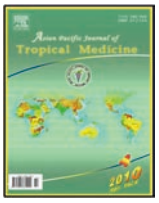
Contents lists available at ScienceDirect

Asian Pacific Journal of Tropical Medicine

journal homepage:www.elsevier.com/locate/apjtm



Document heading

Diagnostic efficacy of Ziehl-Neelsen method against fluorescent microscopy in detection of acid fast bacilli

Soham Gupta[1,2*], Vishnu Prasad Shenoy[2], Indira Bairy[2], Muralidharan S[1]

[1]*Department of Microbiology, St. Johns Medical College, Bangalore, Karnataka, India 560034*
[2]*Department of Microbiology, Kasturba Medical college, Manipal, Karnataka, India 576104*

ARTICLE INFO

Article history:
Received 6 February 2010
Received in revised form 6 March 2010
Accepted 28 March 2010
Available online 6 April

Keywords:
Tuberculosis
Acid fast bacilli
Ziehl-Neelsen
Fluorescent microscopy

ABSTRACT

Objective: To investigate the application of Ziehl-Neelsen (Z-N) and fluorescent microscopy in detection of acid fast bacilli (AFB). **Methods:** Duplicate smears were prepared from 260 sputum samples and stained with Z-N and fluorescent staining (FS) methods. The efficiency of both methods in primary diagnosis of tuberculosis were evaluated. **Results:** The smears were positive for AFB in 15 (5.77%) samples by Z-N staining method and in 16 (6.15%) samples by FS method. The sensitivity and specificity of Z-N staining method against FS method were 93.75% and 100% respectively. **Conclusions:** Though lesser cost-effective than Z-N, FS method is a more sensitive and better case finding tool in detection of AFB.

1. Introduction

Tuberculosis (TB) has a great impact on the morbidity and mortality in the developing world. Diagnosis of pulmonary tuberculosis is mainly done by sputum microscopy and culture. Though culture is considered as a gold standard, the practicability of culture method in controlling tuberculosis in developing countries needs to be considered. Thus sputum microscopy is the main case finding tool in TB control programmes. In India under Revised National Tuberculosis Programme (RNTCP)[1], Ziehl-Neelsen(Z-N) method is the recommended procedure for staining tubercle bacilli.

However, for a better implementation of control programme, there is a need for a better case finding tool. Fluorescence microscopy is a rapid, useful and reliable tool for detection of acid fast bacilli (AFB)[2-5].

The present study deals with the diagnostic efficacy of Z-N method compared to fluorescent staining (FS) method.

2. Materials and methods

Two hundred and sixty sputum samples were collected from suspected cases of tuberculosis at Kasturba Hospital, Manipal, India. Smears were prepared in duplicate of each of the 260 sputum samples: one using Z-N staining and the other using FS following the RNTCP guidelines[1,6].

In Z-N staining the smears were air-dried and heat fixed by flaming. The slides were then flooded with 1% carbol-fuchsin (10 g basic fuchsin, 100 mL methylated spirit, 50 g phenol, distilled water to make a final volume of 1 000 mL) and heated until steaming but not boiling for 5 minutes. The smears were washed in tap water and decolorized with 25% sulfuric acid for 4 minutes. Finally, the slides were rinsed in a gentle stream of tap water, then counter stained with 0.1% methylene blue for 30 seconds, washed and air dried.

In FS method smears from specimens were air-dried and heat fixed by flaming. The slides were then flooded with freshly filtered auramine-phenol (0.3 g auramine in 100 mL 3% phenol) for 7-10 minutes without heating. The smears were washed in running water and decolorized with 3% acid-alcohol for 3-5 minutes. The slides were washed well

*Corresponding author: Mr. Soham Gupta, Junior Research Fellow, Department of Microbiology, St. Johns Medical College, Bangalore- 560034 Karnataka, India.
Tel: +91-80-22065052
E-mail: soham.micro@gmail.com

in running water and counterstained with 0.1% potassium permanganate for 30 seconds, washed and air-dried.

All 260 smears with Z-N and FS staining were randomly numbered separately, therefore the examiner was blind to the identity of the specimen. All slides were examined under oil immersion for Z-N and 40× objective for FS by an experienced examiner. The smears were graded based on RNTCP guidelines as 3+, 2+, 1+, scanty and negative[1,6].

3. Results

A comparison of the smear results obtained with the Z-N staining method against FS method is shown in Table 1.

Table 1
Cross comparison of the Z-N method with fluorescent staining method.

