

Original Research Article

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## Comparison of Rate of Isolation and Characterisation of Mycobacteria by AFB Smear, LJ Medium and MGIT

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### ABSTRACT

Bacteriological confirmation plays a key role in the diagnosis of tuberculosis. Many systems have been developed in the recent years apart from traditional microscopy and culture on Lowenstein Jensen (LJ) medium. The objective of this study is to compare the rate of detection of *Mycobacteria* by acid fast staining, LJ medium and Mycobacteria Growth Indicator Tube (MGIT). Hundred patients with clinical suspicion of pulmonary tuberculosis attending a tertiary care hospital over a period of one and a half years were enrolled in the study. Sputum samples were collected and acid fast smears were prepared. The specimens were decontaminated and cultured on LJ medium and MGIT. Growth obtained was recorded. Out of 100 suspected cases, acid fast bacilli were seen in 21 sputum samples by Ziehl-Neelsen (ZN) staining. In LJ medium culture 21 samples yielded growth and MGIT was positive in 23 samples. The sensitivity and specificity of MGIT in comparison with LJ medium culture were 100% and 97.5% respectively. MGIT culture showed faster rates of isolation compared to LJ medium with a mean detection time of 10.91 days. Among the tuberculosis positive cases, 18 (78.2%) were males and 5 (21.7%) were females, with peak incidence (43.47%) in age group of 31-45 years. Compared to conventional methods, MGIT showed a good sensitivity and specificity and faster rates of isolation. It can be used in the diagnosis of tuberculosis as a rapid and reliable technique.

#### Keywords

Tuberculosis,  
MGIT, Diagnosis

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### Introduction

Tuberculosis is a major health problem worldwide. It is one of the life threatening airborne infectious bacterial disease. The emergence of multidrug resistant and extremely drug resistant tuberculosis has worsened the situation (Negi *et al.*, 2005). India is the highest tuberculosis burden country and accounts for nearly one fifth of global burden of tuberculosis (Park, 2009).

The microscopic examination of sputum to detect presence of Acid Fast bacilli (AFB) has served the world well for past 100 years and is the diagnostic basis of directly observed therapy. Reliance solely upon the limited AFB smear is one of the major factors that contribute to poor tuberculosis control in resource poor countries (Espinal *et al.*, 2000).

The most commonly used conventional Lowenstein Jensen (LJ) medium requires 6-10

weeks of incubation due to slow growth rate of *Mycobacterium tuberculosis complex*. In recent years, the development of rapid and reliable methods for culture detection of acid-fast bacilli has been regarded as worthy of absolute priority. It should be such that the laboratories must be able to isolate *Mycobacteria* easily, quickly and accurately (Styrt *et al.*, 1997, Chitra and Prasad, 2001). Hence, there is need to evaluate newer methods for the early detection of *Mycobacterium tuberculosis complex*, for effective treatment and control.

Rapid diagnosis of Mycobacterial infections is of prime importance. Meanwhile, in the developing countries, where resources are limited, patients continue to undergo diagnosis with acid-fast smears and are subsequently treated with standard anti-tuberculosis regimens. In these countries, the clinical utility of conventional antibiotic susceptibility testing methods has been offset by their long turnaround times and the utilization of more rapid methods such as BACTEC and Mycobacteria Growth Indicator Tube (MGIT) has not proven to be cost effective (Heifets and Cangelosi, 1999). Presumptive diagnosis of tuberculosis can be made on the basis of patient's history, clinical and radiological findings. But definitive diagnosis requires microscopic detection of acid fast bacilli and isolation by culture methods.

The objective of this study is to compare rate of detection of Mycobacteria by acid fast staining, LJ medium and MGIT culture methods.

### **Materials and Methods**

This study was done at a tertiary care hospital over a period of one and a half years. 100 patients suspected of pulmonary tuberculosis based on clinical history, attending the hospital were considered.

### **Inclusion criteria**

Patients with symptoms suggestive of pulmonary tuberculosis such as cough with expectoration of > 2 weeks duration, fever with evening rise of temperature, night sweats, haemoptysis, weight loss, loss of appetite, chest pain were included in the study.

Patients above 16 years of age.

### **Exclusion criteria**

Patients currently on anti-tubercular treatment

Extrapulmonary tuberculosis

Patients in paediatric age group.

### **Sample collection**

Early morning expectorated sputum samples were collected from clinically suspected cases of pulmonary tuberculosis in separate sterile, wide mouthed containers. Quality of the sample was assessed by macroscopic and microscopic examination. Watery sputum samples were excluded. Only samples appearing purulent were taken for the study.

The samples were assessed according to the Bartlett's grading system (Konemen, 1997).

