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Review Article

Comparative Study of the Efficacy of the Abbott ID Now Rapid Assay with Real Time PCR for the Detection of SARS-CoV-2 RNA

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ABSTRACT

Rapid diagnostic tests are of great importance in hospital settings during the current outbreak of SARS-CoV-2. The clinical patient management and spread of infection is critically dependent on molecular assays with shortest possible turn-around time. Here we report performance of a point of care Abbott ID NOW COVID-19 assay in comparison to routinely used real-time RT-PCR assay on 205 clinical specimens.

Overall agreement of ID NOW was found to be 93.7% with Positive Percent Agreement (PPA) of 91.8% and Negative Percent Agreement (NPA) of 95.4%. Based on our findings, low turnaround time, minimal infrastructure need and ease of performing the assay, Abbott ID NOW COVID-19 assay can be considered as a point of care test in hospital settings.

Keywords: SARS-CoV-2, Emergency use authorization, Point of care test, ID Now

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INTRODUCTION

Severe acute respiratory virus coronavirus 2 (SARS-CoV-2) that had emerged in Wuhan, China in December 2019 has penetrated world over (Shereen MA, *et al.*, 2020). This has led to global pandemic of coronavirus disease (COVID-19) with India being the second most affected nations owing to its dense population. Public health efforts to contain the infection were mediated with accelerated large-scale testing by reference laboratories and healthcare centres across the globe. Commercial kit manufacturers and FDA's Emergency Use Authorization (EUA) process ramped up availability of reliable testing kits. Several rapid assays such as ID NOW COVID-19 (Abbott), Xpert Xpress SARS-CoV-2 (Cepheid) and Simplexa™ COVID-19 Direct (Diasorin) offers detection of SARS-CoV-2 RNA in 15 minutes to 90 minutes directly from dry nasopharyngeal swabs or swabs transported in Viral Transport Media (VTM) (Zhen W, *et al.*, 2020). EUA for diagnostic kits is obtained by minimal test validation and hence it is the responsibility of a molecular diagnostic laboratory to carry out in-house verification of the assay prior to clinical use. The aim of our study was to compare the Abbott ID NOW COVID-19 Point of Care Test (POCT) with Real Time RT-PCR-based method to assess its efficacy for patient testing.

MATERIALS AND METHODS

The performance of ID NOW was evaluated on a set of 205 specimens at the molecular diagnostics laboratory of our hospital and the results were compared against Real Time based RT-PCR assay from SD Biosensor. The specimens were collected from 114 individuals presenting in the Outpatient Department (OPD cases) who had signs and symptoms indicative of COVID or had history of contact with COVID-19 positive patient, 57 were from a population of positive patients from COVID ward and 34 were from other hospitalized patients (IPD cases) from non COVID ward. Since the study were conducted as a part of instrument validation and the data obtained was retrospectively analyzed, informed consent was not obtained from the patients.

Two nasopharyngeal swabs (NP) were collected in parallel from each of the subjects for both ID Now and RT-PCR assay. For ID Now assay dry swabs were transported in 15 ml sterile tubes to the molecular diagnostics department within the hospital. These swabs were stored at 4°C and tested on ID NOW within 2 hours of collection, consistent with the manufacturer's instructions. For RT-PCR analysis the swabs were transported in viral transport medium (Himedia; VTM) and were analysed within 24 hours of collection.

ID Now COVID-19 (Abbott Diagnostics) is an automated assay based on isothermal nucleic acid amplification technology that targets RdRp region of the genome. It is a rapid assay that utilizes dry swabs and gives qualitative results within 5 to 13 minutes. The assay method comprises of insertion of orange test base (containing sealed lyophilized reaction) into the orange test base holder, followed by placing the blue sample receiver (containing elution/lysis buffer) into the corresponding blue sample receiver holder. The dry NP swab was vigorously mixed in the sample receiver buffer for 10 seconds. The white transfer cartridge was pressed into the sample receiver, lifted and connected to the Test Base. ID NOW contains an internal control that has been designed to control for sample inhibition, amplification, and assay reagent function.

StandardM nCoV Real-Time Detection (SD BIOSENSOR) target regions of envelope (E) and RNA dependent RNA polymerase (RdRp). The RNA was extracted using 200 µl of VTM by Qiasymphony DSP Virus Pathogen mini kit (Qiagen GmbH, Germany) as per the manufacturer's protocol and 10 µl of eluted RNA was used for RT-PCR reaction. Exogenous internal control provided in the StandardM nCoV kit was spiked in the specimen during extraction. The Real Time PCR was carried out in Rotor-Gene Q platform (Qiagen Inc.) and ORF1ab (RdRp), E Gene and Internal control targets were detected in FAM, HEX and Cy5 channels respectively. The work was carried out inside a Class II Biological Safety Cabinet (BSC) by certified laboratory personnel.

