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A COMPARATIVE STUDY FOR THE DETECTION OF MYCOBACTERIA BY BACTEC MGIT 960, LOWENSTEIN JENSEN MEDIA AND DIRECT AFB SMEAR EXAMINATION

S Rishi, P Sinha, *B Malhotra, N Pal

Abstract

Purpose: To compare BACTEC MGIT 960 (M960) with conventional culture on Lowenstein Jensen (LJ) media and direct acid fast bacilli (AFB) smear examination for the detection of Mycobacteria in clinical samples obtained from suspected cases of pulmonary and extra pulmonary tuberculosis (TB).

Methods: A total of 500 samples were processed for direct AFB smear examination, and culture on M960 and LJ media.

Results: Two hundred fifty-eight out of 500 (51.6%) isolates of Mycobacteria were obtained by combined use of the two culture methods. Two hundred and fifty-three (50.6%) were positive in culture by M960 and LJ media and 28% (140/500) by direct AFB smear examination. The positivity rate of M960 system alone was 34.10% (88/258) and of LJ alone was 1.93% (5/258). Average time to detect growth (TTD) was 9.66 days by M960 and 28.81 days by LJ.

Conclusions: M960 system is a rapid and sensitive method for early diagnosis of pulmonary and extrapulmonary TB. But for maximum recovery of Mycobacteria, a combination of both M960 and LJ media should be used.

Key words: BACTEC MGIT 960, Lowenstein Jensen media

Tuberculosis (TB) is responsible for about one third of preventable deaths worldwide.1 The spread of HIV/AIDS and emergence of multiple drug-resistant TB have further contributed to the worsening impact of the disease.2 In India, 13 million people are infected and 3.5 million are positive for acid fast bacilli (AFB) with 2.2 million new TB cases being added every year.3

Although AFB microscopy and conventional Lowenstein Jensen (LJ) culture remain the cornerstone for the diagnosis of TB, the sensitivity of these traditional methods is quite low, especially in the samples containing small number of organisms.4 There is a need for rapid, sensitive and accurate detection of these organisms in clinical specimens to hasten the administration of appropriate antimycobacterial therapy and prevent the spread of infection in the community.5 A variety of manual and automated systems have been developed specifically to reduce the time to detect and identify Mycobacteria in clinical specimens.5

The present study was carried out to compare one such automated system, i.e., BACTEC MGIT 960 (M960) with conventional culture method, i.e., LJ media and direct AFB smear examination. M960 system is a fully automated, high capacity, non-radiometric, non-invasive instrument, which requires neither needles nor other sharp implements to incubate and monitor 960; 7 mL culture tubes.6 The culture tubes contain Modified middle brook 7H9 media with 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. As the microorganisms grow in the media, oxygen gets depleted, allowing the fluorescence to be detected automatically over time.5

Materials and Methods

A total of 500 clinical samples were obtained from suspected cases of TB from different wards and out patient departments of the SMS and other allied hospitals of SMS Medical College, Jaipur, during the year 2004. The samples included sputum (330), cerebrospinal fluid (49), pleural fluid (41), bronchial washings (29), pus (17), urine (8), ascitic fluid (7), endometrium (5), ovarian cyst fluid (4), gastric aspirate (2), lymph node aspirate (2), bone scrapings (1), discharge from sinus (1), granulation tissue (1), menstrual blood (1), stool (1), and tissue from sinus tract (1).

A BBL MGIT tube (from Becton Dickinson) containing 7 mL modified middle brook 7H9 broth was used. Lyophilized MGIT PANTA (containing polymyxin B, azlocillin, nalidixic acid, trimethoprim, amphotericin B) was reconstituted with MGIT growth supplement (containing oleic acid, albumin, dextrose, catalase, polyoxyethylene stearate), and 0.8 mL of this was added prior to sample inoculation to the M960 tube. LJ media was prepared in the laboratory.

Processing of samples

Within 24 h of receiving the samples, direct smear was made, stained by Ziehl Neelsen method and graded as per RNTCP guidelines. For the purpose of digestion and

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The positivity rate of M960 system alone was 34.10% (88/258) and of LJ alone 1.93% (5/258). Isolation rates of Mycobacteria in pulmonary and extrapulmonary samples were 61.83% (222/359) and 21.98% (31/141) by M960, 44.01% (158/359) and 4.96% (7/141) by LJ, respectively. One hundred and forty-two samples were from patients on anti-tubercular therapy. Out of these, 98.59% (140/142) were positive by M960, 69.01% (98/142) by LJ and 69.71% (99/142) by direct AFB smear examination. Details of isolation rates of Mycobacteria by M960 and LJ with respect to direct AFB smear results are shown in Table 1.

