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Original Research

TRUENAT: A Rapid RT-PCR Test for the detection of SARS CoV2 infection at a tertiary care hospital in J&K

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ABSTRACT:

Background- The rapid spread of SARS Cov2 infection all across the globe has brought the world to a standstill. Early accurate diagnosis and quick management of cases is the need of the hour for the containment of virus. ICMR recommended the use of True Nat machine for Covid19 testing in April 2020. **Method**- The present study was conducted at a tertiary care center, approved by Indian Council of Medical Research for COVID-19 TrueNat testing from July 2020 to June 2021. A total of 350 Patients of all ages and both sexes were included in the study. **Result**- Males (68.57%) constituted majority of our study population. The most common age group involved in the study was between 21-40 years (61.7%). Out of total 69 E gene positive patients, 54 were positive by a confirmatory RdRp gene with the overall positivity of 19.71%. Asymptomatic positive cases accounted for 17.639% of the total positive cases. **Conclusion**- TrueNat machine can be used for rapid confirmation of COVID-19 cases especially in field settings as it demands less technical expertise as well as minimal infrastructural requirements.

Keywords- Confirmatory, Expertise, ICMR, TrueNat Testing

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INTRODUCTION

Severe acute respiratory syndrome corona virus 2 (SARS-CoV-2) has come up as an unprecedented challenge all across the globe. It has spread its reach even to the remotest places of the world. The first case of COVID-19 in India was reported on 30th January 2020. Currently, India has the largest number of confirmed cases in Asia. As of 9th July 2021, India has the second-highest number of confirmed cases in the world (after the United States) with 3.07 crore reported cases of COVID-19 infection and the third-highest number of COVID-19 deaths (after the United States and Brazil) at 4.05 lakhs deaths. At the time of writing this manuscript, Jammu and Kashmir UT has reported 3.18 lakh confirmed cases with 4349 deaths recorded. [1]

WHO's advice to curb spread of Coronavirus was "Test, trace, isolate and treat". Nationwide complete lockdown was implemented in India on 24th March 2020. This time was utilized for strengthening healthcare facilities and enhancing COVID-19 management capacity. The COVID-19 testing laboratories in the country for detection of SARS-CoV-2 were exponentially increased in short span of time. From National Institute of Virology, Pune being the only one authorized laboratory in the country in Jan 2020 today we have scaled upto 1,751 (1059 government labs and 692 private labs) in May 2021 in the entire country.[2] CBNAAT and TrueNat testing were also approved by ICMR for detection of SARS-CoV-2 in addition to conventional rRT-PCR testing. Jammu and Kashmir UT has 13 labs performing rRT-PCR; 19 labs TrueNat tests and 3 CBNAAT labs for COVID-19 diagnosis by June 2021 [2].

TrueNat machines were originally designed for the efficient detection of *Mycobacterium tuberculosis* at the primary health-care level, as these work on batteries contrary to the GeneXpert® machines which need air conditioner for proper functioning [3]. In April, 2020 ICMR issued guidelines for utilization of TrueNat RTPCR machines for COVID-19 testing. [4] This test uses customized cartridges and has quick turnaround time (30-60 minutes), but only 1-4 samples can be tested in one run, limiting the maximum numbers that can be tested to 24-48

samples per day only. The sensitivity of this test is 50-80%, and specificity is 90-95%. Because of closed nature platform and minimums ample handling, this test poses a minimum bio-safety Hazard and have increased access to testing. [5]

The present study was undertaken in Department of Microbiology, GMC Rajouri with the aim to evaluate the performance of Truenat Beta CoV E-gene screening assay and Truenat SARS-CoV-2 RdRp gene-confirmatory assay (Molbio Diagnostics, India) for the detection of SARS CoV 2 infection. As far as our knowledge, this is the first study in the J&K UT on TrueNat testing for COVID-19.

MATERIAL AND METHODS

This was an observational study conducted in ICMR Approved laboratory for TrueNat Covid19 testing, Department of Microbiology, GMC Rajouri from July 2020 to June 2021. A total of 350 patients were included in the study. The patients were selected for testing as per the ICMR guidelines for TrueNat Testing for SARS CoV 2. [3] Samples from other respiratory infections like bacterial pneumonia and tuberculosis were excluded.

TRUENAT PRINCIPAL AND PROCEDURE

SAMPLE COLLECTION

Nasopharyngeal swab specimen was collected as per standard procedures using nylon flocked swab. The swab was inserted into the Viral Lysis Medium. The lysis buffer inactivates the virus if it is present in the sample making it non-infectious. Details of the patients were recorded in Sample Reference form (SRF). All the specimens were processed in Biosafety cabinat II. The biomedical waste items (including the samples) were discarded in red bag after disinfection in 5% sodium hypochlorite.

