

## Comparison of recoveries of *Mycobacterium tuberculosis* using the Automated BACTEC MGIT 960 System and Lowenstein-Jensen Medium in clinically suspected cases of tubercular meningitis in children

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### Abstract

**Background:** Tubercular meningitis (TBM) remains a major complication in children with a considerable mortality and morbidity. Inconsistent and variable clinical features coupled with lack of rapid, sensitive and specific tests poses a diagnostic challenge to the clinical acumen. This study was carried out to diagnose TBM by a combination of direct microscopy by Ziehl-Neelsen (ZN) staining, culture by conventional Lowenstein Jensen (LJ) media and Bactec MGIT 960 system in clinically suspected cases TBM and compare the utility of these diagnostic modalities in the diagnosis of paediatric TBM.

**Methods:** A total of 50 cerebrospinal fluid (CSF) samples from suspected cases of TBM in children aged 0-12 years of age were processed for direct acid fast bacilli (AFB) smear examination, and culture on LJ media and automated Bactec MGIT 960 system.

**Results:** The study comprised of 32 male and 18 female patients in the age group of 0-12 years with a male to female ratio of 1.77: 1. 1 (2%) out of 50 samples was positive for AFB by ZN staining. Overall culture was positive in 15 cases (30%). Culture by LJ media was positive in 4 (8%) of the cases while culture by Bactec MGIT 960 system was positive in 14 cases (28%). The mean time of detection of *Mycobacterium tuberculosis* in LJ media and MGIT were 13.2 days and 32.4 days, respectively.

**Conclusion:** Automated Bactec MGIT 960 system increases the sensitivity of diagnosing TBM in comparison to conventional microscopy and culture and reduces the mean time to diagnosis.

**Keywords:** Tubercular Meningitis, LJ media, Bactec MGIT 960 system.

### 1. Introduction

Tuberculosis (TB) in the paediatric age group continues to be a global health problem especially so in countries with high burden of TB. Tubercular meningitis (TBM) poses as the gravest form of extra-pulmonary tuberculosis (EPTB) afflicting a significant mortality and morbidity. TBM comprises of 20– 45% of all forms of TB among children which is significantly higher when compared to TBM in adults which comprises of only 2.9-5.9% of the overall adult TB cases.[1] In untreated cases the mortality can be as high as 100% and a deference in initiation of therapy may lead to permanent residual neurologic damage.[2]

In developing countries, clinical history and examination remain the primary basis for diagnosis of TBM in as high as 90% of symptomatic cases. [3] The challenges in the microbiological and laboratory diagnosis of TBM in paediatric age group are manifold. The sensitivity of acid fast

bacilli (AFB) microscopy and conventional Lowenstein Jensen (LJ) culture is quite low in cerebrospinal fluid (CSF) which contains a small number of organisms. [2,4] The nucleic acid assays are limited by their high costs and can serve only as adjuncts and not substitutes per se of conventional tests.[5] Immunological and serological assays have not stood the test of confidence to warrant their use in conventional diagnostics.[6]

In the face of such limitations, the need of the hour is for less complicated and more accurate diagnostic tests. Liquid culture media such as that used in the Mycobacterial Growth Indicator Tube (MGIT) (Becton Dickinson) system have been introduced over the years and been extensively evaluated.[7] The BACTEC MGIT 960 system was developed as a non-radiometric, fully automated, continuous monitoring system to serve as an alternative to the radiometric BACTEC 460 for growth and detection of

Mycobacteria.[3] The Bactec MGIT 960 has been shown to be a safe, sensitive and less labour-intensive method as compared to other automated methods.[8]

With this background this study was undertaken to diagnose TBM by a combination of conventional and automated culture methods in clinically suspected cases of paediatric TBM and compare the recovery rates by using conventional methods and the BACTEC MGIT 960 system.

## 2. Materials and Methods

This prospective observational study was conducted in the department of microbiology of a tertiary care hospital over a period of twelve months from December 2012 till November 2013. A total of 50 patients in the age group of 0-12 years who were clinically suspected to have TBM as per the selection criteria were included in the study.

50 cerebrospinal fluid samples from suspected tuberculous meningitis patients in the age group of 0-12 years of age were taken who fulfilled the following selection criteria:

### 2.1 Inclusion Criteria

Fever of more than 2 weeks duration  
Headache  
Signs of meningeal irritation  
Altered conscious level  
Focal neurological deficits

### 2.2 Exclusion criteria:

Patients already on anti-tuberculous drugs  
Patients having co-morbid disease

### 2.3 Processing of samples

CSF aliquots of 3–5 ml were concentrated by centrifugation (3000 ×g for 10 minutes) and the sediments were used to prepare smears for direct smear examination by Ziehl-Neelsen (ZN) stain. CSF was cultured in conventional LJ media along with Bactec MGIT 960 system. LJ media were incubated under an atmosphere containing 5% CO<sub>2</sub> at 37°C and were observed once weekly till 8 weeks, whereas Bactec MGIT 960 cultures were processed till 6 weeks. Due to turbid appearance 4 specimens needed decontamination, 1 of which was blood-stained. Before inoculating into MGIT medium, the specimens were sub-cultured on blood and chocolate agar to check for contamination that was negative for all samples.

