

Comparison of LJ Medium and BACTEC MGIT 960 Culture System for the Diagnosis of Tuberculosis

PINKI KUMARI¹, JIWESH KUMAR THAKUR², PRASHANT KUMAR³, RAKESH KUMAR⁴, DEVAL PAREKH⁵

ABSTRACT

Introduction: Sputum negative pulmonary Tuberculosis (TB) is a major public health problem. So, the emergence of new techniques for a more precise and rapid microbiological identification of *Mycobacterium tuberculosis* in clinical samples is of great importance to improve the management of TB.

Aim: To determine and compare the sensitivity and turnaround time for *Mycobacterium tuberculosis* detection by the BACTEC Mycobacteria Growth Indicator Tube (MGIT) 960 system, Lowenstein Jensen (LJ) medium and Ziehl-Neelsen (ZN) staining.

Materials and Methods: An Institution based, observational, cross-sectional study was conducted at Rajendra Institute of Medical Sciences (RIMS), Ranchi, Jharkhand, India, from July 2013-March 2016. Sputum, pericardial fluid, pleural fluid, peritoneal fluid, pus and endometrial tissue samples were collected from 80 patients of suspected TB cases. All were Acid-Fast stained by ZN staining method and cultured on solid culture LJ medium and on liquid medium (MGIT). Data was analysed using Statistical Package for the Social Sciences (SPSS) software, Version 20.0 (SPSS Inc, Chicago, IL, USA).

Fisher's-Exact test was used to show association of categorical variables. Non-parametric Mann-Whitney U test was used to show median difference of non-normally distributed continuous variables of two groups.

Results: Out of the 80 samples, 41 cases were positive by either of the all methods. The positive specimen for ZN staining, LJ media and MGIT were 21, 29 and 41 cases respectively. The mean Time To Detection (TTD) was shorter for MGIT system than LJ media. Both LJ medium and MGIT 960 detected all cases of sputum smear positive cases and in addition significantly higher number than ZN stain in sputum smear negative cases. MGIT 960 detected significantly higher number of cases of sputum negative cases than LJ Media. The mean TTD was also significantly shorter in case of smear positive cases than the smear negative cases by both the solid and liquid culture mediums.

Conclusion: The use of liquid media (MGIT) is more accurate and rapid method for the diagnosis of TB. The combination of more than one method is also highly recommended for rapid detection and early treatment of TB.

Keywords: Extra pulmonary tuberculosis, Lowenstein-jensen medium, Mycobacteria growth indicator tube system, Pulmonary tuberculosis

INTRODUCTION

History of Tuberculosis dates back to 4000 years [1]. Since ancient times this disease is affecting mankind in different forms. TB is caused by *Mycobacterium tuberculosis* which most often affects the lungs. TB is curable and preventable [2]. TB is a global disease and in 2018, South-East Asian region reported 44% of the total new cases, largest in the world [2]. In 2018, 30 high burden countries reported 85% of the total new cases and out of these 2/3rd of total new cases were reported by eight countries which include China, Philippines, Indonesia, Nigeria, South Africa, Bangladesh, Pakistan and India [2]. People having Human Immunodeficiency Virus (HIV) infection are 15-22 times more likely to develop active TB. HIV and TB co-infection is a lethal combination because each infection fastens the progress of other's infection [2]. TB cases who don't respond to isoniazid and rifampicin are classified as Multidrug Resistant TB (MDR-TB), which poses additional health security threat and remains a public health crisis [2]. While smear microscopy is by far the most popular method to detect mycobacteria in clinical specimen, culture of the aetiological agent remains the accepted "gold standard" for diagnosing mycobacterial infections [3]. However, cultivation on solid media, such as that of LJ, is time-consuming, taking up to 6 to 8 weeks, and has a low sensitivity especially in the samples containing small number of organisms [4,5]. In recent times, non-radiometric liquid culture media such as that used in the MGIT system have been introduced and extensively evaluated [6]. MGIT 960 system is a non-invasive instrument which is fully automated,

high capacity and non-radiometric. It is used to incubate and monitor 7 mL culture tubes [7]. The MGIT uses modified middlebrook 7H9 media. The culture tubes also contain silicon embedded fluorescent growth indicator present at the bottom of each tubes [8]. This compound is quenched by the presence of dissolved oxygen in the broth. As the microorganisms grow in the media, oxygen gets exhausted which allow fluorescence to be detected automatically over time [8].

