

# Review Article on Comparative Study of Different Staining Techniques for Detecting of Mycobacterium Tuberculosis in the Sputum

Sharda Rani (L.T)<sup>1</sup>, Dr. Surinder Pal (Medical Officer)<sup>2</sup>, Mr. Vinesh (DOT provider)<sup>3</sup>.

<sup>1</sup>Department of Biomedical Engineering D.C.R.U.S.T. Murthal, Sonapat

<sup>2</sup>Medical Officer – Distt. TB Control, DRCC Rohini, Sect-7, New Delhi - 85

<sup>3</sup>DOT Provider, Rohin, Sect -18

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## Abstract

### **Introduction of AFB:**

AFB is an Acid Fast Bacilli. Because it does not stain easy he needs heat application.

### **Sample collected:**

For diagnosis of TB. we need three sputum sample with the right time (According to RNTCP guideline)

### **Different Techniques:-**

In this study, we read the three different techniques like- ZN staining, Gabbet's cold staining, fluorescent staining.

### **Discussion:**

We are discussing in all staining methods and his staining principal or History of AFB. **Conclusion:**

After this study, all papers we find out the ZN staining is a good, easy and cheapest, time-saving method. We concluded the both Fluorescence staining techniques or Z-N staining techniques can be used for diagnosis of lung pulmonary TB.

**Keywords:** Auramine rhodamine, Microscope, Mycobacterium, Tuberculosis, Ziehl- Neelsen.

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## INTRODUCTION

TB (tuberculosis) is the biggest problem in the world or in health field (sectors). According to WHO in 1993 this time period is a Tuberculosis global emergency time for the health sector (in world) or major health disease. Approximately counted people of one billion are will be infected with Tuberculosis. Sputum examination is a direct microscopic examination of AFB. In a quantitation scale AFB testing is a good method for Tuberculosis (TB). AFB (microscopic sputum) test is the fastest and cheapest method of Tuberculosis (TB) diagnosis. An AFB technique is an easy technique to compare the culture and other TB tests. Because culture is a slow process of testing (TB Test) . Many factors are effected AFB testing like collection of sample, quality of microscope, (direct/concentrated) staining technique, expertise, etc. On AFB slide at observing big area or lower magnification routine. Ziehl Neelsen (ZN) stained smears detection of Mycobacterium tuberculosis (1, 17). The man is suffering from different diseases, but pulmonary tuberculosis is caused by Mycobacterium tuberculosis. It is called a respiratory system Pulmonary tuberculosis (4, 7, 15). Many countries are fighting with the TB problem. But developing countries are suffering from (TB) major health problems like India. WHO Statistics 2015 are indicated estimated peoples (2.2 million) cases of TB present in India out of total (9.6 million) TB peoples of the world. It is a chronic infectious disease AFB testing of sputum examination is a good and reliable method for the detecting of lungs T.B. It is the quickest or easiest method. According to World health organization in 1993 declared tuberculosis is prevalent in India or single one of

the leading causes of human rising (↑ed) of death. Glass sputum slide microscopy examination is the best techniques to detecting of (TB). Glass sputum slide microscopy process is a good tool of test. We are used the different microscopic techniques to find the AFB in Sputum to detect pulmonary TB is:- fluorescence microscopy and ordinary microscopy or bright field. Ziehl-Neelsen smear method is a quick but cumbersome procedure because it requires the heat application (1, 2,3,4,7, 15, 16, 17, 19 ).

### **AFB: -Acid Fast Bacilli**

According to WHO, RNTCP or any health organization declared TB (tuberculosis) is the biggest health problem for the human being. The microscopic test of Sputum samples is important for TB rapid presumptive diagnosis. Because Acid Fast Bacilli (AFB) are grown in culture very slowly. In other words AFB bacilli slow growth result found in culture. In sputum AFB testing (ZN stain) we save our time and get the best or accurate result. In stained sputum smears 1st stage(early) diagnosis the active TB still presence of(AFB) acid-fast bacilli are depended. This study compares the efficacy of (AO) Acridine orange with (ZN) Ziehl-Neelsen staining or (AR) Auramine rhodamine staining of fluorochrome of tuberculosis (TB) diagnosis. Ziehl- Neelsen (ZN) staining was found more effective than Acridine orange, Auramine rhodamine fluorescent stains for the tuberculosis diagnosis. The microscopic examination of sputum sample is the first step of testing (lungs TB) of AFB (1). In other Techniques like Auramine –o (AO stain) stained sputum smears examination under the LED fluorescent microscopes are

used and 2 min. in 100 fields or 3 horizontal moves (sweeps).we are used lower magnifications to observed the sputum smear with fluorescent stain but in Ziehl Neelsen staining smears we see the 1000x each field observed under fluorescence microscopy. In this examination we see the big area of sputum smears with the help of bright field microscopy (3).

#### **SAMPLE COLLECTED:**

Sample collection or staining is doing according to the RNTCP guideline. When a suspected patient of tuberculosis pulmonary lung infections, we are taking the three sputum samples are collected first is on the spot time, and the second Sample is taking next day early morning. If you are unable to produce sputum, a bronchoscope may be used to collect fluid during a procedure called a bronchoscope (6, 4, 5).

