Comparison of a Point-of-Care Assay and a High-Complexity Assay for Detection of SARS-CoV-2 RNA

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Abstract

Background:

Numerous nucleic acid amplification assays utilizing different target genes of the SARS-CoV-2 genome have received emergency use authorization (EUA) by the United States Food and Drug Administration (FDA). Limited data are available comparing the test performance characteristics of these assays.

Methods:

A diagnostic comparison study was performed to evaluate the performance of the Cepheid Xpert Xpress SARS-CoV-2 assay compared to the Hologic Panther Fusion SARS-CoV-2 assay using clinical nasopharyngeal specimens. Agreement between the two assays was assessed by overall, positive, and negative percent agreement and Cohen's kappa coefficient.

Results:

A total of 104 (54 positive and 50 negative) clinical nasopharyngeal samples were tested by both assays. Using the Panther Fusion as a reference standard, the Xpert demonstrated an overall agreement of 99.0% (95% confidence interval (CI): 94.8 – 100), positive percent agreement of 98.1% (95% CI: 90.1 – 100), and a negative percent agreement of 100% (95% CI: 94.2 – 100). The kappa coefficient was 0.98 (95% CI: 0.94 – 1.0). One sample positive by the Panther Fusion with a cycle threshold (Ct) of 38.6 was found to be reproducibly negative by the Xpert assay.

Conclusions:

The Cepheid Xpert Xpress SARS-CoV-2 assay provides test performance comparable to the Hologic Panther Fusion SARS-CoV-2 assay while offering laboratories rapid, on-demand testing capacity.

Impact statement:

Individuals with suspected or possible COVID-19 are likely to benefit from the results presented in this study. This is particularly applicable to individuals in need of a rapid SARS-CoV-2 diagnosis that can be performed at the point of care, such as pre-operative screening and for the diagnosis of symptomatic healthcare workers. This manuscript provides evidence on the comparison of two commercial assays performed on platforms commonly used in clinical laboratories. The results demonstrate the high performance characteristics of the tested assay, confirming its position as a robust diagnostic option for the point-of-care diagnosis of SARS-CoV-2.

Background

Detection of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) has critical implications in the management of patients and the utilization of limited health care resources, including personal protective equipment for health care workers and negative pressure isolation rooms. Presently, over 50 assays for the detection of SARS-CoV-2 have been granted emergency use authorization (EUA) by the United State Food and Drug Administration (FDA) (1). While high-throughput diagnostic platforms are typically used to meet testing demand, the necessary instrumentation requires a significant financial investment.

There remains a need for rapid diagnostic options with comparable test performance to support distinct clinical needs, especially for laboratories in low-resource settings. The Cepheid Gene Xpert Xpress SARS-CoV-2 (Xpert), is a cartridge-based commercial assay that targets the envelope (*E*) gene and nucleocapsid (*N*) gene (N2 region) with results available in under one hour. As the Xpert has only recently become available for the diagnosis of SARS-CoV-2, there is a need to understand the test performance of this assay in different patient populations and settings. This study aimed to compare the Xpert assay against another EUA assay, the Hologic Panther Fusion SARS-CoV-2 (Panther Fusion).

Methods

Specimen Collection and Selection

This retrospective study was performed at the Stanford Healthcare Virology Laboratory, a northern California laboratory which serves both adult and pediatric tertiary care hospitals. Between March 31st and April 7th, 2020, nasopharyngeal (NP) swabs were submitted in viral transport media

(VTM) (MicroTest M4RT, Remel Inc., San Diego, CA) as part of routine clinical testing. Clinical testing was performed on the Panther Fusion, one of the main assays for routine testing of SARS-CoV-2 in the laboratory. A convenience set of samples from symptomatic and asymptomatic individuals was selected for comparison testing on the Xpert based on the initial Panther Fusion result of detected versus not detected. A sample size of 110 was selected for comparison testing on the Xpert in a manner to represent the full range of cycle threshold values (Ct). All samples selected for testing were frozen at -80°C prior to testing on the Xpert. This study was approved by the Stanford Institutional Review Board (protocol #48973), and individual patient consent was waived.

