

Enhancement of yield of acid fast bacilli in smear negative sputum microscopy with sodium hypochlorite decontamination sedimentation technique

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Abstract

Introduction: To study usefulness of sodium hypochlorite (bleach) decontamination sedimentation technique to increase yield of acid fast bacilli (AFB) in sputum smear negative clinicoradiologically suspected pulmonary tuberculosis patients.

Material & Methods: Cross-sectional prospective study conducted at department of Microbiology of a teaching hospital having RNTCP centre. Screening for AFB is routinely carried out at our RNTCP centre. Patients with 2 smears (spot & morning samples) were selected for study. Their clinicoradiological information & demographic details were collected.

Out of 347 RNTCP registered patients (247 male & 100 Females) during Jan-Dec 2014, 49 (36 Males & 13 Females) (14.12%), AFB positive by RNTCP criteria. Rest of 298 smear negative, 255 included for study, 43 excluded because of salivary nature of sputum samples. Sputum negative smears were processed by Bleach sedimentation technique, left at room temperature for 4 hours. Supernatant discarded & smear prepared from sediment. Smears were heat fixed & Z-N staining done. Reporting done as per American Thoracic Society (ATS) grading.

Result: Out of 255 patients, 26 were positive. Sensitivity increased by 10.19% (p value < 0.01).

Conclusion: Smear positive cases significantly rises after sedimentation at room temperature for 4 hrs with bleach. Sputum pretreatment enhances yield of positive smears. Considering low cost, better sensitivity, liquefaction properties, poor power supply in remote areas, no need of centrifugation (no aerosols), this method is safe and of vital importance for smear negative cases.

Keywords: Bleach Sedimentation, Bleach Smears, Direct Smears, TB Diagnosis, Conventional Microscopy.

Introduction

Sputum microscopy is the most widely used test for the diagnosis of Pulmonary Tuberculosis (PTB) globally. Although alternative diagnostic methods such as culture and molecular tests are more sensitive and specific, they are expensive and often difficult to implement in resource-limited settings.⁽¹⁾

In Revised national tuberculosis control programme (RNTCP), microscopic examination of sputum for AFB plays an important role in the initial diagnosis of TB. It can only detect bacilli when there are $\geq 10^4$ bacilli/ml of sputum,^(2,3,4) and according to the Global Plan to Stop TB 2006-2015 the method needs optimisation.⁽⁵⁾ While studies on optimising microscopy have been performed in reference laboratories, no studies have been reported under routine programme conditions in India.^(1,6)

The use of bleach can be traced back to 1909, where sodium hypochlorite (bleach) was used as a mucolytic before centrifuging sputum samples. This involved the addition of bleach to the sputum sample before the sample was subjected to centrifugation and then processed by direct microscopy after staining with the Zeihl Neelsen method. The bleach method has undergone several modifications and diversifications and has recently received more attention with the advent of the HIV epidemic.^(7,8)

This study compared the yield of and potential benefit for detecting smear-positive PTB by bleach

sedimentation versus direct microscopy under programme conditions in India.

Bacillary concentration after decontamination and liquefaction by 5% sodium hypochlorite is useful in providing increased sensitivity and safety for handling of specimen.⁽⁹⁾

Material & Methods

In this cross-sectional prospective study, carried out from January 2014 to Dec 2014 at R. D. Gardi Medical College, Ujjain, patients were included according to RNTCP criteria. Smear negative patients in RNTCP were screened by Sedimentation and decontamination with 5% sodium hypochlorite. As per national guidelines, two specimens containing ≥ 2 ml of sputum, one spot and morning samples were collected.⁽¹⁰⁾ All the samples were processed and examined by direct microscopy. Then on remaining sputum samples, which were negative by sputum microscopy were processed by bleach sedimentation techniques.

- Study design- Cross-sectional prospective study
- Study setting - Department of Microbiology, having RNTCP centre at R.D Gardi medical college Ujjain
- Study subjects - RNTCP Criteria
- Study material-smear negative sputum samples
- Study period- 1st Jan –Dec 2014

- Patient's with two smears (morning & spot samples) were selected for study.

Bleach sedimentation techniques: After direct smears had been made, the remainder of each sample was processed for bleach sedimentation according to the procedure outlined by Bonnet and colleagues,⁽¹¹⁾ with the exception that samples were manually shaken for 30 seconds instead of being vortexed. An equal amount of household bleach was then added to the sputum sample in a screw-cap tube, shaken for 30 seconds, and allowed to sediment four hours. The following morning, the supernatant was decanted and a smear was made of the sediment. Samples were then processed according to the standard hot Ziehl-Neelsen staining procedure, and the smears were examined for presence of AFB.

An external quality assurance protocol was followed in DMC, including cross-verification of sample slides and random blinded repeat slide reading per Revised National Tuberculosis Control Programme (RNTCP) guidelines.⁽⁶⁾ Both direct and sedimented smears were examined by conventional Ziehl-Neelsen (ZN) microscopy.

Sputum smear-positive TB was defined as at least one specimen with acid-fast bacilli (AFB) detected by ZN microscopy. Only PTB suspects with results available for all four smears (i.e., two direct and two sedimented) were included in the study.

Result

A total of 255 smear negative patients were included in the study. Two smears from each patients were examined. Out of which 26 patients were positive. Sensitivity increased by 7.49%.

Table 1: Direct sputum smear microscopy: by RNTCP

Total Patients	Positive Patients	Percentage
347	49	14.12%
Males-247	Males-36	
Females-100	Females-13	

14.12% were Positive by Direct sputum smear microscopy by RNTCP.

