MYCOBACTERIUM GROWTH INDICATOR TUBE (MGIT) IN COMPARISON TO LOWENSTEIN JENSEN’S MEDIUM FOR ISOLATION OF MYCOBACTERIUM SPECIES FROM URINE SAMPLES.

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Abstract: The purpose of this study was to test the capability, efficiency, and reliability of the Mycobacteria Growth Indicator Tube (MGIT; Becton Dickinson) for the detection of mycobacteria in urine samples, for which the method had not yet been approved. The results were compared with those of the Löwenstein-Jensen (LJ) solid media in terms of recovery of mycobacteria from 307 consecutive urine specimens. The overall sensitivity for recovery of mycobacterium from urine specimens was 90.3% for the MGIT and 16.1% for LJ medium and this difference was found to be statistically significant. The mean time for positivity for MGIT was 19.4 days, while that for LJ medium was 43 days. However, despite the advantages and the higher sensitivity of the MGIT, solid media will still play a role in the recovery of mycobacterium from urine specimens as few of cases were picked by solid media alone. Therefore laboratories should continue to use a combination of liquid broth and solid media for the isolation of mycobacterium.

Keywords: Lowenstein Jensen’s Medium, MGIT, Mycobacterium, Tuberculosis, Urine.

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INTRODUCTION

The increasing incidence of pulmonary tuberculosis and other Mycobacterial diseases has made it essential for the laboratories to quickly detect and identify mycobacterium from human clinical specimens. Rapid, sensitive, and accurate detection of these organisms in clinical specimens can hasten the administration of appropriate anti-Mycobacterial therapy and prevent the spread of infection to susceptible contacts through the use of effective infection control practices. Conventional solid media, such as egg-based Lowenstein-Jensen (LJ) and agar-based Middlebrook 7H10 media, traditionally have been used for the recovery of mycobacterium from clinical materials, however, the slow rate of growth of many pathogenic Mycobacterium species on solid media can substantially delay the identification process. Broth media such as Middlebrook 7H9 have been developed to speed the growth and recovery rate of mycobacterium in the laboratory. With the advent of liquid culture media for the growth of mycobacterium, a variety of manual, semi-automated and automated systems have been developed specifically to reduce the time to detect and identify mycobacterium in clinical specimens. The BACTEC Mycobacterium Growth Indicator Tube (MGIT) is one of the recent methods designed for the rapid detection of mycobacterium in clinical specimens. The system consists of a culture tube containing modified Middlebrook 7H9 medium with a fluorescent growth indicator embedded in silicone on the bottom of each tube. This compound is sensitive to the presence of dissolved oxygen in the broth medium. MGIT, which function on the principle of fluorescence quenching had not been approved by FDA for isolation of Mycobacterium from urine due to non-availability of sufficient data. The aim of the study was to evaluate the clinical use of MGIT for the isolation of mycobacterium from urine samples.

Materials and Methods:

Study Design

One year prospective study (2010-2011) conducted in the department of Microbiology, DMC & H, Ludhiana (Punjab)

Study Protocol

All consecutive urine specimens submitted to the Dept. of Microbiology, for Ziehl-Neelsen (ZN) staining and mycobacterial culture, during the year 2010-11 were included in this study.

Culture medium inoculation, incubation and test duration

The urine was first centrifuged at 3000g for 10 min. A loopful of the resulting sediment was used to make the smear. The remaining sediment was decontaminated by the standard N-acetyl-L-cysteine and sodium hydroxide (3%) method. After 30 min, the suspension was centrifuged again and the sediment was suspended in 1–1.5 ml sterile phosphate buffer (pH 6.8). Exactly
0.5 ml of the digested, decontaminated, concentrated suspension was inoculated into the MGIT tube. Before inoculation, each MGIT tube was supplemented with the PANTA (polymyxin B, amphotericin B, nalidixic acid, trimethoprim, and azlocillin) antibiotic mixture and OADC (oleic acid-albumin-dextrose-catalase) enrichment solution (Becton Dickinson). The tube was recapped tightly, mixed well by inversion, and MGIT cultures were incubated for a total of 6 weeks and monitored daily for increase in fluorescence, before being reported as negative. Approximately 0.1 ml of the concentrated suspension was inoculated onto the LJ slant. The inoculated LJ slants were incubated at 37°C and examined weekly for a total of 8 weeks.10

All positive culture bottles were confirmed by ZN staining for the presence of acid-fast bacilli.

Results:

A total of 307 urine specimens were processed during the study period, and the growth in MGIT was compared with growth on conventional LJ medium. Each individual specimen was considered to represent an independent diagnostic event. The gold standard for the sensitivity and specificity analysis was taken as the total number of positives revealed by either of the method i.e. ZN smear/LJ culture/MGIT culture. Of the 31 positive urine specimens 5 were found to be smear positive leading to a smear positivity of only 16.1%. Out of these two were only smear positive and did not yield the growth of Mycobacterium by any of the culture methods. Comparison of mycobacteria detection by ZN staining and LJ media is depicted in Table1.

