Original Article

COMPARISON OF ZIEHL NEELSEN & AURAMINE O STAINING METHODS ON DIRECT AND CONCENTRATED SMEARS IN CLINICAL SPECIMENS

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Summary

Background: In developing countries like ours with a large number of tuberculosis (TB) cases and limited resources, the diagnosis of TB relies primarily on smear microscopy for Acid Fast Bacilli (AFB) but its sensitivity is limited in paucibacillary cases.

Aim: To evaluate the increase in efficacy of smear microscopy when smears are prepared from clinical samples after concentration by Petroff's method and stained by Auramine O (AO) fluorescent dye as against Ziehl Neelsen (ZN) staining of similar taking culture as the gold standard.

Methods: Smears were prepared from 393 clinical samples both by direct and after Petroff's concentration and examined by fluorescent microscopy and Ziehl Neelsen method .The concentrated material was also cultured on Lowenstein Jensen media and the results of the two microscopy methods were compared with the culture results taken as the gold standard.

Results: Mycobacterial growth was detected in 137(35.77%) specimens, out of which three were non-tubercular mycobacteria. Using culture as the reference method, the sensitivity of direct staining was 55.55% for ZN and 71.85% for AO. Direct fluorescent microscopy detected 9.29% paucibacillary sputum samples that were missed on ZN staining. On concentration, the sensitivity increased by 6.67% for ZN and 11.11% for AO. The sensitivity of AFB smear microscopy increased by 27.41% and was statistically significant (p=<.001) when both methods were combined. The specificity was 99.19% for both ZN and AO.

Conclusion: Fluorescent microscopy has higher sensitivity and comparable specificity which is further enhanced by concentration. Now with the advent of newer inexpensive Light Emitting Diode (LED) based fluorescent microscopes (FM), which are easier to use, fluorescent microscopy can be widely used even in peripheral laboratories where culture facilities are not available. **[*Indian J Tuberc 2011; 58:* 72-76]**

Key words: Ziehl-Neelsen Staining, *Mycobacterium tuberculosis*, Auramine O.

INTRODUCTION

The global burden of disability and death due to tuberculosis (TB) is immense. The expanding HIV epidemic has further increased the morbidity and mortality due to HIV-TB co-infection. India accounts for one- fifth of the world's new TB cases and two-third of the cases in South East Asia [1, 2]. An estimated 1.9 million cases occur annually and around 0.9 million have sputum positive pulmonary TB [3]. In recent years, several automated culture systems and molecular techniques have been developed for the diagnosis of TB which have reduced the turnover time for detection of AFB but are costly and are not suitable for routine use in low-middle income countries[4]. AFB smear microscopy using conventional light microscope still remains the mainstay for diagnosis and monitoring treatment of TB as it is simple, inexpensive, widely applicable and highly specific for TB in endemic countries [5-7], but as the sensitivity of direct AFB smear is low, detecting only if 10^5 bacilli are present per ml, there is an urgent need to improve the sensitivity of AFB smear microscopy. Various techniques like concentration by sodium hypochlorite or sodium hydroxide, sedimentation of sputum using chemicals, fluorescent microscopy, etc., have been tried which have increased the sensitivity by 10-23%[8]. However, these techniques have been tried only one at a time. Therefore, the

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