Section: Microbiology

Original Research Article

UNDERSTANDING THE SINGLE GENE POSITIVE RESULTS OF GENEXPERT FOR SARS-COV-2: IN COMPARISON WITH RT-PCR TESTING

Usha Krishnan K[1], Madhumathy A[2], Therese Mary Dhason[3], Suganthi M[4], Thyagarajan Ravinder[5], Dhamayanthi[6]

[1]Professor, Department of Microbiology, Government Madras Medical College, Chennai, TamilNadu, India.
[2]Assistant Professor, Department of Microbiology, Government Kilpauk Medical College, Chennai, TamilNadu, India.
[3]Associate Professor, Department of Microbiology, Government Kilpauk Medical College, Chennai, TamilNadu, India.
[4]Associate Professor, Department of Microbiology, Government Kilpauk Medical College, Chennai, TamilNadu, India.
[5]Professor & Head, Department of Microbiology, Government Kilpauk Medical College, Chennai, TamilNadu, India.
[6]Tutor, Department of Microbiology, Government Kilpauk Medical College, Chennai, Tamil Nadu, India.

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Corresponding Author:
Dr. Madhumathy A,
Email: madhugstn@gmail.com.

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Abstract

Background: The outbreak of the pandemic of COVID-19 caused by SARS-CoV-2, had a significant impact on the clinical microbiology laboratories. As the crux of control of pandemic was dependent on test, track and trace strategy, rapid and accurate detection of SARS-CoV-2 was crucial. Technically conventional RT-PCR was time consuming, requiring biosafety level 2 laboratory and technical expertise. To help in rapid triaging, GeneXpert a cartridge-based PCR was introduced by the Govt. of India. **Aim:** To compare by parallel testing results of single gene positive samples of GeneXpert assay with the RT-PCR assay. **Material and Methods:** This cross-sectional study was conducted from August 2020 to October 2020 at Government Kilpauk Medical College Hospital, Chennai. Patients with signs and symptoms of SARS-CoV-2 and asymptomatic individuals, whose samples tested positive for single gene (E or N2) by GeneXpert assay were tested by RT-PCR. The continuous variables were analysed as mean and median. The categorical variables were expressed as percentage. **Results:** Of the 1686 samples tested by GeneXpert, 59 single gene positive samples were subsequently tested with RT-PCR. It was observed that 13.6% (8/59) of single gene positive samples were found to be positive by RT-PCR testing. Of the presumptive positive samples with only E gene positive, 0.39% (3/13) of samples were found to be positive by RT-PCR testing. Of the N2 gene target positive samples, 10.8% (5/46) of samples were found to be positive by RT-PCR testing. Visual interpretation of the cycles showed atypical curves among single gene positive samples of GeneXpert assay. **Conclusion:** On the backdrop of a pandemic where rapid triage decisions needed to be taken, GeneXpert, an automated, point of care, run on demand testing, was highly valuable in providing results in 40 minutes. However, interpretation of the single gene positive reports of GeneXpert should not be done only based on interpretive software as these instruments were occasionally overcalling background signals as a positive result.

INTRODUCTION

The outbreak of the current pandemic of COVID-19 caused by Severe Acute Respiratory Syndrome Coronavirus - 2 (SARS-CoV-2), was having a significant impact on healthcare, especially the clinical microbiology laboratories all around the world. As the crux of control of pandemic was dependent on test, track and trace strategy, rapid and accurate detection of SARS-CoV-2 was crucial.

Clinical diagnosis of COVID-19 relied on a combination of chest CT and RT–PCR results. Immunodiagnostic tests like antigen testing were linked to false positive results due to detection of antigens shared among different CoV species, antibody testing had interference due to the

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autoantibodies associated with autoimmune disorders. In addition, immunodiagnostic tests usually turned positive 7-11 days after exposure, thereby making them less reliable in acute infections.[1] Therefore, nucleic acid amplification tests (NAATs) were considered as the gold standard.[2] Technically real-time Reverse Transcription-Polymerase Chain Reaction (RT-PCR) was time consuming, required batch testing, biosafety level 2 laboratory and a high degree of technical expertise. The technique involved sample RNA extraction and amplification taking approximately 6 hours and a turnaround time of 24 hours.[3] However, other nucleic acid amplification-based tests, like Gene Xpert and True NAT had a shorter timeline than usual. These COVID-19 testing methods were validated, implemented and emergency approved under FDA Emergency Use Authorization.[4]

Providing fast results were of critical importance so as to decide upon COVID-19 status of patients to be followed upon by isolation, quarantine and contact tracing. Based on the COVID-19 status, either patients were shifted to covid centres for isolation and further treatment or they were transferred to other non-covid wards of our hospital for speciality care.

