European Journal of Molecular & Clinical Medicine

ISSN 2515-8260 Volume 10, Issue 1, Winter 2023

ORIGINAL RESEARCH

Endorsement of TRUENAT Machine: A Major Tipping Point In Rapid Point Of Care Test For COVID-19

[1]Dr. Priya Gupta, [2]Dr. Deepali Mittal, [3]Dr. Harish Kumar Sagar, [4]Dr. Nidhi Verma, [5]Dr. Abhishake Kumar

[1,2,4,] Department of Pathology, LLRM Medical College, Meerut, Uttar Pradesh, India
[3]Department of Clinical Medicine, ICMR-NJIL&OMD, Agra, Uttar Pradesh, India
[5]Department of Anaesthesia,LLRM Medical College,Meerut,Uttar Pradesh,India

Corresponding author
Dr. Abhishake Kumar
R-18,, LLRM Medical College, Meerut, Uttar Pradesh, India
Email: dr_abhishek1979@rediffmail.com

ABSTRACT

Introduction: COVID- 19 caused by Corovirus, is a global pandemic which our country has witnessed in its most disastrous way. Early diagnosis is always a prerequisite for a country in early detection and decreasing the prevalence in the world. As we have faced the second disastrous wave in India where mortality rate increased upto 10% of detected cases, it is a strict prerequisite to lean on methods encouraging early diagnosis and prompt treatment. TrueNat, a nucleic acid amplification test, offers a fast, point of care diagnostic method which is a reliable, time bound and affordable.

Aims and Objective: The study warrants a compact, easy to approach, alternative diagnostic tool with possible measures like mobile NAAT labs(MiLaN) in a developing country like India which would help in timely diagnosis with approach to distant places in search of hidden cases with use of minimal resources. this study also aimed at the need for capacity building of technician which would further decrease the chances of technical inaccuracies

Materials and Methods: This is the study of 3310 samples received at a tertiary care centre in North India by a chip-based, real-time quantitative PCR system manufactured by Molbio diagnostics, Goa

Results: Comparative analysis of samples with different age groups were done in High detected, Medium detected, Low detected and Very low detected cases.

Discussion: Prompt timely diagnosis with approach to distant places in search of detection of hidden cases with high sensitivity and specificity warrants a compact, easy to approach, alternative diagnostic tool with possible measures like mobile NAAT labs (MiLaN) and capacity building of skilled technicians leading to decrease in technical inaccuracies.

Conclusion: Due to machine portability and accessible technical skills, True nat machine is highly recommended in settings where facilities for tertiary care laboratories are lagging.

Keywords: Truenat, COVID-19, MiLaN

INTRODUCTION

The previous year 2021 had faced severe global pandemic. In late December 2019, there was a surge in cases of pneumonia of unknown cause which were connected to seafood and wet animal market in Wuhan city of China.[1,2,3]

This disease was named as the Corona Virus Disease 2019 (COVID-19) and the etiological agent responsible for this global pandemic was a novel corona virus named severe acute respiratory syndrome corona virus (SARS-CoV-2).[2,3]

This virus bears similarities to the viral agent which were responsible for severe acute respiratory syndrome (SARS) outbreak of 2002-2003 and the Middle East respiratory syndrome (MERS) outbreak of 2012-2013.[4,5]

Coronavirus are positive single stranded enveloped RNA Virus which are divided into four genera: Alpha-, Beta-, Gamma-, and Delta coronavirus.[6]

The genome encodes 27 proteins including an RNA-dependent RNA polymerase (RdRP) and four structural proteins which includes spike surface glycoprotein (S), small envelope protein (E), matrix protein (M), and nucleocapsid protein (N).[7]

The transmission rate of SARS-CoV-2 was found to be much higher than other human corona virus strains. This disease caused fatal damage to the lungs leading to respiratory failure and death. Detrimental effects of the disease was more in individuals over 65 years of age, smokers and people with chronic diseases such as hypertension, diabetes, kidney failure.[8]

Strict measures were implemented by World health organization (WHO) and the mission of "detect, protect and treat" was set to shatter the chain of transmission of SARS-COV -2.

