:** Investigation, formal analysis, writing—review and editing. **Jeannot Dumaresq:** Data interpretation; writing—review and editing. **Carlos Dulcey:** Investigation; writing—review and editing. **Annie-Claude Labbé:** Conceptualization; methodology; formal analysis; data curation; data interpretation; statistical analysis; visualization; writing—review and editing; supervision.

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DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request. Data presented in the manuscript has not been made available.

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