To overcome the demanding situation of rapid triaging for decision making, our center had been provided with GeneXpert equipment, a molecular point of care testing which was already approved for the diagnosis of M. tuberculosis, and the Cepheid Xpert Xpress SARS-CoV-2 assay, a rapid diagnostic NAAT assay (Cepheid, Sunnyvale, CA).[5] This was an automated in vitro diagnostic test for the qualitative detection of nucleic acids targets E and N2 genes of SARS-CoV-2 that could be performed on demand and provide test results in <1 h.[5] As per the GeneXpert, samples with both E and N2 were interpreted as 'positive', the only E gene positive samples were reported as 'presumptive positive' and the N2 gene positives were interpreted as 'positive'. The manufacturer did not recommend repeat testing for single-gene positive results.

This study was planned with the aim of understanding the single gene positive test performance characteristics of the GeneXpert assay. The objective was to compare by parallel testing the single gene positive samples with the gold standard RT-PCR assays approved by ICMR.

MATERIAL AND METHODS

This cross-sectional study was conducted from August to October 2020 on patients attending COVID-19 OP at Government Kilpauk Medical College Hospital, Chennai. A total of 59 single gene positive samples (E or N2) were included for the parallel testing. Institutional ethics committee approval was obtained [Protocol ID number 463: A/2021].

Inclusion Criteria

Patients clinically presenting with signs and symptoms suggestive of SARS-CoV-2 and asymptomatic individuals subjected for universal screening of SARS-CoV-2, whose samples tested positive for single gene (E or N2) by GeneXpert assay were included in the analysis.

Exclusion Criteria

Samples with both the gene (E and N2) positives were excluded.

Sample collection

Nasopharyngeal swab and oropharyngeal swabs were both collected by well-trained laboratory technicians following adequate infection control measures, and biosafety precautions.[6]

The samples were collected in 3ml Viral Transport Medium (VTM) and were transported to the laboratory maintaining the proper cold chain.[7] The samples were subjected to GeneXpert testing.[6] The single gene positive samples were further tested by RT PCR on the same day to avoid freeze thaw variability.

GeneXpert

The Xpert Xpress SARS-CoV-2 (Xpert) test was a rapid, real-time RT-PCR test intended for the qualitative detection of nucleic acid from the SARS-CoV-2 in upper respiratory specimens (i.e., nasopharyngeal, oropharyngeal, nasal, or mid-turbinate swab or nasal wash/ aspirate). This system automated and integrated sample preparation, nucleic acid extraction, amplification, and detection of the target sequences using single-use disposable cartridges that hold the RT-PCR reagents. The cartridge also contained a Sample Processing Control (SPC) and a Probe Check Control (PCC) as control for adequate processing of sample.[5]

The assay targeted the N2 region of the nucleoprotein (N) gene for specific SARS-CoV-2 detection and a conserved region of the structural protein envelope (E) gene for pan-sarbecovirus detection.[8]

Specimen was briefly mixed by rapidly inverting the collection tubes 5 times. 300 µL of sample was transferred to the sample chamber of the Xpert Xpress SARS-CoV-2 cartridge using the transfer pipette provided.[9] After the run time the result was interpreted by the software and displayed in the system monitor.

The detection of N2 and E or only N2 meant positive for SARS-CoV-2. The detection of only E gave a presumptive positive result and presence of only SPC implied a negative test. The failure to detect all markers indicated an invalid test result.[9]

Automated RNA extraction

The HELINI MagPure Viral RNA purification Kit was used for automated purification of viral RNA from the nasopharyngeal and oropharyngeal swabs using HELINI MagPure Instrument. This kit used magnetic particle technology for nucleic acid purification. On completion of the purification, 80µl of elute (nucleic acid) from the elution buffer was transferred into a sterile, fresh 1.5ml centrifuge tube.[10]

STANDARD M nCoV Real-Time Detection kit

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The STANDARD M nCoV Real-Time Detection kit (SD Biosensor Inc.) was a RT-PCR assay for the qualitative detection of SARS-CoV-2 nucleic acids in nasopharyngeal and oropharyngeal swabs. This kit was based on TaqMan probe real-time fluorescent PCR technology.