The various diagnostic tests to diagnose covid19 disease includes Nucleic Acid Amplification Testing (NAAT) such as real-time reverse transcriptase polymerase chain reactions (RT-PCR), serological tests and viral culture.[9,10] Perhaps RTPCR is considered as the gold standard due to its high sensitivity and specificity, still has enough limitations in which protracted time is one of the factor.

The emphasis was laid on early and accurate diagnosis and prompt treatment as the infectivity rate of this disease was alarming and timely medical intervention could save people's life.[9,11]

Oghn 8th April 2020 Indian Council of Medical Research (ICMR) came up with a connatural scaffold, a Truenat machine, based on nucleic acid amplification, which offered a reliable and affordable option to increase the testing capacity in India.[12,13]

Truenat MTB test has already been declared a revolutionary asset for early diagnosis of mycobacterium tuberculosis for detection of tuberculosis, with a good sensitivity and specificity.[14]

Antigen test is another point of care test. Although it is less sensitive, it is rapid and has high specificity, hence patients who are positive can be moved into isolation faster.[15]

This study evaluates theadvantages and limitations of truenat machine for diagnosis of corona virus, which is a remarkable addition in perpetual crusade for a good diagnostic test for COVID 19.

MATERIALS AND METHOD

This is a prospective cohort study of 3310 cases attending the emergency department of LLRM Medical College, Meerut from 1st July 2020 to 31 December 2021. These patients were first subjected to antigen testing by rapid antigen kit, using nasal/oropharyngeal swab. Those cases which came out to be positive were reported as true positive. Cases which were negative by antigen tests but still symptomatic, with complain of fever, sore throat and cough were retested by true nat.

ICMR recommends Truenat test as a two-step test: step one comprises of E gene screening assay (True Beta CoV) for all COVID-19 suspect samples to be followed by step two for the RdRp based confirmatory test (True SARS CoV-2) in all E gene positives (Figure 1).

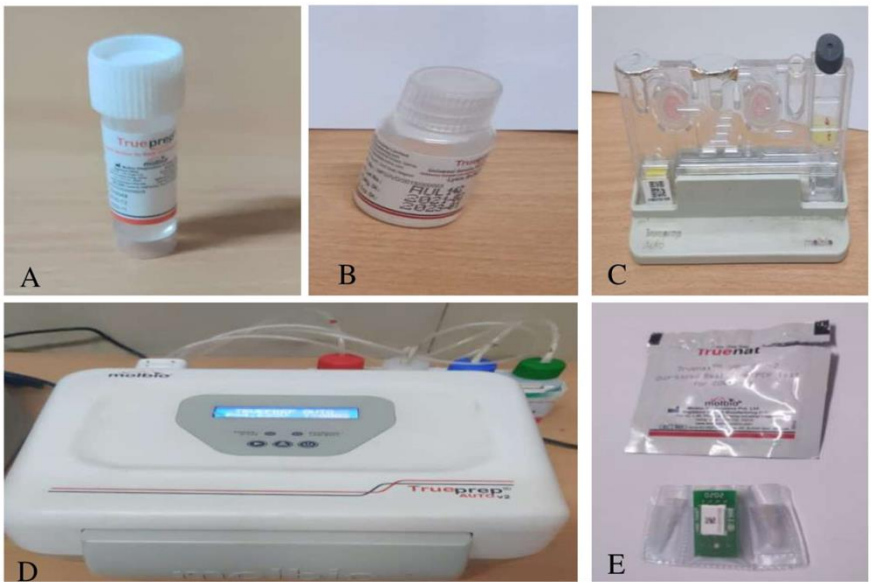

Figure 1: Components of Truenat Machine: Sample preparation kit and RNA extracting machine; A- sample vial; B- Lyse Buffer; C- Trueprep Cartridge; D- RNA extraction machine; E- Beta COV chip

