

## Comparative Study between FM Staining and Z-N Staining in Diagnosing Sputum Smear Positive PTB at S.P. Medical College and Hospital

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### ABSTRACT

The study of sensitivity of Fluorescence staining versus Ziehl-Neelsen fuchsin staining plays an important role in diagnosing sputum smear positive pulmonary tuberculosis. 500 sputum samples collected from 250 suspected cases of pulmonary Tuberculosis attending the microbiology and immunology department between December 2016 to December 2017 were processed and subjected to ZN and FM staining for detection of AFB. Positive smears were graded according to standard RNTCP criteria. Out of 500 sputum smears, 12% and 19.8% were positive by ZN and FM staining respectively. FM staining was found to be superior to ZN staining in detecting TB cases and also FM staining was able to detect more paucibacillary cases than ZN. Our study concludes that FM staining with LED is more efficient over ZN stain in detecting Tuberculosis bacilli in sputum, especially the paucibacillary cases.

### INTRODUCTION

Pulmonary tuberculosis is a disease of the respiratory system, caused by Mycobacterium tuberculosis. Tuberculosis is a predominant infectious cause of mortality today. According to World Health Organization (WHO), tubercular infections are currently spreading at the rate of one person per second per million people. With three millions dying from it, Tuberculosis continues to be a major health problem in our country and is the single largest cause of loss of working hours in the productive age group.<sup>1</sup> There are various methods for bacteriological diagnosis of tuberculosis. Smear examination is believed to be simple, cheap, quick and practicable and effective case finding method for developing countries.<sup>2</sup>

As tuberculosis bacilli are very slow growing organisms, culture results are available after a period of three or six weeks. So, Microscopic examination has the advantage of the giving a result at once. The specimen most commonly examined is sputum and mucous secretion coughed up from the lungs. Microscopic examination of Ziehl-Neelson or auramine stained specimen allows detection of most strains in less than an hour. Ziehl-Neelson is the most extensively used procedure for the demonstration of mycobacterium tuberculosis in smear.<sup>3</sup> Fluorescent staining by Auramine is other methods of staining. In this a smear is made from the specimen and stained with fluorescent stain called auramine. The advantages of fluorescence staining procedure are that it is simpler and can be examined at a

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lower magnification than ZN (40x Vs 100x). It has been estimated that Fluorescent microscope (FM) may take upto 75% less time than ZN<sup>4</sup>.

This advantage would be a tremendous benefit for overburdened laboratory system in many low resource settings. Recently it has been demonstrated that low cost LEDs could be a viable alternative to Mercury vapour lamps used in FM.<sup>5</sup> Therefore the present prospective study was under taken to analyse the sensitivity of Fluorescence staining versus Ziehl-Neelsen fuchsin staining in diagnosing pulmonary tuberculosis.

### MATERIALS AND METHODS

The comparative study was conducted in the Department of microbiology and immunology, Sardar Patel Medical College, Bikaner, Rajasthan over a period from December 2016 to December 2017. Both in- patients and out- patients of all age groups of both sexes presenting with cough and expectoration for 2or >2 weeks with or without symptoms like loss of appetite, loss of weight, chest pain and haemoptysis. A total of 500 sputum samples were collected from 250 patients suspected of pulmonary tuberculosis. Two sputum samples were collected from each patient (one spot and one early morning sample) in clean, sterile, heat proof, wide mouth containers. The processing of samples was carried out in a bio safety cabinet. Each sample was then subjected to Z-N staining and Fluorescence Auramine stain.

**Ziehl–Neelsen Stain**

The reagents used are Ziehl–Neelsen carbol fuchsin, acid alcohol, and methylene blue.

**Fluorescence (Auramine O) Staining**

Reagents used - Auramine-Phenol solution, 1% Acid alcohol, 0.1% Potassium permanganate solution.

