Research article

Evaluation of phenol ammonium sulfate basic fuchsin and auramine O staining by pot technique for the detection of acid-fast bacilli among patients suspected of pulmonary tuberculosis

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(Received: January 2022 Revised: July 2022 Accepted: August 2022)

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ABSTRACT

Introduction and Aim: Tuberculosis (TB) remains a major public health concern with its high incidence and mortality. In developing countries, smear microscopy continues to be the primary diagnostic tool for the diagnosis of TB. Sputum samples processing under resource-limited settings could be hazardous, which urges safe and efficient smear microscopy techniques. This study aimed to evaluate the efficacy of two different pot methods: Phenol ammonium sulfate (PhAS) auramine O and PhAS basic fuchsin in comparison to the conventional Auramine O method.

Materials and Methods: A prospective cross-sectional study was conducted at the Department of Microbiology with 74 sputum samples. All sputum samples were processed using the Auramine O method PhAS auramine O and PhAS basic fuchsin staining methods. Microsoft Office Excel (Microsoft, Redmond, WA, USA) was used to maintain and analyze all the data.

Results: A total of 8 (10.7%) samples were positive for AFB and 67 (89.3%) samples were negative, according to routine auramine O method and pot methods. Pot methods showed a 100% sensitivity and specificity compared to the conventional Auramine O method. In terms of sputum smear grading, the direct smear using auramine O showed better results compared to pot methods.

Conclusion: Pot methods like PhAS basic fuchsin and PhAS auramine O are efficient to detect AFB in sputum smears by reducing the risk of laboratory-acquired infections.

Keywords: Pot method; acid-fast bacilli; pulmonary tuberculosis; sputum; smear microscopy.

INTRODUCTION

Tuberculosis (TB) is an ancient disease, which remains the leading cause of death from a single infectious organism. In 2019, globally an estimated 10 million people were detected with TB and about 1.4 million TB-related deaths occurred (1). Most of the world's tuberculosis cases occur in low-income and middle-income countries, where sputum microscopy is the primary method for diagnosing tuberculosis. Microscopy is a rapid, inexpensive, relatively simple, and highly specific technique in the TB prevalence area (2,3). In low resource and high burden settings, the preparation of direct smear and disposal of clinical samples is hazardous and increases the risk of laboratory-acquired infections (4,5). Adaption of good laboratory practices along with safe and efficient smear microscopy techniques are indispensable for TB diagnosis.

Phenol ammonium sulfate (PhAS) is a potent chemical solution, which can kill mycobacteria efficiently within half an hour (6,7). Phenol is a powerful disinfectant, that denatures proteins without altering the AFB counts and effectively fixes smears for staining (6-8). Ammonium sulfate is an inorganic salt, which precipitates proteins and mucus components of sputum by decreasing protein solubility, termed as salting-out effect (9,10). In this study, we aimed to evaluate the efficacy of two different pot methods: PhAS auramine O and PhAS basic fuchsin in comparison to the conventional Auramine O method.

MATERIALS AND METHODS

Study settings

This prospective cross-sectional study was conducted at the Department of Microbiology for four months from June to September 2020. The study was approved by the Institutional Ethical Committee (IEC:155/2020). Patients, suspected of pulmonary tuberculosis (PTB) were included in this study. A volume of 3-5 ml mucopurulent sputum samples was collected into sterile containers and transported to the microbiology laboratory.

Conventional auramine O staining

A direct smear was prepared on a pre-labelled glass slide for each sample. Slides were stained by the conventional auramine O method as described in the
Revised National Tuberculosis Control Programme (RNTCP) manual and graded accordingly (11).

Pot methods

Phenol ammonium sulfate auramine O method (PhAS auramine O)

Reagent preparation: 0.3% auramine O, 4% ammonium sulfate, 5% methanol, 10% phenol.

