Original Article

Comparative Evaluation of Truenat Reverse Transcription-Polymerase Chain Reaction with Commercially Available Reverse Transcription-Polymerase Chain Reaction Kits for COVID-19 Diagnosis

Anoop Jaiswal, Monica Singh[1], Arindam Chakraborty[1]

Departments of Biochemistry and [1]Microbiology, Motilal Nehru Medical College, Prayagraj, Uttar Pradesh, India

Abstract

Introduction: Early and correct identification of the betacoronavirus is important for effective isolation treatment and case management. Real-time polymerase chain reaction (PCR) are consider as a gold standard for the diagnosis of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2); however, for that, there are a requirement of skilled workforce and elaborate infrastructure. A rapid point of care test known as Truenat Beta CoV and Truenat SARS COV assay were recommended by the Government of India. The aim of the study was to find out the performance of Truenat assay in comparison to four RT-PCR assay kits. **Materials and Methods:** The cross sectional study was conducted in a COVID-19 testing laboratory in Central India. Forty known Truenat positive sample with different viral load were analyze in selected rtPCR kits from 4 different manufacturers. **Results:** Of the total of ten very low viral load samples, BGI kit was able to detect six samples, followed by TruePCR six samples, TaqPath five samples and NIV kit were able to detect three samples. Similarly, in the case of low viral load sample, BGI and TaqPath kit were able to detect all the 10 samples followed by NIV kit five samples and TruePCR nine samples respectably. In the case of medium and high viral load samples, all four reverse transcription-PCR (RT-PCR) kits were shown a 100% detection rate. **Conclusions:** Based on our findings, we believe truenat RT-PCR is a more reliable technique for the detection of SARC-CoV-2. Hence, it should be installed in the healthcare setup for better control of the pandemic.

Keywords: COVID-19, reverse transcription-polymerase chain reaction, severe acute respiratory syndrome coronavirus 2, truenat

Introduction

The severe acute respiratory syndrome coronavirus 2 (SARS-Cov-2) pandemic poses a massive burden on humanity, economic and healthcare systems worldwide, and various actions are being taken to control its spread; one such thrust focus has been to test, trace and treat.[1,2] However, it critically depends on the timely and accurate diagnosis of the virus-infected individual. A real-time reverse transcription-polymerase chain reaction (PCR) is the most sensitive and specific assay; therefore, it is considered the gold standard for the detection of SARS-CoV-2.[3,4] Country like India, where there are limited settings for molecular diagnosis, the main burden of diagnosis was limited to centralised reference laboratories with trained workforce.[5] Apart from this, the test methods are lengthy and time consuming, and results are available to the clinicians with a longer turnaround time, and due to multiple steps involved in the process, there are chances of errors. To overcome those situations, (Indian Council of Medical Research [ICMR]) recommended a closed system Truenat reverse transcription-PCR (RT-PCR) system for the diagnosis of SARS-CoV-2 infection, which can be installed in any setup with minimum infrastructure and workforce. However, there are limited data about the efficiency

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Address for correspondence: Dr. Arindam Chakraborty, Department of Microbiology, Motilal Nehru Medical College, Prayagraj, Uttar Pradesh, India.
E-mail: arindamchakraborty@hotmail.com

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of the Truenat RT-PCR kit in comparison to the commercially available RT-PCR kits. Hence, the present study was planned to compare the Truenat kit with four commercially available RT-PCR kits.

Materials and Methods

Participants and clinical sample

The cross-sectional study was conducted during the period of August 2020 to October 2020 at the state level COVID-19 laboratory in central India after obtaining permission from the institutional ethical committee (ECR/922/Inst/UP/2017). Forty known Truenat beta CoV assay (E gene) and SARS-Cov-2 assay (RDRP) positive samples with a different viral load such as high (computed tomography [CT]: 15+–4), medium (CT: 20+–4), low (CT: 25+–4) and very low (CT: 29+–4) were included in the study. The median age of the study population that tested positive for COVID-19 was 50 years (interquartile range 38–66 years).

