Original Article

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Comparison of Cepheid Xpert Xpress SARS-CoV-2 Assay with Standard RT-PCR Test for Detection of COVID-19 Infection: A Retrospective Cohort Study

Microbiology Section

NITIKA DHURIA[1], NITIN NAGPAL[2], VISHAL SHARMA[3], ARUN KUMAR[4]

ABSTRACT

Introduction: Real time Reverse Transcription-Polymerase Chain Reaction (RT-PCR) test, the gold standard test for Severe Acute Respiratory Syndrome-Coronavirus-2 (SARS-CoV-2) detection, is a tedious process and requires proficient workforce. Accurate and fast test results may permit more efficient use of protective and isolation resources and allow rapid therapeutic interventions.

Aim: To evaluate the analytical performance characteristics of the Cepheid Xpert Xpress SARS-CoV-2 test, a rapid, automated molecular test for SARS-CoV-2 with gold standard RT-PCR test.

Materials and Methods: This retrospective cohort study was conducted in Virus Research and Diagnostic Laboratory (VRDL) in Department of Microbiology at GGS Medical College, Faridkot, Punjab, India, from January to June 2021. A total of 100 nasopharyngeal samples, collected from clinically suspected Coronavirus Diseae-2019 (COVID-19) cases admitted at GGSMC during 1st January-30th June 2021 were tested both by Xpert assay and RT-PCR test simultaneously, taking RT-PCR as the gold standard test. The data was analysed by MedCalc® statistical software version 19.6.4., and sensitivity, specificity, predictive values, likelihood ratios and the agreement between the two tests were calculated.

Results: The mean age of the study participants was 46 years. Of these, 55 were males and 45 were females. The overall sample sensitivity and specificity of the Xpert assay were both 100% and there was perfect agreement across specimens, if authors, set a cut-off Cycle threshold value (Ct value) at 40 cycles for Xpert. Of 100 samples, 32 were positive for SARS-CoV-2 by either of the tests and 68 were negative. Xpert assay could detect 100% positive cases and RT-PCR test could detect 84.37% positive cases. Out of the 32 samples which were positive by Xpert assay, 5 (15.62%) samples had a Ct value greater than 40.

Conclusion: The Xpert assay found to be useful as a point-of-care test in acute scenario, where rapid and authentic diagnosis is essential, but do not have expertise and infrastructure to perform RT-PCR.

Keywords: Coronavirus disease-2019, Point-of-care, Reverse transcriptase-polymerase chain reaction, Severe acute respiratory syndrome-coronavirus-2

INTRODUCTION

A cluster of cases of pneumonia of unknown cause emerged in Wuhan in December 2019. The International Committee of Taxonomy of Viruses (ICTV) identified novel beta coronavirus and named as Severe Acute Respiratory Syndrome-Coronavirus-2 (SARS-CoV-2) and the disease been named COVID-19, by World Health Organisation (WHO). The WHO declared it a public health emergency of international concern on January 30, 2020 and further declared it as a global pandemic on March 11, 2020 [1]. Latest updates by WHO, suggest a total of 45,84,79,635 confirmed cases of COVID-19, including 60,47,653 deaths [2].

This pandemic has created an urgent need for rapid diagnostic tests in controlling the outbreak, and includes serological and molecular assays [3]. The preferred testing method for SARS-CoV-2 virus is the real-time RT-PCR test targeting different genes- N, E, S, Ribonucleic Acid (RNA) dependent RNA polymerase (RdRp) and Observer Research Foundation (ORF) with different recommendations of which target to use [4-7]. Despite being the gold standard test for diagnosis of SARS-CoV-2 infection, the process involves a turnaround time of approximately 12-24 hours and requires trained manpower. Thereby, a high quality rapid point-of-care diagnostic test is needed for early detection of SARS-CoV-2 [8].

Among these, Cepheid Xpert Xpress SARS-CoV-2 (Xpert) assay one of those that received authorisation for emergency use from the US Food and Drug Administration and Indian Council of Medical Research (ICMR) in year 2020 [9,10]. The Xpert assay is an automated in-vitro diagnostic test for qualitative detection of nucleic acid from SARS-CoV-2. The targets are E gene and N2 gene in Xpert with a limit of detection of 250 copies/mL [11]. The study was carried out at a tertiary care hospital with gain in bringing down turnaround time, for diagnosis of SARS-CoV-2 infection, with the objective of assessing the performance of Xpert assay, considering RT-PCR test as the gold standard test.