This fully indigenous diagnostic platform offers a reliable and affordable option to augment SARS-CoV-2 testing capacity in India. The platform comprises of a Truenat machine, in-built RNA extraction system, RT-PCR chips, collection swabs and viral lysis medium (VLM). Oropharyngeal sample was collected in viral transport medium and later on lysis buffer was added to break the cells and remove impurities. Finally, it was transferred into a cartridge where process of RNA extraction occurs.Single assay has a turnaround time of 35-50 minutes for 1-4 samples with a total of 12-48 samples being tested per 8 hours shift, depending upon the model of machine (Figure 2).

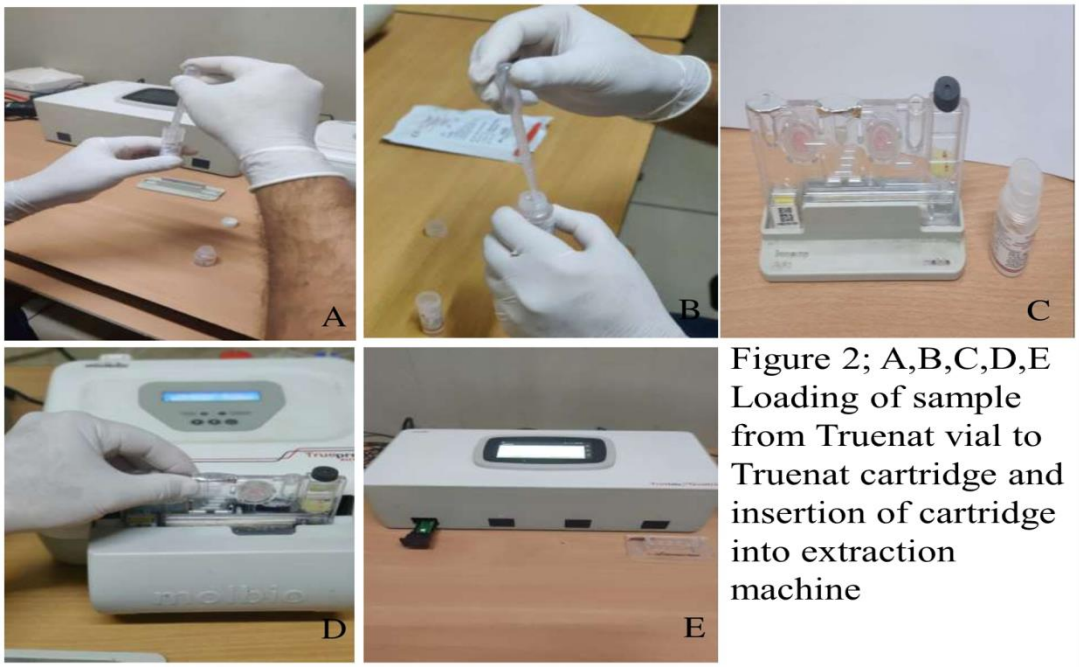

Figure 2; A,B,C,D,E Loading of sample from Truenat vial to Truenat cartridge and insertion of cartridge into extraction machine

True nat machine used is of Molbio diagnostics, Goa. The workstation is a chip-based, real-time quantitative PCR system that is portable, battery-operated, and fully automated, and weighs around 3 kg.

RESULTS AND INTERPRETATIONS

A total of 3310 cases were included in the study. Out of these 492 /3310(14.86%) cases were positive. Remaining 2818/3310 (85.14%) cases were negative for covid 19 infection.

