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TrueNat for COVID-19 detection: An ideal screening option in emergency

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Abstract

Introduction: TrueNat is a chip-based qRT-PCR that shortens the turnaround time and thus helps in prompt diagnosis of COVID-19 and early contact tracing. In tertiary care setups, TrueNat can be utilized in emergency departments to decide the course of management of suspected COVID-19 patients.

Aim: To evaluate the diagnostic performance of the TrueNat Beta CoV E gene Screening assay, by comparing it with RT-PCR kits and assessing its utility in emergency setting.

Materials and methods: We analysed nasopharyngeal swabs of 100 patients over a period of 3 months, extracting their RNA and subjecting these to PCR using TrueNat microPCR Beta CoV E gene screening chips and three commercially available ICMR approved RT-PCR kits.

Results: There was 100% concordance among all the kits for the detection of E gene in all the 100 samples. 40 RNAs showed the presence of E gene of which 34 had a positive result for confirmatory genes. (N gene, ORF gene and RdRp gene).

Conclusion: TrueNat Beta CoV E gene screening assay is beneficial as it has comparable sensitivity and specificity to the RT-PCR kits for detection of E gene.

Keywords: TrueNat, SARS CoV-2, E gene, COVID-19

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Introduction

The effective management of COVID-19 pandemic requires rapid and accurate diagnosis for timely management and preventing transmission. Currently, quantitative real-time PCR (qRT-PCR) is the gold standard method to detect COVID-19. [1] However, it requires specialized and expensive instrumentation and expertise. Therefore such testing can only be performed in dedicated facilities, which were few in our country when the pandemic hit us. [2] Furthermore, the turnaround time for qRT-PCR is slow. The process itself typically requires at least four to six hours generating the results. This does not include the time taken to transfer samples from collection points to the testing facilities, which sometimes could be more than six hours in a hilly state like ours. The COVID-19 report thus could be delayed and aids neither in patient management nor in rapid contact tracing to prevent further transmission. Hence a rapid point-of-care (PoC) test that can identify infected individuals more quickly and accurately is desirable. TrueNAT PoC assays were designed by Molbio diagnostics for the diagnosis of COVID-19, which shorten the turnaround time and provide an added benefit of being used in field setting for diagnosis of COVID-19. [3] These platforms are portable chip-based RT-PCR, which take around 60 minutes for processing of clinical specimens from RNA extraction to amplification. TrueNat is a portable, battery-operated and fully automated system. consisting of Real Time® Truelab® micro PCR Analyzers and True prep AUTO/AUTO v2 Universal Cartridge based Sample Prep Device and room temperature stable micro PCR chips and ®Truenat AUTO Sample Prep kits. (Figure 1)[4]Availability of ready-made master mix preparation and an effective lysis buffer

Table 1. Overview of kits for RTPCR based detection of SARS CoV2 included in study

S No	Manufacturer	Name of kit	Assay type	Storage	Target gene
1.	Molbio diagnostics	TrueNat Beta CoV E	Chip-based	Room Temperature	E gene
2.	3B BlackBio Biotech India Limited	TRUPCR® SARS-CoV-2	Single tube	-20°C	E gene (screening) N gene (confirmatory)
3.	BioMerieux India private limited	SARS-COV-2 R-GENE	Two tube	-20°C	E gene (screening) N gene (confirmatory)
4.	ICMR -NIV Pune (Invitrogen enzyme &2x-MM mix)		Two tube	-20°C	E gene (screening) Orf gene and RdRp gene (confirmatory)

eliminate the requirement of biosafety cabinet to perform the tests. [5] The TrueNat workstation and chips are stable at room temperature thereby making them an ideal option in small laboratories with minimal infrastructure. [6] This workstation has three types of assays namely, TrueNat Beta CoV E gene Screening assay, TrueNat SARS CoV2 RdRp gene confirmatory assay and TrueNat Covid-19 Multiplex assay [7]. We conducted this study to evaluate the diagnostic performance of TrueNat Beta CoV E gene Screening assay, by comparing it with RT-PCR kits approved for diagnosis of COVID-19 and to determine whether a sample having a negative screening assay could have a positive confirmatory PCR thereby assessing the utility of TrueNat in emergency settings.

Materials and methods

The present study was undertaken during the months of October 2020 to March 2021 at Dr Yashwant Singh Parmar Government Medical College, Sirmaur, and Himachal Pradesh. We analysed nasopharygeal swabs of 100 patients by extracting their RNA by True Prep AUTO universal cartridge-based Sample Prep device according to manufacturer's instructions. Then these RNA were subjected to PCR using TrueNat microPCR Beta CoV E gene screening assay. All these RNAs were subjected to RT-PCR using three kits namely SARS-COV-2 R-GENE, TRUPCR® SARS-CoV-2 and TaqManr RT-PCR using ICMR-NIV two tube assay protocol. (Table 1) All these kits are approved by ICMR for molecular diagnosis of COVID-19. In clinical performance studies, SARS-COV-2 R-GENE showed 100% positive agreement and 100% negative agreement, whereas TRUPCR® SARS-CoV-2 had 96.77% and 96.67% positive percent agreement and negative percent agreement respectively based on the information provided in the respective kit inserts. The RT-PCR assays were performed on the CFX96 Touch Real-Time PCR Detection System (Bio-Rad Laboratories, Inc.) according to manufacturer's instructions.

