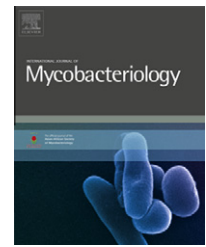


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## Short Communication

## Retrieval of *Mycobacterium tuberculosis* cultures suspended in phosphate buffered saline

V.N. Azger Dusthacker<sup>a</sup>, P. Nagarajan<sup>a</sup>, Dasarathi Das<sup>b</sup>,  
Vanaja Kumar<sup>a</sup>, N. Selvakumar<sup>a,\*</sup>

<sup>a</sup> National Institute for Research in Tuberculosis (ICMR), Chennai, India

<sup>b</sup> Regional Medical Research Centre (ICMR), Bhubaneswar, India

## ARTICLE INFO

## Article history:

Received 26 July 2012

Accepted 2 August 2012

Available online 1 September 2012

## Keywords:

*M. tuberculosis*

Phosphate buffer

Storage

Transportation

MDR-TB

## ABSTRACT

One hundred and twenty-seven of 130 isolates of *Mycobacterium tuberculosis*, suspended in phosphate buffered saline (PBS) and stored at ambient conditions in the laboratory for 14 days, and another 55 of 60 cultures, suspended as above, transported from reference laboratories within 7 days, were successfully retrieved on LJ medium. Considering the maximum retrieval of *M. tuberculosis*, use of PBS can be explored further for transportation of *M. tuberculosis* cultures across laboratories.

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### Introduction

The accuracy of the anti-tuberculosis drug susceptibility testing (DST) methods in different mycobacteriology laboratories across the world is being monitored continuously by Supranational Reference Laboratories (SRL) and National Reference Laboratories (NRL). This requires transportation of *Mycobacterium tuberculosis* cultures from SRL/NRL to Regional/Intermediate Reference Laboratories (IRL) [1]. Transportation of cultures of *M. tuberculosis* to participating laboratories is carried out by suspending the cultures in Middlebrook 7H9 liquid medium supplemented with albumin dextrose catalase

enrichment (7H9) [1]. Preparation and use of such enriched media is appropriate but requires expensive fine chemicals, technical expertise, and it may also suffer a drawback of accidental contamination with environmental microorganisms. So, a suspending medium which is inexpensive, simple to prepare and less prone to contaminating organisms is desired for quick transportation of *M. tuberculosis* across laboratories. Since the loss of viability of *M. tuberculosis* in sputum samples stored at refrigerated temperature and cetylpyridinium chloride/cetylpyridinium bromide solution for up to 2 weeks at ambient conditions was reported to be minimal [2–4], the retrieval of *M. tuberculosis* suspended in phosphate

\* Corresponding author. Address: Division of Mycobacteriology, National Institute for Research in Tuberculosis (ICMR), Chetput, Chennai 600031, Tamil Nadu, India. Fax: +91 44 2836 2528.

E-mail addresses: [selvakumar.nagamiah@gmail.com](mailto:selvakumar.nagamiah@gmail.com), [selvakumarn@hotmail.com](mailto:selvakumarn@hotmail.com) (N. Selvakumar).  
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<http://dx.doi.org/10.1016/j.ijmyco.2012.08.002>

buffered saline (PBS) and stored at ambient conditions is studied and reported.

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## Materials and methods

One hundred and fifty clinical isolates of *M. tuberculosis* were used. As these strains were selected from the National Institute for Research in Tuberculosis's culture repository, the approval of the Institute's ethics committee was not sought.

All chemicals were purchased from Sigma (St. Louis, USA) unless otherwise mentioned. Sterile PBS was used as suspending media. PBS, prepared by mixing 61.1 ml of solution I and 38.9 ml of solution II and adding 0.85 g of sodium chloride, was autoclaved at 121 °C for 15 min. Solution I: 0.067 M anhydrous salt of disodium hydrogen phosphate ( $\text{Na}_2\text{HPO}_4$ ) – 9.470 g in 1000 ml sterile distilled water. Solution II: 0.067 M potassium dihydrogen phosphate ( $\text{KH}_2\text{PO}_4$ ) – 9.07 g in 1000 ml sterile distilled water. PBS had a pH of 7.2.