Ziehl-Neelsen method	Fluorescent staining method					
	3+	2+	1+	Scanty	Negative	Total
3+	4	–	–	–	–	4
2+	–	5	–	–	–	5
1+	–	2	1		–	3
Scanty	–	1	–	2	–	3
Negative	–	–	1	–	244	245
Total	4	8	2	2	244	260

Of the 260 samples, 16 (6.15%) were AFB positive by FS method and 15 (5.77%) were AFB positive by Z-N method. All AFB positive specimens by Z-N method were also positive by FS method, while 1 of the 16 positives, which was read 1+ by FS method was negative by Z-N method.

Since FS method gave a higher positivity, compared to Z-N method, it was taken as the gold standard. The sensitivity and specificity of Z-N staining method was found out to be 93.75% and 100.00%, respectively. The positive predictive value was 100.00% and the negative predictive value was 99.59%. Both showed good agreement between themselves.

4. Discussion

TB poses a great public health challenge in India and there is a need to control the spread of the infection. In developing countries like India, under resource limited setting sputum smear microscopy is the most practicable and cheapest tool for demonstration of AFB in sputum[4]. Demonstration of AFB in smear has a great importance in control of TB, as smear positivity directly correlates with infectiousness. Z-N staining method is the most commonly used worldwide.

Though culture of Mycobacteria is a more sensitive method than smear microscopy but it is time consuming and requires proper laboratory set-ups, which is not possible in remote rural areas with poor resource settings.

Consequently, for proper implementation of RNTCP, we need urgent improvements for the implementation of existing strategies for TB control. FS method has found out to be an effective and more sensitive method than Z-N as indicated by various studies conducted across the globe[2-5]. And so was found out in our study where FS method showed a greater positivity than Z-N. However, in a study conducted by Tansuphashiri *et al*[7] in Thailand, it was seen that sensitivity of Z-N (68.9%) was more than that of FS method (59.7%).

The advantage of FS is that since smears are observed under 250× or 40×, increases the examination area and reduces the examination time. Moreover, Z-N is a cumbersome process due to the heating process involved. With auramine staining the bacilli appears as slender bright yellow fluorescent rods, standing out clearly against dark background. Though sometimes FS method gives false positive results[2,5]. It can be overcome by re-staining the smear according to Z-N method. In our study since we have not performed culture we could not find out whether any of the smears gave false positive result with FS. The major disadvantage of FS method is its higher cost of the excitatory light sources and maintenance.

Though no statistically significant differences were noted, our finding still support previous studies that demonstrated the superior diagnostic performance of fluorescent microscopy compared to conventional light microscopy.

In conclusion, smear microscopy is recommended for case finding in developing countries and fluorescent microscopy can be implemented as a superior tool in TB control programmes.

Conflict of interest statement

We declare that we have no conflict of interest.

References

[1]RNTCP. *Manual for laboratory technicians. director general of health services*. Ministry of Health and Family Welfare. New Delhi, India: RNTCP. Central TB Division. 1999. Available at: http://www.tbcindia.org/LABMANUAL.pdf). Accessed on 3 December 2008.

[2]Ba F, Reider HL. A comparison of fluorescence microscopy with the Ziehl Neelsen technique in the examination of sputum for acid fast bacilli. *Int J Tuberc Lung Dis* 1999; **3**: 1101-5.

[3]Ziaee M, Namaei M, Khazae M, Azarkar G. Comparison of the value of two different sputum staining for diagnosis of acid fast bacilli. *Iranian J Clin Inf Dis* 2008; **3**: 99-102.

[4]Jain A, Bhargava A, Agarwal SK. A comparative study of the two commonly used staining techniques for acid fast bacilli in clinical specimens. *Ind J Tub* 2002; **49**: 161-2.

[5]Ulukanligil M, Aslan G, Tasci S. A comparative study of the different staining methods and number of specimens for the detection of AFB. *Mem Inst Oswaldo Cruz* 2000; **95**: 855-8.

[6]RNTCP. *Manual for sputum smear fluorescence microscopy*. Director General of Health Services, Ministry of Health and Family Welfare. New Delhi, India: RNTCP. Central TB Division. Available at: http://www.tbcindia.org/Fluorescence_Microscopy Manual.pdf. Accessed on 29 September 2009.

[7]Tansuphasiri U, Kladphuang B. Evaluation of sputum staining by modified cold method and comparison with ZN and fluorochrome methods for primary diagnosis of tuberculosis. *Southeast Asian J Trop Med Public Health* 2002; **33**:128-35.