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Original Article

Biochemistry Section

Usefulness of TrueNat: A Chip-based Real-time PCR Test for COVID-19

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ABSTRACT

Introduction: For the containment of growing Coronavirus Disease (COVID-19) pandemic, rapid diagnostic facilities are need of today. Indigenously developed TrueNat assay is a point-of-care assay developed for early diagnosis of Severe Acute Respiratory Syndrome-Coronavirus-2 (SARS-CoV-2). It is a portable, fully automated, chip-based, real-time quantitative polymerase chain reaction system with a turnaround time of 1.5-2 hours.

Aim: To assess the practical utility and diagnostic accuracy of TrueNat testing for COVID-19 in a pandemic situation.

Materials and Methods: A cohort selection cross-sectional study was conducted from July to September 2020 at Department of Biochemistry, Vardhaman Institute of Medical Sciences, Pawapuri, Bihar, India, after obtaining Institutional Ethics Committee (IEC) approval. A total of 296 cases with symptoms of COVID-19 were selected for the study. Assuming real-time Reverse Transcription-Polymerase Chain Reaction (rRT-PCR) to be the gold standard, we collected oropharyngeal swabs from symptomatic COVID-19 suspected cases and tested by both TrueNat and standard RT-PCR. Agreement between both the assays were assessed by overall, Positive Percent Agreement (PPA) and Negative Percent Agreement (NPA) and Cohen's kappa coefficient using Epitools (Ausvet 2020).

Results: Out of 296 oropharyngeal swabs taken from suspected COVID-19 patients, 19 were read as "invalid" and discarded; hence only 277 samples were tested by TrueNat and RT-PCR both. Assuming RT-PCR as standard, TrueNat assay demonstrated an overall percent agreement of 99.64%, PPA of 95.65%, NPA 99.81%. The kappa coefficient was 0.9546.

Conclusion: TrueNat assay offers a rapid, accurate and affordable technique for COVID-19. It may be deployed for mass screening and confirmation of COVID-19 cases in hospitals and remote areas.

Keywords: Coronavirus disease-19, Molecular diagnostic test, Polymerase chain reaction, Point-of-care assay, Rapid test

INTRODUCTION

Coronavirus disease-2019 pandemic is the most devastating event for mankind in recent times. It has jeopardised human activity globally. On second December 2020, there were 63,144,362 confirmed cases of COVID-19 including 1,469,237 deaths in world and 9,499,413 confirmed cases of COVID-19 in India [1]. World Health Organisation (WHO) declared a pandemic of COVID-19 in March 2020 [2].

Provision of the diagnostic test for rapid detection of SARS-CoV-2 is the mainstay for early diagnosis, prompt implementation of infection control measures and epidemiological tracking in both hospital setting and community. WHO recommends RT-PCR test for coronavirus detection and differentiation [3]. Currently, real-time RT-PCR is accepted as the gold standard for detection of SARS-CoV-2 globally [4].

The RT-PCR testing requires centralised reference laboratories, skilled men power and elaborate infrastructure including recommended biosafety measures [3]. It also requires significant financial investment and the results may take several hours to days. It may cause a delay in diagnosis and management of cases. To increase the testing capacity, Indian Council of Medical Research (ICMR) has set a network of Viral Research and Diagnostic Laboratories (VRDL) throughout India which are well-equipped labs for RT-PCR testing with at least Biosafety Level-2 (BSL-2). These VRDL work as State Nodal Centres for coordination of sample collection and shipment for rapid detection and reporting of SARS-CoV-2 cases [5].

To increase the further testing capacity and timely diagnosis and intervention, ICMR approved TrueNat testing for COVID-19 on April 14th 2020. TrueNat is a point of care testing facility which has strengthened the Indian testing capacity in urban as well as rural parts of India. TrueNat is a portable, microchip-based, battery-operated, optical detection, fully automated device developed by Molbio Diagnostics Private Limited, India. [Table/Fig-1]. This device was originally developed for rapid detection of *Mycobacterium tuberculosis* and rifampicin resistance in pulmonary and extrapulmonary tuberculosis. TrueNat is a real-time quantitative micro-PCR testing for coronavirus Ribonucleic acid (RNA) in human oropharyngeal and nasopharyngeal swab specimen. The test detects Nucleocapsid Gene (N2) and Envelope Gene (E) and RNA-dependent RNA polymerase (RdRp) gene. The results are interpreted as positive, negative or invalid [6-8].