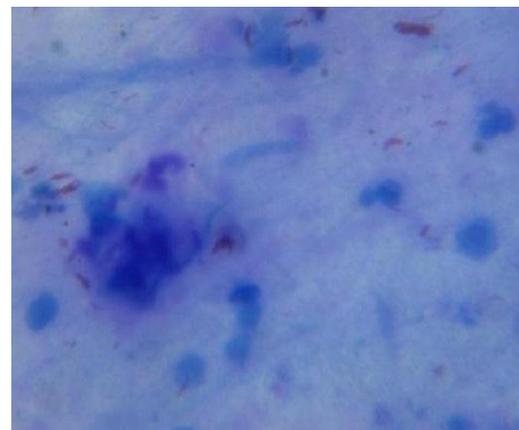
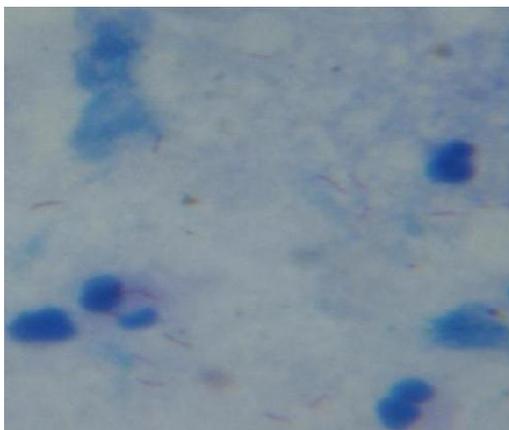
**Table 1: Z-N Grading Chart (Who, luatld, 2007)**

Result (WHO scale) 1000 × field=HPF	Bright fields × 1000 magnification: 1 length = 2cm= 100HPF
Negative	Zero AFB / 100 HPF
Scanty	1-9 AFB/100HPF
1+	10-99 AFB / 100HPF
2+	1-10 AFB /1HPF( on average 50 HPF)
3+	>10 AFB / 1 HPF ( on average 20 HPF)

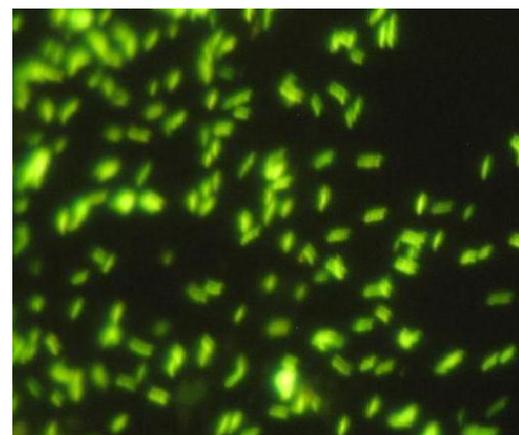
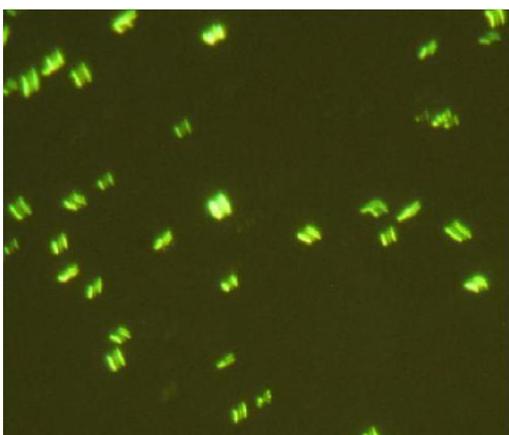
HPF = high power field, AFB = Acid fast bacilli

**Table 2: Grading Chart (Who, luatld, 2007) For Led Fluorescent Microscopy**

Result (WHO scale) 1000 × field=HPF	LED Fluorescent Microscopy (400x:1 length=40fields=200HPF)	Minimum number of fields to be examined
Negative	Zero AFB / 1 length	40
Scanty	1-19 AFB / length	40
1+	20-199 AFB / 1length	40
2+	5-50 AFB / 1field on average	20
3+	>50 AFB / 1 field on average	8



**Fig 1: Photomicrograph of ZN stain sputum smear showing TB Bacilli (1000x) as bright pink to red, beaded or barred forms, while the tissues cells and other organism are stained blue. : (a) AFB++ and (b) AFB +++**



**Fig 2: Photomicrograph of Fluorescent stain sputum smear by LED microscopy (400x).the bacilli are seen as yellow luminous organism in a dark field (a) AFB++ and (b) AFB+++ For the present study 2+ and 3+ were classified as multibacillary and 1+ and scanty as paucibacillary<sup>4</sup>.**

**RESULTS**

Out of 500 sputum samples collected from 250 patients, 60 and 99 sputum samples were found to be positive for acid fast bacilli

by Z-N and Fluorescence staining respectively. The Z-N smear positivity rate and fluorescence staining smear positivity rate in this study was 12% and 19.8% respectively.

