

Comparitive Evaluation of Various Staining Techniques for Identification of Tubercle Bacilli by Direct Microscopy

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Abstract: The aim of this study was to compare Fluorescent(auraminO rhodamine,AO)method ,Ziehl-Neelsen (ZN hot method)and kinyoun(cold method) staining techniques for the diagnosis of Mycobacterium tuberculosis.

Materials And Methods: 186(on 3 consecutive days) sputum samples were collected from 62 suspected pulmonary tuberculosis patients, processed by petroffs method, and subjected to direct microscopy by three methods Fluorescent (auramin O rhodamine) method ,Ziehl- Neelsen (ZN hot method)and kinyoun (cold method) staining techniques for the diagnosis of tuberculosis.

Results: out of 62 patients 30.35%,17.8%and 17.8% were found positive,by AO,ZN and Kinyouns methods respectively. AO was found to be superior to ZN and Kinyoun methods in several aspects. AO was also able to detect more pauci-bacillary cases than ZN.

Conclusion: Direct Microscopy is still the most simple and reliable method for diagnosis of Tuberculosis.In laboratories equipped with Fluorescent Microscope FLUOROCHROME Staining is more sensitive than ACID FAST staining for identification of Tubercle bacilli.

Keywords: Mycobacterium tuberculosis, Ziehl – Neelsen, Auramine O rhodamine,and Kinyoun.

I. Introduction

Tuberculosis is the most infectious disease in the world. TB is global emergency ,more people are dying of TB today than ever before .TB - kills 8,000 people a day, i.e. 2 to 3 million people each year.1/3rd of the world population infected with TB .About 20% of the world s population of tuberculosis patients resides in India. According to WHO expert committee, a case of tuberculosis refers to a person with bacteriologically confirmed disease that is a person who expectorates tubercle bacilli in his sputum..The rate of infection and risk is higher in Asian countries like India with a 35 percent of all cases of world wide alone in India and China. Correct and rapid diagnosis of the disease is very important to control the growing epidemics. laboratory diagnosis of tuberculosis is mainly dependent on the demonstration of acid fast bacilli in sputum by direct microscopy. For developing countries with a large number of cases and financial constraints, evaluation of rapid and inexpensive diagnostic methods has great importance.

II. Materials And Methods

A total of 62 individuals clinically suspected of pulmonary tuberculosis were included in this study. Sputum samples from each patient on 3 consecutive days with maintainence of all sterile conditions were collected(186 samples).Samples were processed by petroffs method and subjected to Direct Microscopy by 3 different staining techniques.

- » Modified Ziehl - Neelsen Acid Fast Technique.
- » Kinyoun Acid Fast Technique.
- » Auramine - O - Rhodamine Flurochrome Staining Technique.

III. Preparation Of Reagents

1. Modified Ziehl – Neelsen acid fast technique

i. Carbofuchsin: Dissolve 3g of basic fuchsin in 10 ml of 90% - 95% Ethanol.Add 90 ml of 5% aqueous solution of phenol.

ii. Acid Alcohol: Add 3 ml of Conc..Hcl to 97 ml of 90% - 95% ethanol.

iii. Methylene Blue Counter Stain: Dissolve 0.3 g of Methylene blue in 100 ml Of distilled water.

2. kinyoun acid fast technique

Carbofuchsin: Dissolve 4 g of basic fuchsin in 20 ml of 90% - 95% Ethanol and add 100ml of a9% aqueous solution of Phenol.

Acid Alcohol: Add 3 ml of Conc.Hcl to 97 ml of 90%-95% Ethanol.

Methylene blue counter stain: Dissolve 0.3g of Methylene blue in 100ml of distilled water.

3. Auramine: O Rhodamine fluorochrome stain

Solution 1. - 0.75 g of Rhodamine and 1.5g of Auramine dissolve in 75 ml of Glycerol.

Solution 2 - Mix 10 ml of phenol in 50 ml of distilled water

Mix 1 and 2 solutions.

Decolorizing Agent: 0.5% Acid Alcohol.

0.5 ml of conc.Hcl+100ml of 70% Ethanol.

Counter stain: 0.5% of KMno4,

0.5 g of KMnO4 in 100 ml of distilled water.

From each sample 3 smears are prepared, and numbered as A,B &C.A smear is stained by modified ziehl – neelsen, B is stained by kinyoun acid fast method and C is stained by Auramine – O Rhodamine fluorochrome method.

IV. Grading

The slides were graded as per the recommendations of the International Union against Tuberculosis and Lung Disease.

V. Results

Out of 62 patients 30.35%,17.8%and 17.8% were found positive by AO, Z - N and Kinyoun methods respectively. Our study shows out of 56 individuals of male patients 17 were positive and females are negative.The age group mostly involved in our study was 20-40yrs.In our study the paucibacillary cases were detected more by AO stain than Z-N and Kinyoun method. Z-N and Kinyoun methods are given equal sensitivity in our study.

