





Performance of Abbott ID Now COVID-19 Rapid Nucleic Acid Amplification Test Using Nasopharyngeal Swabs Transported in Viral Transport Media and Dry Nasal Swabs in a New York City Academic Institution

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ABSTRACT The recent emergence of the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) pandemic has posed formidable challenges for clinical laboratories seeking reliable laboratory diagnostic confirmation. The swift advance of the crisis in the United States has led to Emergency Use Authorization (EUA) facilitating the availability of molecular diagnostic assays without the more rigorous examination to which tests are normally subjected prior to FDA approval. Our laboratory currently uses two real-time reverse transcription-PCR (RT-PCR) platforms, the Roche Cobas SARS-CoV2 and the Cepheid Xpert Xpress SARS-CoV-2. The two platforms demonstrate comparable performances; however, the run times for each assay are 3.5 h and 45 min, respectively. In search for a platform with a shorter turnaround time, we sought to evaluate the recently released Abbott ID Now COVID-19 assay, which is capable of producing positive results in as little as 5 min. We present here the results of comparisons between Abbott ID Now COVID-19 and Cepheid Xpert Xpress SARS-CoV-2 using nasopharyngeal swabs transported in viral transport media and comparisons between Abbott ID Now COVID-19 and Cepheid Xpert Xpress SARS-CoV-2 using nasopharyngeal swabs transported in viral transport media for Cepheid and dry nasal swabs for Abbott ID Now. Regardless of method of collection and sample type, Abbott ID Now COVID-19 had negative results in a third of the samples that tested positive by Cepheid Xpert Xpress when using nasopharyngeal swabs in viral transport media and 45% when using dry nasal swabs.

KEYWORDS SARS-CoV-2, COVID-19, Abbott ID Now, validation, nasopharyngeal and nasal swabs

The novel coronavirus, severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), causing coronavirus disease 2019 (COVID-19) was first identified in Wuhan Jinyintan Hospital, China, in December 2019. Since then, it has spread widely, reaching the status of global pandemic as declared by the World Health Organization on 11 March 2020 (1). As of 25 May 2020, over 5 million cases and over 342,000 deaths have been reported worldwide (2), while in New York City, there were over 196,000 reported cases and more than 16,000 deaths (3, 4).

SARS-CoV-2 is detected in various human samples, including respiratory and fecal samples, but testing has been validated for PCR primarily for nasal, nasopharyngeal, and oropharyngeal specimens. Samples positive for SARS-COV-2 were found for Chinese patients 1 to 2 days prior to symptom onset and persisted for up to 2 weeks in severe cases (1).

While long-term mitigation and prevention goals include prognostic markers, ther-

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apeutics, and vaccines (1), the urgency of this rapidly developing crisis in the United States has prioritized the immediate goal of diagnostic testing, advanced through Emergency Use Authorization (EUA) by the FDA (5, 6). The initiative to expand and accelerate testing has eased the usual scrutiny that new assays would normally undergo prior to release. This obligates clinical laboratories to more carefully assess the overall performance of the various rapidly emerging testing platforms prior to implementation. Several nucleic acid amplification test (NAAT) platforms are currently available. Our laboratory uses two real-time reverse transcription-PCR (RT-PCR) platforms, the Roche Cobas and the Cepheid GeneXpert Dx. These platforms perform comparably in our experience, having similar limits of detection (LOD) of SARS-CoV-2 viral RNA, 100 to 200 and 250 copies/ml, respectively. However, the assay run times of 3.5 h and 45 min, respectively, are still too long for timely decision support in a variety of important clinical situations (e.g., bed assignments for patients being admitted from the emergency department (ED) who may require cohorting by their COVID-19 status). In search for a platform with shorter turnaround time, we sought to evaluate the recently released Abbott ID Now COVID-19 assay (ID Now). The ID Now assay uses isothermal nucleic acid amplification of the RNA-dependent RNA polymerase (RdRp) viral target with a claimed LOD of 125 genome equivalents/ml. The objective of our study was to evaluate the performance of the ID Now test by using the Cepheid-Xpert-Xpress SARS-CoV-2 (Xpert Xpress) on the GeneXpert Dx as the comparator reference method.