#### **STAINING METHODS:-**

##### **PROCEDURE OF Z-N STAINING:-**

According to RNTCP guidelines first, take a clean and dry slide. Then spread the patient sputum on the slide and air dry it room temperature. Then heat-fixed smears with the help of flame two-three times for about two-three seconds (carefully passing the slide over). Then sputum smear is flooded with 1% carbolfuchsin (freshly filtered) and wait for steam rises. Carefully mind it not boiling for five minutes. Then wait for 5 minutes with carbolfuchsin heated stain. Then slide is washed with water. Then decolorize the slide with the help of 25%  $H_2SO_4$  and wait for 4 minutes. Then stain slide is washed with clean tap water. At last finally do counterstain for 30 seconds with 0.1% (methylene blue). We can do take more time if sputum slide is thin. Finally smear slides washed with clean running tap water. Then clean the back of slide and air dried it (AFB smear) room temperature. Then finally see the sputum smear under the microscope with the help of 100x (oil immersion) (1,3).

##### **GABBET'S COLD STAINING METHOD:**

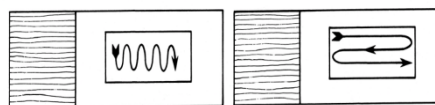
According to RNTCP guidelines first, take a clean and dry slide. Then spread the patient sputum on the slide (near the flame) and air dry it room temperature. Mind it in this method the sputum slide is not heat fixed. Then sputum smear area is fully covered with basic fuchsin phenol stain (freshly filtered) and then waits for ten minutes (without heat applied). Then stain slide is wash with clean running tap water. Then decolorize the slide with the help of  $H_2SO_4$ . Then stain slide is wash with clean running water. At last finally do counterstain with Gabbets methylene blue for 2 minutes. We can do take more time if sputum slide is thin. Then stain slide is wash with clean running water. Then clean the back of slide and air dried it (AFB smear) room temperature (carefully do not blot dry). Then finally see the sputum smear under the microscope with the help of 100x (oil immersion) (1,3).

##### **THE FLUORESCENCE STAINING METHOD**

First, take a clean and grease-free slide. Then make a patient mucopurulent portion sputum smear with the help of broomstick (near the flame). Then air-dried sample slide at room temperature. Then heat fixed the sputum

smear with the help of flame two – three times for about two-three seconds (carefully passing the slide over). Then sputum smear is flooded with auramine – phenol (freshly filtered) without heat application and allowed to stand for 20 minutes. Then glass sputum smears were washed with running water ( $H_2O$ ).Then decolorized by full covering sputum area completely with acid alcohol for allowed standing for three minutes. Then washed with running water and do the counterstain with 0.1%  $KMnO_4$  for allowed to stand for 1minute. Then slides gently rinsed with tap water. Then air dried it (do not blot dry). Then AFB sputum smears were observed under oil immersion objective (with compound microscope) by an experienced examiner (3).

#### **PATTERN OF EXAMINING SLIDES**



The pattern of Examining Slides

#### **DISCUSSION:**

Revised National TB Control Programme (RNTCP) is a good and biggest health program in India. In India RNTCP establish her Primary health centers easily in urban and rural areas and RNTCP sputum smear microscopic examination successfully implemented in health sectors. ZN stain is a carbolfuchsin hot process-based stain. We are used the ZN stain because the wax substances are presence on the cell wall of the tubercle bacilli, in this way we heat the sputum sample smear for to penetration of stain (insert the inside) into the cell wall through its waxy layer barrier. It is a standard method to find out (detection) of T.B bacteria But heat application is a big difficulty in Z-N staining method. Because stain possess is facing the problem like fairly precise control of the heat temperature to the glass slide or regular supply of the liquid LPG & alcohol is required for the fixing and heating of the sputum slide. In different method we are used to basic fuchsin phenol solution (freshly filtered) cold stain detection of AFB (GABBET'S COLD STAINING). (8, 9, 10, 11, 12, 13, 14, 18 ). The fluorescence staining is an expense stain. But in fluorescence technicque the good advantage is fluorescence that glass slides we can be seen under the lower magnification. In this stain we see the big area per unit of time. According to RNTCP guidelines Graded as per flowing :- more than 10 AFB = 3+ (100x /with microscope); 1-10 AFB = 2 + (100x /with microscope); 10-99 AFB = 1+ (100x /with microscope); 1-9 AFB = Scanty (100x /with microscope); No AFB seen = Negative (3,6).

#### **CONCLUSION:**

The study of all papers we found out the Z-N method is the best method of staining. Because India is a big country and it is a developing country. The Indians people are like simplicity in this way he like simplicity method and low-cost processors. The Z-N staining method is a low-cost method, simple method, easiest, short (low) consuming time and fastest, reliable tool for the diagnosis of lunges

T.B. This staining method or technique is easy to apply to any health areas or sectors. Z-N staining method is the best method compared to Gabbet's cold staining methods or fluorescence staining method. Some time fluorescence microscopy gives a false-positive result compared to Z-N staining. Sputum smear examination is the main method of identification of Acid Fast Bacilli (pulmonary tuberculosis). The diagnosis of AFB is dependent on the different things like patient sample collection, guidance of T.B lab. technician (T.B.L.T.), quality of specimen, staining material, storage of required stain (Time period, storage temperature of stain, expiry date of stain), proper stain processor, arrangement of the proper heat application, experienced pathologist / Lab Technician and other lab staff, proper reporting, etc. We concluded that both of fluorescence staining technique or Z-N staining techniques can be used for diagnosis of lung pulmonary TB.

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