Assuming RT-PCR to be the reference method, we calculated the Positive Percent Agreement (PPA) and Negative Percent Agreement (NPA) of the rapid assay in our study population.

RESULTS

In total 205 samples were tested prospectively by Abbott ID Now as well as by StandardM nCoV Real-Time PCR (reference method). SARS-CoV-2 RNA was detected in 97 (47.3%) and 94 (45.8%) cases by RT-PCR and POCT assays respective-

ly. The overall agreement between the two assays was observed in 93.7% (192/205) (95% CI, 89.5 to 96.3%) cases (Table 1). The PPA of ID Now in comparison to StandardM nCoV Real-Time was found to be 91.8% (95% CI, 84.6 to 95.8%) and NPA was 95.4% (95% CI, 89.6 to 98.0%). There were 8 samples that were false negative by ID Now assay. Similarly, StandardM nCoV kit failed to detect SARS-CoV-2 RNA in 5 clinical specimens. Overall, non-concordance of results was observed between the two methods in 13/205 (6.34%) cases.

The mean Ct value for concordant positive samples was 22.0 (95% CI, 20.6-23.3), ranging from 18-33, with a standard deviation of 6.42. The mean Ct for discordant samples (RT-PCR+/ID NOW-) was 27.5 (95% CI, 24-31). The data indicates that majority of the discordant samples (false negative on ID NOW) exhibited comparatively higher Ct values or lower viral load.

The claimed sensitivity of detection of ID NOW is 125 genomic equivalents/ mL which is lower than that of STANDARD M nCoV Real-Time Detection kit which is 250-500 copies/ ml upper respiratory specimen. This is quite surprising as the 3.9% (8 cases) with higher Ct values were missed by ID NOW assay. Similarly there were 5 cases that were missed by Real Time PCR, but were picked up on ID NOW.

DISCUSSION

Accurate results along with rapid turnaround time of testing is of utmost importance for SARS-CoV-2 testing not only for patient management but also to curtail community spread of the infection (Ward S, *et al.*, 2020). There was a huge demand for diagnostic kits and reagents which were made available to the healthcare centers through emergency use approval (EUA) mode. Abbott ID NOW COVID-19 POCT assay claimed to provide accurate results in less than 15 minutes, our study was intended to verify its accuracy and to evaluate its efficacy for clinical use.

Our study showed PPA and NPA of 91.8% and 95.4% respectively. Though testing was done strictly as per the manufacturer's instructions, i.e. dry swab was processed within 1-2 hours of sample collection, discrepancy was observed in 13 of the samples. Out of the 8 samples that were missed on ID NOW, four of them were known COVID positive patients on treatment. The remaining 4 had reported to OPD for testing, three of which had relevant clinical symptoms and one patient had close contact with another COVID-19 positive patient. There were 5 samples that were missed by RT-PCR but picked up by ID NOW assay, 4 of these were again known COVID positive patients on treatment and one was asymptomatic patient admitted in the hospital for some surgical procedure. The discrepancy between the two assays could be attributed to variability in sampling and the fact that majority of the samples were COVID-19 positive patients on treatment and displayed comparatively higher ct values indicating lower viral burden.

Published studies on ID NOW COVID-19 assay have documented PPA of 48.0% to 94.0% and NPA of 98.6 to 100% (Table 2). A study by Basu *et al.* had shown comparison of Abbott ID NOW with Cepheid Xpress Xpert SARS-CoV-2 assay on 101 specimens and had observed low PPA of 54.8% and NPA of 98.6% (Basu A, *et al.*, 2020). The study had raised concerns regarding utility of this assay for diagnostic purpose. There was another study by Lephart *et al.* that had reported very low PPA of 48.0% when compared with composite reference standard (m2000, Simplexa, Xpert) (Lephart PR, *et al.*, 2020). Sensitivity however, improved from 48% to 64% when nasopharyngeal swab in VTM was considered instead of dry swab (Lephart PR, *et al.*, 2020). Thwe *et al* showed overall agreement of 96.2% and PPA of 53.3% when compared against collective data set from all RT-PCR platforms (Abbott RealTime SARS-CoV-2, Panther Fusion SARS-COV-2, Cepheid Xpert Xpress SARS-CoV-2 and a laboratory developed test (Thew PM, Ren P, 2020).

