International Journal of Research
Available at https://edupediapublications.org/journals
p-ISSN: 2348-6848
e-ISSN: 2348-795X
Volume 04 Issue 03
March 2017

# Efficacy of a Biphasic Culture Medium for recovery of mycobacteria in smear negative sputum samples

Dr. Bhawna Sharma[1], Dr. Payal Dutta[2] & Dr. Varsha A Singh[3]
[1] MD Microbiology Senior Demonstrator Department of Microbiology, Kalpana Chawla Government Medical College, Karnal, Haryana
[2] MD Microbiology Senior Demonstrator Department of Microbiology ASCOMS, Jammu, J & K, 180001
[3] MD Microbiology Professor and Head of Department, M.M.I.M.S.R. Mullana, Ambala Haryana 133007

## Abstract:

*Introduction: Tuberculosis (TB) remains a major global health problem worldwide and rank as the second leading cause of death from an infectious disease, after the HIV.*

*The aim of the study: To evaluate the efficiency of a Biphasic culture media for the isolation of Mycobacterium tuberculosis (MTB) in smear negative sputum samples in clinically suspected cases of pulmonary tuberculosis.*

*Material and methods: A total hundred (n=100) LED smear negative sputum samples were collected. Samples were subjected to concentration and decontamination by two methods (N-Acetyl-L-Cysteine (NALC) oxalic acid and Cetyl Pyridium Chloride (CPC) and sodium chloride) and then inoculated on LJ and biphasic media for isolation of MTB.*

*Results: Among hundred LED smear negative sputum samples, 15% (n=15) mycobacterial isolates were obtained on culture. Of these 15 cases, 66.66% (n=10) were positive on LJ media, whereas 93.33% (n=14) were positive for MTB on Biphasic Media. Growth on Biphasic media was exhibited within 2 weeks as compared to LJ media (4 weeks). The rate of Contamination was 5% and 7% on LJ and biphasic media respectively.*

*Conclusion: Biphasic medium was superior to the conventional LJ medium in being rapid, easy to use and interpret, and significantly low time-to-growth detection, but was expensive with higher contamination rate.*

*Key words: Pulmonary tuberculosis, LJ media, Biphasic media*

## Introduction:

The time-tested and Gold Standard method used for years to diagnose pulmonary tuberculosis is the traditional culture of *M. tuberculosis*. Internationally accepted solid media, Lowenstein-Jensen medium has the advantage that it can detect bacterial load as low as 10 bacilli/mL, can be prepared locally, has good buffer capacity, and has a shelf-life of several months when refrigerated, it supports the growth of most mycobacterial species, and contains malachite green used to inhibit the growth of most contaminants.[1]

Liquid media on the other hand, increase the case yield by 10% over solid media, and automated liquid culture systems can significantly reduce the diagnostic delay from weeks to days. However, automated liquid culture systems are expensive and

difficult to operate[2]. Also the rate of contamination is known to be higher in liquid media as compared to solid media.

In the current study a biphasic medium was used which consisted LJ slant medium, in addition with liquid culture medium, Middlebrook 7H9 liquid culture, and a growth indicator. Biphasic media is superior to the conventional LJ Media as its rapid and easy to use and interpret Advantages and limitations of both solid and liquid media were included.

Sputum sample contains normal flora, which may overgrow on culture, and make the detection of mycobacteria difficult, this accentuates the importance of a good decontamination technique before culture, chemical agents employed for this purpose should be able to effectively destroy non-tubercular organisms in sputum and release the intracellular tubercle bacilli from the epithelial cells. The study comprises N-Acetyl-L-Cysteine (NALC) oxalic acid and Cetyl Pyridium Chloride (CPC) and sodium chloride as decontaminants.

## Material and Methods:

A total hundred LED smear negative sputum samples were collected from clinically suspected cases of pulmonary tuberculosis, attending the out patients or in patients departments. Samples were subjected to concentration and decontamination by two methods (NALC oxalic acid and CPC-NaCl) and then inoculated on LJ and biphasic media for isolation of *MTB*. LJ medium was checked twice weekly for first two weeks and then every week for a maximum of eight weeks.Biphasic medium was observed every 2 or 3 days. Any red sediment observed was tested by acid fast staining with auramine O. Ethical clearance for the study was taken from the ethical committee.