Among the positive cases, 263/492 (53.46%) cases were positive by antigen kit and 229/492(46.54%) cases which were negative by antigen testing, were diagnosed by true nat test in two step process including screening by Beta CoV and confirmation by Sars CoV. There was a clear male (297/492; 60.37%) preponderance when compared to females (195/492; 39.63%). Highest incidence among males 63/297(21.21%) was seen in 51 to 60 years age group and among females 41/195(22.02%) was in 21-30 years of age group.

Male: female ratio was 1.5:1

Table 1: Age and sex distribution of the positive cases (n=492)

Age group in years	Males	Females
<10	17	12
11-20	32	19
21-30	46	41
31-40	49	39
41-50	51	27
51-60	63	32
>60	39	25
Total (492)	297	195

Nasal swab of these 3310 cases was first tested by rapid antigen kit method.263 (263/3310; 7.94%) cases came out to be positive. According to ICMR guidelines these were regarded as true positive.

Oropharyngeal swab of the cases which were negative by antigen test, (3047;92.06%) but still symptomatic with history of fever, sore throat and cough, were subjected to 2 step testing for E gene and RdRp gene by True nat machine.

Step 1, screening test showed amplification of E gene in 361 /3047 cases. All these cases underwent step 2, confirmatory test for detection of amplification of Rd Rp gene. Amplification of Rd Rp gene along with E gene was seen in 229/361 cases. These cases were eventually reported as positive.132/361 cases did not show further amplification of Rd Rp gene and were reported as negative. Positive predictive value of step 2 confirmatory test was 63.4%

Table 2: Positive and negative cases after step 1 and step 2

	Positive after step 1and 2	**Negative after step1**
E gene	+	+
RdRp gene	+	-
Total	229	132

Virus concentrations in samples were estimated from cycle threshold (Ct) values of e gene and RdRP gene for all positive cases. Results were recorded under High, Medium, Low and Very low categories which included 41/229,63/229,59/229 and 66/229 cases respectively. Mean CT value for e gene under each category was 16.1,22.4,28.5 and 32.5 and for RdRp gene was 18.4,23.1,28.1 and 32 respectively.

Table 3: Distribution of the cases according to the CT values

Count	**Number of cases**	**Mean Ct value for e gene**	**Mean Ct value for rdrp gene**
High detected	41	16.1	18.4
Medium detected	63	22.4	23.1
Low detected	59	28.5	28.1
Very low detected	66	32.5	32
TOTAL	229		

In order to increase the power of the study, the very low detected samples (28.8%) were cross checked by an alternative RTPCR test at the same institute.

24/66 very low detected cases were found to be positive while 42/66 cases were negative. Positive predictive value was 36.3%.

Table 4: Comparison of positive cases by truenat and alternative RTPCR in cases with very low detected CT value

Total no of Truenat cases	**Truenat positive (very low detected cases)**	**RTPCR positive**	**RTPCR negative**
229	66	24	42

DISCUSSION

COVID-19, a global pandemic currently intensifying the world with increased number of deaths. India a potent contributor to current burden of COVID-19 cases. However, nationwide lockdown has been able to decelerate the spread, the country's ever-increasing population, remarkably high population density and poor socioeconomic conditions are major barriers in India's battle against COVID-19[16]. It is not only the prompt timely diagnosis but laboratory testing and their accuracy which contributes significantly in providing epidemiological parameters in an attempt to access the ongoing burden. So, it is an urgent prerequisite to dig out early diagnostic modality and ensures prompt treatment. Jacqueline Dinnes[17]etal 2020, compiled and reviewed twenty two publications to assess the diagnostic accuracy and point of care testing of rapid antigen based tests and molecular tests and found

that identifies early-stage evaluations of point-of-care tests for detecting SARS-CoV-2 infection, largely based on remnant laboratory samples, however prospective and comparative studies for rapid tests are warranted. Antibody detection plays a pivotal role in diagnosis provided used timely but yields negative results and conveys dissenting opinion about the tests.[18]

