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BRIEF REPORT

Comparison of the value of two different sputum staining for diagnosis of acid-fast bacilli

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ABSTRACT

Background: The aim of this study was to compare the diagnostic value of fluorochrome microscopy (FM) with Ziehl-Neelsen (ZN) staining in the diagnosis of tuberculosis (TB).

Patients and methods: In this study, 920 consecutive patients suspected of having pulmonary TB, referred to TB laboratory, provincial office of health care, Birjand University of Medical Sciences, recruited and a total of 2760 sputum specimens were collected from them. All samples were smeared and stained using both Ziehl Neelsen and auramin-phenol methods as recommended by WHO. All positive smears by fluorescent microscopy were over-stained by ZN technique for confirmation. The sensitivity of ZN staining was also evaluated in different contamination conditions.

Results: A total of 102 out of 920 study subjects had pulmonary TB, of them 68 (66.66%) patients were smear positive by either staining method while others were smear negative. The proportion of positive smears detected was 51% and 57% for the ZN and auramine phenol staining methods, respectively. The sensitivity, specificity, positive predictive value and negative predictive value were 51%,100%,100%, 94% and 57%,100%,100,95% for the ZN and auramine phenol staining methods, respectively.

Conclusion: FM is more sensitive than ZN for diagnosis of TB. However, since FM is more sensitive and rapid, using this method in clinical laboratories with large specimen numbers is recommended.

Keywords: *Mycobacterium tuberculosis, Ziehl Neelsen, Fluorescence microscopy*.
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INTRODUCTION

There are evidences that the worldwide incidence rate of tuberculosis (TB) is increasing. 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 (1).

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Direct microscopy for acid-fast bacilli (AFB) is currently the most microbiological method used for diagnosis and confirmation of pulmonary tuberculosis (PTB) and when positive, defines the more infectious cases (2,3). This method is highly specific and by far the fastest and cheapest method used for detection of AFB in sputum. The only disadvantage of this method is low sensitivity (varying from 50%-80%) relative to culture (3-7). The sensitivity of microscopy is influenced by numerous factors, such as the prevalence and

severity of the disease, the quality of specimen collection, the number of mycobacterium present in the specimen, the method of processing (direct or concentrated), the staining technique, and the quality of the examination (microscope operator expertise, time spent for smear examination, etc.) (2).

There are several reports indicate that fluorochrome staining (either auramin phenol or auramin rhodamine) of smears significantly increases the sensitivity of direct microscopy (4,8,9). The higher sensitivity of this method is attributed to the ease of the detection of a fluorescent rod against a darker background. This allows the examiner to scan the slide at a lower magnification and thus observe a larger area than with carbol fuchsine-stained smears. These factors reduce the time for screening and lead to greater sensitivity. Therefore, it is generally accepted that the fluorescent method should be given preference over the ZN and Kinyoun methods (10).

Although several research groups have investigated the clinical validity and differences in sensitivity between various staining methods, the technical and procedural factors can influence the sensitivity of each staining method (2). Therefore, the aim of this study was to evaluate the routine performance of different acid-fast staining methods in our laboratory.

PATIENTS and METHODS

In this study, 920 consecutive patients suspected of having pulmonary TB, referred to TB laboratory, provincial office of health care, Birjand University of Medical Sciences, Iran, recruited and a total of 2760 sputum specimens were collected from them between April 1996 and April 2004.

All samples were smeared and stained using both Ziehl Neelsen and auramin-phenol methods as recommended by WHO. Two independent experts examined smears microscopically. The American Thoracic Society recommendation was used for reporting the results of smears examination (11) (table 1). All positive smears by fluorescent microscopy were over-stained by ZN technique for confirmation. Positive and negative control slides were included with each staining batch for internal quality control of the staining methods.