SAMPLE PROCESSING

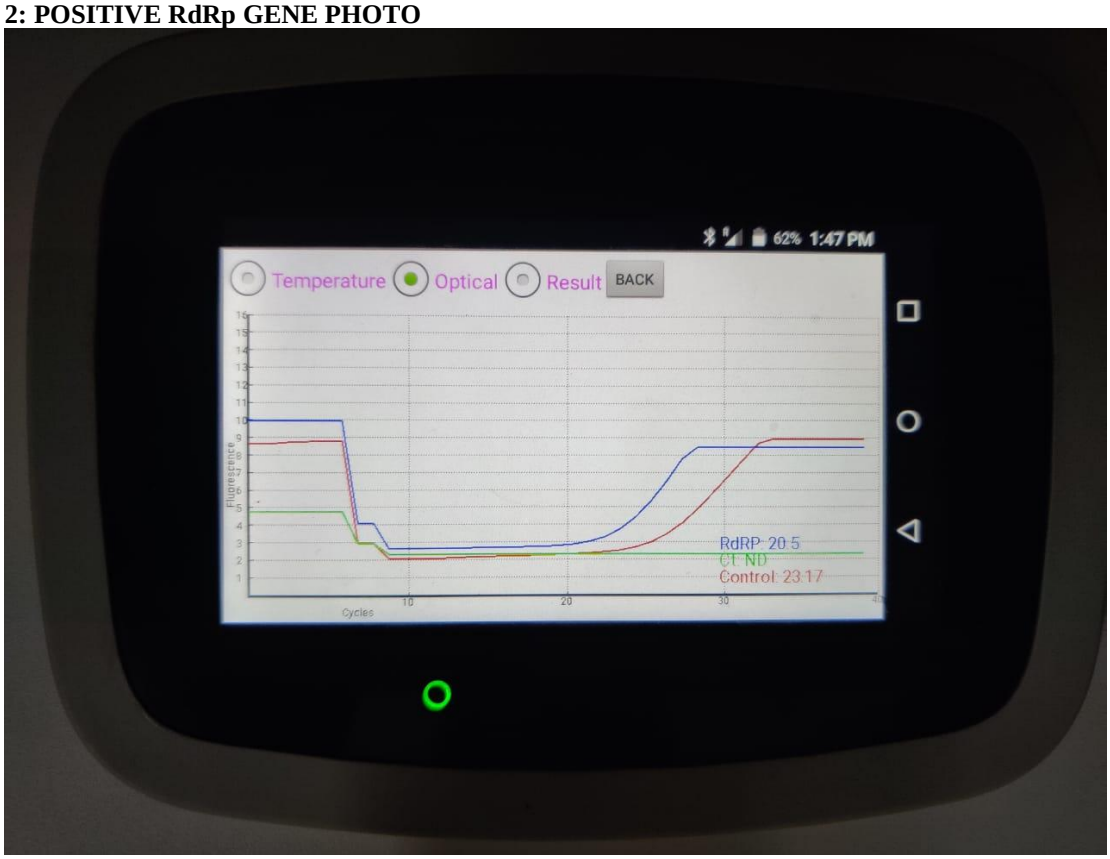
Truenat is a closed system which works on the principal of real-time reverse transcription polymerase chain reaction (rRT-PCR). TruenatTM Beta CoV is a chip-based RT-PCR which detected the screening E gene in the sample. E gene positive samples were then confirmed by using TruenatTM SARS CoV-2 chip-based RT-PCR. It detected the confirmatory RdRp gene. Both screening and confirmatory genes detected samples were taken as positive.

Trueprep AUTO Universal Cartridge based sample preparation device and Trueprep AUTO Universal Cartridge based sample PrepKit were used for the extraction of RNA from the patient sample. The Truenat Beta CoV chip was placed on the chip tray of the Truelab™ Real Time quantitative micro PCR Analyzer. 6 µL of extracted RNA was dispensed into the PCR reagents and allowed to stand for 30 seconds for a clear solution. 6 µL of this solution was then dispensed into the reaction well of the Truenat Beta CoV chip and the chip was inserted into the TruelabTM real time quantitative micro PCR Analyzer.

A positive amplification was indicated by the release of fluorophores in an exponential manner which was displayed as an amplification curve on the screen. The cycle threshold (Ct) is the number of amplification cycles required for the signal to cross the fluorescence threshold, which is inversely proportional to the amount of target nucleic acid in the sample. The results were interpreted as not detected, very low detected, low detected, medium detected and high detected.

