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Evaluation of Rapid Slide Culture Technique for the Diagnosis of Pulmonary Tuberculosis

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Abstract
This study was conducted to evaluate the rapid slide culture technique for the diagnosis of pulmonary tuberculosis. A cross sectional study was carried out in the Department of Microbiology, Mymensingh Medical College and the Reference Laboratory of Damien Foundation at Anantapur in Netrakona, over a period of one year from July 2007 to June 2008. A total of 214 sputum sample were collected purposively from patients attending at the DOT'S corner of Mymensingh Medical College Hospital and DOT'S corner of Netrakona during the study period. Out of 214 samples, 50 (23.36%) & 42 (19.62%) showed growth on Ogawa culture and slide culture positive respectively. On the other hand Ziehl- Neelsen staining (Z-N) was positive in 46 (21.49%) of the samples. Slide culture technique showed 8 false negative and no false positive results but in Z-N staining method showed 9 false negative and 5 false positive results when compared with Ogawa culture. The sensitivity and specificity of slide culture were 84% and 100% respectively which was higher than that of microscopy by Z-N staining (82%) and 96.95% respectively.
Keywords: *Pulmonary tuberculosis; Slide culture; Ogawa culture; Ziehl-Neelsen staining.*

Introduction

Every year more than 9.2 million people develop active tuberculosis and about 1.7 million die and 2 billion harboring latent infection and the South East Asia Region carries 36% of the global TB cases[1].

In 2006, Bangladesh ranked as the sixth highest burden TB countries among 22 countries in the world and MDR-TB rate was 3.6% among new cases and 19% among re-treatment cases.[1] Currently, the only confirmatory criterion for definite diagnosis of TB is the demonstration of the presence of tubercle bacilli in clinical specimens. This is based on microscopy with Ziehl-Neelsen's acid fast stain and culture of *M .tuberculosis* on Lowenstein –Jensen (L-J)

medium.[2] Though time honored and economical, Z-N staining lacks sensitivity and requires a large number of bacilli between 5,000 to 10,000 per ml of sputum only 40 – 60% of truly active pulmonary tuberculosis cases being confirmed even with optimal staining and satisfactory microscopic examination.[3,4] Culture techniques are complex and time consuming, require 4 - 8 weeks to yield growth of *M. tuberculosis.* in Lowenstein –Jensen (L-J) medium though widely used in tuberculosis laboratories have certain difficulties because it requires a well- equipped laboratory and must be prepared the medium at frequent intervals for its short shelf life.[5] For these difficulties culture of *M . tuberculosis* is not done in any of the tertiary level of laboratories in Bangladesh. Consequently, there is a need for a rapid, sensitive and low-cost technique that will be suitable for routine use in the developing world. *M .tuberculosis* grows more rapidly on liquid Sula medium than on solid medium. This medium can be prepared in an ordinary laboratory and it has long shelf life.[6]

Based on these observations a new, efficient, reliable and less expensive method, known as Slide culture technique has been developed at Damien Foundation, Bangladesh by modification of liquid culture method. By this culture technique *M .tuberculosis* can be detected by observing micro colonies within 7 – 10 days.[7]

The present study was carried out to evaluate the slide culture technique in the context of Bangladesh by comparing with the standard culture method in Ogawa medium.

Methods

A total of 214 sputum sample were collected purposively from suspected TB patients attending at DOT'S corner of Mymensingh Medical College Hospital and DOT'S corner of Netrakona during the period from July 2007 to June 2008.

Laboratory procedure

Sputum collected in clean container and processed for microscopic examination and culture in Ogawa medium and Slide culture in liquid Sula medium.

Culture in Ogawa medium

The procedure was described by WHO in 1998.[8] Briefly, sputum was taken in a tube with equal volume of 4% sodium hydroxide and kept for 20 minutes at room temperature. A homogenous mixture of sputum was prepared by vortexing. Then 4 drops of homogenous sputum was inoculated into the medium, kept at inclined position overnight at room temperature and afterwards incubated at 37^0C for eight weeks. The media were checked after two days for contaminations. Then the medium were checked every week for growth.

Slide culture in Sula medium

The technique is used in the Institution reference laboratory, Damien Foundation, Netrakona in 2008.[7] .Briefly, three smears were prepared for every specimen on the non-frosted end of the half of a microscopic slide. Two of the prepared slides were used for plain Sula medium and one for Para nitrobenzoicacid (PNB). Slides were dried, but not fixed. With the help of sterile forceps, slides were placed in the respective sterile 28 ml universal bottle containing 7-8 ml of the appropriate medium. After 2-3 days of incubation all the bottles were checked for contamination. The procedure was repeated for the contamination of specimen, using sputum kept in the refrigerator. After ten days, bottles were opened and slides were removed with the help of forceps, dipped them briefly in a universal container with absolute alcohol for fixation and let them dried on a brown paper in the safety cabinet. After drying they were arranged in a staining dish filled with sufficient 1% carbolfuchsin to immerse the slides completely and kept for 30 minutes. Staining dish was removed from the safety cabinet and Ziehl – Neelsen staining was done as usual. The stained slides were dried in the air and examined under a bright field microscope. Reading and recording were done by observing for micro-colonies under microscope using 10X magnification.