### Retrieval of clinical isolates suspended in PBS for 7 days

In a preliminary experiment, retrieval of 20 *M. tuberculosis* isolates, suspended in PBS, was determined. Of the 20 isolates, 18 were susceptible to isoniazid and rifampicin, and the susceptibility patterns for the other two were not available. From each of the cultures, a suspension of approximately 1 mg moist weight per milliliter of distilled water (McFarland No. 1) was prepared; 100  $\mu\text{l}$  of each suspension was aseptically transferred to 400  $\mu\text{l}$  of PBS in a 2 ml Cryovial. Ten Cryovials with only sterile distilled water formed the controls. The test cultures and controls were coded and stored in ambient conditions (room temperature in a cupboard). From each of the suspensions, 10  $\mu\text{l}$  was inoculated immediately and after 7 days onto two LJ slopes. The growth was recorded after 4 weeks' incubation at 37 °C.

### Retrieval of clinical isolates suspended in PBS for 14 days

In another experiment, retrieval of 130 clinical isolates of *M. tuberculosis* which included 62 multidrug-resistant (resistant to isoniazid and rifampicin-MDR) strains, suspended in PBS and stored in ambient conditions as before for 14 days, was studied.

### Transportation of 60 clinical isolates in PBS from intermediate reference laboratories

To know the retrieval in a real situation, 30 each of fresh (3–4 weeks old on LJ medium) *M. tuberculosis* culture suspensions in PBS, which included 11 MDR strains, from two Regional Medical Research Centres (ICMR)-Bhubaneswar and Dibrugarh, situated respectively 1230 and 2100 km from Chennai, were transported to NIRT within a week, and subcultured on LJ slopes as before.

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## Results

All the cultures, suspended in PBS and stored at ambient conditions for 7 days were retrieved and their culture grades

were comparable to their initial growth. One hundred and twenty-seven of 130 cultures (including 62 MDR strains) suspended in PBS and stored at ambient conditions for 14 days were retrievable with 3+(confluent growth)/2+(2+ = innumerable number of colonies) growth. Fifty-five cultures of the 60 cultures received from intermediate reference laboratories were retrieved with 3+/2+ growth. Three of the remaining five culture suspensions, which yielded contaminants initially, were retrieved after treatment with ceftrimide [5]. The other two which failed to grow were susceptible to isoniazid and rifampicin. The minimum and maximum temperature during the study period was 27.6 and 37.5 °C respectively.

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## Discussion

Middlebrook 7H9 is commonly utilized for transportation of *M. tuberculosis* cultures [1]. Its preparation in the laboratories is tedious and requires clean room facilities, expensive chemicals and expertise of technicians. In addition, it is an enriched medium, and the chances of contamination during its manipulations in the laboratory or transportation is high. A suspending medium with no growth supplements will have advantages over conventional fastidious Middlebrook 7H9 medium for transportation of *M. tuberculosis* cultures.

Generally, the transportation of cultures across laboratories is carried out in ambient conditions or using cool packs. It is the temperature of storage and not the suspending medium that affects the viability of bacilli [6]. It is generally known (data not shown) that MDR strains are lost in delayed transportation, especially with rising ambient temperature above 37 °C. Since the temperature in the present study was well below 37.5 °C, except for a few hours in the daytime, its deleterious effect on the viability of tubercle bacilli, especially on MDR strains, could have been averted resulting in its better survival and retrieval. In addition, the buffering capacity of PBS and the inability of the bacilli to replicate and accumulate toxic metabolites in it during storage could have attributed to prolonged survival and retrieval of tubercle bacilli.

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## Conclusion

Quick transportation of *M. tuberculosis* in PBS across laboratories within and outside the country for bacteriological assessments can be explored further.

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## Conflict of interest

We wish to confirm that there are no known conflicts of interest associated with this publication and there has been no significant financial support for this work that could have influenced its outcome.

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## Acknowledgements

We gratefully acknowledge the laboratory staff of Department of Bacteriology and Miss Shameem, Postgraduate student, Periyar University, Salem, for their excellent technical

support. The financial support from USAID through WHO SEARO New Delhi is gratefully acknowledged.

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