**Table 3: Comparison of Zn and Fluorescence Staining Reports**

Staining method used	No. of Positive smears	No. of Negative smears
ZN stain	60 (12%)	440 (88%)
Fluorescence stain	99 (19.8%)	401 (80.2%)

**Table 4: Distribution of Paucibacillary and Multibacillary Cases Detected By Zn Staining And Led Fluorescent Microscopy**

Staining method	No. of paucibacillary cases	No. of multibacillary Cases	Total no. of positive cases detected
ZN staining	3 (5%)	57(95%)	60
Fluorescence staining	26(26.26%)	73(73.73%)	99

**DISCUSSION**

Tuberculosis (TB) is a major public health problem in India since ages. Despite all the advances made in the treatment and management, still tuberculosis is a public health problem in India with adverse social and economic consequences. Current recommendations for the control of tuberculosis emphasize early case detection so as to allow treatment of patients and thereby limit the transmission of the bacilli. The main stay for its control is the rapid and accurate identification of the infected individuals.<sup>6</sup> The detection of AFB is considered as the evidence of infective stage. The laboratory plays a critical role in diagnosis of pulmonary tuberculosis. A number of alternative diagnostic tests that use molecular and immunological methods have been developed. While molecular methods overcome the insensitivity of smear method, the time required for culture and retrieval of a specimen from the site of infection require a well-prepared laboratory and well-trained personnel. The simplest rapid method is the detection of acid-fast bacilli by microscopy. In developing countries, microscopy of sputum is by far the fastest, cheapest and more reliable method for diagnosis of pulmonary tuberculosis. The estimated detection limit of microscopy is  $10^7$  bacilli/ml of sputum.<sup>8</sup> In immunocompromised patients like HIV infected cases there is major impact on the pathogenesis of tuberculosis.<sup>9</sup>

It directly attacks the critical immune mechanisms involved in protection against tuberculosis. In the early stages of HIV infection, when CMI is only partially compromised, pulmonary tuberculosis presents typically as upper lobe infiltrates and cavitations with high bacillary load in the sputum, whereas in the late stages, primary tuberculosis like pattern with diffuse infiltrates and little or no cavitation is seen resulting in paucibacillary picture of sputum. It is more difficult to diagnose these cases.

ZN stain can detect bacilli when they are in the order of  $10^5$ /ml of the sputum, whereas a more sensitive AO stain can detect in the order of  $10^4$  /ml of sputum. In the current study out of the 500 samples examined, 60(12%) and 99(19.8%) TB cases were detected by ZN and AO staining methods respectively. Similar results have also been reported by studies done by Suria Kumar et al, 2012.<sup>10</sup> Roma Goyal and Anil Kumar conducted a study to compare the efficacy of fluorescence stain with conventional Z-N stain in the diagnosis of pulmonary tuberculosis. The study was conducted in Meerut city in 2011. 388 cases of suspected

pulmonary tuberculosis were included in the study. All samples were screened for Acid Fast Bacilli (AFB) by Z-N & Fluorescent staining methods. Positive samples detected by fluorescent stain were 57(14.69%) when compared to Zn stain 29(7.47%). Compared to Z-N stain (7.47%) fluorescence staining was found to be more efficient (14.69%) in AFB detection from cases of Pulmonary Tuberculosis.<sup>11</sup> Saroj Golia and Sangeetha K.T conducted a comparative study between Z-N and FM staining in the diagnosis of pulmonary tuberculosis in Bangalore from 2012 – 2013. 634 sputum samples collected from suspected cases of pulmonary Tuberculosis were processed and subjected to ZN and Auramine-O (AO) staining for detection of TB. Positive smears were graded according to standard WHO criteria. Out of 634 sputum smears, 10.41% and 16.56% were positive by ZN and AO respectively. AO was found to be superior to ZN in detecting TB cases and also AO staining was able to detect more paucibacillary cases than ZN.<sup>12</sup>

