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Clinical Evaluation of Three Sample-To-Answer Platforms for the Detection of SARS-CoV-2

Running title: Three Sample-To-Answer Platforms for SARS-CoV-2 Detection

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Abstract

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) has now spread across the globe. As part of the worldwide response, many molecular diagnostic platforms have been granted Emergency Use Authorization (EUA) by the Food and Drug Administration (FDA) to identify SARS-CoV-2 positive patients. Our objective was to evaluate three sample-to-answer molecular diagnostic platforms (Cepheid Xpert® Xpress SARS-CoV-2 [Xpert Xpress], Abbott ID NOW™ COVID-19 [ID NOW], GenMark ePlex® SARS-CoV-2 Test [ePlex]) to determine analytical sensitivity, clinical performance, and workflow for the detection of SARS-CoV-2 in nasopharyngeal swabs from 108 symptomatic patients. We found that the Xpert Xpress had the lowest limit of detection (100% detection at 100 copies/mL), followed by the ePlex (100% detection at 1,000 copies/mL), and the ID NOW (20,000 copies/mL). The Xpert Xpress also had highest positive percent agreement (PPA) when compared to our reference standard (98.3%) followed by the ePlex (91.4%) and ID now (87.7%). All three assays showed 100% negative percent agreement (NPA). In the workflow analysis, the ID NOW produced the most rapid time to result per specimen (~17 minutes) as compared to the Xpert Xpress (~46 minutes) and the ePlex (~1.5 hours), but what the ID NOW gained in rapid results, it lost in analytical and clinical performance. The ePlex had the longest time to results and showed a slight improvement in PPA over the ID NOW. Information about the clinical and analytical performance of these assays, as well as workflow, will be critical in making informed and timely decisions on testing platform.

Keywords: SARS-CoV-2, COVID-19, EUA, molecular diagnostics, near patient testing, nasopharyngeal

Introduction:

The outbreak of SARS-CoV-2 and subsequent cases of COVID-19 (1), which began in Wuhan, China by the end of December 2019, has spread to more than 200 countries and territories. As of April 15, 2020, over two million cases have been confirmed, causing over ~133,000 deaths according to the Centers for Disease Control and Prevention (CDC) and database from the Center for System Science and Engineering (CSSE) at Johns Hopkins University (2, 3).

SARS-CoV-2 is the seventh coronavirus known to be transmitted from human to human, has high rates of transmission, and is also relatively stable in aerosols and on surfaces (4, 5, 6). Infection with SARS-CoV-2 can cause mild to severe respiratory illness, including symptoms such as fatigue, shortness of breath, cough, and fever. In addition, some individuals experience rapidly progressive and severe disease. The elderly and those with serious underlying medical conditions (e.g. cardiovascular disease, diabetes, lung disease, and immunocompromised individuals) are most at risk of developing fulminant disease (4). Currently, there are no available specific therapeutics, or vaccines approved by the FDA for treatment or prevention of COVID-19 (7). In addition, the SARS-CoV-2 pandemic has coincided with influenza season in many locations. These challenges have presented a major hurdle for slowing the global spread of disease and have necessitated the need for rapid and accurate SARS-CoV-2 diagnostic testing to implement effective infection control measures.

Currently available molecular diagnostics platforms include several sample-to-answer platforms that have been issued an EUA by the FDA to qualitatively detect SARS-CoV-2 RNA in symptomatic patients. All three sample-to-answer platforms evaluated in this study are individual cartridge-based tests are likely to be widely utilized by hospital laboratories. In addition, both the Xpert Xpress and the ID NOW are also authorized to be used in patient care settings outside of the clinical laboratory environment and are therefore highly likely to be considered for patient testing in the outpatient environment.

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In this study, our objective was to evaluate the analytical and clinical performance, as well as the workflow of these three sample-to-answer platforms for SARS-CoV-2 detection in 108 nasopharyngeal swab specimens from symptomatic patients.

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Materials and Methods

Specimen collection and storage. Nasopharyngeal (NP) swabs were collected from symptomatic patients. A sterile swab made from Dacron, rayon or nylon was used for each collection. The NP swab was then placed into sterile 3 mL universal transport medium (UTM- various manufacturers). Samples were then transported and tested as close to collection time as possible. Storage of specimens occurred at 2-8^{o}C for up to 72 hours. Following routine patient testing, samples were aliquoted and stored at -80^{o}C until comparator testing could occur.

Study design.