Culture in Bactec MGIT 960 system for *Mycobacterium tuberculosis* was done strictly according to the manufacturer's instructions. Lyophilized MGIT PANTA (containing polymyxin B, azlocillin, nalidixic acid, trimethoprim, amphotericin B) was reconstituted with MGIT growth supplement OAEDC (containing oleic acid, albumin, dextrose, catalase, polyoxyethylene stearate), and 0.8 ml of this was added prior to sample inoculation to the MGIT. Smears were made from all positive culture tubes as well as from MGIT 960 negative tubes that had some deposit in them, to confirm the presence or absence of mycobacteria.

Samples positive for AFB either by LJ culture or Bactec MGIT 960 were later subjected to para-nitrobenzoic acid (PNB) test to rule out non-tuberculous mycobacteria (NTM) (PNB resistant) as per standard protocol.

### 2.4 Ethics and Informed consent

Institutional ethical clearance was obtained prior to initiation of the study and informed consent was taken.

### 2.5 Statistical analysis

Data was collected on preformed proformas and entered into Microsoft Excel Sheets. Descriptive statistical analysis was done using the SPSS software system version 16.

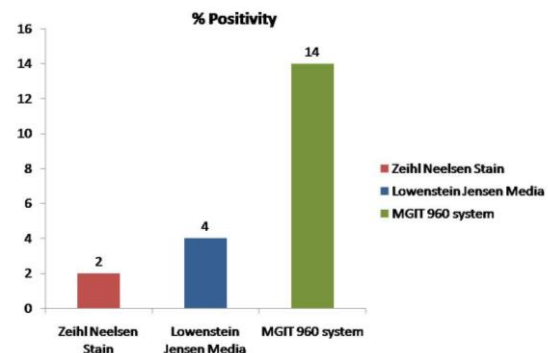
## 3. Results

Of the total 50 samples 32 were male and 18 female in the age group of 0-12 years with a male to female ratio of 1.77: 1. On ZN staining for Acid Fast Bacilli (AFB) only 1 out of 50 samples was positive for AFB. Culture by LJ media was positive in 4 of the cases and all the cases belonged to *Mycobacterium tuberculosis* species without any presence of non-tuberculous mycobacteria. Culture by Bactec MGIT 960 system was positive in 14 cases. Of the 14 cases detected by Bactec MGIT 960 system 3 cases had also shown growth in LJ media. In one sample that was positive result by growth in the LJ media which was not detected by Bactec MGIT 960 system.

As a single test AFB staining could detect only 2% of the cases while LJ media culture could detect 8% of the cases. Culture by LJ media was positive in 8% of the cases. The Bactec MGIT 960 system showed positivity in 28% of the cases. In combination LJ media and Bactec MGIT 960 system showed positivity in 30% of the cases. The results are depicted in Table 1 and Fig 1. The mean time of detection of *Mycobacterium tuberculosis* in LJ media and MGIT were 13.2 days and 32.4 days, respectively.

**Table 1: Showing isolation rates with Lowenstein Jensen Media and MGIT 960 system from CSF samples**

Method	N (Total Number positive out of 50 samples)	%
Lowenstein Jensen Media	4	8
MGIT 960 system	14	28
Combined	15	30



**Figure 1: Showing detection rates by diagnostic method**

#### 4. Discussion

In our study smear positivity for AFB was found to be only 2%. Previously studies have shown the positivity rates using ZN staining for AFB in CSF samples to be below 10%. [9]. The poor positivity rates in CSF samples for AFB have long been a matter of concern. Though recommendations have been made that serial sampling and centrifugation of large volumes of CSF could improve the sensitivity such techniques are impractical considering the difficulties in procuring the sample especially in paediatric cases. [2]

With respect to recoveries by culture the automated Bactec MGIT 960 system displayed a higher rate of recovery of *M. tuberculosis* (28%) than LJ media (8%). Previously authors have showed detection rates using LJ media in the order of from 10.2 to 55.8% for LJ medium, which is in accordance with our study. [4] Similarly the detection rates using the MGIT 960 system has been reported to be ranging from 4.3 to 48.9%. [10] The higher sensitivity of the Bactec MGIT system is probably because of added growth supplements like OAEDC and antibiotics like PANTA which allowed even small number of microorganisms to grow in contrast to LJ media. There was a single culture positive sample which yielded growth only on LJ media and was negative on the MGIT 960 system. While the use of MGIT 960 system definitely has a far better detection rate yet such cases can be missed if conventional methods are not used concurrently thus perhaps indicating use of multi-pronged isolation strategies in cases of paediatric TBM. Besides higher isolation rate, the mean time to detect *M. tuberculosis* was less than half in Bactec MGIT 960 as compared to LJ media (13.2 days versus 32.4 days,) which has also been validated by prior studies. [4]. The Bactec MGIT 960 also offers the additional advantage of utilization for drug susceptibility testing which is of prime importance while facing with drug resistant cases of paediatric TBM which form a significant challenge. Thus our study demonstrates that MGIT 960 system definitely has a better sensitivity for diagnosis of TBM with a caveat that conventional methods of culture and staining should also be used concurrently.

#### 5. Conclusion

Automated Bactec MGIT 960 system increases the sensitivity of diagnosing TBM in comparison to conventional microscopy and culture and reduces the mean time to

diagnosis. The incorporation of Bactec MGIT 960 system can be of substantial aid in the diagnosis of paediatric TBM.

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