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## Microscopic versus culture methods for diagnosis of *Mycobacterium tuberculosis*: Our experience

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### Abstract

**Background:** Tuberculosis is considered as one of the most reason for death worldwide including India. In India, population, destitution, lack of healthy sustenance, and profoundly blocked climate may help for disease with *Mycobacterium tuberculosis*. The finding and treatment of tuberculosis is not in appropriate with regular strategies. Here we analyzed the location of *M. tuberculosis* with two traditional strategies.

**Materials and Methods:** This study was completed to compare and conclude about outcome between the traditional AFB (Acid Fast Bacilli) microscopy and Lowenstein-Jensen (LJ) culture strategy for recognition of *Mycobacterium* spp. in sputums from patients.

**Results:** Among 100 examples, 57 (57%) AFB+ results were seen in microscopy with ZN stain. On LJ culture media, 62 (62%) AFB+ detaches were discovered which uncovers that the way of life could be a highest quality level for conclusion of TB.

**Conclusion:** The AFB smear is fast, modest and explicit test for early finding of TB however Its affectability is low when contrasted with refined strategy in LJ medium which is the gold standard for the confirmation of tuberculosis.

**Keywords:** Tuberculosis, acid fast bacilli, ZN Stain, LJ medium, pulmonary tuberculosis

### Introduction

India has the highest number of Tuberculosis (TB) cases in the world, with over two million TB cases every year<sup>[1]</sup>. Annually, one fourth of the global incident TB cases occur in India. Early and accurate diagnosis is the first critical step in controlling TB. The control of TB is hampered by diagnostic methods with sub-optimal sensitivity, particularly for the detection of drug resistant forms and in patients with human immunodeficiency virus (HIV) infection. Early detection is essential to interrupt transmission and reduce the death rate, but the complexity and infrastructure needs sensitive methods which limit their accessibility and effect. According to WHO global TB report, the estimated incidence of TB (including TB with HIV) is 2.2 million and prevalence is 2.5 million with mortality (excluding TB with HIV) of 0.22 million<sup>[2]</sup>. There were 580,000 estimated new cases of MDRTB (Multi-drug resistant TB) and Rifampicin resistant TB (RRTB); among them 125,000 (20%) were enrolled. India, China and the Russian Federation accounted for 45% of all estimated MDR/RR-TB cases (henceforth to be called as MDR-TB). India, one of the countries with high burden of TB, has an estimated 79,000 MDR-TB cases among notified pulmonary TB cases. The estimated incidence of MDR-TB is 2% among new cases and 15% among re-treatment cases. The sensitivity of smear microscopy and its inability to detect drug resistance limits its impact on TB control. Culture methods and drug susceptibility testing is complex, time consuming, and takes around 6-8 weeks<sup>[3-5]</sup>. While patients await diagnosis, they are likely to receive inappropriate or in effective treatment leading to disease progression. This results in an increased chance of morbidity from tuberculosis. They continue to transmit drug-resistant TB to others; especially for family members and the resistance might have amplified. To address this issue there was a need for a simple and rapid diagnostic tool at least for high-burden countries and a new diagnostic test cartridge based nucleic acid amplification test (CBNAAT) was developed which was rapid, fully automated and was based on polymerase chain reaction (PCR) that detects deoxyribonucleic acid (DNA) directly from the clinical specimens and also detects rifampicin resistance<sup>[2]</sup>.

In TB endemic areas, most of the cases of TB can be diagnosed correctly by simple and cheap methods which are generally available at peripheral hospital level by AFB microscopy. Although acid fast bacilli (AFB) microscopy and Lowenstein-Jensen (L-J) culture remain the cornerstone of the diagnosis of TB but these traditional bacteriological

methods possess several disadvantages. They are either slow or their sensitivity is quite low. In this study we have evaluated the sensitivity of 2 conventional methods (ZN staining and LJ culture) for diagnosis of *M. tuberculosis*.

### Materials and Methods

This prospective study was carried out in the department of pulmonary medicine at IMS and SUM Hospital, Bhubaneswar for a period of 1 year (January to December 2018). Only suspected *M. tuberculosis* patient samples are taken for this study. There are 100 samples are processed for evaluation of sensitivity of ZN staining and LZ.

One spot of morning sputum sample was collected in a container. On the initial hospital visit, the patient was provided a clean, dry, sterile wide-neck, leak-proof container and requested him or her to cough deeply to produce a sputum specimen. Two direct smears were prepared for staining by Ziehl-Neelsen (Z-N) and was examined under microscope with standard operating procedure. For Ziehl-Neelsen staining, smears were prepared from yellow purulent portion of the sputum using a sterile bamboo stick. A good smear was spread evenly, 3 cm by 2 cm in size in the middle part of the slide which was neither too thick nor too thin. The sputum was left for 15-30 minutes for air drying. The smear was fixed by placing the slide over the hot plate at 85 °C for about 3-5 minutes. Lowenstein-Jensen (L-Z) medium is the most widely used matrix for tuberculosis culture. L-J medium containing glycerol favors the growth of *M. tuberculosis*. After decontaminating and concentrating procedure of sputum samples, three or four drops of deposit were inoculated on two slopes of Lowenstein-Jensen media. The media were incubated at 37 °C after inoculation. They were examined within 3-5 days after inoculation for early recognition of rapidly growing *Mycobacterium* and of contaminated cultures, followed by examination once a week for at least 45 days. Culture was reported as positive as soon as colonies of characteristic morphology constituted of acid-fast bacilli were recognized. The report of culture was prepared according to the number of colonies.

