

## COMPARISON OF CONVENTIONAL AND FLUORESCENT STAINING METHODS IN DIAGNOSIS OF PULMONARY TUBERCULOSIS AMONG HIV SEROPOSITIVE INDIVIDUALS

Dr. J. Suria Kumar. Dr. C. Chandrasekar. Dr. S. Rajasekaran

1. Assistant Professor, Department of Microbiology, Institute of Microbiology, Madurai Medical College, Madurai.
2. Superintendent, Government Hospital of Thoracic Medicine Tambaram Sanatorium, Chennai.
3. NACO National Consultant, (ART Quality Management), Chennai.

### CORRESPONDING AUTHOR

Dr. J. Suria Kumar, M.D, Assistant Professor,  
Institute of Microbiology,  
Madurai Medical College, Madurai – 625020.  
E-mail: suryjay@gmail.com, suryjay@yahoo.com,  
Ph: 0091 9894010207.

**ABSTRACT: BACKGROUND:** The diagnosis of Tuberculosis (TB), with its high incidence and atypical manifestations in HIV, demands a reliable, rapid and cost effective diagnostic method. Fluorescent staining (Auramine phenol) carries advantage of easy interpretation of AFB compared to conventional Ziehl - Neelsen staining (ZN). **AIM:** To compare the efficacy between ZN and fluorescent staining in detection of AFB in sputum among the HIV seropositives.

**MATERIAL AND METHODS:** Adult HIV positive individuals, attending GHTM from March to May 2008, with clinical and/or radiological presentations suggestive of Pulmonary TB with no previous exposure to ATT were studied. From 225 clients, early morning sputum sample were subjected to Fluorescent, ZN staining and culture in LJ medium. Results analyzed to determine the sensitivity and specificity of ZN and Fluorescent methods, considering culture as gold standard. **RESULTS :** Of the 225 samples, 43 (19.1%) and 28 (12.4%) were smear positive by fluorescent and ZN stain respectively of which 27 (12%) samples were positive on both stains. The culture turned positive with 44 (19.5%) samples. Based on these reports, the sensitivity and specificity of fluorescent staining was 95.45% and 99.45% respectively and that of ZN stain was 63.64% and 100% respectively. **CONCLUSION:** Fluorescent staining is found superior to ZN staining and this concludes it is a better tool for screening PTB in HIV positives and needs further larger studies to recommend in public health program

**KEY WORDS:** Pulmonary Tuberculosis, HIV, ZN staining, Fluorescent staining, Auramine stain

**MESHTERMS:** Tuberculosis, Pulmonary, HIV

**INTRODUCTION:** In the year 1993, Tuberculosis was declared by World Health Organization (WHO) as a global emergency. Worldwide, one person out of three is infected with Mycobacterium tuberculosis – two billion people in total. TB currently holds the seventh place in the global ranking of causes of death<sup>1</sup>. India has about 1.8 million new cases of tuberculosis annually, accounting for a fifth of new cases in the world - a greater number than in any other country<sup>2</sup>. Tuberculosis is the most common HIV-related opportunistic infection in India, and caring for patients with both diseases is a major public health challenge. Tuberculosis can be expected to develop in more than half of those who are infected with HIV. At present about 5% of new tuberculosis cases in India are occurring in people with HIV infection<sup>2</sup>. HIV and TB form

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a lethal combination, each speeding the other's progress. HIV increases not only the risk but also the rate of progression of recent or latent *M. tuberculosis* infection to disease. This reduces the time available for diagnosis and initiation of treatment. TB is the leading cause of death among HIV-infected persons. Alarmingly, fewer than half of TB cases in HIV-infected patients are diagnosed before death<sup>3</sup>. To complicate the scenario, the clinical presentation of pulmonary tuberculosis is atypical in late stages of HIV challenging the clinical diagnosis at primary and secondary care settings. The resurgence of TB in HIV demands for a reliable, rapid (to identify the organism) and cost effective diagnostic method to facilitate early detection of tuberculosis for early intervention which is the need of the hour. Detection of *M. tuberculosis* by smear microscopy has highest priority in diagnosis of PTB and it correlates well with infectivity<sup>4</sup> and active clinical disease. The bacilli in the sputum can be detected by both the ZN stain and the Fluorescent stain. Each method is having its own sensitivity and specificity. Sensitivity of the ZN method varies from 20% – 80%. Fluorescent method is easy to interpret and the sensitivity is higher and recommended for large programmes<sup>5</sup>. Comparative studies of these microscopy methods in HIV patients are limited, and the present study will give a refined knowledge over the efficacy and accuracy of the two widely followed staining methods. The current study was undertaken 1) To perform both the conventional ZN and Fluorescent microscopy along with mycobacterial culture to diagnose Pulmonary TB in HIV seropositive individuals in the study population and 2) To compare the conventional and Fluorescent microscopy results with Mycobacterial culture results as standard.

**MATERIALS AND METHODS:** The study was conducted at the Government Hospital of Thoracic Medicine (GHTM), Chennai, India. The study population comprised of 225 consecutive adult HIV seropositive individuals having clinical or clinical and radiological suspicion of pulmonary tuberculosis, attending the HIV outpatient department (OPD) of GHTM, from the period of February 2008 to May 2008. Children and individuals with previous exposure to Anti-tuberculous drugs were excluded from the study.

Sputum samples were collected as per the guidelines of Revised National Tuberculosis Control Program and from each early morning sputum sample two smears were made on clean glass slides, air dried and heat fixed. Of the two slides, one each was stained by ZN stain and auramine phenol stain. ZN stained smears were examined under oil immersion objective (x1000) of light microscope and Auramine phenol stained smears were examined by using 40x objective (x400) of fluorescence microscope. Simultaneously the specimens were submitted to culture after decontamination by Petroff's method. The specimen were inoculated on to Lowenstein – Jensen medium under aseptic precautions and incubated at 37°C for eight weeks to look for the growth of the organism.

