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Comparison of positivity rate of Mycobacterium Tuberculosis in Slide Method through Microscopy and Machine Method (Nucleic Acid Amplification Test)

Mahesh Gaba[1] Manjit Kaur[2] Khushi Joshi[3]
Faculty of Allied Healthcare Sciences, Desh Bhagat University[1]
Department of Biotechnology, Rapture Biotech, Dehradun[2]
Department of Microbiology, Rapture Biotech, Dehradun[3]
Corresponding mail: gabamahesh6@gmail

ABSTRACT

Tuberculosis (TB), a disease caused by Mycobacterium tuberculosis (MTB), remains a significant public health challenge in India, which reports the highest incidence globally. Although national programs such as the Revised National Tuberculosis Control Programme (RNTCP) and the National Tuberculosis Elimination Programme (NTEP) have made strides in disease control, timely and accurate diagnosis remains essential to curtail transmission. This study evaluates and compares two diagnostic methods: the traditional Ziehl-Neelsen (ZN) staining technique and the modern Cartridge-Based Nucleic Acid Amplification Test (CBNAAT). While ZN microscopy is cost-effective and widely used in low-resource settings, its diagnostic sensitivity is limited, particularly in cases with low bacillary load. Conversely, CBNAAT offers enhanced sensitivity and specificity by detecting MTB DNA and rifampicin resistance within a short turnaround time. Among 140 sputum samples analyzed, CBNAAT identified a higher number of positive cases than ZN staining, underscoring its diagnostic advantage. However, constraints such as cost and infrastructure still hinder its widespread adoption. This study highlights the importance of a complementary diagnostic approach that leverages the strengths of both methods in varied clinical settings.

Tuberculosis (TB), caused by Mycobacterium tuberculosis (MTB), remains a major concern for public health authorities in India, which bears the world’s highest TB burden (WHO, 2024). Despite ongoing national initiatives like the Revised National Tuberculosis Control Programme (RNTCP) and its successor, the National Tuberculosis Elimination Programme (NTEP), the prompt and accurate diagnosis is crucial for breaking the chain of transmission and initiating effective treatment. This study compares the diagnostic efficiency and positivity rate of the conventional Ziehl-Nelsen (ZN) microscopy and the modern Cartridge-Based Nucleic Acid Amplification Test (CBNAAT) in detecting pulmonary TB from sputum samples. The Ziehl-Nelsen (ZN) microscopy technique has long served as a cost-effective diagnostic approach widely used in resource-constrained settings. Nonetheless, its diagnostic sensitivity is significantly constrained in cases with low bacillary load, extrapulmonary TB, or paediatric TB [1]. In contrast, the Cartridge-Based Nucleic Acid Amplification Test (CBNAAT), which was endorsed by the World Health Organization in 2010, is a rapid molecular diagnostic method that detects MTB DNA and resistance to rifampicin typically within a two-hour timeframe. It is significantly more sensitive and specific, including in smear-negative and non-sputum-producing patients [2].

In this prospective cross-sectional study, 140 sputum samples from patients suspected of pulmonary TB were tested using both ZN microscopy and CBNAAT. ZN staining detected MTB in 15 cases (10.72%), while CBNAAT identified 17 positive cases (12.15%). Notably, two samples undetected by ZN microscopy were reported as “very low” positive by CBNAAT, indicating its superior sensitivity, particularly in paucibacillary samples. No rifampicin resistance was detected among CBNAAT-positive cases, though the tool remains critical for early resistance profiling [3].

The findings underscore the superior diagnostic capability of CBNAAT, particularly in detecting cases that might be missed by microscopy. This has important implications for India’s TB elimination target by 2025, where rapid and accurate diagnosis is a cornerstone. However, widespread implementation of CBNAAT is hindered by higher costs and infrastructure requirements such as electricity and temperature control thereby restricting its deployment in remote or resource-constrained regions. Meanwhile, ZN microscopy, despite its lower sensitivity, continues to be essential due to its low cost and minimal technical demands [4]. A noted limitation of the study is the modest sample size and the exclusion of extra pulmonary and paediatric TB cases, which could have provided more comprehensive evidence of

CBNAAT's utility. Furthermore, CBNAAT only detects rifampicin resistance, not broader multidrug resistance (MDR), necessitating additional drug susceptibility testing where needed [5].

In summary, CBNAAT exhibited enhanced sensitivity and a significantly reduced diagnostic turnaround compared to ZN microscopy. Its application, especially in smear-negative or early-stage cases, offers a valuable diagnostic upgrade. However, a tiered approach using microscopy for preliminary screening and CBNAAT for confirmatory and resistance testing may provide the most practical balance of cost, accessibility, and diagnostic accuracy in India's diverse healthcare landscape [4].

1. Introduction:

India continues to shoulder the highest burden of tuberculosis (TB) globally, accounting for approximately 2.2 million of the 9 million cases reported worldwide each year [6]. The critical need for rapid and reliable TB diagnostics has become increasingly evident to enable early treatment and halt the chain of transmission. Recognizing this, the World Health Organization (WHO) in December 2010 endorsed the use of GeneXpert MTB/RIF, a Cartridge-Based Nucleic Acid Amplification Test (CBNAAT), as a game-changing tool in TB diagnosis and rifampicin resistance detection [7]

GeneXpert is a semi-quantitative, real-time polymerase chain reaction (PCR)-based test. It serves two key purposes:

(1) the detection of *Mycobacterium tuberculosis* (MTB) DNA in expectorated or induced sputum, regardless of smear status.

(2) identification of rifampicin resistance through mutations in the *rpoB* gene, a reliable proxy for multidrug-resistant TB (MDR-TB) [5,8]

Ziehl-Neelsen (ZN) microscopy, while traditional and cost-effective, has limited sensitivity, particularly in smear-negative or paucibacillary cases. In contrast, fluorescent microscopy (FM) recommended by WHO as a replacement for ZN offers approximately 8–10% greater sensitivity and requires less examination time per sample [6,7].

This thesis presents the first study conducted at our institution following the installation of the CBNAAT platform. It aims to compare the positivity rate and diagnostic efficacy of CBNAAT with ZN microscopy in pulmonary TB using sputum samples.

