

## The Bleach Digestion of Sputum: The Method Improves the Detection of Pulmonary Tuberculosis

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### Abstract

*Introduction:* Tuberculosis is a chronic infectious disease caused by mycobacterium tubercular bacilli. It primarily affects lung and it can involve any organ system in the body. There are various methods for detection of acid fast bacilli in the sputum examination for the diagnosis of tuberculosis. *Material and Method:* Our study was done clinically suspected tubercular patients in the tertiary care hospital. On all received samples ZN stain was applied by two method (conventional and bleach). *Result:* 682 cases out of 3962 total cases are positive both in conventional method and bleach method and the 188 cases are positive only in bleach method. *Conclusion:* The majority of positive cases in bleach method showed easily visible and detectable. AFB morphology was observed to be better preserved to in the bleach method. The bleach method is safe, inexpensive and easy to perform and requires no additional equipment.

**Keyword:** Tuberculosis (TB); Mycobacterium Tubercular Bacilli (MTB) Acid Fast Bacilli (AFB); Ziehl- Neelsen (ZN Stain) and Bleach (Sodium Hypochlorite).

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### Introduction

Mycobacterium tubercular bacilli were first described by Koch [1]. Tuberculosis is caused by mycobacterium tubercular bacilli. These bacilli are aerobic or microaerophilic, acid and alcohol fast, non spore forming and non motile bacilli. Tuberculosis is major cause of motility and morbidity in India as well as worldwide. Mycobacterium tuberculosis which primarily affect lung and cause pulmonary tuberculosis.

It can also affect Intestine, meninges, bones, joints, lymphnode, genitourinary system, skin and virtually every organs of the body. The World Health Organisation (WHO) reports approximately 8.8 new cases of pulmonary TB including 3.9 million smear positive cases in 2003 [2] and estimated 1.9 million people died due to tuberculosis including those patients who were co infected with human immunodeficiency virus. There is continuous rise in

new cases so efforts are to be made to diagnose these new cases at the same time these cases to be cured to prevent death [3].

Numerous cytological stains are used to identify MTB on direct smears of expectorated samples using light microscope. MTB is called AFB as it retains certain stains even after being treated with acidic solution. Most common and fast staining is ZN staining which stain AFB as bright red against blue background. ZN staining is most extremely as it is simple, inexpensive and provides rapid results [4].

Microscopy is a valuable tool for AFB detection. New, improved bleach concentration for detection of AFB on light microscope using 100x oil immersion is one of the safest concentration methods compare to the conventional method. Few studies have shown that liquefaction of sputum by bleach and concentration of bacilli through centrifugation will increase positivity of direct microscopy detection of tubercular bacilli by bleach concentration method is a simple technique which required no expertise and is cheap.

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### Aims and Objective

- To analyze detection of AFB positivity between conventional and bleach method.

- To analyze advantage and disadvantage.

## Material and Method

The study was conducted in the pathology department collaboration with pulmonary department from January 2015 to February 2016 year. Minimum volume (1ml) sputum specimen was collected for direct smear microscopy. Each smear was heat fixed and was stain with the ZN method. The residual of the specimen was to be liquefied with the equal volume of 5% sodium hypochlorite (NaOCl) and was place under the room temperature for 20 minutes. Centrifugation was done at 3000 rpm for 15 minutes. After centrifugation, bleach smear was made from sediment and stained by the same ZN method. Culture method is the gold standard for diagnosis of tuberculosis but is not included in our study because of its own limitation. Statistical analysis was made by percentage.

### Procedure

- Prepare the smear from the sputum specimen on a clean glass slide and fixe it by heating on Bunsen burner flame.
- Staining- Place the heat fixed slide on the staining racks or rods and flood the smear with working carbol-fuchsin stain.
- Place the slide over the Bunsen burner flame and heat is gently until the steam raises.
- Avoid boiling and continue heating for about 5 minutes. Do not allow to dry the stain on the slide and add more stains if necessary.
- Wash the stain from the slide with slow running tap water and continue rinsing, until the water turns out the stain colourless.
- Decolourization- Cover the slide with 20% sulphuric acid for about 1 minute. The yellow colour complex should be drained off completely.
- Counter staining- Cover the slide with methylene blue stain for 1 minute.
- Wash with tap water and drain it. Allow it to dry in air or blot carefully.

