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Original Article

Microbiology Section

# Performance of Chip Based Real Time RT-PCR (TrueNat) and Conventional Real Time RT-PCR for Detection of SARS-CoV-2

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## ABSTRACT

**Introduction:** Coronavirus Disease-2019 (COVID-19), caused by the Severe Acute Respiratory Syndrome Coronavirus-2 (SARS-CoV-2) is ravaging the globe due to its rapid spread. Since providing fast results is of critical importance in a time of shortage of medical personnel and beds in isolation wards and to ensure timely treatment for patients, developing high quality rapid Point of Care (POC) diagnostics is essential.

**Aim:** To compare the diagnostic performance of chip based real time Reverse Trancriptase Polymerase Chain Reaction (RT-PCR) (TrueNat) which has a shorter turnaround time compared to conventional real time RT-PCR in samples of suspected COVID-19 patients.

**Materials and Methods:** The present cross-sectional observational study was carried out in a tertiary care hospital in New Delhi, India. Five hundred randomly selected Oropharyngeal (OP) swabs samples received from May-July 2020, were included in the study to compare the diagnostic performance of chip based real time RT-PCR (TrueNat) with conventional real time RT-PCR for diagnosis of SARS-CoV-2 infection. All statistical analysis was performed using STATA version 16.1 software (College station, Texas, USA).

**Results:** The sensitivity of TrueNat test was 100% while the specificity was found to be 99.12% at 95% confidence intervals. The positive predictive value was 91.84% and the negative predictive value was 100%.

**Conclusion:** The short turnaround time, good sensitivity and specificity makes TrueNat a reliable and affordable option to provide rapid results in cases requiring urgent interventions and to augment SARS-CoV-2 testing capacity at peripheral settings where sample load is less.


**Keywords:** Coronavirus disease 2019, Diagnosis, Molecular diagnostics, Pandemic, Reverse trancriptase polymerase chain reaction, Severe acute respiratory syndrome coronavirus-2

## INTRODUCTION

SARS-CoV-2, a novel coronavirus, from the family of SARS-CoV-2 and Middle East Respiratory Syndrome (MERS) coronavirus, was first identified in Wuhan, China, in December 2019 [1]. Its global spread has been rapid, causing the World Health Organisation (WHO) to declare it a Public Health Emergency of International Concern on 30th January 2020. The relentless spread of the disease led to the condition being declared it as pandemic on 11th March 2020 [2]. The virus has disrupted the living and working conditions of billions of citizens worldwide due to different forms of physical distancing and lockdowns in many cities. Since COVID-19 presents a variety of clinical manifestations, ranging from asymptomatic to mild flu-like symptoms to life-threatening complications, efficient testing during the early stages of infection is critical in order to distinguish COVID-19 patients from those with other diseases [3]. Within 5-6 days of the onset of symptoms, patients with COVID-19 demonstrated high viral loads in their upper and lower respiratory tracts [4-7].

Laboratory diagnosis plays an important role, not only in diagnosis of the infection and management of the patients but also in prevention and control of disease. In unravelling the complicated dynamics involved in SARS-CoV-2 infection it is extremely important to have reliable and rapid testing procedures [3]. A Nasopharyngeal (NP) swab and/or an OP swab are often recommended for screening or diagnosis of early infection [5,8,9]. The conventional real time RT-PCR is considered as the gold standard test for detecting cases of COVID-19, targets Envelope gene (*E*-gene) as screening gene and Open Reading Frame 1 ab gene (*ORF*1ab), RNA-dependent RNA polymerase (*RdRp*), and spike gene (*S*-gene) as confirmatory genes. It offers both high accuracy and throughput [10-12]. The Limit of Detection (LoD) was reportedly as 4-8 copies of the virus upon amplification of two or more genes at 95% confidence intervals [11-13]. The samples to be tested by real time RT-PCR require a cold chain maintained at 2-8°C to prevent false negative result. Average time taken is around 5-6 hours from receipt of sample to getting the result. However, setting up conventional RT-PCR laboratory for COVID-19 needs specialised infrastructure with appropriate biosafety measures and is technically demanding.