FIG 1: TRUENAT MACHINE PHOTO

FIG 2: POSITIVE RdRp GENE PHOTO



RESULTS

During the study period of 11 months**,** a total of 350 samples were tested. Of the total samples tested, 69 (19.71%) were found to be positive by TrueNat. 240 (68.57%) males and 110 (31.42%) females were included in this study. Maximum patients were in age group 21-40 years (61.7%) followed by 41-60 years (24%), 61-80 years (8.85%) , <20 years (4.57%) and >80 years (0.8%). [Table1]

Table 1: Age-wise distribution of study population

Age-group	Male	Female	Total
<20 yrs	10	6	16 (4.57%)
21-40 yrs	145	71	216 (61.7%)
41-60 yrs	60	24	84 (24%)
61-80 yrs	23	8	31 (8.85%)
>80 yrs	2	1	3(0.8%)
Total	240	110	350

Data analyzed for SARS CoV2 E gene positive patients showed that maximum positive patients were in age group 21- 40 yrs (n=35), followed by 41-60 yrs of age group in which 18 patients were found positive, 12 in 61-80 years of age. 2 patients were found E gene positive each in < 20 years and > 80 years of age. Overall E gene positivity in our study was found to be 69 (19.71%). [Table 2]

Table 2: Showing age wise distribution of E gene positive and negative patients

Age (Yrs)	Male		Female	
	Egene Positive	Egene Negative	Egene Positive	Egene Negative
<20 yrs	**2**	**8**	**0**	**6**
21-40 yrs	**29**	**116**	**6**	**65**
41-60 yrs	**13**	**47**	**5**	**19**
61-80 yrs	**9**	**14**	**3**	**5**
>80 yrs	**1**	**1**	**1**	**0**
Total	**54**	**186**	**15**	**95**

In 4.57% of the patients E gene was detected very low, 3.42% had low detected E gene, 6% had medium detected E gene and in 5.71% patients, the E gene was detected high. [Table 3]

Table 3: Showing distribution of E gene

Count	350	
Not detected	281	80.28%
Very low detected	16	4.57%
low detected	12	3.42%
Medium detected	21	6%
High detected	20	5.71%
Total	63	

The minimum Ct value of very low detected E gene patients was 30.2 with a maximum of 34.33 and average Ct value of 29.27. In the low detected category, minimum and maximum Ct value was 25.17 and 29.4 respectively with an average Ct value of 27.89. In the medium detected category, the minimum, maximum and average Ct value was 21.0, 33.0 and 21.86 respectively. The minimum Ct value of high detected E gene patients was 9.33 with a maximum of 18.8 with an average of 12.95. [Table 4]

Table 4: Showing Ct value of E gene in different categories

	Very low Detected	Low detected	Medium detected	High detected
Minimum	30.2	25.17	21.0	9.33
Maximum	34.33	29.4	33.0	18.8
Average	29.27	27.89	21.86	12.95

Very low detected cases accounted for 25.39% of all E gene positive patients out of which 62.5% tested positive for confirmatory RdRp gene. Low detected E gene accounted for 19.04% patients with 75% patients positive for RdRp gene. Medium detected were 33.33% of all with 80.95% testing positive with confirmatory test. High detected E gene positive patients accounted for 31.74% of all E gene positive patients out of which 90% were positive by confirmatory test. [Table 5]

Table 5: Showing positivity rate of E gene and confirmatory Assay using RdRp gene

	Very low Detected	Low Detected	Medium Detected	High Detected
Negative	6	3	4	2
Positive	10	9	17	18
Positive %	62.5%	75%	80.95%	90%
Case Distribution	25.39%	19.04%	33.33%	31.74%

Out of the 69 confirmed SARS CoV 2 positive patients, symptomatic cases accounted for 30 (43.47%), followed by contacts of positive patients 18 (26.08%) and asymptomatic 12 (17.639%). Follow-up previously positive patients were found to be 4 (5.79%). Pregnant females and pre-operative patients were seen to be 3 (4.3%) and 2 (2.89%) cases positive respectively. [Table 6]

Table 6: Categories of Patients

Category	Positive	Negative
Symptomatic	30	12
Asymptomatic	12	41
Preoperative	2	74
ANC	3	96
Contact of Positive patient	18	44
Follow-up	4	14
Total	69	281

DISCUSSION

With the surge in cases of COVID-19 in India and over 150 countries across the globe, a rapid point of care assay is considered to be a major goal towards the containment of cases. Molecular assays on PoC platforms have the added advantage of higher applicability in field settings for rapid screening and confirmation of SARS-CoV-2 cases without

compromising the diagnostic parameters. [6] With this objective in mind, performance of Truenat Beta CoV and Truenat SARS-CoV-2 PoC assay for the detection of SARS-CoV-2 suspected cases was evaluated in our Microbiology laboratory.