The final species identification of *M. tuberculosis* was done based on the characteristics such as slow growth, colony morphology, and the typical biochemical features. The identities of the isolates were made by growth rate, colony morphology observations.

### Results

Among one hundred patients, in LJ culture method a total 62 (62%) cases AFB<sup>+</sup> (Fig 1) results were detected and 38 (38%) cases were AFB<sup>-</sup>. Similarly, in ZN staining methods 57 cases were positive and rest 43 were negative (Table 1).

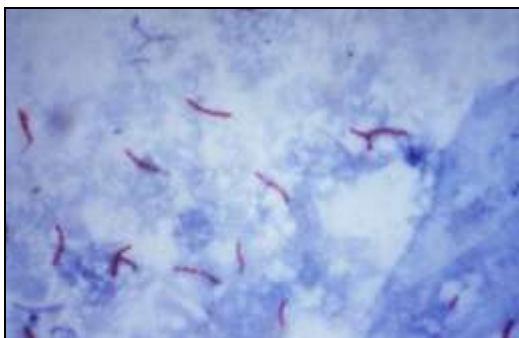


Fig 1: *M. tuberculosis* on ZN stain

Table 1: Results of L-J culture and ZN stain microscopic study for detection of *M. tuberculosis*.

Methods specific for <i>M. tuberculosis</i>	No of samples		P value
	Positive (%)	Negative (%)	
L-J culture	62	38	0.0003
ZN staining microscopy	57	43	0.083

### Discussion

Among communicable diseases, tuberculosis is the second leading cause of death worldwide, killing nearly two million people each year. Most cases are in under developed countries of the world [5]. Another significance of the current work is the statistical analysis on the sensitivity and specificity pattern of AFB microscopic methods. The sensitivity and specificity of BF microscopy was 95.6% and 91.3%. The sensitivity was 92.9% and specificity was 86.9% in conventional fluorescence microscopy in other study. In literature it is found that LED fluorescence microscopy, the sensitivity and specificity was 97.9% and 95.2%, respectively. Thus, LED fluorescence microscopy had higher sensitivity and specificity than others. In a similar study, Githui *et al.* (1999) compared the reliability of fluorescence microscopy (FM) and Ziehl-Neelsen (Z-N) staining method for examination of direct smear in the diagnosis of pulmonary tuberculosis. Culture results were used as the gold standard for assessment [6]. Specificity was 97% and 96% for FM and Z-N methods, respectively. The sensitivity of the FM method was 80% than that of the Z-N method 65%. So, these studies revealed with the present consistency study. Fluorescent microscopy offers well-described benefits, compared with conventional light and fluorescence microscopy, for the evaluation of sputum smear samples for tuberculosis and reduces unnecessary labor.

Thus, this study indicates that in the diagnosis of TB, culture had greater sensitivity and specificity than other microscopic methods. In particular, in case of a single specimen, the diagnostic value of culture was quite significant. It is, therefore, possible to conclude that both microscopy and culture can be used for the diagnosis of TB. If only one or two specimens are available, culture method is preferable. The Z-N method has commonly been used around the world, particularly in developing countries, because of its simplicity and low cost [7-11].

The sensitivity of AFB microscopy (71%) for pulmonary specimens in this study is almost similar to that reported by other studies [12-14]. However one study also reported the high sensitivity of AFB smear microscopy up to 75% [13]. Another study conducted in the same center reported sensitivity of 66.23% for pulmonary specimens [8] which is little low as compared to the present study. This could be due to the fact that most of the specimens received in our study came from patients suspected clinically and radiologically to have pulmonary tuberculosis. Three sputum smears for acid-fast bacilli are recommended for proper diagnosis in pulmonary suspects of TB [15]. However, WHO has proposed two smears for the diagnosis of TB in countries having functional external quality assurance [8]. Culture using LJ medium has been the gold standard for the diagnosis of tuberculosis for many years in the developing countries [7]. An overall AFB culture positivity in the present study was 15.47% and is little higher than the study that revealed the culture positivity of 12.3% [14]. While others

have reported a culture positivity of 48.9% and 47.1% respectively [12, 15]. Culture positivity in the present study is significantly high as compared to AFB smear microscopy as about 5000 to 10000 AFB/ml of specimen is needed to yield positive result by AFB smear microscopy while the advantage of culture on LJ medium is that it has the sensitivity of 80-85%, very specific and being able to detect as few as 10 bacteria per milliliter of specimen [16, 17].

### Conclusions

The prevalence of the disease was 62 %. The smear test was efficient by 57 % in arriving at a positive result with a sample, when its culture test was positive; alternately, it was efficient by 62 % for a negative result, when its culture test result was negative. As found by post-test analysis, both smear and culture tests were dependable by 57–62 % for pulmonary tuberculosis.

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