### **Sample processing**

Smears were prepared and were stained by Ziehl-Neelsen technique. The smear was graded according to RNTCP grading (Staining methods, 2000).

Samples were decontaminated and concentrated using the N-acetyl-L-Cysteine-Sodium hydroxide (NALC-NaOH) method as recommended by the CDC's *Public Health Mycobacteriology: A Guide for the Level III Laboratory*. This was used to inoculate into Lowenstein Jensen medium and Mycobacteria Growth Indicator Tubes.

Cultures on LJ medium were incubated at 37°C for 8 weeks. The slopes were periodically observed for the appearance of growth or contamination for a period of 8 weeks. Any growth detected was confirmed by Gram staining and acid fast staining and characterised by biochemical reactions. Also the time required for the growth to appear was noted.

MGIT Tubes with middle brook's 7H11 broth (7mL) was used (Laboratory procedure, 2005). A lyophilized vial of BBL MGIT PANTA (Polymyxin B, Amphotericin B, Nalidixic acid, Trimethoprim, Azlocillin) Antibiotic Mixture was reconstituted with 15mL of BACTEC MGIT Growth Supplement. 0.8mL of Growth Supplement/MGIT PANTA Antibiotic Mixture was aseptically added to the tubes. 0.5mL of the concentrated specimen suspension prepared was also added. The tube was tightly recapped and mixed well. The tubes were incubated at 37°C for 42 days in the dark and read periodically for positivity. Positive tubes, identified by the BACTEC micro MGIT instrument were sub cultured onto LJ medium and an acid-fast smear was prepared. A Gram stained smear and culture on blood agar was also done to rule out contaminating bacteria.

### **Characterisation of the Mycobacterial growth**

Once growth was obtained on LJ medium, each strain was characterised by the rate of growth, pigmentation and biochemical tests like niacin test, nitrate reduction test, catalase test and aryl sulfatase test.

### **Results and Discussion**

41% of the patients belonged to the age group of 41-60years, 33% were in the age group of 21-40 years and 17% were in the age group of

61-80 years as shown in table 1. The youngest patient was 17 years old and the oldest patient was 88 years old.

Out of 100 patients included in our study, 59% of the patients were male and 41% were females. Male to female ratio was 1.4:1.

### **ZN staining**

Out of the 100 patients, 21(21%) were found to be positive by sputum smear microscopy. Among them 8 patients(38.1%) were of sputum grade 3+, 4 (19%) were of grade 2+, 8 (38.1%) were of grade 1+ and 1 patient(4.8%) was of grade scanty as shown in table 2.

### **Culture on LJ medium**

21% of the samples were positive on culture on LJ medium. The earliest detection time was 5 days and the latest detection time was 32 days. The mean detection time in LJ medium was 22.67 days with a standard deviation of 6.135.

### **Culture in MGIT**

23% of the samples were positive in MGIT method. Earliest growth detected was in 4 days and the latest detection time was 20 days. The mean detection time by MGIT was 10.91 days and the standard deviation was 4.621.

Out of the 23 positive cultures 22 cultures were identified as *Mycobacterium tuberculosis* and 1 culture was identified as non-tuberculous Mycobacteria (*Mycobacterium fortuitum*).

### **Comparison of ZN staining with LJ medium culture**

Table 3 shows that, out of the 100 suspected cases, 20(95.2%) were positive by both ZN staining and LJ medium culture. 1(4.8%)

sample negative by smear microscopy was found to be positive by culture. The sensitivity, specificity, positive predictive value of ZN staining compared to LJ medium culture was 95.2%, 98.7% and 95.2% respectively.

**Comparison of ZN staining with MGIT culture**

Table 4 shows that out of 100 suspected cases of pulmonary tuberculosis, 21 cases were positive by both ZN stain and MGIT culture. In addition 2 cases negative by smear microscopy were positive by MGIT. The sensitivity, specificity and positive predictive value of ZN stain compared to MGIT were 91.3%, 100% and 100% respectively.