Figure 1: Slide culture in sula medium

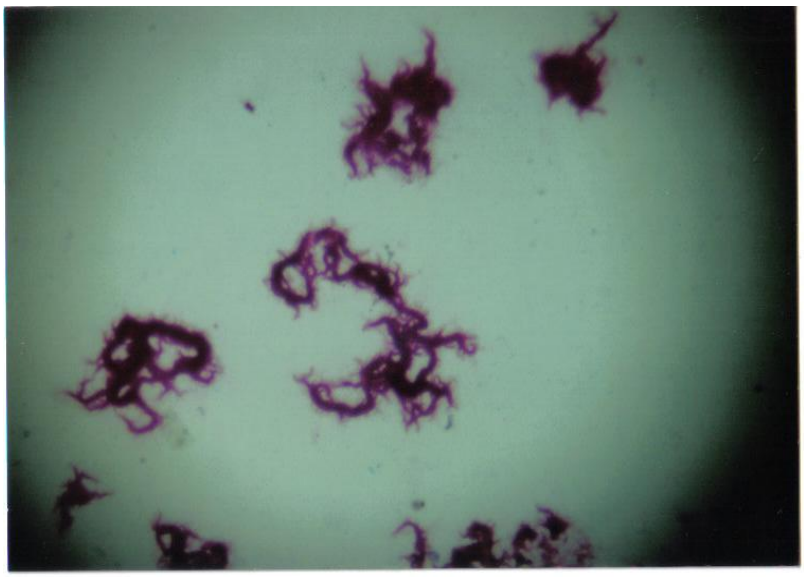

Figure 2: Surpentine cord of *M.tuberculosis*

Results

The mean duration of growth for *M. tuberculosis* was 8.5 days in Sula medium and 24 days in Ogawa medium (Table-1). This difference is statistically significant ('p' <0.001 by 't' test).

Table – 1 Comparison of growth period of *Mycobacterium tuberculosis* on Ogawa and Sula media

Method	Range of detection time in day	Mean detection time in day
Ogawa medium	18 – 30	24
Sula medium	7 – 10	8.5

Calculated value is 10.33 (by independent sample 't' test) which is much greater than tabulated value at 15 d.f. and 5% level i.e. 4.07.

The growth of *M. tuberculosis* in Sula media is significantly rapid than in Ogawa medium.

Table -2 Result of Sputum culture of *Mycobacterium tuberculosis* in Ogawa medium and Slide culture technique

N=214

Test	Positive	True positive	False positive	False negative	True negative	Sensitivity	Specificity	PPV	NPV
Standard culture in Ogawa medium	50	50	0	05	159	99.9%	100%	100%	97%
Slide culture in Sula medium	42	42	0	08	164	84%	100%	100%	95%
Z-N staining	46	46	05	09	159	83.6%	96.9%	90.2%	94.6%

Table -3 Performance of sputum culture in Ogawa medium and Slide culture technique

Description	Standard culture	Slide culture	Z-N staining
Sensitivity	99.9%	84%	83.6%
Specificity	100	100	96.9%
PPV	100	100	90.2%
NPV	97	95	94.6%

Out of 214 sputum samples, 50 (23.4%) showed growth in Ogawa medium and 42(19.6%) in Sula medium. There is no significant statistical difference between the two methods. ('p' is >0.05 by chi-square test). The sensitivity, specificity, PPV and NPV of Ogawa and Sula medium were 99.9%, 100%, 100%, 96.9% and 84%,100%, 100%, 95.3% respectively (Table-2)

Discussion

The data show that rapid slide culture technique was similar in sensitivity and specificity like that of standard culture in solid Ogawa medium but with an advantage of rapid growth within 10 days. The preparation of medium, easy storage and its long shelf life are other advantages for its use in developing countries like Bangladesh.

There are several methods for isolation of mycobacteria. Methods based on solid media are most commonly used in developing countries. Although less costly, these methods are labour intensive and time consuming. Sophisticated and automated broth based culture methods are rapid but too expensive to be used routinely in TB laboratories of developing countries. Slide culture technique is a liquid based rapid, less expensive, less time consuming and effective method for isolation of *M. Tuberculosis*.[9]

In a study by Sula L. from Czechoslovakia in 1968 showed that the culture positive in Sula medium were (30.55%) and in Ogawa medium were (33%)[7] (Sula L. 1968) in smear positive cases.

Conclusion

We can therefore, conclude that Rapid slide culture technique will be suitable for routine mycobacterial culture in developing countries like Bangladesh and direct drug susceptibility test in the same method can be an added advantage in the perspective of rising concern for MDR-TB.

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