MGIT 960 system is an excellent diagnostic method for the TB and many researchers have published on this topic [3-6]. However, the research which compares between the conventional and MGIT 960 media are very limited in eastern part of the country especially in resource poor state like Jharkhand. This study was planned with an objective to compare the diagnostic sensitivity of ZN Stain, LJ culture medium and MGIT 960 culture medium at a tertiary level health care facility of Jharkhand, India.

MATERIALS AND METHODS

An Institution based, observational, cross-sectional study was conducted at Rajendra Institute of Medical Sciences (RIMS) Ranchi, Jharkhand, India, from July 2013-March 2016. This study was ethically cleared by Institutional Ethics Committee (IEC) of RIMS (Memo No-10: IEC/IAEL/RIMS/Ranchi, Date-13.03.15). Specimens were collected after getting written informed consent from all the study participants. Non-probability, consecutive sampling method was used and all those who came during data collection period and consented to participate in study were

included in study. Total 80 study participants consented and samples were collected from them.

Samples were collected from patients suspected to be suffering from TB who visited the "Department of Microbiology, RIMS" during data collection period. All the suspected cases of pulmonary TB (persistent cough for ≥ 2 weeks, unexplained low grade fever specially with evening rise of temperature, unexplained weight loss etc.) as well as Extrapulmonary TB (Non-resolving lymph node swellings, pleural effusion, abdominal pain with low grade fever and weight loss, etc.) were included in the study. All the samples were Acid-Fast stained by ZN method and cultured on solid medium (LJ) media and on liquid media (M960).

Specimen preparation and culture methods: All specimens were processed following conventional methods for mycobacterial isolation, digestion and decontamination by the N acetyl-L-cysteine-NaOH procedure [5].

Briefly, equal volume of the freshly prepared MycoPrep NALC-NaOH solution was added to the sputum specimen, mixed on vortex and left to stand at room temperature for 15 minutes. The mixture was completed to double its volume with sterile phosphate buffer (pH 6.8) and centrifuged at 3000 x g for 15 minute. The supernatant was separated and the sediment was used for Acid-Fast Bacilli (AFB) microscopy (ZN stain) and for cultures {(one solid medium (LJ) and one liquid medium MGIT (BACTEC MGIT 960, Becton Dickinson))}.

- 1) **MGIT 960:** MGIT consists of liquid broth medium which gives better and faster growth of mycobacteria. It contains 7 mL of modified Middlebrook 7H9 broth base. A growth supplement MGIT OADC (Oleic acid, Albumin, Dextrose and Catalase) which is essential for the growth of many mycobacteria is added to make the medium complete. The medium is sterilised by autoclaving. MGIT PANTA (Polymyxin B, Amphotericin B, Nalidixic acid, Trimethoprim, and Azlocillin) is added to suppress contamination [8]. Each tube is inoculated with 0.5 mL of the processed specimen and then the tubes were kept into the MGIT 960 instrument at 37°C and were monitored automatically after every one hour for increase in fluorescence for a maximum of six weeks. Any sample, which showed signal of growth, is taken out from the instrument. From the positive tube, a smear preparation is done for microscopic examination of AFB. A blood agar plate inoculated for sub-culture on LJ slant to detect any contamination for the BACTEC MGIT 960 culture media. No growth was observed in blood agar plate.
- 2) **Solid media:** For inoculation onto solid LJ slant, 0.1 mL of the suspension was used. Solid media was incubated at 37°C in a slant position for 24-48 hours, then in upright position for a further eight weeks and read daily for the 1st week then twice weekly. Colonies of *Mycobacterium tuberculosis* is identified by its rough, crumbly, waxy, buff colored appearance which develops after 2-3 weeks after inoculation. The colony with doubtful morphology was confirmed by ZN staining [9]. All positive, liquid and solid media are sub-cultured onto Columbia agar with 5% sheep blood to check for contaminants [5]. No growth was observed onto Columbia agar with 5% sheep blood in present study.