1.5 g auramine O and 20 g ammonium sulfate were dissolved in 25 ml methanol. Fifty milliliters of molten phenol and distilled water were added to make up to 500ml. The solution was filtered (Whatman No. 4 filter paper) and stored in a brown bottle for a maximum of one week.

Staining process: An equal amount of phenol ammonium sulfate auramine O solution was added to the container containing sputum and gently vortexed for a few seconds. It was left at room temperature (22° to 26°C) for an hour. Smear was prepared from this PhAS auramine O solution treated sputum, which was air-dried and heat-fixed. Smears were decolorized using 1% acid-alcohol for 2 minutes and counterstained with 0.1% potassium permanganate (KMnO₄) for 30 seconds same as the routine auramine O staining method.

Phenol ammonium sulfate basic fuchsin (PhAS basic fuchsin) method

Reagent preparation: Two percent basic fuchsin, 4% ammonium sulfate, 5% methanol, and 10% phenol. The method of preparation was similar to the method described above, except that 10 g basic fuchsin was added instead of auramine O. After preparing the direct smear the remaining sputum sample was divided equally into two containers.

Staining process: The first step of this method was similar to the PhAS auramine O method described above, except that phenol ammonium sulfate basic fuchsin was added instead of the PhAS auramine O solution. In this method, 25% sulphuric acid was used as the decolorizer (2-3 min) and counter-stained with 0.1% methylene blue (30 sec) following the routine ZN staining method. To remove the observer’s bias, all the smears were evaluated and graded by two independent observers.

Table 1: Comparison of smears stained by pot methods with conventional auramine O staining

<table>
<thead>
<tr>
<th>Variables</th>
<th>PhAS auramine O method</th>
<th>PhAS basic fuchsin method</th>
</tr>
</thead>
<tbody>
<tr>
<td>True positive</td>
<td>8</td>
<td>8</td>
</tr>
<tr>
<td>False positive</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>True negative</td>
<td>67</td>
<td>67</td>
</tr>
<tr>
<td>False-negative</td>
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<tr>
<td>Sensitivity (%) (95% CI)</td>
<td>100 (63-100)</td>
<td>100 (63-100)</td>
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<tr>
<td>Specificity (%) (95% CI)</td>
<td>100 (94.6-100)</td>
<td>100 (94.6-100)</td>
</tr>
<tr>
<td>Positive predictive value (%)</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>Negative predictive value (%)</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>Accuracy (%) (95% CI)</td>
<td>100 (95.2-100)</td>
<td>100 (95.2-100)</td>
</tr>
</tbody>
</table>

Table 2: Comparison of AFB sputum smear grading by conventional auramine O method and pot methods (PhAS auramine O and PhAS basic fuchsin)

<table>
<thead>
<tr>
<th>Smear grading</th>
<th>Scanty</th>
<th>1+</th>
<th>2+</th>
<th>3+</th>
<th>Negative</th>
<th>Total</th>
</tr>
</thead>
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<tr>
<td>PhAS auramine O method</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Scanty</td>
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<td>0</td>
<td>0</td>
<td>0</td>
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<tr>
<td>1+</td>
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<tr>
<td>2+</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>2</td>
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<tr>
<td>3+</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>4</td>
<td>0</td>
<td>4</td>
</tr>
<tr>
<td>Negative</td>
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<td>0</td>
<td>0</td>
<td>0</td>
<td>67</td>
<td>67</td>
</tr>
<tr>
<td>Total</td>
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<td>1</td>
<td>1</td>
<td>6</td>
<td>67</td>
<td>75</td>
</tr>
<tr>
<td>PhAS basic fuchsin method</td>
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<td></td>
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<tr>
<td>Scanty</td>
<td>0</td>
<td>1</td>
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<td>0</td>
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<td>1+</td>
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<td>0</td>
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<tr>
<td>2+</td>
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<tr>
<td>Negative</td>
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<td>0</td>
<td>0</td>
<td>67</td>
<td>67</td>
</tr>
<tr>
<td>Total</td>
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<td>1</td>
<td>1</td>
<td>6</td>
<td>67</td>
<td>75</td>
</tr>
</tbody>
</table>
Statistical analysis

Microsoft Office Excel (Microsoft, Redmond, WA, USA) was used to maintain and analyze all the data. The sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV) of the smear microscopy results by pot methods were calculated in comparison with direct auramine O smear.

