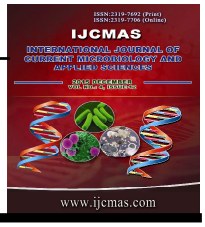
Int.J.Curr.Microbiol.App.Sci (2015) 4(12): 536-540

International Journal of Current Microbiology and Applied Sciences
ISSN: 2319-7706 **Volume 4 Number 12 (2015) pp. 536-540**
http://www.ijcmas.com



Original Research Article

Evaluation of Different Staining Methods for the Detection of Acid Fast Bacilli in Sputum Samples

Hemant Krishnaji Kulkarni[1*], Maria Pinto Joao Wiseman[1], T. Jayaprakash[1], Anup Banur[1], K.G. Basavarajappa[2], V.L.Jayasimha[2], K.G. Raghu Kumar[2], S. SathishPatil[2], S. Chethan Kumar[2] and C.S. VinodKumar[2]

[1]Department of Pulmonary Medicine, S. S. Institute of Medical Sciences and Research Centre, NH-4, Bypass Road, Davangere-577005, Karnataka, India
[2]Department of Microbiology, S. S. Institute of Medical Sciences and Research Centre, NH-4, Bypass Road, Davangere-577005, Karnataka, India
**Corresponding author*

Keywords

Ziehl Neelsen, Acridine orange, Auramine rhodamine, *Mycobacterium tuberculosis*

A B S T R A C T

Microscopic examination of sputum samples is of special importance for the rapid presumptive diagnosis of tuberculosis because of slow growth of *Mycobacterium tuberculosis* in culture. The early diagnosis of active tuberculosis still depends on the presence of acid fast bacilli (AFB) in stained sputum smears. This study compares the efficacy of Ziehl Neelsen (ZN) staining with Acridine orange (AO) and Auramine rhodamine (AR) fluorochrome staining in the diagnosis of tuberculosis (TB). A total of 2715 sputum samples were included and all samples were smeared and stained by using ZN staining and positive smears were subjected to fluorochrome staining AO and AR. Results of the study revealed that out of 2715 samples, 373 (13.73%) were smear positive by ZN staining while others were negative. The proportion of positive smears detected was 12.85% and 10.82% for the AO and AR staining methods respectively. ZN staining was found more sensitive than fluorescent stains (AO and AR) for the diagnosis of TB.

Introduction

Tuberculosis (TB) is the major health problem in the world since 1993 when declared as a global emergency by world health organization (WHO). It is estimated that nearly one billion people will be infected with TB, 200 million develop the disease, and 35 million will die from TB during 2000- 2020 (Floyd, 2002). Direct microscopic examination of acid fast bacilli (AFB) is currently the most microbiological method used for diagnosis and preliminary confirmation of tuberculosis (TB) and also defines the more infectious cases in positive smears in grading (quantitation scale) (Masood, 2008). The microscopic method of detection of AFB in sputum sample is fastest and cheapest as compare to culture and other techniques (Masood, 2008). The only disadvantage of this method is low sensitivity (50-80%) relative to culture

(Masood, 2008, Bruchfeld, 2000). The sensitivity of microscopy is influenced by many factors like method of sample collection, processing (direct/concentrated) staining technique, quality of microscope and expertise etc (Masood, 2008, Bruchfeld, 2000). The previous studies indicated that fluorescent staining of smears significantly increases the sensitivity of direct microscopy (Masood, 2008, Murray, 2003).

The higher sensitivity of this method is attributed to the easy detection of fluorescent rod against dull/darker background, this allows the expert to screen the slide at a lower magnification and observe large area than with routine Ziehl Neelsen stained smears (Masood, 2008). The objective of this study was to evaluate the routine performance of different staining methods for the detection of *Mycobacterium tuberculosis* in our laboratory.

Materials and Methods

Ethical Clearance

Study protocol was reviewed and approved by Institutional Ethics Committee, S. S. Institute of Medical Sciences and Research Centre, Davangere, Karnataka.

Study was conducted as per the RNTCP guidelines and standard microbiological techniques for the diagnosis of TB.

Study Type

Prospective cross sectional

***Study duration*:** April 2013 to October 2015.

Inclusion criteria

New clinically diagnosed cases of TB visiting RNTCP units at Davangere district.