Figure 1: TB Bacteria

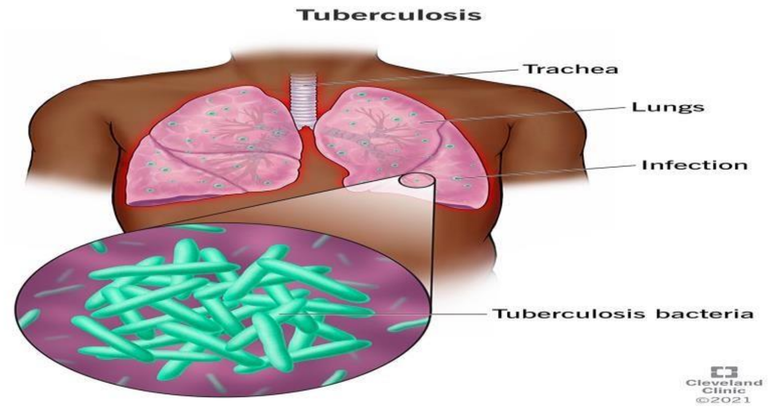


Figure 1 the image illustrates the pathology of tuberculosis (TB), focusing on its pulmonary form, which is the most common manifestation of the disease. It presents a frontal view of the human thoracic region, highlighting the lungs and trachea, with a specific emphasis on the infection site within the lungs. The infected lung tissue is visually marked with small greenish lesions, indicating the areas colonized by *Mycobacterium tuberculosis* (MTB), the causative agent of TB. A magnified inset at the bottom of the image provides a closer view of the tuberculosis bacteria, depicted as rod-shaped bacilli. These bacteria are acid-fast and are typically identified in clinical settings using techniques such as Ziehl-Neelsen (ZN) staining or Cartridge-Based Nucleic Acid Amplification Test (CBNAAT). The image clearly demonstrates how the pathogen resides and proliferates within the pulmonary structures, leading to symptomatic manifestations such as a persistent cough, fever, night sweats, and weight loss.

The diagram is educational and supports understanding of the pathophysiology and diagnostic targets in TB, making it suitable for both clinical and academic use. It visually reinforces the importance of detecting the bacteria early using reliable diagnostic tools, especially in symptomatic patients suspected of pulmonary TB. The illustration is credited to [9] and provides a clear and informative representation of the disease process.

1.1 DISTRIBUTION:

Tuberculosis (TB) is a bacterial infection that typically affects the lungs. They can also spread to other organs. While pulmonary TB is the most prevalent form, extra pulmonary TB can also develop, involving other organs such as the kidney, brain, spine, lymph nodes, and bones, which can be treated with medications, but can be fatal if not treated [10].

1.2 Morphology:

- Morphology of Mycobacterium Tuberculosis.
- Straight or slightly curved thin rod-shaped bacilli.
- Non-sporing, non-motile, and non-capsulated bacteria.
- Acid-fast bacilli, neither gram +ve nor gram -ve.
- During acid-fast stain, they appear bright red to intensive purple with green/blue background.
- They measure 0.5 micro-meter- 3 micro-meter.
- The cell wall is rich in lipid and waxes.
- They are wrapped together due to the presence of fatty acid.
- Capable of intracellular growth.
- They are resistant to disinfectants: detergents, common antibiotic, dyes, stains, osmotic lysis, lethal oxidation, etc. [11].

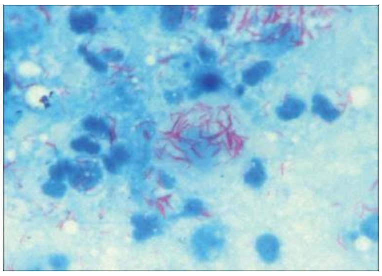


Figure 2 Mycobacterium (ZN stain)

The image provided is a microscopic view of a Ziehl-Neelsen stained sputum smear, commonly used for the diagnosis of tuberculosis (TB). In this stain, acid-fast bacilli (AFB) such as *Mycobacterium tuberculosis* appear as bright red or pink rod-shaped organisms against a contrasting blue background composed of non-acid-fast material, such as epithelial cells and mucus.

The red rods visible in clusters and scattered throughout the field confirm the presence of acid-fast bacilli, indicating a positive result for TB. The intensity and density of bacilli suggest that this may correspond to a moderate to high bacterial load, possibly consistent with a grading of 2+ or 3+ in smear microscopy terms, depending on the field count. The clarity of the bacilli and the background staining further support that the staining technique was appropriately conducted.

This image is a classic representation of smear-positive pulmonary tuberculosis, where the identification of AFB under the microscope plays a critical role in early diagnosis and initiation of treatment, especially in resource-limited settings where more advanced diagnostics like CBNAAT may not be immediately available.

1.3 Pathogenesis of *MT*B:

- Infection in a healthy individual begins with inhalation of air droplets containing MTB bacilli.
- The bacteria are phagocytosed by the alveolar macrophages.

- The formation of phagolysosomal complexes in macrophages kills bacteria.
- Few bacteria escape the host innate immune response and replicate in the macrophages.
- This induces adaptive immune response causing release of pro-inflammatory cytokines and recruitment of other immune cells.
- The immune cells form granuloma to contain the infection at primary site (latent TB infection).
- Under immune compromised condition the granuloma ruptures causing release of MTB to other sites (active TB infection) [12].

1.4 Diagnostic methods for detection of *MT*B:

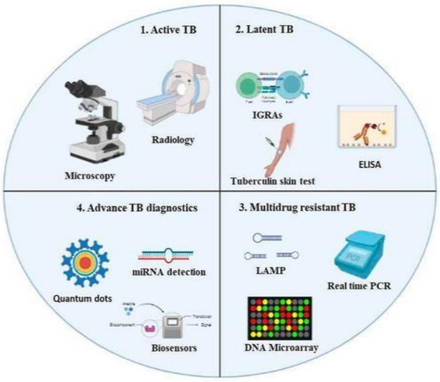


Figure 3 Diagnostic methods

1.4.1 Active TB:

Early diagnosis of TB is performed using microscopy, in which the presence of active bacilli is visualized under a microscope and radiology, where the chest X-ray is read to detect lesions.