MATERIALS AND METHODS

The retrospective cohort study was conducted at VRDL in Department of Microbiology at GGSMCH, Faridkot, Punjab, India, from 1st January to 30th June 2021. Analysis of data was done in February 2022. Institutional Ethical Committee (IEC) approval was not taken as study was conducted in a reference laboratory, all samples tested with Xpert assay were also subjected to RT-PCR considering it as gold standard, but for study purpose only 100 samples were taken, for whom Ct values of all genes were available and data was collected retrospectively from year 2021.

Inclusion criteria: Patients admitted in GGSMCH with acute respiratory infection (influenza-like illness) with fever of ≥38°C, cough, and onset within the last 10 days, were included in the study.

Exclusion criteria: Samples not sent in proper storage conditions or leaked, were excluded from the study.

Study Procedure

A total of 617 nasopharyngeal samples were collected for testing with Xpert assay between given time period. The collected nasopharyngeal swabs were transported immediately to the molecular laboratory in HiViralTM Transport Medium (HiMedia Laboratories Pvt. Ltd., Mumbai, India) at 2° to 8°C. Of these 617 samples, 100 non random convenient samples were included in the study. Though, all samples tested with Xpert assay were also subjected to RT-PCR considering it as gold standard, but for study purpose only 100 samples were taken, for whom Ct values for all genes were available and data was collected retrospectively from year 2021.

GeneXpert assay: The Xpert Xpress SARS-CoV-2 test is an automated in-vitro diagnostic test for qualitative detection of nucleic acid from SARS-CoV-2 on GeneXpert instrument system which performs automated specimen processing, Ribonucleic Acid (RNA) extraction, RT-PCR of SARS-CoV-2 RNA, and amplicon detection in a single run. Results were analysed using E gene, N2 gene and Sample Processing Control (SPC) and the detection of both genes or N2 gene alone is considered positive, and the detection of E gene alone is considered presumptive positive (Ct value <45) [9].

RT-PCR test: Using MagMaxTM viral/pathogen nucleic acid isolation kit, viral RNA from nasopharyngeal sample was extracted following manufacturer's instructions. RT-PCR assay was performed using applied biosystems TM 7500 fast Dx real-time PCR instrument. Extracted RNA samples were amplified using Genes2Me Viral Detect-|| Multiplex RT-PCR kit for COVID-19 targeting Envelope (E), Nucleocapsid (N) and RdRp genes. RNAse P gene was used as an amplification control. The assay was run for 40 cycles and amplification data was interpreted based on cut-off cycle threshold (Ct) values i.e., samples with Ct value ≤37 were considered positive and those with >37 negative for SARS-CoV-2 infection. The percent positivity for SARS-CoV-2 with Xpert assay was 20.74% [Table/Fig-1].

S. No.	Result	Number	Total	Percentage (%)
1.	Positive	128	617	20.74
2.	Negative	489	617	79.25

[Table/Fig-1]: Percent positivity of SARS-CoV-2.

STATISTICAL ANALYSIS

Data was analysed using Microsoft excel. Using MedCalc® Statistical Software version 19.6.4 (MedCalc Software Ltd., Ostend, Belgium), sensitivity, specificity, Negative Predictive Values (NPV) and Positive Predictive Values (PPV), Positive Likelihood Ratio (PLR) and Negative Likelihood Ratios (NLR) were calculated. Agreement between the two tests was assessed by Cohen's Kappa coefficient (κ). The association was explored through correlation coefficients and scatter plots using IBM ® Statistical Package for the Social Sciences (SPSS) version 20.0.

RESULTS

A total of 100 non random convenient samples were included in the study from all age groups with a mean age of 46 years. Of these, 55 were males and 45 were females. Total 100 non random convenient samples were tested with both Xpert assay and RT-PCR by taking RT-PCR as a gold standard. Comparison between the sensitivity, specificity, PPV, NPV, PLR and NLR among Xpert assay and RT-PCR at different cut-off Ct values is shown in [Table/Fig-2]. Of 100 samples, 32 were positive for SARS-CoV-2 by either of the tests and 68 were negative. Xpert assay could detect 100% positive cases and RT-PCR test could detect 84.37% positive cases. Out of the 32 samples which were positive by Xpert assay, 5 (15.62%) samples had a Ct value greater than 40.

