Comparative Evaluation of the BACTEC MGIT 960 (Mycobacteria Growth Indicator Tube) System with LJ Solid Medium for Diagnosis of Pulmonary Tuberculosis

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ABSTRACT

Tuberculosis (TB) is responsible for about one third of preventable deaths worldwide. Accurate and rapid diagnosis of tuberculosis is essential for controlling the spread of the disease caused by Mycobacterium tuberculosis. The aim of this study was to evaluate the Mycobacteria Growth Indicator Tube (MGIT 960 (M960)) which is a fully automated, non-invasive, system for growth and detection of mycobacteria and Lowenstein-Jensen (LJ) media for the recovery of Mycobacterium tuberculosis from sputum samples. Out of 67 specimens processed, 60 isolates (89.5%) were recovered by M960 media while, 50 isolates (74.6%) were obtained by LJ. M960 as a single system detected 11 (16.4%) isolates more than LJ media while; LJ media detected 2 (2.9%) isolates (revealed no growth in MGIT 960). In total, Out of these 67 specimens, 48 (71.6%) were positive by all methods (Z-N smear, culture on both LJ and MGIT broth) and 48 (71.6%) isolates also were obtained by the combined use of both culture methods. Average detection time of M960 and LJ media was 14.6 days (2-38) and 30.6 days (7-58) respectively. The contamination rates in our study were (2.9 %) for M960 and (1.49 %) for LJ media. In conclusion, comparable to LJ media, M960 system is a rapid and efficient method for diagnosis of pulmonary tuberculosis. But for maximum recovery of mycobacteria, a combination of both M960 and LJ media should be used.

Keywords: Mycobacterium Tuberculosis, MGIT, LJ

INTRODUCTION

It was estimated that, the global pandemic of tuberculosis (TB) have caused disease in 8 million and killed 1.6 million persons in 2006.1 TB is an increasing health problem worldwide, especially in developing countries. The spread of HIV/AIDS and emergence of multiple drug-resistant TB have further contributed to the worsening impact of the disease.2 For detection of mycobacteria in a clinical specimen, smear microscopy is by far the most popular among all the methods currently employed worldwide. Culture of the etiologic agent remains the accepted "gold standard" for diagnosing mycobacterial infections.3 However, cultivation on solid media, such as that of Lowenstein-Jensen (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

Recently, nonradiometric liquid culture media such as that used in the Mycobacteria Growth Indicator Tube (MGIT; Becton Dickinson) system have been introduced and extensively evaluated.6 MGIT 960 (M960) system is a fully automated, high capacity, non-radiometric, non-invasive instrument, used to incubate and monitor 960; 7 mL culture tubes.7

The present study was carried out to compare such automated system, MGIT 960 (M960) with conventional culture method, LJ media.

MATERIAL AND METHODS

In this study, Sixty seven sputum specimens were collected from patients with newly diagnosed cases of active pulmonary tuberculosis, (12 females and 55 males), were selected from Sohag university and chest hospitals.

Each patient was subjected to full history taking, clinical examination, radiological examination and bacteriological study including: microscopic examination of Ziehl-Neelsen stained smears, culture on Lowenstein-
Jensen media (LJ), and liquid medium \{Mycobacteria Growth Indicator Tube (MGIT) (M960)\} followed by biochemical Identification of isolates.

**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 using the MycoPrep specimen digestion/decontamination kit (BBL MycoPrep, Becton Dickinson, and BD). 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 min. The mixture was completed to double its volume with sterile phosphate buffer pH 6.8 and centrifuged at 3000 x.g for 15 min. The supernatant was decanted and the sediment was used for AFB microscopy (Ziehl-Neelsen stain, which is the routine method in our laboratory), and for cultures \{one solid medium (Löwenstein-Jensen) and one liquid medium (Mycobacteria Growth Indicator Tube (MGIT) (BACTEC MGIT 960, Becton Dickinson)\}).