RESULTS

In this study, a total of 75 sputum samples were included to observe the presence of AFB using three different staining methods. A total of 8 (10.7%) samples were positive for AFB and 67 (89.3%) samples were negative, according to the routine auramine O method and pot methods. No observable difference was found between the three different methods, further details are shown in Table 1. In terms of sputum smear grading, the direct smear using auramine O showed better results compared to pot methods, as it detected 6 as 3+, 1 as 2+ and 1 as 1+. However, using both the pot methods we observed at least one smear which was scanty. Similarly, for the 3+ grading, a smaller number of samples were detected using the pot methods in comparison to the conventional auramine O method (Table 2).

DISCUSSION

In this present study, a similar proportion of smear positivity for AFB was noted for the PhAS basic fuchsin and PhAS auramine O methods in comparison to routine auramine O. The sensitivity and specificity of the pot methods were found to be 100% in comparison to conventional auramine O staining. Previous studies from India had reported similar findings, with 96.8% - 100% sensitivity and 91.7% - 99.6% specificity (12-14). In this present study, the smear grading with 3+ AFB bacilli was comparatively less in pot methods than the conventional auramine O method. There were discrepancies between previous studies that used pot methods. Two studies from India reported an increase in 3+ grading smears using pot methods, whereas another study reported a decrease in 3+ grading smears (12,14,15). These variations could be due to the dilution of sputum samples by adding an equal volume of PhAS-stain solution, and also the quality of sputum used while preparing the smear. The quality assessment of stained smear showed a satisfying colour of AFB using PhAS basic fuchsin and conventional auramine O method. Although the colour retention of AFB using PhAS auramine O was poor compared to the other two methods. The phenol ammonium sulfate complex helps to break down the highly mucoid consistency of sputum samples to obtain a more uniform smear. But in the case of fluorescent stains like auramine O, the unstable uptake of stain may lead to a lower grading of smear for AFB. To overcome this problem further studies are required to make the PhAS auramine O method more efficient and acceptable for routine use in laboratories.

Advantages of pot staining methods are smear preparations become much safer and non-hazardous. The staining process is initiated in the container itself and it is safe to dispose of the sputum containers without any further biomedical waste management procedures to follow. A disadvantage of the pot method is that the samples subjected to staining cannot be used for culture (13).

The limitation of the present study was the sample size which in turn has been reflected in the low positive samples being tested in the study, hence more elaborate studies with adequate testing of sputum specimens of suspected Pulmonary Tuberculosis are needed to confirm the usefulness of these staining procedures.

CONCLUSION

The pot methods like phenol ammonium sulfate basic fuchsin and phenol ammonium sulfate auramine O are efficient to detect AFB and are comparable to the conventional method of staining used in national tuberculosis elimination programs. Extensive studies in different health care settings should be conducted to understand the feasibility and utility of these methods in the screening of acid-fast bacilli which remains a key tool in the presumptive diagnosis of tuberculosis.

ACKNOWLEDGMENTS

The authors are grateful to MAHE, Manipal for providing support from Student Research Fund and assistance from Mrs. Jyothsna, the Technical staff, Microbiology department for this study.

CONFLICT OF INTEREST

The authors declare there is no conflict of interest.

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DOI: https://doi.org/10.51248/v42i4.1472
Druti et al: Evaluation of phenol ammonium sulfate basic …… suspected of pulmonary tuberculosis


DOI: https://doi.org/10.51248/v42i4.1472

Biomedicine- Vol. 42 No. 4: 2022