The overall positivity rate in our study was found to be 19.71% as compared to national positivity rate of 8% as on June 24, 2021. This is higher as compared to national data, which may be because most of the patients enrolled in our study were symptomatic and contacts of positive patients.

Majority of the cases were in the age group of 21-40 years with male predominance. Similar results were seen in studies conducted by Kashyap B *et al* [7] and Wang *et al* [8]. This may be because males had higher chances of exposure to infection due to active lifestyle and testing was more in males as compared to females.

In Truenat RT PCR, E gene detected the numerous Coronaviruses including SARS CoV2 while RdRp gene only detected SARS CoV 2 which is used as a confirmatory test. E gene was found to be positive in 69 patients out of which 54 (78.26%) were confirmed by RdRp gene. This is in concordance with study conducted by Alagarasu K et al [9] where positivity by RdRp gene was found to be 79.2%.

In our study, asymptomatic positive cases accounted for 17.639% of the positive cases. Similar findings were observed by Sadhna S *et al* [10]. This shows that asymptomatic carriers are a huge challenge for any country for the simple reason that people infected with the virus don't even know they have it and therefore unknowingly may infect many others. Rapid and accurate detection of COVID-19 is pivotal in controlling outbreaks in the community and hospitals.

CONCLUSION

The current pandemic of Covid-19 has revolutionized laboratory medicine. Rapid and accurate Covid-19 testing is the need of the hour in early management of cases. Nucleic acid amplification test (NAAT) and Reverse Transcriptase Polymerase Chain Reaction (RT-PCR) are the gold standard diagnostic tools for dealing with COVID-19. However, they have several technological challenges. Truenat Beta CoV and Truenat SARS-CoV-2 PoC assays, targeting *E* and *RdRp-2* genes may be recommended for screening and confirmation, respectively, of suspected cases of COVID-19. These assays would be of value in rapid confirmation of COVID-19 cases especially in field settings as it demands less technical expertise as well as minimal infrastructural requirements.

REFERENCES

1. Ministry of Health and Welfare, Government of India. COVID-19 India. Available from: https://www.mohfw.gov.in. [Last accessed on 2021 June 12].
2. Indian Council of Medical Research Department of Health Research, Ministry of Health and Family Welfare, Government of India. Total Operational (Initiated Independent Testing) Laboratories Reporting to ICMR. Available from:https://www.icmr.gov.in/pdf/covid/labs/COVID_Testing_Labs_16092020.pdf. [Last accessed on 2021 June 18].
3. Indian Council of Medical Research Department of Health Research, Ministry of Health and Family Welfare, Government of India. Guidance on the Use of TruenatTM Beta CoV. Available from:https://www.icmr.nic.in/sites/default/files/upload_documents/Guidance_TrueNat_14042020.pdf. [Last accessed on 2020 Sep 04].
4. Ravichandran K, Anbazhagan S, Singh SV, *et al*. Global status of COVID-19 diagnosis: An overview. J Pure Appl Microbiol 2020; 14:879-2.
5. Mohanty A, Kabi A, Mohanty A.P ,et al. Laboratory Diagnosis of COVID-19 Infection: Current Issues and Challenges: An Indian Perspective; JAMMR, 32(14): 10-17, 2020; Article no.JAMMR.60513
6. Basawarajappa SG, Rangaiah A, Padukone S,et al: Performance evaluation of truenat sars-cov-2 poc assays. Indian J Med Res 153, January & February 2021, pp 144-150.
7. Kashyap B, Jhamb R, PS Narendra, et al: Socio-Demographic and Clinical Profile of Health Care Workers Diagnosed for COVID-19 by Truenat at a Tertiary Care COVID Hospital. Hospital Practices and Research 2021; 6(1):11-17.
8. Huang C, Wang Y, Li X, Ren L, Zhao J, Hu Y, et al. Clinical features of patients infected with 2019 novel coronavirus in Wuhan, China. *Lancet*. 2020 ; 395:497–506.
9. Alagarasu K, Choudhary ML, Lole KS, Abraham P, Potdar V, et al. Evaluation of RdRp & ORF-1b-nsp14-based real-time RT-PCR assays for confirmation of SARS-CoV-2 infection: An observational study :Indian. *J Med Res*. 2020; 151(5):483–5.
10. Sadhna S, Hawaldar R. Evaluation of Truenat RT PCR for diagnosis of SARS CoV2 infection- An observational study. *Indian J Microbiol Res* 2020; 7(3):265-269.