ORIGINAL ARTICLE

Performance of Xpress Xpert SARS-Cov-2 Assay for Diagnosis of COVID-19 at a Tertiary Care Centre

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ABSTRACT

Introduction: The Expert Xpress SARS-Cov-2 assay is an automated real-time high-performance rapid PCR assay encompassing specimen processing, nucleic acid extraction, amplification and amplicon detection in a single cartridge. On March 20th 2020, it received an emergency authorization for use from the U.S. Food and Drug Administration for diagnosis of SARS-CoV-2.

Materials and Methods: This study was conducted in the virology section of Microbiology at Sher-i-Kashmir Institute of Medical Sciences from February to April 2023. A total of 73 nasopharyngeal samples that we received in our virology lab were subjected to RT-PCR and Xpert Xpress SARS-CoV-2 assay.

Results: Considering RT-PCR as the gold standard, the sensitivity of Xpert Xpress SARS-CoV-2 assay was 100%, specificity was 88.88%, positive predictive value was 96.49% and negative predictive value was 100%.

Conclusion: Based on the interpretation of our results, we find Xpert Xpress SARS-CoV-2 assay an excellent tool for diagnosis of COVID-19 especially in emergency cases and in situations where fewer samples need to be tested.

Key words: SARS-CoV-2, Covid-19, RT-PCR, Xpert Xpress SARS-CoV-2 assay.

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INTRODUCTION

The abominable pandemic that took the world by surprise was tackled by experts with brisk measures, be it in terms of diagnosis or prevention. COVID-19 was declared a global pandemic by WHO on 11th March 2020 and called off on 5th May 2023. Globally, as of 18th August 2024, there have been 776,007,137 confirmed cases of COVID-19, including 7,059,612 deaths. In India, 45 Million confirmed cases of COVID-19 with 534,000 deaths have been reported to WHO as of 18th August 2024 [1].

SARS-CoV-2 is an enveloped, non-segmented, positive sense, single-stranded RNA virus which belongs to the coronaviridae family. This virus is the seventh coronavirus which has been reported to infect humans[2]. SARS CoV-2 has a genome size of 29,903 bp. The viral genome contains six major (ORF1a, ORF1b, S, M, E and N) and many accessory ORFs (ORF 3a/b, ORF6, ORF7a/b, ORF8, ORF9b/c and ORF10). ORF1a and ORF1b account for two-thirds of the viral genome. ORFs encode 29 proteins that fall into two categories: nonstructural and structural proteins.

For diagnosis of SARS-CoV2, rRT-PCR (real-time reverse transcription-polymerase chain reaction) is one of the finest and most accurate laboratory methods. In PCR assays various molecular targets used in identification are: Nucleocapsid (N), Envelope (E), Spike glycoprotein (S), Helicase (hel), Hemagglutinin -esterase (HE), Open reading frames ORF 1a and ORF1b and RNA-dependent RNA polymerase (RdRp) which are used in different combinations by various manufacturers[3]. Although RT-PCR is a gold standard test for diagnosis of SARS CoV-2, the test

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needs an expert to perform it and the approximate turn around time is around 4-5 hrs.

With a rapid surge in cases around the globe, a rapid point-of-care test was considered to be a major weapon in containing the cases.

The Cepheid GeneXpert system was initially introduced in the market to boost tuberculosis diagnosis. On March 20th 2020, it received an emergency authorization for use from the U.S.Food and Drug Administration for diagnosis of SARS-CoV-2[4]. On May 9th2020, the Indian council of Medical Research also issued an advisory recommending its use for SARS-CoV-2 testing in India as well[5]. The Xpress Xpert SARS-Cov-2 assay is an automated real-time high-performance rapid PCR assay which encompasses specimen processing, nucleic acid extraction, amplification and amplicon detection in a single cartridge. It targets two genes of SARS CoV-2: Sarbeco specific E gene and SARS CoV-2 specific N2 gene and includes internal processing controls. The time of detection is around 50 minutes. The advantages over RT-PCR are that no specific set-up is required and doesn't require fine technical expertise[6]. The current study was done with the aim of checking the performance of Xpress Xpert SARS-Cov-2 assay for diagnosis of Covid-19 by comparing it with the gold standard RT-PCR test for SARS-CoV-2.

MATERIALS AND METHODS

This study was conducted in the virology section of Microbiology at Sher-i-Kashmir institute of Medical Sciences from February to April 2023. The study was approved by the institutional ethics board.

A total of 73 samples were included in this study. Among them, we included 54 samples retrospectively that were already confirmed SARS-CoV-2 positive by RT-PCR. In addition, we also compared 19 prospective samples of clinically suspected patients.

The nasopharyngeal samples that we received in our virology lab were subjected to RT-PCR and Xpert Xpress SARS-CoV-2 assay simultaneously.

Xpert Xpress SARS-CoV-2 assay (Cepheid, Sunnyvale, CA, US) was performed as per the manufacturer's instructions. Briefly, the cartridge was removed from the package. The specimen was mixed by rapidly inverting the specimen transport tube 5 times. The sample was transferred using the provided pipette, avoiding bubble formation in the cartridge. After filling in patient details and scanning the bar code, the cartridge was loaded and the test was started. The results were interpreted automatically by the GeneXpert System and interpreted as SARS CoV-2 positive, SARS CoV-2 presumptive positive, SARS CoV-2 negative and invalid depending on various combinations of genes detected (cut-off Ct as $\leq$ 40 cycles).

