

Original Research Article

## Sensitivity and Specificity of Conventional Fluorescent Microscopy to diagnose Pulmonary Tuberculosis among Pulmonary tuberculosis symptomatics in Karimnagar

S. Kelamane<sup>1</sup>, P. Mispah<sup>1,2</sup><sup>1</sup>Department of Microbiology, Prathima Institute of Medical Sciences, Karimnagar<sup>2</sup>Department of Microbiology, Kakathiya Medical College, Warangal

### \*Corresponding author

Dr. Santosha Kelamane

Email: [santoshak2020@gmail.com](mailto:santoshak2020@gmail.com)

**Abstract:** The conventional sputum microscopy is the standard method for detection of pulmonary tuberculosis in suspected cases. ZN staining is specific but less sensitive compared to fluorescent microscopy. The objective of the present study was to evaluate the sensitivity and specificity of ZN microscopy compared with conventional Fluorescent microscopy for examination of sputum in suspected pulmonary tuberculosis cases. A spot and early morning sample was collected in a sterile container from suspected cases of pulmonary tuberculosis. The sputum was processed under ZN staining, fluorescent staining and inoculated in Lowenstein Jensen media after decontamination. Of the 383 cases, culture results were available for all of them. Mycobacterium tuberculosis isolated in 102 (27%) of cases, no growth found in 269 (70%) cases, contamination observed in 12 (3%) patients. No Non tuberculous mycobacteria isolated. More than half 53 out of 102 cases of the culture positive samples were of 2 + culture grading. Under solid culture 26.6% (102 cases) were positive and 22.2% (85 cases), 17.8% (68 cases) reported conventional fluorescent microscopy and ZN microscopy respectively. The sensitivity and specificity of ZN microscopy to culture was 62.7% and 98.5% respectively similarly when the sensitivity and specificity of conventional fluorescent microscopy with culture was compared the results were 78.14% of Sensitivity and 98.14% of Specificity were obtained. Ziehl - Neelsen microscopy is less sensitive compared to fluorescent microscopy. By replacing the existing ZN microscopy and utilizing fluorescence microscopy, smear positive pulmonary tuberculosis case detection may be enhanced as the fluorescent microscopy is 16% more sensitive compared to ZN microscopy. The fluorescent microscopy is found to reduce the examination time by half and will help in screening more number of slides in high work load microscopy centers.

**Keywords:** sputum microscopy, pulmonary tuberculosis, fluorescent microscopy, ZN staining

### INTRODUCTION

Tuberculosis, or TB, is an infectious bacterial disease caused by Mycobacterium tuberculosis, which most commonly affects the lungs (Pulmonary TB) but can affect other sites as well (Extra pulmonary TB). It is transmitted from person to person by droplet infection from patients with active tuberculosis. Each active TB disease patient will on average infect 10 – 15 people every year which continues the transmission [1]. Despite advances in treatments TB remains a major global health challenge. In 1993, the World Health Organization (WHO) declared TB a global public health emergency, at a time when an estimated 7–8 million cases and 1.3–1.6 million deaths occurred each year. In 2010, there were an estimated 8.5–9.2 million cases and 1.2–1.5 million deaths (including Deaths

from TB among HIV-positive people). India is the highest TB burden country accounting for one fifth (21%) of the global incidence and India is 17th among 22 High countries in terms of incidence rate [2, 3]. TB is the second leading cause of death from an infectious disease worldwide (after HIV, which caused an estimated 1.8 million deaths in 2008) [2, 4]. The Revised National Tuberculosis Control Program in India to combat TB scourge conceptualized in 1993 after critical review of existing National Tuberculosis Control Program and the same has been implemented in a phased manner from 1999. The program is covering the entire nation since March 2006 reaching over a billion populations (1164 million) in 632 districts/reporting units. One of the main objectives of the program is to One of the main objectives of the