Results

100 RNAs extracted by TruePrep AUTO universal cartridge-based Sample Prep device were analysed. 40 of them showed the presence of E gene by TrueNat and in 60, E gene was not detected. All these RNA were subjected to RT-PCR using SARS-COV-2 R-GENE, TRUPCR® SARS-CoV-2 and TaqMan RT-PCR using ICMR-NIV two tube assay protocol. The results of the detection of E gene by all 4 platforms are depicted in Table 2. There was 100% concordance among all the kits for the detection of E gene in all the 100 samples.

Table 2. Detection of E gene of SARS CoV2 by all diagnostic RT PCR kits as a screening assay

	TrueNat E Gene	
	DETECTED (40)	NOT DETECTED (60)
ARGENE E Gene	40	60

We also performed the confirmatory PCR using ICMR-NIV Pune confirmatory protocol and SARS-COV-2 R-GENE kits (TRUPCR® SARS-CoV-2 assay performed in first stage only, being

a single tube screening and confirmatory assay) on these RNAs. The cycle threshold (Ct) values of 40 RNAs, showing the presence of E gene is shown in Table 3. None of the RNAs, which were negative for E gene, showed the presence of confirmatory genes.

Table 3: Ct values by RT PCR using 3 commercially available kits of 40 RNAs having positive E gene on TrueNat

S.No	TrueNAT	TRUPCR		Argene		ICMR-NIV two tube assay		
	E gene	E gene	N gene	E gene	N gene	E gene	Orf	RdRp
1	26	25	25	24	25	25	27	29
2	25	25	24	25	25	25	26	28
3	22	23	22	21	21	22	23	25
4	18	19	19	18	18	18	19	22
5	18	18	18	18	16	17	18	20
6	29	28	28	27	29	28	32	Nd
7	20	20	21	21	21	21	21	23
8	23	23	21	22	22	22	24	26
9	30	30	Nd	31	Nd	31	Nd	Nd
10	16	16	17	17	17	17	18	20
11	29	28	29	28	28	29	31	33
12	29	28	27	28	28	29	31	Nd
13	20	19	19	19	20	20	21	23
14	18	18	20	18	19	18	20	21
15	28	28	29	28	28	28	29	31
16	26	26	25	26	26	26	27	29
17	19	18	20	20	19	19	20	22
18	18	18	18	20	20	19	20	22
19	20	19	19	20	21	20	22	24
20	22	23	22	21	22	22	23	25
21	21	22	21	21	19	21	22	24
22	18	18	18	17	21	19	20	22
23	29	28	28	28	30	28	30	31
24	30	30	Nd	31	Nd	31	Nd	Nd
25	32	31	Nd	31	Nd	31	Nd	Nd
26	22	22	21	22	20	21	22	25

S.No	TrueNAT	TRUPCR		Argene		ICMR-NIV two tube assay		
27	25	25	24	23	23	24	26	29
28	26	25	26	25	25	25	27	29
29	27	26	28	28	26	26	28	30
30	22	21	22	22	23	22	23	25
31	27	27	28	28	26	27	30	31
32	28	27	29	28	29	29	31	Nd
33	22	21	23	22	23	22	24	26
34	21	21	23	22	22	21	23	25
35	30	30	Nd	32	Nd	31	Nd	Nd
36	16	15	17	18	17	17	18	20
37	31	31	Nd	30	Nd	30	Nd	Nd
38	18	19	19	18	18	19	20	23
39	31	32	31	30	31	30	32	Nd
40	32	32	Nd	31	Nd	31	Nd	Nd

Nd - not detected

There were 6 RNA, which did not have positive confirmatory gene on any of the three confirmatory PCRs performed. Additionally, 4 RNAs did not have a detectable RdRp gene when tested using ICMR NIV confirmatory assay. Therefore, 34 of 40 RNAs were confirmed to be positive for SARS-CoV-2. (Table 4) Six patients wherein only screening assay was positive, were advised repeat sampling after 96 hours of the first sample. Only four of these patients gave repeat samples. Two of these patients turned out to be positive and two became negative for E gene as well.