Panther Fusion SARS-CoV-2 Assay

The Panther Fusion SARS-CoV-2 Assay (Hologic, Inc., San Diego, CA) is a high-throughput, automated multiplex real-time RT-PCR assay which occurs in a single tube. A volume of 500 μL of clinical sample is transferred into a lysis tube and loaded into the instrument. In a series of automated steps, target nucleic acid from the lysed cells undergo oligonucleotide capture hybridization, wash purification, and elution. Eluted nucleic acid is transferred to a reaction tube for target amplification by RT-PCR. Two regions of open reading frame 1ab (ORF1ab) are targeted by the assay and detected in the same fluorescence channel (2). The Panther Fusion assay result was interpreted based on the manufacturer's cycle threshold cut-off value.

Cepheid Xpert Xpress SARS-CoV-2 Assay

The Cepheid Xpert Xpress SARS-CoV-2 Assay (Cepheid, Sunnyvale, CA) is an automated multiplex real-time RT-PCR assay housed in single-use disposable cartridges. Clinical samples

are loaded into the sample chamber of the cartridge by use of a kit transfer pipette. Following this loading step, sample processing, nucleic acid extraction and amplification, and target sequence detection occurs in an automated, self-contained process. Both the envelope (*E*) gene and nucleocapsid (*N*) gene (N2 region) are targeted by the assay (3), and are considered detected based on the manufacturer's cycle threshold cut-off values. Detection of N2, irrespective of the detection of the *E* gene, is considered positive for SARS-CoV-2 on the Xpert platform. Detection of the *E* gene in isolation is considered a presumptive positive for SARS-CoV-2.

Statistics

Overall percent agreement, positive percent agreement, negative percent agreement, and Cohen's kappa coefficient with associated 95% confidence intervals (95% CI) were determined for the result interpretation of detected versus non-detected. Cohen's kappa values greater than 0.81 were interpreted to indicate excellent agreement (4). Statistical analysis was performed using Stata v15.1.

Results

A total of 110 clinical nasopharyngeal samples previously tested by the Panther Fusion, representing 60 positive samples and 50 negative samples, were selected for testing by Xpert. Six positive samples showed insufficient quantity for additional testing, leaving a total of 54 positive samples and 50 negative samples for evaluation in this study. The 54 positive samples selected spanned a range of cycle threshold (Ct) values on the Panther Fusion, with a median ORF1ab Ct of 30.5 (interquartile range (IQR): 26.4 – 33.1) (Figure 1). One Panther Fusion positive sample was repeated on the Xpert due to an initial interpretation of no result, and was detected upon repeat.

The overall agreement, positive percent agreement (PPA), and negative present agreement (NPA) between the two assays were 99.0% (95% CI: 94.8 – 100), 98.2% (95% CI: 90.1 – 100), and 100% (95% CI: 94.2 – 100), respectively. Cohen's kappa coefficient was 0.98 (95% CI: 0.94 – 1.0), indicating excellent agreement between the two methods. *E* and N2 for the Xpert demonstrated median Ct values of 29.1 (IQR: 25.1 – 32.0) and 31.6 (IQR: 27.9 – 36.3), respectively (Figure 1).

Of 10 samples for which the Panther Fusion ORF1ab Ct value was >35, the Xpert detected 7 with both *E* and N2 targets. The Xpert *E* gene was not detected in 2 samples, with detectable N2 (Xpert) and ORF1ab (Panther Fusion) Ct values of 41.2 and 38.9, respectively, for one sample and 43.1 and 37.0, respectively, for the other sample. There were no presumptive positive results on the Xpert in this study. For one sample originally tested by Panther Fusion with an ORF1ab Ct value of 38.6, the Xpert was reproducibly not detected for both the *E* and N2 targets. This sample was subsequently retested on the Panther Fusion and found to be negative.