(i) **MGIT 960.**
The MGIT 960 culture tubes contain 7 ml of Middlebrook 7H9 broth base, to which an enrichment supplement-ADC was added according to the instructions of the manufacturer, as well as a mixture of antibiotics consisting of polymyxin B, amphotericin B, nalidixic acid, trimethoprim, and azlocillin (MGIT PANTA). After inoculation of each tube with 0.5 ml of the processed specimen, the tubes were entered into the MGIT 960 instrument. The vials were incubated at 37°C and were monitored automatically every 60 min for increase in fluorescence for a maximum of 6 weeks. Any sample, which showed signal of growth, was removed from the instrument. From the positive tube, a smear was prepared for examination of AFB. Furthermore, a blood plate was inoculated and a subculture was made on an LJ slant.

(ii) **Solid media.**
For inoculation onto conventional solid LJ slant, 0.1 ml of the suspension was used for it. Solid media were incubated at 37°C in a slant position for 24-48 hours, then in upright position for a further 8 weeks and read daily for the 1st week then twice weekly. An individual medium was considered positive upon appearance of colonies on the surface, confirmed by positive AFB smear.

**Identification procedures:**
Smears from suspected colonies were stained with Z.N. stain for acid-alcohol fast bacilli. All mycobacterial isolates were identified using conventional methods of identifications (based on their rate of growth and pigmentation on LJ) and on biochemical tests as niacin accumulation, nitrate reduction and sensitivity to thiophene-C carboxylic acid hydrazide (TCH).

**RESULTS**
In the present study, 67 sputum specimens revealed 53 positive Z-N smears.
Out of these 67 specimens processed, 60 isolates (89.5%) were recovered (positive culture) by M960 media (50 of them were smear positive) while, 51 isolates (76.1%) were recovered by LJ media (all of them were smear positive). (Table 1)
The isolated strains were identified as being *M. tuberculosis* according to their rate of growth (slow growers), pigmentation (no pigment production), niacin accumulation (niacin positive), nitrate reduction (nitrate positive) and sensitivity to thiophene-C carboxylic acid hydrazide (TCH) (insensitive to TCH).
No isolates were recovered from the 14 negative smear specimens by L.J media while, 9 isolates were grown using M960 media. Three smear negative specimens were also culture negative by both methods.
In total, Out of these 67 specimens, 48 (71.6%) were positive by all methods (Z-N smear, culture on both LJ and MGIT broth) and 48 (71.6%) isolates also were obtained by the combined use of both culture methods. (Table 2)
The detailed comparison of both culture methods showed that MGIT as a system detected 12 (17.9%) isolates more than LJ media, while, LJ media detected only 2 (2.9%) isolates (not recovered from MGIT 960). (Table 3)
We found that detection time was significantly shorter in the MGIT than in the LJ. Moreover, detection time was significantly shorter in smear positive specimens than in smear negative ones for both LJ and M960.
Average periods for the detection of *M. tuberculosis* for M960 and LJ medium were 14.6 days (2-38) with M960 and 30.6 days (7-58) with LJ media respectively.
The mean detection time for MGIT was 14.7±9.6 days and for LJ 30.6±13.1 days.
Unpaired t test was used for comparison and detected extremely significant difference with p value < 0.0001.

The contamination rates in our study were 2 media (2.9 %) for M960 and 1 medium (1.49 %) for L.J.

