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RESEARCH ARTICLE

Comparitive studies on preparation of Panel Test (AFB) Smears by BAS and NALC Treated Sputum samples

<sup>1</sup>Sherafin Jancy Vincy, <sup>2</sup> Vincent .S, <sup>3</sup> Prabakaran .M, <sup>\*</sup><sup>1</sup>Chandrasekar. M,  
<sup>4</sup>John Regulus Gunanithy, <sup>1</sup>Rajalakshmi .R, Bashir Ali Siyad<sup>1</sup>

<sup>1</sup>Department of Medical Laboratory Technology, Loyola College (Autonomous), Chennai 600034,  
Tamil Nadu, India

<sup>2</sup>Centre for Environmental Research and Development, Loyola College (Autonomous), Chennai 600034,  
Tamil Nadu, India

<sup>3</sup>Department of Radiology and Imaging, Balajee Medical College and Hospital, Chennai 600044,  
Tamil Nadu, India

<sup>4</sup>Surgical Operating Suites, University Medical Center Groningen, The Netherlands

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ABSTRACT

Panel testing (PT) slides were prepared by Bleach Ammonium sulphate (BAS) and N-acetyl L- Cysteine (NALC) methods. A total of 150 slides were prepared, 80 slides were stained and two different technologists validated 60 slides. Consistency was found true when compared with standard consistency table for all grades in both methods and M $\pm$ 2SD was within the limits. This study suggests that BAS method is equally good as NALC sputum concentration method.

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INTRODUCTION

Tuberculosis is among the top ten causes of death worldwide (Lopez *et al.*, 2006; Dye *et al.*, 2008). Panel testing or Proficiency testing (PT) consists of staining and reading centrally prepared slides with known numbers of Acid fast bacilli (AFB) (Martinez *et al.*, 2003). In National level laboratories, sputum is concentrated with Sodium hydroxide (NaOH) and N-acetyl L-cysteine (NALC) methods (Iseman, 2000; Karen *et al.*, 2006; Kent and Kubica, 1985) and quality control (QC) slides are prepared as per the World Health Organization (WHO) and the International Union against Tuberculosis and Lung disease (IUATLD) guidelines. Other concentration method includes Phenol Ammonium sulphate (PhAS) (Sherafin Jancy Vincy *et al.*, 2007) and Bleach Ammonium Sulphate (BAS) (Chandrasekar *et al.*, 2008) methods. In order to achieve the required technical quality with the preparation of smears, standard techniques for digestion of sputum is needed. Standard sputum concentration method for manufacturing smears in PT improves the quality of slides and it remains a priority for effective training. Hence, we compared Bleach Ammonium sulphate (BAS) with N-acetyl L- cysteine (NALC) sputum digestion methods for preparation of PT smears.

MATERIALS AND METHODS

BAS (5%) reagent was prepared by dissolving 5g of bleaching powder and 4g ammonium sulphate (E. Merc, Mumbai, India) in 100 ml of distilled water (Chandrasekar *et al.*, 2008). For NALC reagent, solution A was prepared by dissolving 200 mg NALC in 10 ml distilled water and solution B was prepared by dissolving 290 mg sodium citrate in 10 ml distilled water. Equal volume of solution A and solution B were mixed and used whenever required (Aziz *et al.*, 2002). Sputum samples were collected from the Institute of Thoracic Medicine and Tuberculosis Hospital, Chennai. Negative samples from different patients with 20 or more white blood cells per field were collected, 3+ positive samples having a bacillary load of approximately 50 AFB per field was collected. Initially direct smears were taken from sputum samples and they were stained with Ziehl Neelsen (ZN) stain. The number of cells and bacilli in 100 fields were counted and recorded in standardized forms containing 100 boxes, for both negative and positive samples simultaneously. The pooled positive and negative sputum samples were aliquated into two portions of 3 to 5 ml, so that these two portions were approximately equal in volume. The two portions were randomly allocated first to BAS method and second to NALC method. BAS positive stock: A sample of 3 ml of sputum was taken to which an equal amount of reagent was added, incubated overnight to concentrate the bacilli, and the supernatant was discarded (Chandrasekar *et al.*, 2008). The sputum deposit was vortexed for about 5 minutes to get BAS positive stock solution. NALC positive stock: A sample of 3 ml positive

\*Corresponding author: vince\_mlt@yahoo.co.in,  
shekar\_mlt@yahoo.co.in, bashka1@yahoo.com

3+grade sputum was taken in McCartney bottle and an equal volume of NALC reagent was added. After 30 minutes the supernatant was discarded and the deposits were mixed well. This deposit solution was considered as NALC positive stock solution.