The chip based real time RT-PCR, which is modified real time RT-PCR is easy to perform, has rapid turnaround time, requires minimal infrastructure and hence can be performed in small healthcare setups. One such test is TrueNat Beta CoV test by Molbio Diagnostics Private Limited, India which is a POC molecular diagnostic test and has been approved by Indian Council of Medical Research (ICMR) on 14th April 2020 [14] for diagnosis of COVID-19. The cost of the test is more than that of RT-PCR but for rural areas where healthcare infrastructure is very poor, TrueNat is the test of choice. It has rapid turnaround time, does not require biosafety cabinet and staff with minimal training can perform the test. In TrueNat RT-PCR, *E*-gene detects the numerous coronaviruses including SARS-CoV-2 while *RdRp* gene only detects SARS-CoV-2 which is used as a confirmatory test [15]. The present study aims to compare the diagnostic performance of chip based real time RT-PCR (TrueNat) with conventional real time RT-PCR for diagnosis of SARS-CoV-2 infection.

## MATERIALS AND METHODS

A cross-sectional observational study was carried out in Department of Microbiology, ABVIMS and Dr. RML Hospital, New Delhi, India.

Five hundred randomly selected OP swab samples received from May-July 2020 were included in the study. This study was approved by the Institutional Review Board (IRB) (438(87/2020). Waiver of consent was obtained by the IRB, as the study was carried out on left over samples, which were stored at appropriate temperature to prevent the damage of samples, identified by a laboratory generated number with no traceability to the patients. The comparative results were not used in the clinical decision making.

**Inclusion criteria:** Samples stored at appropriate temperature that were tested by real time RT-PCR were included.

**Exclusion criteria:** Insufficient sample and samples which were not stored at appropriate temperature were excluded.

### RNA Extraction

TrueNat™ SARS-CoV-2 works on the principle of real time RT-PCR based on TaqMan chemistry. The RNA from the patient sample is first extracted using Trueprep® AUTO/AUTO v2 Universal Cartridge based Sample Prep Device and Trueprep® AUTO/AUTO v2 Universal Cartridge based Sample Prep Kit and assayed using TrueNat™ Beta CoV test [16].

### Real Time RT-PCR Assay

1. **Chip based real time RT-PCR (TrueNat):** This is a two-step assay. The procedure was followed as per manufacturer's instructions as follows:

**Step 1:** All the samples were first tested by *E*-gene for Sarbecovirus screening assay which takes 40 minutes to complete. All negatives were considered as true negatives. All positive samples were subjected to confirmation by step 2 assay.

**Step 2:** All the samples that tested positive by *RdRp* gene for SARS-CoV-2 confirmatory assay was considered as true positive. The results were available in 40 minutes.

2. **Conventional real time RT-PCR Test:** ICMR approved PathoDetect™ Coronavirus (COVID-19) PCR kit (Mylab Discovery Solutions, Pune, India) was used for performing quanitative RT-PCR test for the amplification and detection of *E*-gene of Sarbecovirus and *RdRp* gene of SARS-CoV-2 [17]. The test was carried out as per the manufacturer's instructions. The PCR was put up in CFX96 Touch BioRad (Hercules, California, USA) real time PCR system. Thermal profile for RT-PCR was followed as per the manufacturer's instructions which completes in 2.5-3 hours. Samples which detected both *E*-gene and *RdRp* were considered as positive.

## STATISTICAL ANALYSIS

All the results were entered in Microsoft Excel sheet and correlation tables were made. Performance of TrueNat test was compared with conventional real time RT-PCR for detection of SARS-CoV-2 genes. Sensitivity, specificity, positive predictive value, and negative predictive value were calculated. All statistical analysis was performed using STATA version 16.1 software (College station, Texas, USA). The p-value was calculated by Pearson's correlation with bonferroni correction.

## RESULTS

Performance of chip based real time RT-PCR (TrueNat) was assessed using the conventional real time RT-PCR as a benchmark. A total of 500 samples were screened.

**Chip based real time RT-PCR (TrueNat) results:** Out of 500 samples, 49 were TrueNat positive and 451 were TrueNat negative.

