



Original Research Article

TrueNat: A feather in the cap of India's rapid testing capacity and its scope in dentistry

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ARTICLE INFO

Article history:

Received 05-05-2021

Accepted 10-06-2021

Available online 03-08-2021

Keywords:

Truenat

Tuberculosis

COVID-19

ABSTRACT

Tuberculosis is a major cause of morbidity and mortality throughout the world. Major population of patients suffering from Tuberculosis belong to such areas of the community where the traditional tests of Tuberculosis are not practical to set up because of the extensive infrastructural requirements like continuous power supply. TrueNat is a new testing facility that uses a portable battery-operated device that tests the presence of the tubercle bacilli in the sputum as well as test the Rifampicin resistance of the bacillus in about an hour's time, by the use of rapid molecular diagnostics. WHO endorsed this test for the initial testing of Tuberculosis and ICMR allowed the use of TrueNat for COVID-19 testing. Contagious diseases can impose a serious health hazard to dental staff due to the aerosolizing procedures being carried out in dental office. TrueNat testing before such procedures can help in significant risk reduction and quicker results which will help in avoiding delay in dental treatment. Due to higher accuracy, reliability, same day testing and reporting by TrueNat, it can play a pivotal role in the future of diagnostic abilities of infectious diseases.

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1. Introduction

Infectious diseases constituted the most serious health issue in the world until the beginning of the 20th century when chronic degenerative diseases began to dominate this scenario in developed countries.¹ Tuberculosis (TB) is a contagious infection that usually attacks the lungs. It can also spread to other parts of the body, like the brain and spine. A type of bacteria called Mycobacterium tuberculosis causes it. Presently, tuberculosis (TB) is among the leading causes of death from a single infectious pathogen globally.^{2,3} TB remains the world's most deadly infectious disease; it claims more than a million lives each year and affects millions more, with enormous impacts on families and communities.⁴ Worldwide, TB is one of the top 10 causes of death and the leading cause from a single infectious agent (above HIV/AIDS). In 2019, an estimated 10 million people fell ill with tuberculosis (TB)

worldwide. 5.6 million men, 3.2 million women and 1.2 million children.⁴ In India alone, approximately 2.8 million cases are being reported annually making our country having the highest incidence of this disease in the world. According to WHO Global Tuberculosis Report, 2020, the three countries with the largest share of the global burden were India (27%), China (14%) and the Russian Federation (8%).⁴ Over 25% of the patients seeking care in our public sector are neither diagnosed properly nor started on treatment because of the widespread use of diagnostics with low sensitivity, for example, the sputum smear microscopy.⁵ Smear microscopy often detects only 45% cases of tuberculosis,⁶ yet it remains the most practical test available in India. Rapid molecular diagnostics could dramatically increase the tuberculosis detection which is a key component to WHO End Tuberculosis Strategy and India National Strategic Plan for TB Elimination 2017-25.^{4,7} Currently the Xpert MTB-RIF (Cepheid, Sunnyvale, CA, USA) assay, which is a WHO endorsed test, is able to detect both the presence of Tubercle bacilli and Rifampicin

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resistance of the bacillus.^{4,8} There has been an interest in deploying this test in peripheral laboratories, for widening the testing facilities, but due to the extensive infrastructure requirement which includes the continuous power supply and air conditioning,⁹ its decentralization has become limited.

These very limitations led to the development of TrueNat, which is developed by MolBio Diagnostics Pvt Ltd, Goa.¹⁰ It is a chip-based, micro real-time polymerase chain reaction test that detects the tubercle bacilli in sputum samples in about an hour's time. An 'add-on' chip may then be used after a positive test result to detect Rifampicin resistance of the bacillus. The testing device is a portable, battery-operated device, therefore it can be used in peripheral laboratories as well as in primary health care centers in India, where continuous electricity supply and air-conditioned settings are not available.¹⁰ (Figure 1).

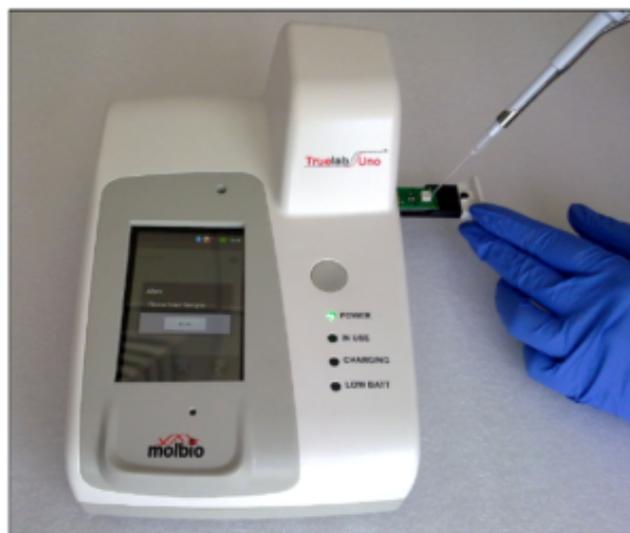


Fig. 1: TrueLab PCR analyser (Reprinted from Reprinted from molbio diagnostics product listing https://www.molbiodiagnostics.com/product_details.php?id=1)

2. WHO Endorsement

Recently, on 2nd July 2020, WHO endorsed the Truenat Assay as the initial test to identify Tuberculosis and detect Rifampicin resistance,¹¹ which would help Universal access to TB molecular diagnostics in countries with endemic TB, which would eventually enable the low and middle-income countries in easy procurement of this assay. This will have a great impact on reducing life-threatening delays in the diagnosis and treatment of TB.