[Table/Fig-1]: Truenat device developed by Molbio Diagnostics Private Limited, India.

The TrueNat beta COV screens through E gene while TrueNat SARS-COV-2 RdRp gene for confirmation. A multiplex assay combining E-gene screening and Orf1a-gene confirmatory assay has been validated recently. It simultaneously amplifies and detects the target Deoxyribonucleic acid (DNA) during every cycle, thus the presence of target DNA and its quantity in each reaction is accurately detected

without the need to analyse post PCR product separately. There is no need to prepare master mix and no need for clean hood. As a result, the peripheral laboratories with minimal infrastructure and minimally trained technician can easily perform these tests routinely in their facilities and report PCR results in less than an hour [6-9].

MATERIALS AND METHODS

This cross-sectional study conducted at Department of Biochemistry, Vardhaman Institute of Medical Sciences, Pawapuri, Bihar, India, from July to September 2020. After receiving approval by the Institutional Ethics Committee (Vide no. 1015, dated 29.06.2020), 296 cases with symptoms of COVID-19 were selected for the study. Informed consent was obtained from every patient.

Inclusion criteria: Suspected cases of SARS-CoV-2 infection who meets the following clinical criteria: Acute onset of any three or more of the signs or symptoms- fever, cough, general weakness/ fatigue, headache, myalgia, sore throat, coryza, dyspnoea, anorexia nausea/vomiting, diarrhoea, altered mental status and patients with severe acute respiratory illness recent onset of anosmia or ageusia in the absence of any other identified cause were included in the study. Epidemiological criteria like subjects residing or working in an area with high risk of transmission of virus, residing or travelled to an area with community transmission anytime within the fourteen days prior to symptom onset or subjects who were working in any health care setting were included in the study.

Exclusion criteria: Patients suffering from other respiratory infections such as history of chronic respiratory illness, known bacterial infections and pulmonary tuberculosis were excluded from the study.

Study Procedure

Oropharyngeal samples were taken using nylon flocked swab following standard protocols as per the instructions given by the manufacturer and transported to the laboratory in viral transport media within two hours. The extraction and amplification of the viral genome was done according to standard procedure.

The test was run on TrueNat machine as per the guidelines given in the brochure. The result was read as "detected" for positive results and "not detected" for negative results. Nineteen samples were read as invalid and hence discarded from the study. All the 277 patient samples were also tested by standard RT-PCR.

TrueNat SARS-CoV-2 works on the principle of real-time RT-PCR based on Taqman chemistry. First, the RNA is extracted from the patient sample using Trueprep AUTO/AUTO V2 universal cartridge-based sample prep device and sample prep kit. RNA obtained is then assayed using TrueNat beta COV test. If sample tests positive, then it is tested for SARS-CoV-2 in TrueNat lab [9].

STATISTICAL ANALYSIS

Statistical analysis was performed using Epitools (Ausvet 2020). The statistical guidance on reporting results from studies evaluating diagnostic tests issued by the US Department of Health and Human Services were followed [10]. Proportion positive agreement, proportion negative agreement, Overall proportion agreement and Cohen's kappa coefficient with 95% confidence intervals were determined [Table/Fig-2]. Kappa value 0.81-1.00 was considered as almost perfect agreement.

Positive percent agreement=100%×a/(a+c)

Negative percent agreement=100%×d/(b+d)

Overall percent agreement=100%×(a+d)/(a+b+c+d)

Calculation of Cohen's kappa may be performed according to the following formula:

κ=Pr(a)-Pr(e)/1-Pr(e)

Where Pr(a) represents the actual observed agreement, and Pr(e) represents chance agreement.

Test performed		RT-PCR test	
Truenat	Positive	Positive	Negative
		a	b
	Negative	c	d
Total		a+c	b+d

[Table/Fig-2]: Common 2×2 table format for reporting results comparing a new test to a standard test.

RESULTS

A total of 296 oropharyngeal swabs were taken from suspected COVID-19 patients. As 19 patients were read as invalid there were 277 sample size was considered for the study. Out of 277 patients, 202 were males and 75 were femles. The mean age for males, females and total sample was 35.6, 37.3 and 33.9 years respectively. The demographic data of total sample was presented in [Table/Fig-3].