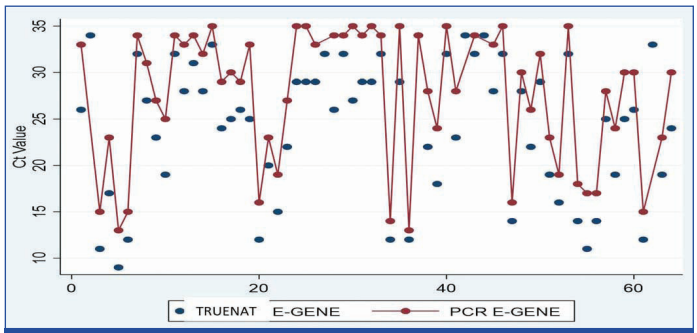
**Comparison with conventional real time RT-PCR:** Out of 500, 45 samples were positive and 455 were negative by real time RT-PCR. All the samples negative by TrueNat were also negative by real time RT-PCR. Out of 49 TrueNat positive samples, 45 were positive by real time RT-PCR and 04 samples were negative by real time RT-PCR [Table/Fig-1].

| | RT-PCR +ve | RT-PCR -ve | Total |
|---|---|---|---|
| TrueNat +ve | 45 | 04 | 49 |
| TrueNat -ve | 0 | 451 | 451 |
| Total | 45 | 455 | 500 |

**[Table/Fig-1]:** Comparison of test results of RT-PCR and TrueNat.

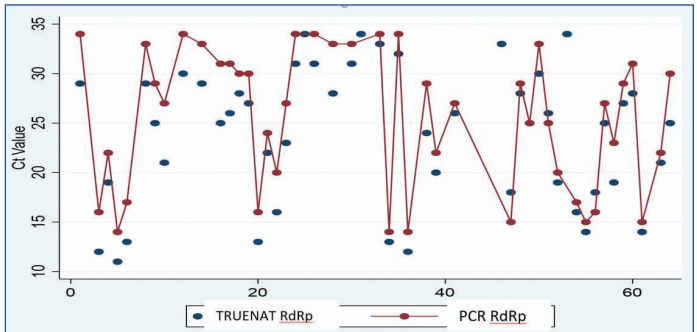
The sensitivity of TrueNat test was 100% while the specificity was found to be 99.12% at 95% confidence intervals. The positive predictive value was 91.84% and the negative predictive value was 100%. The turnaround time of TrueNat was 75 minutes for samples that were negative for *E*-gene and approximately two hours for samples positive for both *E*-gene and *RdRp* gene whereas the turnaround time for processing a sample by real time RT-PCR was around 4-6 hours.

Among the 64 *E*-gene positives detected by TrueNat, 59 were found to be positive by conventional real time RT-PCR method for the same. There was a very high correlation i.e., 97% among the Ct values between TrueNat and real time RT-PCR (p<0.001) [Table/Fig-2]. The average Ct value for *E*-gene by TrueNat was 21.62±6.64 vs real time RT-PCR was 26.02±7.19.




**[Table/Fig-2]:** Line diagram showing comparison of Ct values between TrueNat and RT-PCR where correlation coefficient=0.97 (n=64) (p<0.001). X-axis represents number of samples positive for E-gene.

Among the 49 *RdRp* gene detected positive by TrueNat, 45 were found to be positive by real time RT-PCR for the same. There was a very high correlation i.e., 96% among the Ct values between TrueNat and real time RT-PCR (p<0.001) [Table/Fig-3]. The average Ct value for *RdRp* gene by Ct TrueNat was 23.40±6.68 vs real time RT-PCR method was 25.96±7.20.




**[Table/Fig-3]:** Line diagram showing comparison of Ct values between TrueNat and RT-PCR where correlation coefficient=0.96 (n=49) (p<0.0001). X-axis represents number of samples positive for RdRp gene.

