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A Microbiological Analysis Of Smear Microscopy And Truenat Pcr Method In The Diagnosis Of Pulmonary Tuberculosis In A Tertiary Care Centre

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ABSTRACT

Fast and accurate diagnostic tests serve as vital role for detecting active tuberculosis, particularly in endemic countries like India. Since 2020, the World Health Organization is urging the usage of Truenat MTB Plus assay, a quick molecular test, as a first step in the detection of tuberculosis (TB). In order to guide future recommendations, the WHO emphasized the necessity of further assessing assay performance, including in individuals with HIV. The Truenat MTB/RIF molecular method is operated on rechargeable batteries, point-of-care, chip-based Real Time Polymerase Chain Reaction (RT-PCR) microdevice. In contrast to smear microscopy and solid culture, the Truenat MTB/RIF screening test's diagnostic accuracy in detecting pulmonary tuberculosis is to be assessed in this study. Over the period of a year prospective cross-sectional study is carried, where samples from suspected TB cases that met the inclusion criteria were subjected to Truenat RTPCR for MTB and Ziehl Neelsen (ZN) staining for smear microscopy. The study was conducted in Govt. Chest disease Hospital, Jammu, J&K. This study is carried on 248 patients with suspected pulmonary TB. The sensitivity, specificity and diagnostic accuracy for the diagnosis of tuberculosis were calculated for Acid Fast Bacilli (AFB) smear microscopy and the Truenat. Out of the total 248, 64 (16.66.%) patients were TB positive by TrueNat, and 51 (13.28%) as per smear microscopy. In low-resource environments, the Truenat MTB test is an affordable, quick molecular test that has good sensitivity and specificity for diagnosing pulmonary tuberculosis.

Keywords: *Mycobacterium tuberculosis, diagnostics, smear microscopy, TrueNat.*

1. INTRODUCTION

Tuberculosis (TB), caused by *Mycobacterium tuberculosis* (MTB), is the leading cause of death by an infectious disease. It remains a serious public health problem over the globe. Tuberculosis 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 (3). With approximately 2.8 million cases annually, India has the world's highest incidence of tuberculosis (4). Treating tuberculosis is extremely challenging if the bacteria that cause the disease are resistant to first-line drugs. If they are resistant to rifampicin, the disease is termed rifampicin-resistant tuberculosis (RR-TB), and if they are also resistant to isoniazid, the disease is termed multidrug-resistant tuberculosis (MDR-TB). Microbiological confirmation is recommended for diagnosing pulmonary tuberculosis (PTB) and extra-pulmonary tuberculosis (EPTB). The miniaturized forms of PCR tests have the advantages of a reduction in the cost of instruments and faster turnaround times in poor resource settings. The micro-PCR devices have the added advantages of better diagnostic sensitivity and portability. They are widely used in India and other South-East Asian countries (7). GeneXpert was endorsed by the World Health Organization (WHO) to be used in India as part of the National TB control programme (8). Cartridge Based Nucleic Acid Amplification Test (CBNAAT) is a test which detects TB bacilli and also screens for rifampicin drug resistance (9).

In contrast, the present study aimed to assess the rate of inconclusive Truenat results in real-world situations and to determine their root causes. This knowledge is needed in order to optimize the testing performance of Truenat MTB-RIF, to ensure timely diagnosis, and thereby to reduce the magnitude of undiagnosed TB cases, not only in India but also in other countries that have rolled out Truenat as a molecular point-of-care tool to strengthen the TB diagnostic care cascade in national TB programs. This study is also evaluate the diagnostic accuracy of Truenat MTB assay in pulmonary (PTB) tuberculosis cases in a tertiary care hospital of Govt CD Hopsital Jammu in Jammu & Kashmir India.

2. MATERIAL & METHODS

This was a prospective cross-sectional study which was conducted in a tertiary care centre, Govt. Chest Disease Hospital, Jammu J&K India during the period from December 2023 to December 2024. Total 248 pulmonary clinical samples were obtained during this period. Patients who were enrolled in the study gave their signed and informed consent.

Inclusion criteria: The patients who were having cough, fever and unintentional weight loss, were included in this study.

Exclusion criteria: Salivary samples were excluded.

Sample Size and Sampling technique: Sample size 248.

The sample size is determined using a single population proportion formula. Sample size is calculated by cochran W.G (1977) sampling technique(3rd) New York:John Wiley & sons technique

Formula S $= Z^2 * p * (1-p) / M^2$

Study Procedure:

Specimen collection and processing: Sterile environments and leak-proof, sterile containers were used to collect the sputum samples. The samples were processed as quickly as feasible. If there was a delay, they were stored at 4°C for no more than 24 hours before being processed without delay. Every sample is handled within a class II A2 biosafety cabinet**.** Sample collection, Smear Microscopy and the Truenat MTB PCR tests were performed at Intermediate Reference Laboratory (IRL), Govt Chest Disease Hospital, Jammu, J&K, India.

