Comparison of three sample-to-answer RT-PCR testing platforms for the detection of SARS-CoV-2 RNA in positive nasopharyngeal and nasal swabs

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Abstract

Introduction

The COVID-19 pandemic has strained clinical microbiology laboratories due to testing supply allocations. As a result, laboratories have had to invest in multiple COVID-19 assays performed on different testing instruments. Comparing the results achieved by testing positive samples between in-use assays can provide insights into which platforms may be interchangeable for testing in times of supply chain emergencies.

Methods

Nasopharyngeal and nasal swab specimens collected in viral transport media that tested positive on the Xpert® Xpress SARS-CoV-2 assay were tested on the ePlex® SARS-CoV-2 and BD SARS-CoV-2 Reagents for BD Max™ assays. Positive percent agreement was calculated using the Xpert® Xpress SARS-CoV-2 assay as the reference method.

Results

We tested 78 positive swabs, resulting in a positive percent agreement (PPA) of 92% [CI 84-97%] for the BD SARS-CoV-2 assay and 58% [CI 47-70%] for the ePlex® assay. Following development of a new workflow for the ePlex®, we detected SARS-CoV-2 in 7 additional samples, resulting in a new PPA of 68% [CI 56-78].

Conclusions

During times of supply allocation and shortage of the Xpert® Xpress SARS-CoV-2 assay, the BD SARS-CoV-2 assay is well suited for test substitutions due to its high positive percent agreement.

Impact Statement

Shortages and allocations of SARS-CoV-2 testing supplies and reagents have resulted in clinical microbiology laboratories validating tests on multiple instruments to maintain testing demand. Due to the emergency nature of SARS-CoV-2 test development and short supply, comparisons between assays are important to help laboratories understand which tests may be easily interchanged. In this report, we examine three SARS-CoV-2 assays common in clinical microbiology laboratories and their agreement for the detection of positive samples.

Introduction

Allocation of SARS-CoV-2 testing reagents has required that laboratories place large purchase orders for reagents for multiple instruments to simply keep up with testing demand. Many of these tests are performed on sample-to-answer instruments that require very little manipulation and generate results in just a few hours. Due to ordering restraints, our laboratory procured reagents for the Xpert® Xpress SARS-CoV-2 (Cepheid; Sunnyvale, CA), ePlex® SARS-CoV-2 (GenMark; Carlsbad, CA), and BD SARS-CoV-2 Reagents for BD Max™ (BD; Franklin Lakes, NJ) assays. Although the individual performance of sample-to-answer SARS-CoV-2 assays such as the Xpert® (1–3) and ePlex® (2, 4–6) assays has been established, a study directly comparing these two instrument's ability to detect positive samples along with that of the BD Max™ has not yet been performed.

Methods

We assessed the interchangeability of the Cepheid GeneXpert® Xpress, BD Max™, and ePlex® SARS-CoV-2 assays by calculating the positive percent agreement (PPA) of the assays using the

Xpert® Xpress SARS-CoV-2 assay as the reference method due to it being standard of care in our hospital during the study period. Between April 22nd, 2020 and May 15th, 2020, 78 patient specimens (74 nasopharyngeal and 4 anterior nares swabs) that tested positive by the Xpert® Xpress SARS-CoV-2 assay were automatically reflexed to the ePlex® SARS-CoV-2 assay and BD SARS-CoV-2 assay as reagents and instrument space allowed. Tests were performed according to the package insert of each assay except that anterior nares swabs were tested on the ePlex® assay although not a part of their instructions for use. Results and Ct values for the samples on each instrument were collected, when available. Specifically, the Ct values for the E gene target and N2 gene target were collected from the Xpert® Xpress SARS-CoV-2 assay and the N1 and N2 gene targets collected from the BD SARS-CoV-2 assay. Ct values could not be collected from the ePlex® SARS-CoV-2 assay as the assay detects a N gene target through DNA hybridization which does not generate Ct values. Similar to the study performed by Rhoads et al (7), the exact Clopper-Pearson 95% confidence intervals were calculated using the freely available online Medcalc tool (https://www.medcalc.org/calc/diagnostic_test.php). This study was submitted to an approved by the University of Maryland, Baltimore Institutional Review Board as Not Human Subjects Research under the protocol number HP-00095950.

Results

Following testing, we found that the BD SARS-CoV-2 assay detected viral RNA in 72 of the 78 swabs (PPA 92% [CI 84-97%]), but only 46 were detected by the ePlex® (PPA 58% [CI 47-70%]) (Table 1). These 46 samples were also positive on the BD Max™, for a total of 46 of the 78 samples positive by all three methods. Upon examination of the results, it was found that 14 did not have a detected E gene probe by the Xpert® Xpress SARS-CoV-2 assay, with a

corresponding N2 probe Ct range of 39.2-42.8. Of these 14, six were not detected at all by both the BD Max™ or ePlex® assays. Chart review of the associated patients found that three of the specimens were repeats from previously positive patients, suggesting true positives that were below the limits of detection for the BD Max™ and ePlex® assays. The other three specimens came from patients with no signs or symptoms of SARS-CoV-2 infection, suggesting that these were likely false positives results. Further chart review was performed on the 26 other discrepant samples in which RNA was detected on both the GeneXpert and BD Max but not the ePlex® assay. The analysis revealed that 2 samples were from patients with no symptoms, 1 sample was from a patient with symptoms that developed the day of presentation, and 23 samples were from known positive patients either with positive tests at an outside hospital or earlier during their course at our hospital. This suggests that concentrations of viral RNA in the nasopharynx of these patients may have been low, perhaps due to early course of disease or due to resolution of disease in those who had previously known disease.