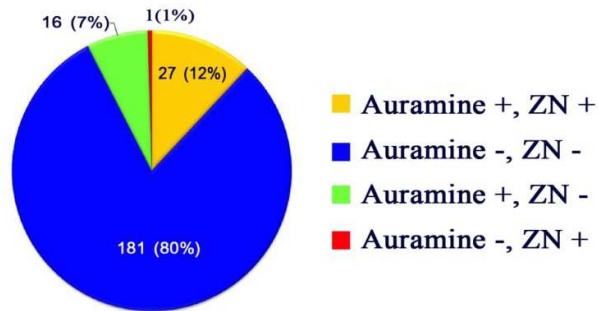
On identifying eligible clients from the OPD, the clients were oriented about the study. As per the practice of the hospital, elicitation of clinical history and proper clinical examination were done from which necessary data was collected in the data sheet created for the study along with the basic demographic data. Subsequently the lab results were compiled on each client's data sheet. The results were analyzed to determine the sensitivity and specificity of ZN and Fluorescent method, considering Mycobacterium culture as a gold standard by using Epi-info software.

**RESULTS:** Of the 225 samples studied, 181 samples turned negative by both of the staining methods as well as by culture. Of the remaining 44 samples which gave positive result by

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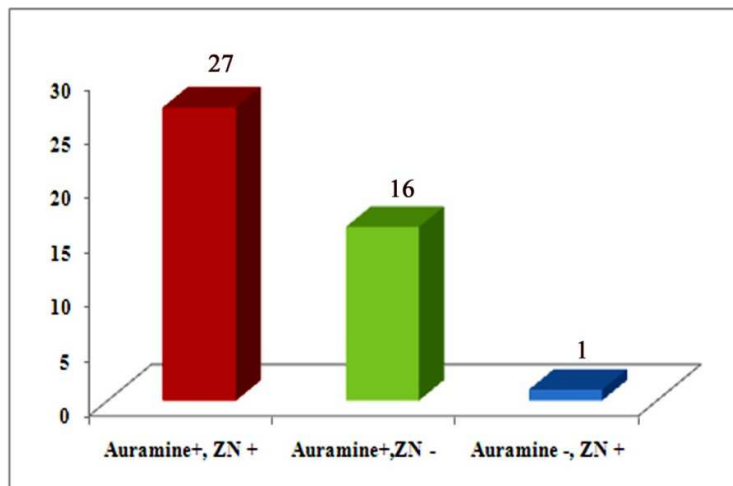
staining method, 43 samples (i.e. 19.1%) showed bacilli by fluorescent method and 28 (ie, 12.4%) samples were smear positive by ZN stain. And out of the 44 positive samples, 27 (12%) samples were positive by both of the stains.

Overall test results of the samples by staining methods



Of the 44 samples that gave positive result by the staining methods, all of them turned culture positive with 100% concordance. Taking the culture result as the standard for reference, the sensitivity and specificity of both the staining methods were calculated. The sensitivity and specificity of fluorescent staining was found to be 95.45% and 99.45% respectively and that of ZN stain was found to be 63.64% and 100% respectively. The p value, as calculated by the McNemar test, for the Fluorescent staining is 1.00 and for ZN staining is < 0.0001.

Chart depicting the comparison of positive results by the staining methods:



**DISCUSSION:** In this study, analysis was done on the lab results obtained by the staining and culture methods. The p value, as calculated by the McNemar test, for the Fluorescent staining is 1.000 which states that this difference is considered to be not statistically significant and for ZN staining are < 0.0001 states that the difference is significant compare with the gold standard. The sensitivity of Auramine staining is 31.6% higher than the ZN staining. This is well correlates with a similar study by Kivihya- Ndugga et al 2003<sup>6</sup>, which shown the sensitivity of the Fluorescence microscopy is 37% higher than ZN staining. Another study by K Prasanthi et al, 2005<sup>7</sup> without using culture as a reference test showed that the sensitivity of Auramine staining

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is 26% higher than the ZN staining in HIV positive individuals. But the specificities of both staining methods were almost similar (i.e. 99.5% with Auramine stain and 100% with ZN stain). These findings are supported by Karen R Steingart et al, 2006<sup>8</sup>, stating that the mean specificity estimates of fluorescence microscopy and conventional ZN microscopy were similar. The major strength of the study was comparing the staining methods with culture which is considered as gold standard for the diagnosis of mycobacterial infection. The sensitivity and specificity of both the staining methods was calculated comparing the culture result, which adds to the strength to the evidence of the values. Very limited data are available on comparison of sputum microscopic findings with culture result, as reference standard in HIV setting. This study did not address the immune status of the patient, differences in the expertise of microscopists and cost effective issues which may be considered as the limitation of the study.

**CONCLUSION:** Considering the sensitivity and specificity of the Fluorescent staining in detection of Mycobacterium tuberculosis, with its additional advantage of easy visibility and identification of the organism, fluorescent stain can be considered superior to ZN staining and may conclude Fluorescent staining as a better tool for screening PTB in HIV positives. The relative shorter time consumption for identification of AFB adds further value to the fluorescent method especially for the labs handling high number of specimen. It may be concluded that before recommending for the large scale roll out of fluorescent microscopy in the TB program in India as a screening tool, similar study with larger sample size as well as further study to detect cost efficacy and efficiency costing exercise may be undertaken.

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