1.4.2 Latent TB:

Latent TB infection is diagnosed using tuberculin skin test, ELISA, and IGRA. These tests are based on the inflammatory reaction initiated by the host in the presence of the MTB pathogen [11].

1.4.3 Multidrug-resistant TB:

For the diagnosis of multidrug-resistant TB, more specific and sensitive assays are needed, such as RTPCR, DNA microarray, and LAMP. These methods specifically determine mutations in genes, allowing for the identification of drug resistance [5,13]

Advances in diagnosis with the exploitation of nanoparticles, miRNA, and CRISPR-Cas have led to a revolution in TB diagnosis [14].

1.5 Radiology for pulmonary TB:

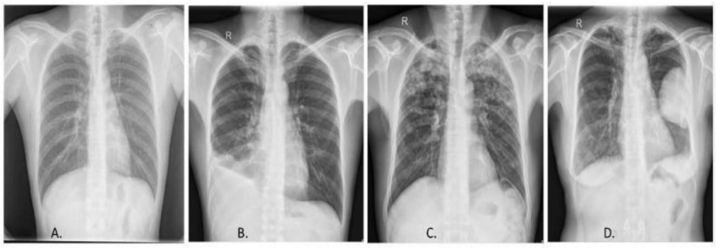


Figure 4 ***Chest X-ray***

1.5.1 Radiological diagnosis of TB, Chest X-ray:

(A) Normal chest X-ray. X-ray displaying different manifestations of TB (B)

Pleural effusion.

(C) Infiltrates.

(D) Cavity lung lesion [13,14].

1.6 Tuberculin Skin Test (TST):

1.6.1 Injection:

A small amount (0.1 mL) of purified protein derivative (PPD) was injected under the forearm skin.

1.6.2 Wait:

After 48 to 72 h, you must return to a healthcare provider for the test site to be examined.

1.6.3 Reading the Result:

- Measured the size of the induration (raised and hardened areas).
- Redness is not measured, only the firmness (induration)

Induration size
≥5 mm

If it is positive, further evaluation is required.

- Chest X-ray.

  Possibly sputum testing

- A positive TST does not indicate active TB, which indicates TB exposure or latent TB infection.

1.7 Interferon-gamma Release Assays (IGRAs):

IGRAs are blood tests that measure immune response to TB-specific antigens. They are used to detect latent TB infections (LTBI) and, in some cases, help support the diagnosis of active TB [11,17].

1.7.1 Work:

- A blood sample is taken.
- Blood is mixed with TB-specific antigens (which are not found in BCG).
- If the immune system recognizes TB, T cells release interferon-gamma (IFN-γ).
- The amount of IFN-γ released is measured.

1.7.2 Who Should Get an IGRA?

- People who received the BCG vaccine.
- Those unlikely to return for a second TST appointment.
- Healthcare workers, immune compromised patients, or those in high-risk settings.
- People born or living in countries with high TB prevalence.

1.7.3 IGRA Limitations:

- Cannot distinguish between latent and active TB.
- May be less reliable in young children (<5 years).
- More expensive than TST.

1.8 Urinary lipoarabinomannan test:

The urine lipoarabinomannan (LAM) test is a rapid diagnostic test used to detect tuberculosis (TB), especially in people with advanced HIV infection. It detects lipoarabinomannan (LAM), a TB cell wall antigen that is shed into urine when TB bacteria are present in the body. It is non-invasive and gives results quickly (within 25–30 minutes) [3,4,5].

1.8.1 The World Health Organization (WHO) recommends the test only for:

- People with HIV who have signs or symptoms of TB.

- Have CD4 count <200 cells/µL.
- Are seriously ill, regardless of CD4 count.

It’s not recommended for use in HIV-negative people, as the sensitivity is much lower.

1.8.2 Works:

- Urine sample is collected
- A dipstick test detects the LAM antigen.
- A visible band appears if positive — similar to a pregnancy test.

1.8.3 Advantages:

- Fast (results in less than 30 minutes)
- No sputum needed (helpful for patients who cannot produce sputum) Useful in severely ill or immune compromised patients.
- Can help reduce TB-related deaths in HIV-positive hospitalized Patients.

Table 1: Factor And Limitation

Outlines the limitations associated with a particular diagnostic method, likely Ziehl-Neelsen (ZN) staining, commonly used for detecting *Mycobacterium tuberculosis*. One key limitation is its low sensitivity, particularly in individuals who do not have advanced HIV infection. This is significant because TB often co-exists with HIV, and reduced bacterial load in early-stage HIV can lead to false negative results. The specificity of the test is also noted to be variable, depending on the clinical and epidemiological setting. This variability may stem from differences in laboratory practices, sample quality, or the prevalence of non-tuberculous mycobacteria in a region. Furthermore, the test's use case is limited, as it is not suitable for general population screening. This is due to its inability to reliably detect TB in asymptomatic individuals or in cases with low bacillary load, thereby restricting its application primarily to symptomatic individuals in clinical settings.

Factor	**Limitation**
Sensitivity	Low in patients without advanced HIV
Specificity	Variable depending on setting
Use case	Not for general population screening

1.9 Habitat:

The primary habitat of MTB, the bacterium that causes tuberculosis, is within human, it is facultative intercellular parasite, meaning it can live both inside and outside of the cells, but it primarily resides inside macrophages within the human body. Although it is often found in the lungs, it can also infect other parts of the body [15].

Reservoir: Humans are the primary and only known reservoirs of MTB.

Infection: MTB enters the body through inhalation of respiratory droplets.

Intracellular Parasitism: MTB is a facultative intracellular parasite, meaning it can survive and multiply within macrophages.

Location: It is commonly found in well-aerated upper lobes of the lungs.

Other sites: primary found in lugs, MTB can infect other parts of the, body including the bones, CNS, and lymphatic system.

Environmental survival: Due to its waxy cell wall MTB survive. In the environmental for extended period (months) and more resistance to disinfectants compared with other bacteria.