program is to achieve and maintain the case detection of at least 70% of estimated New Smear Positive cases in the community by quality assured sputum smear microscopy facilities which are available through more than 13000 sputum microscopy laboratories in the health system across the country. In 2008 1.51million patients were put on treatment and in 2009- 1.53 million patients and in2010 (Only from Jan-Sep 2010) 1.52 million patients have been placed on treatment. Since its inception, the Program has initiated more than 12.8million patients on treatment, thus saving nearly 2.3 million additional lives [5]. The diagnosis of TB in any TB Control Program is aimed at early identification and prompt treatment of infectious cases of TB in the community. It is to break the chain of transmission in the community in order to bring down the problem of tuberculosis. Diagnosis of TB depends on the history, physical and radiographic evidence or the presence of Acid Fast Bacilli (AFB) in acid fast smears and cultures [6]. Detection of AFB in direct smears prepared with sputa, urine and specimens of other body fluids has considerable clinical and epidemiological value and remains the most widely used rapid diagnostic test for TB in most developing countries. Direct (un-concentrated) smear microscopy using Ziehl-Neelsen staining is still the mainstay of TB diagnosis in most high burden countries, including India, having remained essentially unchanged for over 100 years. This method is rapid and inexpensive and highly specific for *Mycobacterium tuberculosis* in developing countries [7]. However the main limitation of the direct sputum microscopy using ZN stain is its low sensitivity in programmatic settings, particularly in human immunodeficiency virus (HIV) co-infected patients. New radiometric and molecular diagnostic techniques have been developed and are widely used in the developed world, it is estimated that between 60% and 70% of all TB cases are diagnosed by means of sputum smear examination [8]. Conventional Mercury Vapour Lamp Fluorescence microscopy (FM) has been documented to have higher sensitivity (10%) than ZN microscopy [9] and can be performed in less time. We tried to evaluate the Sensitivity and specificity of conventional fluorescent microscopy and ZN microscopy compared with culture as standard.

## MATERIALS AND METHODS

The present study was carried in Prathima Institute of Medical Sciences [PIMS] Karimnagar Telangana from March 2010 to Feb 2011. Pulmonary TB symptomatics referred for sputum examination in “Revised National Tuberculosis Control Program designated Microscopy Center of PIMS Hospital Karimnagar” were enrolled in the study. Ethical committee permission was obtained for the study and informed consent was taken from all participants in this study. The cases were selected using following Inclusion Criteria and Exclusion criteria.

### Inclusion criteria

Pulmonary tuberculosis symptomatics referred for sputum examination and diagnosis in “Revised National Tuberculosis Control Program designated Microscopy Center of PIMS Hospital Karimnagar” irrespective of their previous treatment history.

### Exclusion criteria

- Cases of suspected extra pulmonary tuberculosis.
- Tuberculosis treatment defaulters.

### Sample collection

The sample is collected in a sterile screw capped wide neck containers. The sample was transported into PIMS tuberculosis laboratory immediately after the collection without adding any transport medium along with the clinical information form. The sample is processed as early as possible. If any delay is anticipated in processing, they were stored at 4°C. The sputum samples collected from the patients are serially recorded in the tuberculosis laboratory register and processed as follows

### Microscopy

TWO smears were prepared from the thick mucopurulent portion of sputum on a new unscratched slide on an area of 2 x 3 cm. They were air dried for 30 minutes and fixed the slide by heating it briefly over the flame 3–5 times, for 3–4 seconds each time and these smears were labeled with their respective laboratory serial numbers. One smear was subjected to ZN staining as recommended by RNTCP<sup>1</sup>. The second slide was subjected to fluorescent staining auramine rodamine stain and examined under fluorescent microscope. The stained smears were graded (Table 1) using WHO recommendation<sup>2</sup>.

