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Research Article

Optimising Pulmonary Tuberculosis Detection: A Comparative Study of Modern and Traditional Diagnostic Methods In A Tertiary Healthcare Centre of Telangana

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Abstract
Background: Tuberculosis (TB) has afflicted humanity for millennia. In 1882, Robert Koch identified *Mycobacterium tuberculosis* as the causative agent. Despite this early discovery, TB remains a major global health challenge, largely due to inadequate case detection. Early and accurate diagnosis is critical for timely treatment and effective TB control.
Methods: This study compared the diagnostic performance of smear microscopy, Cartridge-Based Nucleic Acid Amplification Test (CBNAAT/GeneXpert), and Truenat in detecting pulmonary TB. A total of 2,875 patients were screened over two years (2022–2024). Of these, 196 were clinically and radiologically suspected of TB, and CBNAAT microbiologically confirmed 123.
Results: Among the 123 CBNAAT-positive cases, 71 were men and 52 were women. The highest prevalence (21%) was seen in the 21–30 and 51–60-year age groups, followed by 19% in the 31–40-year group. Of the 82 Truenat-negative samples, nine were CBNAAT-positive, while CBNAAT confirmed all 114 Truenat-positive cases. Among 93 AFB smear-negative samples, 21 were CBNAAT-positive, and 102 AFB smear-positive samples were also CBNAAT-positive. Using CBNAAT as the gold standard, Ziehl-Neelsen (ZN) staining demonstrated 82.93% sensitivity, 98.63% specificity, 99.03% positive predictive value (PPV), 77.42% negative predictive value (NPV), and 88.78% accuracy. In contrast, Truenat exhibited 92.68% sensitivity, 100% specificity, 100% PPV, 89.02% NPV, and 95.41% accuracy.
Conclusion: Truenat outperformed ZN staining in diagnostic accuracy and reliability for pulmonary TB detection. Integrating molecular diagnostics such as Truenat and CBNAAT with conventional microscopy within a unified diagnostic algorithm can enhance TB case detection and improve differentiation of Mycobacterium tuberculosis from non-tuberculous mycobacteria. Such an approach will be vital in strengthening TB control strategies and advancing toward the goal of ending TB by 2035.

Keywords: CBNAAT, Truenat, Tuberculosis, *Mycobacterium,* ZN staining.

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Introduction

Tuberculosis (TB) has afflicted humanity for millennia. In 1882, Robert Koch identified *Mycobacterium tuberculosis* as the causative agent of the disease [1,2]. Despite its early discovery, TB remains a primary global health concern, with an estimated 10.6 million new cases and 1.6 million deaths reported in 2022, according to the Global TB Report 2023 [3]. The burden is particularly high in countries such as India, where TB continues to pose a significant public health challenge. Pulmonary TB, accounting for over 80% of all TB cases, is the most infectious form of the disease. If left untreated, a single individual can transmit the infection to 10–15 others per year through close contact. Early detection and accurate diagnosis are therefore critical for effective disease control and timely initiation of treatment. However, inadequate case detection continues to impede global TB control efforts [4]. Sputum smear microscopy has long been the cornerstone of pulmonary TB diagnosis due to its simplicity, affordability, and reliability [5]. Although microscopy provides high specificity, it offers only a presumptive diagnosis and is limited in detecting cases with low bacillary load [6]. Culture methods, considered the gold standard for TB diagnosis, demonstrate superior sensitivity and specificity compared to smear microscopy. Nevertheless, their widespread application is restricted by long turnaround times (21 days to 8 weeks for solid and liquid cultures), high costs, biosafety requirements, and the need for specialised laboratories and skilled personnel [7–9]. To overcome these challenges, molecular diagnostic tools such as the Cartridge-Based Nucleic Acid Amplification Test (CBNAAT/GeneXpert) and Truenat have emerged as promising alternatives. The WHO's End TB Strategy emphasises the importance of early diagnosis and universal Drug Susceptibility Testing (DST), highlighting the critical role of rapid molecular methods in detecting both TB and drug resistance [10]. CBNAAT (GeneXpert®, Cepheid) is a semi-quantitative, hemi-nested PCR-based system that detects *M. tuberculosis* and rifampicin resistance by targeting mutations in the *rpoB* gene [11]. Truenat, developed by Molbio Diagnostics (India), is a portable, battery-operated real-time micro-PCR system using chip-based assays such as Truenat MTB (*nrdB* target), MTB Plus (*nrdZ* and *IS6110*), and MTB-RIF Dx (*rpoB* target for rifampicin resistance) [12,13]. In 2020, the WHO endorsed molecular rapid diagnostic tests as the primary tools for presumptive TB detection, replacing smear microscopy due to their superior speed and accuracy [14]. Despite these advances, the comparative diagnostic performance of CBNAAT, Truenat, and conventional microscopy remains an area of ongoing research. The present study aims to evaluate and compare the **accuracy, sensitivity, and specificity** of CBNAAT, Truenat, and smear microscopy in diagnosing pulmonary tuberculosis.

