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Performance Evaluation of Truenat rtPCR assay with COBAS -6800 in SARS COV-2 detection: A diagnostic accuracy study

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Abstract

Context: Diagnostic testing plays a critical role in early and accurate detection of SARS-COV-2. Real-time PCR is the Gold standard test for the diagnosis of SARS-COV-2 which requires well equipped BSL-2 Lab along with skilled manpower. There is a need for a rapid point of care test for diagnosis. TrueNat is one such testing system that is approved by Govt. of India.

Aims: To find out the performance of the TrueNat assay in comparison to cobas 6800.

Settings and Design: The Cross-sectional study was conducted in a COVID 19 Testing Laboratory in Central India.

Methods and Material: A total of 122 COVID-19 positive samples in cobas 6800 were analysed in TrueNat assay.

Results: Of the total of cobas positive122 sample TrueNat system were able to detect 116 samples with the viral load of high(n=18), medium(n=28), low(n=44),very low(n=26). Considering cobas 6800 as a Gold standard assay TrueNat test showed a sensitivity and specificity of 95% and 100%respectively.

Conclusions: Based on our findings we believe that TrueNat assay is equally effective for the diagnosis of SARS-COV-2 compared to a highly sophisticated instrument such as cobas 6800. Hence, it should be installed in all healthcare setups with a limited setting for rapid and reliable diagnosis of SARS-COV-2.

Keywords: cobas 6800, TrueNat, COVID-19, Point of care test.

INTRODUCTION:

The SARS-COV-2 (COVID-19) pandemic has faced significant diagnostic challenges worldwide [1] and showed serious concern for the healthcare system. The detection of SARS-COV-2 in the laboratory by rtPCR is the gold standard method in the acute phase of infection.[2] At the beginning of the COVID-19 pandemic, a molecular diagnosis such as rtPCR was very limited in developing countries especially in India because of the requirement of infrastructure and trained manpower. To control the spread of the infection there was a need of high surveillance testing in the field hence there were requirements for rapid and simple assays with high sensitivity and specificity. To overcome the situation govt of India and ICMR validated and approved many newer diagnostic tools and kits for early detection. One such rapid point of care test was TrueNat assays which target the Beta CoV E gene and SARS-COV-2 Rdrp gene and can be installed in limited settings. The study aimed to find out the performance of TrueNat assay with a highly sophisticated close system automatic rtPCR system known as cobas 6800 (Roche Molecular Systems, Pleasanton,CA, US)

SUBJECTS AND METHODS:

Study setting and population: This cross-sectional study was conducted during the period from March 2021 to May 2021 in a state-level BSL-3 reference laboratory in central India. The study was conducted after obtaining the permission from Institutional ethical committee. A total of 250 non-repeated patients of all age groups who were admitted to the triage ward with symptoms of fever breathlessness, SpO2<95%, pain in the chest, seizures, weakness, or numbness on the face were included in the study.

Sample collection and transportation to Lab: Oropharyngeal and nasopharyngeal Samples were collected in a viral transport media as per the guideline provided by ICMR. All these samples were packed and transported in triple-layered to the lab in cold chain as per guidelines provided by ICMR.[3]

Sample processing: All the samples were processed in the BSL3 labs with standard precaution and the sample was stored at -80 ° c for further analysis. For TrueNat analysis, specimens are transferred into the cartridge provided by the manufacturer and processed in class II biological safety cabinet.[4]

Ribonucleic acid extraction:

COBAS 6800:A total 600 of µl of each sample was collected in a barcode label test tube provided by the manufacturer. Then the sample was transferred to the sample supply module from where it is automatically transferred to its processing module and nucleic acid extraction was performed automatically.[5]

TrueNat: For TrueNat analysis 500µl specimens are transferred into the cartridge and inserted into the extractor machine and waited for 20 min, the extracted RNA was collected in a sterile container provided by the manufacturer for further analysis.[1,4]

Nucleic acid amplification: For detection of SARS-COV-2 in cobas 6800 two specific genes of the virus were targeted, containing ORF1 a non-structural region (target-1), and a conserved region in the structural protein envelope E gene (target-2). The test utilizes RNA internal control for sample preparation and PCR amplification.

In TrueNat 6µl of extracted RNA is added to a PCR tube consisting of real-time PCR reagents and the mixture is added into a disposable microchip containing the Beta CoV E gene and SARS-CoV-2 Rdrp gene. The loaded microchip was inserted into the amplifier machine for 40 minutes, and a total of 40 cycles were run for viral RNA amplification.

Interpretation of results: After two and half hours of process, the results were displayed in the monitor for positive results both ORF-1 (target-1) and E (target-2) should be amplified or only the ORF 1 gene amplification was considered. In the case of positivity for the E gene only (target2), the result should be reported as SARS-COV-2 presumptive positive.