Table 4: Confirmatory RT PCR results for E gene positive RNAs

PCR kit	E gene	N gene	Orf	RdRp
TRUPCR® SARS-CoV-2	40	34	NT	NT
SARS-COV-2 R-GENE	40	34	NT	NT
ICMR - NIV Pune (two tube assay)	40	NT	34	30

Discussion

TrueNat is a state-of-art technology that has proved to be beneficial for testing in far- flung areas as well as emergency departments of health-care facilities. Considering a high transmissibility of SARS-CoV-2 even from asymptomatic carriers [8], rapid and accurate diagnosis has significance in judicious treatment and breaking the chain of transmission. Early diagnosis of COVID-19 by using a PoC test guides immediate isolation of positive patient and effectual contact tracing. [9]

The results of E gene screening by all kits showed 100% concordance. This implies that the use of TrueNat for screening of COVID-19 is as good as performing any conventional RT-PCR using ICMR approved kits. All RNAs that were negative in TrueNat screening did not show any positive results in either screening or confirmatory assays performed using RT-PCR kits. Though there have been concerns that some RNAs, which are negative for E gene, could have the presence of confirmatory genes in certain studies, but we did not find such discordance in our study. [10] TrueNat screening negative patients can be given timely reports and further the cost of confirmatory PCR can be curtailed. RT- PCR can be used to confirm only samples showing a positive E gene. There were four RNAs, which did not have a detectable RdRp gene whereas they were positive for ORF gene. Such patients have a chance of being missed if tested with a kit that has only RdRp as a confirmatory target. Similar findings have been reported earlier also by Chan *et al.* [11] Though WHO recommended that E gene can be used as screening assay followed by a confirmatory assay using RdRP gene for the detection of COVID-19 cases [12] but using a single gene for conformation can miss a significant number of cases which can further fuel the epidemic. Therefore, it is advisable to use at least two confirmatory targets to confirm any RNA having a positive E gene, especially keeping in view a possible genetic drift in SARSCoV2. An ideal design would include at least one conserved region and one specific region to avoid missing any variants resulting due to mutations. [13]. As for the other targets, namely N gene and ORF gene, there was similarity between the positive RNAs wherein all RNAs, which were positive for N gene, were positive for ORF gene also. The presence of E gene in the absence of confirmatory genes indicates that Sarbecovirus RNA is detected but SARS-CoV-2 specific RNA targets are not detected. Such patients should be sampled again to confirm the presence of SARS-CoV-2. For samples with the same result on a repeated test, additional confirmatory testing should be conducted using an alternative method. A study by Ghoshal et al on 1807 samples, TrueNat had demonstrated a sensitivity, specificity and diagnostic accuracy of 69.5%, 90.9% and 89.2% respectively which is quite less than our observation.[14] Another study by Swarn et al on 277 samples, TrueNat demonstrated a positive percent agreement of 95.65%, negative percent agreement of 99.8% with overall percent agreement of 99.64%, which is comparable to our results. [15] Both these studies compared the confirmatory SARS CoV-2 chips of TrueNat with the RT PCR kits whereas we only evaluated the TrueNat as a screening assay to rule out the SARS CoV 2 infection in patients with respiratory illness.

The use of TrueNat in hospital settings in mainly in emergency departments to decide the course of management of suspected COVID-19 patients. In our hospital, we mainly employ TrueNat firstly, for pregnant females who are in labour. In case, these patients are to be operated, a timely report guides the gynaecologists and team to plan for usage of personal protective equipment (PPE) to be used during operation. This avoids wasteful consumption of PPE in times when there is already scarcity of PPE and decreases the redundant biomedical waste generation. At the advent of the COVID-19 pandemic, shortage of PPE was reported worldwide due to rise in demand, panic buying and irrational use. Similar crisis was faced during Ebola outbreak of 2014-2016 in West Africa, which resulted in higher number of health care professionals being infected. [16] Therefore appropriate and rational use of PPE was advised by WHO by giving recommendations for optimizing the availability of PPE. [17]

Secondly, we use TrueNat for samples of patients who are either brought dead or die in the emergency wards. Such bodies are to be handed over to the relatives only after confirming their COVID-19 status so that appropriate precautions can be taken during cremation of the body or during autopsy if indicated. A swift report prevents unnecessary delay and stress to already bereaved family members. Though we were not a Dedicated Covid Hospital (DCH) still all suspected patients had to be managed especially pregnant females as every time a referral to DCH was not possible. Third group of patients were those screened preoperatively or prior to admission to hospital. The implications of delayed reports are significant for such patients, including delay of urgent surgery and transfer to COVID-19 designated units and sometimes administration of inappropriate treatment.

Lastly, are the patients requiring COVID-19 reports for joining new jobs as a part of routine medical testing. Here also, giving these patients a screening negative report should suffice the purpose and avoid the performance of confirmatory PCR. As evident by our findings, none of the patients having a negative screening test, turned out to have a positive confirmatory PCR. Thus already limited resources could be saved. One disadvantage of TrueNat is its high cost but reduced transmission from faster diagnosis, and isolation could improve the cost-effectiveness of PoC testing at a broader scale.

The limitation of our study was the less number of samples tested, but we performed the detection of E gene with three commercially available RT PCR kits to support the use of TrueNat as emergency screening assay. In conclusion, use of TrueNat is advantageous in terms of its ease of use, prompt results and comparable sensitivity and specificity to the RT-PCR kits. This PoC test could be used in distant sites aiding early detection on one hand and reducing the load of testing samples from suspected COVID-19 patients on main hospitals on the other hand. It is truly advantageous in emergency settings saving precious time and aiding rapid management of suspected patients. Using TrueNat could decentralize the rapid molecular diagnosis of COVID-19 and thus it is conducive to daily epidemic control.

Conflicts of interest: none

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