### Primary Isolation of *Mycobacteria*, Culture on L J Medium

All the samples were processed for solid culture in LJ medium prepared as follows. Two loops full of decontaminated deposit was inoculated on the entire surface of two LJ slopes which were prepared in our laboratory as per standard in apre-sterilized inoculation hood taking necessary aseptic precautions. Date of inoculation was noted. The slopes were incubated at 37°C for a maximum period of 8 weeks. The slopes were inspected daily for the growth or for the contamination. In case of growth of *Mycobacteria*, date of appearance of first colony was noted and slopes were further incubated for more growth. In case of contamination, the slopes were removed. Each strain was subjected to following of tests for species identification: Rate of growth, Growth at different temperatures, Pigmentation, Niacin test, Nitrate reduction test, Catalase test

**Quality Control Procedures adopted**

All the procedures performed in the study were strictly in accordance to Standard quality control procedures.

**Table-1: Showing the grading of smears ZN stains**

Number of AFB	Result	Grading	Number of fields to be examined
>10 AFB per oil immersion field	Positive	3+	20
1-10 AFB per oil immersion field	Positive	2+	50
10-99 AFB per 100 oil immersion field	Positive	1+	100
1-9 AFB per 100 oil immersion field	Positive	Scanty	100
No AFB in 100 oil immersion field	Negative	Negative	100

**RESULTS**

Out of the 383 cases enrolled in the study males were 238 (62.1%) compared to females who were 145 (37.9%). The Male: Female ratio was 1.65:1 see table 2. The youngest patient enrolled for this study was

5 years and oldest patient is 80years. The average age of the patients was 44.13 years. 67 (17.5%) patient were found in 41-50 years age group followed by 63 (16.4%) patients found in 21-30 and 51-60 years age group.

**Table-2: Sex wise distribution of TB suspected enrolled for the study.**

Sex	No. of cases	Percentage
Male	238	62
Female	145	38
Total	383	100

Out of 383 cases by ZN microscopy for AFB 18% (68 patients) was positive and 82% (315 patients) reported negative. The positive cases were distributed

according to smear grading and frequency given in table 3.

**Table-3: Distribution of ZN Positive cases**

Smear Grading	Frequency	Percentage
3+	10	14.5
2+	7	10.1
1+	49	72.5
Scanty	2	2.9
Total	68	100

Out of 383 cases AFB seen under Mercury vapor lamp fluorescent microscope in 85 (22.2%) cases

and no AFB (negative) were seen in 298 (77.8%) cases given in table 4

**Table-4: Conventional fluorescent microscopy positive smear grading**

Smear Grading	Frequency	Percentage
3+	12	14.1
2+	29	34.1
1+	44	51.8
Scanty	0	0
Total	85	100

Of the 383 cases, culture results were available for all of them. Mycobacterium tuberculosis isolated in 102 (27%) of cases, no growth found in 269 (70%) cases, contamination observed in 12 (3%) patients. No Non tuberculous mycobacteria isolated. More than half 53 out of 102 cases of the culture positive samples were of 2 + culture grading. Under solid culture 26.6% (102

cases) were positive and 22.2% (85 cases), 17.8% (68 cases) reported conventional fluorescent microscopy and ZN microscopy respectively.

**DISCUSSION**

The objective of the present study is comparison of the fluorescent microscopy with the ZN