Variable	Total		Male		Female	
No. of cases	277		202		75	
Mean age of participants (years)	35.6 Range (4-71)		37.3 Range (5-71)		33.9 Range (4-65)	
Religion	H	M	H	M	H	M
	251	26	190	12	61	14
Residence	R	U	R	U	R	U
	157	120	113	89	44	31

[Table/Fig-3]: Demographical data of participants.
H: Hindu; M: Muslim; R: Rural; U: Urban

Of the 277 cases, 12 were tested positive by TrueNat while RT-PCR gave only 11 positive results. A 265 samples were tested negative by TrueNat and RT-PCR gave 266 negative results [Table/Fig-4]. PPA, NPA and overall percent agreement between the two methods were 95.65%, 99.81% and 99.64% respectively. The kappa coefficient was 0.9546 [Table/Fig-5].

	RT-PCR		
Truenat	Positive	Negative	Total
Positive	11	1	12
Negative	0	265	265
Total	11	266	277

[Table/Fig-4]: Comparison of results.

Statistics	Value
kappa	0.9546
SE kappa 0	0.0600
P (kappa)- one tailed	0.000
Proportion positive agreement	0.9565
Proportion negative agreement	0.9981
Overall proportion agreement	0.9964
Mc Nemar's chi-sq	0
P (chi-sq)	1
Absolute diff. in proportions	0.0036
Relative diff. in proportions	0.0038
SE for non-zero kappa	0.0452
Kappa lower 95% limit	0.866
Kappa upper 95% limit	1.0433

[Table/Fig-5]: Statistical analysis of Truenat vs RT-PCR test results.

DISCUSSION

Accurate and timely diagnosis of SARS-CoV-2 is crucial for policy-making, implementation of control measures, identification, isolation and contact tracing of patients and containment of people coming in contact with infected patient [8]. RT-PCR is the gold standard for diagnosis of SARS-CoV-2. The TrueNat beta COV screens through

E gene while TrueNat SARS-CoV-2 RdRp gene for confirmation. This is quicker as compared to rRT-PCR, which takes around 4-6 hour for the entire process. It has turn-around time 1.5-2 hours and run-time of only 45 minutes and is a very cost-effective procedure [6,7,9].

The present study was done to assess the practical utility and diagnostic accuracy of TrueNat testing for SARS-CoV-2 in a pandemic situation. Similar studies done in India has exhibited 100% sensitivity and specificity while this study showed PPA, NPA and overall percent agreement 95.65%, 99.81% and 99.64%, respectively [6,8,9]. This study has further confirmed TrueNat to be highly accurate method for diagnosis of SARS-CoV-2 as a good alternative to RT-PCR. Other studies were done on confirmed cases of COVID-19 while the subjects in the present study and researchers both were blind. This difference in results may be due to sampling or procedural errors [6,8,9].

The samples were collected in viral lysis buffer and hence biosafety and biosecurity requirements for use of TrueNat are minimal. The study has shown TrueNat to be the simple, reliable and useful method for the case-to-case screening and confirmation of SARS-CoV-2. It is particularly helpful for small size sample testing especially for the hospital cases awaiting outpatient as well as inpatient surgical procedures and medical emergencies. Due to availability of TrueNat the burden of the testing centres doing RT-PCR will reduce [6,8,9]. During the pandemic, a large number of samples require to be tested daily for screening and diagnostic purposes. While RT-PCR machines can test 96 samples at a run, TrueNat can test only four samples at a time. So, TrueNat can be used to test only small number samples in a day.

Limitation(s)

The sample size of the present study was small and the tests were performed only on the oropharyngeal swab. Studies with larger sample size performed in field settings are required to further validate the test. RNA degradation during storage or freeze thawing may occur leading to inability of TrueNat technique to successfully detect SAR-CoV-2 RNA but this problem can be solved with testing done at collection sites or nearby places.

CONCLUSION(S)

Authors recommend this testing procedure for community testing centres and hospitals, especially during emergencies. Being a simple, accurate and affordable technique, it may be useful for COVID-19 pandemic across the globe. Inclusion of TrueNat at testing centres will increase the testing capacity, decrease the turnaround time and hence will hasten the process of early diagnosis, management and containment of COVID-19 Pandemic.

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