Animal reservoirs: some animal species like cattle can also be infected with related strains such as M. bovis.

1.10 Spread of TB:

TB bacteria spread through air when someone with active TB coughs, sneezes, speaks or sings releasing tiny droplets containing the bacteria.
When someone inhales these droplets, the bacteria can settle in the lungs, where they can multiply and cause infection.
In some cases, the bacteria can spread to other organs through the blood stream [6,16].

1.11 Objective of the research:

The objective is to compare CBNAAT (Gene Xpert MT / RIF) with ZN microscopy in detection of Pulmonary Tuberculosis using sputum Sample.

2. MATERIAL AND METHOD

2.1 Study Design:

This is prospective cross-sectional study conducted to compare the positivity rate of Mycobacterium Tuberculosis using Ziehl-Neelsen (ZN) staining and Cartridge-Based Nucleic Acid Amplification Test (CBNAAT) in suspected TB patient.

2.2 Sample Size:

In total, 140 clinical samples (for example, Sputum) where collected from patient Suspected pulmonary TB.

2.3 Inclusion Criteria:

Patients presenting with clinical symptoms suggestive of TB (e.g., cough > 2 weeks, weight loss, fever, night sweats).

- Patient aged (e.g., more than 12 years)
- Both new and previous TB suspects.

2.4 Exclusion Criteria:

- Patient already on anti-tubercular treatment (ATT) for more than 7 days
- Inadequate or contaminated samples.

2.5 Sample Collection:

Sputum sample: Early morning, deep coughed sputum collected in a sterile, leak-proof container. Other samples (if included): processed according to the standard protocol depending on the specimen type.

2.6 Materials:

2.6.1 Equipment's:

- Microscope.
- CBNAAT Machine.
- Refrigerator.
- Air conditioner.

2.6. 2 Reagents:

- Carbol Fuchsin
- Sulphuric acid
- Methylene Blue

- Immersion oil
- Phenol

2.6.3 Miscellaneous:

- Broom sticks.
- Burner.
- Sputum Cup.
- Slide.
- Falcon Tube.
- Mask.
- Gloves.

2.8 Method /Procedure ZN Stain:

The Ziehl-Nelsen (ZN) microscopic staining procedure is a method used to identify acid-fast bacteria, particularly Mycobacterium tuberculosis, by staining them with a primary stain, decolorizing with acid alcohol, and then counterstaining with a contrasting dye. The acid-fast bacteria retain the primary stain, while other bacteria are decolorized and stained with the counterstain.

2.8. 1 Smear Preparation:

A small amount of the sample is mixed with a drop of water on a clean slide, spread thinly, and allowed to air dry completely.

2.8.2 Heat Fixation:

The dried smear is passed through a flame 3-4 times to adhere the bacteria to the slide.

2.8.3 Primary Staining:

Flood the smear with carbol fuchsin, a primary stain, and gently heat until steam rises, maintaining the steam for 5 minutes, or until the stain is sufficiently penetrating.

2.8.4 Decolorization:

Wash the slide with water and then apply acid-alcohol to the smear, allowing it to sit for a few minutes, or until the smear is pale pink.

2.8.5 Counterstaining:

Rinse the slide with water and then counterstain with methylene blue or malachite green for a few minutes.

2.8.6 Washing:

Rinse the slide with water to remove excess stain.

2.8.7 Drying and Examination:

Allow the slide to air dry and examine under the oil immersion lens of a microscope.

2.9 Method /Procedure CBNAAT:

2.9.1 Sample Collection:

Sample is collected first; collect at least 5 ml of sputum sample.

2.9.2 Sample processing:

Use a vortex (buffer) to liquefy sample for processing. Then incubated for 15 min. room temp.

2.9.3 Sample Loading:

The liquefy sample is then loaded in to the cartridge.

2.9.4 Cartridge Loading:

Scan the Barcode of the cartridge, loaded with liquefy sample and start the test.

2.9.5 Automated Testing:

The machine automatically processed the sample and performs Nucleic acid amplification or Drug sensitivity.

2.10 Bio Medical Waste Generated:

BMW generated through this process generally discarded through following:

Table 2: Bio Medical Waste Generated

Table 2 Outlines the biosafety and biomedical waste (BMW) management protocols for various laboratory items used in the processing of clinical specimens, such as sputum, particularly in the context of tuberculosis diagnostics. Each item is treated according to established infection control guidelines to minimize the risk of cross-contamination and environmental exposure.

Broomsticks are disinfected using 5% phenol solution, a common chemical used for surface and instrument decontamination. For sputum cups, after overnight soaking (24 hours) in 5% phenol, they are disposed of in a red-colored dustbin, while the residual waste water is directed to a common sewage treatment plant (STP), ensuring no direct environmental release.

Slides used for smear preparation follow a similar disinfection protocol with 5% phenol for 24 hours, after which they are disposed of in a blue BMW (Biomedical Waste) bag, adhering to color-coded waste segregation norms. Likewise, falcon tubes undergo phenol soaking and are also discarded in the red dustbin, with wastewater routed to the STP.

For personal protective equipment (PPE), masks are disposed of in yellow BMW bags, which typically indicate waste requiring incineration, while gloves are placed in red BMW bags, designated for plastic waste that can be disinfected and then shredded or autoclaved.

This structured protocol ensures effective infection control and environmental safety, complying with national biomedical waste handling standards and minimizing the risk of TB transmission in the laboratory setting.

Broom sticks	5% Phenol Solution.
Sputum Cup	5% Phenol Solution for overnight socking (24hr) and then Sputum cup are dumped in Red coloured dustbin and other waste water to common STP plant.
Slide	5% Phenol Solution for overnight socking (24hr) and then Slide are dumped in Blue Coloured BMW Bag and other waste water to common STP plant.
Falcon Tube	5% Phenol Solution for overnight socking (24hr) and then Falcon Tube are dumped in Red coloured dustbin and other waste water to common STP plant.
Mask	Yellow BMW Bag.
Gloves	Red BMW Bag.

3. RESULTS

3.1: ZN Stain:

Two slides for the Microscopy are taken for one Patient primarily to improve the accuracy and sensitivity of diagnose.