Table 1: Performance of Abbott ID NOW in comparison to StandardM nCoV Real-Time PCR (SD Biosensor) Assay

StandardM nCoV real-time PCR					
Molecular assay	Positive	Negative	PPA (± 95% CI)	NPA (± 95% CI)	OPA (± 95% CI)
Abbott ID NOW					
Positive	89	5	91.80%	95.40%	93.70%
Negative	8	103	(84.6-95.8)	(89.6-98.0)	(89.5-96.3)

Table 2: Performance of Abbott ID NOW Assay for detection of SARS-CoV-2 against other Molecular Methods

Sr. No.	Reference	No. of cases	Reference method	PPA	NPA
1	Current study	205	SD biosensor (Real Time PCR)	91.80%	95.40%
2	Basu *et al.*, 2020	101	Cepheid Xpert Xpress SARS-CoV-2	54.80%	98.60%
3	Smithgall, *et al.*, 2020	113	Roche Cobas Assay	73.90%	100.00%
4	Harrington, *et al.*, 2020	524	Abbott RealTime SARS-CoV-2	74.73%	99.00%
5	Rhoads, *et al.*, 2020	96	Modified CDC assay (RT-PCR)	94.00%	NA (Not available)
6	Mitchell, *et al.*, 2020	61	Real-Time PCR	71.70% (33/46)	100.00% (15/15)
7	Zhen, *et al.*, 2020	108	Hologic panther fusion SARS-CoV-2 assay	50/57 (87.7%)	50/50 (100%)
8	Lephart, *et al.*, 2021	88	Composite reference standard	48.00%	100.00%
9	Cradic, *et al.*, 2020	184	Consensus standard	91.00%	100.00%
10	Thwe, *et al.*, 2020	129	Panther fusion® SARS-COV-2	53.30%	100.00%
11	Moore, *et al.*, 2020	200	Modified CDC assay (RT-PCR)	80.30%	100.00%
		200	Abbott Molecular Real-Time SARS-CoV-2 assay	75.20%	100.00%

Smithgall *et al.* had evaluated performance of ID NOW assay using specimens in transport media and not direct nasal swabs and had demonstrated PPA of 73.9% and NPA of 100% with Roche Cobas as the reference method (Smithgall MC, *et al.*, 2020). Authors concluded that PPA was 100% for high and medium viral load, however, it dropped considerably (34.3%) for lower viral load samples with CT values >30. Another study by Harrington *et al.* on large number of samples (n=524) showed lower PPA 74.73% against Abbott Real Time SARS-CoV-2 assay. In addition they also conducted in-house study on limit of detection and concluded that low PPA was attributed to higher limit of detection on ID NOW assay and preanalytical sampling error (Harrington A, *et al.*, 2020). Moore *et al* compared ID NOW assay on 200 samples against two different assays-Modified CDC assay and Abbott Molecular RealTime SARS-CoV-2 assay with positive percent agreement of 80.30% and 75.2%) respectively (Moore NM, *et al.*, 2020).

Couple of studies demonstrated comparatively good PPA of greater than 90%. (Rhoads DD, *et al.*, 2020; Cradic K, *et al.*, 2020) The study by Rhoads on 96 NPS collected in normal saline showed PPA of 94% when compared against modified CDC assay (Rhoads DD, *et al.*, 2020). The authors conclude that besides PPA, limit of detection of the assay along with other variables should be considered while implementing molecular assays for clinical use. Cradic *et al.* had used consensus standard to evaluate Abbott ID NOW assay in Nasopharyngeal Swab Specimens Collected in Universal Viral Transport Medium and demonstrated PPA of 91% (Cradic K, *et al.*, 2020). Also, Abbott ID NOW was evaluated on matched specimens-swabs collected in UVT and dry swab and no difference in the performance was noted.

Our study has shown an improved PPA of 91.80% in comparison to most of the other studies. The current study has a couple of limitations-the discrepancy between the two assays was not discerned by utilizing the third assay and Limit of Detection (LoD) assay for ID NOW was not determined. However, we could resolve discrepant results through detailed review of clinical records.

CONCLUSION

Overall, the performance of ID NOW COVID-19 assay was found to be satisfactory and comparable to RT-PCR assay. In addition, it is a POC test that offers exceptionally good turnaround time of testing and can be considered not only in the healthcare set up but also as a screening tool for travelers.

AUTHOR'S CONTRIBUTION

All the authors were involved in designing the study, analyzing and interpretation of the data, and drafting and writing the paper.

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