MATERIALS AND METHODS

Study Design and Setting: This cross-sectional study was conducted between August 2022 and August 2024 at a tertiary care hospital in Hyderabad, India. All patients clinically suspected of having pulmonary tuberculosis (TB), irrespective of age, sex, or HIV status, who were referred to the Department of Microbiology for mycobacterial analysis, were included. A total of 2,875 sputum samples were processed for Ziehl-Neelsen (ZN) staining and molecular testing. Samples lacking relevant clinical information and extrapulmonary specimens were excluded to avoid bias. Ethical permission was obtained from the Institutional Ethics Committee (IEC) via letter dated (IEC/MAMS/2024/040), and research progress reports were submitted every 6 months. Statistical analysis was performed using GraphPad Prism, version 10.6.1.

Specimen Collection and Processing: Patients were instructed in proper sputum collection procedures and asked to provide approximately 5 mL of sputum in a sterile, leakproof container. For hospitalised patients unable to expectorate sputum, bronchoalveolar lavage (BAL) specimens were collected under aseptic conditions by trained personnel. Each specimen was labelled with the patient's identification details and promptly transported to the microbiology laboratory. Specimens were processed immediately upon receipt. In cases of unavoidable delay, samples were stored at 4°C for up to 24 hours. All specimen handling was performed in a Class II A2 biosafety cabinet, in accordance with standard biosafety protocols. Ziehl-Neelsen (ZN) Staining: Smears were prepared on clean glass slides from each sample and stained using the conventional Ziehl-Neelsen technique. Slides were examined under oil immersion (100×) using a binocular microscope. The presence of red, slender, slightly beaded acid-fast bacilli (AFB) was considered positive for *Mycobacterium tuberculosis* (Fig. 5) [15]. Truenat MTB Assay: For the Truenat MTB test, 0.5 mL of homogenised sputum was mixed with 3 mL of lysis buffer and processed using the Trueprep Auto Universal cartridge-based system (Molbio Diagnostics, India) for nucleic acid extraction. A 5–6 µL aliquot of extracted DNA was then analysed using the TruNAT MTB assay (Molbio Diagnostics, India) as per the manufacturer's instructions. The assay employs real-time PCR using proprietary reagents and a preprogrammed microchip profile. The device automatically interpreted results as *"Invalid," "Error," "MTB not detected,"* or *"MTB detected"* [16,17]. Cartridge-Based Nucleic Acid Amplification Test (CBNAAT/GeneXpert): CBNAAT was performed according to WHO-recommended protocols. For each sample, 3–5 mL of sputum was mixed with twice its volume of reagent containing sodium hydroxide (NaOH) and isopropanol. The mixture was incubated for 10 minutes with intermittent shaking to liquefy and decontaminate the sample. Following incubation, 2 mL of the processed specimen was loaded into a GeneXpert cartridge and analysed using the GeneXpert system (Cepheid, USA). The assay provided results within 2 hours, reporting both detection of *Mycobacterium tuberculosis* and rifampicin resistance status. The bacterial load was semi-quantitatively categorised as *very low, low, medium,* or *high* [18,19].