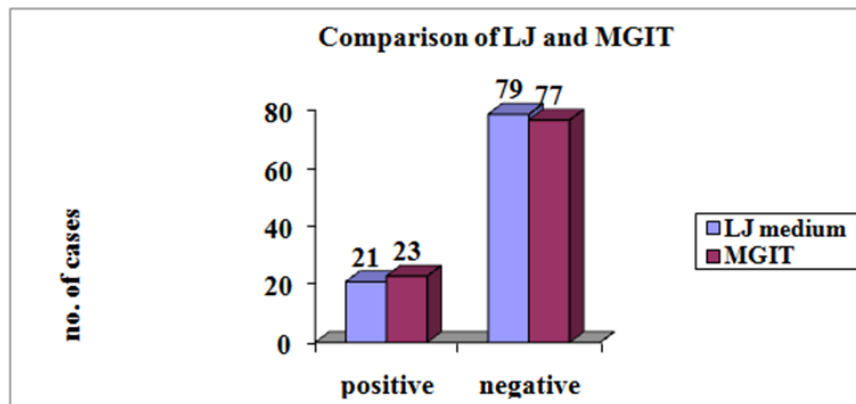
**Comparison of MGIT and LJ medium culture**

Totally MGIT culture was positive in 23 cases and LJ medium culture was positive in 21 cases (as shown in table 5 and figure 1). So the rate of isolation on LJ medium was 91.3%.

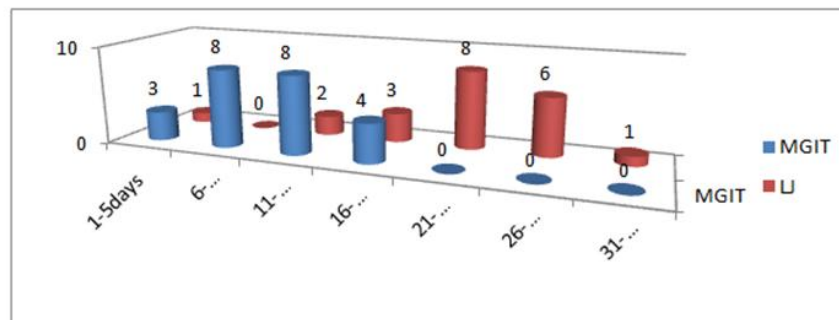
Out of the 23 strains isolated by MGIT, 2(8.7%) strains were isolated exclusively in MGIT whereas no (0%) strains were isolated only on LJ medium. 77(97.5%) cases were negative by both culture techniques.

The sensitivity, specificity and positive predictive value of MGIT compared to LJ medium culture were 100%, 97.5% and 91.3% respectively.

**Fig.1** Comparison LJ medium and MGIT



**Fig.2** Duration for isolation by MGIT and LJ medium



**Table.1** Age distribution

Age group(years)	Number of patients	Percentage
20 and below	7	7
21-40	33	33
41-60	41	41
61-80	17	17
Above 80	2	2
Total	100	100

**Table.2** Distribution of cases according to sputum smears grading

Sputum grade	Number of patients (N=21)	Percentage
3+	8	38.1%
2+	4	19%
1+	8	38.1%
Scanty	1	4.8%

**Table.3** Comparison of ZN staining with LJ medium culture

	LJ medium positive	LJ medium negative	Total
ZN stain positive	20	1	21
ZN stain negative	1	78	79
Total	21	79	100

**Table.4** Comparison of ZN staining with MGIT culture

	MGIT positive	MGIT negative	Total
ZN stain positive	21	0	21
ZN stain negative	2	77	79
Total	23	77	100

**Table.5** Comparison of MGIT and LJ medium culture

	LJ medium positive	LJ medium negative	Total
MGIT positive	21	2	23
MGIT negative	0	77	77
Total	21	79	100

**Table.6** Duration for isolation by MGIT and LJ medium culture

Number of days	No. of positive MGIT	No. of positive LJ
1-5	3	1
6-10	8	0
11-15	8	2
16-20	4	3
21-25	0	8
26-30	0	6
31-35	0	1

**Table.7** Mean time to detection by MGIT in different studies

Study series	Detection time by MGIT
S. Rishi <i>et al.</i> , 2007	11.23days
C.Rodrigues <i>et al.</i> , 2009	16 days
Bohy ME <i>et al.</i> , 2009	18.4 days
Tortolli <i>et al.</i> , 1999	13.3 days
Pfyffer GE <i>et al.</i> ,1997	9.9 days

### Duration for isolation

Table 6 and figure 2 show the duration for isolation by MGIT and LJ medium cultures. MGIT culture showed faster rates of isolation compared to LJ medium with mean detection time being 10.91 days.

It was also noted that among the 23 tuberculosis positive cases, peak incidence (43.47%) was seen in age group of 31-45 years of age. The age of the patients ranged from 18 years to 73 years. Among the 23 cases of pulmonary tuberculosis, 18 (78.2%) were males and 5(21.7%) were females. Male to female ratio was 3.6

The objective of our study was to find the rate of isolation of *Mycobacteria* by MGIT liquid culture and conventional methods. The MGIT culture method showed a faster detection rate compared to the conventional LJ medium culture technique. The mean detection time by MGIT culture was 10.91 days with a standard deviation of 4.621.