Exclusion criteria

Patients on anti-tubercular treatment were excluded

Methodology

A prospective study was carried out in the Department of Pulmonary Medicine from April 2013 to October 2015. The sputum samples included in the study were obtained from the patients attended to RNTCP Laboratories which comes under Davangere district. The sputum samples were collected preferably early morning in wide mouthed, sterile, properly labeled plastic container. All samples were smeared and stained using Ziehl Neelsen (ZN) staining. The positive smears by ZN staining were subjected to two different fluorescent stains such as Acridine orange (AO) (Smithwick. 1995, Sethi, 2010) and Auramine rhodamine (AR) (Sethi, 2010, Vildan, 2011) for comparison. The positive and negative control slides were included with each staining batch for internal quality control.

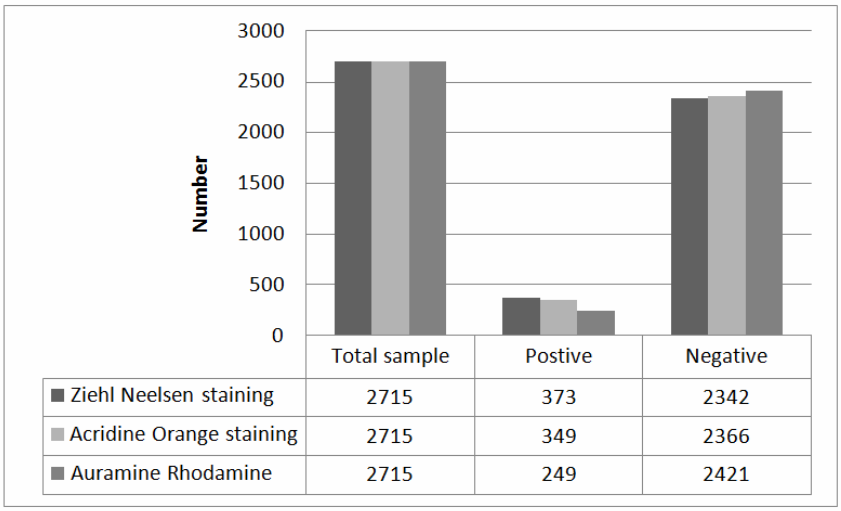
All three staining procedures were done according to the standard protocol (Smithwick. 1995, Sethi, 2010, Vildan, 2011). In ZN staining *Mycobacterium tuberculosis* appears pink stained bacilli on blue background. AO staining orange/red fluorescing bacilli dull black background. AR staining yellow/green fluorescing bacilli on black background.

Results and Discussion

A Total of 2713 samples were collected during the study period. The results obtained from ZN stained smears and fluorescent (AO and AR) stained smears were compared together (Fig.1).The result of our study revealed that AO (12.85%)was nearly as sensitive as ZN(13.73%) and fared better than AR (10.82%).

Smear microscopy plays an important role in early diagnosis of Mycobacterial infections as the method is highly specific, rapid and cheapest method used for detection of AFB in sputum. The only disadvantage of this method is low sensitivity (varying from 50% to 80%) relative to culture (Bruchfeld 2000, Murray 2003, Masood, 2008). The sensitivity of microscopy is influenced by the quality of specimen collection, the number of mycobacterium present in the specimen, the method of processing, the staining technique, and the quality of the examination (Somoskovi, 2001). To achieve maximum sensitivity of this diagnostic test, it is essential to have a good quality sputum sample, i.e. the sample should contain mucoid or mucopurulent material and the volume should be at least 3 mL (TB India 2009).

Fig.1 The comparison of different methods of detection of AFB in sputum samples



The ZN method has commonly been used around the world, particularly in developing countries, because of its simplicity and low cost. Though the fluorescence microscopy was on average 10% more sensitive than ZN (Steingart, 2006), but a real disadvantage of the fluorochrome method is that fluorescence fades with time along with the cost of the microscope. For this reason, the slides must be read within 24 hours. Fluorescence microscopy method can give "false positives" as compared to ZN staining, so it is recommended that the positive fluorescence microscopy smears should be confirmed by over staining the smear by ZN method (Somoskovi 2001, Steingart 2006, Ulukanligil 2000).

The sensitivity and specificity of ZN staining reported by various authors have been shown to range between 61% to 86.4% (Ulukanligil, 2000, Tansuphasiri, 2002, Jain 2002, Guthui, 1993) and 96.2% to 100% respectively (Tansuphasiri, 2002, Jain 2002,

Guthui, 1993) whereas with respect to fluorescence microscopy, reports of sensitivity range between 59.7% to 83% [13-15] and that of specificity from 85.5% to 99% (Tansuphasiri, 2002, Jain 2002, Guthui, 1993). Two different studies conducted by Smithwick et al and katila et al have showed that results of AO were comparable to those of the AR method in four laboratories. Another study was conducted in Europe also found that AO out performed AR staining in six different laboratories (Katila, 1982). In other study by Masood et al. had reported higher sensitivity and rapidity of the fluorochrome technique as compared to ZN staining (Masood, 2008). In the present study, results of AO was nearly as sensitive as ZN.