A total of 108 nasopharyngeal samples (50 negative and 58 positive specimens) tested between March to April of 2020 were selected for this study and included symptomatic patients of all genders and ages. This work was conducted as a quality improvement activity in order to complete each assay validation. The 108 specimens included 88 retrospective samples initially tested on the ePlex and then immediately aliquoted and frozen at -80^{o}C, remaining frozen until this study was performed. Retrospective samples were thawed and immediately tested on the Hologic Panther Fusion® SARS-CoV-2 assay (Reference standard), ID NOW and Xpert Xpress assays. The prospective 20 specimens were performed fresh on each platform at the time of patient testing. The specimens selected represented our true positivity rate at the time this study was performed (50 - 60%) and also included positive specimens spanning the range of positivity, including those with low viral loads (characterized by high cycle threshold (Ct) values obtained by the reference method).

Cepheid Xpert® Xpress SARS-CoV-2 assay. The Xpert Xpress assay is a molecular *in vitro* diagnostic test utilizing widely-used real time RT-PCR amplification technology to detect the nucleocapsid gene (N2) and the envelope gene (E) in upper respiratory specimens and is performed on GeneXpert instrument

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system. All testing was performed according to the manufacturer's instructions. Briefly, the specimen collection tube is mixed by rapidly inverting five times and then 300 µL of NP specimen is transferred to the sample chamber of the assay cartridge. The lid is then closed and the cartridge is loaded onto the GeneXpert platform, which performs automated sample processing, and real time RT-PCR for viral RNA detection.

Abbott ID NOW™ COVID-19 assay. The ID NOW is a rapid molecular *in vitro* diagnostic test utilizing isothermal nucleic acid amplification technology to detect the RNA-dependent RNA polymerase (RdRp) gene segment of the SARS-CoV-2 virus and is performed on the ID Now instrument. It consists of a Sample Receiver containing elution/lysis buffer, a Test Base, and a Transfer Cartridge for transfer of the eluted sample to the Test Base, and ID NOW instrument. All testing was performed according to the manufacturer's instructions. Briefly, a Test Base and a Sample Receiver are inserted into the ID now instrument. When instructed via on-screen instructions, 200 µL of NP specimen is added to the Sample Receiver and then immediately transferred to the Test Base using the provided Transfer Cartridge, initiating target amplification.

GenMark ePlex® SARS-CoV-2 assay. The ePlex assay is an *in vitro* diagnostic test that targets the N gene of SARS-CoV-2 and uses combined electrowetting and GenMark's eSensor® technology for the extraction, amplification and detection using competitive DNA hybridization and electrochemical detection. All testing was performed according to the manufacturer's instructions. Briefly, the specimen is initially vortexed and 200 µL of NP specimen is added to the sample delivery device (SDD) provided with the ePlex SARS-CoV-2 kit and vortexed for 10 seconds. The entire volume of the SDD is dispensed into the sample loading port of SARS-CoV-2 test cartridge, followed by pushing down the cap to seal the sample delivery port. The cartridge is bar-coded and scanned with the ePlex® instrument barcode

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scanner, then is loaded into an available ePlex bay, which performs extraction, amplification, and detection.

Hologic Panther Fusion® SARS-CoV-2 assay (Reference Standard assay). The Fusion SARS-CoV-2 assay was used as the reference standard for all three assays evaluated in this study and was performed according to the manufacturer's instructions for use. NP specimens are lysed by transferring 500 µL of specimen into a Specimen Lysis Tube containing 710 µL lysis buffer and loaded onto the instrument. An Internal Control is added to each specimen by the working Panther Fusion Capture Reagent-S and hybridized nucleic acid is then separated using a magnetic field. Following wash steps, 50 µL of purified RNA is eluted. Then 5 µL of eluted nucleic acid is transferred to a Panther Fusion reaction tube. The Fusion® SARS-CoV-2 assay amplifies and detects two conserved regions of the ORF1ab gene in the same fluorescence channel, with amplification of either or both regions leading to a fluorescent ROX signal. Reporting of a positive specimen requires only one of the two targets to be detected (ORF1a or ORF1b gene).

Analytical Sensitivity. Limit of detection (LoD) was performed using the Exact Diagnostics synthetic RNA quantified control (SARS-CoV-2 Standard) containing five gene targets (E, N, ORF1ab, RdRP and S Genes of SARS-CoV-2 (SKU COV019, Fort Worth, TX). A starting concentration of 200,000 copies/mL control was used to prepare a serial dilution panel. The control material was prepared using Ambion® RNA Storage Solution (Catalog No. AM7001, ThermoFisher Scientific) to limit the potential of degradation of the RNA transcript and aliquoted for testing to obtain replicates at 20,000, 10,000, 5,000, 2,000, 1,000, 500, 100, 50, and 5 copies/mL (with replicates ranging from 1-10, as shown in **Table 1**). Positive rate was defined as the lowest dilution at which all replicates were positive at a 100% detection rate and was used to evaluate the analytical sensitivity of all three sample-to-answer platforms.