Results

A total of 2,875 patients were screened over a two-year period (August 2022–August 2024). Of these, 196 patients were clinically and radiologically suspected of having pulmonary tuberculosis (TB). Microbiological confirmation was obtained in 123 (62.8%) of these cases using CBNAAT, which was used as the gold standard. Among the 123 CBNAAT-positive patients, 71 (57.7%) were men and 52 (42.3%) were women (*Fig. 1*). The highest prevalence of TB was observed in the 21–30-year and 51–60-year age groups (each 21%), followed by 19% in the 31–40-year group (*Fig. 2*). Comparative Performance of Diagnostic Tests, of the 196 clinically suspected TB patients: 123 (62.8%) were CBNAAT-positive**,** 114 (58.2%) were Truenat-positive**,** and 103 (52.6%) were AFB smear (ZN stain)-positive (*Fig. 3*). Among the 82 Truenat-negative samples, 9 (11%) were CBNAAT-positive. Conversely, all 114 Truenat-positive samples were confirmed positive by CBNAAT. Similarly, among the 93 AFB smear-negative samples, 21 (22.6%) tested positive by CBNAAT, while 102 smear-positive samples were also CBNAAT-positive**.** Truenat detected 114 true positives**,** no false positives**,** and nine false negatives (*Fig. 4*). Comparison with the Gold Standard (CBNAAT): Using CBNAAT as the gold standard, ZN staining detected 102 true positives**,** one false positive, and 21 false negatives (*Fig. 5*). Truenat demonstrated higher sensitivity, NPV, and overall accuracy than ZN staining, with perfect specificity and PPV. Receiver Operating Characteristic (ROC) Analysis: The ROC curves further validated the excellent diagnostic performance of both tests ZN staining**:** AUC = 0.9187, indicating a 91.87% ability to distinguish TB-positive from TB-negative cases. Truenat: AUC = 0.9634**,** indicating a 96.34% diagnostic accuracy. Both AUC values suggest excellent diagnostic utility, with Truenat outperforming ZN staining. (*Fig 6, Table 1, Image 1*).

Figure 1. Gender distribution among confirmed pulmonary TB cases. Out of 123 CBNAAT-positive patients, 71 (58%) were male and 52 (42%) were female.

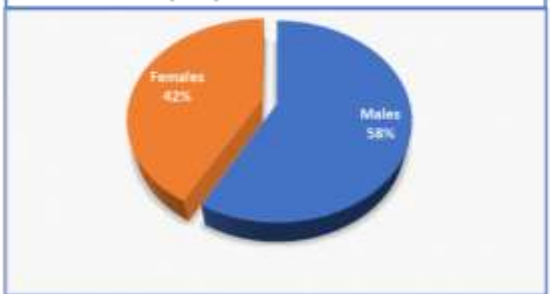

Figure 2. Distribution of pulmonary TB cases according to age group

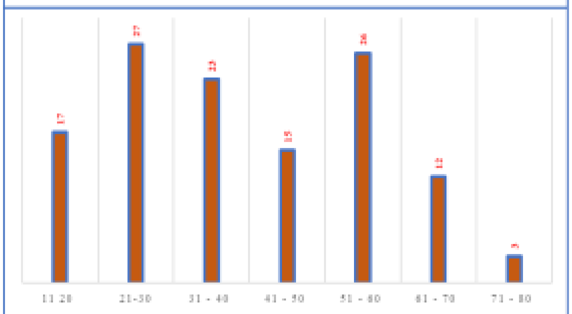

Figure 3 - Comparison of diagnostic positivity among CBNAAT, Truenat, and AFB smear microscopy.

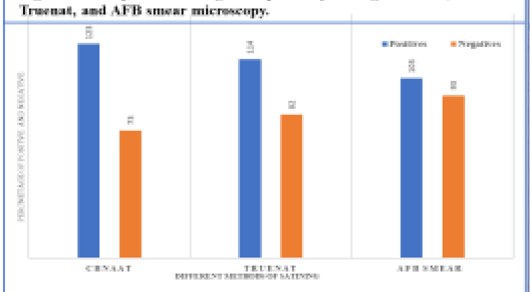

Figure 4 - Comparison of Truenat results among CBNAAT-positive and CBNAAT-negative cases.

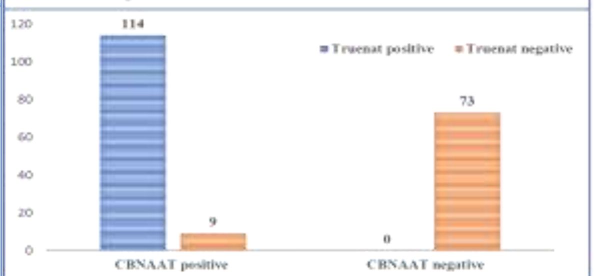

Figure 5 - Comparison of AFB smear results among CBNAAT-positive and CBNAAT-negative cases.