RESULTS:

Of the total of 250 samples, 122 were positive by both genes (ORF and E gene) in cobas 6800. Of the 122 positive samples, 68(55%) were male and 54(44.2%) as female, and the mean age group of ±49. Among the positive patients on analysis of clinical symptoms fever with mean body temperature 100^0c± 1 followed by dyspnoea 25%, Breathlessness (13%), Spo_2(<75%) in 25% of patients. On Hematological analysis of severe patient TLC, Neutrophil was high in 35% of the patient along with the higher value of Hb and D dimer and a decrease in lymphocytes.

On analysis of Ct value in cobas 6800 it is found that high Ct values (10-20) were observed in 20 patients. Similarly, in 28 patients the range of Ct value was (21-24). 74 patients have lower Ct values with the range from 25-37. (Table 1)

In TrueNat assay out of 122 samples, 16 samples were detected High. Twenty-six samples have medium value and 6 samples that were above cutoff value were shown positive in cobas 6800. The details of Ct value in cobas and TruNnat results are summarised in table no 1.

Table 1: Comparison of COBAS 6800 positive samples with Truenat assay.

CT value of COBAS 6800	Interpretation of Truenat assay with level of viral load					
	High	Medium	Low	Very Low	Above target cut off	Total
≥10-20	16	00	4	00	00	20
≤21-24	02	26	00	00	00	28
≤25-29	00	02	38	00	00	40
≤30-34	00	00	02	26	00	28
≤35-37	00	00	00	00	6	6
Total	18	28	44	26	6	122

DISCUSSION:

To control the pandemic, there is a necessity for mass testing, and accurate and timely diagnosis of SARS-COV-2, for that ICMR has recommended a portable point of care TrueNat rtPCR system for the detection of SARS-COV-2. Which can be installed and performed the test with limited manpower and setting.[6,7]

In the present study, we have found one in two patients visited in the Triage area during the pandemic were positive for SARS-CoV-2 and need hospitalization. This is in concordance with the study done by B. Ajayi et.al.[8] where they found a higher rate of hospital admission among COVID-19 patients.

In our study population, we found that age was an important risk factor for susceptibility to infection with SARS-COV-2. Patients with a mean age group ± 49 years were more prone to the infection. This is similar to a multicentric study done by the ICMR COVID-19 group where they reported 50-69 years age group was more prone to the diseases in the Indian population. However, a study from London has reported elderly patients in the age group >60 years were more vulnerable to the

infection and need hospitalization. We have also observed a higher proportion of male patients compared to females (55% Vs 45%). This finding is similar to other studies done by Nigam et al[9] and Jimeno S et al.[10]

We are not the first to observe that fever, breathlessness, dyspnoea, and decrease SpO_2 level were some of the common symptoms in our study subject which is in concordance with the study done by Nigam et al.[9]

On analysis of hematological parameters among the study population, we have observed higher values of Hb, Neutrophil, TLC and lower values of lymphocytes and platelets which are similar to the study done by Sun s et al [11], Chen et al[12], and Toledo S et al[13]. It has been found that leukocytosis, lymphopenia, and thrombocytopenia are associated with greater severity and even fatality in COVID-19 cases.[13]

In the present study, we have found that the TrueNat system can detect various concentrations of viral load in COVID 19 patients. This finding was in accordance with the study conducted by Sadhna et al.[14] In our previous study we also reported the same where we found TrueNat is equally effective for the diagnosis of SARS-COV-2 compared to conventional rtPCR assay .[15]

On analysis of the cobas 6800 results with TrueNat assay, we found 95% of the cobas 6800 positive samples were also showing positive results with different viral load in TrueNat assay remaining 5% positive samples of cobas 6800 were displayed negative with above target cutoff (ct >32) in TrueNat device. On analysis of the sensitivity and specificity of the TrueNat device in compared to cobas 6800 we found 95.5% sensitivity and 100% specificity.

We also observed there were not many differences in the threshold level of cobas 6800 and TrueNat assay. Where we have found 38 samples with a range of Ct Value 25-29 and 26 samples with ct value of 30-34 in cobas 6800 were shown “detected low” and “detected very low” in TrueNat assay.

In the further analysis, we observed that nearly (5%) of positive samples in cobas 6800 with the Ct range of 35-37 were negative with the comment of " above target cut off" in TrueNat. Nevertheless, on analysis of clinical parameters, they were found positive based on clinical and radiological findings. It has been reported in the later stage of COVID-19 the viral load used to be very less in the upper respiratory tract sample due to the elimination of the viruses by the immune system. Due to this many molecular techniques were not able to detect the viruses there is a need for repeat testing.

Considering our findings we believe in TrueNat essay there is a need to increase the cut-off value (ct ≤35) so that it can be more effective for the diagnosis of COVID 19 compared to cobas 6800 which is a very sophisticated closed system RTPCR instrument with higher sensitivity and specificity.

Hence, we suggest more TrueNat machines should be installed in all the laboratories, especially in the periphery where there are limited resources for early and accurate diagnosis of covid 19 to reduce the morbidity and mortality of the disease.

Limitation of our study: Our study has a certain limitation, We consider cobas 6800 as 100% sensitive hence we have not validated cobas 6800. We believe more number of samples should be tested for further validation of the assay.

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