STATISTICAL ANALYSIS

All the data were codified and entered in MS Excel spread sheet. Data were analysed using SPSS software, Version 20.0 (SPSS Inc,

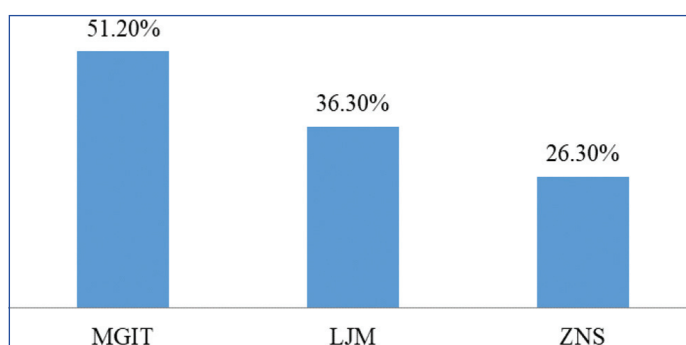
Chicago, IL, USA). Fisher's-Exact test was used to show association of categorical variables. Non-parametric Mann-Whitney U test was used to show median difference of non-normally distributed continuous variables of two groups. The p-value ≤ 0.05 was considered as statistically significant.

RESULTS

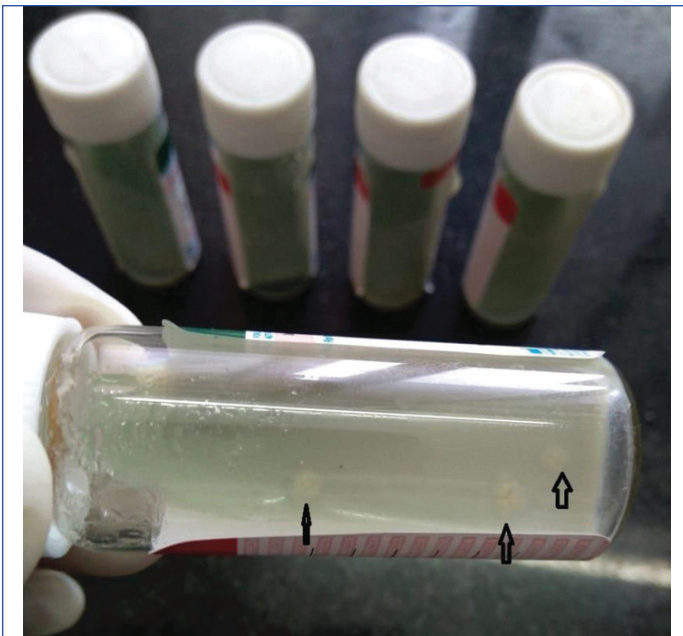
Mean age of the study population was 51.63 ± 12.9 years. A 47.5% of study population belonged to age group of 41-60 years followed by 30.0% and 22.5% who belonged to age group of ≥ 61 years and 20-40 years, respectively. Total 80 samples were processed using ZN stain, LJ medium culture and MGIT 960 culture medium. An 80.0% of sample was sputum followed by pus (7.5%), peritoneal fluid (5.0%), pleural fluid (3.75%) and lymph node aspirates (3.75%). Two of the study population was HIV positive [Table/Fig-1]. The isolates were identified as *Mycobacterium tuberculosis* on the solid culture media by the rate of growth, pigment production and further Acid-Fast staining and from the M960 tubes by confirmation by the BD MGIT 960TbC ID test strips. Out of the 80 samples under study, total 41 samples (51.2%) were found to be positive by any of the three methods viz., ZN Stain, LJ Media or MGIT 960. All 41 were detected by MGIT 960 method followed by 29 and 21 by LJ media and ZN staining, respectively [Table/Fig-2]. Out of the 59 smear negative cases, culture on LJ media detected eight cases [Table/Fig-3] while M960 system detected 20 cases. All 21 sputum positive cases on ZN stain [Table/Fig-4] were detected by both LJ media as well as by MGIT 960 media [Table/Fig-5].