Sample collection

Nasopharyngeal and oropharyngeal swabs from the known SARS-Cov-2 Truenat assay-positive patients were collected in viral transport medium as per the guideline provided by the ICMR.[6]

Processing of samples

All the samples were processed in BSL3 labs with standard precaution, and proper cold chains were maintained for the storage (–80°C) of the sample.[7]

Ribonucleic acid extraction

Viral Ribonucleic acid (RNA) for RT-PCR was extracted in an automated 96 well Thermo Fisher extractor with the use of Thermo Fisher reagents and plasticware which includes (1) Tip comb plate, (2) elution plate containing 100 µl of elution buffer/well, (3) Wash II plate containing 1000 µl 80% ethanol/well, (4) Wash I plate containing 500 µl wash buffer/well and (5) sample plate containing 200 µl sample + master mix (265 binding solutions + 10 µl magnetic beads + 5 microliter proteins k).[8] Viral RNA for Truenat assay was extracted with the help of the truenet extractor machine.[5]

Amplification of viral ribonucleic acid

Truenat nucleic acid amplifier was used for the amplification of viral RNA; extracted RNA was loaded E gene and RDRP loaded chip and inserted in the Truenat amplifier machine and amplification were performed as per the manufacturer instruction, a total of 40 cycles were run for viral RNA amplification.

Extracted viral RNA from automated extractor was run in Quantstudio 5 Real-time PCR system manufactured by Thermo Fisher scientific by the use of four RT-PCR kits provided by ICMR, India, which includes TRUPCR SARS-CoV-2 RT qPCR kit, BGI's Real-Time Fluorescent RT-PCR Kit, TaqPath™ COVID-19 Combo Kit from Applied Biosystems™ and NIV Pune RT-PCR kit for COVID-19. Details of the kits were summarised in Table 1.

Results

Of the total of forty samples, 11 were female (27.5%) and 29 were male (72.5%), and the mean age was ± 50. Of the forty samples with different viral load (Very low: 10 Nos, Low: 10 Nos, Medium: 10 Nos and High: 10 Nos) were positive by Truenat Beta CoV for E gene and Truenat SARS-CoV-2 for RDRP gene with valid CT value.

Ten samples show a very low viral load while comparing with four sets of commercially available RT-PCR kits. It found that in the BGI kit, Open reading frame (ORF) genes were detected in six samples with CT values range from 34 to 38. In the case of TaqPath assay, ORF was amplified in five samples, and N gene and S gene were detected in six samples with valid CT values. It was found that in the NIV kit, E genes were detected in five samples and RDRP and orf1b were detected in three samples. Similarly, in the case of the True PCR kit, N/RDRP genes were detected in six samples, and E genes were detected in all 10 samples.

Of the total of ten samples with the low viral load as per Truenat assay in BGI kit, ORF was amplified in all the 10 samples, followed by TaqPath kit (ORF gene: 10Nos, N gene: 10Nos, S gene: 10Nos); TRUE-PCR kit (E gene: 10 Nos, N gene: 9Nos), respectively. However, in the case of NIV kits, E genes were amplified in seven samples and in the case of RDRP, and orf1b was amplified in three samples.

Amongst the detected medium and detected high viral load samples BGI, TaqPath, TRUE PCR and NIV kits are able to detect all of the targeted genes, which includes E gene, ORF, S, N RDRP and orf1b genes with valid CT value [Table 2].