Statistical methods. The reference standard was established as the result obtained from the Hologic Panther Fusion® SARS-CoV-2 assay. Percent positive agreement (PPA), percent negative agreement (NPA), positivity rate, Kappa, and two-sided (upper/lower) 95% confidence interval (CI) were calculated using Microsoft® Office Excel 365 MSO software (Microsoft, Redmond, WA). Cohen's kappa values (κ) were calculated as a measure of overall agreement, with values categorized as almost-perfect (>0.90), strong (0.80 to 0.90), moderate (0.60 to 0.79), weak (0.40 to 0.59), minimal (0.21 to 0.39), or none (0 to 0.20) (8-9). The dose-response 95th percentile (with 95% confidence interval [CI]) model was assessed using the Finney and Stevens calculations (10).

Results

Analytical Sensitivity.

LoD was determined by preparing serial dilutions ranging from 20,000 to 5 copies/mL using a known concentration of the Exact Diagnostics SARS-CoV-2 control panel and was defined as the minimum concentration with detection of 100% by positive rate. The LoD established by percent positive rate and the manufacturer's interpretation algorithm for each assay was determined to be 20,000 copies/mL for the ID NOW, 1,000 copies/mL for the ePlex, and 100 copies/mL by the Xpert Xpress assay (including presumptive positive results) (**Table 1**).

Clinical performance

Clinical testing was performed on 108 retrospective and prospective clinical specimens, and was compared to the reference standard. The Xpert Xpress demonstrated a PPA of 98.3%, followed by the ePlex at 91.4% and the ID NOW at 87.9%. NPA was also calculated and was 100% for each platform evaluated (**Table 2**). One sample was invalid on the ID NOW and was not included in the calculations for this platform. When distribution of positive results was further evaluated across all three platforms, the Xpert Xpress detected a total of 57 positive results, followed by the ePlex at 53 and the ID NOW at 50. The ePlex also detected 3 positive results that were not detected by the ID NOW and the ID NOW detected 1 positive result that was not detected by the ePlex, but all 4 of these positive results were detected by the Xpert Xpress, as well as 4 additional positive results that were only detected by the Xpert Xpress. The ePlex and the ID NOW did not detect any additional results that were not detected by the Xpert Xpress. One specimen that was positive on Panther fusion was not detected on all 3 platforms. A total of eight discordant samples were found among the three sample-to-answer platforms evaluated, with ID NOW having the most discordant results (n=7), followed by the ePlex (n=5), and the Xpert Xpress (n=1). All discordant results were negative results as compared to a positive result from the reference method. When evaluating cycle threshold (Ct) values obtained from the reference method, A-24, which

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167 was the only discordant specimen by the Xpert Xpress assay, had a Ct value of 38.5, which would be
168 considered a low viral load positive specimen. The ePlex exhibited negative results with specimens that
169 had Ct values ranging from 33.1- 38.5, while the ID now ranged 32- 38.5 **(Table 3)**.

170 Hands on time (HoT), run time, and total turnaround time (TAT) per specimen were evaluated.
171 The Xpert Xpress HoT is approximately one minute per specimen, while the ID NOW and the ePlex both
172 had a HoT of approximately two minutes per specimen. The ID NOW had the shortest overall TAT of ~17
173 minutes for one specimen. Xpert Xpress TAT was ~ 46 minutes and ePlex TAT was ~1.5 hours for one
174 specimen, with the majority of TAT on each assay being assay run time. The ID NOW turnaround time
175 can also differ for positive specimens, which can be as low as 5 minutes, including HoT **(Table 4).**

Discussion

Clinical confirmation of COVID-19 is at the core of our strategy to stop the current spread of infection. It has recently been shown that SARS-CoV-2 has a basic reproduction number (R_0) of 2.2, meaning that an infected person, on average, can spread the infection to two additional persons (5, 6). Vulnerable patient populations are especially at risk, such as people with pre-existing medical conditions, immunocompromised individuals, and the elderly, especially people living in a nursing home or long-term care facility (11, 12). With this in mind, it is critical that patient results are as accurate as possible and are also available in a rapid fashion to stop the spread of infection in real-time.

