Comparison of the Automated Mycobacteria Growth Indicator Tube System (BACTEC 960/MGIT) With Löwenstein-Jensen Medium for Recovery of Mycobacteria From Clinical Specimens

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Abstract

We examined whether the BACTEC/Mycobacteria Growth Indicator Tube (MGIT) system alone could supplant the use of a supplemental Löwenstein-Jensen (LJ) slant for routine recovery of Mycobacterium species from clinical specimens. A total of 6,062 specimens were included in the study. Of these, 273 specimens were positive for 278 mycobacterial isolates while 15 specimens were smear positive but culture negative using both media. Further analysis showed that 143 (51.4%) of the 278 total isolates were recovered from both the MGIT and LJ media. An additional 106 isolates (38.1%) were recovered from the MGIT only, while 29 (10.4%) isolates grew only on the LJ slant. The overall sensitivities of the MGIT and LJ media were 86.5% and 59.7%, respectively, for the recovery of mycobacteria from clinical materials. This study shows that although the MGIT system demonstrates better sensitivity for the recovery of mycobacteria from clinical specimens, both media types are necessary to maximize the sensitivity of detection.

Key Words: Mycobacteria; Löwenstein-Jensen (LJ) slant; MGIT; BACTEC 960

The clinical microbiology laboratory has a critical role in the detection and control of infection caused by clinically significant Mycobacterium species, such as Mycobacterium tuberculosis complex and Mycobacterium avium complex (MAC) organisms. Rapid, sensitive, and accurate detection of these organisms in clinical specimens can hasten the administration of appropriate antimycobacterial therapy and prevent the spread of infection to susceptible contacts through the use of effective infection control practices. Conventional solid media, such as the egg-based Löwenstein-Jensen (LJ) and agar-based Middlebrook 7H10 media, traditionally have been used for the recovery of mycobacteria 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 mycobacteria in the laboratory.

A variety of manual and automated systems have been developed specifically to reduce the time to detect and identify mycobacteria in clinical specimens. Examples of the manual approach include the biphasic Septi-Chek AFB (Becton Dickinson, Sparks, MD) and the MB-Redox (Biotest AG, Dreieich, Germany) systems. Advances in automation and novel growth detection methods have led to the development of the radiometric BACTEC 460TB (Becton Dickinson), the fluorometric BACTEC MB9000 and BACTEC MGIT (Mycobacteria Growth Indicator Tube) 960 systems (Becton Dickinson), the carbon dioxide–sensing MB/BacT ALERT 3D System (Organon Teknika, Durham, NC), and the pressure-sensing ESP Culture System II (Trek Diagnostic Systems, Westlake, OH).
The BACTEC MGIT 960 system is one of the more recent automated detection systems designed for the rapid detection of mycobacteria in all types of clinical specimens except blood. 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. Clinical specimens are processed and inoculated into the MGIT tube. As microorganisms grow, the oxygen in the medium is depleted with a subsequent increase in the fluorescence of the indicator. The enhanced fluorescence in tubes can be monitored automatically over time using the BACTEC 960 detection module. The difference between the BACTEC MB9000 system and the BACTEC MGIT 960 system is mainly in total specimen capacity.

Recently, Sharp et al\(^5\) reported that mycobacterial cultures using the BACTEC MB9000 system alone provided for the recovery of more than 99.7% of clinically relevant isolates without the need for supplemental solid media such as LJ media. The present study was initiated to determine whether the BACTEC MGIT 960 system was equally sensitive for the recovery of mycobacteria from clinical samples, thereby eliminating the need for supplemental solid medium.

Materials and Methods

All clinical specimens submitted to Barnes-Jewish Hospital Microbiology Laboratory (St Louis, MO) for culture of mycobacteria from December 1999 through January 2001 were included in the study. All contaminated specimens, such as sputum, bronchial washings, and bronchial alveolar lavages, were digested and decontaminated within 24 hours of receipt. Tissue specimens (unless from a sterile site) were homogenized before decontamination and digestion. Other specimens from presumed sterile sites were inoculated directly to growth media after concentration and digestion. Other specimens from presumed sterile sites were inoculated directly to growth media after concentration by centrifugation. All specimens were examined for the presence of acid-fast bacilli using the auramine-rhodamine stain.\(^6\)

Processing Specimens With Bacterial Contamination

Decontamination and digestion of contaminated specimens was performed essentially as previously described.\(^7\) Briefly, an equal volume of MycoPrep reagent (BBL, Cockeysville, MD) containing N-acetyl-L-cysteine and sodium hydroxide was added to each specimen in a 50-mL conical centrifuge tube. The suspension then was mixed for 20 seconds and permitted to stand for 15 minutes. If the specimen was on a swab, it was removed at this point. Phosphate buffer (0.067-mol/L concentration, pH 6.8) was added to make a final volume of 40 mL with adequate mixing to stop the digestion process. The tubes were centrifuged for 20 minutes at 3,000g. The supernatant fluid was decanted carefully, and the sediment was retained. The sediment was suspended in 1 to 3 mL of sterile phosphate buffer, pH 6.8.

