Validation of the Cepheid Xpert® Xpress SARS-CoV-2 using upper and lower respiratory tract specimens

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ABSTRACT
This study validated the performance of the reverse transcriptase-polymerase chain reaction (rRT-PCR) based Cepheid Xpert® Xpress SARS-CoV-2 assay against the TIB MOLBIOL E-gene/EAV, a standard laboratory rRT-PCR SARS-CoV-2 assay. Upper and lower respiratory tract samples (nasopharyngeal and nasal swabs, bronchoalveolar lavage, and tracheal aspirate) were obtained from patients suspected to have contracted COVID-19. Results from the Xpert® Xpress and standard rRT-PCR assays were compared for positive and negative agreement and analyzed for precision, reproducibility, 95% confidence intervals, and coefficients of variation. The Xpert® Xpress assay demonstrated 100% agreement with the standard lab rRT-PCR for both upper and lower respiratory tract samples. Both the Xpert® Xpress and lab rRT-CPR identified weakly positive (Ct values 35–39) sample replicates with 100% reproducibility and showed 100% precision in identifying triplicates of upper respiratory tract samples. The single-cartridge Xpert® Xpress system has a short turnaround time and can be employed to improve patient management and hospital bed allocation. Further verification of the system is required before implementation and consideration must be paid to its higher cost and impracticality for high-throughput use.

INTRODUCTION
The ongoing COVID-19 pandemic, caused by the severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2), has claimed 5,285,888 lives worldwide as of December 2021 [1]. Rapid and reliable testing remain crucial for managing outbreaks and reducing the spread of disease. As such, microbiology laboratories continue to seek ways of increasing testing speed and capacity while working to overcome obstacles posed by the supply chain. The widespread circulation of new variants of concern also presents a challenge as mutations to the SARS-CoV-2 virus may impact the reliability of some diagnostic tests, resulting in false negatives [2]. Multiple testing methods have been developed throughout the course of the pandemic including serological assays, antigen detection and nucleic acid amplification tests [3]. The current gold-standard for detection of SARS-CoV-2 virus is real time reverse transcriptase-polymerase chain reaction (rRT-PCR). This process can be fully automated and scaled up for high throughput testing and is noted for its high sensitivity and specificity [4]. Numerous rRT-PCR assays and systems are currently on the market, targeting different regions of the SARS-CoV-2 genome and offering various levels of speed, throughput, and convenience. Assays targeting multiple gene sequences can be more robust against mutation-associated false negative results compared to assays targeting only one sequence [2]. Here we assess the validity of the Cepheid Xpert® Xpress SARS-CoV-2 for testing both upper and lower respiratory tract samples from suspected
COVID-19 patients. Performance was compared to the TIB MOLBIOL E-gene/EAV assay using the Roche RNA Virus Master mix.

The Xpert® Xpress is a self-contained, single-cartridge rRT-PCR assay run on the automated GeneXpert® System that targets the bat virus derived SARS associated E gene and SARS-CoV-2-specific N2 region of the N gene. The Xpert® Xpress system is a random-access instrument, with a quick turnaround time making it a useful tool for rapid SARS-CoV-2 detection.

METHODS

Upper respiratory tract specimens

Nasopharyngeal and nasal swabs from suspected COVID-19 patients were analyzed using our laboratory’s standard real-time rRT-PCR assay. Patient samples that had been collected and tested within the past month and stored at −80 °C were included for this validation study. To assess the accuracy of the Xpert® Xpress assay, 14 positive samples which included four samples positive for variants of concern (N501Y and E484K positive) and 10 negative samples were randomly selected for testing. Each cartridge was inoculated with 300 μl of sample. The following media types were also included: MANTACC Miraclean (Cat. No. MBT-010), Roche Cobas (Cat. No. 06466281190), Deaou (Cat. No. T330-01) and Starplex Viral Transport Media (Cat. No. 22-046-420). Two positive samples with two different Ct values (15 and 30) and one negative sample, were each tested in triplicate on the two instruments to determine assay precision. Samples were run on different days/shifts. Two concentrations of a positive sample with a Ct value of 35–39 were also tested in replicates on both instruments. A total of 20 replicates were performed to assess reproducibility on both instruments.

