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Comparison of Xpert Xpress SARS-CoV-2 assay and RT-PCR test in diagnosis of COVID-19

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Abstract:

Background*: In the context of SARS-CoV-2 pandemic, early and rapid case detection is of paramount importance to impact patient management. Routine real time RT-PCR test, the gold standard test for SARS-CoV-2 detection, is time consuming and requires skilled manpower. With number of molecular assays receiving emergency use authorization from US-FDA, Cepheid Xpert Xpress SARS-CoV-2 (Xpert) assay, an automated, rapid molecular point-of-care test, has received emergency use authorization for COVID-19 testing on 20th March 2020.*

Materials and Methods*: The present study was conducted in the molecular laboratory of a tertiary care hospital. A total of 102 nasopharyngeal samples, collected from clinically suspected COVID-19 cases, were tested by Xpert assay and real time RT-PCR test following manufacturer's instructions. The performance characteristics of the Xpert assay were calculated considering RT-PCR as the gold standard test. Sensitivity, specificity, predictive values, likelihood ratios and the agreement between the two tests were calculated using MedCalc® Statistical Software version 19.6.4.*

Results*: The overall sensitivity, specificity, positive predictive value, and negative predictive value of Xpert assay was 65.52%, 93.15%, 79.17% and 87.18%, respectively. Both the assays show a substantial agreement with each other at a cut-off Ct value of 35. Probably because of higher cycle threshold value and lower detection limit, Xpert assay detected a greater number of positive cases than RT-PCR test.*

Conclusion: *With a shorter turnaround time and minimal technical expertise, the Xpert assay is useful as a point-of-care test in acute-care settings where rapid and accurate diagnosis is of critical importance.*

Key Word*: COVID-19 diagnostics, SARS-CoV-2, Cepheid Xpert Xpress, RT-PCR, Point-of-care test.*

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I. Introduction

Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2), a novel coronavirus, emerged in Wuhan, China in December 2019 [1]. Since then, the disease has spread rapidly across the world causing more than 77 million confirmed cases and approximately 1 million deaths worldwide till date [2]. World Health Organization (WHO) announced COVID-19 outbreak as a pandemic on 11 March 2020 [3].

This pandemic has created an urgent need for rapid diagnostic testing which is one of the major pillars of public health preparedness for early case detection, timely management, and control of the disease [4]. Diagnosis of COVID-19 is based on a combination of clinical symptoms with or without radiological imaging and is confirmed by reverse transcriptase polymerase chain reaction (RT-PCR), the gold standard test for diagnosis of COVID-19 [5][6]. The RT-PCR test detects the virus by targeting the pan-sarbecovirus Envelope gene (E gene) in combination with polymerase gene (RdRp) or Nucleoprotein gene (N) or Spike protein (S) or Open Reading Frame gene (ORF) which are specific to SARS-CoV-2. Though it is the gold standard test for diagnosis of SARS-CoV-2 infection, its sensitivity and specificity are estimated to be approximately 70% and 95% respectively [7]. The process is also time consuming with a turnaround time of approximately 12-24 hours and requires skilled manpower. Therefore, it is essential to develop high quality rapid point-of-care diagnostic tests for early detection of SARS-CoV-2 and for rapid case management and contact tracing [5].

Cepheid GeneXpert Instrument Systems, which was introduced in December 2010 to upscale the capacity of tuberculosis diagnostic testing [8], received authorization for emergency use from U.S. Food and Drug Administration for SARS-CoV-2 testing on 20 March 2020 [9]. Indian Council of Medical Research (ICMR) also issued an advisory on 9th May 2020 recommending its use for COVID-19 testing in India [10]. The Xpert Xpress SARS-CoV-2 (Xpert) assay (Cepheid, Sunnyvale, CA, US) is an automated, rapid real-time PCR for qualitative detection of nucleic acid from SARS-CoV-2. This cartridge based nucleic acid amplification test, integrates specimen processing, nucleic acid extraction, RT-PCR amplification of SARS-CoV-2 RNA, and

amplicon detection in a single cartridge. It detects the virus in approximately 45-50 minutes and does not have any specific pre-requisites for its set-up and requires little technical training [11].

Therefore, the present study was carried out at a tertiary care hospital with the objective of assessing the performance of Xpert Xpress SARS-CoV-2 test in diagnosis of SARS-CoV-2 infection, considering RT-PCR test as the gold standard test.

