Original Article

DOI: 10.7860/JCDR/2022/55009.16400

Microbiology Section

SARS-CoV-2 Positivity using Closed System RT-PCR at a Tertiary Care Medical Institute in Manipur, India

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ABSTRACT

Introduction: Rapid cost effective, Point-of-Care (PoC) Truenat assay for the diagnosis of Severe Acute Respiratory Syndrome Corona Virus 2 (SARS-CoV-2) have been developed to shorten the Turn Around Time (TAT) of reporting with a wireless data transfer system.

Aim: To explore the SARS-CoV-2 positivity using closed system Reverse Transcriptase Polymerase Chain Reaction (RT-PCR).

Materials and Methods: An observational cross-sectional study was carried out in Molecular Laboratory of, Department of Microbiology, Jawaharlal Nehru Institute of Medical Sciences, Imphal, Manipur, India, using Truenat RT-PCR (Molbio diagnostics) and data was entered from May 2020 to April 2021. Manufacturer's literature was followed while performing the test. Screening of sample was done with Envelope (E) gene test and confirmed with RNA-dependent RNA polymerase gene (*RdRP*) gene test. Statistical analysis was done using Microsoft Excel sheet by calculating the percentage, proportions.

Results: A total of 1,528 individuals were tested for SARS-Cov-2 and 73 tests were reported positive. The positivity rate by age was highest among 21-30 years. The positivity rate was higher among males than females. Among 1,105 asymptomatic individual, 27 (2.4%) were positive and among 423 symptomatic, 46 (10.9%) were positive.

Conclusion: Using Truenat, positivity rate among symptomatic Coronavirus Disease 2019 (COVID-19) suspected persons was about four times more than positivity rate among exposed contact persons who are asymptomatic.

Keywords: Coronavirus, Point-of-care, Reverse transcriptase nucleic acid amplification test

INTRODUCTION

On 9th January, 2020, Chinese Center for Disease Control and Prevention (CDC) reported a novel coronavirus as the causative agent of this outbreak and on 11th February 2020, World Health Organisation (WHO) named the disease had COVID-19. From China, the disease spread to other parts of the globe, including Europe, USA, the middle East and parts of Asia including India [1,2]. By 11th March 2020, WHO declared SARS-CoV-2 as a global pandemic [3]. The first few cases of SARS-CoV-2 in India were reported from Kerala, among three Indian medical students who had returned from Wuhan [4-6]. The infection had spread dynamically over India, with cases linked to people with a travel history from affected countries [7].

On March 24, 2020, Manipur in India's North East was in abundant panic among its public with the confirmation of its 1st SARS-CoV-2 positive case, a 23-year-old student returning from the United Kingdom. Since then, the state has recorded a gradual rise in cases of the coronavirus as migrant workers returned to their home state by special trains and buses in April and May [8].

The outpouring of SARS-CoV-2 cases in India and across the world demands a rapid and sensitive molecular assay. Truenat is a rapid PoC diagnostic assay developed for detection of COVID-19 which is expected to shorten the TAT of reporting the results. Truenat machine is a portable indigenous chip-based rRT-PCR (real-time reverse transcriptase polymerase chain reaction) designed for rapid diagnosis of infectious diseases, including COVID-19. The processing of samples and RNA extraction to amplification can be achieved in <60 min. Availability of ready-made master mix prep adds additional benefit in gearing up the process [9]. For conventional RT-PCR, allowing for sample handling and processing, typically takes 4–6 hours to complete, and the transportation of clinical samples can often increase the TAT to more than 24 hour, potentially resulting in delays in diagnosis and inappropriate infection-control precautions. To the best of the knowledge this is so far the first study in Manipur regarding analysis of Truenat SARS-CoV-2 positivity rate. The data is evolving with the upcoming trend.

The aim of the study was to find SARS-CoV-2 positivity rate using closed system RT- PCR among symptomatic patients suspected of COVID-19 and asymptomatic contacts/exposed person.