MATERIALS AND METHODS

Abbott ID Now platform versus Cepheid platform using nasopharyngeal swabs in VTM. All samples were obtained from patients who presented to the NYU Langone Hospitals (Manhattan) emergency department for whom a physician ordered a test for SARS-CoV-2. Nasopharyngeal (NP) samples were obtained using flocked NP swabs (NPS) and transported to the laboratory in universal viral transport media (VTM) (Cepheid Xpert viral transport medium [SWAB/B-100] or BD Universal VTM/2250527) within 1 to 2 h of collection. All samples were NPS transported to the lab in VTM.

Initial verification of the ID Now platform was accomplished by using two previously tested positive patients' samples using the Xpert Xpress assay, one with a cycle threshold (C_T) value 30.8 for N2 and another with a C_T value of 19.3 (i.e., high viral load). Both samples were diluted 1:2, 1:5, 1:10, 1:20, 1:50, and 1:100 in VTM. All the dilutions were tested in replicates on both platforms.

We assessed the performance of Abbott ID Now on 15 consecutive positive (by Xpert Xpress) NP specimens submitted in VTM from patients seen at the emergency department with suspected COVID-19. Each specimen was tested on the ID Now platform.

ID Now performance in relation to N2 C_T values obtained by the Xpert Xpress assay was assessed by testing a set of 8 previously tested specimens using the Xpert Xpress assay that had increasing N2 C_T values ranging from 32.6 to 41.8. All samples were tested on the ID Now and the results compared to those previously obtained using the Xpert Xpress.

In response to our initial findings of lower positive agreement of the ID Now compared to the Xpert Xpress, Abbott communicated to us that despite the indications in the package insert, their assay was not optimized for testing NPS in VTM; the best performance would be from dry nasal swabs obtained and tested at the point of care (POC). They indicated that they would be modifying their package insert to reflect this change (ID Now COVID-19 product insert, IN190000 Rev.3 2020/04:6-8).

Abbott ID Now platform using dry nasal swabs versus Cepheid platform using nasopharyngeal swabs. We compared the Abbott ID Now platform using paired dry nasal swabs (both nares) to the Cepheid platform using NPS in VTM. We collected paired nasal swabs and nasopharyngeal swabs in parallel from patients who presented to the NYU Langone Hospitals (Manhattan) emergency department for whom a physician ordered a test for SARS-CoV-2. These patients had their ED visit between 22 and 24 April 2020. Collection instructions for the nasal swabs provided by Abbott were communicated to ED care providers. Nasal samples were obtained with swabs supplied with the Abbott assay (Puritan Medical Products; 25-1506 IPF100). While the sequences of collecting the two sample types varied, all NPS were obtained through one nostril and nasal samples were obtained from both nares. Nasopharyngeal swabs were collected as described above.

Although Abbott indicated that the dry swab could be transported in the same sleeve as that in which the swabs were packaged, we chose to transport the samples in a sterile tube in order to minimize loss of sample on the sleeve and ensure safety during transport. A total of 101 paired samples collected during 12-h shifts over 3 consecutive days were included.

Dry nasal swabs were transported to the laboratory at room temperature in a sterile tube along with the corresponding NPS in VTM. The dry nasal swabs were tested within 2 h of collection or kept refrigerated at 4 to 8°C for up to 24 h before testing as per package insert instructions.

Instruments. ID Now COVID-19 (Abbott Diagnostics Scarborough, Inc., Scarborough, ME) is a rapid test that qualitatively detects SARS-CoV-2 viral nucleic acids from nasal, nasopharyngeal, and throat swabs. It is an automated assay that utilizes isothermal nucleic acid amplification technology. The assay

TABLE 1 Initial verification of ID Now^a

Patient specimen ^b	Dilution	No. of replicate runs	No. of Abbott results in agreement with reference method (%)
Sample 1	1:2	1	1 (100)
	1:5	1	1 (100)
	1:10	4	4 (100)
	1:20	3	3 (100)
	1:50	2	2 (100)
	1:100	2	2 (100)
Sample 2	1:2	1	1 (100)
	1:5	1	1 (100)
	1:10	4	3 (75)
	1:20	4	2 (50)
	1:50	3	2 (67)
	1:100	3	1 (33)

^aPositive agreement when testing two progressively diluted patient specimens with low and high Xpert Xpress N2 C_T values. All sample dilutions were detected by Xpert Xpress.