Media Inoculation and Smear Preparation

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 (Becton Dickinson). Exactly 0.5 mL of the digested, decontaminated, concentrated suspension was inoculated into the MGIT tube. The tube was recapped tightly, mixed well by inversion, and placed into the BACTEC 960 detection module. MGIT cultures were incubated for a total of 6 weeks before being reported as negative. Approximately 0.1 mL of the specimen then was inoculated onto the LJ slant. The inoculated LJ slants were incubated in carbon dioxide at 37°C for a total of 8 weeks. For skin, superficial abscesses, ulcers, extremities, or other superficial wounds or tissues, a second set of media was inoculated (LJ, MGIT tube, and chocolate slant) and incubated at 30°C in 8% to 10% carbon dioxide with humidity. The LJ tubes were examined twice weekly for 6 weeks and then at 8 weeks before discarding. When growth was detected, smears were prepared and stained using the Kinyoun carbolfuchsin stain to determine the presence or absence of acid-fast bacilli.

Cultures positive for acid-fast bacilli isolates were evaluated for the presence of *M tuberculosis* complex– or MAC-specific sequences by probe hybridization (AccuProbe, Gen-Probe, San Diego, CA). *Mycobacterium* species other than *M tuberculosis* complex or MAC were forwarded to the Missouri Department of Health Mycobacteriology Laboratory (Mt Vernon, MO) for identification.

Statistical Analysis and Recovery Comparison

Exact McNemar \(P\) values were calculated using STATA release 7.0 statistical software (College Station, TX). For the purpose of this study, the sensitivity of a culture method was defined as the percentage of total mycobacterial isolates (MGIT and LJ slants) recovered by a single culture method (MGIT or LJ slants).

Results

During the study period, a total of 302 of 6,062 specimens were positive for acid-fast organisms by smear, culture, or both. The majority (78.1%) of specimens were from respiratory tract sources, including sputum, bronchial washings, tracheal aspirates, bronchoalveolar lavages, and transbronchial
biopsies. Non–respiratory tract specimens included tissue (lymph node, lung, liver, skin and soft tissue, bone, bone marrow, and wound; n = 42), urine (n = 4), stool (n = 7), blood (n = 9), drainage from the chest wall and an unspecified site (n = 2), and pleural fluid (n = 2). Fourteen specimens were contaminated by bacterial overgrowth (4 MGIT and 10 LJ slant) and were excluded from the study. Of the remaining 288 positive specimens, 273 produced 278 mycobacterial isolates in one or both media, while 15 specimens were smear-positive only.

Of the 278 mycobacterial isolates, 143 (51.4%) were recovered from both MGIT and LJ media, while an additional 106 (38.1%) and 29 (10.4%) were recovered only from MGIT or LJ medium, respectively. The overall sensitivity for recovery of mycobacteria from clinical specimens was 86.5% for the MGIT and 59.7% for LJ medium (P < .0001) Table 1. Of the 278 positive cultures, 65 (23.4%) and 105 (37.8%) were identified as *M. tuberculosis* complex and MAC, respectively, by probe hybridization (Gen-Probe). *M. tuberculosis* complex was recovered from both MGIT and LJ media in 44 (68%) of 65 positive cultures, while 17 (26%) and 4 (6%) of the 65 cultures positive for *M. tuberculosis* complex were recovered from MGIT or LJ medium alone, respectively. The calculated sensitivities of MGIT and LJ media for the recovery of *M. tuberculosis* complex were 94% and 74%, respectively (P = .0072). MAC was isolated from 56 (53.5%) of 105 positive cultures from both MGIT and LJ media, while 36 (34.3%) and 13 (12.4%) of the 105 positive cultures were obtained only from MGIT or LJ medium, respectively. The calculated sensitivities of MGIT and LJ media for the recovery of MAC were 87.6% and 65.7%, respectively (P = .0014) Table 2.