- Negative - 298 patients, 43 excluded due to salivary nature of sputum.
- 255 patient's samples processed by bleach sedimentation method.

Table 2: Result by Bleach sedimentation method

Total	Positive	Percentage
255	26	10.1 9%

7.49% increase in sensitivity by Bleach sedimentation techniques.

Overall Positivity is 21.61%.

Table 3: Distribution of bleach sedimentation positive samples

Patients diagnosed by bleach sedimentation technique (26)	Status by RNTCP Method	Status of bleach sedimentation method	Grading by ATS
14	Smear negative	One smear positive (Morning sample)	1+
10	Smear negative	Two smear positive (morning - spot)	2+
02	smear negative	Two smear positive (morning- spot)	3+

Table 4: Distribution of Positive sputum samples by Bleach sedimentation method

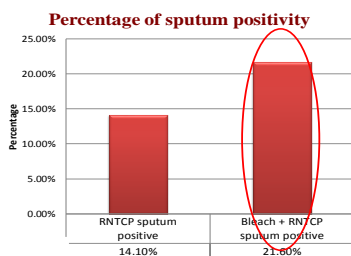
Age groups in years	Patient's (255)	Sputum samples (510)	Positive Patients (26)
<15yrs	05	10	01
16-24yrs	11	22	03
25-34yrs	21	42	01
35-44yrs	30	60	07
45-54yrs	104	208	05
55-64yrs	35	70	05
>64yrs	49	98	04

More Positive Patients were from age group of 35-44 yrs.

Table 5: ATS (American Thoracic Society) scale

Quantified Result	Interpretation by ATS Scale (Threshold at 1/100 fields)
Negative	Negative
1-2 AFB/300 fields	Doubtful
1-3 AFB/100 fields	Positive 1+
4-9 AFB/100 fields	Positive 1+
1-9 AFB/10 fields	Positive 2+
1-9 AFB/field	Positive 3+
>10 AFB/field	Positive 4+

Graph 1



Discussion

In the developing countries, the microscopy of the specimen is by far the fastest, cheapest and the most reliable method for the detection of AFB. In the late 1940s, sputum liquefaction with NaOCl (which is readily available at low costs as household bleach) and then its concentration by centrifugation before acid fast staining was implemented to improve the smear positivity for the detection of AFB.⁽¹²⁾

Sputum concentration by homogenisation and microscopic examination of the sediment can increase the detection rate of AFB. Several concentration techniques using sedimentation or centrifugation have been reported.^(6,7) Bleach homogenisation followed by overnight sedimentation can significantly increase detection of AFB compared with direct microscopy.^(6,8)

One other important point of view regarding the success of the bleach method study is use of ATS scale. The Revised National Tuberculosis Control Programme (RNTCP) aim to improve case detection rates of tuberculosis to facilitate prompt recognition and treatment. The low case detection rates in the programme can be directly attributed to failure to screen patients with suspected tuberculosis and the low sensitivity of the direct smear microscopy method to detect cases among the fraction of patients that are screened. Apart from low sensitivity, this method also has other disadvantages including the increased risk of infection transmission to technicians. There are several methods that can be used to improve sensitivity, but their applicability in a national programme 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.

In our study samples were manually shaken for 30 seconds instead of being vortexed. An equal amount of household bleach was then added to the sputum sample in a screw-cap tube, shaken for 30 seconds, and allowed to sediment four hours. We add equal amount of Household bleach to sputum sample and kept for sediment for 4 hours instead of overnight sedimentation, so that reports of Positive patients were dispatched in same day.

The results showed that there was a significant increase in the sensitivity with the use of 5% NaOCl. The increase in the 7.19% smear positivity with the use of 5% NaOCl with the Sedimentation method was very encouraging as compared to that of the direct smears. In present study among 255 PTB (sputum negative) cases, 26 (7.19) were detected by bleach sedimentation vs. 49 (14.12%) by direct microscopy.

PH. Vishnu⁽¹³⁾ found among 3168 PTB suspects, 684 (21.6%) were detected by bleach sedimentation vs. 625 (19.7%) by direct microscopy, with a proportional overall agreement of 96% ($\kappa = 0.88$).

Bleach-sedimentation has been hypothesized to concentrate acid-fast bacilli in sputum specimens and in support of this hypothesis a recent meta-analysis reported that bleach-sedimentation caused a 9% increase in tuberculosis diagnostic sensitivity compared to conventional smear-microscopy.^(14,15) In present study we find 7.19% increase in the sensitivity of microscopy compared to that by examination of direct smears, and was comparable with Mindolli et al.⁽¹²⁾

This suggests that NaOCl digests the sputum, which when followed by the concentration of bacilli by either centrifugation or sedimentation, greatly increases the number of bacilli per microscopic field, which explains the increase in the sensitivity. There was increase in the 23.14% smear positivity with the use of 5% NaOCl with the centrifugation method as compared to that of the direct smears by Preeti B. Mindolli et al.⁽¹²⁾

Before coming to a conclusion that centrifugation is the better method when compared to overnight sedimentation, operational considerations such as cost, technical and feasibility factors which exist in the installing of a centrifuge to peripheral laboratories, and issues such as frequent power cuts or power failures in resource limited settings must be considered. The risk of exposure to bio-hazardous material is also a concern while using centrifugation.

Conclusion

There is statistical significant rise in smear positive cases after concentration with 5% sodium hypochlorite solution. We have kept for sedimentation for 4 hours and dispatched reports same day without delay. Considering its low cost, decontaminating & liquefaction properties with better sensitivity, this method is safe and can be of vital importance at least for smear negative cases.

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