3. METHODS

ZN staining: The ZN staining method was performed following the established technique[8]**,** after performing direct and concentrated acid-fast bacillus (AFB) microscopy (Ziehl-Neelsen [ZN] staining), sputum was processed using 2% N-acetyl-L-cysteine and sodium hydroxide (NALC-NaOH) and centrifuged (10).

TrueNat MTB test: DNA extraction was done using Trueprep-MAG kit instructions. According to the manufacturer's instructions. Untreated sputum specimens were liquefied with 2drops of liquefaction buffer, 500 µL was then introduced to the sample pretreatment tube. All buffers and reagents used for nucleic acid extraction and all mastermixes used for PCR are proprietary components of the Truenat MTB kit (11).

TrueNat PCR chip test: A lyophilized master mix and 5 µL of isolated DNA were tested for real-time PCR using a preprogrammed profile on the TrueNat Mycobacterium tuberculosis microchip. Results were shown on the screen (12).

4. RESULT

A total of 248 suspected cases were studied over a period of one year from December 2024 to December 2025. Samples were obtained from various departments like Surgery, Medicine, Orthopaedics, Paediatrics, , Pulmonary Medicine and Gynaecology. There was an almost equal distribution of males and females among the study population with 126 males (50.8%) and 122 females (49.2%). Out of the 248 samples tested, 44 (17%) were positive for MTB by Truenat. Out of the different positive samples, three were Sputum (33.3%). The prevalence of positive samples in our study is about 17%.