Table 1. Quantitation scale for acid-fast bacillus smears according to stain used

Carbolfuchsin (X1,000)	Fluorochrome (X 250)	Quantity reported
No AFB/300 fields	No AFB/30 fields	No AFB seen
1-2 AFB/300 fields	1-2 AFB/30 fields	Doubtful (repeat test)
1-9 AFB/100 fields	1-9 AFB/l 0 fields	Rare (1 +)
1-9 AFB/10 fields	1-9 AFB/field	Few (2+)
1-9 AFB/field	10-90 AFB/field	Moderate (3+)
>9 AFB/field	>90 AFB/field	Numerous (4+)

Active TB was diagnosed in a patient when two sputum specimens were positive for AFB by smear. Smear negative patients with history of prolonged fever, weight loss and/or cough, and radiological evidence suggestive of TB, were also considered to have clinically diagnosed TB.

The ethical committee of Birjand University of Medical Sciences approved the study.

The results obtained from ZN smears and the auramine-phenol methods were compared together. Sensitivity, specificity, and positive and negative predictive values for each method, with their 95% confidence intervals, were calculated using SPSS 10 software.

RESULTS

A total of 102 out of 920 study subjects had pulmonary TB, and among them 68 (66.66%) patients were smear positive by either staining method while others were smear negative. The smear results obtained from both staining methods are compared in table 2. The agreement in grading (one grade above and one below) between the two methods was 93.2%.

Table 2. Comparison of case yield respectively from ZN staining and FM staining techniques

	Auramin phenol staining					
	Negative	1+	2+	3+	4+	Sum
Ziehl Neelsen staining						
Negative	34	8	4	2	0	50
1+	2	26	2	6	0	34
2+	6	0	6	2	0	14
3+	2	0	0	2	0	4
4+	0	0	0	0	0	0
Sum	44	34	12	12	0	102

The proportion of positive smears detected was 51%and 57% for the ZN and auramine phenol staining methods, respectively: ZN method missed 16 (27.6%) of the 58 slides found positive by the auramine phenol method while auramine phenol method missed only 10 (19.2%) of the 52 slides found positive by the ZN method.

The performance of the ZN method (table 2) and auramine phenol method were evaluated using a combination of smear result and clinical picture of patients as the "gold standard." The proportion of smear negative patients was higher with the ZN (49.02%) than with auramine phenol method (43.14%). The ZN method missed 6 more patients than the auramine phenol method did.

The sensitivity, specificity, positive predictive value and negative predictive value were 51%,100%,100%,94% and 57%,100%,100,95% for the ZN and auramine phenol staining methods, respectively.

We also compared the sensitivity of ZN staining in different contamination conditions. Our results showed that in 1+, 2+ and 3+ contamination, the sensitivity of ZN staining was 70%, 67%, , and 83%, respectively.

DISCUSSION

Smear microscopy plays an important role in early diagnosis of mycobacterial infections. ZN and fluorochrome staining are two staining techniques, commonly used in clinical laboratory for acid-fast bacilli smear examination. In laboratories where large numbers of sputum are tested for pathogenic mycobacteria, fluorochrome staining is used in conjunction with the ZN technique. The advantage of using fluorochrome staining is that slides can be scanned under lower magnification. While a ZN prepared slide must be examined under oil immersion (100X magnification), fluorochrome staining slides can be examined with 40X or 60X magnification; thus detection of acid-fast organisms with the fluorochrome stain takes less time than with the ZN stain (4,7,8).

The aim of this study was to compare two conventionally used acid-fast staining methods, ZN and fluorochrome staining. Our results showed that the sensitivity of fluorochrome staining method was more than ZN method. This is in agreement with other studies (8,12,13). Many reports showed that sensitivity of ZN ranged from 32% to 94%, and fluorescence microscopy was on average 10% more sensitive than ZN (14).

Habeenzu et al study reveled that among the 488 specimens, 152 were positive for acid-fast bacilli using fluorochrome staining while only 66 were positive by ZN method (15). The sensitivity of these two techniques was also compared by Mustafa Ulukanligil et al (16) and they found higher sensitivity in fluorochrome (85.3%) than ZN (67.6%) in HIV patients.

