

## A comparative study of sensitivity of sputum microscopy by direct method versus sodium hypochlorite concentration method at RNTCP Centre

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### Abstract

In revised national tuberculosis control program (RNTCP), microscopic examination of sputum for AFB plays an important role in the initial diagnosis of tuberculosis. Decontamination and liquefaction with 5% sodium hypochlorite increases sensitivity and safety for handling specimen. In this prospective hospital based cross sectional analytical study, patients of all ages and either sex coming to RNTCP centre were included according to RNTCP criteria. From each patient, two samples of sputum were collected one spot and one morning. One set of smears was prepared by RNTCP guidelines and remaining samples were concentrated by using 5% NaOCl, followed by centrifugation and staining with ZN stain. Both set of smears were observed by two observers to remove observer's bias. A total of 882 patients were included in the study with 577 (65%) males and 305 (35%) females. A total of 172(19.5%) patients were positive by RNTCP method whereas 201(22.79%) patients were positive by concentration method diagnosing 29 additional patients. This constitutes a rise of 3.29%. This constitute 16.86% rise in case detection over RNTCP method. There is a rise in smear positive cases after concentration with 5% sodium hypochlorite solution. The bleach method has advantage over routine RNTCP method as it is simple, does not require any additional expertise, is safe, reagents are also affordable and easily available and could diagnose additional cases.

**Keywords:** Tuberculosis, Sodium hypochlorite, Sputum microscopy.

### Introduction

Tuberculosis (TB) remains a worldwide public health problem despite the fact that the causative organism was discovered more than 100 years ago.<sup>(1)</sup> The major objective of the TB control programs is to identify and treat the patients with infectious pulmonary tuberculosis, the diagnosis of which relies on a bacteriological examination of the sputum. Direct microscopy with Ziehl Neelsen staining is still most widely used and it is specific, fast and cheap method. The program has approved LED microscopes but these are not provided at every centre. The culture of Mycobacterium is the reference method for the detection of the tubercle bacilli, but it is prohibitively slow and it requires special safety procedures in laboratories.<sup>(2)</sup> Many automated culture systems and molecular techniques have been developed which require less turnaround time but are costly.

In revised national tuberculosis control program (RNTCP), microscopic examination of sputum for AFB plays an important role in the initial diagnosis of tuberculosis. The microscopic examination requires 10<sup>(4)</sup> bacilli per milliliter of sputum in order to be detected on smear. Considering the amount of sputum material that is examined in oil immersion field, chances of missing the organism are high thus reducing the sensitivity. Much of the transmission of TB can occur even before the concentration in sputum reaches a critical level when it is diagnosed. A negative smear does not exclude the diagnosis of tuberculosis, as about 55% of pulmonary tuberculosis cases worldwide harbors low bacillary load. It has also been established

that sputum smear microscopy is less sensitive in HIV – TB co infection where sputum smear tends to be negative.<sup>(3,4,5)</sup>

The concentrations of 2-5% of Sodium hypochlorite (NaOCl) digest the sputum products and they inactivate the mycobacteria without altering their structures, so that even when they are killed, they can still be stained and observed. This provides a greater security for laboratory use. Further centrifugation or sedimentation concentrates the acid fast bacilli (AFB) in the mixture and it increases the rate of the positivity.<sup>(6)</sup>

With this background an operational research was planned to look for increase in sensitivity of sputum smear microscopy by concentration after pre-treatment with sodium hypochlorite over routine RNTCP method.

### Materials and Methods

This prospective hospital based cross sectional analytical study was carried out as an operational research in the Department of Microbiology, NKPSIMS & RC, Nagpur after obtaining permission from Institutional Ethics Committee. Patients of all ages and either sex coming to RNTCP centre were included according to RNTCP criteria, having:

- Cough for two weeks or more duration
- Known contacts of sputum smear positives irrespective of duration of cough
- Extra pulmonary TB irrespective of duration of cough.

After obtaining informed consent, two sputum samples (1spot and 1 morning sample) from each

patient were collected in a clean, wide mouth labeled container. Two sets of sputum smears were prepared from each sample.

For the first set the mucopurulent portion of the sputum was taken on a new, clean and grease free glass slide and the smears were heat fixed and stained by modified ZN staining using 25% H<sub>2</sub>SO<sub>4</sub> as a decolourizer.