These studies demand an urgent requisite of a rapid, cheap, affordable and accurate point of care testing which could alleviate and assess the spectrum of testing population primarily to the carriers and asymptomatic contacts. Nucleic Acid Amplification Test (CBNAAT and Truenat) are major diagnostic tools in detection of small amounts of viral RNA. ICMR has validated Truenat as a substitution and created an option of aborting prolonged detection of test positivity by RT-PCR, however meticulous sensitivity and specificity is always a stumbling block in false negativity and false positivity. For epidemiological studies, serological screening is a useful asset however nucleic acid amplification tests (NAAT) remains the standard reference for diagnosis at very early stages due to their high sensitivity. Commendation to NAAT make an accessibility to provide laboratory services with early access to patients as well as contacts. Another deflection which could have an influence in using NAAT practices is mobile NAAT lab (MiLaN). Mobile NAAT labs (MiLaN) are the home care laboratories which can be employed for home diagnostics for detection of carriers as well as asymptomatic contacts. (Figure 3)

Figure 3: Mobile NAAT Lab: A prospective model of mobile vehicle for installation and assembling of Truenat Machine

Truenat, a Semi quantitative detection of Beta Coronavirus (Sarbeco) has been recommended in detection of RNA in human oropharyngeal and nasopharyngeal swab specimen. It is approved for use as a first line screening test for COVID-19. Samples testing positive by Truenat Beta CoV should be confirmed using confirmatory tests for SARS CoV 2.[19]

The biosafety and biosecurity requirements are minimal in view of the sample being collected in viral lysis medium (VLM), which inactivates the virus. Safety is further augmented by the closed nature of this platform and minimum sample handling. These features have facilitated use of these tests at the district and Primary health center levels as well, increasing access to testing.[20]

Perhaps owing to respiratory tropism of SARS COV, the best samples for increasing the sensitivity of test is from nasopharangeal swab due to higher and longer persistence of viral loads as compared to oropharangeal swabs.[21]

Another point of consideration for erroneous results in very low detected cases could be attributed to technical incompetence and difference in kits with different gene target. So skilled sampling is imperative in elucidating sensitivity in nucleic acid detection. Another consideration to testing is microbial flora present at site of sample collection which may play a considerable role in viral presentation.

Efforts should be made in elucidating technical skills,expanding technical manufacturing and marketing of cartridges and NAAT machines by central government funding in order to transit diagnostic services from door to door.

Finally looking to this global outlook of pandemic, NAAT results might influence in decreasing the burden in a country where people are low to housing standards and door to door screening necessitates in decreasing prevalence.

CONCLUSION

In our study, we recommend that Truenat machine is a boon in an emergency situation, where timely diagnosis can initiate prompt intervention. It is not only cost effective, but with the help of skilled technical manpower can be considered as Mobile NAAT labs (MiLAN).

In our study, we have also found that very low category of results with increased CT values could pose a deceptive view. We suggest that this category of result should be reconfirmed by an alternative test before shifting the suspected patient either to COVID wards or grey zone area. In this way, we could evade unnecessary exposure of a normal patient with COVID positive patients. Emphasis should also be laid down upon employing only the trained and skilled technical staff in True nat lab as errors including defective sampling could also be a cause of contamination which could further lead to erroneous results.