3. Accuracy of TrueNat for Diagnosis of Tuberculosis

The performance of Truenat MTB, MTB Plus and MTB-RIF Dx assays (all products trademarked by MolBio

Diagnostics) show comparable accuracy to the Xpert MTB/RIF and the Xpert Ultra for TB detection (Truenat MTB and Truenat MTB Plus, respectively) and for sequential rifampicin resistance detection (Truenat MTB-RIF Dx).¹⁰ The Truenat MTB and MTB Plus assays also show comparable accuracy to the TB-LAMP assay as replacement tests for sputum smear microscopy. The data for Truenat MTB-RIF shows similar accuracy to WHO-approved commercial line probe assays.¹² A validation study in India reports that the TrueNAT MTB test has good sensitivity and specificity for case detection of TB as compared with Xpert with hands-on time less than 3 h.¹³

4. Testing Procedure

For testing tb, a sputum sample is taken from the patient. The quantity of the sample required is 0.5ml whereas the GeneXpert machine requires about 1ml. Purified DNA is extracted from the sample which is amplified using real-time polymerase chain reaction (PCR), which is then tested for tubercle bacilli using a chip. Automated battery-operated devices are used for the extraction of DNA (Trueprep Auto device) [Figure 2], amplification of DNA (TrueNat MTB chip) [Figure 3] and reading the presence of specific genomic sequences (TrueLab PCR analyser) [Figure 1] rifampicin resistance (RR) is detected by doing a second RTPCR (Reverse transcription polymerase chain reaction).



Fig. 2: Trueprep auto device (Reprinted from molbio diagnostics product listing https://www.molbiodiagnostics.com/product_details.php?id=1)

TrueNat machine is both battery operated and portable which makes it point of care machine. It is advantageous in the areas where there is problem of electricity and transportation of sample to lab is a herculean task. The device has a potential in detecting resistant cases in a limited resource setting and curtailing cost of transporting samples to district hospitals or treatment units (TUs) where GeneXpert machines are placed.¹⁴



Fig. 3: TrueNat MTB chip (Reprinted from molbio diagnostics product listing https://www.molbiodiagnostics.com/product_details.php?id=1)

5. Time taken for the Test

It takes about 25 minutes to do the DNA extraction. It takes another 35 minutes to diagnose Tubercle bacilli. It takes an additional one hour for testing for rifampicin resistance.

6. TrueNat for the Diagnosis of COVID-19

6.1. Use for COVID-19 diagnosis

The rapid diagnosis of coronavirus disease 2019 (COVID-19) is a significant step towards the containment of the virus. The rapid surge of COVID-19 cases in India necessitates the use of rapid molecular diagnosis of the disease for early onset of treatment and prevention of transmission of infection. Currently, the most widely used method is the real-time polymerase chain reaction (RT-PCR).¹⁵

6.2. ICMR approval and Guidelines for use of TrueNat for COVID-19 testing

TrueNat can be used for testing of Covid-19 cases. It was approved by Indian Council of Medical Research(ICMR) in May 2020 for screening and confirmation of covid - 19.^{16,17} It works by detecting the SARS-CoV-2 E-gene and the gene for the RNA-dependent RNA polymerase (Rdrp gene), which help to build the envelope of the virus and enable it to replicate respectively.

All the samples that are tested positive by E gene assay (using Truenat Beta Cov chip) are further tested for the Rdrp gene (using the Truenat SARS-COV-2 chip) which is

a confirmatory gene for SARS-CoV-2. The samples that are tested positive by this final assay for Rdrp are considered as True positive or Confirmed positive and there is no need of further RT PCR testing for these positive samples. Recently, Multiplex Truenat Assay has also been developed and it has been approved by ICMR. This assay uses E gene as screening and Orf1a as confirmatory gene for COVID 19 detection in a multiplex format.¹⁸

In the traditional RT-PCR tests, the sample collected has to be transported to the laboratory maintaining the proper cold chain. As SARS-COV 2 being a RNA virus, it needs a cold environment to be stable so that it can be detected in Real time PCR.¹⁹ The final result may take days to give the results. On the contrary, TrueNat test is automated, and the results can be obtained within an hour.²⁰