## Results

**Table I: Comparison of culture positivity on LJ media and Biphasic media.**

| Total culture positive | LJ media | Biphasic media | P* value |
|---|---|---|---|
| 15 | 10 (66.66%) | 14 (93.33%) | 1.000 |

**Table II: Co relation of culture with LED microscopy and concentration and decontamination techniques**

| Culture media | Culture positive samples | LED microscopy | | | |
|---|---|---|---|---|---|
| | | After CPC NaCl | | After NALC Oxalic acid | |
| | | Positive | Negative | Positive | Negative |
| LJ Media | 10 | 6(60%) | 4(40%) | 4(40%) | 6(60%) |
| Biphasic Media | 14 | 6(46.25%) | 8(57.14%) | 4(30.76%) | 10(71.42%) |

**Table III: Week wise distribution of mycobacterial isolates on LJ and Biphasic media**

| | | 1st week | 2nd week | 3rd week | 4th week | 5th week | 6th week | 7th week | 8th week | Total |
|---|---|---|---|---|---|---|---|---|---|---|
| LJ media | After CPC NaCl | | | | 5 | 3 | 2 | | | 10 |
| | After NALC oxalic acid | | | | 2 | 2 | 1 | | | 5 |
| Biphasic media | After CPC NaCl | | 2 | 5 | 5 | 2 | | | | 14 |
| | After NALC oxalic acid | | 1 | 3 | 2 | | | | | 6 |

**Table IV: Frequency of nature of contamination on LJ and biphasic media**

| Nature of contamination | LJ MEDIUM | BIPHASIC MEDIUM |
|---|---|---|
| Gram Positive organisms | 3(60%) | 3(42.85%) |
| Gram Negative organisms | 1(20%) | 4(57.14%) |
| Fungus | 1(20%) | NIL |
| Total | 5 | 7 |

## Results:

In the present study a total hundred LED smear negative sputum samples were collected from clinically suspected cases of pulmonary tuberculosis, of these only 15% mycobacterial isolates were grown on culture. Out of these15 cases, 66.66% were on LJ media, whereas 93.33% on Biphasic Media. (**Table I**). of 10 isolates on LJ media, 60% were both smear and culture positive after CPC NaCl, whereas after NALC Oxalic acid 40%. Similarly, out of 14 isolates on biphasic media, both culture and smear positive were 46.25% after CPC NaCl, whereas 30.76% were after NALC Oxalic acid.**(Table II)** Biphasic media exhibited the growth within 2 weeks as compared to LJ media i.e. 4 weeks.(**Table III**)

The rate of Contamination observed on LJ and biphasic media was 5% and 7% respectively, furthermore, the nature of contaminating organism, on LJ media

predominantly was gram positive (60%),followed by gram negative (20%) & fungus (20%). On the contrary on biphasic medium gram negative (57.14%) organisms showed the predominance, followed by gram positive organisms (42.85%). (**Table IV**)

## Discussion:

Culture is the ultimate diagnostic tool for tuberculosis, in the present study only 15% mycobacterial isolates were obtained on culture media. This was in accordance with **Farzana rahman et al[3]** and **Ben J. Marais et al[4]** who observed 11 % and 19% culture positivity respectively. The reason for less isolation may be because the present study includes only the paucibacillary cases, i.e. LED smear negative.

LJ is the internationally accepted media. In the present study a new biphasic medium has been used. It combines the characteristics of liquid culture medium and LJ slant. Compared to LJ medium, the biphasic medium is provided with 10% OADC, which has glycerol and dextrose present in liquid medium imparting additional nutrition, providing more conducive environment for the growth of mycobacteria. **M.Ghatole et al[5]** in there study concluded that LJ and biphasic media, had isolation rates of 66.6% and 91.66% respectively, as compared to it in the present study 66.66% & 93.33% showed growth on the LJ & biphasic medium, correspondingly**(Table I) (p value= 1.000 which was not statistically significant) (Sensitivity 64.29%,Specificity 98.83%,Positive PV 90%,NPV 94.44)** while **Zhenling Cui et al**[6] in their study indicated that 33.46% & 66.53% were culture positive from 830 smear negative samples on the LJ media & biphasic media. The sensitivity, specificity, PPV and NPV of biphasic medium for the recovery of mycobacteria were 98.3%, 84.4%, 73.5%, and 99.1% respectively. Higher percentage (91.66%) of growth on biphasic media in the study done by **M.Ghatole et al[5]** is due to the fact that they used Middlebrook biphasic medium system.

