

## Evaluation of BACTEC Micro MGIT with Lowenstein Jensen Media for Detection of Mycobacteria in Clinically Suspected Patients of Extra Pulmonary Tuberculosis in a Tertiary Care Hospital at Mullana (Ambala)

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### Abstract

**Background and objectives:** Tuberculosis continues to be an important public health problem in India and globally. Extra pulmonary mycobacterial infections are more often smear negative than pulmonary cases which makes the diagnosis difficult. With this in background, this study aimed at the isolation of Mycobacteria from clinical specimens of patients suspected of extra pulmonary tuberculosis using BACTEC Micro MGIT, Lowenstein Jensen (LJ) media and Direct acid fast bacilli smear examination. **Methods-** A total of 200 samples were processed for direct AFB smear examination, and culture on M960 and LJ media. Acid fast staining of the specimens was done using the Ziehl-Neelsen method. **Results and Interpretation -** Of the 24 positive specimens, the highest rate of Mycobacterial recovery was by MGIT (91.6%) as compared to LJ media (58.3%). From smear negative samples LJ and MGIT media detected 6.06 % and 10.10 % isolates respectively. For smear-positive specimens, the mean turnaround time was 8 days by MGIT whereas on LJ medium, it was 36 days. For smear-negative specimens, the same was 18 days for MGIT and 40 days for LJ medium. **Conclusion-** The MGIT system is a good system for, especially in the diagnosis of extra pulmonary samples. The MGIT system was found to be more sensitive in detecting *mycobacterium* in smear-negative samples. The MGIT system detected *mycobacterium* significantly earlier than the LJ medium.

**Keywords:** *Mycobacterium tuberculosis*; MGIT; Ambala; Lowenstein Jensen (LJ) media

### Introduction

The medical microbiology laboratory has a decisive role in the identification and management of infections caused by clinically significant *Mycobacterium tuberculosis*. The precise and prompt analysis of suggestive patients forms the foundation of global strategies for tuberculosis control. Though pulmonary form is the commonest presentation, the Extra Pulmonary Tuberculosis (EPTB) is also an important emerging clinical problem [1].

Extra pulmonary tuberculosis infections are more often smear negative than the pulmonary cases which makes its diagnosis difficult to establish [2]. Extra pulmonary tuberculosis comprises 10-50% of all tuberculosis in HIV negative patients and about 35-80% in HIV infected patients. WHO fact sheet dated March 2010 on tuberculosis stated that overall one-third of the world's population is currently infected with the TB bacillus [3]. The vast majority of the economic burden of tuberculosis in India is caused by the loss of life rather than by morbidity [4].

The diagnosis of EPTB is not always promising with conventional methods, due to the longer time required for the cultivation and the paucibacillary nature of samples [5]. Therefore for a definitive diagnosis, an array of manual and automated systems has been developed to trim down the time to detect and identify bacteria in the clinical specimens. In this series the *Mycobacterium* Growth Indicator Tube (MGIT) system have been launched and extensively evaluated [6,7].

In this study we had compared the performance of Bactec Micro *Mycobacterium* Growth Indicator Tube (MGIT) culture (containing modified Middlebrook 7H9 broth base) against conventional Lowenstein Jensen (LJ) media and direct AFB smear examination

in extra pulmonary tuberculosis specimens. A MGIT has an oxygen sensor embedded at its silicon bottom which contains a modified Middlebrook 7H9 broth which fluoresces following the oxygen reduction which is induced by aerobically metabolizing bacteria within the medium. The amount of fluorescence is inversely proportional to the oxygen level in the culture medium, indicating the consumption of oxygen due to the growth of organisms in the vials. Until now, the focus of tuberculosis program has been on pulmonary variety and the extra pulmonary variety is now beginning to emerge at a very fast pace. So, to best of our knowledge there are very few published report on the evaluation of this new system from this part of our country which focus especially on extra pulmonary paucibacillary samples.