The remainder of the 108 mycobacterial isolates included *Mycobacterium kansasi* (n = 31), *Mycobacterium gordonae* (n = 27), *Mycobacterium fortuitum* (n = 18), *Mycobacterium mucogenicum* (n = 13), *Mycobacterium cheloneae* (n = 12), *Mycobacterium lentiflavum* (n = 2), *Mycobacterium xenopi* (n = 1), *Mycobacterium marinum* (n = 2), *Mycobacterium terrae* (n = 1), and *Mycobacterium flavescens* (n = 1) species. Of these, 43 were recovered from both MGIT and LJ media, while 53 and 12 isolates were recovered from MGIT or LJ medium alone, respectively. The overall sensitivities for the recovery of *Mycobacterium* species other than *M. tuberculosis* complex and MAC were 89% and 51% for MGIT and LJ, respectively (P < .0001).

### Discussion

The results of this study demonstrated that the MGIT system consistently provided better recovery of all *Mycobacterium* species from a variety of clinical specimens than did the traditional LJ slant. This observation is consistent with the findings of previous studies regarding the performance of the MGIT system. Indeed, the MGIT system provided a 20% or greater improvement in total recovery rate for all mycobacteria (249 vs 172 of 278), *M. tuberculosis* complex (61 vs 48 of 65), MAC (92 vs 69 of 105), and miscellaneous *Mycobacterium* species (96 vs 55 of 108) over a traditional LJ slant.

However, the MGIT system did not achieve 100% sensitivity for the isolation of *M. tuberculosis* complex and MAC organisms. Six percent of total *M. tuberculosis* complex isolates and 12% of total MAC isolates were recovered only from the LJ slant, while the MGIT remained negative for growth after 6 weeks of incubation. Of the 13 MAC isolates detected by LJ medium alone, 6 (46%) were isolated from patients with a primary diagnosis of MAC disease. If the LJ slant had been eliminated from the mycobacterial culture

### Table 1

Comparison of Mycobacteria Recovery From the BACTEC 960 Mycobacteria Growth Indicator Tube System* and Löwenstein-Jensen Culture Medium

<table>
<thead>
<tr>
<th>Mycobacteria Growth Indicator Tube</th>
<th>Positive</th>
<th>Negative</th>
<th>Total Isolates</th>
</tr>
</thead>
<tbody>
<tr>
<td>Löwenstein-Jensen</td>
<td>143</td>
<td>29</td>
<td>172</td>
</tr>
<tr>
<td>Negative</td>
<td>106</td>
<td>15</td>
<td>121</td>
</tr>
<tr>
<td>Total isolates</td>
<td>249</td>
<td>29</td>
<td>278</td>
</tr>
</tbody>
</table>

* For proprietary information, see the text.

### Table 2

Mycobacterial Isolates Detected by the BACTEC 960 Mycobacteria Growth Indicator Tube (MGIT)* and Löwenstein-Jensen (LJ) Methods

<table>
<thead>
<tr>
<th>Organism</th>
<th>Total No. of Isolates</th>
<th>MGIT and LJ</th>
<th>MGIT Alone</th>
<th>LJ Alone</th>
<th>P (MGIT vs LJ)</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Mycobacterium tuberculosis</em> complex</td>
<td>65</td>
<td>44 (68)</td>
<td>17 (26)</td>
<td>4 (6)</td>
<td>.0072</td>
</tr>
<tr>
<td><em>Mycobacterium avium</em> complex</td>
<td>105</td>
<td>56 (53.3)</td>
<td>36 (34.3)</td>
<td>13 (12.4)</td>
<td>.0014</td>
</tr>
<tr>
<td>Other</td>
<td>108</td>
<td>43 (39.8)</td>
<td>53 (49.1)</td>
<td>12 (11.1)</td>
<td>&lt;.0001</td>
</tr>
<tr>
<td>Total</td>
<td>278</td>
<td>143 (51.4)</td>
<td>106 (38.1)</td>
<td>29 (10.4)</td>
<td>&lt;.0001</td>
</tr>
</tbody>
</table>

* For proprietary information, see the text.
procedure, the diagnosis of 17 cases of *M tuberculosis* complex or MAC would have been missed.

In this study, 15 specimens were smear-positive and culture-negative in either MGIT or LJ media. Nine of these specimens were obtained from patients with previous positive cultures who were currently receiving antimycobacterial therapy, presumably explaining the false-negative culture results.

Although the automated MGIT system demonstrated better sensitivity than the traditional LJ slant for the recovery of mycobacteria from clinical specimens, our study indicated that approximately 10% of clinically significant *Mycobacterium* species would be missed by the use of this system alone. The MGIT system is not yet sufficiently sensitive to warrant the elimination of supplemental solid media.

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References