^bSample 1 Xpert Xpress N2 C_T , 19.3 (undiluted); sample 2 Xpert Xpress N2 C_T , 30.8 (undiluted).

amplifies a unique region of the RdRp genome with a manufacturer's claimed LOD of 125 genome equivalents/ml. Positive results are available within 5 to 13 min and negative results within 13 min.

The Xpert Xpress SARS-CoV-2 test (Cepheid, Sunnyvale, CA) is a rapid, real-time RT-PCR that detects SARS-CoV-2 RNA in nasopharyngeal swab and/or nasal wash/aspirate specimens. The assay amplifies 2 nucleic acid targets, namely, N2 (nucleocapsid) and E (envelope), wherein N2 is more specific for SARS-CoV-2. The LOD for this assay is claimed by the manufacturer to be 250 copies/ml. SARS-CoV-2 genomic load is assessed using the number of amplification cycles needed for a positive PCR test (i.e., the C_T value). C_T values have an inverse relationship to viral loads and thereby provide a surrogate measurement of the viral genomic load (7). The Cepheid GeneXpert instrument interprets the results automatically based on detection of N2 and E targets. For a positive detection, the C_T values must be within a valid range and endpoint above the minimum setting as determined by the manufacturer software.

Both assays are used only under the Food and Drug Administration's Emergency Use Authorization (EUA) (8).

The performance evaluation of the Abbott ID NOW assay was done as a routine laboratory quality assessment as normally conducted before introducing any new assay.

Statistical analysis. Qualitative method comparison was performed using EP Evaluator (Data Innovations, VT), Evidence-Based Medicine Diagnostic Toolbox (<https://ebm-tools.knowledgetranslation.net/calculator/diagnostic/>), and the correlation coefficient using Excel 2010.

RESULTS

Abbott ID Now platform versus Cepheid platform using nasopharyngeal swabs in VTM. Results of the initial verification of the ID Now revealed that there was good positive percent agreement (PPA) with Xpert Xpress when a specimen (sample 1) with a low N2 C_T value (19.3) was progressively diluted up to 1:100. However, this was not the case for a specimen (sample 2) with an initial higher N2 C_T value (30.8) (Table 1). Lower positive percent agreement between the two platforms was noted at a 1:10 dilution level and beyond, with only a 33% agreement at a 1:100 dilution.

Comparison of results from our first study of Abbott ID Now obtained on 15 consecutive NP positive specimens in VTM that was previously tested by Xpert Xpress produced 5 negative results by ID Now (PPA of 66.7%). The Xpert Xpress N2 C_T values of the discordant specimens ranged from 36.3 to 44.3.

In order to confirm that the lower sensitivity of the ID NOW platform was related to the C_T value obtained on the Xpert Xpress, we tested a series of previously positive NP specimens with increasing N2 C_T values. Similar to the case with the dilution studies, the performance of the ID Now started to decline as the Xpert Xpress N2 C_T values increased throughout the series, although at a higher C_T value (38.0) than that seen with the earlier-tested consecutive positive specimens from ED patients (36.3).

The distribution of the Xpert Xpress C_T values of 1,439 positive samples submitted from different areas of our institution in the previous 6 weeks revealed that 15.1% of total positive samples had C_T values above 38 (data not shown), the percentage increasing over more recent weeks.

TABLE 2 Comparison of ID Now using dry nasal swabs and Xpert Xpress using NPS in VTM on 101 paired samples

ID Now result	No. of swab with indicated Xpert Xpress result		Total
	Positive	Negative	
Positive	17	1	18
Negative	14	69	83
Total	31	70	101

Abbott ID Now platform using dry nasal swabs versus Cepheid platform using nasopharyngeal swabs. For the comparison of dry nasal swabs versus NP samples in VTM, with testing in ID Now and Xpert Xpress, respectively, we included samples from 101 ED patients (ages, 28 to 90 years), all suspected of COVID (onset of symptoms 1 day to 1 month) except 1 patient who was tested prior to surgery for ectopic pregnancy as per emergency department protocols. As seen in Table 2, the ID Now using dry nasal swabs agreed in 17 of 31 positive Xpert Xpress samples, with a PPA of 54.8% (95% confidence interval [CI], 37.8 to 70.8%). The remaining 14 samples were not detected by ID Now. The ID Now results matched 69 of the 70 negative Xpert Xpress results, with 1 detected by ID Now but not by Xpert Xpress, with a negative percent agreement (NPA) of 98.6% (95% CI, 92.3 to 99.7%). Overall, there was 85.1% agreement (95% CI, 76.9 to 90.8%) and a positive predictive value (PPV) and negative predictive value (NPV) of 94.4% (95% CI, 74.3 to 99.0%) and 83.1% (95% CI, 73.7 to 89.7%), respectively.

