A SIMPLE COLD STAINING METHOD FOR ACID FAST BACILLI

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ABSTRACT

Sputum from 105 cases of pulmonary tuberculosis were studied. Direct and post concentration smears were stained by Ziehl - Neelsen (ZN) and cold staining methods. The cold staining method is simple, because it eliminates heating of stain. For direct smear, the correlations of cold staining procedure with conventional ZN method was 93% and for post concentration smear it was 100%.

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KEY WORDS : Acid fast bacilli; Mycobacterial cold staining.

Introduction

The Ziehl Neelsen (ZN) method of staining acid fast bacilli has been in vogue for more than hundred years. In the ZN method [1], the basic fuchsin phenol dye is used hot there by melting the unsaponifiable waxy substance on the surface of the cell wall. However, because of disadvantages like controlled heating and non-availability of spirit for lamps there have been attempts to develop a cold staining method for tubercle bacilli.

In the cold staining technique, Kinyoun used higher concentrations of basic fuchsin and phenol [2]. Tan Thia Hok [3] devised a method by combining the staining techniques of Kinyoun and Gabbet, and found it quicker than ZN method. Vasantha Kumari *et al* reported a variation of ZN method that permitted cold staining of tubercle bacilli by avoiding the use of heat and by increasing the staining time. We report here a study of this method using sputum from 105 cases of pulmonary tuberculosis.

Material and Methods

Sputum samples were obtained from 105 patients clinically and radiologically suspected to be suffering from pulmonary tuberculosis. Two direct smears were prepared from the thickest portion of each sputum sam-

ple. One slide was stained by the ZN method [1] while the other was stained by the cold staining method [2]. Thereafter each sputum sample was subjected to decontamination and concentration using Petroff's method [4]. Concentrated deposits were stained by ZN and cold staining methods.

Solutions for cold staining method

(i) Ziehl - Neelsen basic fuchsin - phenol solution.

- Basic fuchsin 10 gm
- Absolute alcohol 100 ml
- 5% aqueous phenol 1 ltr.

Dissolve the dye in alcohol and add to the phenol solution.

(ii) Gabbet's methylene blue

- Methylene blue 1gm
- Sulfuric acid (AR) 20 ml
- Absolute alcohol 30 ml
- Distilled water 50 ml

Procedure for cold staining method [2].

Smear was flooded with basic fuchsin phenol stain and allowed to stand at room temperature for 10 minutes. Smear was then washed in running tap water and counter stained with Gabbet's methylene blue for 2 minutes. Subsequently, it was washed in tap water and air dried. All smears were then

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examined and graded microscopically under oil immersion as per recommendation of the American Thoracic Society (Table 1) [5].

TABLE 1
Microscopic grading of the sputum smears

Number AFB observed	Report
None	Negative for AFB
1-2/300 fields	Actual number seen
1-9/100 fields	+
1-9/10 fields	++
1-9/field	+++
> 9/field	++++

Results

Of the 105 direct smears examined 59 (56.2%) were positive by the cold staining methods in contrast to 65 (61.8%) by the conventional ZN method (Table 2). Statistically using the unpaired students "T" test method there was no significant difference between the results of the two method ($p > 0.5$). The number of bacilli seen was more by the ZN method since 13 out of 65 smears showed +++ bacilli as compared to only 7 such smears by cold staining method (Table 3). After concentration by the Petroff's method, both ZN and cold staining method showed equal positivity of 68.6% and negativity of 31.4% (Table 4). Of the 72 smears positive by either of two methods, 11 were negative by alternate method.

Discussion

Staining of acid fast bacilli depends on the ability of the dye to penetrate the waxy fatty surface coat of tubercle bacilli. Successful staining technique depends on the ability of the dye to uniformly penetrate the cell wall through the waxy surface barrier, while leaving intact the acid fast character of the bacilli.

In the conventional ZN method, this is achieved by precise controlled heating by the spirit lamp in the hands of trained personnel adept at this techniques. For identification and follow up of cases of tuberculosis, this is a very important investigation. However,

TABLE 2
Comparison of the results of direct smears examination of sputum by ZN method and cold staining method

ZN Method	Cold Staining Method		
	Positive	Negative	Total
Positive	52 (49.5%)	13 (12.4%)	65 (61.9%)
Negative	7 (6.7%)	33 (31.4%)	40 (38.1%)
Total	59 (56.2%)	46 (43.8%)	105 (100%)

TABLE 3
Comparison between ZN and cold staining methods in relation to bacilli seen on direct smear

No. of bacilli in Microscopy	ZN Method	Cold Staining Method
+++	13	7
++	24	12
+	28	40
Total	65	59

TABLE 4
Comparison of the results of smear examination by ZN and cold staining methods after concentration and decontamination by Petroff's method

ZN Method	Cold Staining Method		
	Positive	Negative	Total
Positive	61 (58.2%)	11 (10.4%)	72 (68.6%)
Negative	11 (10.4%)	22 (21.0%)	33 (31.4%)
Total	72 (68.6%)	33 (31.4%)	105 (100%)

often this method cannot be undertaken due to absence of trained personnel or shortage of spirit for the lamps. Untrained workers either underheat the dye leading to unsatisfactory penetration of the dye or overheat the dye so as to char the entire smear.

Attempts by various workers [3,6,7] in the past to develop a cold staining method as an alternative to the ZN method for acid fast bacilli have not been very successful. In the method attempted here, exposure of the bacilli to the dye for a longer period favours uniform penetration through the cell wall and hence the identification of the acid fast bacilli. It has shown good correlation with the

ZN method. One hundred and five direct smears and post concentration smears were stained by the ZN and cold staining methods in this study. Of the direct smears, 65 were positive by the ZN method and 59 by the cold staining method (Table 2). Sixty-one of the post concentration smears were positive by both methods (Table 4). The difference in results obtained by both methods was not very significant and compared well with results of other workers [3,6,7]. The intensity of cold staining method was much less. Hence only 7 smears showed +++ bacilli by cold staining method as compared to 13 by the ZN method. The cold staining method has the distinct advantage of being a simple procedure which can be carried out easily without the need of trained personnel and it does not require spirit for heating purposes. Although the cold staining method is a longer procedure as compared to the ZN method, its simplicity, low cost and efficiency make it a

useful alternative technique in laboratories for early identification of acid fast bacilli.

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