II. Material And Methods

The present study was conducted in the molecular laboratory of department of microbiology at Byramjee Jeejeebhoy Government Medical College and Sassoon General Hospitals (BJGMC and SGH), Pune, Maharashtra, India.

A total of 2,611 nasopharyngeal samples were collected from SARS-CoV-2 suspected patients admitted in Sassoon hospital between 1st October to 31st October 2020. Of these 2,611 samples, 102 samples were randomly selected and were included in the study. The collected nasopharyngeal swabs were transported immediately to the molecular laboratory in HiViral™ Transport Medium (HiMedia Laboratories Pvt Ltd, Mumbai, India) at 2^{o} to 8^{o} C.

Ethical concerns: Present study was reviewed and approved by the Institutional Ethics Committee, BJGMC, Pune**.**

Methodology:

The collected nasopharyngeal samples were subjected to RT-PCR and Xpert Xpress SARS-CoV-2 (Cepheid, Sunnyvale, CA, US) assay. The technicians performing the RT-PCR test were blinded from Xpert Xpress SARS-CoV-2 assay results and vice-versa. The RT-PCR test was considered as the gold standard test for SARS-CoV-2 detection.

The Xpert Xpress SARS-CoV-2 assay was performed as per the manufacturer's instructions (Cepheid, Sunnyvale, CA, US). The assay targets two genes, the E gene (Sarbeco specific gene) and N2 gene (SARS-CoV-2 specific gene) for detection of SARS-CoV-2 virus. The results of Xpert assay were interpreted as per the algorithm recommended by the kit manufacturer i.e., a sample was considered positive for SARS-CoV-2 if it was positive for both E and N2 gene or if it was positive for N2 gene only. Similarly, it was considered negative for SARS-CoV-2 when both E and N2 genes were negative. A sample was considered presumptive positive for SARS-CoV-2 if only E gene was positive.

The viral RNA was extracted using MagMax™ Viral/Pathogen Nucleic Acid Isolation Kit (Thermofisher Scientific Inc., Waltham, US) following manufacturer's instructions. Extracted RNA was stored at -80^{o}C prior to testing.

Master mix for the PCR was prepared using Multiplex Single Tube real-time PCR kit, Version 3, for detection of 2019 novel coronavirus (2019-CoV) developed by ICMR-NIV, Pune, India following manufacturer's instructions.

Real-time multiplex PCR assay was performed using Applied Biosystems™ 7500 Fast Dx real-time PCR instrument (Thermofisher Scientific Inc., Waltham, US). The recommended thermal cycling parameters include Uracil-N-Glycosylase activation (UNG incubation) at 25^{o}C for 2 minutes, reverse transcription at 53^{o}C for 10 minutes, Polymerase activation at 95^{o}C for 2 minutes followed by 40 cycles of amplification at 95^{o}C for 15 seconds and 60^{o}C for 1 minute (data acquisition). The target genes used for detection of SARS-CoV-2 are Envelope (E gene) gene and Open Reading Frame - 1ab (ORF-1ab) gene. B-Actin gene was used as both extraction and amplification control. The amplification data was interpreted based on cut-off cycle threshold (Ct) values recommended by ICMR-NIV, Pune, India, i.e., samples with Ct value ≤ 35 were considered positive and those with >35 were considered negative for SARS-CoV-2 infection.

Statistical analysis: Data was analyzed using Microsoft Excel. Sensitivity, specificity, negative and positive predictive values, positive and negative likelihood ratios were calculated using MedCalc® Statistical Software version 19.6.4 (MedCalc Software Ltd, Ostend, Belgium). Cohen's Kappa co-efficient (κ) was used to measure the inter-test reliability and the agreement between the two tests.

III. Result

A total of 102 patients were included in the study from all age groups with a mean age of 34.2 years. Of these, 51 (50%) were males and 51 (50%) were females. Patients were categorized as per the ICMR guidelines (SRF V_11) for COVID-19 testing.

Correlation between Xpert Xpress assay and RT-PCR test

Overall, there were 47 (46.08%) samples which were positive for SARS-CoV-2 by either of the tests and 55 (53.92%) were negative. Xpert assay could detect 39/47 (82.98%) positive cases and RT-PCR test could detect 29/47 (61.70%) positive cases.

