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Diagnostic Accuracy of the True Nat MTB/RIF Test and Comparison with the Smear Microscopy for Diagnosis of Pulmonary Tuberculosis in a Tertiary Care Centre

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ABSTRACT

Background*: The need for fast, precise diagnostic tests to identify active tuberculosis is essential, mainly in endemic nations such as India. The TrueNat MTB Plus assay is a rapid molecular test that has been recommended by the World Health Organization since 2020 as an initial test to detect tuberculosis (TB). The WHO highlighted the need to further evaluate assay performance to inform future recommendations, including in people living with HIV. TrueNat MTB/RIF (rifampicin) is a novel method, which is battery operated, point-of-care and chip-based Real Time Polymerase Chain Reaction (RT-PCR) micro device. The purpose of this study is to evaluate diagnostic accuracy of the TrueNat/MTB/RIF as a screening test in the diagnosis of Pulmonary Tuberculosis in comparison with smear microscopy.*

Materials and Methods*: A prospective cross-sectional study was carried out over a year in which samples from suspected cases of TB fitting in the inclusion criteria were subjected to Ziehl Neelsen (ZN) staining for smear microscopy, and PCR for MTB by TrueNat.*

Results*: A prospective cross-sectional comparative study on 394 patients with suspected pulmonary TB was conducted from January 2021 to December 2021 in a tertiary care hospital at Muzaffarnagar Medical College, Muzaffarnagar, UP India. The sensitivity, specificity, PPV, NPV, and diagnostic accuracy for the diagnosis of tuberculosis were calculated for Acid Fast Bacilli (AFB) smear microscopy and the TrueNat, and compared with each other. Statistical analysis of the data was conducted with Statistical Package for the Social Science (SPSS) version 20.0.Out of the total 394, 83 (21.%) patients were TB positive by TrueNat, and 75 (19%) as per smear microscopy.*

Conclusion*: Truenat MTB test is a cost-effective rapid molecular test with good sensitivity and specificity for the diagnosis of pulmonary tuberculosis in low resource settings.*

Keywords: *Mycobacterium tuberculosis, diagnostics, smear microscopy, molecular methods*

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I. Introduction

Tuberculosis (TB) is a significant global health challenge, with an estimated 10 million people becoming sick with the disease and 1.5 million deaths due to TB in 2020.[1] The global public health response for TB has been complicated by challenges to diagnose and link people to care. In 2019, an estimated 2.9 million people with TB were undiagnosed and unreported[2], and in 2020 this estimate increased to more than 4 million people undiagnosed and unreported for TB during the COVID-19 pandemic[1]. Ensuring that better diagnostic tools are developed and made accessible to people to be evaluated for the disease are key priorities for ending TB[3]. Rapid, inexpensive, and sensitive point-of-care tests that can be used to diagnose active TB disease from more readily obtainable, nonsputum specimens are in development and may greatly improve TB elimination efforts[4,5]. However, such tests are not yet widely available, and at present, diagnostic testing for TB is still typically sputum and hospital based. The currently recommended algorithms to diagnose TB include the use of molecular-based tests to detect TB and drug-resistant TB[6.7]. The Xpert MTB/RIF assay (Cepheid, USA), a rapid molecular diagnostic tool for TB, was recommended by the World Health Organization (WHO) in 2010, and the Xpert MTB/RIF Ultra assay, a more sensitive test, was recommended in 2017. Use of the GeneXpert system with both the Xpert and Ultra assays has been widely scaled up; however, uptake in some settings has been

