Comparison of the MB/BacT and BACTEC 460 TB Systems for Recovery of Mycobacteria from Various Clinical Specimens

FRANCESA BRUNELLO,¹ FLAVIO FAVARI,¹ AND ROBERTA FONTANA¹,²*

Servizio di Microbiologia dell’ Azienda Ospedaliera di Verona,¹ and Istituto di Microbiologia dell’Università,² Verona, Italy

Received 27 July 1998/Returned for modification 2 September 1998/Accepted 6 January 1999

A total of 1,830 specimens (75.7% respiratory and 24.3% nonrespiratory) were cultured in parallel with the MB/BacT and BACTEC 460 TB systems and on Lowenstein-Jensen (LJ) medium. Mycobacteria were identified from 173 (6.5%) specimens. The most common species recovered were Mycobacterium tuberculosis complex (65.9%), Mycobacterium avium complex (22.5%), and Mycobacterium chelonae (9.2%). The recovery rates by individual systems were 96.5, 99.4, and 95.9% for MB/BacT, BACTEC 460 TB, and LJ medium, respectively, for all mycobacteria; the recovery rates were 99.1, 100, and 98.2%, respectively, for M. tuberculosis complex alone. The difference among the recovery rates for all mycobacteria and those for individual species was not significant. The BACTEC 460 TB system detected M. tuberculosis isolates more rapidly than the MB/BacT system (8 versus 11.8 days for smear-positive specimens [P < 0.01] and 18 versus 21 days for smear-negative specimens [P < 0.05]), whereas the MB/BacT system more rapidly detected the nontuberculous mycobacteria (17.1 versus 12.7 days [P < 0.01]). These results indicate that the nonradiometric MB/BacT system is a rapid, sensitive, and efficient method for the recovery of M. tuberculosis and nontuberculous mycobacteria from both pulmonary and extrapulmonary clinical specimens.

The continuing global threat of tuberculosis has led to an urgent need to design more effective diagnostic procedures and develop improved antituberculosis therapies (2, 3, 19). Despite the introduction of DNA techniques which have provided a new approach to the rapid diagnosis of diseases caused by mycobacteria, the definitive diagnosis of tuberculosis continues to depend on cultures of the microorganisms (3, 17). Recently, turnaround times not exceeding 21 days for the isolation and identification of Mycobacterium tuberculosis complex were recommended (1, 13, 14).

A significant advance in culturing methods was obtained with the introduction of a rapid radiometric mycobacterial detection system (BACTEC 460 TB; Becton Dickinson Diagnostic Instruments Systems, Sparks, Md.). The use of this method has reduced the average detection time of both smear-positive and -negative samples by nearly 50% (5, 6, 11). The BACTEC 460 TB system has also been adapted to differentiate M. tuberculosis from other mycobacteria, as well as to perform antimicrobial susceptibility testing, particularly of M. tuberculosis.

Recently, several new growth-based strategies have been developed for the rapid detection of mycobacteria which circumvent the main disadvantage of the radiometric system, which is the disposal of radioactive wastes. Among these are the Mycobacteria Growth Indicator Tube (MGIT) 960 and BACTEC 9000 MB (both marketed by Becton Dickinson), which use a fluorescence-based detection system (8, 9, 10, 16); the ESP culture system II (AccuMed, Chicago, Ill.), based on the detection of pressure changes by gas production or consumption due to microbial growth (15, 18); and the MB/BacT system (Organon Teknika, Turnhout, Belgium), which relies on a colorimetric CO2 detection device to indicate mycobacterial growth in a closed system (12). All are fully automated technologies which provide the almost continuous monitoring of bacterial growth. This avoids the cumbersome handling of vials during incubation, which is an additional drawback of the radiometric system.

The performance parameters (sensitivity and detection time) of the nonradiometric systems in comparison with those of BACTEC 460 TB and traditional culture systems have been evaluated in several recently published studies (8–12, 15, 16, 18). The main features highlighted by these studies are (i) the detection time of the new methods compared to that of traditional cultures on solid media; (ii) the lower sensitivity and detection time of the new methods when compared to those of the radiometric method, which, however, remain within clinically acceptable limits; (iii) the advantages of nonradioactive waste; and (iv) the noninvasive reading of growth. However, before considering the new automated methods as a valid alternative to the radiometric system for the culturing of mycobacteria in liquid media, additional, comparative studies need to be performed.