We evaluated three sample-to-answer platforms currently in use in our health system for the detection of SARS-CoV-2, including the Xpert Xpress and ID Now, which are designed to be performed in near patient testing environments and outside of the clinical laboratory environment. LoD determination, correlation of clinical results, and performance comparisons, including HoT and overall TAT for each assay were done as part of our evaluation. This information is especially critical at the current moment, where accurate and rapid results are at the center of clinical decision-making, both in the outpatient clinics and in the hospital. All three of these platforms are designed to produce rapid test results and each platform is a sample-to-answer system designed to run one patient per test cartridge. This makes the comparison of these platforms especially pertinent as decisions are made for testing in both the inpatient and outpatient environments.

When we compared all three platforms, the Xpert Xpress out-performed both the ID NOW and the ePlex, exhibiting the lowest LoD of all three platforms at 100 copies/mL, whereas the ID NOW and ePlex had higher LoDs of 20,000 and 1,000 copies/mL, respectively **(Table 1)**. We also observed that in each case, the manufacturer's stated LoD differed from our findings, with the ePlex having a much lower LoD than that stated in their EUA submission (1,000 RNA copies/mL vs. EUA-listed 100,000 RNA transcript copies/mL), while the ID NOW had a much higher LoD than what was stated in their EUA

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submission (20,000 RNA transcript copies/mL vs. EUA-listed 125 genome equivalents/mL). Xpert Xpress had the closest manufacturer's stated LoD (100 RNA transcript copies/mL, including presumptive positives vs. EUA-stated 250 copies /mL). This may be due to different quantified materials being used by each manufacturer. Our LoD findings also correlated with the clinical sensitivities, which ranged from a high of 98.3% for the Xpert Xpress to a low of 87.9% for the ID Now, with the ePlex falling in the middle at 91.4% **(Table 2)**. A closer analysis of positive results showed that while the majority of positives were detected by all three platforms, the Xpert Xpress also detected four results that were missed by both the ID NOW and the ePlex, and also detected additional results singly detected by either the ID NOW, or the ePlex. All three assays had 100% specificity and did not exhibit false positive results.

When it comes to the HoT and TAT of the three platforms, each platform has specific advantages. The Xpert Xpress is the easiest to use with the least technical interventions, which include loading the sample and the cartridge. The ID NOW has the shortest sample to answer time at ~17 minutes maximum to final result. The ePlex has the ability to tests more patients at once on a random access 6 bay tower. Both the Xpert Xpress and ePlex platforms can also be expanded by adding modules/bays for more capacity in certain models of instrumentation, while the ID NOW is limited to 1 sample testing port per instrument.

Some limitations of this study are that this is a single-center study and the majority of specimens were initially tested on the ePlex system and were then stored frozen. While this is the case, the ePlex had sensitivity performance considerably lower than that of the reference standard (Panther Fusion) and the Xpert Xpress, yet had the competitive advantage as the assay that was initially performed on fresh specimens. Considering this workflow limitation, the results of our study in regards to the sensitivity of the ePlex are even more telling, since the ePlex results do not contain testing after one freeze-thaw of retrospective specimens, such as was the case for the Xpert Xpress and the ID NOW (as well as the Reference standard).

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In addition, while the number of specimens included in the clinical correlation was only 108, these specimens were chosen to span the positivity range of clinical specimens, including those specimens with a low viral load. Also, the percentage of positive specimens in our study actually reflected our overall true positivity rate (50-60% SARS-CoV-2 positive) for this time period.

In summary, we evaluated three sample-to-answer platforms for the detection of SARS-CoV-2 using NP specimens, including two platforms that are designed to be done in the near patient testing environment, the Xpert Xpress and the ID NOW. Our results showed that the Xpert Xpress performed well and had the lowest LoD and highest sensitivity, while the ePlex and ID NOW had lower sensitivities and missed several positive patient specimens. The lack of sensitivity in both the ID NOW and ePlex is particularly concerning in the midst of this current pandemic, where identifying new infections is the bedrock of limiting spread. While the ID NOW is the most rapid of the three platforms tested, taking ~17 minutes to complete from beginning to end, it missed 12.3% of positive patients tested, exhibiting a sensitivity of 87.7% in our study. The ePlex also missed 8.6% of positive patients and had a sensitivity of 91.4%, and also takes ~1.5 hours to perform. In contrast, the Xpert Xpress missed 1.7% of positive patients, showing a sensitivity of 98.3% and takes ~46 minutes to perform. In conclusion, while the ID NOW gives the most rapid result, both the ID NOW and the ePlex (which takes substantially longer to result) lack sensitivity as compared to the Xpert Xpress. These parameters will need to be considered when deciding which testing platform should be implemented for COVID-19 testing.

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284 **Table 1.** Summary of Limit of Detection results.