Shortly after the completion of these experiments, GenMark released a new workflow for their ePlex® SARS-CoV-2 test in which patient specimens were directly inoculated into the test cartridge rather than using the specimen delivery device, increasing analytical sensitivity to 750 copies/mL compared to 1,000 copies/mL as stated for the previous workflow (GenMark ePlex® SARS-CoV-2 EUA, Effective Date: June 2020). Indeed, the new direct work flow did increase analytical sensitivity as we observed a limit of detection of 500 copies/mL in our laboratory with SeraCare reference materials. We reassessed 29 of the 78 specimens that had sample remaining using the new ePlex® workflow and were able to detect 7 positive samples the test had previously called negative, resulting in an increased PPA of 68% [CI 56-78]. The samples had a

GeneXpert® Ct value range of 30.7-35.8 (1 not detected) for the E gene and 33.6-40.1 for the N2 gene target, and BD Max™ Ct value ranges of 27.6-33.0 (2 not detected) and 27.6-31.5 (2 not detected) for the N1 and N2 gene targets, respectively. Although Ct values can not be directly compared between qualitative tests (8), these results suggest the new workflow does increase sensitivity of the ePlex® assay.

Discussion

In summary, we found that the BD SARS-CoV-2 assay produced a higher PPA than the GenMark ePlex® SARS-CoV-2 assay when using the GeneXpert® Xpress SARS-CoV-2 assay as the reference method, and thus may be a better assay to use in combination or interchangeably with the GeneXpert® assay to distribute test loads when supplies are limited. Analysis of the Ct values for which the BD assay did not agree with the GeneXpert® assay suggest that these samples may have contained quantities of viral RNA beneath the BD assay's limit of detection, which is stated to be 640 genetic copies/mL by the manufacturer. In comparison, studies have shown that the limit of detection for the GeneXpert® Xpress SARS-CoV-2 assay to be 100 copies/mL (2). This raises the concern that the BD assay will result in more false negatives than the GeneXpert® assay. However, as we found in our study, specimens that were not detected by the BD Max were from patients who either were determined to be recovered from infection (suggesting a very low viral load in the nasopharynx or anterior nares) or were asymptomatic and never developed signs and symptoms of SARS-CoV-2 infection. In this regard, the PPA between the BD and GeneXpert® assays is likely higher, resulting in good interchangeability between assays. To allow for more precise assay comparison, the FDA developed and shared a reference panel of heat inactivated SARS-CoV-2 virus with test developers (9). Interestingly, as published

in Table 2A of this study, both the BD and GeneXpert® assays were found to have a limit of detection of 5,400 NAAT Detectable Units/mL, further suggesting good interchangeability between the BD and GeneXpert® assays.

In contrast to the BD SARS-CoV-2 assay, our study demonstrated that the GenMark ePlex® SARS-CoV-2 assay did not strongly agree with the GeneXpert® Xpress SARS-CoV-2 assay, suggesting that the ePlex® assay may not be a good substitution should there be supply chain concerns for either the BD or GeneXpert® assays. However, the lack of agreement by the ePlex® assay likely is due to the lower analytical sensitivity of the assay in comparison to the GeneXpert® assay. Further, we found that the direct workflow developed by GenMark did increase analytical sensitivity and resulted in an increase of PPA. Although not as interchangeable as the BD assay, the ePlex® assay may be most useful in testing samples from patients who are exhibiting signs and symptoms of SARS-CoV-2 infection while a different assay is used to screen those who may be asymptomatic and have lower viral loads in their nasopharynx.

As this data was collected in the initial stages of the pandemic in 2020, it is important to consider whether variants that have emerged in 2021 would further create false negatives on the assays utilized in this study. As of May 4th, 2021, the FDA currently lists four assays that could be impacted by SARS-CoV-2 mutations (10), including the Xpert® Xpress SARS-CoV-2 assay that was analyzed in this study. As identified by the FDA, a single point mutation in the region of the N2 target area may lead to decreased detection by this target. However, true positive patients can still be detected as the assay also utilizes an E gene target for SARS-CoV-2 detection. In

contrast, neither the BD nor ePlex® assay are noted by the FDA to be affected by currently known viral mutations.

Author Contributions: *All authors confirmed they have contributed to the intellectual content of this paper and have met the following 4 requirements: (a) significant contributions to the conception and design, acquisition of data, or analysis and interpretation of data; (b) drafting or revising the article for intellectual content; (c) final approval of the published article; and (d) agreement to be accountable for all aspects of the article thus ensuring that questions related to the accuracy or integrity of any part of the article are appropriately investigated and resolved.*

Authors' Disclosures or Potential Conflicts of Interest: *No authors declared any potential conflicts of interest.*

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Table 1. SARS-CoV-2 RNA detection by the ePlex® SARS-CoV-2 and BD SARS-CoV-2 assays from 78 positive specimens as detected by the Xpert® Xpress SARS-CoV-2 assay.

	Positive by Xpert Both Targets	Positive by Xpert E Target Only	Positive by Xpert N2 Target Only
Positive by ePlex	46	0	0
Positive by Both BD N Gene Targets	62	0	5
Positive by BD N1 Target Only	1	0	3
Positive by BD N2 Target Only	0	0	1