Equal volume of 5% Sodium hypochlorite was added to the test tube and kept at room temperature for half an hour. The test tube was shaken intermittently after which approximately 8 ml of distilled water was added to the test tube and it was centrifuged at 3000 G for 15 min. The supernatant was carefully discarded and smears prepared from the sediments.<sup>(7)</sup> The smears were heat fixed and stained similarly.

To remove observer's bias slides prepared by both the methods were observed by two experienced microbiologist separately by bright field microscopy and graded according to the RNTCP guidelines. In view of potential aerosol formation during manipulation of sputum samples for second set of smears, Bio-Safety Cabinet was used. All the data was managed in Microsoft Excel and statistical analysis was done using Epi-info. Pearson's chi-square test was used for comparative evaluation between two groups.

## Results

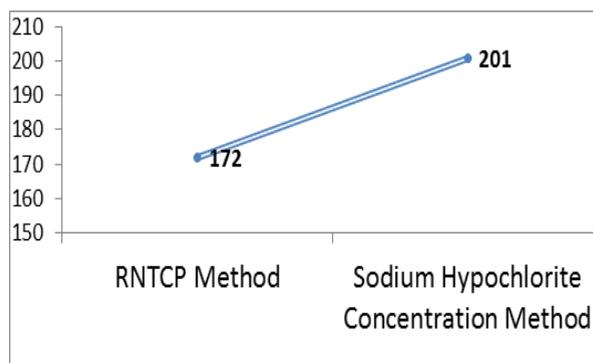
A total of 882 patients were included in the study with 577 males (65.42%) and 305 females (34.58%), a ratio of 1.89. Total samples collected and processed from 882 patients were 1764. From each patient two samples were collected one spot and one morning sample. Of the 882 patients, 172 (19.5%) were positive by routine method employed by RNTCP and 201 (22.79%) were found positive by Sodium hypochlorite concentration method. This constitutes an increase of 3.29% over routine method (P= 0.0908) (Table 1 and Fig. 1). These additional 29 cases diagnosed by hypochlorite concentration method constituted 16.86% rises in case detection when compared to RNTCP method. Thus concentration method is found to be more sensitive.

Another advantage reported by both the observers was clear fields with less debris in smears processed by sodium hypochlorite concentration method thus making it less strenuous for the observers.

**Table 1: Comparative evaluation of sodium hypochlorite concentration method over routine RNTCP method**

	Positive cases	Negative cases	Total	
Sodium hypochlorite Concentration Method	201	681	882	Chi square value=2.859
RNTCP method	172	710	882	P=0.0908

Total	373	1391	1764	
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**Fig. 1: Increase in number of cases detected by Conc. Method**

## Discussion

Sputum smear microscopy is still the backbone for diagnosis of pulmonary tuberculosis. It is rapid, inexpensive and highly specific method for detection of AFB in sputum specimen. However the disadvantage is its low sensitivity. There are several methods that can be used to improve sensitivity, but their applicability in a national program and in resource limited settings are limited. Bleach processing of sputum smears prior to microscopy may be a cheap and effective way to improve on the sensitivity of the direct smear.<sup>(8)</sup>

Pretreatment with sodium hypochlorite is not labour intensive and can be easily carried out by the existing technical team with only centrifuge machine as additional requirement. Sodium hypochlorite increases sensitivity of test and makes sample safer for handling. Bleach itself is inexpensive and readily available almost everywhere. The half-life of NaOCl is about 12 months; it is likely to be reduced by 1 month if the bottle is opened and by about 3 months if the ambient temperature is high (around 30°C).<sup>(9)</sup> This has to be kept in mind.

In this study additional 29 patients were diagnosed using concentration method which otherwise would not have been diagnosed by RNTCP method and would have gone undetected as open cases. This constitute 16.86% rise in case detection over RNTCP method. Further 25 out of 29 (86.20%) of these cases were diagnosed after concentration from a spot sample. Only 4 cases needed the morning sample.

At least the samples which are negative by routine RNTCP method can be retested by sodium hypochlorite concentration technique. If centrifuge is available results can be given on the same day as the procedure is not lengthy.

## Conclusion

There is significant rise in smear positive cases after concentration with 5% sodium hypochlorite solution. Considering its low cost, decontaminating and liquefaction properties with better sensitivity, this

method is safe and can be of vital importance; at least for smear negative cases. Can the sample size be reduced to one with this concentration method which is also safe for laboratory workers? We need large scale metacentric studies.

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