Out of the 47 positive samples, 21 (44.68%) were positive by both RT-PCR and Xpert assay, 18 (38.29%) were positive by Xpert assay only and 08 (17.02%) were positive by RT-PCR test only.

Out of the 39 samples which were positive by Xpert assay, 16 (41.03%) samples had a Ct value greater than 35 and 6 (15.38%) samples were positive for E gene only and hence were considered as presumptive positive by Xpert assay.

Considering 35 as the cut-off Ct value for both the assays, there were 34 (33.33%) samples positive for SARS-CoV-2 by either of the tests. Of these 34 samples, 19 (55.88%) were positive by both RT-PCR and Xpert Xpress SARS-CoV-2 assay. 24/34 (70.59%) samples were positive by Xpert Xpress SARS-CoV-2 assay and 29/34 (85.29%) were positive by RT-PCR test.

Correlation between Xpert Xpress SARS-CoV-2 assay and RT-PCR results considering two different cut-off Ct values for Xpert assay have been shown in **Table 1** and **Table 2**.

Table 1. Correlation between Xpert Xpress SARS-CoV-2 assay and RT-PCR test results with cut-off Ct value of 45 for Xpert assay and 35 for RT-PCR test.

	COVID-19 Positive by RT-PCR	**COVID-19 Negative by RT-PCR**	**Total**
COVID-19 Positive by GeneXpert	21	18	39
COVID-19 Negative by GeneXpert	08	55	63
Total	29	73	102

Table 2. Correlation between Xpert Xpress SARS-CoV-2 assay and RT-PCR test results with cut-off Ct value less than and equal to 35 for both the assays*.

	COVID-19 Positive by RT-PCR	**COVID-19 Negative by RT-PCR**	**Total**
COVID-19 Positive by GeneXpert	19	05	24
COVID-19 Negative by GeneXpert	10	68	78
Total	29	73	102

*Considering Ct value 35 as the cut-off Ct value for both the assays, 16 samples with Ct value greater than 35 by Xpert assay, were considered negative.

Sensitivity, Specificity, Positive Predictive Value (PPV), Negative Predictive Value (NPV), Positive Likelihood Ratio (PLR), Negative Likelihood Ratio (NLR), Cohen's Kappa co-efficient (κ)

The comparison between the sensitivity, specificity, PPV, NPV, PLR and NLR considering two different cut-off Ct values for Xpert assay is shown in **Table 3**. A moderate agreement was seen between Xpert assay and RT-PCR test with cut-off Ct value of 45 ($\kappa = 0.43$) for Xpert assay. A substantial agreement was seen between the two tests considering cut-off Ct value of 35 ($\kappa = 0.62$) for Xpert assay.

From here on, all discussions will be based on results obtained after considering 35 as the cut-off Ct value for both the assays.

Table 3. Comparison of performance characteristics of Xpert assay considering two different cut-off Ct values.

Performance characteristics	With Cut-off Ct value of 45 cycles for Xpert and 35 cycles for RT-PCR (95% CI)	With Cut-off Ct value less than or equal to 35 for both the assays (95% CI)
Sensitivity	72.41% (52.76% to 87.27%)	65.52% (45.67% to 82.06%)
Specificity	75.34% (63.86% to 84.68%)	93.15% (84.74% to 97.74%)
Positive Predictive Value (PPV)	53.85% (42.42% to 64.88%)	79.17% (61.03% to 90.21%)
Negative Predictive Value (NPV)	87.30% (78.98% to 92.64%)	87.18% (80.40% to 91.85%)
Positive Likelihood Ratio (PLR)	2.94 (1.85 to 4.65)	9.57 (3.94 to 23.21)
Negative Likelihood Ratio (NLR)	0.37 (0.20- 0.67)	0.37 (0.22- 0.61)
Cohen's Kappa Coefficient (κ)	0.43 (0.25- 0.61)	0.62 (0.45- 0.79)

IV. Discussion

The GeneXpert platform has a widespread availability at every district health care centre in India, as it is being widely used for diagnosis of tuberculosis under the RNTCP program [12]. Therefore, optimizing the use of this pre-existing platform for rapid and prompt diagnosis of COVID-19 in acute conditions would be lifesaving and should be considered in areas with difficult access to laboratories performing RT-PCR tests. As per the advisory issued by Indian Council of Medical Research (ICMR), in hospital settings, Xpert assay can be used as a choice of test for testing all symptomatic ILI cases, SARI patients, and all pregnant women in/near labor [13]. Therefore, in the present study, this new platform was evaluated against the conventional RT-PCR test.