### Microscopy Examination

Under oil immersion objective microscope, we directly observed both direct and bleach smear. The cell wall of mycobacterium has lipid content which shows unique capability of binding with these fuchsin dye so that it does not get distained by acid alcohol. This acid fast staining reaction of microbacteria along with their characteristic size and shape is valuable aid in the early detection of infection and monitoring the therapy from the microbacterium diseases. The property of acid fastness is due to the thick, waxy capsule that surrounds the mycobacterial cells. For the aqueous carbol-fuchsin to penetrate in wax, the capsule must be softened. This is obtained with the heat in the ZN procedure which is much like to melt the paraffin in hot rays of sun. Dye which is penetrated, heat the softened capsule and binds to the cell wall. As soon as heat is removed bacterial cell cool down the wax again hardens protecting the bound dye from the action of acid alcohol decolourizer (acid-fast). They were observed under direct microscopy after staining with the ZN method.

### Result

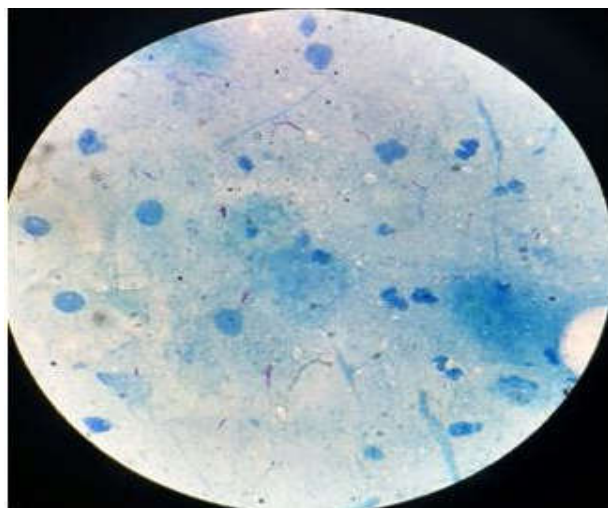
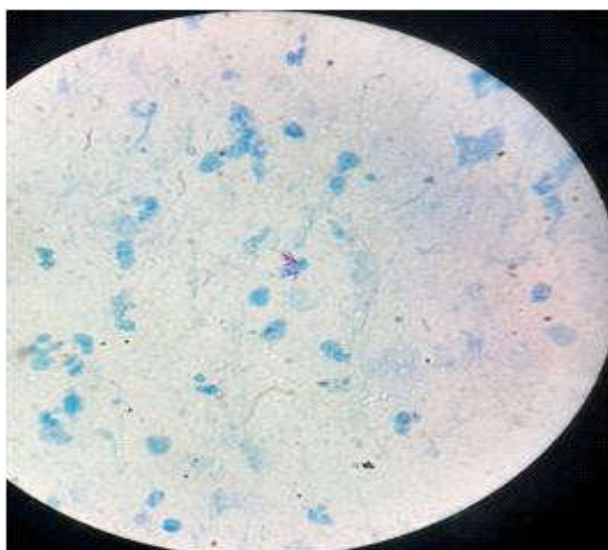
This study was carried out on 3962 patients who were clinically suspected by pulmonary tuberculosis. Further study was conducted on clinically suspected pulmonary tuberculosis patients of the all age groups. 2751 (69.43%) males were reported with male:female ratio=2.27:1. Total numbers of 3962 specimen were collected and proceed by both bleach and conventional method. Smears were stain by ZN method and searched for acid fast bacilli under the light microscope (100x). Among these specimens 2020 (50.98%) were purulent, 896 (22.61%) were mucoid, 396 (10%) were bloody and 650 (16.41%) were salivary. In 3962 patients, 682 cases were found positive both in conventional ZN method and bleach concentration method and the 188 cases were positive only in bleach method. (Table-1) AFB positivity by bleach method was more in comparison with the routine conventional ZN stain.