Considering 40 as the cut-off Ct value for both the assays, there were 27 samples positive for SARS-CoV-2 by either of the tests. A perfect agreement was seen between the two tests considering cut-off Ct value of 40 (κ=1) for Xpert assay [Table/Fig-3,4].

Performance characteristics	With cut-off Ct value of 45 cycles for Xpert (95% CI)	With cut-off Ct value of 40 cycles for Xpert (95% CI)
True positive	27	27
True negative	68	73
False positive	5	0
False negative	0	0
Sensitivity	100% (87.23-100%)	100% (87.23-100%)
Specificity	93.15% (84.74-97.74%)	100% (95.07-100%)
Positive Predictive value (PPV)	84.37% (69.86-92.64%)	100% (0)
Negative Predictive value (NPV)	100%	100%
Positive Likelihood Ratio (PLR)	14.60 (6.27-34.02)	-
Negative Likelihood Ratio (NLR)	0.00(-)	0.00(-)

[Table/Fig-2]: Comparison of diagnostic test performance measurement and statistical analysis of the Xpert assay with RT-PCR for the detection of SARS-CoV-2 RNA at different cut-off Ct values.

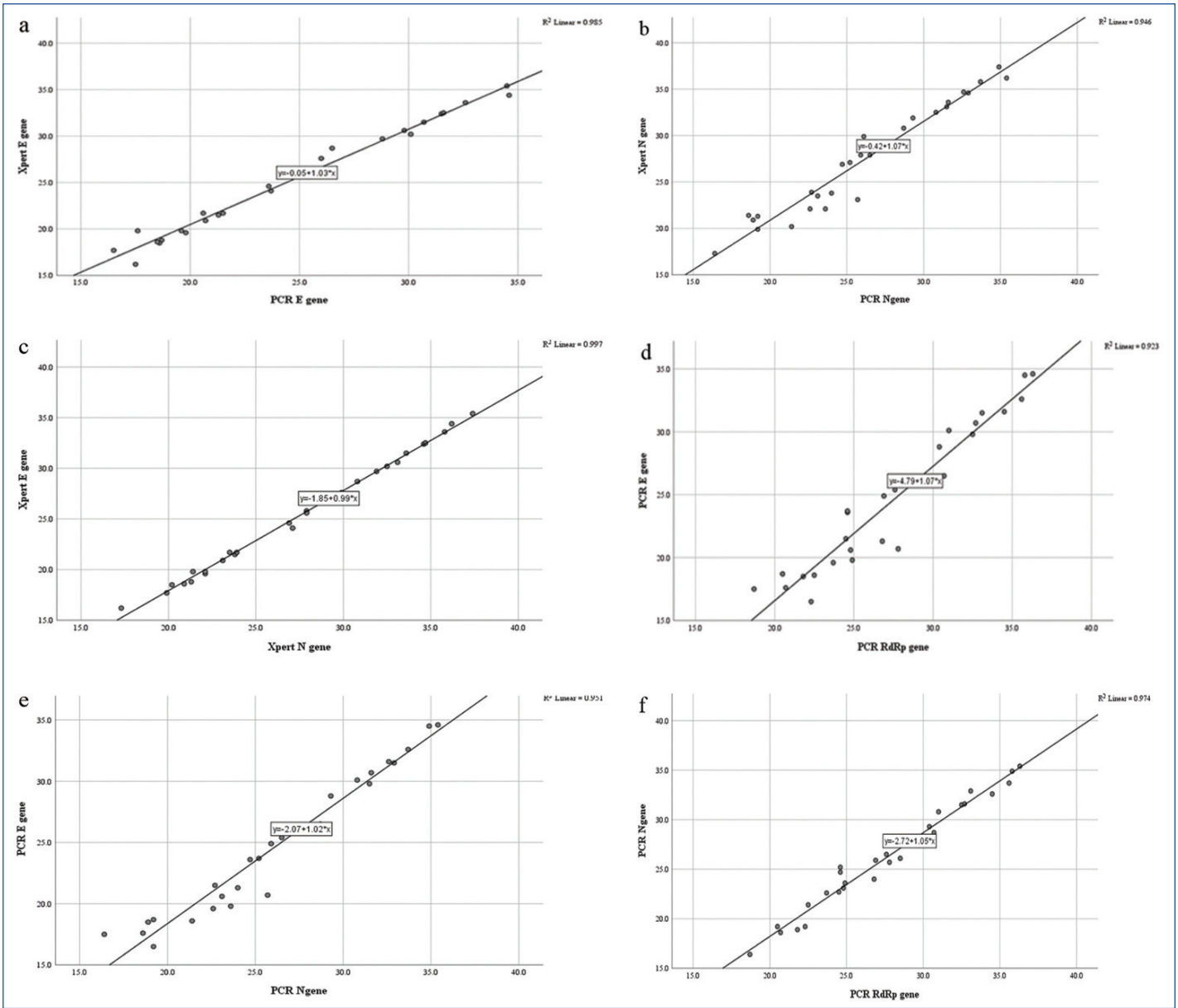
S. No.	Xpert E gene	Xpert N gene	PCR E gene	PCR N gene	PCR RdRp gene
1	17.7	19.9	16.5	19.2	22.3
2	21.5	23.8	21.3	24.0	26.8
3	29.7	31.9	28.8	29.3	30.4
4	18.8	21.3	18.7	19.2	20.5
5	16.2	17.3	17.5	16.4	18.7
6	19.6	22.1	19.8	23.6	24.9
7	21.7	23.5	20.6	23.1	24.8
8	18.5	20.2	18.6	21.4	22.5
9	32.4	34.6	31.5	32.9	33.1
10	30.6	33.1	29.8	31.5	32.5
11	19.8	22.1	19.6	22.6	23.7
12	20.9	23.1	20.7	25.7	27.8
13	24.6	26.9	23.6	24.7	24.6
14	28.7	30.8	26.5	28.7	30.7
15	31.5	33.6	30.7	31.6	32.7
16	33.6	35.8	32.6	33.7	35.6
17	25.8	27.9	24.9	25.9	26.9
18	32.5	34.7	31.6	32.6	34.5
19	24.1	27.1	23.7	25.2	24.6
20	34.4	36.2	34.6	35.4	35.3
21	27.6	29.9	26	26.1	28.5
22	35.4	37.4	34.5	34.9	35.8
23	18.6	20.9	18.5	18.9	21.8
24	21.7	23.9	21.5	22.7	24.5
25	25.6	27.9	25.4	26.5	27.6
26	19.8	21.4	17.6	18.6	20.7
27	30.2	32.5	30.1	30.8	31.0

