EVALUATION OF TRISODIUM PHOSPHATE AS A TRANSPORT MEDIUM AND ITS UTILITY IN A SINGLE-STEP DECONTAMINATION TECHNIQUE FOR THE CULTURE OF *M. TUBERCULOSIS*

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**Summary**

**Background**

Sputum specimens were received from various out-patient departments of a tertiary care referral hospital to acid-fast staining and mycobacterial culture.

**Material & Methods**

A simple, one-step decontamination and concentration method was adopted before subjecting the samples to acid-fast staining and mycobacterial culture. Trisodium phosphate, a cheap, "soft" decontaminant-cum-liquefying agent was used to treat the sputum specimens before Ziehl-Neelsen's (ZN) acid-fast staining and Lowenstein-Jensen’s (LJ) medium culture. The sputum samples were collected directly into trisodium phosphate containing screw capped McCartney bottles (Day-0). The bottles were vortexed and left overnight at room temperature. On the subsequent morning (Day-1), the supernatants were discarded and smears made from deposits were stained by ZN stain.

**Results**

A total of 30 consecutive samples, which showed smear positivity by ZN technique, were selected for the present study. Deposits from these smear positive cases were cultured onto duplicate slants of LJ medium and incubated at 37°C (Day-1). Duplicate slants of LJ media were inoculated from each of these preserved deposits on 2nd, 3rd, 4th, 6th and 8th days. Culture bottles were incubated at 37°C for 8 weeks and positive growths were recorded. Culture retrieval was possible from 29 (96.7%) samples from deposits of Day –1 to Day-3. The culture positivity, however, had dropped to 26 (86.7%), 18 (60%) and 6 (20%) from deposits of Day-4, Day-6 and Day-8, respectively. All the isolates were identified as *M. tuberculosis* and there was minimal contamination (0.83%). The culture retrieval dropped significantly only after Day-3.

**Conclusion**

This method is, therefore, suitable for transportation, preservation and decontamination of sputum samples before staining and culture, up to 3 days after collection. This will be helpful especially for collection of sputum samples from distant places and their transportation to nearest mycobacteriology laboratory as also for sputum samples arriving late in a working day’s schedule. [Indian J Tuberc 2004; 51:137-140]

**Key Words:** Trisodium Phosphate, *M. Tuberculosis*, Transport Medium, Decontamination Technique

**INTRODUCTION**

Although there has been significant progress in combating the global tuberculosis epidemic over the last decade, advances in some parts of the world have been offset by increase in tuberculosis due to human immunodeficiency virus (HIV) epidemic, and by the emergence and increase in spread of multi-drug resistant strains of *M. tuberculosis*. Bacteriological examination of sputum is, as a rule, the only way by which the diagnosis of pulmonary TB can be confirmed. It is well known that concentration of sputum yields a higher proportion of positive results and that culture is a more sensitive method than smear examination. However, complicated and laborious nature of conventional concentration and decontamination techniques keep them beyond the reach of many laboratories, particularly the smaller ones.

Decontamination and concentration of sputum specimen by modified Petroff’s concentration method is one of the most commonly used methods in our country for *M. tuberculosis* culture. The method is an age-old one with proven efficacy. However, it is fraught with a number of disadvantages - (a) it is a lengthy procedure involving many steps making it time consuming; (b) NaOH

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used in the method is a “hard” reagent, therefore unavoidable prolonged exposure of specimen to NaOH while processing a large number of specimens or an electricity failure during the procedure will certainly decrease the number of viable bacilli available for culture; (c) need for elaborate infrastructural laboratory facilities and skilled technicians is mandatory for this procedure; (d) contamination is not completely excluded (varies from 3-5%); (e) repeated opening of the screw cap of the specimen container for washing off excess NaOH after each bout of centrifugation increases chances of formation of aerosols containing mycobacteria; (f) direct handling of sputum specimens which are often purulent, foul smelling and blood tinged is disagreeable to most of the laboratory staff. Because of these factors, attempts to adopt a simple decontamination/concentration method for sputum was made with an objective to overcome the practical problems generally experienced during smear and culture procedures for *M. tuberculosis*.