Discussion

Testing strategy at the beginning of the SARS-CoV-2 pandemic largely focused on implementing high-throughput platforms to meet the demands of increasing test volume. While such testing is efficient from a volume standpoint, these platforms require significant financial investment, infrastructure support, and can have turnaround time to results which may vary between several hours to days. As more rapid diagnostics receive FDA EUA, there has been significant interest in leveraging these methods to enable timely and appropriate clinical management and infection control measures. Clinical scenarios where a need for rapid and accurate SARS-CoV-2 diagnosis has been identified include patients presenting for transplantation or high-risk surgical procedures,

active labor, and for symptomatic healthcare worker screening. In this study, we demonstrated comparable test performance between the Cepheid Xpert Xpress SARS-CoV-2 assay and the Panther Fusion SARS-CoV-2 assay, with an overall agreement of 99%. These findings are consistent with several studies which reported positive percent agreements between the Xpert and various standard reference assays as ranging between 98.3 and 100% (5-10). There was a single sample missed by the Xpert which had a high Panther Fusion Ct consistent with low viral burden. Further evaluation of the sample by repeat testing on the Panther Fusion demonstrated a negative result. The original result on the Panther Fusion may have been a false positive; alternatively RNA degradation may have occurred during storage or freeze-thaw of the primary sample leading to the subsequent inability of both the Xpert and the Panther Fusion to successfully detect SARS-CoV-2 RNA.

The Xpert offers several advantages to laboratories. Firstly, it provides a rapid diagnostic method from viral transport medium with high accuracy to confidently rule-in or rule-out SARS-CoV-2. This is an important benefit given the low performance reported for two other molecular point-of-care tests for SARS-CoV-2, the Abbott ID NOW and the Mesa Biotech Accula. When compared to a reference standard, the Abbott ID NOW has shown positive percent agreements ranging between 73-80% (8, 11, 12). Similarly, the Mesa Biotech Accula demonstrated low positive percent agreement of 68% when compared against a laboratory-developed test for SARS-CoV-2 (13). Indeed, the dual target approach may increase sensitivity for detection of samples with low viral burden, as shown by two samples in this study which were detected by N2 and not detected by the *E* gene. Secondly, given the Xpert Xpress is CLIA-waived, this testing can be considered in near-patient settings by trained non-laboratorians to facilitate prompt uptake of results and

minimize patient inconvenience. Thirdly, the only processing step required is transfer of the sample to the cartridge eliminating the need for large extraction instrumentation; subsequently, the reaction mixture processing is entirely contained within a single use cartridge. However, institutions may require sample transfer to proceed only in a biosafety cabinet to minimize the risk of aerosol generation. Finally, this assay makes use of the Xpert diagnostic system, which is a platform staff in many laboratory settings are already familiar with given its use for the diagnosis of other infectious agents. Given the global presence of the Xpert system, rapid increases in SARS-CoV-2 catridges would extend testing capabilities to many laboratories in need of testing options.

Several limitations are present in this study. Samples were frozen prior to testing on the Xpert which could have decreased detection, although this did not appear to cause a significant detrimental effect based on the high agreement observed. In addition, the Xpert assay has received EUA for upper respiratory samples only. This study does not assess the utility of the Xpert assay for lower respiratory tract samples, a specimen type authorized for use on the Panther Fusion platform. Finally, the assay comparison was limited to the Xpert and Panther Fusion at a single institution, and does not include the numerous other assays which vary in methodology and target selection. However, multiple studies from both the United States and Europe now show similar performance of the Xpert when compared to other SARS-CoV-2 assays (5-10). Understanding the performance of the Xpert in additional settings worldwide would help to determine the overall utility of the platform as the pandemic continues to progress.

In summary, this study showed that the Cepheid Xpert Xpress SARS-CoV-2 assay performed with high overall agreement compared to a reference standard. While high-throughput instruments are

critical for laboratories to provide sufficient testing capacity, instrumentation investement may be a limiting factor in certain settings. The Xpert provides laboratories rapid and accurate SARS-CoV-2 testing with comparable performance to high-throughput platforms and can be implemented in near-patient settings globally.