For RT-PCR, viral RNA was automatically extracted using The GeneMag Viral DNA/RNA Purification Kit prefilled (Genetix Asia Pvt.Ltd.).

Master mix for PCR was prepared using Meril One-step RT-PCR Kit (Meril Diagnostics, Pvt.ltd) which targets Orf 1ab gene, Nucleoprotein N gene of SARS-CoV-2. Real-time multiplex PCR was performed using Applied Biosystems 7500 Fast Dx real-time PCR instrument (Thermofischer Scientific Inc., Waltham, US). Results were interpreted according to the kit literature where the cut-off Ct was taken as $\leq$35 for all the genes.

STATISTICAL ANALYSIS

The data were entered into a Microsoft Excel sheet. Statistical analysis was performed using the open Epi software version 3.01, which is freely available online. Continuous variables, such as age and gender, were presented as mean or median, while categorical variables were interpreted as numbers and percentages.

RESULTS

Among the 73 samples that were included in this study 42(57.5%) were males and 31(42.6%) were females. The mean age of the patients was 45.67 yrs.

The 54 samples that were selected from the retrospective samples which were all positive by RT-PCR and were stored at -80°C were run on the Xpert Xpress SARS-CoV-2 assay system, all the samples tested positive by Xpert Xpress SARS-CoV-2 assay as well showing 100% concordance. Among the prospective samples, two cartridges were aborted by the Xpert Xpress SARS-CoV-2 assay due to some system error. Among the remaining 17, 1 was positive by RT-PCR and 3 by Xpert Xpress SARS-CoV-2 assay(Table 1). Considering RT-PCR as the gold standard, the sensitivity of Xpert Xpress SARS-CoV-2 assay was 100%, specificity was 88.88%, positive predictive value was 96.49% and negative predictive value was 100%(Table-2).

Table 1: Comparison of results of SARS-CoV-2 samples by both assays

	Covid-19 positive by RT-PCR	Covid-19 negative by RT-PCR	Total
Covid-19 positive by Xpert Xpress SARS-CoV-2 assay	55	2	57
Covid-19 negative by Xpert Xpress SARS-CoV-2 assay	0	14	14
Total	55	16	71

Table 2: Table showing performance characteristics of Xpert Xpress SARS-CoV-2 assay

Performance Characteristics	Percentage
Sensitivity	100%
Specificity	88.88%
Positive predictive value	96.49%
Negative predictive value	100%

DISCUSSION

The GeneXpert system has widespread availability even in the remotest areas of India due to being used under the National Tuberculosis Elimination Programme. It can be used at those places also where setting up a lab for PCR won't be feasible. The Xpert Xpress SARS-CoV-2 assay received emergency approval at a time when there was huge demand for a rapid point-of-care reliable test. This test combines an extraction that's cartridge-based and a real-time micro-PCR analyzer.

The Xpert Xpress SARS-CoV-2 assay has the advantage of running an individual sample in case of an emergency and has a shorter turnaround time.

In our study, we found a good correlation between RT-PCR and Xpert Xpress SARS-CoV-2 assay. There were only two discordant results and those two also had Ct values which are negative by RT-PCR standards and positive by Xpert Xpress SARS-CoV-2 assay. The reason for the two discordant results is the high Ct value of the samples (37.6,35and 39.6and36.2 respectively) which is positive by the cut-off value of Xpert Xpress SARS-CoV-2 assay but not by RT-PCR. The higher cut-off values for Xpert Xpress SARS-CoV-2 assay might pose a problem of picking up false positives. A study published in the Lancet by Rakotosamimanana N also suggested the potential false positive cases picked up by Xpert Xpress SARS-CoV-2 assay might just end up overburdening the health care system[7]. Therefore standardization of cut-off value in Xpert Xpress SARS-CoV-2 assay is required.

In our study, the sensitivity and negative predictive value both were 100% followed by positive predictive value of 96.49% and specificity of 88.88%. These results are comparable with the results of Rakotosamimanana N et al[7] and Singh et al[8] where in they found a sensitivity of 100% and 99% respectively and specificity of 80% and 88% respectively. This test is feasible when only a small number of samples are to be tested.

Based on the interpretation of our results, we find Xpert Xpress SARS-CoV-2 assay an excellent tool for diagnosis of COVID-19 especially in emergency cases and in situations where only a couple of samples need to be tested. Because of the rapid turnaround time of Xpert Xpress SARS-CoV-2 assay, it can be used as a point-of-care test in emergency rooms which will help in rapid diagnosis and management of patients in terms of isolation, antimicrobial stewardship and targeted treatment.

LIMITATION

The limitation of our study was a small sample size. To comment upon considering a change in cut-off values of Xpert Xpress SARS-CoV-2 assay we need to have a huge number of samples.

CONCLUSION

Based on the observations of our study the Xpert Xpress SARS-CoV-2 assay is an excellent tool for diagnosis of COVID-19 especially in times of emergency when some samples need to be tested on high priority and in areas where RT-PCR facility is not available.

Conflict of Interest: None

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Written Consent: Taken

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