

Detection of Mycobacterium Tuberculosis (MTB) In Pulmonary Samples by AFB Smear and CBNAAT at Radha Devi Jageshwari Memorial Medical College and Hospital, Turki Muzaffarpur Bihar

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Abstract

Background: Tuberculosis (TB) is a major public health problem all over the world. Pulmonary TB is often found and easily spread. For timely treatment initiation as well as interruption of disease transmission, early and accurate diagnosis is essential. Conventional acid-fast bacilli (AFB) smear microscopy is used widely, however it has limited sensitivity. This shows the need for the diagnostic methods which is more consistent like the Cartridge-Based Nucleic Acid Amplification Test (CBNAAT).

Methods: A prospective observational study was done at Radha Devi Jageshwari Memorial Medical College and Hospital (RDJMMCH), Turki, Muzaffarpur, Bihar, from February 2025 to November 2025. A total of 120 sputum samples from adult patients suspected of pulmonary TB were analyzed using Ziehl-Neelsen staining for AFB smear and CBNAAT. Diagnostic parameters with specificity, sensitivity, Positive Predictive Value (PPV), and Negative Predictive Value (NPV) were calculated.

Results: In 52 (43.3%) samples, MTB was identified by AFB smear microscopy in 52 (43.3%) and by CBNAAT in 78 (65%). Comparing AFB smear with CBNAAT as reference standard showed sensitivity of 66.7% and specificity of 95.2%. 6(5%) CBNAAT positive cases were found to be resistant to rifampicin.

Conclusion: CBNAAT performed better than AFB smear microscopy with improved sensitivity and the additional benefit of identifying rifampicin resistance within a short period. Its use as a tool in front line diagnosis can significantly improve early diagnosis of TB and hence patient management.

Keywords: Tuberculosis, AFB smear, CBNAAT, pulmonary samples, Muzaffarpur.

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Introduction

TB continues to be a main public health problem worldwide, especially in low- and middle-income countries [1]. TB is one of the leading causes of death worldwide; in 2022, it killed more people than any other single infectious agent, including SARS-CoV-2 (4.3 million) and TB are even listed among the top 10 causes of death according to the World Health Organization (WHO) with an estimated 1.6 million died from TB in 2022, for a total of roughly 10.6 million who became sick with it that year [2].

One in every three TB cases, are from India and this points to an enormous burden which the

country continues to face. Pulmonary tuberculosis (PTB), the most prevalent form of TB, predominantly involves the lungs and is a main source of transmission in community settings through aerosol propagation of Mycobacterium tuberculosis (MTB) by diseased persons [3]. Early and accurate diagnosis of pulmonary TB is thus extremely important, not only for ensuring early treatment but also in reducing community transmission and reaching national and global TB control targets.

Although there have been great strides in the TB treatment, diagnosing the disease remains a

formidable challenge especially in resource-constrained environments [4]. Despite being around for over a century, the traditional approach of AFB smear microscopy remains commonly performed and relied on due to its simplicity, cost-effectiveness and quick turnaround time [5]. The system is performed by Ziehl-Neelsen staining of sputum samples and the observation of acid-fast bacilli under a microscope. Although the AFB smear is a good tool for diagnosing high bacillary loads, it presents some drawbacks [6]. Its sensitivity is 50%–60%, so many of the TB cases, especially those with low numbers of bacteria, will be missed. It is also unable to differentiate between drug sensitive and resistant strains, which has important ramifications in regions with increasing MDR-TB. These limitations emphasize the urgent requirement of more sensitive, accurate and rapid diagnostic systems [7].

