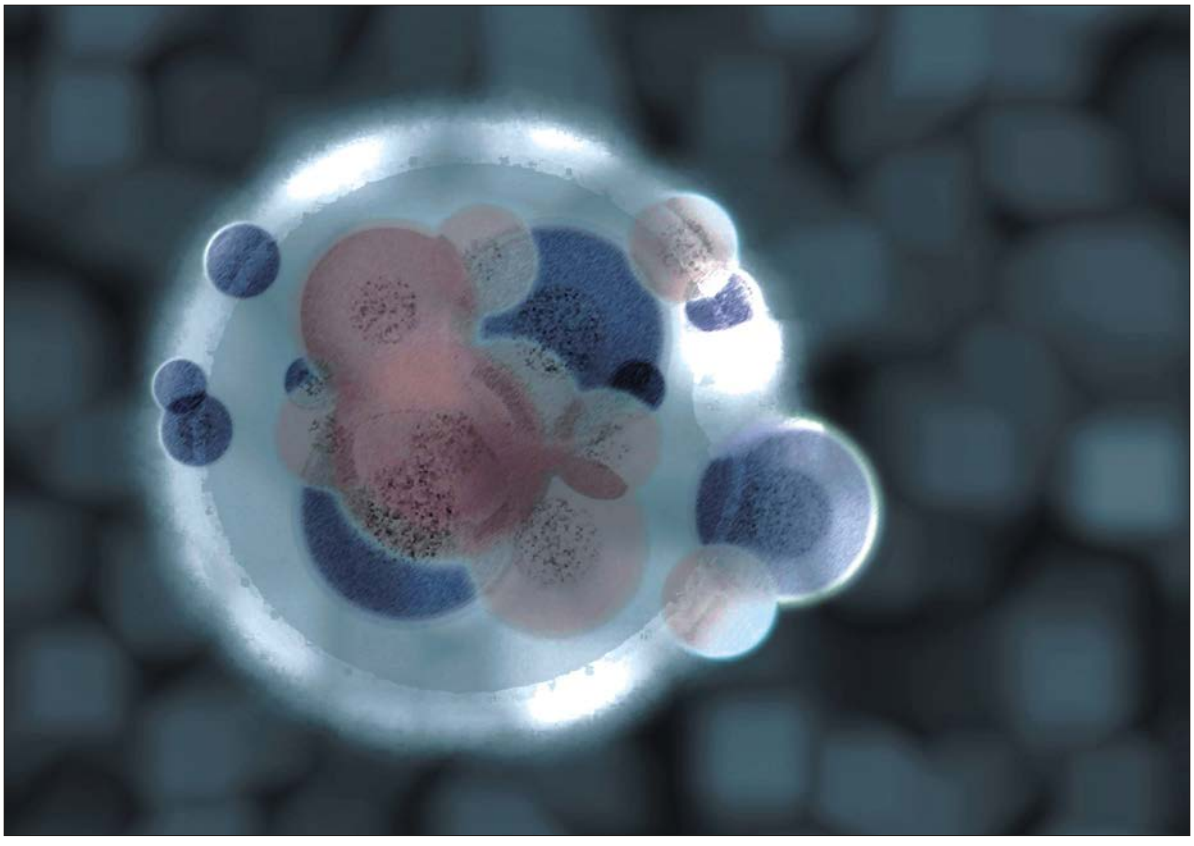

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A Comparison of Direct and Concentrated Flurochrome-Stained Smears for the Detection of *Mycobacterium* sp. in Clinical Respiratory Specimens

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Abstract: The objective of this study was to compare characteristics of Direct and Concentrated Flurochrome (auramine-rhodamine) smears for the detection of *Mycobacterium* sp. and the direct microscopically examination, the concentration method and culture. A total of 900 sputum specimens from patients diagnosed with pulmonary tuberculosis were tested for the presence of mycobacteria. In this study we used the direct staining and an improved technique after treating the smears with *N*-acetyl-L-cysteine, NaOH and sodium citrate and concentration of bacteria by centrifugation. Specimens were stained using the auramine-rhodamine technique. The gold standard was defined as a positive Löwenstein-Jensen culture, or a positive AFB smear of material obtained from a negative culture after 60 days. Fifty two specimens were positive according to the gold standard definition. Decontamination and concentration of the sample increased the sensitivity of the direct microscopically examination from 71.1 to 88.4%. The concentration method adds significantly to the sensitivity of the direct microscopic examination, without much extra input.

Key words: Flurochrome, concentration, direct smear

INTRODUCTION

Epidemiologically, the let up of tuberculosis transmission by early treatment of cases of infectious tuberculosis remains the advantage for tuberculosis programmes[1].