Chart 1: Distribution of Samples from various Clinical departments

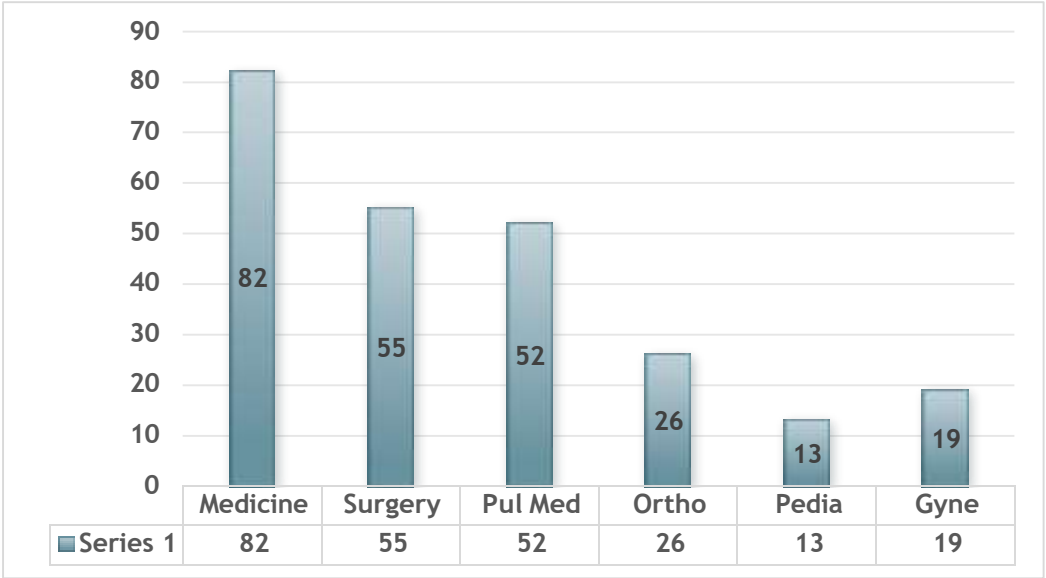


Chart 2: Symptomatology of study population

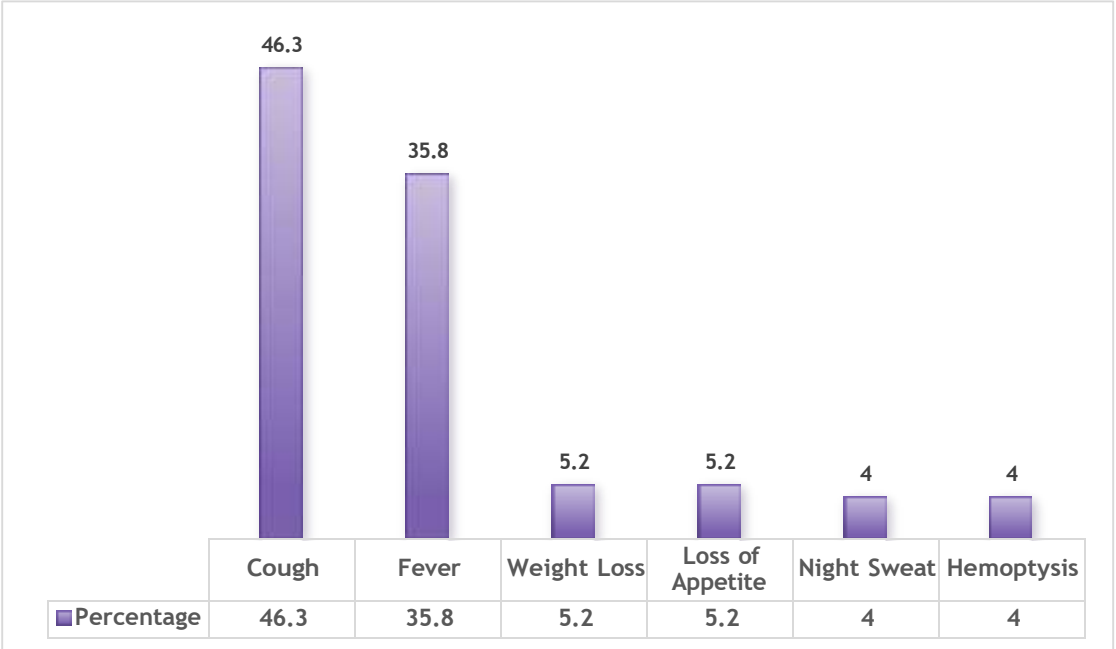
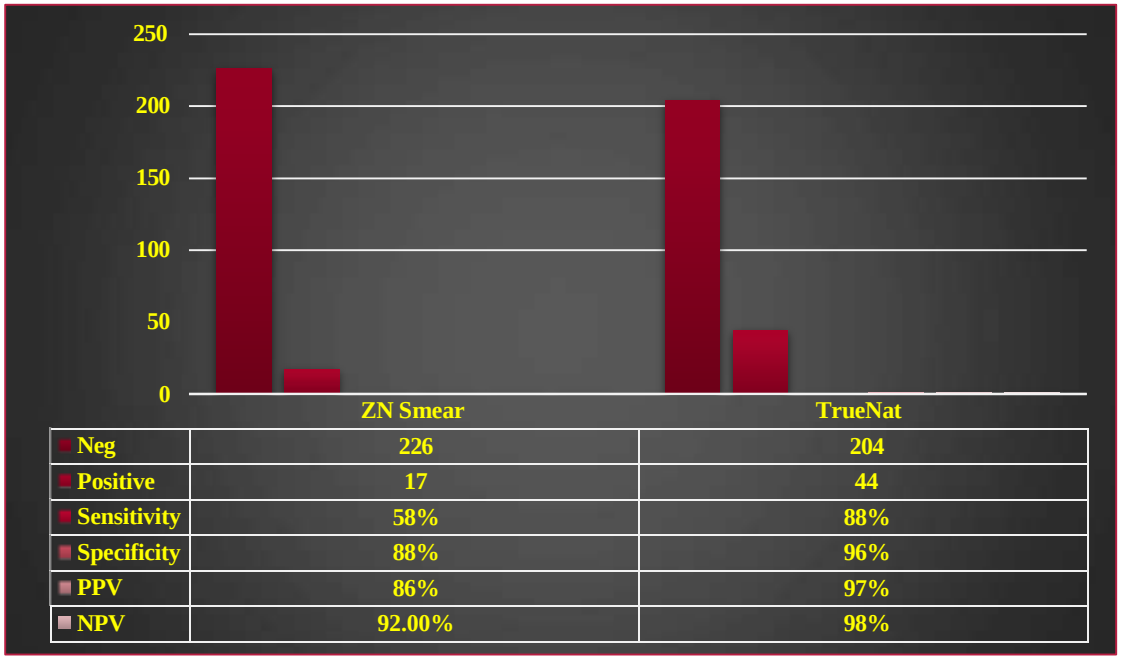


Chart 4: Shows comparison of diagnostic accuracy of ZN Smear and TrueNat



	ZN Smear	TrueNat
Neg	226	204
Positive	17	44
Sensitivity	58%	88%
Specificity	88%	96%
PPV	86%	97%
NPV	92.00%	98%

5. DISCUSSION

A major WHO priority for TB diagnostics is to implement a rapid, sputum-based molecular test to replace smear microscopy at the peripheral level (i.e., microscopy centers and attached primary healthcare facilities) [11, 12]. Our model-based analysis shows that in India, Truenat PCR system is a cartridge based test for the rapid diagnosis of tuberculosis along with the detection of rifampicin resistance which is developed in India. With the extensive availability of this economically viable diagnostic method in resource poor country like India will not only help in the early diagnosis but also in the appropriate management and infection control measures and controls the spread of this deadly disease. Truenat will improve linkage-to-care and increase life expectancy also. The prevalence rate of mtb positive samples in our study is about 17%.