Lower respiratory tract specimens

Bronchoalveolar lavage (BAL) and tracheal aspirate (TA) samples were collected from suspected COVID-19 patients and heat inactivated at 56 °C for 30 min before treatment with dithiothreitol at a ratio of 1:1. Negative samples spiked with 50 μl of known positive analyte in UTM were included. Following testing with our laboratory rRT-PCR, the accuracy of the Xpert® Xpress assay was determined by testing 20 positive (15 TA and 5 BAL) and 10 negative (5 TA, 5 BAL) samples. Two concentrations of a positive sample with a Ct of 35–39 were also tested in replicates on both instruments for a total of 30 replicates to assess reproducibility on both instruments.

Cepheid Xpert® Xpress SARS-CoV-2

The Cepheid Xpert® Xpress SARS-CoV-2 (XPRSARS-COV2-10) was performed on the automated GeneXpert® System following the manufacturer’s instructions.

TIB MOLBIOL E-gene/EAV assay

For the control samples, extraction was performed on the Hamilton Vantage using the Promega extraction kit. The TIB MOLBIOL E-gene/EAV assay (Cat No. 40-0776-96) with Roche RNA Virus Master mix (Cat No. 06754155001) was carried out following manufacturer instructions. rRT-PCR was performed on the on LightCycler 480 II instrument, while assay analysis was conducted using an in-house validated diagnostic algorithm.

Statistical analysis

Positive and negative agreement, precision, reproducibility, 95% confidence intervals (CI), and coefficients of variation (CoV) were calculated using Microsoft Excel (Table 1).

RESULTS

The Xpert® Xpress demonstrated 100% positive agreement (CI 76.84%–100.00%) and 100% negative agreement (CI 69.15%–100.00%) with our standard rRT-PCR in identifying the 14 positive and 10 negative upper respiratory tract samples (Table 2). Both assays accurately identified all 20 replicates of the weakly positive samples (Ct values 35–39), indicating 100% reproducibility (CoV 2.7%). Moderate, weakly positive, and negative samples tested in triplicate with each assay were also identified with 100% precision (CoV 0.9%). For lower respiratory tract specimens (BAL and TA), the Xpert® Xpress similarly demonstrated 100% positive agreement (CI 83.16%–100.00%) and 100% negative agreement (CI 69.15%–100.00%) with our laboratory’s rRT-PCR results, accurately identifying the 20 positive and 10 negative samples (Table 3). Both assays also showed 100% reproducibility (CoV 4.5%) from testing the 30 replicates of weakly positive samples.

Table 1. Acceptance criteria for analytical performance characteristics determined during validation of the Xpert® Xpress assay in comparison with our laboratory rRT-PCR.

| Sample Type | Parameter | Acceptance criteria |
|-------------|-----------|---------------------|
| Upper respiratory tract | Accuracy | >90% |
| | Precision | CoV <5% |
| | Reproducibility | 95% with CoV <5% |
| Lower respiratory tract | Accuracy | >95% |
| | Reproducibility | 95% with CoV <5% |

Table 2. Xpert® Xpress assay results on upper respiratory tract specimens compared to expected results from our laboratory rRT-PCR.

| Xpert® Xpress | Lab rRT-PCR |
|---------------|-------------|
| Pos | 14 | 0 |
| Neg | 0 | 10 |
Table 3. Xpert® Xpress assay results on lower respiratory tract specimens compared to expected results from our laboratory rRT-PCR

| Sample type | Xpert® Xpress | Lab rRT-PCR |
|-------------|---------------|-------------|
| TA          | Pos           | 15 0        |
|             | Neg           | 0 5         |
| BAL         | Pos           | 5 0         |
|             | Neg           | 0 5         |

Note: For two of the samples that tested positive, the E gene target was not detected but the N2 target was detected. One such sample was weakly positive with a Ct of 37.9 while the other returned a Ct of 26.3.