In the present study, both the assays have a comparable performance at a cut-off Ct value of 35. But samples with Ct values greater than 35 and samples with no amplification for E gene but Ct values greater than 40 for N2 gene were considered positive by Xpert assay. Study by Niaina Rakotosamimanana et al. has similar findings and state that these might be potential false positive cases which may unnecessarily overburden the healthcare systems [14]. There were 16 (34%) such samples which had Ct value greater than 35 by Xpert of which only 3 (18.8%) were positive by RT-PCR test. These patients were mostly asymptomatic high-risk contacts of confirmed cases. This indicates that those could be probably low positive samples with few copies of viral RNA or with residual viral particles which were detected by Xpert assay due to higher cut-off Ct value. Also, there are studies which have shown a strong correlation between Ct value and sample infectivity. It is observed that patients with Ct values equal to or greater than 34 are less likely to transmit infection as no virus could be cultured in such cases [15][16]. Therefore, Ct values must be interpreted with caution. The question, whether higher Ct values i.e., Ct value greater than 35, have any impact on the clinical outcome is still a matter of debate and requires further study. Therefore, in the present study, 35 was considered as the cut-off Ct value for both the assays for statistical ease.

There were 13 samples which had discrepant results i.e., 5 and 8 samples were positive by Xpert and RT-PCR only, respectively. This finding can be attributed to multiple factors. First, multiple genetic variations in the viral genome at the primer/probe binding sites have been reported which result in mismatches and lower the sensitivity of target genes [17]. Even the American Society for Microbiology, in their COVID-19 International Summit held on 23 March 2020, has recommended that routine verification of mutations in primer/probe binding sites of viral genome should be done for optimal virus detection [18]. Secondly, both the systems use different genes to detect SARS-CoV-2 virus. The RT-PCR test used ORF-1ab gene which is the

most conserved gene. However, it presents with lowest sensitivity in comparison to other target genes. On the other hand, gene targeted by the Xpert assay, i.e., N gene, is a less conservative gene and is found to be more sensitive as a target [17]. Thirdly, the target gene may have failed to amplify due to low copy numbers of target sequence to primer.

Considering 35 as the cut-off Ct value, both the assays show a substantial agreement with each other. The sensitivity and specificity of Xpert assay is found to be 65.52% and 93.15%, respectively. On the contrary, various studies have shown an agreement of 100% and 99% between Xpert assay, in-house RT-PCR assays, and Roche Cobas 6800 assay, respectively [5][1][19][20]. However, a good negative predictive value (87.18%) suggests that samples negative by Xpert assay are true negatives and a positive likelihood ratio of 9.57 suggests that in those who are positive by Xpert assay, the probability of having the disease is likely to be increased by approximately 40-45% [21].

Despite the advantages of being a rapid and easy to perform test, the lower throughput of the Xpert assay compared to other high throughput assays limit its use during the pandemic. Also, the cost-per test is significantly higher, thus making it less affordable than conventional RT-PCR tests when large number of samples need to be tested [20]. However, the marginally high cost is justifiable when weighed against the number of lives that can be saved in acute care settings. Its use is also subjected to uninterrupted supply and continuous availability of the cartridges due to high global demand for testing. This can be addressed if we follow the same system of supply management as that practiced under the RNTCP program.

Limitation of this study includes a small sample size of SARS-CoV-2 specimens used for comparison between Xpert assay and RT-PCR test, as we had limited supply of cartridges which were used only for testing of critically ill patients within our institution. Secondly, in case of samples with discrepant results, correlation between the clinical condition of the patient and the test results could not be established due to loss to follow-up.

V. Conclusion

To summarize, the performance of this cartridge based diagnostic test, with a run-time of 50 minutes and requiring minimal technical expertise, is comparable to that of RT-PCR test when the standard cut-off Ct value of 35 is considered for both the assays. It is ideal as a point-of-care molecular test at specific settings. It should be best used in acute-care settings, where rapid triage decisions are needed to be made regarding patient discharge, isolation and initiation of potentially lifesaving treatment. It can also enable decentralized testing for SARS-CoV-2, thereby allowing laboratories to use both the assays at the same time as per the local testing strategies.

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