**Table 1:** Comparison of the Conventional ZN method with the Bleach method for detection of AFB

Conventional ZN Method	Bleach Method	Total
	Positive	Negative
Positive	682	00
Negative	188	3092
Total	870(21.96%)	3092(78.04%)

**Table 2:** Comparison of AFB positivity in different studies by conventional and bleach method

Authors	Conventional Method	Bleach Method
Marline Bonnet et al(10)	16-19.9%	19.3-24.5%
Ongkhammy S et al(9)	12.30%	16.42%
Anagaw B et al(16)	12.5%	23.2%
Preeti B Mindolli et al(8)	9.80%	32.94%
Present study	17.21%	21.96%

**Fig. 1:** Acid fast bacilli in conventional ZN stain method shows AFB with thick background**Fig. 2:** Acid fast bacilli in bleach ZN stain method shows AFB with thin background due to the reduction of debris

## Discussion

TB is major health problem in developing countries like India as this disease is very contagious and can affect any organ of the body. Measures for early diagnosis of TB and early treatment would enable not only cure of the patient but at the same time control the spread of infections to others in community.

Various tests available for diagnosis of TB like use of specific stains to identify organism, culture and PCR. However, culture is necessary for definitive diagnosis but culture is time consuming and expensive. On the contrary PCR is rapid but costly to be routinely used in developing countries. ZN stain method used for detection of acid fast bacilli placed important role in diagnosis as well as monitoring of treatment in TB [5]. Its major disadvantage is low sensitivity (9-46%) [6-7].

It is observed that liquefaction of specimen with sodium hypochlorite (bleach method) increases the detection of AFB might be due to changes in surface properties of the AFB or denaturation of specimen leading to flocculation and increased sedimentation rate of AFB. The increased smear positivity by bleach method is due to high density of bacilli per microscopic field obtained by this method and liquefaction of debris leaving a thin background for light microscopy compared to thick background in conventional method. The bleach method reduces the time required for examination of the slides to detect AFB. This method is safest tool for improving the positivity of direct microscopy for detection of AFB. In our study 682/3962 (17.21%) cases are positive both in conventional method and bleach method and 188/3962 (21.96%) cases are positive only in bleach method. The AFB positivity increased in bleach method compare to conventional method in our study which are similar to the findings of previous studies [8-16] reported by Preeti B Mindolli et al. Ongkhammy S et al, Marline Bonnet et al and Anagaw B et al respectively (Table-2) [8-10,16].

There are many benefits of this technique over routine ZN staining. Bleach can effectively kill mycobacteria so lower the risk of laboratory infection. This makes the specimen safe to handle, but not suitable for micobacterial culture. The bacilli can be easily visible against the clear background due to liquefaction and making the screening process easier, faster and less strenuous on the eye.

Smear microscopy and sputum culture are important tool for diagnosing TB. Sputum culture being the gold standard is more sensitive method compared to microscopy. But major pitfall is a slow growth rate of micobacteria in culture leads to a delay

of 4-6 weeks in obtaining the definitive diagnosis. So sputum microscopy is the cornerstone of tuberculosis diagnosis. This study suggests that the bleaching method can improve the detection of AFB under the light microscopy for diagnosis of TB.

### Conclusion

- Conventional ZN staining for acid fast bacilli detection is having low positivity.
- By the bleach method, the majority of positive cases showed easily visible and detectable. AFB morphology was observed to be better preserved in the bleach method. The bacilli can be easily detected against the clear background.
- The bacilli were seen in clumps in a thin background, making the screening process easier, faster and less strenuous on eye.
- Sodium hypochlorite can effectively kill mycobacteria. This makes the specimen safe to handle.
- The bleach method for detection of tubercle bacilli in sputum, smear is more delicate than the conventional ZN method. Moreover, the bleach method is safe, inexpensive and easy to perform and requires no additional equipment.
- Bleach method for sputum, smear samples significantly increased the detection rate of smear positive patients compared to the conventional method.