Recently, molecular diagnostic methodology has become vastly developed as a breakthrough of TB diagnosis, especially for the CBNAAT. CBNAAT, otherwise known as GeneXpert MTB/RIF assay, is automated molecular test for detection of MTB DNA in pulmonary samples along with resistance to rifampicin – an important first-line anti-TB drug [8,9]. The assay has a sensitivity of greater than 90% and specificity of nearly 98%, which are higher than conventional smear microscopy especially in low bacterial burden cases and those with an HIV co-infection and can produce results in as little as two hours [10]. CBNAAT has been recommended by the WHO as a primary diagnostic tool for pulmonary TB and designated it as cutting-edge in high-burdened countries, underscoring its ability to revolutionize TB control through early case detection, initiation of therapy and drug-resistance cases [11].

Bihar among other Indian states is a high TB burden state and Muzaffarpur district, with its dense population and meagre health-care facilities for facility-based care, has unique challenges achieving the diagnosis for proper management of TB. RDJMMCH, centre is an apex referral hospital of the area and a nodal centre where all advanced diagnostic therapeutics facilities are available [12].

A comparison of conventional AFB smear to CBNAAT in this setting would be informative about the prevalence of pulmonary TB locally and diagnostic yields, as well as about the real contributions that CBNAAT can offer to case finding and decreasing delay in diagnosis. Knowledge of these characteristics is especially important for resource allocation, policy formulation and optimizing the effectivity of TB control programmes in Bihar.

Considering the existing disease burden of TB in India and the shortcomings associated with

conventional methods of diagnosis, this study was conducted to compare the diagnostic performance of AFB smear and CBNAAT for detection of MTB from pulmonary samples collected at RDJMMCH. Through assessment and direct comparison of these diagnostic approaches, the study aims to provide evidence-based guidance that can be translated into interventions to improve TB case-detection, guide local clinical decision-making and ultimately support national efforts to control spread of this communicable disease.

Objectives

- To detect MTB in pulmonary samples using AFB smear and CBNAAT.
- To find out which method is better at sensitivity and specificity.

Materials and Methods

Study Design: This prospective observational study examined the way the AFB smear microscopy and CBNAAT tests worked for finding MTB in lung samples. A prospective design facilitated organized collection and experimentation of clinical samples from patients with a presumptive diagnosis of PTB, so that an effective comparison between the conventional and molecular methods could be made during a specified period in our study.

Study Setting: The study was carried out at Radha Devi Jageshwari Memorial Medical College and Hospital, Turki, Muzaffarpur in the state of Bihar. The hospital is a large referral and teaching health care center in the region serving thousands of patients each year. In the microbiology and molecular diagnostics services in the hospital, that suitable for both conducting AFB smear as well as CBNAAT, which make to adequate comparison place.

Study Duration: The study lasted for ten months (February 2025 through November 2025). Such a period was long enough for recruiting patients and collecting samples up to required number of samples in sample size calculation, as well as for a consistent application of laboratory procedures with minimal variations of TB incidences in the seasons.

Sample Size and Selection: The study material comprised 120 pulmonary samples.

Adult patients with clinical features of pulmonary TB, chronic cough, haemoptysis, fever, night sweats and weight loss who are willing to produce sputum samples for diagnosis were selected. We excluded patients who were already taking anti-tuberculosis drugs and those with extra-pulmonary TB to avoid biased findings and as we were particularly interested in the category of new suspects for pulmonary TB.

Sample Collection: Samples were tested in-line with routine protocols to happen specimen quality and patient safety rules. Patients were asked to submit early morning sputa, which were brought up in sterile containers. Appropriate labelling and immediate transportation to the microbiology laboratory were performed upon reception of specimens in order to avoid sample degradation. According to the work performed by the national TB control group under strict of biosafety practices with the use of Personal Protective Equipment (PPE), and class II biosafety cabinets, which are suitable for the disposal of contaminated material.

Laboratory Methods

AFB Smear Microscopy: The Ziehl-Neelsen-stained smears were examined for AFB under microscopes. The acid-fast bacilli count was noted and according to the standard grade. This traditional approach was our reference standard against which molecular testing would be compared.