hindered by issues such as the relatively high cost of the tests, lack of availability of the required infrastructure needs for the equipment, and technical challenges to keep the instruments running[8,9]. Until 2020, the Xpert system was the only WHO-recommended option for rapid molecular detection of TB and rifampin-resistant TB; however, it is beneficial for end-users to have access to multiple options for diagnostic testing[10]. The TrueNat MTB Plus assay (Molbio Diagnostics, India) is a more recently available molecular test that runs on the portable, battery-operated Truenat platform and, to date, has been used primarily in India[11,12,13,14,15,16]. In 2020, the WHO recommended the TrueNat MTB or MTB Plus assay as the initial diagnostic test for TB rather than smear microscopy/culture, along with the Truenat MTB-RIF Dx for detection of rifampicin resistance in people with a positive Truenat MTB or MTB Plus result[17]. This recommendation followed a multicenter evaluation of the Truenat assays conducted by the Foundation for Innovative New Diagnostics (FIND) at seven sites in four countries[18]. The WHO has highlighted the need for additional evaluations of the diagnostic accuracy of these assays in a variety of settings and populations, including for people living with HIV, to inform future recommendations[6].Globally, the annual incidence of TB estimates about 10.0 million people of which 2.7 million cases are reported from India[1]. Rapid, sensitive tests for TB detection have the potential to greatly improve TB diagnosis and care in this setting. Therefore, it is important to estimate the ability of Truenat MTB to diagnose TB correctly. The present study was aimed to evaluate Truenat MTB test in comparison with microscopy for the diagnosis of Pulmonary tuberculosis.

II. Materials And Methods

This was a prospective cross-sectional study which was done in a tertiary care centre in Muzaffarnagar Medical College, Muzaffarnagar, India during the period from January 2021 to December 2021. Total 394 pulmonary clinical samples were obtained during this period. Informed and written consent was obtained from patients enrolled in the present study.

Inclusion criteria: All the patients who were referred to the Department of Microbiology for a Mycobacteriology study by ZN staining and molecular detection of pulmonary TB were included in this study.

Exclusion criteria: Samples that were obtained without a clinical history were excluded.

Study Procedure:

Specimen collection and processing:

As necessary, sputum samples were collected under sterile conditions and also in leak proof, sterile containers. In the shortest time possible, the samples were processed. In the case of a delay, they were kept at 4°C for no longer than 24 hours before even being processed immediately. All samples were handled in a class II A2 biosafety cabinet.

ZN staining: The ZN staining method was performed following the established technique[19]. A smear was made from each sample and spread on a label, clear glass slide. The slide was then stained by ZN stain and observed under immersion oil after that the smear had been allowed to air-dry and also fixed by heat (X100). Acid fast bacteria had a bright red appearance and a beaded look.

TrueNat MTB test: DNA extraction was done using Trueprep-MAG kit instructions. According to the manufacturer's instructions[20,21], fresh specimens (sputum) from untreated individuals were processed, with a starting volume of 500 µL added to the sample pretreatment tube. The Trueprep-MAG Sputum and TrueNAT Mycobacterium tuberculosis kits contain only proprietary master mixes for PCR as well as all buffers and reagents essential for nucleic acid extraction.

Real-time PCR on chip: A preprogrammed profile on the TrueNat Mycobacterium tuberculosis microchip was used to conduct realtime PCR with 5 µL of extracted DNA and lyophilised master mix. The screen displayed the results. Unique primers and an Mycobacterium tuberculosis-specific probe were included in the lyophilised master mix.

STATISTICAL ANALYSIS: The data was recorded in a master chart using a Microsoft excel spreadsheet, and correlation was checked. SPSS version 20.0 was used to statistically analyse the data. The sensitivity, specificity, PPV, NPV, and diagnostic accuracy for the diagnosis of TB was calculated for AFB smear microscopy and TrueNat, and compared with each other.

III. Results

Out of the total of 394 cases of suspected TB, 240(61%) were males and 154(39%) were females. Of which 83 (21%) patients were found to be TB positive by TrueNat Technique, out of which males were 63 (76%) whereas females were 20 (24%). However, in the case of ZN smear microscopy, 75 (19%) were positive.Out of 83 TB patients who tested positive with the gold standard i.e., TrueNat, 75 patients also tested positive for ZN stain, respectively and were hence labelled as true positives while out of eight patients who tested negative with the gold standard i.e., TrueNat, eight patients also gave negative result on ZN stain, respectively and were categorised as true negatives. However, considering the total patients who tested positive and negative, respectively using ZN staining and TrueNat, i.e., test positives and test negatives, it was observed

that 8 samples and 8 samples turned out false positive and false negative, respectively with ZN staining. Sensitivity, specificity, PPV, NPV, and accuracy of ZN stain in the detection of pulmonary TB in sputum samples were 90.36%, 97.43%, 90.36%, 97.43%, and 95.94%, respectively. [Table/Fig-1].