Grouping the Xpert Xpress N2 C_T values arbitrarily into low, medium, and high ranges showed that all 6 dry nasal swabs with their corresponding NPS in VTM having a N2 C_T value of <33.5 by Xpert Xpress tested positive by ID Now. But the PPAs of ID Now were 54.5% (6 of 11) and 35.7% (5 of 14) when N2 C_T values obtained by Xpert Xpress were between 33.7 and 38 and >38 , respectively.

The average time between collection and testing of the samples with discrepant results and those testing positive by the ID Now was approximately 90 min.

There was no correlation between N2 C_T and days of onset of symptoms ($R^2 = 0.097$). Nineteen of the 31 patients whose samples tested positive for SARS-CoV2 RNA by Xpert Xpress were admitted. Of the 14 ID Now-negative/Cepheid Xpert Xpress-positive patients, 1 was admitted to hospice and 5 were admitted to NYU Langone Health-Tisch Hospital; 1 of these patients was admitted for ectopic pregnancy surgery. Thirteen of 14 patients whose samples were ID Now negative/Cepheid Xpert Xpress positive had clinical or radiological evidence of COVID.

DISCUSSION

The urgency of the COVID-19 pandemic and the U.S. government's subsequent use of the EUA has placed an unusual onus of responsibility on the clinical laboratories to substantiate the clinical value and performance of the COVID-related tests they introduce. Accurate diagnostic testing and subsequent isolation of infected individuals are crucial to mitigating continued transmission. The appeal of the Abbott technology stemmed from our intention to provide rapid and accurate results in our institution, especially for our emergency department.

Our results showed progressively lower PPA with lower viral load (higher N2 C_T values) as determined by Xpert Xpress, but this was not consistent. In our comparison studies of paired dry nasal swabs and NP swabs in VTM, there were samples testing positive by Abbott platform even when Xpert Xpress N2 C_T values were above 38, which might suggest better sampling using the dry swab in these cases. A study by Zhen et al. has also shown discordant results comparing these two assays against the Hologic Panther Fusion SARS-CoV-2 assay as the reference method (9). The ID Now produced 7 false negatives out of 57 positive NP samples, resulting in a PPA of 87.7%, compared to 98.3% on Xpert Xpress. Their study, however, included a total of 108 NPS transported in VTM, which the manufacturer has now noted decreases the sensitivity of

their assay. These authors also determined that the Xpert Xpress had the lowest LOD at 100 viral copies/ml, while the ID Now had an LOD of 20,000 copies/ml (9), well above the limit of detection stated in the Abbott ID Now COVID-19 package insert (125 genome equivalents/ml).

Smithgall et al. found overall PPAs of 73.9% with ID Now and 98.9% with the Xpert Xpress compared against the Cobas Roche assay (10). Similar to our study, these authors found that the performance of the ID Now decreased for samples with high C_T values. The study by Rhoads et al. on 96 NPS, however, found a PPA of 94% for ID Now, compared to 96% with Simplexa Diasorin against a modified CDC assay. This study also showed a correlation with C_T values ($R^2 = 0.89$ to 0.91). This would suggest that the sensitivity of both the ID Now and the Simplexa was reduced as the C_T values exceeded 34.5 (11).

Although the original Abbott package insert had stated that 0.5 to 3.0 ml of viral transport medium was acceptable for use in their assay, it also recommended minimizing dilution of the sample, as it might result in decreased test sensitivity. The use of dry swabs was recommended by the manufacturer for optimal test performance. Contrary to manufacturer expectations, however, our parallel study showed that the PPA of ID Now using dry nasal swabs (54.8%) was actually lower than when using NPS in VTM (66.7%) on 15 consecutive positive samples as determined by Xpert Xpress in this study.