### Material and Methods

#### Study design and period

This cross-sectional study was carried out during the period January 2010 to December 2011 at M. M. Institute Of Medical Sciences and Research, Mullana. A total of 216 suspected cases of extra pulmonary

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**Received** February 01, 2013; **Accepted** March 18, 2013; **Published** March 20, 2013

**Citation:** Bungar R, Singh VA, Avneet, Mehta S, Pathania D (2013) Evaluation of BACTEC Micro MGIT with Lowenstein Jensen Media for Detection of Mycobacteria in Clinically Suspected Patients of Extra Pulmonary Tuberculosis in a Tertiary Care Hospital at Mullana (Ambala). J Med Microb Diagn 2: 123. doi:10.4172/2161-0703.1000123

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tuberculosis attending the OPD and Indoor were included in the study. 16 patients were excluded at screening as they were previously treated with anti tubercular medications. The clinical samples included Ascitic fluid (6), Bone scrapings (2), Bone marrow (4), CSF (8), Cyst wall (2), Endometrial biopsy (112), Gastric aspirate (2), Lymph node aspirate (28), Menstrual blood (4), Ovarian cyst fluid (4), Pericardial fluid (2), pleural fluid (8), Pus (14), synovial fluid (2) and urine samples (2). Informed and written consent was taken from patients before enrollment.

### Culture medium inoculation, incubation, and test duration

All specimens were processed by standard N-acetyl-L-cysteine NaOH (NALC-NaOH) digestion decontamination technique [8,9]. A final concentration of 4% NaOH was used for decontamination. After the centrifugation, the sediment was resuspended in 1.5 ml of sterile phosphate buffer. Following processing, AFB smear were prepared and stained with the ZN Staining method. The grading of smears was carried out as described by Kent and Kubica [9].

**MGIT tube:** The A BBL MGIT tube (from Becton Dickinson) containing 7 mL modified middle brook 7H9 broth was used, to which an enrichment supplement as well as a mixture of antibiotics consisting of polymyxin B, amphotericin B, nalidixic acid, trimethoprim, and azlocillin were added. After inoculation, the tubes were incubated at 37°C. Readings were taken daily for the first three weeks and once a week thereafter for culture positivity until the end of six weeks using the BBL Micro MGIT system. All the positive tube was further confirmed by ZN staining and a sub culturing on blood agar plate and a LJ slant. The time to detection (TTD) of *Mycobacteria* was based on the date of the earliest instrumental indication of positivity.

**LJ Media Tubes:** LJ slant was inoculated with the 0.1 ml of the processed sample and were incubated at 37°C for a maximum of 8 weeks. They were checked twice weekly for first two weeks and then once every week for maximum period of 8 weeks. Bacterial contamination was detected by performing Gram staining from the suspected colonies.

**Para-nitro-benzoic acid (PNB) testing:** Confirmation of M tuberculosis complexes was made by (PNB) testing [10].

**Quality control:** Strict Quality control was maintained as per the manufacturer protocol. It was carried out using STD H 37 RV and ATCC strains of *M. tuberculosis* complex and NTM.

### Results

Out of 200 extra pulmonary samples, *Mycobacterium tuberculosis* complex was found to be positive in 24 (12%) cases by any of the three methods i.e. MGIT media, LJ medium and ZN Staining. Only 2 samples (8.33%) were found to be AFB positive by Z.N staining method. The comparison of the Micro MGIT media with the LJ medium for detection of *mycobacterium* in 200 specimens is shown in table 1. Of the 24 positive specimens, the highest rate of *Mycobacterial* recovery was by MGIT i.e. 22 out of 24 (91.6%) as compared to LJ media 14 out of 24 (58.3%). There were 10 samples (41.66%) which were found positive only by Micro MGIT, while 2 samples (8.33%) were found positive by LJ only.

The Micro MGIT system was found to be more sensitive in detecting mycobacteria in smear-negative samples table 2. LJ media and Micro MGIT System detected all isolates from smear positive samples whereas from smear negative samples LJ and Micro MGIT media detected 12/198 (6.06%) and 18/198 10.10% isolates respectively. The Micro MGIT system detected mycobacteria significantly earlier than the LJ

Löwenstein-Jensen (LJ) medium.	Mycobacteria Growth Indicator Tube(MGIT)			
		Positive	Negative	Total Isolates
	Positive	12	2	14
	Negative	10	176	186
	Total Isolates	22	178	200

**Table 1:** Recovery Rae of *Mycobacterium tuberculosis* by BACTEC Micro MGIT and LJ Medium.

AFB Smear	Detection by LJ Medium			Detection by Micro MGIT System		
	Number	TTD (Days)	Range (Days)	Number	TTD (Days)	Range (Days)
(N=2)	2	36	15-40	2	8	7-10
Negative						
(N=198)	12	40	32-42	20	18	15-44

AFB - Acid fast bacilli

LJ- Lowenstein Jensen media

**Table 2:** The Time to Detection (TTD) of *Mycobacteria* for smear positive and smear negative isolates in BACTEC MGIT and LJ Medium.

medium. For smear-positive specimens, the mean turnaround time was 8 days by Micro MGIT whereas on LJ medium, it was 36 days. For smear-negative specimens, the same was 18 days by Micro MGIT and 40 days by LJ medium table 2.

