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Improved Sensitivity of Direct Microscopy for Acid-Fast Bacilli: Sedimentation as an Alternative to Centrifugation for Concentration of Tubercle Bacilli

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There is a great need for improved methods for the diagnosis of tuberculosis by techniques that are appropriate for control programs in low-income countries. Liquefaction of sputum with sodium hypochlorite followed by concentration of bacilli through overnight sedimentation significantly increases the sensitivity of direct microscopy, and this method could be an alternative for diagnostic centers not equipped with a centrifuge.

The diagnosis of pulmonary tuberculosis (TB) relies on the bacteriological examination of sputum. However, microscopy of smears made directly from sputum has a low sensitivity, especially when performed in overburdened control programs (1). Previous studies have shown that liquefaction of sputum with sodium hypochlorite (NaOCl) and concentration of bacilli through centrifugation will significantly increase the sensitivity of direct microscopy (2, 4, 6). One major disadvantage of this method is the need for a centrifuge. The aim of this investigation was to compare the efficiency of concentration of mycobacteria from NaOCl-treated sputum samples by centrifugation with that by sedimentation and to compare the results achieved by these methods with those obtained with the direct smear.

Preparation of slides. Five hundred and fifty sputum samples were collected from suspected pulmonary TB patients at the Addis Ababa Tuberculosis Demonstration and Training Centre, Addis Ababa, Ethiopia. Slides for direct sputum smears were prepared by taking a small portion of the purulent part of the sputum with a sterile loop (5). The remaining sputum was mixed on a Vortex and divided into two equal parts in 10-ml conical screw-cap tubes. An equal volume of NaOCl (5%) was added to each tube. The tubes were incubated at room temperature for 15 min and shaken by hand at regular intervals. After addition of 8 ml of distilled water, one tube was centrifuged at 3,000 × *g* for 15 min and the other tube was left on the bench at room temperature overnight (15 to 18 h). The supernatant of each tube was carefully poured off, the sediment was mixed with the remaining fluid, and 1 to 2 drops were transferred with a sterile pipette to a slide. The slides were coded, dried in air, heat fixed, and stained by the Ziehl-Neelsen technique.

The coded slides were examined by bright-field microscopy (magnification, ×1,000) independently by two experienced microscopists. The number of acid-fast bacilli (AFB) in 100 microscopic fields was graded by using Ridley's logarithmic scale (7). The results were compared at the end of the study. Statistical analysis was performed with the Mann-Whitney rank sum test.

Results. Five samples were excluded because of technical errors in preparation of the slides, and the comparison of results obtained by Ziehl-Neelsen staining by the three methods was made on 545 samples. One hundred and twenty samples (22%) were AFB positive in the hands of at least one microscopist by one of the methods. The numbers of slides examined by technicians A and B and found to be positive for AFB by the three methods are summarized in Table 1.

For both technicians, there was a statistically significant difference between the results of smears prepared directly from sputum and those of smears prepared after concentration of tubercle bacilli through centrifugation or sedimentation (Table 1). A statistical comparison of results obtained from smears prepared after centrifugation and those obtained after sedimentation showed no significant difference between the two concentration methods.

There was a significant increase in the average number of AFB seen per microscopic field in the smears prepared after NaOCl treatment and concentration of the bacilli. Concentration through centrifugation yielded the highest average number of AFB per microscopic field (Fig. 1).

Discussion. Direct microscopy for AFB is currently the only microbiological method for confirmation of pulmonary TB applicable to control programs in low-income countries. Direct microscopy is a rapid, inexpensive, and highly specific method for detection of AFB in sputum. The major disadvantage of this technique is a discouragingly low sensitivity when used in overburdened control programs, often not more than 20 to 40% compared with culture (1, 4). The low sensitivity is often reflected by high rates of smear-negative pulmonary TB cases.

The NaOCl method has a special advantage in overburdened control programs, where the technicians, because of the large workload, cannot endure or afford to spend the required 5 to 10 min on the examination of one slide. A meticulous preparation and examination of smears made directly from sputum gives a sensitivity of 55% compared with culture (3), whereas the sensitivity of the NaOCl method is close to 70% (4). A similar ratio between smears prepared directly from sputum and smears prepared after concentration of bacilli was also obtained in this study. The increased sensitivity of the NaOCl method is attributable to the significantly higher density of bacilli per microscopic field obtained by this method and

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TABLE 1. Comparison of results of Ziehl-Neelsen staining of smears prepared directly from sputum and of smears prepared after liquefaction of sputum with NaOCl and concentration of bacilli by centrifugation or sedimentation

Method	No. of positive slides (*P* value[a]) as determined by:	
	Technician A	Technician B
Direct smear versus NaOCl + sedimentation	91/114 (0.015)	94/112 (0.046)
Direct smear versus NaOCl + centrifugation	91/115 (0.008)	94/114 (0.027)
NaOCl + sedimentation versus NaOCl + centrifugation	114/115 (0.913[b])	112/114 (0.825[b])

[a] Determined by the Mann-Whitney rank sum test.
[b] Not a statistically significant difference.

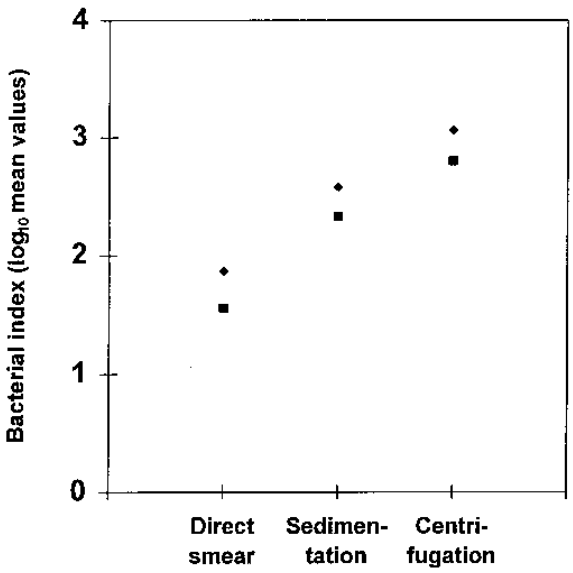

FIG. 1. Average numbers of AFB found in 100 microscopic fields by technician A (◆) and technician B (■) in 122 positive sputum samples examined after sample preparation by three different methods. The numbers of AFB were graded by using Ridley's logarithmic scale and are presented as $\log_{10}$ mean values.

the reduction of debris, leaving a clear field for microscopy. As a potent disinfectant, NaOCl also has the advantage of limiting the risk of laboratory infection. The technology is appropriate for control programs, and NaOCl is readily available at low cost as household bleach.

The increasing workload at TB laboratories is of major concern to program managers. The preparation of samples by the NaOCl method reduces the time needed for examination of the slides. However, the overnight delay in obtaining the results may be a drawback of the sedimentation method. The effect of using the NaOCl method on pooled sputum samples from one patient is currently being investigated. This approach could potentially reduce the workload at TB laboratories.

Although there was no statistically significant difference between the number of AFB-positive samples obtained by the sedimentation method and the number achieved by the centrifugation method, the average numbers of bacilli per microscopic field indicate that centrifugation is more efficient for concentration of tubercle bacilli.

The results of this study show that liquefaction of sputum with NaOCl followed by concentration of bacilli through overnight sedimentation significantly increases the sensitivity of direct microscopy. This technique for preparation of slides could be an alternative method in diagnostic centers not equipped with a centrifuge, but further studies are needed to validate the applicability under field conditions.

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