During the PCR reaction, the fluorescence signal were detected by the instrument: FAM channel qualitatively detected the new coronavirus (2019-nCoV) ORF1ab (RdRp) gene, HEX channel qualitatively detected the coronavirus E gene, and CY5 channel detected internal reference. The kit used dUTP and UNG enzymes to prevent contamination of amplification products

Interpretation of Results

Interpretation of the clinical specimen test results was performed after the positive and negative controls were examined and determined to be valid. If the controls were not valid, the patient results were not interpreted. The cycle threshold (CT) value of the test results was analysed based on the cut-off provided for each fluorescent channel as per the manufacturer's guidelines.[11]

Statistical Analysis

The continuous variables were analysed as mean and median. The categorical variables were expressed as percentage.

RESULTS

During the three-months study period the total number of samples collected and subjected to GeneXpert was 1686. Out of the 1686 samples, 59 samples which were single gene positive were subsequently tested with RT-PCR and the results were compared. RT-PCR was taken as the gold standard.

The performance of GeneXpert in the detection of SARS-CoV-2 is shown in. [Table 1]

Out of the 1686 samples tested, 59 (3.4%) of the samples were single gene positive. Of which 46 (2.72%) were N2 gene positive and 13 (0.7%) were E gene positive.

Comparison of GeneXpert single gene positive results with RT-PCR results is illustrated in. [Table 2]

Out of the 59 samples tested, 8 (13.5%) of the samples were positive by RT-PCR and 51 (86.4%) were negative by RT-PCR.

Comparison of GeneXpert with E-negative, N2-positive reports with the RT-PCR positive results are discussed in. [Table 3]

Median Ct value of N2 single gene positive samples by GeneXpert was 42.2.

Comparison of GeneXpert presumptive positive results with RT-PCR is depicted in. [Table 4]

Median Ct value of the GeneXpert presumptive positive samples was 37.6

Atypical reaction curves (a, b, c) along with a typical sigmoid curve (d) for the N2 gene positive samples have been depicted in. [Figure 5]

The Figure shows the N2 reaction curve as generated by GeneXpert. Legends (red curve) a), b), c) represents N2 positive curves from samples which tested negative by RT-PCR. Legend (red curve) d) represents N2 curve from sample which tested positive by RT-PCR. (Green curve represents SPC.)

The atypical reaction curves and typical reaction curves of presumptive positive samples (E gene positive) is depicted in. [Figure 6]

The figure shows the E reaction curve as generated by GeneXpert. Legends (blue curve) a), b), c) represents E curves from samples which tested negative by RT-PCR. Legend (blue curve) d) represents curve from sample which tested positive by RT-PCR. (Green curve - SPC)

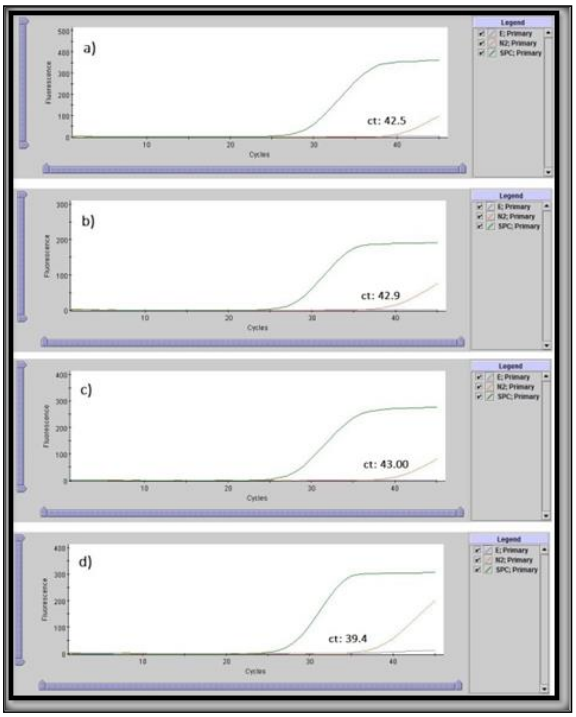

Figure 5:

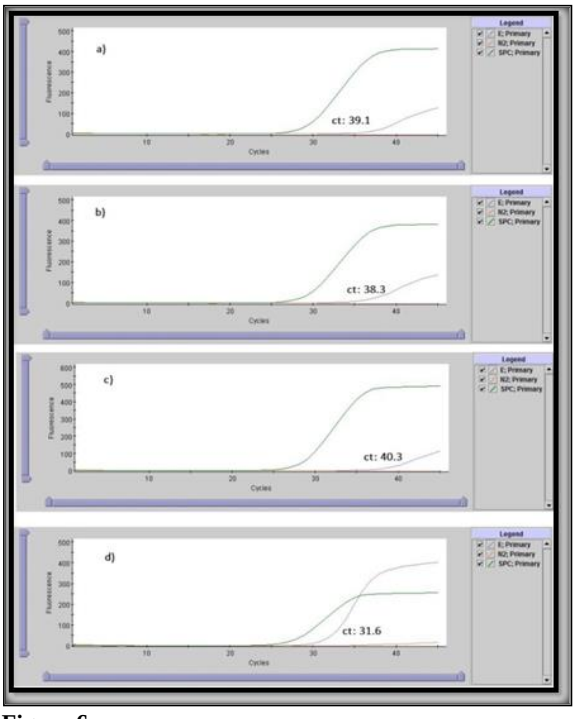

Figure 6:

Table 1

GeneXpert		N= 1686
E gene	N2 gene	

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Negative	Negative	1297 (76.92%)
Positive	Positive	330 (19.57%)
Negative	Positive	46 (2.72%)
Positive	Negative	13 (0.7%)

Table 2

GeneXpert (n =59) E gene / N2 gene	RT PCR	
	Positive	Negative
Negative / Positive (n=46)	5 (10.8%)	41 (89.1%)
Positive / Negative (n=13)	3 (23.1%)	10 (76.9%)
Total	8 (13.5%)	51(86.4%)

Table 3

Specimen ID	GeneXpert Ct values	RT PCR Ct values	
	N2 gene	E gene	RdRp gene
31563	42.5	33.7	32.4
38194	38.6	11.5	12.18
39014	42.2	-	27.0
42057	44	27.37	28.03
44330	39.4	34.39	33.63

Table 4

Specimen ID	GeneXpert Ct values	RT PCR Ct values	
	E gene	E gene	RdRp gene
21828	37.6	32.26	31.21
43856	41.4	33.82	33.32
44537	31.6	27.07	27.54

DISCUSSION

In this cross-sectional study, the results of the single gene positive GeneXpert samples were compared with results of the RT-PCR assay. It was observed that 3.4% (59/1686) of samples tested single gene positive on GeneXpert. Out of which, 0.7% (13/1686) of the samples were positive for envelope (E) gene target and negative for the nucleocapsid (N2) target. These samples were interpreted as presumptive positive. Among the samples 2.7% (46/1686) were negative for the envelope (E) gene target but positive for the nucleocapsid (N2) target. This is similar to the study by Mahdi et al., in which authors have observed that 3.9 % (44/1123) of SARS-CoV-2 positive results were positive for the nucleocapsid (N2) gene but negative for the envelope (E) gene target.[12] Out of the 59 samples tested, 8 (13.5%) of the samples were positive by RT-PCR and 51 (86.4%) were negative by RT-PCR.

In this study, for the five N2 single gene positive samples, Ct value range in GeneXpert assay was observed as 38.6 to 44.9 with median Ct value of 42.2. This was similar to a study in which for the N2 single gene positive samples, the median Ct value observed was 41.6 with the range 38.8–44.9.[12] For the three presumptive positive samples, Ct value range in GeneXpert assay was observed as 31.6 to 41.4 with median Ct value 37.6.

On visual interpretation of the results, atypical curve was observed in all these samples. A standard RT-PCR amplification curve normally should have 4 different phases: linear ground, early exponential, log-linear, and plateau.[13] Curve d in Fig 5 and 6 which were typical sigmoid shaped amplification curves starting with a slow upward trend, then strong upward swing followed by plateau for N2 or E gene were interpreted as “detected” by the software.

An atypical curve usually showed slight deflection followed by a flattened plateau phase. Curves a, b, c in Fig 5 and 6 showed atypical curves with proper amplification of the internal control. This type of atypical curves observed were also interpreted as “detected” by the interpretive software. Nonspecific amplification of background nucleic acid could have been one of the reasons for potential positivity of single gene positive results in GeneXpert assay.[14]

CONCLUSION

On the backdrop of a pandemic where rapid triage decisions needed to be taken, GeneXpert, an automated, point of care, run on demand testing, was highly valuable in providing results in 40 minutes. However, interpretation of the single gene positive reports of GeneXpert should not be done only based on interpretive software as these instruments were occasionally overcalling background signals as a positive result. Interpretation of single gene positive results irrespective of the Ct value should be done by the visual inspection of amplification curves. Retesting of single gene positive samples with atypical amplification curves with a real time PCR may help us to avoid potential false positive results and to establish laboratory interpretation guideline. Every laboratory needs to establish their standards for the interpretation of such results.

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Limitation: The conclusion from our study was limited by a small number of single gene positive cases and the Ct value of the individual gene were not analysed.

Contribution: All authors contributed to this journal

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