Statistic	Value	95%CI
Sensitivity	90.6%	81.89% to 95.75%
Specificity	97.43%	94.99% to 98.88%
Positive Likelihood Ratio	35.13	17.66 to 69.87
Negative Likelihood Ratio	0.10	0.05 to 0.19
Disease prevalence	21.07%	17.14% to 25.43%
Positive Predictive Value	90.36%	82.50% to 98.65%
Negative Predictive Value	97.43%	95.14% to 98.65%
Accuracy	95.94%	93.49% to 97.66%

IV. Discussion

In the current study, the diagnostic accuracy of TrueNat to detect Mycobacterium tuberculosis in pulmonary specimens was evaluated and compared with AFB smear staining by ZN staining.

ZN staining of sputum sample: In the present study, 19% and 81% of the subjects were detected to be ZN stain positive and negative, respectively on sputum samples. Truenat was used as a gold standard to test the diagnostic efficacy of ZN staining. TrueNat detected 83 positive specimens whereas ZN staining detected only 75 positive specimens. Sensitivity, specificity, PPV, NPV, and accuracy of ZN stain in the detection of pulmonary TB in sputum samples were 90.36%, 97.43%, 90.36%, 97.43%, and 95.94%, respectively. Chandora AK and Chandora A, screened 100 patients for pulmonary TB observed sensitivity, specificity, PPV, NPV of Sputum microscopy was 22.22%, 78.38%, 63.64% and 37.18%, respectively[22]. Padmaja GV et al, reported the sensitivity and specificity of ZN staining on sputum samples to be 50-80% and 98%, respectively[23].

Limitation of ZN stain: Although ZN stain is quick, inexpensive, and simple, it needs atleast 10,000 bacilli per millilitre of sputum to prove TB. This test only has a sensitivity range of 20-80% and a 99% specificity. Smear microscopy's primary flaw is its inability to identify rifampicin resistance[24]. Misdiagnosis of smears could be the reason for the false positive cases. Technical mistakes such as insufficient slide preparations, poor staining technique, observational inaccuracy, etc. could be to blame for the false negative cases. A repeat sample is advised or TrueNat should be performed to confirm the diagnosis in the event of a false positive or suspected case in order to avoid needless medical intervention, pharmacological side effects, and stress on the patient and their family.

TrueNat of Sputum sample: In the present study, 21% and 79% of the subjects were detected positive and negative, respectively on TrueNat using sputum samples. Ngangue YR et al., reported the sensitivity and specificity of TrueNat for pulmonary TB on different hospitalised patients to be 91% and 96%, respectively[25]. PennNicholson A et al., 2021 reported the sensitivity and specificity of TrueNat in primary healthcare to be 84% and 95%, respectively[26].

V. Limitation of detection of TrueNat[27]

The assay is not advised for patients receiving antituberculosis treatment, who are being monitored for bacterial cure and response to therapy. Improper or insufficient sample collection especially during transportation may affect the quality of the results giving rise to false negative results. The possibility of isolating Mycobacterium tuberculosis-complex from the sputum sample is not disregarded by a negative test result. Positive outcomes may not always mean that a living entity is present. The Mycobacterium tuberculosis-complex species are not distinguished by the Xpert Mycobacterium tuberculosis/RIF assay. To determine whether Mycobacteria Other Than Tuberculosis complex (MOTT) is present in addition to Mycobacterium tuberculosis-complex, a culture must also be done.

VI. Conclusion:

The findings reported here provide support for the use of the Truenat MTB Plus assay as a sensitive diagnostic test for TB. As the NPV is high, this will be an ideal test for screening of TB. In the current study, Truenat assay showed a high concordance with other studies on molecular diagnostic tests. Thus, this assay might be a potential, accurate, and rapid method for the detection of pulmonary tuberculosis cases in low resource settings.

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