Chi square test was used to compare the negative and positive results between M960 and LJ. We found a significant statistical difference with P value = 0.04. (Table 4)

Table 1: Summary of culture results:

<table>
<thead>
<tr>
<th>Parameter</th>
<th>No. of Samples</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total samples tested</td>
<td>67</td>
</tr>
<tr>
<td>Negative cultures for M. tuberculosis</td>
<td>5</td>
</tr>
<tr>
<td>Positive cultures for M. tuberculosis</td>
<td>62</td>
</tr>
</tbody>
</table>

Table 2: Combination of M. tuberculosis positivity in the various mycobacterial diagnostic tools included in this study:

<table>
<thead>
<tr>
<th>Smear</th>
<th>M960</th>
<th>L. J media</th>
<th>Number</th>
</tr>
</thead>
<tbody>
<tr>
<td>N</td>
<td>N</td>
<td>N</td>
<td>3</td>
</tr>
<tr>
<td>P</td>
<td>P</td>
<td>P</td>
<td>48</td>
</tr>
<tr>
<td>P</td>
<td>N</td>
<td>P</td>
<td>2</td>
</tr>
<tr>
<td>N</td>
<td>P</td>
<td>N</td>
<td>9</td>
</tr>
<tr>
<td>P</td>
<td>P</td>
<td>N</td>
<td>3</td>
</tr>
</tbody>
</table>

N: Negative    P: Positive
M960: (MGIT 960) Mycobacteria growth indicator tube 960, LJ: Löwenstein-Jensen

Table 3: Comparison of the results between M960 and LJ

<table>
<thead>
<tr>
<th>Parameter</th>
<th>M960</th>
<th>L. J</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Positive</td>
<td>60</td>
<td>50</td>
<td>0.04</td>
</tr>
<tr>
<td>Negative</td>
<td>7</td>
<td>17</td>
<td></td>
</tr>
</tbody>
</table>

Table 4: Detailed results of M960 compared to L J in smear positive and smear negative specimens.

<table>
<thead>
<tr>
<th>Smear results</th>
<th>n</th>
<th>LJ culture results</th>
<th>M960 results</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Positive</td>
</tr>
<tr>
<td>Smear positive</td>
<td>53</td>
<td>Positive</td>
<td>48</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Negative</td>
<td>2</td>
</tr>
<tr>
<td>Smear negative</td>
<td>14</td>
<td>Positive</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Negative</td>
<td>2</td>
</tr>
</tbody>
</table>

DISCUSSION

The present study demonstrated that M960 system provided better isolation rate of M. tuberculosis (89.5%) from the clinical samples than LJ media (74.6%).

In comparison to other studies, various authors have reported similar findings ranging from 80 to 100% for M960 and from 59.7 to 87.2% for LJ. 7,9

While other studies revealed high positivity rate for LJ in comparison to M960 as out of 75 clinical specimens which were found positive for AFB in smear microscopy, 73 were grown in M960, and 74 on the LJ slants.10

Other studies revealed very low positivity rate for LJ in comparison to M960 (the positivity rate of M960 system was 34.10% (88/258) and of LJ was 1.93% (5/258). It was explained that 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.

Others reported a very low positivity isolation rate for both M960 and LJ (19.5%) for M960 and (15.1%) for LJ.

In our study, the expected reasons for low isolation rate by LJ culture could be explained due to many factors as the inoculums size, the quality of the media, and technical experience.

Average periods for the detection of \( M. \) \( tuberculosi \) for M960 and LJ medium were 14.6 days (2-38 days) with M960 and 30.6 days (7-58 days) with LJ media respectively.

In our study, besides high isolation rate, we found that detection time was significantly shorter in the M960 than in the LJ. Moreover, detection time was significantly shorter in smear positive specimens than in smear negative for both LJ and M960.

Various studies have reported similar findings. Rishi and his colleagues reported that average time to detect growth was 9.66 days by M960 and 28.81 days by LJ, while, Zwolska et al. estimated that detection mean time of mycobacterial growth in smear-positive specimens were 15.4 days for M960 and 28.2 days for LJ medium.

Others found that the times to detection obtained with the M960 system were 13.3 days while that with LJ medium were 25.6 days.

Moreover, Chan and his group found that the mean time of positivity for M960 was 19.3 days while that for LJ medium was 35 days.

Our results revealed higher average periods in comparison to other studies which estimated lower average periods for the detection of mycobacteria (10.9 days and 19.4 days) for MGIT systems and LJ medium respectively.