		Positive Rate % [a] No. of replicates detected at each dilution (copies/mL)									Final LoD[b] copies/mL
Molecular Assay		20,000	10,000	5,000	2,000	1,000	500	100	50	5	
Xpert Xpress	N2	1/1 (100%)	N/A	N/A	10/10 (100%)	**9/9 (100%)**	9/10 (90%)	7/10 (70%)	4/8 (50%)	0/5 (0%)	**100[c]**
	E	1/1 (100%)	N/A	N/A	10/10 (100%)	9/9 (100%)	10/10 (100%)	**10/10 (100%)**	7/8 (87.5%)	0/5 (0%)	
ID NOW	RdRp	**5/5 (100%)**	8/10 (80%)	5/10 (50%)	5/10 (50%)	0/8 (0%)	0/1 (0%)	0/1 (0%)	0/1 (0%)	0/1 (0%)	**20,000**
ePlex	N	10/10 (100%)	N/A	N/A	10/10 (100%)	**9/9 (100%)**	7/10 (70%)	1/10 (10%)	1/4 (25%)	0/4 (0%)	**1,000**

285 [a] The limit of detection by positive rate for each gene target is highlighted in bold

286 [b] The final LoD was based on each manufacturer's results interpretation algorithm

287 1. [c]Also includes presumptive positive results

288 **Table 2.** Clinical performance comparison of three sample-to-answer EUA molecular assays for the

Molecular Assay		Reference Standard [a] Positive	Negative	(± 95% CI) [bc] Kappa (κ)[d]	PPA	NPA
Xpert Xpress						
	Positive	57	0		98.3%	100%
	Negative	1	50	0.98 (1-0.95)	(0.91-1)	(0.93-1)
ID NOW[e]						
	Positive	50	0		87.7%	100%
	Negative	7	50	0.87 (0.96-0.78)	(0.76-0.95)	(0.93-1)
ePlex						
	Positive	53	0		91.4%	100%
	Negative	5	50	0.91 (0.99- 0.83)	(0.81-0.97)	(0.93-1)

289 detection of SARS-CoV-2 (n = 108).

290 [a]Reference standard was the Hologic Fusion assay.

291 [b] ±, upper/lower 95%

292 [c] CI, confidence interval

293 [d] Almost-perfect (>0.90), strong (0.80 to 0.90), moderate (0.60 to 0.79), weak (0.40 to 0.59), minimal

294 (0.21 to 0.39), or none (0 to 0.20).

295 [e]ID NOW had one Invalid that was removed from the analysis, which was positive by the reference

296 standard and the other two methods

297 **Table 3.** Details of discordant samples.

	SARS-CoV-2 sample-to-answer molecular assay results and Ct values[ab]					
SAMPLE ID	Reference Method		Xpert Xpress		ID NOW	ePlex
		Ct value		Ct value E/ N2		
A-10	**POS**	33.1	POS	32.8/35.8	POS	**NEG**
A-12	**POS**	33.2	POS	31.7/34.6	**NEG**	**NEG**
A-14	POS	34	POS	33.3/35.5	**NEG**	**NEG**
A-15	POS	32.6	POS	32.2/35.4	**NEG**	POS
A-16	POS	33.2	POS	33.6/36.4	**NEG**	POS
A-24	POS	38.5	**NEG**	N/A	**NEG**	**NEG**
A-26	POS	36.2	POS	36.6/39.5	**NEG**	**NEG**
A-103	POS	32	POS	31.1/34.3	**NEG**	POS

298 [a] Discordant sample results are highlighted in bold

299 [b] Ct, Cycle threshold

300 **Table 4.** Basic performance characteristics of three sample-to-answer EUA molecular SARS-CoV-2 assays

301 evaluated.

	Xpert Xpress® SARS-CoV-2	**ID NOW™ COVID-19**	**ePlex® SARS-CoV-2**
Manufacturer	Cepheid	Abbott	GenMark
Sample type	NPS, nasal, mid-turbinate swab, nasal wash, nasal aspirate	NPS, NS, TS	NPS
Sample volume required (µl)	300	200	200
Extraction required	Yes (automated)	No	Yes (automated)
Detection platform/System	GeneXpert®, Xpress, Infinity	ID NOW™	ePlex®
Target region of SARS-CoV-2	N2, E	RdRp	N
Analytical sensitivity per claim	250 copies /mL	125 genome equivalents/mL	100,000 RNA transcript copies /mL
Maximum Throughput	4 per instrument (4-module configuration)	1 per instrument	6 per tower
Hands-on Time (per specimen)	~1 min	~2 min	~2 min
Assay Run Time	~45 min	<15 min	~ 90 min
User Results Interpretation	No	No	No
Overall Turn-around Time (per specimen)	~46 min	~17 min	~1.5 hr

302