Processing of sputum with subsequent concentration by centrifugation or sedimentation may increase the sensitivity of smear microscopy; hence some investigators have recommended sputum processing and concentration as a global standard. In the present study both smear and LJ culture positive isolates were 60% & 40% after CPC & NALC oxalic acid respectively. Similarly the isolates that showed growth on biphasic media and smear positivity, 46.25% and 30.76% following CPC & NALC oxalic acid. **Statistical analysis of NALC Oxalic acid: Sensitivity 100%, Specificity 94.44%, PPV 66.66%,NPV 100% on LJ media; Sensitivity 100%, Specificity 90.68%, PPV 63.63%, NPV 100% on biphasic media (Table II)** Similar results were obtained by studies done using different methods of decontamination**, B.V.Peerapur et al[7]** showed improved smear positivity after 3.5% NaCl, by 76% in culture positive isolates. **Adithya chattamachi et al[8]** showed 25% improvement in smear positivity after treatment with 2% NaCl, in culture positive samples.

The best method to study any microorganism is culture; isolated colonies are needed to identify and characterize the specific bacteria. Mycobacterium is a slow growing organism, with generation time of 15 hrs. Hence it takes weeks for colonies to appear. Various culture media are employed for the isolation of mycobacteria; both liquid and solid media can be used. Solid media requires more time but provides exact colony morphology for identification of the species. Whereas liquid media contributes to early growth but has higher rate of contamination. Hence the ideal media should result by combination of both, a media showing faster growth & less contamination i.e. a biphasic medium.

In present study biphasic medium provides early growth of isolates i.e. 2-4 weeks as compared to LJ medium within 5-6 weeks. **(Table III).** This is attuned with **Zhenling Cui et al[6]** in their study on smear-negative specimens, the mean time to detect mycobacteria on LJ media was 4 weeks (3-5

p-ISSN: 2348-6848
e-ISSN: 2348-795X
Volume 04 Issue 03
March 2017

weeks) and for biphasic media was 3 weeks, (2-4 weeks). In the study of **A. J. Van Griethuysen et al[9]** it was 3weeks on biphasic media and (4weeks) on LJ media. Similar results were obtained by **M.Ghatole et al[5]**in their research on smear negative cases, concluded that 4 weeks and 6 weeks were required by biphasic system and LJ medium respectively

In this study, a growth indicator TTC was added to the biphasic medium which helped to detect mycobacterial growth using the color change. The determination of the results is more objective than with other methods. Only a few bacilli are needed in the biphasic medium, bacterial colonies give visible pink granule sediments that are detectable with the naked eye. The biphasic medium cannot provide quantitative data as accurately as other culture media. However, it can provide a rough estimate in a much speedy manner.

Contamination is a major obstacle in the diagnosis of tuberculosis. The effectiveness of culture systems is greatly undermined by contamination with bacteria and fungi, contamination reduce the proportion of interpretable results, thereby limiting the diagnostic value of culture systems at additional cost to public health systems, which delays or ultimately prevents TB diagnosis.

Present study revealed that the contamination rate for LJ was lower i.e. 5%, as compared to 7% of the biphasic media for the same specimens. **Zhenling Cui et al[6]** observed almost comparable rate of contaminations on cultures i.e. 4.0% and 4.6% in LJ & biphasic medium respectively. Similarly a lower rate of contamination on LJ was observed by **Isenberg et a[10] & A. J. Van Griethuysen et al[9]** i.e. septi-chek biphasic medium 4.5% and LJ medium 2%. And 6.5% of LJ media and 7.6% of septi-Chek biphasic media. On the other hand **M Ghatole et al[5]** observed higher rate of contamination 4.63% on LJ medium and only 1.3% on biphasic system.