**MATERIAL AND METHODS**

A total of 30 consecutive sputum samples which showed smear positivity by ZN technique were selected for the present study. Sputum specimens were received from various out-patient departments of a tertiary care referral hospital for acid-fast staining and mycobacterial culture. A simple, one-step decontamination and concentration method using trisodium phosphate, a cheap “soft” agent was adopted before subjecting the samples to acid-fast staining and mycobacterial culture.

**Preparation of trisodium phosphate liquid transport medium:** The transport medium used was similar to the one described by Vasanthakumari but was enriched with 5% mineral salt solution. This consisted of trisodium phosphate (Na₃PO₄.12H₂O) 200 g; ammonium sulphate 5 g; magnesium sulphate 500 mg; ferric ammonium citrate 250 mg; mineral salt solution 25 ml and distilled water 975 ml. Penicillin powder was not added to the medium. Mineral salt solution used was exactly the same as used in preparation of LJ medium. The chemicals were dissolved in the water by heating; the solution was filtered and dispensed into sterile screw-cap McCartney bottles in aliquots of about 7-8 ml and thereafter bottles were autoclaved at 121°C for 15 minutes. The bottles were then stored at room temperature. Each bottle thus prepared, constituted a single unit of transport medium.

The bottles with medium were supplied to the patients who were instructed to expectorate early morning sputum into them and to close them immediately with the help of the screw cap.

The bottles received in the laboratory were vortexed and left overnight at room temperature (Day 0). On the subsequent morning (Day-1), the supernatants were discarded and from the deposit smears were prepared on-to glass slides for ZN stain. Deposits from smear positive cases were cultured onto duplicate slants of LJ medium and incubated at 37°C (Day-1). The deposits of these 30 consecutive samples were then preserved at room temperature. Duplicate slants of LJ media were inoculated from each of these preserved deposits on 2nd, 3rd, 4th, 6th and 8th days. Culture bottles were incubated at 37°C for 8 weeks and positive growths were recorded thereafter. All the isolates were identified and culture bottles were checked for contamination.

**RESULTS**

Culture retrieval was possible from 29 (96.7%) of the 30 smear positive samples from Day-1 to Day-3. The culture positivity, however, had dropped to 26 (86.7%), 18 (60%) and 6 (20%) from samples of Day-4, Day-6 and Day-8 respectively (Table). All the isolates were identified as *M. tuberculosis* and there was contamination detected in only 3 out of total 360 bottles inoculated (0.83%).

**DISCUSSION**

Identifying acid-fast bacilli from specimens by staining methods is in practice the only sure means of establishing a diagnosis of TB, and culturing bacilli the only means of confirming the diagnosis and testing for drug resistance. Due to the laborious and
complicated nature of the conventional bacteriological methods used in tuberculosis bacteriology, only a handful of specialized laboratories have such facilities. Therefore, technically simpler methods practicable in smaller and less equipped laboratories have been tried in the past. Use of trisodium phosphate as a “soft” decontaminating agent has been well documented\(^4\). This has been improvised by Corper and Stoner\(^7\) and subsequently improved by Vasanthakumari\(^4\). The enriched solution used in this study was just the same as used by Vasanthakumari to which 5% mineral salt solution was added\(^6\). Use of mineral salt solution causes an increase in the size of the colonies resulting in a more luxuriant growth at the end of 4 weeks incubation; it also facilitates and enhances the viability of the mycobacteria during prolonged incubation with the medium. These have been confirmed by the author in an earlier publication\(^6\). As neutralization and at least 3 repeated washings / centrifugation in Petroff’s method is not required here, the method therefore does not demand high technical skill; this makes the procedure practicable for laboratory technicians with limited training. It also makes it possible for a single technician to process a large number of specimens within a short period of time. The reagents are not very expensive and are readily available even in relatively smaller places.