Interestingly, a recent study by Harrington et al. compared the results of 524 dry nasal swabs tested on the ID Now with paired nasopharyngeal swabs in VTM run on the Abbott m2000 RT-PCR system (12). The specimens were submitted from 3 emergency departments and 2 immediate care centers; about two-thirds of the dry swabs were tested at the point of care. The ID Now showed an overall 74.7% positive agreement with the m2000 and 70.6% for the group tested at the point of care.

The literature provides no strong consensus regarding the influence of transport medium and storage conditions on viral load stability. One study looking at differing viral transport media and storage conditions using the Quest and Roche EUA assays showed consistent detection of SARS-CoV-2 RNA after 7 days at room temperature and up to 14 days refrigerated or frozen (13). The ID Now package insert also directs clinicians to test swabs as soon as possible after collection and, if this is not possible, to hold it in its original package for no more than 2 h prior to testing or to refrigerate for up to 24 h prior to testing. Outside of inpatient hospital laboratories, such as in outpatient point-of-care testing, independent validation should be performed to assess performance in different settings, including preanalytic variables.

Extrapolating from our results from 101 patient samples, if the ID Now assay were to be used as a first-step screening, confirmation of more than 80% of the tested samples would be required to be confident that the negative results are truly negative. While there was one apparent false-positive result in our parallel study, the overall high PPV (94.4%) and NPA (98.6%) do support the added value that in 5 to 13 min a positive result can be interpreted as a true positive. We could not confirm that the apparent false-positive result obtained with the Abbott ID Now was due to sampling variability.

Based on our findings, the ID Now has some utility as a rapid rule-in test for COVID-19 with samples at high viral load; however, we advise caution with its use as a singular rule-out test, especially in the setting of samples with lower viral loads. In the case of the positive samples missed by ID Now, 13 of the 14 patients had clinical or radiologic evidence of COVID-19. The remaining patient was an asymptomatic patient admitted for an ectopic pregnancy. Currently we cannot assume that patients with low viral loads are unable to transmit the virus, as a false negative can significantly affect subsequent clinical and epidemiologic decision-making. It is noteworthy that the ID Now exhibited higher target detection when using NPS in VTM than the dry nasal swabs. Patients having lower levels of SARS-CoV-2 in the nasopharynx might harbor even lower concentrations in the nasal cavity, leading to a lower level of detection by the ID Now.

The lower PPA between the ID Now and Xpert Xpress could be attributed to several

factors. The sample population included in our study during the latter part of April 2020, although having a broad spectrum of Xpert Xpress-determined N2 C_T values, did have a significant number of samples with C_T values above 38, which ID Now did not detect consistently. Differences in viral RNA detection could be related to the differences in NAAT technology used. Although isothermal nucleic acid amplification test (INAAT) is a promising tool, it has been found to have lower sensitivity than RT-PCR for SARS-CoV-2 RNA detection (14). The molecular chemistry of INAAT is more complex than that of RT-PCR, involving more primers and intricate conformational rearrangements through enzymatic strand displacement, emphasizing the importance of target selection and primer design to performance.

Another possible explanation is the potentially poorer performance of the RdRp target used in the Abbott assay than of other RNA targets as has been described previously (15, 16). Recent reports indicate that mutations in the RdRp domain have been emerging (17). It is unknown at this point if the genomic region targeted by the Abbott assay has changed significantly as the virus has spread throughout the world.

Limitations of our study include relatively small sample size, inability to control for sampling variability, and the lack of an additional comparator method to discern the discrepancies. In our laboratory, we periodically perform correlation studies between our 2 platforms, Cobas and Cepheid, and results consistently match. Another limitation was transporting the dry swabs specimens to the laboratory for testing rather than testing directly in a point-of-care setting as intended for the Abbott ID Now platform. Nevertheless, this factor might not have influenced our results, since the samples were handled and tested in ID Now within the appropriate time and conditions stipulated in the package insert.

Overall, our study revealed low PPA of ID Now compared with Xpert Xpress irrespective of use of viral transport media or sample type, which raises concerns regarding its suitability as a diagnostic tool. An assay that detects a broad range of the SARS-CoV-2 RNA levels is highly desirable since it is difficult to control the sample quality and, as a consequence, variations in viral concentrations. At this point, the significance of low viral levels in disease or transmission is largely unknown.

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We have no conflicts of interest to declare.

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