Whereas, the mean detection time by LJ medium culture was found to be 22.67 days with standard deviation of 6.135.

Varying mean time to detection has been observed in different studies.

Our results regarding mean time for isolation are comparable to those of Rishi *et al.*, (2007), Tortolli *et al.*, (1999) and Pfyffer *et al.*, (1997).

Rodrigues and co-workers (2009) observed that in highly smear positive specimens (3+) a MGIT culture scored almost 99% recovery in 48-72hrs of culture inoculation and in case of smear negative specimen an average detection time was found to be 16 days compared to 48 days by LJ method. Also, they found the system to be more advantageous in paucibacillary specimens like cerebrospinal fluid, other body fluids and fine needle aspiration cytology samples. Rishi *et al.*, (2007) showed positivity rate of MGIT960 system alone was 34.10% and of LJ alone 1.93% and isolation rates of *Mycobacteria* in pulmonary and extra pulmonary samples were 61.83% and 21.98% by MGIT, 44.01% and 4.96% by LJ respectively. But for maximum recovery of *Mycobacteria*, a combination of MGIT960 and LJ media should be used. The use of one liquid medium and one solid medium is recommended by the Centres for Disease Control and Prevention, and nowadays the use of such a combination is acknowledged worldwide.

In our study 2 strains were isolated exclusively in MGIT. In this study, increased yield in MGIT could be partly due to bigger size of the inoculums used and partly due to addition of nutritional supplement in MGIT.

Dongsi Lu *et al.*, (2002) showed in their study that although automated MGIT system demonstrated better sensitivity than traditional LJ slant for recovery of *Mycobacteria* from clinical specimens, approximately 10% of clinically significant

*Mycobacteria* species would be missed by use of this system alone. MGIT system is not yet sufficiently sensitive to warrant elimination of supplemental solid medium. Bohy *et al.*, (2009) found that MGIT tubes detected 2 isolates of *Mycobacterium tuberculosis* which were missed by BACTEC 460 and LJ medium, while BACTEC 460 also detected 2 isolates missed by MGIT and LJ medium. On the other hand solid media alone detected 1 isolate missed by the liquid media. Also they found that, the earliest growth of *M. tuberculosis* occurred in 15 days.

In our study, LJ medium (6 cultures) showed a higher contamination rate compared to MGIT (5 cultures). In the study by Tortolli *et al.*, (1999), it was found that the BACTEC MGIT 960 system had a contamination rate of 10.0%, intermediate between those of the radiometric system (3.7%) and the egg-based medium (17.0%). In various studies the rate of contamination depended on the concentration of sodium hydroxide, delay between specimen collection and processing due to longer specimen shipment times at some sites and different patient populations.

In our study among the 23 tuberculosis positive cases, peak incidence (43.47%) was seen in age group of 31-45 years of age. The age of the patients ranged from 18 years to 73 years. Our study showed male preponderance among the cases of pulmonary tuberculosis. Among 23 cases, 18 (78.2%) were males and 5 (21.7%) were females. The male preponderance may be because male is more likely to come in contact with an active TB case while at work. Also male is more likely to develop habits like alcoholism and smoking.

The results of this study demonstrated that the MGIT system provided better recovery of *Mycobacteria* than the traditional LJ slant. However, in other studies, MGIT did not

achieve 100% sensitivity for isolation of *Mycobacterium tuberculosis complex*. Dongsu Lu and co-workers (2002) found that 6% of the strains were isolated only on LJ slants. Rishi and co-workers (2007) isolated 1.93% of the strains on LJ alone in their study. Rodrigues and co-workers (2009) found in their study 15 of the isolates grew only on LJ medium. These results do not go along with our study, probably due to the small number of positive cultures obtained in our study.

Liquid broth cultures require 1 to 3 weeks of incubation for detection of organisms. Solid media require 3 to 8 weeks. However, solid media allow examination of colony morphology, detection of mixed cultures, and quantification of growth. Further occasional strains may grow only on solid media. For these reasons experts suggest using liquid and solid media in conjunction, with inoculation of at least one solid medium culture (Mandell, 2005). Culture still remains indispensable in a clinical Mycobacteriology laboratory. Rapid, sensitive and accurate detection of *Mycobacterium* species in clinical specimens can hasten the administration of appropriate anti mycobacterial therapy. Culture on solid medium such as LJ medium is insensitive and may take several weeks. Newer techniques which use liquid medium are able to detect *Mycobacteria* in less time. But they are labour intensive and have other limitations like high cost. The rapidity by which *Mycobacteria* are detected is the most obvious advantage of MGIT culture method. MGIT culture method is a simple, rapid and reliable method of Mycobacterial culture.

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