6.3. Advantages of TrueNat

As the TrueNat test is rapid and portable as compared to conventional RT-PCR testing, it can be advantageous in Random Testing of population in areas with high positivity rate and in containment zones. It requires less technical expertise, does not require any special infrastructure and can be done in any basic health care facility. In turn, this can reduce the burden on testing sites performing RT-PCR and shorten the turnaround time of reporting of results.²¹

A study was done to evaluate the performance of Truenat assays for screening and confirmation of SARS-CoV-2, and the results indicated that the Truenat Beta CoV and SARS-CoV-2 assays were highly sensitive and specific for the detection of SARS-CoV-2 RNA.²¹ The sensitivity and specificity of TrueNat is comparable to RT-PCR.²⁰

6.4. Disadvantages of TrueNat²⁰

The major disadvantage of TrueNat is that only one sample can be processed by one Truenat machine at a time, so it is not an ideal instrument for a high throughput laboratory. Another disadvantage is that a specialized vial is to be used for processing of sample for TrueNat testing and the sample once put in that vial cannot be used for conventional RT-PCR. In some rare circumstances of mutations within some areas of target genome, TrueNat may show false negative results.

6.5. Use of TrueNat in other diseases

Even though an increasing number of studies are being done to validate the use of TrueNat for the diagnosis of Tuberculosis and COVID-19, Molbio Diagnostics has various other trademarked products for use in other diseases. A study done in Andhra Pradesh, India suggested the use of TruenatTM H1N1 rapid diagnosis of the swine flu cases.²² The other diseases TrueNat may be used for are Malaria (TruenatTM Malaria Pv/Pf), Dengue (TruenatTM Dengue), Chikungunya (TruenatTM Chikungunya), Typhoid

(Truenat™ Salmonella), Rabies (Truenat™ Rabies), Hepatitis (Truenat™ HBV, Truenat™ HCV, Truenat™ HAV, Truenat™ HEV), AIDS (Truenat™ HIV-1), Influenza (Truenat™ Influenza A/B), Papilloma (Truenat™ HPV-HR) and Urinary Tract Infection (Truenat™ CT/NG).

7. Scope of TrueNat in Dentistry

Airborne infections transmit microorganisms via fine mist, dust, aerosols and liquids.²³ Dental procedures are a source of aerosols being produced in the dental clinic. Dental procedures like ultrasonic cleaning by ultrasonic scalers, ultrasonic oscillating irrigation of root canals, use of high speed airrotors for caries-removal, use of air-water syringe are all a great source of aerosols and splatter. These aerosols can land on skin and mucous membranes of people and can be inhaled into lungs, causing serious infections.²⁴ Thus, a dental professional is prone to infections that are transmitted by respiratory aerosols like tuberculosis and COVID-19.

It is easier to identify patients who are infected and show symptoms of the disease. In case of COVID-19, there have been people who do not develop symptoms of the disease, yet harbor a similar viral load as compared to symptomatic patients, suggesting similar potential for viral transmission.^{25,26} Thus, it becomes necessary to identify such patients by efficient diagnostic testing facility to be made available for every single patient who visits the dental office for a dental procedure. In addition to this, patients needing emergency dental treatment will be benefitted by quick and sensitive diagnostic testing facility.

Rapid molecular testing, like TrueNat may play a significant role in such a scenario. TrueNat provides good sensitivity for the pathogen and sensitivity in diagnosing asymptomatic cases as well. The whole process takes about an hour's time, making it feasible to be used before emergency dental treatment. Surface sampling can also be done for dental operatory surfaces for testing the presence of SARS-CoV-2 virus, since the disease can also spread via contaminated surfaces.²⁷ A wet swab may be used to collect the sample, which is then processed to test for the virus.

Therefore, role of TrueNat in dentistry to test patients for infectious diseases is significant and would prevent the spread of such infections from patient to doctor and vice-versa.

8. Conclusion

TrueNat is battery operated, cheaper, and quicker than other RT-PCR tests. They can be used to test the samples in the containment zone and for creating mobile testing centers in cases of Covid-19 screening. With minimal training, both sample collection and analysis can be performed. TrueNat is one such promising indigenous equipment which is cost effective and can be used to diagnose COVID-19 infection in small laboratories and community centers with minimum infrastructure. This will help in reducing the burden on

government laboratories. Since every new invention has a few shortcomings, TrueNat also has certain disadvantages like it being impractical to be used in high throughput laboratories. Further research is required for optimal use of TrueNat in the field of dentistry and to make judgement regarding its feasibility and practicality in dental set-ups. In the coming years, TrueNat is expected to play a very significant, dominant role in the global molecular diagnostic segment.

9. Source of Funding

None.

10. Conflict of Interest

The authors declare that there is no conflict of interest.

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Cite this article: Singla L, Goel V, Kanwar A, Mohapatra S. TrueNat: A feather in the cap of India's rapid testing capacity and its scope in dentistry. *International Dental Journal of Student's Research* 2021;9(2):77-81.