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### Reference

1. David HL: bacteriology of the mycobacterioses. US department of health, education and welfare, PHS, CDC, Washington DC, 1976.
2. Murray P.R, Baron E.J, Jorgensen J.H, Landry M.L., Pfaller M.A. Mycobacterium: General characteristic, Laboratory detection and Staining procedures, chapter 36. In: Manual of clinical Microbiology: 9<sup>th</sup> ed 2007;1:543-548.
3. Corbet L, Raviglione M. Global Burden of Tuberculosis: past, present and future: In Cole S.T, Eisenach K.D, Mc Murray D.N, Jacob W.R. Journal on

- Tuberculosis and the Tubercle Bacillus 2005:3-12.
4. Ganoze C.A. Gault E, Delon S. Conventional method of staining sputum samples for Mycobacterium Tuberculosis. Journal of Medical Microbiology 2008;87:1094-1098.
  5. Friede T. Tuberculosis is Case detection, treatment, and monitoring questions and answer. 2<sup>nd</sup> ed. Geneva:WHO; 2004.p.14-20.
  6. Daniel TM. Rapid diagnosis of tuberculosis: laboratory techniques applicable in developing countries. Rev Infect Dis 1989;2:471-8.
  7. Balows A, Hausler WJ, Herrmann KL, Shadomy HJ. In: Manual of clinical microbiology. 5<sup>th</sup> ed. Washington, D.C: American Society for Microbiology; 1991:308-11.
  8. Preeti B Mindolli, Manjunath P Salmani, Prashant K Parandekar. Making an improved Diagnosis of pulmonary tuberculosis with the use of the bleach microscopy method. Journal of Clinical and Diagnostic Research. 2013;7(7):1336-1338.
  9. Ongkhammy S, Amstutz V, Barennes H, Buisson Y. The bleach method improves the detection of pulmonary tuberculosis in Laos. Int J Tuberc Lung Dis. 2009;13(9):1124-9.
  10. Marline Bonnet, Andrew Ramsay, Willie Githui, Laramie Gagnidze, Francis Varaine and Philippe J. Guerin. Bleach Sedimentation: An Opportunity to Optimize Smear Microscopy for Tuberculosis Diagnosis in Settings of High Prevalence of HIV. Clinical Infectious Diseases 2008;46:1710-6.
  11. Ramsay A, Squire SB, Siddiqi K, Cunningham J, Perkins MD. The bleach microscopy method and case detection for tuberculosis control. Int J Tuberc Lung Dis 2006;10:256-258.
  12. Aung W W, Nyein M M, Ti T, Maung W. Improved method of direct microscopy for detection of acid-fast bacilli in sputum. Southeast Asian J Trop Med Public Health 2001;32:390-393.
  13. Yassin M A, Cuevas L E, Gebrexabher H, Squire S B. Efficacy and safety of short-term bleach digestion of sputum in case finding for pulmonary tuberculosis in Ethiopia. Int J Tuberc Lung Dis 2003;7:678-683.
  14. Bruchfeld J, Aderaye G, Palme B, Bjorvatn B, Kallenius G, Lindquist L. Sputum concentration improves diagnosis of tuberculosis in a setting with a high prevalence of HIV. Trans Roy Soc Trop Med Hyg 2000;94:677-680.
  15. Angeby KAK, Alvarado-Galvez C, Pineda- Garcia L, Hoffner S E. Improved sputum microscopy for a more sensitive diagnosis of pulmonary tuberculosis. Int J Tuberc Lung Dis 2000;4:684-687.
  16. Anagaw B, Mulu A, Abate E, Anagaw B, Belay T, Gelaw A, Alemayehu M, Belyhun Y, Biadlegne F, Schon T. Improved detection of acid-fast bacilli in sputum by the bleach-concentration technique at Gondar University Teaching Hospital, northwest Ethiopia. Ethiop Med J. 2012;50(4):349-54.