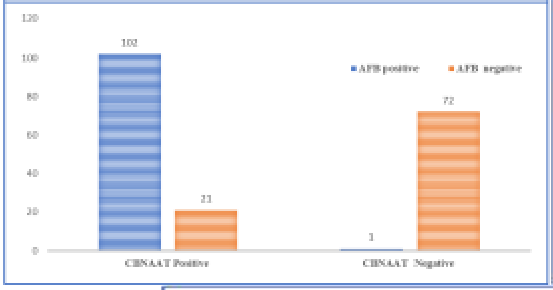

Figure 6 - ROC curve comparing diagnostic performance of AFB smear and Truenat tests.

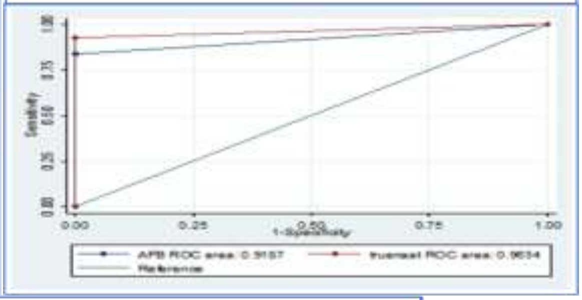

Image 1: ZN Stain showing red colored, thin, beaded, acid-fast bacilli.

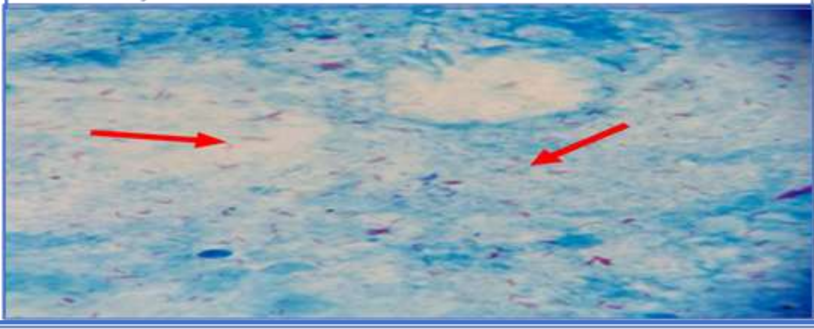

Table 1 - Comparison of diagnostic performance of Zn stain and Truenat for detecting tuberculosis (TB)

	Zn stain		Truenat	
Statistic	Value	95% CI	Value	95% CI
Sensitivity	82.93%	75.09 to 89.11%	92.68%	86.56 - 96.60%
Specificity	98.63%	92.60 to 99.97%	100.00%	95.07 - 100%
Positive predictive value	99.03%	93.56 to 99.86%	100.00%	96.82 - 100%
Negative predictive value	77.42%	69.88 to 83.51%	89.02%	81.22 - 93.83%
Accuracy	88.78%	83.50 to 92.83%	95.41%	91.46 - 97.88%