microscopy which is the standard diagnostic procedure in urban, semi urban and rural settings of India as prescribed by Revised National Tuberculosis Control Program. The ZN positive cases were 68 (17.6%) out of 383 cases. The sputum smear grading has been done as per WHO guidelines. Of the 68 positive cases 2 scanty cases (0.5%), 49 (12.8%) were 1+, 7 (1.8%) were 2+ and 10 (2.6%) were 3+. Among the newly identified TB suspects Unchalee T *et al.*; in 2002 reported positivity rate of 22.6% under ZN microscopy with 14% cases 1+ grading, 4.3% cases 2+ and 4.3% of cases 3+ grading. [10] The study by Paramashivan C.N. and coworkers reported 76.7% patients had 1(+) grade. [11] In our study more than 3 in 5 cases (73%) cases were paucibacillary as per ZN report. Conventional mercury vapor lamp fluorescent microscopy the RNTCP fluorescent microscopy grading has been used for grading the positive samples. 85 patient's samples (22.2%) was positive under conventional mercury vapor lamp. In our study, 44 (11.5%) 29 (7.6%) and 12 (5.7%) cases were found to be 1+, 2+, and 3+ grading. No scanty sample was found under mercury vapor lamp fluorescent microscopy. Our findings are contrary to the findings of Unchalee T *et al.* in 2002 reported positivity rate of 19.5% under ZN microscopy with 5.9% cases 1+ grading, 45.4% cases 2+ and 8.2% of cases 3+ grading. [10] Bell WJ in 1962 reported positivity rate 24% with 12.1% cases of 1+ grading, 6.1% cases of 2+ grading and 4% cases of 3+ grading. [12] In our study almost 1 in 2 cases (51%) were of 1+ grading.

Number of sputum samples collected and methodology of collection exerts its influence on rate of isolation. Jena *et al.* [13] (85.6%) have used three consecutive samples while Narang P. *et al.*; [14] (56.23%) have used 2 samples per patient, one was spot collection and other was overnight collection like other study. Rate of isolation varies with the method of decontamination used. Damale and Kaundinyahave found that Nassau's swab method of decontamination was superior to Pettroff's method and NALC method in giving positive cultures. Studies by Claudio Peirsioni *et al.*; have also reported variation in culture yield when different decontamination methods were used [15]. Previous antitubercular treatment and its duration is an important determining factor of isolation of MTB. Jena, Panda B. *et al.*; have conclusively proved that rate of isolation decreases as the duration of anti-tuberculosis treatment increases [16]. In our study 78% of pulmonary TB suspects not had any previous history of tuberculosis treatment.

To calculate sensitivity and specificity of Ziehl-Neelsen stained and fluorochrome-stained microscopy smears, we cross-tabulated each result against culture. Sensitivity refers to the proportion of culture-positive sputum samples that are identified as positive by the staining method in question specificity

refers to the proportion of culture-negative sputum samples that are identified as negative by the same smear method. For calculation of these measures, we excluded contaminated culture results like other studies. The sensitivity, specificity, positive predictive value and negative value of ZN microscopy is 62.7%, 98.5%, 94.11 and 0.013 respectively compared to conventional fluorescent microscopy and LED fluorescent microscopy which reported 78.43%, 98.14%, 94.11, 0.017 and 80.43%, 98.5%, 94.11 and 0.013 respectively. In our study we used Auromine O as primary stain in fluorescent microscopy and Fluorescent microscopy is generally believed to increase the sensitivity of AFB microscopy, particularly for paucibacillary smears. [17] Our study showed that the sensitivity of fluorescent microscopy is more compared to ZN microscopy. The conventional fluorescent microscopy showed additional 16% positive cases. This is similar to findings of a recent systematic review by studies in which conventional fluorescence microscopy was on average 10% more sensitive than conventional light microscopy [18].

## CONCLUSIONS

Ziehl Neelsen microscopy is less sensitive compared to fluorescent microscopy. By replacing the existing ZN microscopy and utilizing fluorescence microscopy, additional yield of more than 16% of positive cases can be detected. This will help the treating physicians/revised national tuberculosis control program to initiate prompt treatment which in turn reduce air borne infection transmission. The fluorescent microscopy is also found to reduce the examination time by half and will help in screening more number of slides in high work load microscopy centers.