The contamination rate of BACTEC Micro MGIT and Lowenstein Jensen media was found to be 6% and 8% respectively.

### Discussion

An array of manual and automated systems has been developed for the faster and more accurate detection of acid fast bacilli from various clinical samples. The Micro MGIT has been extensively evaluated, but very few researches have been carried solely on extra pulmonary specimen. The studies done in the past MGIT has shown higher isolation rates reason being the type of sample (both respiratory and non respiratory samples) included under those studies [11]. The Centers for Disease Control and Prevention recommends the use of one liquid medium and one solid medium for diagnostic tuberculosis [11]. Reason for the higher isolation rate is the type of sample (includes both respiratory sand non respiratory samples) under study. So the Micro MGIT system is found to be more useful in paucibacillary specimens where LJ media gives very scanty or no growth. In this study total positivity rate of *Mycobacteria* was 12%, which was higher than Hillemann et al. [12] (8%). Very high positivity rates were reported by Rishi et al. [13] (51.6%) and Rodrigues et al. [14] (42%). This could be due to the fact that both the author had included both pulmonary and extra pulmonary tuberculosis samples

The combination of both Micro MGIT and LJ method (100%) was more than the Micro MGIT alone 5 (41.6%) and LJ alone 1 (8.3%).

The recovery of *Mycobacteria* from clinical specimens was compared for the combinations of Micro MGIT with LJ media versus LJ media alone. Similar results for the percentage positivity were demonstrated by Dongsu Lu et al. [15] who had reported that 143 of the 278 total isolates were recovered from both the MGIT and LJ media. An additional 106 isolates (38.1%) were recovered from the MGIT only, while 29 (10.4%) isolates grew only on the LJ slant. Although none of the methods could isolate all of the mycobacteria, therefore the combined use of Lowenstein Jensen media and Micro MGIT could be justified for the maximum recovery.

Grading of smears gives an idea regarding the bacterial load. It depends upon various factors such as time of collection, number of samples taken, nature of sample, treatment with anti tuberculous drugs and its duration and method of grading used. In present study 8.3% cases were found to be AFB positive with ZN Microscopy. The positivity rate of 11% was reported by Hillemann et al. [12] (extra pulmonary samples). Whereas Huang et al. [16] isolated around 20.6% of the isolates. This could be attributed to the type of samples (both pulmonary and extra pulmonary samples) the author had considered for the study [16]. Conventional bacterial culture by LJ medium is routinely applied in the developing countries but its utility is limited because of the low positivity in the smear-negative cases, slowly growth rate (6-8 weeks) and lesser sensitivity than liquid medium. Newer automated methods using has drastically shortened the detection period to 1–3 weeks and also decrease the workload. But in contrast to solid media it is expensive, contamination rates are more, requires more infrastructures, need purchase of specific equipments and maintenance is needed. As one system covers the drawbacks of other, the combination of Micro MGIT with primary solid yielded a higher recovery of *Mycobacteria* compared to primary solid medium than that could be obtained with either of the techniques alone. In present study, the combination isolated total of around 12 *Mycobacteria* species, out of which Micro MGIT alone detected 41.6% and LJ alone only 8.3%.

In the present study average time to detection of *Mycobacteria* growth according to the smear positivity noted to be significantly shorter for BACTEC MGIT 960 (7-10 days for AFB positive and 8-44 days for AFB negative) compared to LJ media (15-34 days for AFB positive and 32-42 days for AFB negative) in this study. This is further supported by Douglas et al. [17] reported longer TTDs of 19.3 days and 35 days by Bactec MGIT 960 and LJ media. This may be because of the pauci bacillary nature of the samples [17]. Higher contamination rate is the major drawback of the fully automated system but interestingly the contamination rate was found to be lower with MGIT system (6%) as compared to LJ medium (8%). The possible reason for the significant reduction in the contamination rate could be the use of the N-acetyl cysteine sodium hydroxide (NALC-NaOH) method and the addition of the MGIT PANTA mixture [7,15,18].

In the end it is concluded that since tuberculosis still remains a major global health problem, so there is a need for a rapid, sensitive and accurate detection system like Bactec Micro MGIT for culturing the microorganism in clinical specimens. This would hasten the administration of appropriate antimycobacterial therapy thereby decreasing morbidity and mortality as well as preventing the spread of infection in the community.

## Acknowledgment

This work was supported by RNTCP Research Grant.

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