MATERIALS AND METHODS

This was an observational cross-sectional study carried out in the Molecular Laboratory of Jawaharlal Nehru Institute of Medical Sciences, Imphal, Manipur, India from May 2020 to April 2021. A total of 1,528 patients irrespective of age and gender, who came for testing and fulfilled the ICMR criteria during that period were included in the study. Informed consent was taken before participating in the study. In our setup, ICMR approved TRUELAB Uno Dx PCR analyser machine from Molbio Diagnostics was used for performing Beta CoV and SARS-CoV-2 assays. The ICMR advisories were followed for selection and sampling of patients and exposed persons [10]. Approval of ethical committee was obtained from the Institutional Ethical Committee JNIMS.

Inclusion criteria

1) Symptomatic Influenza Like Illness (ILI) symptoms individuals with history of international travel in the last 14 days.
2) Hospitalised patients who develop ILI (ILI is defined as one with acute respiratory infection with fever ≥38°C and cough) symptoms.
3) Patients of Severe Acute Respiratory Infection (SARI).
4) Asymptomatic direct and high-risk contacts of a confirmed case tested once between day 5 and day 10 of coming into contact.

Exclusion criteria: Samples from other respiratory infections like bacterial pneumonia and tuberculosis were excluded.

A person is said to be COVID-19 suspect would be defined as:

A- A patient with acute respiratory illness (fever and at least one sign/symptom of respiratory disease, e.g., cough, shortness of breath), and a history of travel to or residence in a location reporting community transmission of COVID-19 disease during the 14 days prior to symptom onset;
OR **B**-A patient with any acute respiratory illness and having been in contact with a confirmed or probable COVID-19 case in the last 14 days prior to symptom onset [11-14];

OR **C-**A patient with severe acute respiratory illness (fever and at least one sign/symptom of respiratory disease, e.g., cough, shortness of breath; and requiring hospitalisation) and in the absence of an alternative diagnosis that fully explains the clinical presentation [15].

Contact person: A person who had experienced any one of the following exposures during the 2 days before and the 14 days after the onset of symptoms of a probable or confirmed case:

1) Face-to-face contact with a probable or confirmed case within 1 meter and for >15 minutes;
2) Direct physical contact with a probable or confirmed case;
3) Direct care for a patient with probable or confirmed COVID-19 disease without using proper personal protective equipment [16].

Sample collection and nucleic acid extraction: Oropharyngeal and nasopharyngeal swab specimens were collected from the patients following standard protocols of CDC with nylon flocked swabs [17]. The swabs with the specimen were inserted into the Viral Transport Medium (VTM) tube and transported to the Molecular Lab. The sample was processed inside biosafety cabinet level 2. Then swab was repeatedly twirled to mix the specimen with the buffer solution. The swab was then gently broken at a point which was provided in the swab stick and the swab was left in the tube containing the transport medium with the lid tightly capped to prevent spillage. The transport medium used lyses and decontaminates the virus so that it can be easily transported and stored without posing a hazard.

Swab sample of 0.5 mL was transferred into the lysis buffer bottle using 1 mL transfer pipette. Then, the entire content of the lysis buffer tube were transferred to the sample chamber (black cap) of cartridge using 3 mL transfer pipette. The cartridge was inserted into the extraction machine, beep sound at the end of the process (20 min) indicated the finish of extraction.

The elute was collected in elute collection tube, then 6 µL of it was dispensed into microtube containing freeze dried RT-PCR reagents. It was incubated for 30 seconds at room temperature to obtain a clear solution. Using the same pipette and tip the clear solution was dispensed into the white reaction well of the microchip and the PCR amplification machine starts. Single assay had a TAT of 60 mins.