[Table/Fig-3]: Positive samples Ct value for two different molecular assays by the target gene of SARS-CoV-2.

The association of Ct values of genes of Xpert assay and GENES2 ME Viral Detect-|| Multiplex RT-PCR Kit (27 were positive by both assays) were explored using Pearson's correlation coefficient which depicted a statistically strong significant association between Ct value among different genes of both assay and were as follows:

Xpert E gene Ct vs Genes2Me Viral Detect-|| Multiplex Real Time PCR kit E gene Ct, r=0.992, p<0.001,

Xpert N gene Ct vs Genes2Me N gene Ct, r=0.0.973, p<0.001,



[Table/Fig-4]: Correlation of Ct values between different assays and targeted SARS-CoV-2 genes for the positive samples (n=27). a) Simple scatter of Xpert E gene by PCR E gene. b) Simple scatter of Xpert N gene by PCR N gene. c) Simple scatter of Xpert E gene by Xpert N gene. d) Simple scatter of PCR E gene by PCR RdRp gene. e) Simple scatter of PCR E gene by PCR N gene. f) Simple scatter of PCR N gene by PCR RdRp gene.

Xpert E gene Ct vs Xpert N gene Ct, r=0.0.998, p<0.001,

Genes2Me E gene Ct vs Genes2Me N gene Ct gene Ct, r=0.0.975, p<0.001,

Genes2Me E gene Ct vs Genes2Me RdRp gene Ct, r=0.0.961, p<0.001,

Genes2Me N gene Ct vs Genes2Me RdRp gene Ct, r=0.987, p<0.001

DISCUSSION

Early diagnosis of COVID-19 is often deterimental in not only decision making for quarantine/isolation but also can help in early initiation of therapy in acute severe disease. RT-PCR is recognised as benchmark for COVID-19 testing, but this test requires well equipped laboratory facilities, highly skilled technologists and multiple reagents. Emergency approval was given both by WHO and United States Food and Drug Administration (US FDA) for use of Xpert assay platform for COVID-19 testing which is a closed nature platform, requires minimum sample handling, pose minimum biosafety hazard and has less turnaround time. This has been dependably used for diagnosis of Tuberculosis (TB) under Revised National TB Control Programme (RNTCP) and the facilities were already available across India [12].