| Clinico-social characteristics | n (%) |
|--------------------------------|-----------|
| Age group (years) | |
| 20-40 | 18 (22.5) |
| 41-60 | 38 (47.5) |
| ≥ 61 | 24 (30.0) |
| Gender | |
| Male | 55 (68.8) |
| Female | 25 (31.2) |
| Types of sample | |
| Sputum | 64 (80.0) |
| Pus | 6 (7.5) |
| Peritoneal fluid | 4 (5.0) |
| Pleural fluid | 3 (3.75) |
| Lymph node aspirate | 3 (3.75) |
| HIV status | |
| HIV Negative | 78 (97.5) |
| HIV Positive | 2 (2.5) |

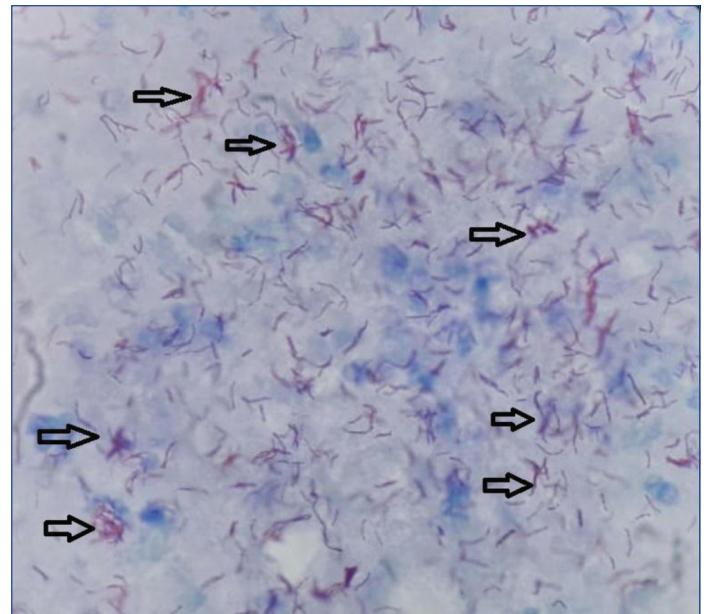
[Table/Fig-1]: Clinico-social characteristics of study population, n=80.



[Table/Fig-2]: Simple bar diagram showing percentage of samples tested positive by different media, n=80. MGIT: Mycobacterial growth indicator tube; LJ: Löwenstein jensen medium; ZNS: Ziehl neelsen stain



[Table/Fig-3]: Colonies of *Mycobacterium tuberculosis* (arrow) on L-J medium.



[Table/Fig-4]: ZN staining of mesenteric lymph node aspirate from HIV positive patient showing Acid-Fast Bacilli (arrow).

| Diagnostic test | Total positive | Total negative | Sputum positive diagnosed by LJ media | Sputum negative diagnosed by LJ media | Sputum positive diagnosed by MGIT 960 | Sputum negative diagnosed by MGIT 960 | LJ medium positive diagnosed by MGIT 960 | LJ medium negative diagnosed by MGIT 960 |
|-----------------|----------------|----------------|---------------------------------------|---------------------------------------|---------------------------------------|---------------------------------------|--|--|
| ZN stain | 21 | 59 | | | | | | |
| LJ medium | 29 | 51 | 21 | 8 | | | | |
| MGIT 960 | 41 | 39 | | | 21 | 20 | 29 | 12 |