The basic diagnostic methods of Pulmonary Tuberculosis (PTB) in developing countries are still sputum microscopy. Although the acid-fast smear is less sensitive than culture, it is essential and rapid to diagnose of tuberculosis. A single smear of a respiratory specimen has a reported sensitivity of between only 22 and 43%, when multiple specimens are examined, the detection rate improves and as many as 96% of patient with PTB may detected by acid fast smear examination. In a high HIV prevalence area, the ratio of smear-negative PTB patients is higher[2]. This has led to a need for more sensitive methods for sputum microscopy of PTB. This test must also be feasible in hospitals with limited resources and basic laboratory equipment[3-5]. It should be pointed out, however that although in a survey of public health laboratories, 29 of respondents preferred the Zihle-Neelsen or kinyoun stain, the use of a flurochrome dye such as auramine-rhodamine is superior[6]. Because flurochrome-stained smear are viewed under high dry magnification rather than the oil immersion magnification required by fuchsin-stained smears, they may be examined more rapidly, simpler and efficiently. Furthermore, smear examined by fluorochrome method have been found to have a greater senility predictive value of a negative result and efficiently than those stained with fuchsin[7,8]. Since the maintenance of higher sensitivity of Flurochrome compare characteristics of Direct and Concentrated Flurochrome smears for the detection of *Mycobacterium* sp.

MATERIALS AND METHODS

The sputum obtained from all suspected pulmonary TB patients. No induction was practiced. From these patients three sputum samples was taken before any TB drugs were administered for microscopically examination and culture. Flurochrome staining and culture were performed at the Microbiology Laboratory in the

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Department of Microbiology, Medical Faculty, Tehran University of Medical Sciences, Tehran, Iran during the period 2002 to 2004. These specimens included sputum samples from all patients tested for mycobacterial disease, without regard to HIV infection, or other diagnostic status. For the concentration method, the respiratory specimens were first liquefied and decontaminated with N-acetyl-L-cysteine, 2% NaOH and 1.45% Sodium Citrate. Following vortexing, the specimens were concentrated by centrifugation at 3,000×g for 15 min. The processed specimens were inoculated on Löwenstein-Jensen medium (0.1 to 0.2 mL of sediment) and incubated for 6 weeks. Because of proven high sensitivity of culture, this technique was used as the gold standard in this study. All samples that gave a positive culture were checked by re-staining them with the Truant's stain (auramine-rhodamine stain) technique, to confirm the growth of Acid Fast Bacilli (AFBs). Three drops of sediment from a glass pipette (approximately 0.15 mL) were placed on a slide and heat fixed on a slide warmer at 75°C for approximately 2 h. The slide was stained with auramine-rhodamine stain and examined with an American Optical Microscope with a 25 × objective under a standard fluorescence UV filter. Oil immersion (100 × objective) microscopy was used when confirmation of morphology was required. Each slide was examined for the presence of fluorescent bacilli by making three to four passes along the long axis of the slide. No quantification for the microscopy results was practiced: at least one bacillus in any examination was rated as positive[9].

RESULTS

Nine hundred sputum samples were investigated with the three methods.

Out of these, 52 specimens were positive according to the gold standard definition, 37 specimens were contaminants and other was negative (Table 1).

All smears that were found positive by the simple direct microscopy technique were confirmed with the concentration method. However, 9 smears that were considered negative with the first technique were positive after concentration. One patient positive by both direct and concentrated smear techniques and 3 patients positive by concentrated smear techniques failed to grow mycobacterium on LJM.

Decontamination and concentration of the sample increased the sensitivity from 71.1 to 88.4%, a 17.3% improvement. Calculated Concentrated Flurochrome-Stained Smears Positive Predictive Value (PPV) and Negative Predictive Value (NPV) were 93.8 and 99.2%, respectively. As for direct smear, the calculated PPV and NPV were 97.5 and 98.4%, respectively (Table 2).

Table 1: Test characteristics of the direct and concentration microscopy

Culture		Positive	Negative	Total
Direct micro	Positive	37	1	40
	Negative	13	848	861
	Total	52	849	901
Concentration	Positive	46	3	49
	Negative	6	848	854
	Total	52	851	903

Table 2: Sensitivity and specificity of direct and concentration microscopically techniques

	Directmicroscopy (%)	Concentration microscopy (%)
Sensitivity	71.1	88.4
Specificity	99.8	99.6
PPV	97.5	93.8
NPV	98.4	99.2

DISCUSSION

Present results indicate that the concentration method more sensitive than direct microscopic examination. Although it required a slightly longer period of time for detection, the procedure is simple and can easily be applied in a district laboratory with basic equipment and staff training. The only disadvantage is the possible increased risk of contamination for the laboratory personnel during the centrifugation, but if safety cabinets are available this risk can be reduced to an acceptable minimum. Grebe showed that the use of the concentration method increased the number of samples positive for acid-fast bacilli by more than 100%[10]. In study of Peterson *et al.*[11] they found that the same method increased the sensitivity of the Ziehl-Neelsen stain from 81% to 91%. Apers *et al.*[12] reported that concentration of the sample increased the sensitivity of the direct microscopically examination from 65.8 to 86.8%. Paul[8] reported that flurochrome-stained smears were positive for Acid-Fast Bacilli (AFB) in 63% of specimens growing *M.tuberculosis* and 56% of specimens growing the four most common species of NTM.

Present results confirm that the sensitivity of the direct microscopically technique is lower than the concentration method. In conclusion, we recommend that the concentration method for diagnosis of pulmonary tuberculosis.

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