Acid-fast bacteria (like M. tuberculosis) will appear red or pink due to the carol fuchsin stain, while other bacteria will appear blue or green due to the counterstain.

Table 3: Cases for Microscopy

Parameter	No. of cases for Microscopy
Total Patients	140
Male	85
Female	55
Above 40 years	98
Below 40 years	42
Positive slide	30
Negative slide	250
1+, 2+, 3+ positive slide	30

Table 3 The table presents data related to microscopy-based diagnosis of tuberculosis using Ziehl Nelsen (ZN) staining among a group of 140 patients. Of these, 85 were male and 55 were female, indicating a slightly higher prevalence of suspected cases among males. In terms of age distribution, the majority of the patients (98 cases) were above 40 years, while 42 patients were below 40, suggesting a higher burden or suspicion of TB in the older age group.

Regarding diagnostic outcomes, 30 slides were reported as positive, indicating the presence of acid-fast bacilli (AFB), while 250 slides were marked as negative. The number of negative slides exceeds the number of patients, implying that multiple samples were likely taken per patient, which is consistent with standard TB diagnostic procedures that often require more than one sputum sample for confirmation.

All 30 positive slides were categorized as 1+, 2+, or 3+, reflecting the grading scale used to quantify the bacillary load in smear microscopy. This distribution suggests that among the positive cases, the presence of TB bacilli was sufficient to be graded, which helps in assessing the severity and infectiousness of the disease.

Overall, the data highlights a moderate positivity rate in the tested population and suggests that older adults and males were more represented in the sample. It also reinforces the importance of multiple sample analysis to improve diagnostic sensitivity in microscopy-based detection of tuberculosis.

Table 4: Result ZN Stain.

Table 4 The table summarizes the microscopy results of Ziehl-Nelsen (ZN) staining for detecting *Mycobacterium tuberculosis* among 140 patients, breaking down the frequency and percentage of each grading category. The vast majority of samples, 125 out of 140 (89.28%), were negative, indicating no visible acid-fast bacilli (AFB) under microscopy. This high percentage of negative results could be attributed to low bacillary load, early-stage disease, or non-tubercular respiratory conditions.

Among the 15 positive cases (10.71%), the distribution of bacillary load varied. A single sample (0.71%) was categorized as scanty, which refers to the detection of very few bacilli, typically 1–9 AFB per 100 fields, reflecting a very low bacterial burden. Eleven samples (7.85%) were graded as 1+, indicating a slightly higher but still relatively low concentration of bacilli. Two cases (1.42%) fell into the 2+ category, suggesting a moderate bacterial load, while only one case (0.71%) was classified as 3+, which represents a heavy infection with numerous AFB observed.

This distribution indicates that most positive cases had low to moderate bacillary counts, which could reflect early diagnosis or less severe disease. The data also reinforces the limited sensitivity of microscopy, especially in cases with scanty or low-grade positivity, highlighting the need for more sensitive diagnostic tools like CBNAAT in clinical practice.

No.	**Microscopic Result**	**F(frequency)**	**Percentage**
1	Negative	125	89.28%
2	Scanty	1	0.71%
3	1+	11	7.85%
4	2+	2	1.42%
5	3+	1	0.71%
Total		**140**	**100**

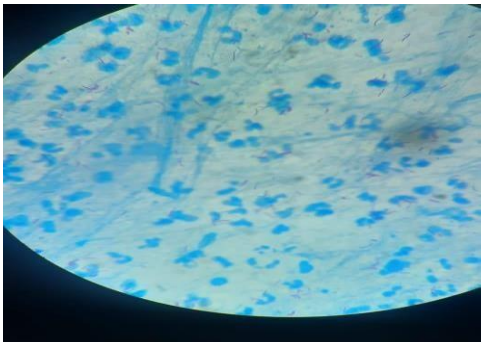
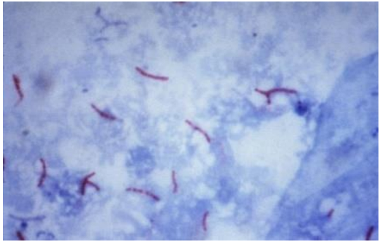


Figure 5: 3+ positive slide through ZN Microscopy



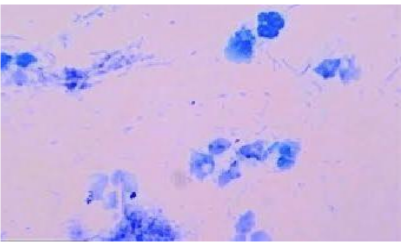


Figure 6: Positive slide ZN stain

Figure7: Negative slide ZN stain

Table No 5: Grading Chart

Result (WHO scale) 1000 x field=HPF	**Bright Field x 1000 magnification : 1 length = 2cm=100HPF**
Negative	Zero AFB / 100 HPF
Scanty	1-9 AFB / 100 HPF
1 +	10-99 AFB / 100 HPF
2+	1-10 AFB / 1 HPF (on average 50 HPF)

3+	>10 AFB / 1 HPF (on average 20 HPF)

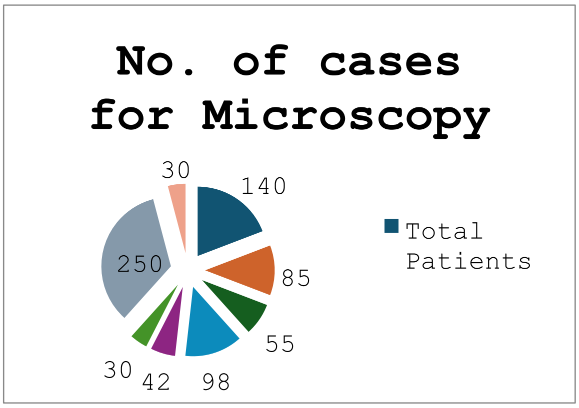


Figure 8: Graphical representation of Microscopy sample (Age wise)

Figure 8 The pie chart titled "No. of cases for Microscopy" illustrates the distribution of patients and their test results using sputum smear microscopy for tuberculosis diagnosis. A total of 140 patients were included in the study. Of these, 85 were male and 55 were female, once again indicating a male predominance in the tested population.