As the decontaminating agent, trisodium phosphate is a “soft” reagent, inadvertent delays due to sudden electricity failure during processing of large number of specimens, do not affect the viability of the mycobacteria. This is the precise reason the method can be practised in relatively less equipped smaller laboratories without standby generator facility.

In this procedure there is no handling of untreated specimens involved because the specimens are directly collected into the transport medium, which makes it less disagreeable to the staff. As the specimens are subjected to culture the day after receipt, samples of sputum received in the last moment of a working day can easily be accepted without any problems. This is useful for patients coming from distant places and reaching late. As repeated opening of the bottles during washings / centrifugations of specimens as done in Petroff’s method is avoided, it is relatively safe due to lesser chance for aerosol formation. As the method uses relatively inexpensive chemicals, does not need the use of a centrifuge and yields excellent results, it ultimately works out to be cheaper and can be practised in smaller laboratories. This increases the rate of bacteriological confirmation of cases and also provides better bacteriological monitoring of response to therapy.

In the present study, on diligent history taking and perusal of the medical documents, it was found that the only one patient with smear positive sputum, who failed to yield culture positivity, was on anti-tubercular treatment started 11 days prior to sample collection. Thus, it is clear that this simple single step decontamination technique does indeed

### Table: Culture retrieval depending on day of inoculation

<table>
<thead>
<tr>
<th>Day of culture from transport medium</th>
<th>Total number of specimens</th>
<th>Number isolated (culture positive)</th>
<th>% isolated (culture positive)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Day-1</td>
<td>30</td>
<td>29</td>
<td>96.7</td>
</tr>
<tr>
<td>Day-2</td>
<td>30</td>
<td>29</td>
<td>96.7</td>
</tr>
<tr>
<td>Day-3</td>
<td>30</td>
<td>29</td>
<td>96.7</td>
</tr>
<tr>
<td>Day-4</td>
<td>30</td>
<td>26</td>
<td>86.7</td>
</tr>
<tr>
<td>Day-6</td>
<td>30</td>
<td>18</td>
<td>60.0</td>
</tr>
<tr>
<td>Day-8</td>
<td>30</td>
<td>6</td>
<td>20.0</td>
</tr>
</tbody>
</table>

Note: There is significant drop in culture positive results after the third day (\(\chi^2\) for linear trend = 59.38; p<0.001).
have a significantly high positive yield.

Excellent culture retrieval up to three days of collection of sputum samples in trisodium phosphate transport medium and minimal culture contamination were the highlights of this study. The technique will be very useful for transporting sputum samples from distant places to nearest mycobacteriology laboratory when it is not possible on the part of the patient to come there physically. Furthermore, samples can be collected into the transport medium during any time of the day, hence patients reaching the laboratory late can also be accommodated. Being a “soft” reagent, mycobacteria can thrive in the medium for at least three days without affecting their viability, hence samples can be collected during holidays in the transport medium and processed on the next working day.

Chauhan et al5 have also used trisodium phosphate medium and have demonstrated in 300 smear positive cases and 299 smear negative cases that mycobacteria could be recovered after 4th and 8th day with 93.4% and 83.6% positivity in smear positive cases and 11% and 1.9% positivity in smear negative cases in contrast to modified Petroff’s method where the recovery in the two groups were 78.7% and 74% and 7.4% and 5% respectively.

Cetyl pyridinium chloride is also one of the recommended methods for decontamination of sputum but detailed evaluation at one of the leading Tuberculosis research centres in India has shown it to be less sensitive than results obtained using trisodium phosphate medium4.

Extreme simplicity of this one-step technique of decontamination, utilizing a “soft” decontaminant, trisodium phosphate, and high rates of isolation of M. tuberculosis make this procedure of transport and decontamination a useful alternative to modified Petroff’s method in resource-crunch laboratories of developing countries.

REFERENCES

Indian Journal of Tuberculosis