DISCUSSION

The Xpert® Xpress assay demonstrated very high agreement with our lab standard rRT-PCR in detecting SARS-CoV-2 in upper and lower respiratory tract samples. Testing of replicates also indicated 100% reproducibility of the assay results at weak Ct values (35–39). Previous studies have presented similar findings on the performance of the Xpert® Xpress assay with nasopharyngeal specimens [5, 6]. Here we demonstrate that the assay is also able to accurately detect SARS-CoV-2 in TA and BAL specimens. Generally, these samples are collected in a hospital setting from admitted patients who are in more urgent need of care. As such, the assay’s ability to rapidly produce diagnostic results make it particularly beneficial for bed management and highly time-sensitive cases such as transplant patients. We propose that the Xpert® Xpress can best be used to assist in decreasing test turnaround time in order to facilitate hospital bed utilization or patient movement. Furthermore, testing can be considered for critical care and transplant patient populations. It has been particularly useful during peak community transmission with patients being transferred between hospitals. This has enabled the effective management of existing bed capacity within the southwestern Ontario region.

Drawbacks of the single-cartridge system include the low throughput capacity and high cost per test, rendering it an impractical choice for large volume testing. These constraints made it difficult to scale up testing capacity in order to meet the large volume testing demand necessitated at the peaks of the COVID-19 pandemic. Our current standard diagnostic rRT-PCR throughput can be increased to 10,000 samples/day to meet these demands. Nevertheless, we have found that the Xpert® Xpress to be an accurate and reliable diagnostic tool that enables a fast, targeted testing approach when necessary for both upper and lower respiratory tract specimens.

As with all molecular tests, the Xpert® Xpress SARS-CoV-2 cannot rule out other causes of infections due to bacteria or other viruses. Positive results only indicate the presence of SARS-CoV-2 RNA and must be correlated with patient’s current medical history and presentation to determine relevance of the result. Clinical correlation is also required for negative specimens to rule out, among others, poor specimen collection technique, inadequate transport conditions, or very low concentration of the relevant RNA targets in the specimen.

Notably, the U.S. Food and Drug Administration has identified potential impacts of viral mutations on the performance of the Xpert® Xpress SARS-CoV-2 assay [2]. Mutations within the regions targeted by the assay could negatively affect primer and or probe binding, resulting in failure to detect the presence of the virus. Reports have noted that single point mutations in the N2 target region can reduce the sensitivity of the Xpert® Xpress test. However, since the assay also targets the E gene, this mutation would not produce a false negative result [2]. Out of the 14 positive upper respiratory tract specimens in our study, four were variants of concern (N501Y and E484K positive) and the Xpert® Xpress assay was able to accurately identify all four as positive.

Limitations of our study include the small sample size given the limited number of kits we had available at the time of validation. As well, no samples with known absolute copy numbers were included as reference in our assessment. Another limitation is the absence of clinical data for the samples tested as they were randomly selected and de-identified. However, considering that the evaluation included samples with low RNA target concentrations as indicated by a Ct value of more than 35, we do not anticipate results to change dramatically with a larger sample size.

In conclusion, Xpert® Xpress SARS-CoV-2 shows a very high agreement with our current lab-based rRT-PCR for COVID-19 virus detection. It can be used for both upper and lower respiratory tract specimens in hospitalized patients but still requires thorough methodical verification and validation prior to implementation, which this study cannot replace. The assay should be restricted to situations where rapid testing is required for better patient management or to help with bed allocation. A sample including a greater number and variety of mutations is needed to better ascertain their effect on test performance.

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**Data availability:** Deidentified participant data are available upon request.

**Patient and public involvement:** Patients and the public were not involved in this study.

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