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References

1. U.S. Food and Drug Administration. Emergency use authorizations. 2020.

2. U.S. Food and Drug Administration. Panther fusion sars-cov-2 emergency use authorization. https://www.fda.gov/media/136156/download (Accessed May 27 2020.

3. U.S. Food and Drug Administration. Xpert® xpress sars-cov-2. https://www.fda.gov/media/136314/download (Accessed May 27 2020.

4. Landis JR, Koch GG. The measurement of observer agreement for categorical data. Biometrics 1977;33:159-74.

5. Lieberman JA, Pepper G, Naccache SN, Huang ML, Jerome KR, Greninger AL. Comparison of commercially available and laboratory developed assays for in vitro detection of sars-cov-2 in clinical laboratories. J Clin Microbiol 2020.

6. Zhen W, Smith E, Manji R, Schron D, Berry GJ. Clinical evaluation of three sample-to-answer platforms for the detection of sars-cov-2. J Clin Microbiol 2020.

7. Moran A, Beavis KG, Matushek SM, Ciaglia C, Francois N, Tesic V, Love N. The detection of sars-cov-2 using the cepheid xpert xpress sars-cov-2 and roche cobas sars-cov-2 assays. J Clin Microbiol 2020.

8. Smithgall MC, Scherberkova I, Whittier S, Green DA. Comparison of cepheid xpert xpress and abbott id now to roche cobas for the rapid detection of sars-cov-2. J Clin Virol 2020;128:104428.

9. Wolters F, van de Bovenkamp J, van den Bosch B, van den Brink S, Broeders M, Chung NH, et al. Multi-center evaluation of cepheid xpert(r) xpress sars-cov-2 point-of-care test during the sars-cov-2 pandemic. J Clin Virol 2020;128:104426.

10. Loeffelholz MJ, Alland D, Butler-Wu SM, Pandey U, Perno CF, Nava A, et al. Multicenter evaluation of the cepheid xpert xpress sars-cov-2 test. J Clin Microbiol 2020.

11. Harrington A, Cox B, Snowdon J, Bakst J, Ley E, Grajales P, et al. Comparison of abbott id now and abbott m2000 methods for the detection of sars-cov-2 from nasopharyngeal and nasal swabs from symptomatic patients. J Clin Microbiol 2020.

12. Hogan CA, Sahoo MK, Huang C, Garamani N, Stevens B, Zehnder J, Pinsky BA. Five-minute point-of-care testing for sars-cov-2: Not there yet. J Clin Virol 2020;128:104410.

13. Hogan CA, Garamani N, Lee AS, Tung JK, Sahoo MK, Huang C, et al. Comparison of the accula sars-cov-2 test with a laboratory-developed assay for detection of sars-cov-2 rna in clinical nasopharyngeal specimens. J Clin Microbiol 2020.

Table 1. Comparison of the Cepheid Xpert Xpress SARS-CoV-2 assay and the Panther Fusion SARS-CoV-2 assay

		Panther Fusion		
		Detected	**Not Detected**	**Total**
Cepheid Xpert	**Detected**	53	0	53
	Not Detected	1	50	51
	Total	54	50	104

Positive Percent Agreement	98.1%	(95% CI: 90.1 – 100)
Negative Percent Agreement	100%	(95% CI: 94.2 – 100)
Overall Agreement	99.0%	(95% CI: 94.8 – 100)
Cohen's Kappa Coefficient	0.98	(95% CI: 0.94 – 1.0)

SARS-CoV-2: severe acute respiratory syndrome coronavirus 2
CI: confidence interval

Figure 1. Frequency Distribution of Cycle Threshold Values for the Panther Fusion SARS-CoV-2 assay (ORF1ab) and the Cepheid Xpert Xpress SARS-CoV-2 assay targets; *E* gene and *N* gene (N2 region)