In terms of age distribution, 98 patients were above 40 years of age, while 42 patients were below 40, which reflects a trend similar to the CBNAAT data, showing that older individuals form a major portion of the tested cases.

Microscopy results reveal that 30 patients had positive slides, confirming the presence of acid-fast bacilli indicative of TB. In contrast, a significantly larger group—250 slides—were negative, suggesting that the majority of tested patients did not have smear-detectable TB. This imbalance may be attributed to the relatively lower sensitivity of microscopy, especially in cases with low bacterial load.

Interestingly, 30 slides were categorized as 1+, 2+, or 3+ positive, indicating varying degrees of bacterial load among the positive cases. This grading is crucial in TB diagnosis, as it helps in assessing the severity of infection and guiding treatment decisions.

In summary, the pie chart shows that although a relatively small proportion of patients had smear-positive TB, the test remains a valuable tool for detecting and grading TB infection. The predominance of negative slides highlights the need for complementary diagnostic methods such as CBNAAT, particularly in cases where microscopy may miss low-burden infections.

3.2 Result CBNAAT:

Rapid Results:

CBNAAT offers significantly faster turnaround times compared to traditional culture-based methods, allowing for quicker initiation of appropriate treatment.
On completion of a test run, the Cartridge-based Nucleic Acid Amplification Test (CBNAAT) gives the following results:

01) MTB NOT DETECTED.
02) MTB DETECTED; Rif Resistance DETECTED.
03) MTB DETECTED; Rif Resistance NOT DETECTED.
04) MTB DETECTED; Rif Resistance INDETERMINATE.

Table 6: Cases through CBNAAT

Table 6 The data presented outlines the diagnostic outcomes of 140 patients who were tested through CBNAAT (Cartridge Based Nucleic Acid Amplification Test). Among the total cases, there is a male predominance, with 85 males and 55 females undergoing the test. In terms of age distribution, a majority of the patients (98) were above 40 years, while the remaining 42 patients were below 40 years of age, indicating that older individuals constituted the larger proportion of the tested population.

Regarding the test results, the vast majority of cases (123 out of 140) were found to be negative for *Mycobacterium tuberculosis* (MTB), suggesting no detectable infection in these patients. Among the 17 patients in whom MTB was detected, none were found to have rifampicin (RIF) resistance, indicating the absence of multidrug-resistant tuberculosis in this group. Furthermore, no cases exhibited intermediate resistance to RIF. All 17 MTB-positive cases were classified under "MTB Detected, RIF Not Detected," suggesting drug-sensitive tuberculosis in these patients.

In summary, the analysis reveals a predominance of older and male patients in the tested group, a high rate of MTB-negative results, and an absence of rifampicin resistance among the MTB-positive cases, reflecting a favourable resistance profile within the study population.

Parameter	**No. of Cases Through CBNAAT**
Total Patients	140
Male	85
Female	55
Above 40 years	98
Below 40 years	42
MTB NOT DETECTED	123
MTBDETECTED, RIF RESISTANCE DETECTED	0
MTB DETECTED, RIF. NOT DETECTED	17
MTB DETECTED RIF. INTERMEDIATE	0

Table 7: Result CBNAAT

Table 7 The CBNAAT results for 140 patients provide insight into the frequency and distribution of *Mycobacterium tuberculosis* detection among the tested population. A substantial majority of the patients, 123 out of 140, representing 87.85%, had results indicating "MTB Not Detected," suggesting no presence of tuberculosis in these individuals. This highlights a low overall prevalence of MTB in the sample group. Among the 17 patients who tested positive for MTB, the bacterial load varied across different levels. Only one case (0.71%) showed a high bacterial load ("MTB Detected High"), while two cases (1.42%) demonstrated a medium load. The majority of the MTB-positive patients—12 cases, accounting for 8.57%—were classified as having a low bacterial load. Additionally, two cases (1.42%) fell under the "MTB Detected Very Low" category, indicating minimal bacterial presence.

Overall, the data suggests that while MTB detection was relatively uncommon in this cohort, most of the positive cases exhibited a low to very low bacterial burden, with only a minimal number showing medium or high loads. This may reflect early detection, effective immune response, or low infectivity among those who tested positive

No.	**CBNAAT Result**	**F(frequency)**	**Percentage**
1	MTB Not Detected	123	87.85%
2	MTB Detected High	01	0.71%
3	MTB Detected Medium	02	1.42%

4	MTB Detected Low	12	8.57%
5	MTB Detected Very Low	02	1.42%
Total		**140**	**100**


GeneXpert PC 05/28/25 11:13:05

Test Report

Patient ID:
Sample ID: 92
Test Type: Specimen
Sample Type:

Assay Information

Assay	Assay Version	Assay Type
Xpert MTB-RIF Assay G4	6	In Vitro Diagnostic

Test Result: MTB NOT DETECTED

Analyte Result

Analyte Name	Ct	EndPt	Analyte Result	Probe Check Result
Probe D	0.0	1	NEG	PASS
Probe C	0.0	4	NEG	PASS
Probe E	0.0	0	NEG	PASS
Probe B	0.0	2	NEG	PASS
SPC	27.7	257	PASS	PASS
Probe A	0.0	3	NEG	PASS
QC-1	0.0	0	NEG	PASS
QC-2	0.0	0	NEG	PASS

User: <None>
Status: Done
Expiration Date*: 10/11/26
S/W Version: 5.3
Cartridge S/N*: 9361286977
Reagent Lot ID*: 94493
Notes: baldev singh s/o kachru ram, Tanchi, chadhiar
Error Status: OK
Start Time: 02/24/25 11:50:04
End Time: 02/24/25 13:30:25
Instrument S/N: 832993
Module S/N: 664626
Module Name: A2

Figure 9: MTB Not Detected report Report


GeneXpert PC 05/28/25 11:12:07

Test Report

Patient ID: 101443719
Sample ID: 106
Test Type: Specimen
Sample Type:

Assay Information

Assay	Assay Version	Assay Type
Xpert MTB-RIF Assay G4	6	In Vitro Diagnostic

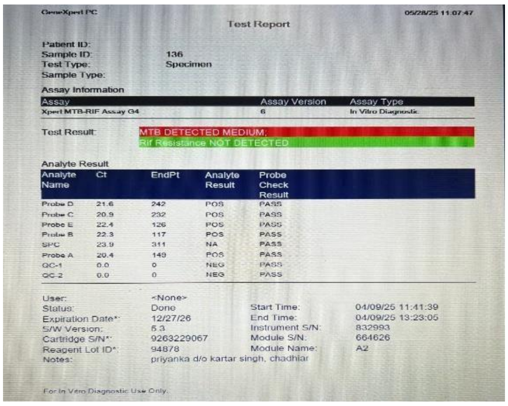
Test Result: MTB DETECTED VERY LOW;
Rif Resistance NOT DETECTED

Analyte Result

Analyte Name	Ct	EndPt	Analyte Result	Probe Check Result
Probe D	31.0	134	POS	PASS
Probe C	30.2	144	POS	PASS
Probe E	32.5	104	POS	PASS
Probe B	29.9	119	POS	PASS
SPC	23.1	272	NA	PASS
Probe A	30.9	97	POS	PASS
QC-1	0.0	0	NEG	PASS
QC-2	0.0	0	NEG	PASS

User: <None>
Status: Done
Expiration Date*: 10/11/26
S/W Version: 5.3
Cartridge S/N*: 9361286945
Reagent Lot ID*: 94493
Notes: kulwant kumar 65 m vill majhera po kachhera
Start Time: 03/11/25 11:19:53
End Time: 03/11/25 13:00:43
Instrument S/N: 832993
Module S/N: 664626
Module Name: A2

Figure 10: Very Low Detected CBNAAT


GeneXpert PC 05/28/25 11:07:47

Test Report

Patient ID:
Sample ID: 136
Test Type: Specimen
Sample Type:

Assay Information

Assay	Assay Version	Assay Type
Xpert MTB-RIF Assay G4	6	In Vitro Diagnostic

Test Result: MTB DETECTED MEDIUM;
Rif Resistance NOT DETECTED

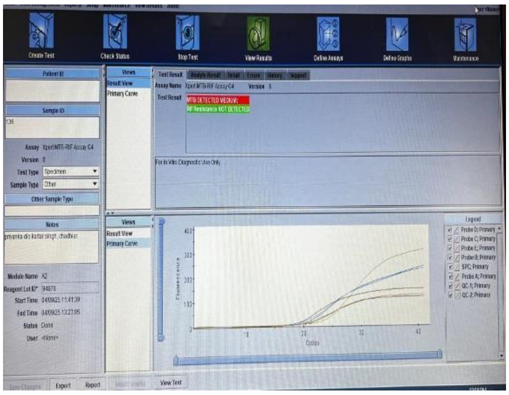
Analyte Result

Analyte Name	Ct	EndPt	Analyte Result	Probe Check Result
Probe D	21.6	242	POS	PASS
Probe C	20.9	232	POS	PASS
Probe E	22.4	126	POS	PASS
Probe B	22.3	117	POS	PASS
SPC	23.9	311	NA	PASS
Probe A	20.4	149	POS	PASS
QC-1	0.0	0	NEG	PASS
QC-2	0.0	0	NEG	PASS

User: <None>
Status: Done
Expiration Date*: 12/27/26
S/W Version: 5.3
Cartridge S/N*: 9263229067
Reagent Lot ID*: 94878
Notes: priyanka d/o kartar singh, chadhiar
Start Time: 04/09/25 11:41:39
End Time: 04/09/25 13:23:05
Instrument S/N: 832993
Module S/N: 664626
Module Name: A2

For In Vitro Diagnostic Use Only.

Figure 11: Medium Detected CBNAAT Report



Patient ID:
Sample ID: 136
Assay: Xpert MTB-RIF Assay G4
Version: 6
Test Type: Specimen
Sample Type: Other
Other Sample Type:
Notes: priyanka d/o kartar singh, chadhiar
Module Name: A2
Reagent Lot ID*: 94878
Start Time: 04/09/25 11:41:39
End Time: 04/09/25 13:23:05
Status: Done
User: <None>

Assay Name: Xpert MTB-RIF Assay G4 Version: 6
Test Result: MTB DETECTED MEDIUM;
Rif Resistance NOT DETECTED

For In Vitro Diagnostic Use Only

Figure 12: Medium Detected CBNAAT Report with Graph Curve

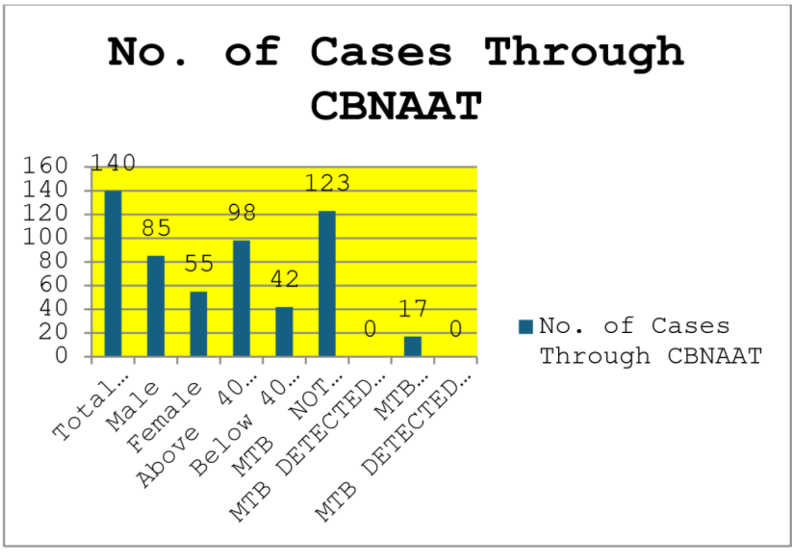


Figure 13: Graphical representation of CBNAAT sample (Age wise)

Figure 18 The bar graph titled "No. of Cases Through CBNAAT" visually represents the distribution of patients tested through the CBNAAT method based on various demographic and diagnostic categories. A total of 140 patients underwent testing. Among these, 85 were male and 55 were female, indicating a higher proportion of male patients in the tested group.

