Title: Save the COVID-19 point of care nucleic acid test swab after testing to identify variants of concern

Running Head: COVID-19 ID NOW residual swab for variant testing

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Nonstandard Abbreviations: SARS-CoV-2, Severe acute respiratory syndrome-coronavirus-2; rRT-PCR, reverse transcriptase real-time polymerase chain reaction; COVID-19, coronavirus infectious disease-2019; VoC, variants of concern; POCT, point of care testing, NPS,

nasopharyngeal swab; rSwab, residual ID NOW swab; NPS-dxPCR, diagnostic SARS-CoV-2 PCR done on NPS; rSwab-dxPCR, diagnostic SARS-CoV-2 PCR done on NPS

Point of care testing (POCT) for severe acute respiratory syndrome-coronavirus-2 (SARS-CoV-2), such as the Abbott ID NOW™ isothermal nucleic acid test, improve the turnaround time of laboratory results and make testing more widely available. Since the beginning of the coronavirus infectious disease-2019 (COVID-19) pandemic, typing of SARS-CoV-2 has been an important component to the pandemic response. With the emergence of variants of concern (VoC), typing has become a critical tool utilized by public health and infection prevention and control for case management. Although POCT has many benefits, one downside is that unless a second swab is collected, no sample is available to the laboratory for further analysis. Using a prospective study design, we determined if the ID NOW swab could be used for confirmatory PCR or variant testing after mixing in the sample receiver to perform the POCT.

People with symptoms of COVID-19 or those considered as asymptomatic close contacts to a confirmed case as per Alberta Health guidelines (1) were eligible for POCT ID NOW testing at community collection and test (assessment) centres in the province of Alberta, Canada. Two swabs were collected by health care workers: a throat swab for ID NOW and a nasopharyngeal swab (NPS) in universal transport media (UTM; GDL Korea Co. Ltd, Anyang, South Korea) for confirmation of negative results or VoC typing of positive results. As per manufacturer instructions, the ID NOW swab was mixed in the sample receiver buffer on the ID NOW instrument and then discarded after testing. When collecting swabs and testing on the ID NOW, contact and droplet precautions (gown, gloves, and eye/face mask) were used. For this study,

after POCT ID NOW testing, the residual throat swab (rSwab) was saved in a sterile container or original packaging and, if positive, the swab was added to a tube with 2 mL of UTM. The rSwab and NPS were shipped to the laboratory at room temperature and refrigerated upon arrival until testing within 48 h of receipt. SARS-CoV-2 diagnostic PCR on the rSwab and NPS SARS-CoV-2 PCR (rSwab-dxPCR; NPS-dxPCR) was initially tested on the Cobas 6800 (Roche, Basel, Switzerland) or using the Alberta Public Health Lab (ProvLab) E gene PCR (2). If SARS-CoV-2 dxPCR positive, samples were tested with a ProvLab B.1.1.7 (Alpha) VoC PCR (vPCR) (3). The vPCR detected the routine diagnostic E gene target and two S gene mutations (N501Y and the 69/70 deletion). Samples were considered positive for the Alpha VoC if positive for both the N501Y mutation and 69/70 deletion, wild type if the 69/70 deletion alone was positive or both targets were negative, or presumptive variant of concern if the N501Y target alone was positive. Samples negative for the E gene target or with a cycle threshold (Ct) >35 were deemed "unresolved". Next generation sequencing was only performed on presumptive VoC (N501Y positive and no 69/70 deletion) and other select samples if the Ct was <32 (due to high likelihood of next generation sequencing failure at low viral loads). Technologists performing PCR testing were unaware of the ID NOW results.

Swabs were collected from April 19-29, 2021. Compared to the ID NOW result, the positive agreement for rSwab-PCR was 101/113 (89.4%; 95% CI 82.4-93.8) and for NPS-dxPCR, 98.2% (95% CI 93.7-99.7) (Table 1). The difference between rSwab-dxPCR and NPS-dxPCR was statistically significant (p=0.0104, Fisher's exact). The positive agreement of rSwab-dxPCR compared to NPS-dxPCR was 90.1 % (95% CI 83.1-94.3, p=0.008).

Fifteen rSwab and NPS samples were not tested by vPCR due to laboratory error. Excluding these 15 samples (total 98 paired samples), 79.6% (78/98) rSwab gave a vPCR result of positive or negative for the Alpha VoC compared to 95.9% (94/98) from the NPS (Table 2). Other samples that did not give a result were either not eligible for vPCR because they were negative by initial PCR screen or unresolved by the vPCR.

Only 83.2% (94/113) of the rSwabs would have been whole genome sequencing eligible (Ct <32) compared to 93.8% of the NPS (106/113). The median Ct for the rSwab was significantly higher than the paired NPS at 27.0 (IQR 24.1-30.4) vs 21.8 (IQR 17.7-26.2), respectively ($p<0.0001$, Wilcoxon-signed rank). This included negatives as zero and excluded indeterminates or positives without a Ct value (n=108). Linear regression yielded a correlation coefficient (r^2) of 0.0746.

Using the rSwab is not as sensitive as collecting second NPS and reduces the number of samples with variant result. It does, however, offer an option when a second swab cannot be obtained and a 20% reduction in the ability to identify variants in positive cases is acceptable.

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Table 1. SARS-CoV-2 PCR results from paired residual point of care testing ID NOW positive swabs (rSwab-PCR) and nasopharyngeal swabs.

		Nasopharyngeal Swab	
		Positive	Negative
rSwab	Positive	101	1
	Negative	10	1

Table 2. Variant of Concern PCR results from paired residual point of care testing ID NOW positive swabs (rSwab) and nasopharyngeal swabs.

			Nasopharyngeal Swab					
	SARS-CoV-2 PCR Result		Positive				Negative	
rSwab		VoC Result	α	γ	WT	Unr	N/A	**Total**
	Positive	α	64	1	0	1	1	67
		WT			10			10
		Unresolved	8					8
		Pres. Pos		1				1
		Lab error	10	2	3			15
	Negative	N/A	7		1	2	1	11
	Indeterminate	N/A	1					1
		Total	89	4	15	3	2	113

Whole genome sequencing identification of the gamma (γ) VoC (P1 lineage) was only performed on nasopharyngeal swabs. Both gamma VoC were presumptive positive ("Pres. Pos.", N501Y target only positive on VoC PCR) . α, Alpha VoC (B.1.1.7 lineage); Unr, unresolved.