Sputum smear examination plays a key role in routine diagnosis and treatment of pulmonary tuberculosis. The sensitivity of sputum microscopy is influenced by the number of mycobacterium present in the specimen, technique/s and quality of specimen. Fluorochrome microscopy is rapid and easy visualization of AFB and positive smears were further confirmed by ZN staining. From the findings of the present study it can be concluded as ZN stain is more sensitive and still remains the standard method against other AR and AOstaining methods.

Acknowledgement

Authors would like to thank the technicians of RNTCP centres for their co-operation.

References

Bruchfeld J, Aderaye G, Palme IB, Bjorvatn B, Kullenius G, Lindquist L, Sputum concentration improves diagnosis of tuberculosis in a setting with a high prevalence of HIV, Trans R Soc Trop Med Hyg, 2000; 94: 677-80.

Floyd K, Blanc L, Raviglione M, Lee JW. Resources required for global tuberculosis control. Sci 2002; 295:2040-41.

Guthui W, Kitui F, Juma ES, Obvana DO, MwaiJ, Kwamanga D. A comparative study on the reliability of the fluorescence microscopy and Ziehl-Neelsen method in the diagnosis of pulmonary tuberculosis. East Afr Med J 1993;70:263-6.

Jain A, Bhargava A, Agarwal SK. A comparative study of two commonly used staining techniques for acid fast bacilli in clinical specimens. Indian J Tuberc 2002;49:161-2.

Kalich R, Bennert G, Fiedler T, Kubin M, Mezensky L, Turzova M, et al, Comparison of auraminerhodamine B and acridine orange for staining of acid fast bacteria, J HygEpidemiolImmunol, 1979; 23: 307-17.

Katila ML, Mantyjarvi RA, Acridine orange staining of smears for demonstration of *Mycobacterium tuberculosis*, Europe Journal of Clinical Microbiology, 1982; 1: 351-53.

Masood Z, Mohammad N, Majid K, Ghodsieh A, Comaparison of the value of two different sputum staining for diagnosis of acid fast bacilli, Iranian Journal of Clinical Infectious Diseases, 2008; 3(2): 99-102.

Murray SJ, Barrett A, Magee JG, Freeman R, Optimization of acid fast smears for the direct detection of Mycobacteria in clinical samples, Journal of Clinical Pathology, 2003; 56: 613-15.

Murray SJ, Barrett A, Magee JG, Freeman R. Optimisation of acid fast smears for the directdetection of mycobacteria in clinical samples. J ClinPathol 2003;56:613-5.

Sethi S, Chatterjee SS, Bedi C, Sharma M,

Evaluation of Acridine orange staining for rapid detection of Mycobacteria in primary specimen, Indian Journal of Pathology and Microbiology, 2010; 53(4) :894-95.

Smithwick RW, Bigbie MR, Forguson RB, Karlix MA, Wallis CK, Phenolic Acridine orange fluorescent stain for Mycobacteria, Journal of Clinical Microbiology, 1995; 33(10):2763-64.

Somoskovi A, Hotaling JE, Fitzgerald M, O'Donnell D, Parsons LM, Salfinger M. Lessons from a proficiency testing event for acid-fast microscopy. Chest 2001;120:250-7.

Steingart, K.R., Henry M, Ng V, Hopewell PC, Ramsay A, Cunningham J, et al. Fluorescence versus conventional sputum smear microscopyfor tuberculosis: a systematic review. Lancet Infect Dis 2006;6:570-81.

Tansuphasiri U, Kladphuang B. Evaluation of sputum staining by modified cold method and comparison with Ziehl-Neelsen andfluorochrome methods for the primary diagnosis of tuberculosis. Southeast Asian J Trop Med Public Health 2002;33:128-35.

TB INDIA 2009. RNTCP Status Report. Central TB Division. Directorate General of Health Services. http://tbcindia.nic.in/pdfs/TB%20India%202009.pdf

Ulukanligil M, Aslan G, Tasci S. A comparative study on the different staining methods and number of specimens for the detection of acid fast bacilli. MemInstOswaldo Cruz 2000;95:8558.

Vildan AO, Nurbanu S, Aygun O, Nur Y, Suheyla S, Ayse Y, A comparison of two different fluorochrome stains for the detection of acid fast bacilli in sputum specimens, Turkey. Journal of Medical Sciences, 2011, 41(3): 411-17.