[Table/Fig-5]: Distribution of test results as per the different diagnostic test results (n=80).
MGIT: Mycobacterial growth indicator tube; LJM: Löwenstein jensen medium; ZNS: Ziehl neelsen stain

While the sensitivity of MGIT 960 culture was 100.0%, the sensitivity of LJ medium and ZN stain was found to be 70.7% and 51.2%, respectively [Table/Fig-6]. LJ culture medium detected eight more case than ZN stain and MGIT 960 detected 20 more cases than ZN stain. Higher detection rate of TB infection by LJ Media and MGIT 960 Media than ZN stain was found to be statistically significant by Fisher's-Exact test [Table/Fig-7]. In addition to identifying all the LJ medium positive samples, MGIT 960 diagnosed 12 more cases which were found to be negative by LJ Medium. Higher detection rate of MGIT 960 than LJ medium was also found to be statistically significant [Table/Fig-8].

| Test | n | True Positive (TP) | False Negative (FN) | True Negative | Sensitivity (TP/TP+FN) |
|----------|----|--------------------|---------------------|---------------|------------------------|
| ZN stain | 80 | 21 | 20 | 39 | 21/41×100=51.2% |
| LJ media | 80 | 29 | 12 | 39 | 29/41×100=70.7% |

[Table/Fig-6]: Sensitivity of ZN stain and LJ media in detecting Mycobacterium tuberculosis as compared to "Gold standard" MGIT 960 media (n=80).
MGIT: Mycobacterial growth indicator tube; LJM: Löwenstein jensen medium; ZNS: Ziehl neelsen stain; TP: True positive; FN: False negative

| Culture media | Sputum | | Total n (%) | p-value |
|-----------------|--------------|--------------|-------------|---------|
| | Positive (%) | Negative (%) | | |
| LJ Media | | | | |
| Positive | 21 (72.4) | 8 (27.6) | 29 (100.0) | |
| Negative | 0 | 51 (100.0) | 51 (100.0) | <0.05 |
| MGIT 960 | | | | |
| Positive | 21 (51.2) | 20 (48.8) | 41 (100.0) | |
| Negative | 0 | 39 (100.0) | 39 (100.0) | <0.05 |

[Table/Fig-7]: Fisher's-Exact test showing association between sputum positivity and different culture media (n=80).
MGIT: Mycobacterial growth indicator tube; LJM: Löwenstein jensen medium

The median TTD was 9.0 days {Inter Quartile Range (IQR)}: (8.0-10.0) and 33.0 days (IQR: 28.0-34.0) in MGIT 960 media and LJ

| Culture media | LJ medium | | Total n (%) | p-value |
|-----------------|--------------|--------------|-------------|---------|
| | Positive (%) | Negative (%) | | |
| MGIT 960 | | | | |
| Positive | 29 (70.7) | 12 (29.3) | 41 (100.0) | |
| Negative | 0 | 39 (100.0) | 39 (100.0) | <0.05 |

[Table/Fig-8]: Fisher's-Exact test showing association between LJ media and MGIT 960 (n=80).
MGIT: Mycobacterial growth indicator tube; LJM: Löwenstein jensen medium

media, respectively. Mean Rank of LJ Media was 14.79 and 49.65 in case of sputum smear positive and sputum smear negative samples, respectively. Similarly, mean rank of MGIT 960 was 25.17 and 45.96 in case of sputum smear positive and sputum smear negative samples respectively. Low mean rank in case of sputum smear positive cases as compared to sputum smear negative cases was statistically significant in both LJ media culture and MGIT 960 culture method [Table/Fig-9].

| Test | Sputum smear | Number | Mean rank | Median (IQR) days | Mann-Whitney U | p-value |
|-----------------|--------------|--------|-----------|-------------------|----------------|---------|
| LJ media | Positive | 21 | 14.79 | | | |
| | Negative | 59 | 49.65 | 33.0 (28.0-34.0) | 79.5 | <0.05 |
| MGIT 960 | Positive | 21 | 25.17 | | | |
| | Negative | 59 | 45.96 | 9.0 (8.0-10.0) | 297.5 | <0.05 |