Z. Khatun and K. Kamal conducted similar studies in Bangladesh in 2011. 300 sputum samples were collected from suspected pulmonary tuberculosis patients and processed for Z-N and AO staining. In this study out of 300 samples 10.4% and 17.8% were positive by Z-N and AO staining respectively. So FM is superior to Z-N in detecting Mycobacterium Tuberculosis.<sup>13</sup> Suria Kumar, C.Chandrasekhar and Rajasekharam conducted a study of comparison of Z-N and FM Staining methods in diagnosing PTB in 2012 in Madras. A total of 400 sputum samples collected from suspected PTB patients and processed for Z-N and AO staining. Out of 400 samples 11% and 17% are positive by Z-N and AO Staining respectively. The results in current study are similar to studies done by others (table no.8) showing the sensitivity of fluorescence staining versus Z-N staining in diagnosing sputum smear positive pulmonary tuberculosis. The high consistency of the two methods resolves a pre eminent question of quality assurance, using a system of internal quality control without recourse to culture or external proficiency testing. Nevertheless, recourse to culture could be valuable, particularly for microscopically low scanty results, both for confirmation of microscopic findings and for internal quality control. It was observed that a total no. of 39 sputum smears which were negative by ZN method were positive by AO staining method and that AO staining with LED Microscopy was more efficient over ZN

stain in determining paucibacillary cases have also been proved in current study. AO staining could detect 26 paucibacillary cases, whereas ZN staining detected only 3 of them. This was in concordance with studies done by Saroj Golia et al 2012. Failure to detect and hence to treat paucibacillary cases can be effectively prevented by the use of fluorescent LED microscopy. Further all sputum smears positive for AFB by Z-N staining were also found positive by fluorescent method. Sputum culture is widely regarded to be the most sensitive and specific test for the detection of pulmonary TB, but its routine use in resource- limited settings is hampered by excessive cost, slow turnout, and the need for adequate laboratory infrastructure. In practice, improvements in direct sputum sample evaluation that result from improved sensitivity and improved access to decentralized diagnostic services remain highly relevant. The need for rapid smear results and effective treatment of the most infectious TB cases remains paramount. The efficacy of LED fluorescence microscopy proved to be much higher than conventional fluorescent microscopy and bright field microscopy and comparable to that of culture in other studies. In current study, Auramine O (AO) stained sputum smear has been found to improve significantly the sensitivity and efficiency. So, LED microscopy of sputum by AO staining can be used effectively for the diagnosis of pulmonary tuberculosis instead of doing difficult and time consuming culture method. Finally, in current study fluorescence Auramine staining was found to be 7.8% more effective than ZN staining. This shows that fluorochrome staining of sputum smears in comparison to that of ZN staining is a better method of microscopy.<sup>14</sup> FM LED therefore appears to be a more sensitive technique than ZN due to its ability to detect the bacilli load even in HIV positive subjects with little or no cavitation. However Fluorescence staining has been added in Revised National Tuberculosis Control Programme (RNTCP) because of more sensitivity and rapid results and is used in all medical colleges in our country.

## CONCLUSION

Sputum examination for the tubercle bacilli is usually conducted for patients clinically and radio logically suspected of pulmonary tuberculosis. However, the routine method of sputum examination, that is, ZN staining is not sensitive enough and same suspected cases are not confirmed. Moreover they remain undiagnosed and fail to get treatment. Hence our study concludes that Fluorochrome staining with LED is more efficient over ZN stain in detecting Tuberculosis bacilli in sputum, especially the paucibacillary cases and also FM LED has been found to be less time consuming as compared to ZN method (1000x) in the diagnosis of TB. FM with LED is easier to use, quicker and cheaper especially in centers where large numbers of sputum specimens are processed. The only disadvantage with Fluorescent staining is that the cost of LED microscope is relatively high and may not be affordable for developing countries. Whenever it is possible, sputum smear examination should be done by Fluorescent method by gradually replacing the traditional microscopes with LED Fluorescent ones.

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