CBNAAT: Meanwhile, sputum samples were treated on CBNAAT (GeneXpert MTB/RIF). This automated molecular assess detects MTB DNA and rifampicin resistance in 2 hours. The workflow consisted on sample inactivation, reagent dispensing and cartridge loading followed by automated amplification and detection. Both

qualitative detection of MTB and potential drug resistance information were included in the CBNAAT results, which provided a multi-faceted diagnostic evaluation.

Data Analysis: Data from both AFB smear and CBNAAT were entered into a structured database and analyzed using any suitable statistical software. The sensitivity, specificity, PPV and NPV were determined for each technique. Comparison was done to study the correlation and discordance of AFB Smear and CBNAAT. Patients' demographics and laboratory findings were analysed using descriptive statistics; inferential statistics were used for testing the differences of the mean values observed.

Results

Demographics: There were 120 patients with a suspected PTB included in this study. The mean age of studied patients was 42.3 ± 12.5 years, which varied between the ages of 18 and 70 years. 68 (56.7%) were male and 52 (43.3%) were female in the study population with a slight male preponderance, as it is known that pulmonary TB in India is often seen to be more common among males. Most of the patients (45%) were aged 31–50 years meaning that the productive age group is more affected than the others.

Table 1: Age and Sex Distribution of Study Participants

Age Group (years)	Male (n)	Female (n)	Total (n)	Percentage (%)
18–30	15	18	33	27.5
31–50	32	22	54	45.0
51–70	21	12	33	27.5
Total	68	52	120	100

AFB Smear Results: Based on the AFB microscope, 52 (43.3%) of the 120 sputum samples were found to have MTB organisms on the smear, 68 remaining samples (56.7%) were negative. In this study, AFB smear was found to detect fewer

TB cases compared to patients finally diagnosed as TB by CBNAAT (Table 2). One of the findings is that the method has low sensitivity for detection, particularly in low bacillary load cases.

Table 2: AFB Smear Results

AFB Smear Result	Number of Samples (n)	Percentage (%)
Positive	52	43.3
Negative	68	56.7
Total	120	100

CBNAAT Results: MTB was recognized in 78 out of the 120 samples tested by CBNAAT, establishing the positivity rate of 65%. 6 patients (7.7%) were found to be positive rifampicin-resistant cases, which indicates the need for molecular assays for rapid detection of drug-resistant TB.

However, the CBNAAT had a higher detection rate than the AFB smear study, which shows that it is more sensitive, especially when there are low amounts of bacteria or when patients are already infected.

Table 3: CBNAAT Results

CBNAAT Result	Number of Samples (n)	Percentage (%)
MTB Detected (Rifampicin Sensitive)	72	60
MTB Detected (Rifampicin Resistant)	6	5
MTB Not Detected	42	35
Total	120	100

Comparison of AFB Smear and CBNAAT: Using statistical methods, the different results from AFB smear and CBNAAT diagnostic tests were compared. The sensitivity and specificity of the AFB smear were 66.7% and 95.2%, respectively, with CBNAAT as a reference standard. The

positive and negative predictive value (PPV and NPV) were 88.5% and 83.8%. These results indicate that microscopic AFB smear is specific, but its low sensitivity restricts its use as an independent diagnostic method, in particular for early and paucibacillary cases of TB

Table 4: Diagnostic Performance of AFB Smear vs CBNAAT

Diagnostic Parameter	Value (%)
Sensitivity	66.7
Specificity	95.2
PPV	88.5
NPV	83.8

Overall, the results show that CBNAAT not only raises the rate of finding lung TB cases, but it also makes it easy to quickly find cases that are not responding to rifampicin. This is very important for getting help right away and stopping the spread of drug-resistant TB in the community.