Table 1: Details of real-time-polymerase chain reaction kits

Assay	trueNat	BGI	TaqPath				NIV			TruePCR	
	Targeted gene										
	E	*RDRP*	*ORF*	*ORF*	*N*	*S*	*E*	*RDRP*	*Orf1b*	*N/RDRP*	*E*
Very low viral load	10	10	6	5	6	6	5	3	3	6	10
Low viral load	10	10	10	10	10	10	7	3	3	9	10
Medium viral load	10	10	10	10	10	10	10	10	10	10	10
High viral load	10	10	10	10	10	10	10	10	10	10	10

PCR: Polymerase chain reaction

Table 2: Total number of gene amplified in different real-time-polymerase chain reaction kits

RT-PCR KIT	Targeted gene	Total cycle	Ct value cut off
Truenat Beta CoV	*E*	40	35
Truenat SARS-CoV-2	*RDRP*	40	35
TaqPath	*Orfab, N* and *S*	45	35
BGI	*E, Orfab*	45	37
TruePCR	*E, RDRP/N*	38	35
NIV	*E, RDRP, orf1b*	45	37

PCR: Polymerase chain reaction, RT-PCR: Real-time-PCR, SARS-CoV-2: Severe acute respiratory syndrome coronavirus 2

Discussion

The responsibility of the COVID testing laboratory is to maintain accurate and timely diagnosis of SARS-CoV-2, which is crucial for policymaking, implementation of control measures, identification isolation and contact tracing of patients and containment of people coming in contact with infected patients. However, the most difficult task is to set up a molecular diagnostic laboratory in remote settings to overcome the situation, which and ICMR have recommended an indigenous rapid point-of-care Truenat RT-PCR system for the diagnosis of SARS-COV-2, which can be installed in any laboratories with limited settings.[9,10]

In our study population, we found that age was an important risk factor for susceptibility to infection with SARS-CoV-2. The late forties and fifties patients were more susceptible to infection when compared with any other age group. Several investigators also reported the same.[5,11] We also found a higher proportion of males with COVID-19 infection compared to females. (72.5% vs. 27.5%). This finding is similar to other studies done by other investigators where they have found that males face higher odds of both intensive therapy unit admission and death compared to females.[11,12]

In the present study, we have found that the Truenat system can able to detect the various concentration of viral load in nasopharyngeal and oropharyngeal swabs in COVID-19 patients. This finding was in accordance with the similar study conducted by Sadhna and Hawaldar.[5] As Truenat is a closed system, so the chances of contamination are less, and the degradation of the genetic material of the viruses are minimum. Hence, the sensitivity and specificity of the assay are well reliable in comparison to conventional RT-PCR.[9]

In the present study, we evaluate the performance of the Truenat assay for the detection of SARS-COV-2 in comparison to four commercially available RT-PCR kits. We are the first to observe that in the case of a very low viral load sample, 3 of 4 RT-PCR kits were able to detect 60% of orf and N genes and 100% of E genes. We also observed that, in the case of low viral load samples, the sensitivity rate was higher in Truenat assay in comparison to RT-PCR assay as we found that around 90% of samples shown amplification of at least one confirmatory gene, which include orf, N and S gene. However, 100% amplification was observed in the E gene. It was interesting to know that, amongst Truenat detected medium (n = 10) samples, BGI, TaqPath, Trupcr and NIV kits are able to detect all of the targeted genes (orf, N, S, RDRP and E gene). Results of our study also shown that, in the case of high viral load samples, all four RT-PCR kits were able to diagnose all the targeted genes with high accuracy.

Conclusions

Considering our finding, we believe that all of the commercially available RT-PCR kits included in this study were not able to detect very low and low viral load samples in comparison to the Truenat RT-PCR assay. This discrepancy may be because of low viral load as the patients might be in a later or early stage of the disease. Hence, we suggest that patients who are later stage or early stage of disease or symptomatic with RT-PCR negative report can be re-tested with Truenat assay and more number of Truenat machine should be installed in all the laboratory, especially in the periphery where there are limited resources by this we can able to minimise the human error, false-negative cases, morbidity and mortality in the community.

Limitations of the study

Our study had a certain limitation: we considered Truenat finding as 100% sensitive hence, we have not done the validation of the Truenat RT-PCR kit. Furthermore, no clinical data were collected from our study population. Thus, it is difficult to comments on the causes of different viral loads amongst the participants.

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Conflicts of interest

There are no conflicts of interest.

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