Our study also showed a relation between ZN missed positive smears and density of bacilli on FM stained smears. This is in agreement with other studies (14,16). The use of FM greatly improves the diagnostic value of the sputum smear especially in patients with a low density of bacilli that are likely to be missed on ZN stained smears.

False positive results are a problem in diagnostic AFB smear. False positive results should be considered when the specimen is from a patient receiving anti tuberculosis drugs and also when specimen is bloody (2). Therefore, it is recommended that positive or doubtful fluorochrome staining smear results are better to be

confirmed by ZN stained smear or a second examiner (2,16).

In conclusion, because of the higher sensitivity and rapidity of the fluorochrome technique compared to ZN, in clinical laboratories with large specimen numbers, at first it is preferred to evaluate smears with fluorochrome staining and then positive specimens should be confirmed by ZN staining.

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REFERENCES

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

2. 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;120:250-57.

3. Bruchfeld J, Aderaye G, Palme IB, Bjorvatn B, Kallenius 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.

4. Murray SJ, Barrett A, Magee JG, Freeman R. Optimization of acid fast smears for the direct detection of mycobacteria in clinical samples. J Clin Pathol 2003;56:613-15.

5. Peterson EM, Nakasone A, Platon-DeLeon JM, Jang Y, de La Maza LM, Desmond E. Comparison of direct and concentrated acid-fast smears to identify specimens culture positive for Mycobacterium spp. J Clin Microbiol 1999;37:3564-68.

6. Woods GL, Pentony E, Boxley MJ, Gatson AM. Concentration of sputum by cytocentrifugation for preparation of smears for detection of acid-fast bacilli does not increase sensitivity of the fluorochrome stain. J Clin Microbiol 1995;33:1915-16.

7. Saceanu CA, Pfeiffer NC, McLean T. Evaluation of sputum smears concentrated by cytocentrifugation for detection of acid-fast bacilli. J Clin Microbiol 1993;31:2371-74.

8. Prasanthi K, Kumari AR. Efficacy of fluorochrome stain in the diagnosis of pulmonary tuberculosis co-infected with HIV. Indian J Med Microbiol 2005;23:179-81.

9. Garg SK, Tiwari RP, Tiwari D, Singh R, Malhotra D, Ramnani VK, et al. Diagnosis of tuberculosis: available technologies, limitations, and possibilities. J Clin Lab Anal 2003;17:155-63.

10. Tenover FC, Crawford JT, Huebner RE, Geiter LJ, Horsburgh CRJr., Good RC. The resurgence of tuberculosis: is your laboratory ready? J Clin Microbiol 1993;31:767-70.

11. No authors listed. Diagnostic Standards and Classification of Tuberculosis in Adults and Children.This official statement of the American Thoracic Society and the Centers for Disease Control and Prevention was adopted by the ATS Board of Directors, July 1999. This statement was endorsed by the Council of the Infectious Disease Society of America, September 1999. Am J Respir Crit Care Med 2000;161:1376-95.

12. Cheng AG, Chang A, Farwell DG, Agoff SN. Auramine orange stain with fluorescence microscopy is a rapid and sensitive technique for the detection of cervical lymphadenitis due to mycobacterial infection using fine needle aspiration cytology: a case series. Otolaryngol Head Neck Surg 2005;133:381-85.

13. Sethi S, Sharma M, Sengupta C, Mohandas K, Sharma SK. Enhanced detection of Mycobacteria stained with rhodamine auramine at 37 degrees C. Indian J Pathol Microbiol 2003;46:521-23.

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

15. Habeenzu C, Lubasi D, Fleming AF. Improved sensitivity of direct microscopy for detection of acid-fast bacilli in sputum in developing countries. Trans R Soc Trop Med Hyg 1998;92:415-16.

16. 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. Mem Inst Oswaldo Cruz 2000;95:855-58.