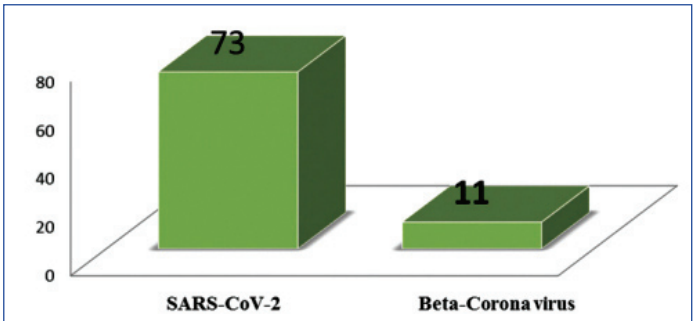
Amplification test and interpretation: At the end of 60 minutes, a graphical representation of the data with three amplification curves was displayed on the Real Time PCR analyser screen to indicate the progress of the test. Both the target and the Internal Positive Control (IPC) curves will take a sheer, expanding path when the fluorescence will cross the threshold value in case of positive samples. The Cycle threshold (Ct) of the specimen will depend on the number of virus copies in the sample [18]. There will be a straight curve throughout the test duration and the curve will take a augmented path in case of negative samples. In case the IPC curve remains horizontal, the test is considered as Invalid. At the end of the test run, the results screen will display "DETECTED" for Positive result or "NOT DETECTED" for Negative result. The result screen in the truenat machine would also display the viral load as "HIGH" (Ct<20), "MEDIUM" (20≤Ct<25),"LOW" (25≤Ct<30) or " VERY LOW" (Ct≥30) for positive sample. It also displays the validity of the test run as "VALID" or "INVALID" as per kit instructions. Invalid samples have to be repeated with fresh specimen from the sample preparation stage [18].

STATISTICAL ANALYSIS

Data was analysed using Microsoft Excel sheet by calculating the percentage, proportions.

RESULTS

During the study period, a total of 1,528 nasopharyngeal swab samples were collected and processed for SARS-CoV-2. Out of these, 73 samples (5%) were SARS-CoV-2 positive. Of the 1,528 samples, a total of 84 samples were found to be positive. Out of 84 total positives for *E* gene, 73 samples found to be confirmed positive by *RdRp* gene test. Rest 11 samples were found negative for *RdRp* gene, hence they were Beta-Corona virus. So, total 73 samples were found positive for SARS CoV-2 [Table/Fig-1].



[Table/Fig-1]: Histogram showing comparison between SARS-CoV-2 and Beta Coronavirus.

The highest positivity (21.9%) was observed in the 21-30 years age group followed by 51-60 years (20.6%). The gender wise distribution showed males 45 (62%) outnumber the females 28 (38%) [Table/Fig-2].

Age group (in years)	Total no. of positivity, n (%)	Male	Female
0-10	2 (2.7)	1 (2.2)	1 (3.6)
11-20	2 (2.7)	2 (4.4)	0
21-30	16 (21.9)	12 (26.7)	4 (14.3)
31-40	12 (16.5)	8 (17.8)	4 (14.3)
41-50	13 (17.8)	6 (13.3)	7 (25)
51-60	15 (20.6)	9 (20)	6 (21.4)
61-70	6 (8.2)	3 (6.7)	3 (10.7)
71-80	5 (6.9)	3 (6.7)	2 (7.1)
81-90	2 (2.7)	1 (2.2)	1 (3.6)
Total (%)	73 (100)	45 (62)	28 (38)

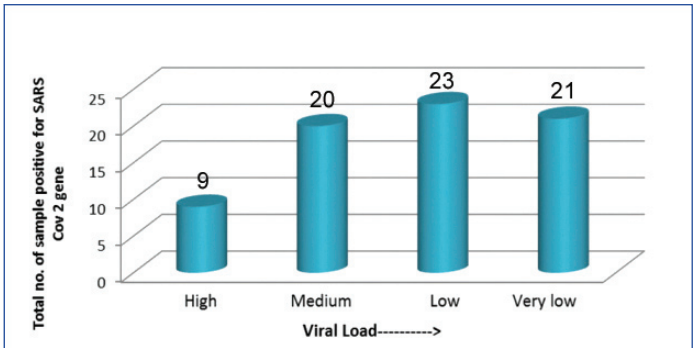
[Table/Fig-2]: Age group and gender distribution among COVID-19 positive samples.

The [Table/Fig-3] depicts distribution of SARS-CoV-2 gene among contact person and COVID-19 suspect. Of 1105 contact person, 27 were positive and among 423 suspect, 46 were positive.

Contact person	COVID-19 suspect	Positive among Contact person	Positive among COVID suspect
1105	423	27 (2.4%)	46 (10.9%)

[Table/Fig-3]: Distribution of SARS-CoV-2 gene.

Analysis of distribution of SARS-CoV-2 gene among the positive samples showed that out of the 73 positive samples, nine of them were detected very high viral load while 20 were detected medium viral load [Table/Fig-4].