**Conflict of interest:** None

**Source of support:** Nil

**Ethical Permission:** Obtained

## REFERENCES

1. Revised National Tuberculosis Control Program, India Medical Officer Training Module 1-4 Central TB Division, Directorate General of Health Services, Ministry of Health and Family Welfare, Nirman Bhavan, New Delhi; Module 1- Course Introduction; 1-14
2. International statistical classification of diseases and related health problems, 10th revision (ICD-10), 2nd ed. Geneva, World Health Organization, 2007; WHO TB 2011; 15-30
3. International statistical classification of diseases and related health problems, 10th revision (ICD-10), 2nd ed. Geneva, World Health Organization, 2007; <http://apps.who.int/ghodata>. These HIV

- deaths include 0.4 million deaths from TB.WHO TB 2011; 35-36 [Accessed on 20-10-2016]
4. Powell DA, Hunt WG. Tuberculosis in children: an update. *Adv Pediatr.* 2006;53: 279-322
  5. RNTCP TB India Report 2011. Chapter 1- Tuberculosis Burden; RNTCP TB India Report 2011; 5-10
  6. Truffot-Pernot C, Véziris N, Sougakoff W. Modern diagnosis of tuberculosis. *Presse Med* 2006;35 (11 Pt 2):1739-46.
  7. Goel MM, Ranjan V, Dhole TN, Srivastava AN, Mehrotra A, Kushwaha MR, et al. Polymerase chain reaction vs. conventional diagnosis in fine needle aspirates of tuberculous lymph nodes. *ActaCytol* 2001;45:333-40.
  8. Revised National Tuberculosis Control Program, EQA Module, Central TB Division, Directorate General of Health Services, Ministry of Health and Family Welfare, NirmanBhavan, New Delhi; 5-30.
  9. Revised National Tuberculosis Control Program; Manual for Sputum Smear Fluorescence Microscopy Central TB Division Directorate General of Health Services Ministry of Health and Family Welfare, NirmanBhavan, New Delhi; 4-16.
  10. Unchalee Tansuphasiril and Booncherd Kladphuang, evaluation of sputum staining by modified cold method and comparison with ziehlneelsen and fluorochrome methods for the primary diagnosis of tuberculosis southeast Asian j trop med public health 2002;33(1):128-135.
  11. Paramasivan CN, Bhaskaran K, Venkatraman P, Chandrasekaran V, Narayanan PR. Surveillance of drug resistance in tuberculosis in the state of Tamil Nadu. *Ind J Tub*2000 ; 47 : 27-33.
  12. Bell WJ, Brown PP. Fluorescence microscopy in the laboratory diagnosis and assessment of pulmonary tuberculosis. *Cent Afr J Med* 1962; 8: 4-9.
  13. Jena J. Assessment of trisodium phosphate (TSP) penicillin transport medium and single step method of culture. *Ind J Tub* 1997;3:176.
  14. Narang P, Mendiratta DK, JyotiKannathe. Characterisation of M. tuberculosis variants in Wardha district. *Ind J Tub* 1997;44 : 21-26.
  15. Clandio Piersimoni, ClandiaScarpato, Annapaola Callegaro et al. Comparison of MB/BacT ALERT 3D system with radiometric BACTEC system and LJ medium for recovery and identification of mycobacteria from clinical specimens : a multicenter study. *J. Clin. Microbiol.*2001; 39(2): 651-57.
  16. Jena J, Panda BN, Nema SK, Misra AK. Impact of previous chemotherapy on culture of Mycobacterium tuberculosis from sputa of freshly diagnosed smear positive cases of pulmonary tuberculosis. *Ind J Tub* 1997 ; 44 : 191-94.
  17. Karen R Steingart, Megan Henry, Vivienne Ng, Philip C Hopewell, Andrew Ramsay, Jane Cunningham, Richard Urbanczik, Mark Perkins, Mohamed Abdel Aziz, MadhukarPai; Fluorescence versus conventional sputum smear microscopy for tuberculosis: a systematic review; <http://infection.thelancet.com> Vol 6 2006; 570-581.
  18. Trusov A, Bumgarnar R, Valijev R, Chestnova R, Televski S, Vragoterova C, Neelev ES; Comparison of Lumin LED fluorescent attachment, fluorescent microscopy and ZiehlNeelsen for AFB diagnosis; *Int J Tuberc Dis* 2009; 13(7): 836-41.