REFERENCES

1. Hussain A.Rothans, Siddappa N.Byrareddy.The epidemiology and pathogenesis of coronavirus disease (COVID-19) outbreak.J Autoimmun.2020 May;109:102433.
2. Zhai P,Ding Y,Wu X,Long J,Zhong Y,Li Y.The epidemiology,diagnosis and treatment of COVID-19.*Int J Antimicrob Agents.*2020 May;55(5):105955.
3. Fan BE, Chong VCL, Chan SSW, et al. Hematologic parameters in patients with COVID-19 infection. Am J Hematol. 2020; 95:E131-E134.
4. Frater JL, Zini G, d'Onofrio G, Rogers HJ: COVID-19 and the clinical
5. hematology laboratory. Int J Lab Hematol. 2020, 42:11-18.
6. Zhu, N., Zhang, D., Wang, W., Li, X., Yang, B., Song, J., Zhao, X., Huang, B., Shi, W., Lu, R., Niu, P., Zhan, F., Ma, X., Wang, D., Xu, W., Wu, G., Gao, G. F., & Tan, W. (2020). A novel coronavirus from patients with pneumonia in China, 2019. New England Journal of Medicine, 382(8). https://doi.org/10.1056/NEJMoa2001017
7. Ciotti, M, Angeletti, S, Minieri, M, Giovannetti, M, Benvenuto, D, Pascarella, S et al. 2019. COVID-19 outbreak: an overview. Chemotherapy 64:215–23.
8. Udugama B, Kadhiresan P, Kozlowski HN, Malekjahani A, Osborne M, Li VYC, Chen H, Mubareka S, Gubbay JB, Chan WCW. Diagnosing COVID-19: The Disease and Tools for Detection. ACS Nano. 2020 Apr 28;14(4):3822-3835.
9. Sarıgul F, Doluca O, Akhan S, Sayan M. Investigation of Compatibility of SARS-CoV-2 RT-PCR Kits Containing Different Gene Targets During COVID-19 Pandemic. medRxiv; 2020.

10. Padhi A, Kumar S, Gupta E, Saxena SK. Laboratory Diagnosis of Novel Coronavirus Disease 2019 (COVID-19) Infection. Coronavirus Disease 2019 (COVID-19). 2020 Apr 30:95–107.
11. Abduljalil JM. Laboratory diagnosis of SARS-CoV-2: available approaches and limitations. New Microbes New Infect. 2020 Jun 14;36:100713.
12. PMathuria JP, Yadav R, Rajkumar. Laboratory diagnosis of SARS-CoV-2 - A review of current methods. J Infect Public Health. 2020 Jul;13(7):901-905.
13. Website: http://www.icmr.nic.in newspaper truenat
14. Kerala true nat can be final words
15. Nikam C, Kazi M, Nair C, Jaggannath M, M M, R V, Shetty A, Rodrigues C. Evaluation of the Indian TrueNAT micro RT-PCR device with GeneXpert for case detection of pulmonary tuberculosis. Int J Mycobacteriol. 2014 Sep;3(3):205-10.
16. COVID-19 testing: How antibody, antigen, RT-PCR, TrueNat tests differ, their strengths and limitations. kavya
17. Pal R, Yadav U. COVID-19 Pandemic in India: Present Scenario and a Steep Climb Ahead. *Journal of Primary Care & Community Health*. January 2020.
18. Dinnes J, Deeks JJ, Adriano A, Berhane S, Davenport C, Dittrich S, Emperador D, Takwoingi Y, Cunningham J, Beese S, Dretzke J, Ferrante di Ruffano L, Harris IM, Price MJ, Taylor-Phillips S, Hooft L, Leeflang MMG, Spijker R, Van den Bruel A. Rapid, point-of-care antigen and molecular-based tests for diagnosis of SARS-CoV-2 infection. Cochrane Database of Systematic Reviews 2020, Issue 8. Art. No.: CD013705.
19. Ravichandran K, Anbazhagan S, Singh SV, et al. Global Status of COVID-19 Diagnosis: An Overview. J Pure ApplMicrobiol. 2020;14(suppl 1):879-892.
20. http://www.molbiodiagnostics.com/product_details.php?id=54
21. Press Release (21 May 2020) & Advisory (23 June 2020), Indian Council of Medical Research, Delhi
22. Zou L, Ruan F, Huang M, Liang L, Huang H, Hong Z, Yu J, Kang M, Song Y, Xia J, Guo Q. SARS-CoV-2 viral load in upper respiratory specimens of infected patients. New England Journal of Medicine. 2020 Mar 19;382(12):1177-9.