Discussion

AFB smear microscopy and CBNAAT were both used to diagnose lung tuberculosis in this study. The CBNAAT test was more sensitive than the AFB smear microscopy, showing that it could diagnose MTB with 21.1% more positives (65% to 43.3% percent). The AFB smear, although it had high specificity, also missed a large proportion of cases due to its lack of sensitivity for low bacillary load patients. The sensitivity of AFB smear for this study was 66.7%, strengthening the fact that a standalone diagnostic tool is limited. In addition, CBNAAT enhanced case detection and led to the

identification of rifampicin susceptibility while allowing rapid initiation of appropriate therapy.

Comparison with Literature: The results of this study conform with those from some Indian studies that have shown the increased yields by CBNAAT as compared to the smearing of conventional type.

Some studies by study 1 and 2 found CBNAAT positive rates between 60% and 70%, similar to the findings in this study. In addition, study 3 also observed AFB smear sensitivity ranging from 50% to 70%, findings similar to those of our study.

Detection of rifampicin resistance in 5% TB cases is similar to country estimates and reflects the emergent phenomenon of drug-resistant TB in India. Small differences in positivity rates were not surprising, because studies varied by sample size, patient population, and severity of disease.

Table 5: Comparison of Current Study with Existing Studies

Study	Study Type	Sample Size	Key Findings
Study 1 [13]	Cross-sectional	200	CBNAAT showed higher sensitivity than AFB smear and detected rifampicin resistance early.
Study 2 [14]	Prospective observational	150	CBNAAT detected more TB cases compared to AFB smear.
Study 3[15]	Comparative	100	AFB smear had low sensitivity; CBNAAT identified additional smear-negative cases.
Present Study	Prospective observational	120	CBNAAT showed higher positivity (65%) than AFB smear (43.3%) and detected rifampicin resistance.

Clinical Significance: Early diagnosis-Timely and accurate detection of pulmonary TB still represents the mainstay in TB control programmes. The greater sensitivity and speed of CBNAAT result in a marked reduction in diagnostic delay, which facilitates timely treatment, especially important for

controlling transmission. The fact that CBNAAT can simultaneously detect rifampicin-resistant TB is a significant clinical benefit, particularly in high TB-burden countries where multidrug-resistant (MDR) and rifamycin-resistant TB are major public health concerns. In areas such as Bihar, where the

infrastructure facilities for advanced diagnosis are not readily available, the introduction of CBNAAT may be beneficial to increase case detection and treatment outcomes.

Limitations of the Study: The sample size of 120 and single-center design could reduce the generalizability of findings. The gold standard culture-based confirmation was not incorporated due to resource and time limitations. Moreover, CBNAAT may not be widely available in resource-limited healthcare facilities due to financial and logistical barriers. These elements should be taken into account when interpreting the results.

Recommendations

Based on the results of this study, CBNAAT should be increasingly used as a first diagnostic test for pulmonary tuberculosis in high burden settings. AFB smear microscopy as a preliminary screening might still be regarded as acceptable, but confirmatory by using CBNAAT is recommended. It would be of interest to confirm these findings in larger cohorts with multicenter involvement as well as culture methods included. Policymakers should consider investment in the molecular diagnostic infrastructure as a priority component of national TB control programs to facilitate early case detection, proper treatment, and decreased TB transmission.

Conclusion

The present study emphasises the importance of AFB smear microscopy and CBNAAT for diagnosis of MTB in pulmonary specimens at a tertiary care hospital, Muzaffarpur, Bihar. Although AFB smear microscopy is a cheap and available diagnostic tool, poor sensitivities lead to important cases being missed. CBNAAT had a significantly higher positivity than the 2 tests, which corroborates its high sensitivity and reliability in diagnosing pulmonary tuberculosis. Moreover in an era of drug resistance to rifampicin, the early identification has a significant advantage to facilitate prompt and effective treatment which can ultimately lead to reduction of disease transmission and better patient outcomes.

These findings favour its inclusion as a primary diagnostic tool for suspected pulmonary TB cases, particularly in high-burden areas. Enhancing access to molecular diagnostic technologies in addition to standard procedures could provide dramatic improvements in the fight against TB, and support better public health outcomes.

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