Table 1: Isolation rate of Mycobacteria in relation to acid fast bacilli smear

<table>
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<tr>
<th>Type of sample</th>
<th>ZN smear</th>
<th>LJ positive</th>
<th>%</th>
<th>Avg. TTD (in days)</th>
<th>M960 positive</th>
<th>%</th>
<th>Avg. TTD (in days)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pulmonary samples</td>
<td>Positive (137)</td>
<td>116/137</td>
<td>84.67</td>
<td>27.09 (7-48)</td>
<td>135/137</td>
<td>98.54</td>
<td>6.79 (2-30)</td>
</tr>
<tr>
<td></td>
<td>Negative (222)</td>
<td>42/222</td>
<td>18.91</td>
<td>30.02 (14-48)</td>
<td>87/222</td>
<td>39.18</td>
<td>13.35 (3-32)</td>
</tr>
<tr>
<td>Extra pulmonary</td>
<td>Positive (3)</td>
<td>2/3</td>
<td>66.66</td>
<td>28 (28)</td>
<td>3/3</td>
<td>100</td>
<td>8.33 (4-14)</td>
</tr>
<tr>
<td>samples</td>
<td>Negative (138)</td>
<td>5/13 8</td>
<td>3.62</td>
<td>32.5 (7-48)</td>
<td>28/138</td>
<td>20.28</td>
<td>17.89 (4-39)</td>
</tr>
</tbody>
</table>

Avg. TTD - average time to detection of growth, Z.N - Ziehl Neelsen, LJ - Lowenstein Jensen media

Discussion

The present study demonstrated that M960 system provided better isolation rate of Mycobacteria (98.06%) from a variety of clinical samples than the LJ media (63.95%). Various authors have reported similar findings ranging from 80 to 100% for M960 and from 59.7 to 87.2% for LJ. In our study, isolation rate by M960 system was 34.11% more than that by LJ method. The additional positivity was seen both in pulmonary and extrapulmonary samples, not only in smear negative but in smear positive samples also. There was a low positivity rate shown by LJ method in our study in comparison to around 69-87.2% reported in literature. This could be because of the fact that samples that were grossly contaminated on LJ were considered negative, whereas in M960, since the smears were made from all instrument positive MGIT tubes, it was found that there were samples, which had both contaminants, as well as Mycobacteria grown in them. Such tubes were considered positive by M960.

Besides higher isolation rate, even the time to detect Mycobacteria was shorter on M960 than on LJ, average being 9.66 days (2-39) with M960 and 28.81 days (7-48) with LJ media. Similar findings have been reported in the literature. However in our study, time to detect Mycobacteria by M960 was lesser than that reported by others (11.6-14.4 days). This is probably because large number of samples (28%) were smear positive and incidence of TB is also higher in our country.

Time to detect Mycobacteria was directly related to the grade of positivity in direct AFB smear examination as seen in Table 2. In smear positive samples, growth of Mycobacteria was detected in average 6.79 days (2-30) by M960.
The break through contamination rates in our study (13.4% for M960 and 27.2% for LJ) were higher in comparison to other studies (3.7-10% by M960 and 1.2-21.1% by LJ).6,8,11,12 This may be attributed firstly to the hot climatic conditions in our country and secondly to the longer transport time of the specimen to the laboratory in some cases, leading to overgrowth of the contaminants.

Thus, M960 was found to be most rapid and efficient system to isolate Mycobacteria. However, for maximum recovery of Mycobacteria, it is important to use both types of media as 1.93% isolates could be detected by LJ only.

As Mycobacteria can be isolated on an average within a week in smear positive samples, the antibiotic susceptibility tests can then be done and results would be available in another 4-13 days (as per BD protocol). This is in contrast to the time taken by LJ method, average 29 days for growth and another 30 days for sensitivity tests. This has great implications in monitoring the treatment failure and multidrug resistant TB patients. Though the cost of M960 culture system is approximately 8-10 times more than that for LJ media, the sensitivity and rapidity of the system are the major advantages that benefit not only the individual patient but also the community by controlling the disease and ultimately its transmission in the society.

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