Discussion

In our study, females exhibited a higher rate of TB positivity compared to males, which contrasts with findings from studies conducted in India and other countries where men were more frequently infected [20, 21]. This observation may indicate that women in our study population are more proactive in seeking healthcare. Apart from socio-economic and cultural influences, biological factors could also play a role in gender-related differences in TB susceptibility [20]. Analysis of TB-positive cases across different age groups revealed the lowest prevalence among children below 10 years**,** whereas adults exhibited the highest infection rate. The protective effect of Bacillus Calmette–Guérin (BCG) vaccination could partially explain the reduced TB prevalence among younger children [22]. However, pediatric TB remains underdiagnosed and underreported**,** owing to nonspecific clinical features, low bacillary loads, and a lack of reliable biomarkers. Further research is warranted to assess the long-term efficacy of childhood BCG vaccination in preventing pulmonary TB. Our study compared the diagnostic performance of Ziehl-Neelsen (ZN) staining-based AFB smear microscopy and Truenat**,** using the GeneXpert MTB/RIF assay as the reference standard for detecting *Mycobacterium tuberculosis* in pulmonary samples. In the present study, ZN staining identified 52.56% of suspected pulmonary TB samples as positive, while 47.44% tested negative among the screened population. The test demonstrated an overall accuracy of 88.78%, with a sensitivity of 82.93%, specificity of 98.63%, positive predictive value (PPV) of 99.03%, and negative predictive value (NPV) of 77.42%. These findings are consistent with the results reported by Agrawal M et al., who evaluated 170 pulmonary samples [23]. The high specificity (98.63%) observed in our study aligns with the findings of Sabyasachi Mandal et al. and Padmaja GV et al [24,25], but contrasts with the lower specificity (57.14%) reported by Akhtar S et al. [26]. Conversely, the study by Chandora AK and Chandora A et al., involving 100 patients, reported markedly lower performance (sensitivity 22.22%, specificity 78.38%, PPV 63.64%, and NPV 37.18%) [27], which clearly deviated from our results. In our analysis, ZN smear microscopy demonstrated a PPV of 99.03%, confirming that a positive result strongly indicates pulmonary TB. However, the NPV of 77.42% suggests that a negative result cannot reliably exclude the disease. The receiver operating characteristic (ROC) curve analysis positioned ZN smear microscopy close to the upper-left corner, with an area under the curve (AUC) of 0.9187, indicating intense diagnostic discrimination between GeneXpert-positive and GeneXpert-negative specimens. Comparable ROC analyses with slightly lower AUC values were reported by Mavenyengwa et al. and Umair M et al. [28, 29]. This study assessed the diagnostic accuracy of Truenat in detecting pulmonary disease. Truenat detected 58% of the samples as positive, while 42% were negative. While CBNAAT, the gold standard, detected 123 positive cases, Truenat identified 114. Additionally, CBNAAT confirmed nine specimens as positive that Truenat had classified as negative. Sensitivity, specificity, PPV, NPV, and accuracy of Truenat in the detection of sputum specimens were 92.68%, 100%, 100%, 89.2%, and 95.41%, respectively, in the present study, which is in concurrence with 97.53%, 23.08%, and 92.00%, which were the sensitivity, specificity, PPV, NPV, and accuracy of Truenat for the samples tested by Akhtar S et al., except for the NPV value [26]. The Truenat MTB assay demonstrated 92.68% sensitivity and 100% specificity in our study, consistent with findings by Ngangue YR et al., who reported 91% sensitivity and 96% specificity for Truenat in TB detection. Penn-Nicholson A et al. reported Truenat's sensitivity and specificity as 84% and 95%, respectively. In comparison, Akthar S et al. reported sensitivities of 94.05% and specificities of 42.86%, both of which differ slightly from our study [21, 26, 30]. In this study, ZN smear microscopy failed to detect 20 cases (16.4%) that were accurately detected by both Truenat and GeneXpert MTB/RIF, a finding consistent with reports from other studies [31, 32, 33, 34]. AFB smear microscopy has lower sensitivity due to its higher detection threshold (5,000 bacilli/mL) compared to TRUENAT and GeneXpert MTB/RIF, which detect as few as 100 and 131 bacilli/mL, respectively. CBNAAT identified 123 positive specimens, outperforming ZN staining, which detected only 103, underscoring CBNAAT's superior sensitivity in tuberculosis diagnosis. CBNAAT detected 20 smear-negative specimens. A delayed or missed diagnosis of smear-negative pulmonary tuberculosis can impose substantial financial strain on individuals, their families, and the nation as a whole. Achieving our goal of TB elimination by 2035 requires intensified action of accurate diagnosis, proper treatment, and follow-up to reduce incidence by 16% annually [35]. Despite its cost-effectiveness, sputum smear microscopy's sensitivity is suboptimal.

Conclusion:

Our study emphasises the need for an integrated diagnostic algorithm that combines advanced molecular diagnostics with conventional methods, thereby enhancing the specificity of NAATs through the distinguishable detection of Non-Tubercular Mycobacteria. This approach will enable us to strengthen TB control strategies and move closer to the goal of ending TB by 2035.

Limitations of the study:

This study did not evaluate GeneXpert 's ability to detect rifampicin resistance, as it was beyond its scope due to the absence of requisitions for rifampicin sensitivity testing for all samples. The study's robustness could have been enhanced by identifying all isolates to the species level. The limited sample size and the single-centre design highlight the need for further research with a larger cohort to validate our findings.

Autros Contribution

1,2, and 3 Formulation of Hypothesis, 4 and 5 Preparation and execution of methodology, 6 and 7 Review and statistical analysis preparation of the manuscript.

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