Normal flora of mouth contain various gram positive and negative organisms, there growth should be effectively inhibited on culture to avoid contamination. Higher rate of contamination with gram positive organisms was observed in LJ media, may be because it contains malachite green which inhibits gram negative organisms and hence gram positive contamination is common on it as in the present study it is observed that on LJ media rate of contamination by gram positive organisms (60%) was highest, as compared to gram negative organisms and fungi. Whereas biphasic media contains PANTA (polymixin, amphotericin B, nalidixic acid, trimethoprim, and azlocillin) which has limited anti-pseudomonas activity therefore gram negative contamination (57.14%) was most frequent as compared to gram positive and fungus. **(Table IV)**

## Conclusion:

For the preliminary diagnosis of tuberculosis, staining is still the key step, LED Microscopy after fluorescent staining yields better results than the conventional ZN staining and is recommended by WHO too in being >10% sensitive than the conventional staining, but it comes with a drawback of being evaluated by LED Microscope which is expensive and requires an expert eye.

Culture being the gold standard in the definite diagnosis of tuberculosis, biphasic media; was superior to the conventional LJ Media as it was rapid and easy to use and interpret, whereas LJ media had lesser contamination rate and lower cost, because the liquid media contains growth supplement OADC, and antibiotic mixture PANTA, which are expensive. Sensitivity Specificity, PPV, NPV of biphasic media for the recovery of mycobacteria was 64.29%, 98.83%, 90%, 94.44% respectively. Hence for rapid and effective diagnosis, both these medium should be employed.

## References:

I. World Health Organization. The use of liquid medium for culture and DST. World Health Organization, Geneva, Switzerland. 2007.Available from: http://www.who.int/tb/dots/laboratory/policy/en/index3.html

II. Rothlauf NV,Brown GL, Blair EB. Isolation of mycobacteria from undecontaminated specimens with selective 7H10 medium. J Clin Microbiol 1998;13:76-9.

III. Rahman F, Munshi SK, Kamal SM. Comparison of different microscopic methods with conventional TB culture. Staf J Microbiol 2011; 1(1): p 18.

IV. Marais BJ, Brittle W, Painczyk K, Hesseling AC, Beyers N, Wasserman E et al. Use of light-emitting diode fluorescence microscopy to detect acid-fast bacilli in sputum. Clin Infect Dis. 2008 15; 47(2): 203-7.

V. Ghatole M, Sable C, Kamale P, Kandle S, Jahagirdar V, Yemul V. Evaluation of biphasic culture system for mycobacterial isolation from the sputum of patients with pulmonary tuberculosis. Indian J Med Microbiol 2005; 23 (2): 111-3.

VI. Cui Z, Wang J, Zhu C, Huang X, Lu J, Wang Q et al. Evaluation of a Novel Biphasic Culture Medium for recovery of mycobacteria: a multi- center study. PLOS ONE 2012; 7(4): e36331.

VII. Naveen G, Peerapur BV. Comparison of the Lowenstein-Jensen Medium, the Middlebrook 7H10 Medium and MB/BacT for the Isolation of Mycobacterium Tuberculosis (MTB) from Clinical Specimens. J Clin Diagn Res 2012; 6(10): 1704-9.

VIII. Cattamanchi A, Dowdy DW, Davis JL, Worodria W, Yoo S, Joloba M et al. Sensitivity of direct versus concentrated sputum smear microscopy in HIV-infected patients suspected of having pulmonary tuberculosis. BMC Infect Dis. 2009 6; 9: 53.

IX. Van Griethuysen AJ, Jansz AR, Buiting AG. Comparison of fluorescent BACTEC 9000 MB system, Septi-Chek AFB system, and Lowenstein-Jensen medium for detection of mycobacteria. J Clin Microbiol 1996; 34(10): 2391-4.

X. Isenberg HD, D'Amito RF, Heifets L, Murray PR, Scardamaglia V, Jacob M et al. Collaborative feasibility study of a biphasic system (Roche Septi chek AFB) for rapid detection and isolation of mycobacteria. J Clin Microbiol 1991; 29: 1719-922.

XI.