ISSN: 0975-8585

Research Journal of Pharmaceutical, Biological and Chemical Sciences

Study Of Definitive Results Of TrueNat PCR Over Open System RT-PCR At Rural Centre During Covid -19 Pandemic With Specific To Inconclusive Results.

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ABSTRACT

The molecular diagnostic techniques used in Covid-19 pandemic, were restricted to various target gene detection, many times imposing a dilemma for reporting. The present study highlights the results by open system RT PCR and chip based real time PCR (TrueNat) in the same patients collected at the same time. Naso-pharyngeal/ Oro-pharyngeal specimens received in the molecular laboratory, were screened both for E gene and Orf1 gene first by open system real time RT-PCR (My-lab Pathodetect kit). Further, inconclusive result specimens (From open system RT PCR) were subjected to same target gene detection by TrueNat Covid-19 (chip-based Real time Duplex PCR) on the same day.. Result interpretation was done according to, guidelines led by ICMR. Out of total 4543 Naso-pharyngeal/ Oro-pharyngeal specimens included in the study period of six months, 2947(64.86%) were positive and 1540 (33.89%) were negative, by open system RT PCR. Rest 56 specimens which showed inconclusive/invalid results were further run by TrueNat PCR test. We could report additional 52 definitive results by TrueNat technique in a shorter duration. Though open system RT PCR is the gold standard molecular technique for Covid-19, the present study highlights the usefulness of the additional test with good sensitivity, with short turnaround time and with more definitive results was practically helpful for the microbiologist in the field.

Keywords: SARS CoV2 TrueNat test, Open system RT-PCR, E gene, orf1 gene, inconclusive result

https://doi.org/10.33887/rjpbcs/2024.15.1.58

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ISSN: 0975-8585

INTRODUCTION

SARS–Cov 2 (Covid-19), a novel corona virus, first identified in Wuhan (China) in December 2019. It globally spread in a short period and WHO declared it a public Health Emergency of international concern on 30th January 2020. The relentless spread of the disease led to the condition being declared as pandemic on 11th March 2020. Laboratory diagnosis of Covid -19 played an important role, not only diagnosis of infection and management but also in prevention and control of disease [1].

At that juncture, the Covid-19 virus was top priority pathogen to deal with, because of high transmissibility, severe illness and associated mortality. Every molecular laboratory soon was ready for the must needed equipments and training of staff knowing and updating the bio-risk in the handling the specimens, test procedures, preservation and disposal of specimens [2].

The molecular diagnostic techniques used in Covid-19 pandemic, were restricted to various target gene detection. The open system real time RT PCR is being considered as the gold standard test detecting various target genes of Covid-19. The chip based real time RT-PCR was also introduced and found as rapid, easy technique with shorter duration to report the result. In India, that was huge task to augment testing of Covid-19 in underserved areas and most of health care facilities. A combination of different tests and testing platforms were used to augment capacity to 1.2 million tests for as of sept. 2020. The Truelab workstation included sample preparation, an RNA extraction system, an RT-PCR machine, and disposable kit components. This portable, battery operated, automated and low weight machine could be used in remote areas with network data transferability [3].

Conventional open PCR system was almost used by every laboratory and found valuable during he said pandemic time. But target detection genes were always not detected on first testing due to various reasons and posed dilemma to clinicians specially in emergency cases.

The present study was undertaken to get definitive results by PCR assay (chip based real time PCR) on those specimens, results of which came inconclusive/ invalid by open system RT PCR in the patient's specimens collected at the same time.

MATERIAL AND METHODS

This six month (January 2021 to June 2021) retrospective study was conducted in Dept. of Microbiology, Dr BVP Rural Medical College, Loni (PIMS-DU). Test was performed on virus lysis media containing only oropharyngeal swab while open system RTPCR was performed on viral transport media(VTM) containing both oropharyngeal/ Nasopharyngeal swab. Both samples were collected simultaneously. Specimens which received in the molecular laboratory, were screened both for E gene and Orf1 gene first by open system real time RT-PCR (Quant Studio 5 analyser and My-lab Pathodetect kit).

Further, inconclusive/ invalid result specimens (From open system RT PCR) were subjected to same target genes detection by SARS CoV-2 assay [a chip-based Real time Duplex PCR (Truelab Quattro) system] on the same day. [4,5] Result interpretation was done according to, guidelines led by ICMR time to time.

OBSERVATIONS AND RESULTS

A total of 4543 specimens were received for screening of Covid-19 infection during the study period.

Table 1: Results of suspected Covid -19 specimens run by open system RT PCR test

TAL SPECIMENS	NEGATIVE	POSITIVE	INCONCLUSIVE / INVALID
n=4543	1540	2947	56
	(33.89%)	(64.86%)	(1.23%)

ISSN: 0975-8585

Table 2: Distribution of TrueNat PCR results of specimens with inconclusive outcome by open system RT PCR

TOTAL SPECIMENS	NEGATIVE	POSITIVE	INCONCLUSIVE
n=42	14	25	03

Table 3: Distribution TrueNat PCR results of specimens with invalid outcome by open system RT PCR

TOTAL SPECIMENS	NEGATIVE	POSITIVE	INCONCLUSIVE
n=14	11	02	01

Table 4: Detection of E gene and Orf 1 gene by PCR in specimens with inconclusive / invalid outcome by open system RT PCR