The isolation rate of Mycobacterium species using both LJ and MGIT culture was 93.5% (29/31). The overall sensitivity for recovery of mycobacterium from urine specimens was 90.3% (28/31) for the MGIT and 16.1% (5/31) for LJ medium and this difference was found to be statistically significant. ($\chi^2 = 22.728; p<0.001$) (Table 2)

The mean detection time for recovery of Mycobacterium species by MGIT and LJ slants was 19.4 days and 43 days respectively. This difference was found to be statistically significant. ($t = 5.246; p<0.001$) (Table 3)

Discussion

Out of 307 urine specimens processed, 31(10.1%) were positive for mycobacterium. However, Chan et al.10 and Hillemann et al.5 have reported a positivity of 4.1% and 2.6% from Singapore and Germany, respectively. Higher positivity rate in the present study could be due to the higher incidence of tuberculosis in India. Among rapid techniques of Mycobacterial culture MGIT has found its place as a
standard method for the isolation of mycobacterium in recent years.\textsuperscript{[7,12-14]} Since the introduction of the MGIT, it has been extensively evaluated but most of the data available with the recovery of mycobacterium is from pulmonary specimens.\textsuperscript{[6-7,9,15-16]} Recovery of mycobacterium from urine specimens has not been included in the claims from the manufacturers of MGIT, as not enough specimens were processed in the clinical trials.\textsuperscript{[5,10]} In order to test whether the MGIT is adequately applicable for the recovery of mycobacterium from urine specimens, we compared the MGIT with conventional solid media, i.e. LJ media. In our study we processed 307 specimens out of which 29 were positive for Mycobacterial culture (9.4%). Among the total culture positive, MGIT missed only (1/29) 3.4%, while solid media missed (24/29) 82.7%. The results of our study clearly demonstrated that MGIT system yields better recovery of \textit{Mycobacterium species} from urine specimens than did the traditional LJ slant. Theses observation are consistent with the findings of previous studies regarding the performance of the MGIT system.\textsuperscript{[5,10]}

Dongsi (2002) has reported more than 20% improvement in recovery of mycobacterium species using MGIT in comparison to traditional LJ slants.\textsuperscript{[1]}

In the present study though an increase in isolation rate of \textit{Mycobacterium species} was observed using MGIT, however, MGIT did not achieve 100% sensitivity and if the LJ slant had been eliminated from the Mycobacterial culture procedure, the diagnosis of 1 cases of Mycobacterium would have been missed. Based upon above findings authors also support the use of both liquid as well as traditional solid media for culture of \textit{Mycobacterium species} as recommended by others.\textsuperscript{[1,17]} In our study MGIT could detect \textit{Mycobacterium species} about 2 weeks prior to detection of growth on LJ media. This advantage would enable clinicians to establish a definitive diagnosis and institute appropriate therapy within the shortest possible time. Similar findings have also been obtained by Hillman and Chan\textsuperscript{[5,10]} from urine specimens. They have reported that growth of MAC was detected at least 2 weeks sooner and >80% of these isolates were detected within the first 4 weeks in liquid media compared with only 56% on LJ. The increases in the detection rate and decrease in the recovery time of \textit{Mycobacterium species} have also documented in respiratory specimens using MGIT.\textsuperscript{[18-20]}

Conclusions

To conclude, MGIT provide a suitable growth and detection method to culture \textit{Mycobacterium} species from urine specimen. Although the MGIT demonstrated better sensitivity than the traditional LJ slant for the recovery of \textit{Mycobacterium} species from urine specimens, our study indicated that few of the renal tuberculosis cases would be missed by the use of this system alone.
Therefore, instead of resorting to MGIT alone, laboratories should use a combination of liquid broth and solid media for the isolation of Mycobacterium species from urine specimens. MGIT has been proven to be dependable, highly efficient and rapid system for the recovery of Mycobacterium species.

Table 1. Comparison of mycobacteria detection by ZN staining and LJ Media.

<table>
<thead>
<tr>
<th></th>
<th>ZN Positive</th>
<th>ZN Negative</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>LJ Positive</td>
<td>3</td>
<td>2</td>
<td>5</td>
</tr>
<tr>
<td>LJ Negative</td>
<td>2</td>
<td>300</td>
<td>302</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>5</strong></td>
<td><strong>302</strong></td>
<td><strong>307</strong></td>
</tr>
</tbody>
</table>

Table 2. Comparison of mycobacterium recovery from MGIT and LJ media

<table>
<thead>
<tr>
<th></th>
<th>LJ Positive</th>
<th>LJ Negative</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>MGIT Positive</td>
<td>4</td>
<td>24</td>
<td>28</td>
</tr>
<tr>
<td>MGIT Negative</td>
<td>1</td>
<td>278</td>
<td>279</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>5</strong></td>
<td><strong>302</strong></td>
<td><strong>307</strong></td>
</tr>
</tbody>
</table>

Note: Statistical Analysis: $\chi^2 = 22.728; \ p<0.001$ (Significant)

Table -3: Comparison of mean detection time of Mycobacterium by MGIT and LJ media

<table>
<thead>
<tr>
<th></th>
<th>Positive</th>
<th>Mean Days±SD</th>
<th>Minimum (Day)</th>
<th>Maximum (Day)</th>
</tr>
</thead>
<tbody>
<tr>
<td>MGIT</td>
<td>28</td>
<td>19.43±9.69</td>
<td>9</td>
<td>42</td>
</tr>
<tr>
<td>LJ</td>
<td>5</td>
<td>43±5.38</td>
<td>35</td>
<td>48</td>
</tr>
</tbody>
</table>

Note: Statistical Analysis: $t = 5.246; \ p<0.001$ (Significant)
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