Initially smear was taken from the deposit and suspension of BAS and NALC positive stock solution respectively. These smears were stained by ZN stain (Sherafin Jancy Vincy *et al.*, 2008) and validated for 100 boxes to assess the average bacilli/ field, which was found to be 80 and 30 bacilli/ field for BAS and NALC method respectively. Negative stock solution was prepared by directly adding 10% formalin to per ml of negative sputum and vortexed.

Negative grade suspension smears were prepared directly from the negative stock. In order to obtain positive (Scanty, 1+, 2+, 3+) grade suspension, the stock solution of positive AFB sputum prepared by both BAS and NALC sedimentation was diluted with the negative stock solution respectively. For calculation of the dilution factor, the following formula was used:  $N = (DC/AC) \times A$ , where  $N$  is the number of drops of positive sputum to be added,  $DC$  is the desired AFB concentration,  $AC$  is the actual AFB concentration and  $A$  is the number of drops in a given volume. To know the number of drops per ml, a Pasteur pipette was used.  $AC$  was obtained in a smear made with two drops of the each grade suspension prepared (Mark *et al.*, 2000).

Each grade suspension prepared by the above described procedures was vortexed for five minutes and 25 slides were prepared from each grade (3+, 2+, 1+, Scanty and Negative) suspension. From 25 slides randomly 8 slides were selected and stained by ZN stain. Then from 8 stained slides 6 slides were randomly selected and validated (Chandrasekar and Venkatesan, 2007). Data were entered and processed using Microsoft Excel. The mean (M), standard deviation (SD), and consistency (M±2SD) was calculated in order to assess the equality of BAS method with NALC method for manufacturing PT slides.

## RESULTS AND DISCUSSION

Tables 1 and 2 shows the results of validation of manufacturing PT slides by BAS and NALC methods respectively. Table 1 shows SD for 3+, 2+, 1+, scanty and negative as 5, 1, 14, 1, 0 respectively. Table 2 shows SD for 3+, 2+, 1+, scanty and negative as 3, 1, 13, 1, 0 respectively. M±2SD were found to be within the limits irrespective of the used methods (BAS and NALC). Consistency was found to be true for 3+, 2+, 1+, scanty and negative grades in both methods.

**Table 1. Validation Log for BAS method (Cons: True)**

Bleach Ammonium Sulphate (BAS)										
Grade	Average Slide Test Results							SD	M-2SD	M+2SD
	1	2	3	4	5	6	M.			
3+	27	37	24	25	28	26	28	5	8	37
2+	5	3	3	4	4	3	4	1	2	5
1+	54	78	91	71	78	58	71	14	44	99
SC	6	8	6	5	6	4	6	1	3	8
NEG	0	0	0	0	0	0	0	0	0	0

**Table 2. Validation Log for NALC method (Cons: True)**

N-Acetyl L- Cysteine (NALC)										
Grade	Average Slide Test Results							SD	M-2SD	M+2SD
	1	2	3	4	5	6	M.			
3+	14	21	17	17	20	17	17	3	12	23
2+	5	7	4	3	4	4	4	1	2	7
1+	78	64	43	74	56	55	61	13	35	87
SC	4	4	7	3	6	5	5	1	2	8
NEG	0	0	0	0	0	0	0	0	0	0

**Smear results:** 3+: More than 10 AFB per oil immersion field in at least 20 fields; 2+: 1 to 10 AFB per oil immersion field in at least 50 fields; 1+: 10 to 99 AFB per 100 oil immersion fields; Scanty: 1 to 9 AFB per 100 oil immersion fields. M: Mean, SD: Standard

Deviation, Cons: Consistency The procedure recommended by WHO for manufacturing slides for PT was to process the sputum with NALC (Aziz *et al.*, 2002). NALC avoids the clumping of bacteria and provides uniform distribution with pink background. The limitations of this method are the pink background and the shortage of NALC reagent. The present study involved the use of BAS reagent for sputum processing in PT slides preparation.

The manufactured smears by the NALC and BAS methods were screened by two readers; however, the smears randomly coded were such that the reader who reads the slide was unable to identify which is BAS method and NALC method processed from the same sample. Because the BAS and NALC methods are distinct in appearance, it was not possible to blind the reader from the type of smear. Both the readers preferred the BAS method over the NALC method. Reasons stated for preferring the BAS method included ease of processing, ease to read with well-defined margins and with distinct AFB against a healthy blue cells background. Further, the BAS method is less expensive than the NALC method. The main limitation of the BAS method is that it necessitates an overnight sedimentation, which delays the processing time for PT slides.

This study suggests that BAS method could be used as standard method for PT slide preparation along with conventional NALC method. In National level laboratories where large numbers of technicians are trained, the BAS method can substantially increase the efficiency of preparing PT slides. However, a comparison of BAS method with NaOH or PhAS concentration method in preparation of PT smears is desirable.

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