Table -1 Sex wise distribution of total No of individuals & Positive individuals (n=62)

Sex	Total no of individuals	%	Total number of positive individuals	%
Male	56	90.32	17	30.35
Female	6	9.67	0	-

Table - 2Percentage of positivity by different staining techniques (n=56)

Sex	Mod.Z-N	Kinyoun	Fluorochrome
Male	17.8%	17.8%	30.35%
Female	0	0	0
Total	17.8%	17.8%	30.35%

Table – 3Age wise distribution of smear positive cases (n=17)

Age (yrs)	Z-N	Kinyoun	Fluorochrome
10-20	1	1	2
21-40	8	8	12
41-60	1	1	3
Total	10	10	17

Table – 4 Grading of sputum smears (n=17)

Grading	Z -N	Kinyoun	Fluorochrome
1+	-	-	5
2+	5	5	6
3+	3	3	4
4+	2	2	2

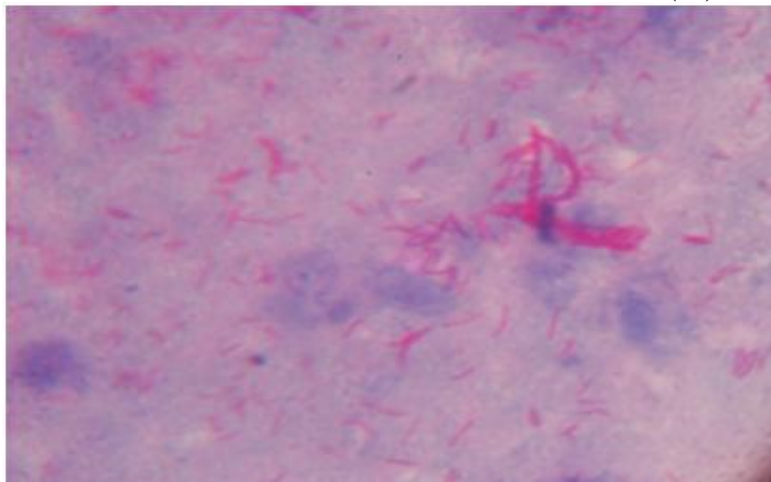
Table - 1 shows the total number of positive cases by three methods and sex wise distribution.

Table - 2 shows the percentage of positivity by three methods

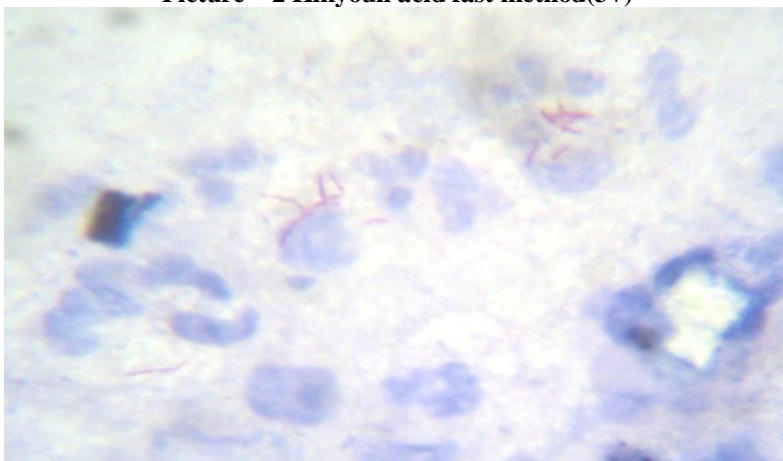
Table – 3 shows the age wise distribution of positive individuals

Table – 4 shows the grading of sputum smears

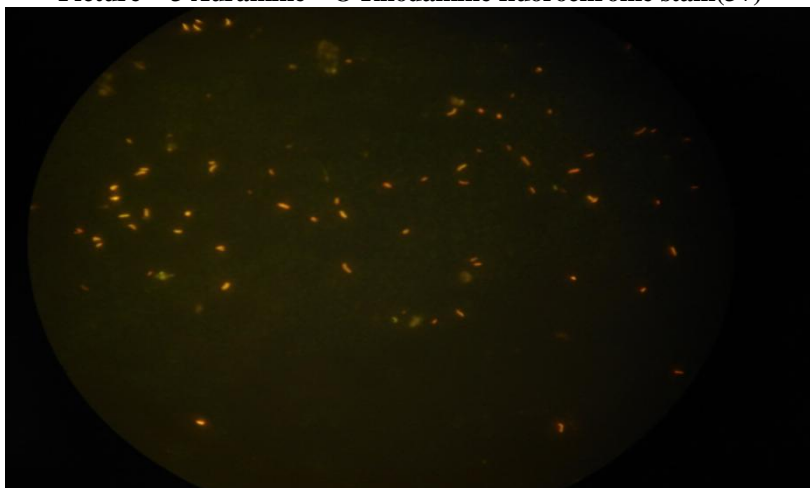
Picture – 1 Modified Ziehl – neelsen acid fast method(4+)



Picture – 2 Kinyoun acid fast method(3+)



Picture - 3 Auramine – O Rhodamine fluorochrome stain(3+)



Picture 1 shows the modified Ziehl –Neelsens method which is graded as 4+

Picture 2 shows the kinyoun method of stained smear of grade 3+

Picture 3 shows the AO method of stained smear of grade 3+

VI. Conclusion

The present study includes 62 individuals suspected of pulmonary tuberculosis were tested. Among them 56 were males and 6 were females. 17 were positive by fluochrome stain and 10 were positive by both modified Ziehl – Neelsen and Kinyoun staining techniques respectively. Direct microscopy is still the most simple and reliable method for diagnosis of tuberculosis. It also detects more number of paucibacillary cases. In laboratories equipped with fluorescent microscope fluochrome staining is more sensitive than acid fast staining for identification of tubercle bacilli.

Our priority in this study was to find a rapid and cost- effective method for the diagnosis of TB. We conclude that the FM method is quite economical in terms of both time and expense and it is recommended for laboratories handling large number of sputum specimens. We conclude that FM is more reliable than ZN and kinyoun method.

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