As per manufacturer's recommendation in Xpert assay, sample with no amplification for E gene but Ct value upto 45 for N2 gene is considered positive. This suggests that automatic interpretation of the results by Xpert assay software may lead to high number of false positives thus affecting its specificity, as in the present study we ended up getting 32 positives by Xpert assay and 27 positive by RT-PCR. Due to low copy numbers of target sequence to primer, the gene failed to amplify in RT-PCR test, resulting in five discordant samples.

But, when authors took a Ct value of 40 as cut-off the results became comparable with RT-PCR and hence, improved specificity by decreasing the false positives. This was further supported by statistical analysis and both the assays showed perfect agreement at a cut-off value of 40. Similar observations were purported by Rakotosamimanana N et al., in their study, in which of 40 nasopharyngeal specimens that were previously confirmed as positive (n=20) or negative (n=20) using Da An Gene RT-PCR test were tested on the GeneXpert platform using the Xpert Xpress SARS-CoV-2 assay and found sensitivity and specificity of the Xpert Xpress SARS-CoV-2 assay to be 100% (20 of 20) and 80% (16 of 20), respectively, due to different limits-of-detection of the two assays but positive specimens showed similar Ct values individually, which favours similar analytical sensitivity. By using an arbitrary cut-off at 40 cycles, improves the specificity and does not affect the sensitivity of the test or increase the risk of getting a false negative result [13]. However, different authors from various geographical

regions have reported agreement at different Ct value. Das R et al., suggested a substantial agreement at a cut-off Ct value of 35 with sensitivity and specificity of Xpert assay to be 65.52% and 93.15%, respectively [14].

Various authors suggested a good agreement among different assays even at a Ct value of 45. The overall sample sensitivity and specificity were both 100% between Xpert assay and Roche Cobas 6800 assay and observed very high coefficients of determination of the different viral gene targets [15]. The test performance of the GeneXpert assay compared to the Liferiver RT-PCR showed that, sensitivity and specificity were both 100% [16]. Singh K et al., also considered cut-off value more than 40 as insignificant [17]. A Ct value more than 40 in positive samples may carry few copies of viral RNA which may be insignificant in disease transmission and overall reduce the patient burden in already exhausted healthcare facilities during the peak of COVID-19 second wave. Moreover, the extended turnaround time associated with reference laboratory testing can be avoided as this technology has utilitarian in acute care hospitals in high-prevalence settings, where testing can be done on demand and rapid triage decisions can be made regarding patient disposition and isolation and the targeted use of personal protective equipment for healthcare workers and potentially lifesaving treatment.

Limitation(s)

As different studies suggest different cut-off for Ct values, a larger sample size with prospective double blinding is desirable to establish a cut-off value of 40 as standard. Also, comparison of viral load with Ct value was not done. Another limitation was thought to be that samples were processed, when second deadly wave was at their peak and microbiology staff was overburdened with handling, processing and reporting of COVID-19 samples and after requiescence for some time, sample load again was on rise impending third wave, leads to delay in compilation of data.

CONCLUSION(S)

Despite the advantages of being a rapid and easy to perform test, the available system can only process limited samples in a day. Also, the cost per test is relatively higher, hence, the authors recommend its routine use in patients presenting with acute severe illness, where it can be detrimental in early initiation of therapy and thus, can be helpful in reducing morbidity and mortality. In the future, further advanced Xpert assay testing system, with a high throughput, can further widen its application.

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PARTICULARS OF CONTRIBUTORS:
1. Assistant Professor, Department of Microbiology, GGSMCH, Faridkot, Punjab, India.
2. Professor, Department of General Surgery, GGSMCH, Faridkot, Punjab, India.
3. Associate Professor, Department of Microbiology, GGSMCH, Faridkot, Punjab, India.
4. Research Scientist, Department of Microbiology, GGSMCH, Faridkot, Punjab, India.

NAME, ADDRESS, E-MAIL ID OF THE CORRESPONDING AUTHOR:
Dr. Nitika Dhuria,
Assistant Professor, Department of Microbiology, GGSMCH, Faridkot, Punjab, India.
E-mail: drnitikadhuria@gmail.com

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