Table 2: Comparison of ZN Smear & TrueNat Testing by various studies

Arora D, Dhanashree B at al in 2020[13]	Reported ZN & Truenat 13.8% and 37.1%
Albert H, Nathavitharana RR, Isaacs C, et al. in 2016. [14]	Reported ZN smear positive about 14% and TrueNat 35% in MTB isolates.
Beall SG, Cantera J, Diaz MH, et al. In 2019. [15]	find that the ZN smear about 16% and TrueNat is about 40% positivity rate.
Chakravorty S, Simmons AM et al. In 2017. [16]	observed that AFB smear positive 18% and Rapid PCR 27.2% reported in pulmonary specimen.
Nikam C, Jagannath M, Narayanan MM, et al. in 2013 [17]	observed that ZN smear is about 17% and Rapid pcr about 15.6% in MTB isolates.
Kasat S, Biradar M, Deshmukh A et al in 2018 [18]	Find that the ZN smear in MTB isolates about 16.5% and TrueNat PCR about 9.5%.

TrueNat, when replacing smear microscopy and used at point-of-care, increases the number of TB cases correctly detected and linked to care by 590 per 10,000 individuals with presumptive TB. It also increases life expectancy by nearly 0.4 years and is cost-effective. While Truenat DMC was economically inefficient among the four strategies, it was cost-effective when compared directly to SSM. The cost-effectiveness of Truenat POC, compared to SSM, was consistent across a wide range of clinical and cost parameter values. The WHO's target product profile (TPP) of the "smear replacement test" includes a set of minimal and optimal requirements [7,8]. Truenat fits many minimal TPP standards, including battery-powered operation and less than 2 hours to result [19].

Majority of the cases were in the age group of 60-69 years with male predominance. This was in agreement with studies conducted by Subbarao et.al (20) and Desai K et al. The most common symptoms in our study were cough (69.75%) and fever (56.27%). Similar findings were seen in study conducted by Avashia et al (21) as they found fever (69.4%) and cough (72.2%) as the main symptom. Our analysis shows that scaling up molecular diagnostics will increase the required budget but the majority of the cost will be from MDR-TB treatment. A recent economic analysis for India similarly found that full replacement of smear microscopy with Xpert would substantially increase budget requirements but would result in lower cost per MDR-TB case initiated on treatment [22]. As the NTEP plans its NSP budget for 2020–2025, it should consider MDR-TB treatment costs as much as, if not more than, the prices of diagnostic tests.

Truenat MTB test has a higher sensitivity than other conventional diagnostic tests like smear microscopy or culture for MTB. The present study stresses the importance of this new tool, which is indigenous, economical and convenient to use in a low resource setting like India. This study paves and opens windows for larger studies for replacing other molecular diagnostic tests. The performance of Truenat has been evaluated extensively by various researchers and has been compared with conventional culture based as well as with other molecular diagnostic methods. Nikam et al., from Hinduja Hospital and Medical research centre, Mumbai evaluated the performance of Truenat RTPCR in comparison with GeneXpert on sputum samples from Pulmonary TB cases and found a high concordance (96%) with GeneXpert.

6. CONCLUSION

This study was conducted in the Govt Chest Disease Hospital Jammu (J&K). A total 248 clinical samples were collected and tested for mycobacterium tuberculosis infection and drug resistance by TrueNat a rapid PCR system and ZN smear method from December 2024 to December 2025 about one year. The prevalence rate is about 17% in our study.

Truenat MTB-RIF assay (Truenat), a nucleic acid amplification test (NAAT), is a real-time polymerase chain reaction (RT-PCR) chip-based assay that can detect Mycobacterium tuberculosis (Mtb) and rifampicin (RIF) drug resistance using portable, battery-operated devices. The National TB Elimination Program (NTEP) in India introduced this novel tool at the district and subdistrict level in 2020. This study aimed to assess the level and causes of inconclusive results (invalid results, errors, and indeterminate results) in MTB and RIF testing at NTEP sites and the root causes of these in the programmatic setting.

The World Health Organisation (WHO) has endorsed the use of TrueNat PCR as a rapid diagnostic test for the diagnosis of TB and prioritized areas like drug-resistant TB, paediatric TB, TBHIV co-infection, extra-pulmonary TB, and sputum smear negative PTB for use of TrueNat. This study concludes that in less accessible and resource limited rural settings where establishing a sophisticated laboratory for culture to the prescribed biosafety levels is difficult, TrueNat PCR system provides a very good detection laboratory method. Extensive use of this assay thereby facilitates early treatment decisions and curbing transmission of Tuberculosis. Our study highlighted the usefulness of the TrueNat over and above the traditional smear microscopy for significantly higher positive results. It also has an added advantage of detection of multi-drug resistant cases, thus contributing as a milestone in 'End TB' strategy.

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