RESULT BY PCR	POSITIVE (27)		INCONCLUSIVE (4)	NEGATIVE (25)
TOTAL (n=56)	Both E gene and Orf 1 gene detected	Only Orf 1 gene detected	Only E gene detected	Both E gene and Orf 1 gene not detected
Inconclusive (42)	20	5	3	14
Invalid (14)	1	1	1	11
Total	21	6	4	25

Out of total 4543 total specimens received, (33.8%) were negative and 2947(64.8%) were positive, by open system RT PCR (Table 1) Rest 56 (1.2%) specimens which showed inconclusive/ invalid results were further run by TrueNat PCR assay. Out of 56 specimens, a total of 42 specimens which showed inconclusive results were further run by TrueNat PCR assay. It detected 25 specimens as positive and 14 as negative and three as inconclusive (Table 2). Fourteen specimens with invalid results by open system RT PCR were further run by TrueNat PCR assay 13 definitive results came (2 positive and 11 negative) while one specimen still showed inconclusive result (Table 3). Out of total 27 positive specimens Both E gene and Orf1 gene were detected in 21 positive specimens and only Orf 1 gene was detected in 6 positive specimens. In all these 25 negative specimens both E gene and Orf1 gene were not detected by TrueNat PCR assay. Four inconclusive results showed presence of only E gene, yet not providing definitive result (Table 4). In these cases, fresh samples were requested after 48 hours.

DISCUSSION

In Covid -19 pandemic, for everyday considerations, open system RT PCR was a principle method and is known 'Gold Standard' method. Every molecular laboratory was performing the tests regularly in large batches and at affordable cost. Overall, we could report 98.75% of conclusive result using open system RT PCR molecular assay. On the other hand, the factors affecting the molecular assay like quality of specimen, stage of disease, technical errors, bound to give the inconclusive /invalid outcomes on few occasions.

Further, Covid -19 cases surge in India and abroad required a rapid and sensitive molecular assay. Rapid point of care(PoC) assays like TrueNat Beta CoV and TrueNaT SARS-CoV2 were soon developed and proved expected readily result with short turnaround time. Basawarajappa SG et al in their study from Bengaluru revealed 100% concordance with clinical sensitivity, clinical specificity, positive predictive value and negative predictive value, when TrueNat Beta CoV and TrueNaT SARS-CoV2 results were compared to reference standard rRT-PCR. Their study confirmed this by detection of valid ct values in log IVT dilutions assay. Further , limit of detection (LOD) for TrueNat assay was 10^2 copies/ µl for the said target than of rRT-PCR 10^3 copies/ µl indicating higher sensitivity [6].

In the above study, 56 specimens were reported as inconclusive / invalid by open system RT PCR molecular assay. We could report additional 52 definitive results (by TrueNat PCR assay on the same day which proved to be beneficial to clinicians planning the treatment. These point of care assays exhibited

the advantage of higher applicability in field settings for rapid screening and confirmation of SARS CoV2 cases without compromising the diagnostic parameter.

Amiyabala Sahoo et al study showed a high concordance with the RT–PCR test with sensitivity of 100% and specificity of 99.12% by assay. Though, four samples which were negative by RT PCR were positive by TrueNat system. With this highest sensitivity and specificity, the study concluded TrueNat assay as reliable and affordable option to provide rapid result [1].

Ujjala Ghoshal et al study revealed sensitivity(69.55%), specificity(90.9%) and diagnostic accuracy(89.2%) for Covid -19 diagnosis and commented as it will be game changer of molecular diagnostics in future especially in areas with poor infrastructure [7].

Study by Sadhna S and Hawaldar R observed a sensitivity of 96.5% while study by Alagarasu K et al observed sensitivity of 81.8% by RdRP assay for detection of SARS-CoV-2 [8-9]. Similarly, Sandhya rani Pagidirani et al reported TrueNat Beta CoV assay, sensiivity and specificity as 82.6 % and 96.7% respectively , in initial period of pandemic and advised confirmation by conventional RT-PCR [10].

Comparative analysis by Mamta Sharma et al in their study reported the assay was able to detect target genes in the specimens from individuals with mild form of disease which were persistently negative by RT PCR indicating its better performance [11]. Rodriguez I A et al reported four samples which were negative by RT PCR but positive by TrueNat system and further study concluded, TrueNat system exhibited early detection of the virus suggested by a lower ct value in comparison to Real time PCR [12].

CONCLUSION

Rapid definitive diagnosis was a remarkable step towards the containment of the Covid-19 virus spread. Diagnostic dilemma in the emergency situations could be solved to some extent by availability of such an additional assay. The readily conclusive outcomes of the TrueNat assay were helpful for screening of the covid cases in emergency, elective surgeries, labours. Home isolation and quarantine issues were also streamlined in time.

Such point of care assays relieved the greatly burden on molecular laboratories and overall increased the testing capacity of laboratories, with open system RT PCR testing .

The above study reveals the significance of availability of an additional assay like TrueNat assay along with open system PCR and its role in conclusive result outcome. Certainly, this has enhanced an impact on clinical management of patient in Covid -19 pandemic situation. Genuinely, in rural place, with such facility we could confirm the inconclusive results which made difference in clinical decision.

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