Letter to the editor

Saliva for use with a point of care assay for the rapid diagnosis of COVID-19

Dear Editor

Access to rapid and accurate testing is essential to limit the community spread of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) and curtail COVID-19 resurgence. The development of point of care (POC) testing platforms can facilitate rapid clinical decision making and alleviate testing backlogs in centralized laboratories [1]. The Abbott ID NOW (Abbott Diagnostics) is a POC isothermal amplification-based platform that detects SARS-CoV-2 in approximately 5 min, and is used in pharmacies, hospitals, and outpatient settings in all 50 states [2].

Recently, concerns regarding ID NOW sensitivity compared to RT-PCR assays have been raised [3]. In response, the manufacturer no longer endorses the use nasopharyngeal swabs (NPS) collected in viral transport media (VTM). However, any reflexive or confirmatory testing requires recollection of an additional specimen. This not only poses additional risk of exposure to the patient and health care workers, but also burdens already thin resources.

Because of these limitations, laboratories continue to explore the utility of alternative specimen types for COVID-19 testing. Saliva does not require specialized collection materials, collection is non-invasive, and samples can be self-collected limiting healthcare worker exposure and improving user convenience and comfort. As the optimal sample type for POC testing is unsettled, we examined the performance of the ID NOW COVID-19 assay in comparison to traditional RT-PCR using 114 total symptomatic patient saliva specimens. Testing supply chain constraints and clinical demand limited the number of samples we were able to analyze; the US Food and Drug Administration Emergency Use Authorization benchmark of a minimum of 30 positive and 30 negative samples was used to guide study design and sample size.

We evaluated 83 symptomatic patient saliva samples with paired NPS in VTM simultaneously collected (Table 1). ID NOW saliva testing results were compared to paired NPS specimens tested by either the Xpert® Xpress SARS-CoV-2 (Cepheid) or Abbott RealTime SARS-CoV-2 (Abbott Molecular) RT-PCR assays. All RT-PCR testing was performed per manufacturer's instructions, and 200 μl of saliva was used for ID NOW.

We found an 82% (32/39) positive percent agreement (PPA) and

100% (44/44) negative percent agreement (NPA) for POC saliva testing compared with NPS in VTM tested by RT-PCR (Table 1). Most false-negative (FN) results on the ID NOW platform occur with specimens which exhibit higher cycle number (CN, Abbott) or cycle threshold (Ct, Cepheid) values suggestive of lower viral RNA levels [3]. The limit of detection for saliva specimens on the ID NOW was similar to previous reports on NPS (2000 copies/mL, SARS-CoV-2 standard, Exact Diagnostics) [4]. FN saliva samples were also associated with increased NPS CN (Abbott, N2: 30.44) or CT values (Cepheid, E: 33.0, 36.5, 40.6, 43.3, 30.1, 31.2; N2: 35.9, 36.1, 38.5, 39.0, 32.7, 34.0). This suggests patients with FN results have decreased levels of viral RNA in both saliva and NP specimens.

We recently validated the Cepheid Xpert® Xpress SARS-CoV-2 assay for clinical use with saliva at our institution [5], and compared the performance of ID NOW to this assay using 59 saliva specimens (Table 1). In this comparison, we observed 82% (19/23) PPA and 100% NPA (35/35). As before, FN samples by ID NOW exhibited higher CT values (E: 36.4, 36.5, 42.7, 43.3; N2: 36.1, 37.6, 39.0, 41.2).

Chart review of all FN samples in this work revealed a majority (n = 6/9) of patients were tested > 2 weeks after symptom onset. Four patients already received a diagnosis of COVID-19 over 1 week before this test, while 2 patients tested positive by NPS on a qPCR assay within 2 days of the saliva sample being collected. One patient lacked specific symptoms onset time but had close contacts who were COVID-19 positive, so they may have been infected much earlier. A liver transplant recipient was 10 days from symptom onset. The last patient had no previous COVID-19 diagnosis, but had a two-week atypical presentation of weakness and diarrhea before coming to the ED. Radiographic exam revealed subtle hazy opacities of the lung base indicative of minor respiratory disease.

Saliva contains digestive enzymes which could potentially affect specimen stability during self-collection and transport. To investigate this, six SARS-CoV-2-positive saliva specimens were evaluated daily by the ID NOW for 5-days post-collection stored at room temperature (20–22 °C). All specimens remained positive during serial testing over that time, further demonstrating the utility of this sample type.

Resurgence in COVID-19 cases necessitates expanded accessibility

Table 1
Saliva as sample type for ID NOW COVID-19 assay performance comparison against the Abbott RealTime SARS-CoV-2 and Cepheid Xpert® Xpress SARS-CoV-2 Real-Time PCR assays.

Sample type	(n)	TP	FP	TN	FN	IN	PPA % (95%CI)	NPA % (95%CI)	PPV* %	NPV* %
ID NOW Saliva Vs NPS RT-PCR	83	32	0	44	7	0	82 (67–93)	100 (92–100)	100	99
ID NOW Saliva Vs Saliva (Cepheid Xpert® Xpress SARS-CoV-2)	59	19	0	35	4	1	82 (61–95)	100 (90–100)	100	98

 * PPV and NPV calculated based upon an institutional prevalence of 7.32% as determined through the analysis of all positive SARS-CoV-2 tests at this institution from 3/8–7/6/2020. TP: True positive, FP: False positive, TN: True negative, FN: False negative and IN: Invalid.

to molecular testing and rapid POC devices. Easily collected specimens like saliva are one solution. Many FN patients presented after 2 weeks of symptom onset, when viral loads are decreasing [6]. Therefore, the FN rate may be minimized by testing patients within 2 weeks of symptom onset. Specimen stability and RT-PCR comparison studies indicate saliva as a matrix is stable, and sensitivity is only limited by the analytic performance of the ID NOW COVID-19 assay. We therefore propose further investigation of saliva as an alternative sample type for POC testing is warranted.

Acknowledgments

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