[Table/Fig-4]: Distribution of viral load among SARS-CoV-2 gene.

DISCUSSION

Truenat is an ICMR approved real time RT-PCR system for diagnosis of SARS-COV-2 in India. It is a disposable, chip-based RT-PCR test which is based on TaqMan and uses the *E*-gene from Beta-coronavirus for screening of infected individuals followed by confirmatory test using the *RdRp* gene of SARS-CoV-2. Truenat was found to have 100% sensitivity and 99% specificity in recently published data by A Sahoo et al., [19].

In current study, the overall positivity rate was 5% as compared to the national average of 7.3% [9]. It was observed in present study that males outnumbered the females, making up 62% of the total positive samples which was similar to the studies by Indian study like Kushwaha S et al., [20]. Bakshi AS et al., [21], which was also observed in other country studies, Huang C et al., (73%), Xu XW et al., [22,23]. The reason for this male preponderance may be due to higher sampling in males, higher susceptibility and greater chances of exposure. It can also be noted that in the study published by Jin JM et al., report of high protein expression of Angiotensin-Converting Enzyme 2 (ACE2) receptor in specific organs correlated with specific organ failures, indicated by corresponding clinical parameters in SARS patient [24]. The circulating ACE-2 levels are higher in male than in females as well as in patients with diabetes or cardiovascular diseases [23].

It was also observed that the highest positivity rate was distributed to age group between 21-30 and the lowest was observed in the extremes of the ages i.e, 0-10 and 81-90. This may be due the fact that these age groups are not exposed as much as the young adults. As there is a belief in the society that in older age groups this disease is more fatal and in younger ones less severe and non-fatal. It might be talked and discussed much regarding the severity in old age in various social platforms like radio, facebook, local cable channel and older age people (>80 years) usually most of them stays at home because of their ill health and during COVID times they were more cautious so exposure might be less on these groups. In a study done by ICMR, the highest number of cases were those aged 50-59 and 60-69 years with the highest attack rate in males [25].

In present study, out of the 1528 samples, a total of 73 samples were found to be positive for both *E*-gene and *RdRp* gene. 11 (0.71%) samples showed only *E*-gene positivity with negative *RdRp*. 18 samples out of the 1528 gave invalid result. Among 1105 asymptomatic individual, 27 (2.4%) were positive and among 423 symptomatic 46 (10.9%) were positive for SARS-CoV-2. The cause of the invalid report could be attributed to various reasons such as poor execution of sample collection leading to poor quality of sample, extraction error, and failure to read the chip memory by the analyser or a faulty chip, failure of amplification of the internal control. In a study done by Sadhna S and Hawaldar R, 93 samples were *E*-gene positive and of which 73 were positive with a confirmatory test [26]. As Truenat will detect the virus early, it becomes the investigation of choice when there is urgent need.

Limitation(s)

The Truenat positive COVID-19 samples were not compared with conventional RT-PCR.

CONCLUSION(S)

Using Truenat, positivity rate among symptomatic COVID-19 suspected persons was about four times more than positivity rate among exposed contact persons who were asymptomatic. As Truenat is an indigenous tool for detection of COVID-19 it can be used along with conventional RT-PCR to shorten the turn over time.

Acknowledgment

Authors would like to acknowledge RNTCP (Revised National Tuberculosis Control Programme) Manipur Branch for providing our college with Truenat Machine.

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PLAGIARISM CHECKING METHODS: [Jain H et al.]
- Plagiarism X-checker: Jan 19, 2022
- Manual Googling: Apr 25, 2022
- iThenticate Software: Apr 27, 2022 (18%)

ETYMOLOGY: Author Origin

AUTHOR DECLARATION:
- Financial or Other Competing Interests: None
- Was Ethics Committee Approval obtained for this study? Yes
- Was informed consent obtained from the subjects involved in the study? Yes
- For any images presented appropriate consent has been obtained from the subjects. NA

Date of Submission: **Jan 18, 2022**
Date of Peer Review: **Feb 03, 2022**
Date of Acceptance: **Apr 25, 2022**
Date of Publishing: **May 01, 2022**