In terms of age distribution, 98 patients were above 40 years of age, while 42 were below 40, showing a significant skew toward older individuals in the sample. This could suggest that tuberculosis screening via CBNAAT is more commonly performed in older populations, possibly due to increased vulnerability or symptomatic presentation in this age group.

The diagnostic outcomes reveal that 123 patients (out of 140) had results of “MTB NOT DETECTED,” which forms the largest category and indicates no evidence of *Mycobacterium tuberculosis* in the majority of tested individuals. MTB was detected in 17 patients, and all of them fell under the category “MTB DETECTED, RIF. NOT DETECTED,” meaning these individuals had tuberculosis without resistance to rifampicin. Notably, no cases were found to have rifampicin resistance (RIF DETECTED) or intermediate resistance, suggesting a complete absence of drug-resistant TB in this sample.

Overall, the graph illustrates a predominantly male and older patient group, a high rate of negative MTB results, and importantly, no detection of rifampicin resistance, which reflects an accessibility drug susceptibility pattern in this patient population.

4. DISCUSSION

After analysing the 140 samples through ZN microscopy, 15 samples found positive with +1, +2 and +3 results.

While the same samples analysed through CBNAAT method, 17 samples found positive showing that 2 samples (MTB detected very Low) which cannot seen by microscopy ZN method came positive in CBNAAT method showing that CBNAAT method is more accurate and result oriented than the ZN Microscopy method.

The present study compared the diagnostic efficacy of Ziehl-Nelsen (ZN) staining with the Cartridge. Based Nucleic Acid Amplification Test (CBNAAT) for the detection of Mycobacterium tuberculosis. ZN staining, though cost-effective and widely available, demonstrated lower sensitivity than CBNAAT, which is consistent with previous findings. While ZN staining is limited to detecting acid-fast bacilli and is more dependent on bacterial load, CBNAAT can detect even low levels of M. tuberculosis DNA and identify rifampicin resistance, providing a significant advantage in both diagnosis and management. Our findings show that CBNAAT outperforms ZN staining in terms of sensitivity and rapid turnaround time, making it a more suitable diagnostic tool, particularly in high-burden or drug-resistant TB settings. However, the limited accessibility and high cost of CBNAAT remain challenges, particularly in resource constrained environments. Therefore, the role of ZN staining continues to be relevant as a preliminary screening tool, particularly in peripheral health centres.

The discrepancies observed between the two methods in some cases could be attributed to differences in detection thresholds and technical variability. CBNAAT is an automated molecular test that reduces observer bias, whereas ZN staining remains highly user-dependent.

5. Conclusion

Tuberculosis (TB) continues to be a significant global health issue, with India bearing the highest burden of the disease. Timely and accurate diagnosis plays a critical role in TB control and elimination efforts, particularly under initiatives like the National Tuberculosis Elimination Programme (NTEP). This study aimed to evaluate and compare the diagnostic effectiveness of two methods Ziehl-Nelsen (ZN) staining, a conventional microscopy-based technique, and Cartridge-Based Nucleic Acid Amplification Test (CBNAAT), a rapid molecular method in detecting *Mycobacterium tuberculosis* in suspected pulmonary TB patients.

From a total of 140 sputum samples analysed, ZN microscopy detected 15 positive cases (10.72%), whereas CBNAAT detected 17 positive cases (12.15%). Two cases that were undetectable by microscopy were found positive with a “very low” bacterial load through CBNAAT, clearly indicating its higher sensitivity. Importantly, none of the CBNAAT-positive cases showed rifampicin resistance, though the tool’s capability to detect such resistance is a major advantage in early MDR-TB management.

The results of this study underscore the limitations of microscopy, which, despite its low cost and widespread availability, struggles with reduced sensitivity especially in smear-negative and low bacillary-load cases. CBNAAT, while costlier and infrastructure-dependent, offers superior diagnostic performance, rapid turnaround, and drug-resistance profiling, making it highly valuable in TB control strategies.

However, challenges remain. CBNAAT’s high operational costs, requirement for uninterrupted electricity and temperature control, and limited penetration in rural and peripheral health centres restrict its universal application. ZN staining, despite its shortcomings, remains indispensable in such settings due to its simplicity and affordability.

This study contributes region-specific data to the growing body of evidence supporting the integration of molecular diagnostics into routine TB care. The findings advocate for a tiered diagnostic approach using microscopy for preliminary screening and CBNAAT for confirmation and drug-resistance detection. Such a strategy could optimize resource use while maximizing diagnostic accuracy.

In conclusion, CBNAAT outperforms traditional microscopy in sensitivity, specificity, and clinical utility. For India to achieve its ambitious goal of eliminating TB by 2025, greater accessibility to CBNAAT and similar technologies must be prioritized, while continuing to utilize microscopy where molecular tools are not feasible. Strengthening laboratory infrastructure, training personnel, and ensuring policy-level support are essential steps toward this goal.

drug resistance. There is also a need for broader regional studies and cost-effectiveness analyses to evaluate its feasibility in national programs.

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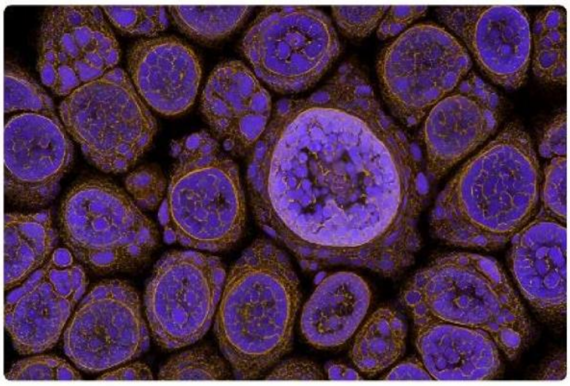

Plot no 977, GMS Road, near Balliwala Flyover, opposite Cubic Plaza,
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