The overall contamination rates in our study were 2.9 % for M960 and 1.49 % for LJ. Other studies reported a contamination rate ranging from 3.7 to 10% for M960 and 1.2 to 21.1% for LJ.

We also agree with other study which concludes that the rates of contamination were: 3.8% for MGIT 960 and 2.9% for LJ.

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.

To avoid a high contamination rate, we propose an intensification or repetition of the decontamination process.

Thus, M960 was found to be rapid and efficient system to isolate \( M. \) \( tuberculosi \). However, for maximum recovery of \( M. \) \( tuberculosi \), it is important to use both types of media as 2.9 % isolates could be detected by LJ only.

REFERENCES


مقارنة تقييمية لجهاز أنبوب دليل النمو لميكروب الدرن (BACTEC MGIT 960) وطريقة الزراعة على المزارع الصلب (وسط لوينستين جنسن الصلب) للتشخيص حالات الدرن الرئوي

ندا عبد الغني، هاني أحمد مرسى، إيمان نبت، أقسام الميكروبولوجيا الطبية والمناعة، البيئولوجيا الإكلينيكية، الأطفال، الأمراض الباطنة، كلية طب سوهاج، جامعة سوهاج

يشكل مرض الدرن مشكلة صحية واجتماعية كبيرة في العالم ومختلف الدول النامية، ويعتبر مرض الدرن مرضًا معد يسببه جراثيم تدعى الميكرون، مثل مصيدة عصبات صادمة الحمض (AFB) أو مزعة عصبات السل، على وسط لوينستين جنسن الصلب. هذه الدراسة هي تقييم ومقارنة طريقة وسط لوينستين جنسن الصلب لزراعة عصبات السل بطريقة أنبوب دليل النمو (BACTEC MGIT 960) أوتروماتيكية الجهاز، وهي متعددة على محساسة من قاع أنبوب المزودة، التي تصبح متقدمة إذا استهدفت الأجسام من الوسط خلال نمو البكتريا وينتقل فيها الجهاز أوتروماتيكية بالتنبؤ إلى العينات الإنجابية. وذلك لعزل ميكروبات الدرن من الصلاف في حالات الدرن الرئوي. وقد أظهر الدراسة أن 27 عينة نجا، واستخدمت في الدراسة تم عزل 40 عينة في حالة من العينات (angled على مكينة) وتم عزل 16 عينة درنية (ميكروب الدرن) بطريقة أنبوب دليل النمو لميكروب السل (BACTEC MGIT 960) بوسط لوينستين جنسن الصلب، كما تم عزل 11 عينة درنية بطريقة أنبوب دليل النمو لميكروب السل لم تنمو على وسط لوينستين جنسن الصلب. وقد بلغ عدد العينات التي أظهرت نمو ميكروبات الدرن بواسطة استخدام الطرق الثلاثة معاً (مزعة عصبات صادمة الحمض (AFB) أو مزعة عصبات السل على وسط لوينستين جنسن الصلب وطريقة أنبوب دليل النمو لميكروب السل) 48 عينة (%71.4) وأيضاً استخدمت هذه الدراسة أن متوسط الوقت المطلوب لميكروب السل لم تنمو على وسط لوينستين جنسن الصلب (301 يوم) بينما استغرقت (141 يوم) بطريقة أنبوب دليل النمو لميكروب السل. وقد بلغ متوسط لقاح المزارع السائل (طريقة أنبوب دليل النمو لميكروب السل) أثناء فترة الزرع إلى 25% من إجمالي العينات بينما بلغ 44% من إجمالي الزراعة على المزارع الصلب. وخلاصة تظهر تتيح طريقة أنبوب دليل النمو لميكروب السل طريقة فعالة وسريعة لعزل ميكروبات الدرن مقارنة بطريقة الزراعة على المزارع الصلب. ويدعو باستخدام الطرقين مجتمعًا للحصول على أفضل نتيجة لعزل الميكروبات.