[Table/Fig-9]: Mann-Whitney U test showing median Time To Detection (TTD) and mean rank of different culture media in sputum smear positive and negative samples (n=80).
MGIT: Mycobacterial growth indicator tube; LJM: Löwenstein jensen medium

DISCUSSION

The present study demonstrated that M960 system provided better isolation rates of *Mycobacterium tuberculosis* (100%) from the clinical samples than the LJ media (70.7%) or the simple acid fast staining by the ZN Method (51.2%). Results of this study are in agreement with few other studies which also

reported that the BACTEC MGIT 960 had higher recovery rate for *Mycobacterium tuberculosis* than solid media [7,10-13]. Bunker R et al., reported 91.6% recovery of *Mycobacterium tuberculosis* by MGIT 960 system as compared to only 58.3% by LJ medium [14]. However, a study by Siddiqui MAM et al., reported a low detection rate of 15.0% for both LJ Media and MGIT 960. This might be due to either improper sample collection or higher contamination rate [15]. In this study, all the ZN stained smear which were positive by direct microscopy were also positive by the growth on LJ media, and also out of the total positive cases of TB (41 cases), the LJ media showed growth in 28 cases implying the isolation rate or the sensitivity of LJ media to be 70.7%. An almost similar detection rate of *Mycobacterium tuberculosis* by LJ medium was reported by Almazini MAI et al., (72.6%) and Aziz NA et al., (76.1%) [16,17]. A bit higher detection rate of 81.8% by LJ medium was reported by Somoskovi A et al., [18]. The isolation rate was also higher in smear positive samples than the smear negative samples. These slight variations in the sensitivity of LJ medium to detect TB may be due to the method and quality of sample collection or due to the difference in clinical case presentation.

In present study, the *Mycobacterium tuberculosis* detection rate was significantly higher (100.0%) for MGIT 960 than LJ culture media (70.7%) and ZN staining method (51.2%). Many other research articles have reported almost similar and significant higher detection rate of *Mycobacterium tuberculosis* by MGIT 960 than LJ culture media and ZN staining method [7,10-13]. Higher detection rate with MGIT 960 medium may be because of the less contamination rate with the MGIT 960 system [19,20]. However, there are studies who reported a lower detection rate of *Mycobacterium tuberculosis* by MGIT 960 [21-23]. Somoskovi A et al., reported a detection rate of 96.4% by MGIT 960 [18]. Few other studies reported a detection rate of 75.3%-95.1% for MGIT 960 culture medium [21-23].

In this study, the median TTD was 9.0 days and 33.0 days in MGIT 960 media and LJ media respectively. Mean rank of both LJ media culture and MGIT 960 culture method were significantly lower in sputum smear positive cases. The median TTD by both LJ media and MGIT 960 media was significantly lower in sputum smear positive samples. In present study, median TTD was 9 days for MGIT 960 culture media which was shorter than Tortoli E et al., (19.6 days) Somoskovi A et al., (15.8 days) [10,18]. Lower median TTD in present study might be due to less contamination, precise sample collection and inclusion of more clinically relevant cases. Lower mean TTD for sputum smear positive samples than smear negative samples were also reported by Almazini MAI et al., Aziz NA et al., Somoskovi A et al., and Piersimoni C et al., [16-18,24]. MGIT 960 culture medium is a rapid and more sensitive diagnostic test for *Mycobacterium tuberculosis* than the traditional ZN stain and LJ culture medium.

Limitation(s)

Limitation of the study includes non-probability sampling technique and failure to compare the MGIT 960 with other liquid culture medium like BACTEC12B.

CONCLUSION(S)

The use of liquid media (MGIT-960) is more accurate and rapid method for the diagnosis of TB than the conventional solid culture method using LJ media. This early and appropriate diagnosis of TB can aid in the usage of appropriate drugs and prevent the emergence of MDR-TB and hence reduce the overall morbidity and mortality due to the disease.

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