## DISCUSSION

Rapid diagnosis is a remarkable step towards the containment of the COVID-19 virus. The surge of COVID-19 cases in India and across the globe requires a rapid and sensitive molecular assay. Getting accurate, convenient, and rapid testing for widespread dissemination will help eradicate the silent spread by asymptomatic viral carriers of COVID-19. TrueNat is a chip based real time

RT-PCR test used for diagnosis of SARS-CoV-2 in India. It is a temperature stable test based on TaqMan chemistry and detects *E*-gene from Sarbecovirus for screening followed by *RdRp* gene of SARS-CoV-2 for confirmation. The viral lysis buffer that comes with the COVID-19 cartridges inactivates the virus and poses minimum biosafety hazard. Safety is further augmented by the closed nature of this platform and minimum sample handling. The LoD for *E*-gene is 486 genome copies/mL and *RdRp* gene is 407 genome copies/mL (Molbio Diagnostics) [18]. Testing by TrueNat requires limited infrastructure as compared to conventional real time RT-PCR. It does not require cold chain maintenance which enables easy transportation of samples and reagents. The turnaround time for reporting negative results after screening assay is one hour and for reporting positive result by confirmatory assay is another one hour.

Practical considerations, however, still position conventional real time RT-PCR as the principal method as: 1) conventional real time RT-PCR has for decades been the gold standard and has a well-developed supply chain for reagents and equipment; 2) conventional real time RT-PCR is simpler in the primer design and requires fewer additives, which brings down the cost per test; 3) in clinical laboratories where large batches of samples are processed, conventional real time RT-PCR easily makes up for the speed advantage of TrueNat; and 4) conventional real time RT-PCR is compatible with different kits being designed as the disease evolves, whereas TrueNat is proprietary product [19].

In the current study, the TrueNat assay showed a high concordance with the RT-PCR test with a sensitivity of 100% and specificity of 99.12%. With a negative predictive value of 100%, TrueNat has an advantage to test asymptomatic cases, patients requiring urgent surgery or any other intervention, antenatal patients who land in emergency with labour and mortuary samples where rapid reporting is required. Similar findings were also revealed in a study by Basawarajappa SG et al., which showed a clinical sensitivity, clinical specificity and overall concordance to be 100% [20]. A study by Alagarasu K et al., observed a sensitivity of 81.8% by *RdRP* assay for detection of SARS-CoV-2 [21]; while another study by Sadhna S and Hawaldar R observed a sensitivity of 96.5% [15].

In this study, there were four samples that were positive by TrueNat but negative by conventional real time RT-PCR. A systematic review by Rodriguez IA et al., on the accuracy of COVID-19 tests reported false negative rates between 2-29% (equating to sensitivity of 71-98%), based on negative conventional real time RT-PCR tests which were positive on repeat testing [22]. Due to multiple steps in conventional real time RT-PCR there are chances of errors. Moreover, the requirement of sophisticated laboratory and specifically trained personnel adds to the limitations posed by conventional real time RT-PCR making it unsuitable for use in peripheral settings. However, a positive RT-PCR result for COVID-19 test has more significance than a negative result owing to its high specificity. Thus, a negative result by RT-PCR cannot rule out the possibility for the patient of not having the disease.

Also, an interesting feature that TrueNat exhibits was the early detection of the virus suggested by a lower Ct value in comparison to the conventional real time RT-PCR [Table/Fig-2,3]. As this feature will aid in detecting the virus early, hence TrueNat becomes the investigation of choice in circumstances where urgent intervention is required.

### Limitation(s)

Even though TrueNat exhibits a high sensitivity and specificity, the high cost limits its use in urban areas where real time RT-PCR test, being the gold standard test for detection SARS-CoV-2, is easily available and affordable. A larger sample size could have further helped to obtain better estimates of the performance of TrueNat.

## CONCLUSION(S)

The gold standard method for laboratory diagnosis of SARS-CoV-2 infection is RT-PCR, which is a time tested method with high sensitivity and specificity but from sample collection to sample processing and interpretation of data, it necessitates certain strict requirements and skill. While the indigenous TrueNat is a chip based real time RT-PCR test kit that provides a viable and economical solution for supplementing SARS-CoV-2 testing capability in India at remote locations with low sample loads. It is not very technically demanding, routine staff with minimal training can perform the test. The good sensitivity and specificity of TrueNat for case detection of COVID-19 along with shorter turnaround time takes the advantage over conventional real time RT-PCR in cases requiring urgent interventions.

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