In this study we evaluate the performance of the MB/BacT system as a means of detecting mycobacterial growth in comparison with the BACTEC 460 TB system and a conventional solid medium, with a broad range of specimens of both respiratory and nonrespiratory origins.

(These results were partially presented at the 8th European Congress of Clinical Microbiology in Lausanne, Switzerland, 1997, and at the Xth International Congress of Infectious Diseases in Boston, Mass., 1998.)

A total of 1,830 specimens submitted for detection of mycobacteria were collected from 689 patients (2.6 cultures/patient) and included sputum (846 specimens), bronchoalveolar lavage fluid (540 specimens), urine (58 specimens), blood (174 specimens), cerebrospinal fluid (CSF) (89 specimens), and 123 miscellaneous samples. Specimens were processed according to standard methods (4, 7). Blood specimens were collected in isolator tubes (Wampole Isolator; Oxoid, Milan, Italy). The medium used in the MB/BacT system was a modified Middlebrook 7H9 broth supplemented with growth factors and an
antimicrobial mixture, MAS, consisting of amphotericin B (0.018% [wt/vol]), azlocillin (0.0034% [wt/vol]), nalidixic acid (0.04% [wt/vol]), polymyxin B (10,000 U), and trimethoprim (0.0105% [wt/vol]). After processing, 0.5 ml of each specimen was inoculated into one MB/BacT bottle, which was placed in the MB/BacT instrument for up to 8 weeks at 37°C. Another 0.5 ml aliquot of the sample was inoculated into one BACTEC 460 TB bottle which contained 4 ml of modified Middlebrook 7H9 broth, casein hydrolysate, bovine serum albumin, catalase, [14C]palmitic acid, and an antimicrobial mixture, PANTA (polymyxin B [2,000 U/ml], amphotericin B [200 µg/ml], nalidixic acid [800 µg/ml], trimethoprim [200 µg/ml], azlocillin [200 µg/ml]). The BACTEC 460 TB bottles were monitored by the instrument every 2 days during the first week and weekly thereafter for the next 7 weeks. Finally, a tube containing a conventional medium (Lowenstein-Jensen [LJ] medium; Biostest, Heidelberg, Germany) was inoculated with 0.2 ml of the same treated specimen, incubated at 37°C, and inspected weekly for 8 weeks. Growth on solid medium was detected by the visual observation of colonies, while in the radiometric BACTEC 460 TB medium, a growth index (GI) of 10 or more was considered positive. Growth in the MB/BacT bottles was detected through the CO2 that is released during metabolic processes. The CO2 produced color changes in the pH indicator mixture at the bottom of the culture bottles, and these color changes were monitored and recorded by the instrument every 10 min. The acid fastness of the cultures was verified by the Ziehl-Neelsen stain. Identification tests were performed on colonies growing on LJ medium by routine biochemical methods (7) or by nucleic acid probes (Gen-Probe, San Diego, Calif.) on samples removed from BACTEC 12B vials (GI > 999) or from MB/BacT vials when signalled as positive. Statistical analysis of recovery rates was determined by the chi-square test with a P value of 0.05 or less as significant. The comparison of isolation times was performed by the Student t test.

Mycobacteria were identified in 173 cultures (79 patients) from all three culture-based assays: 114 Mycobacterium tuberculosis complex isolates, 39 Mycobacterium avium complex (MAC) isolates, 16 Mycobacterium chelonae isolates, 1 Mycobacterium gordoniae isolate, 1 Mycobacterium kansasi isolate, 1 Mycobacterium scrofulaceum isolate, and 1 Mycobacterium xenopi isolate. Most species and strains were isolated from respiratory specimens. For nonrespiratory samples, Mycobacterium tuberculosis complex was isolated from CSF (n = 1), gastric fluid (n = 1), pleural aspirate (n = 2), pericardial liquid (n = 1), urine (n = 1), and biopsy specimens (n = 1). MAC was isolated from blood (n = 34), CSF (n = 2), and urine (n = 1).

Contamination rates were 4% for the MB/BacT system, 3% for the BACTEC 460 TB system, and 5% for LJ medium. No false-positive signals were detected.

The recovery rates of the mycobacteria isolated in this study by the three different methods were not significantly different (Tables 1 and 2). The MB/BacT and BACTEC 460 TB systems detected 96.5 and 99.4% of all isolates, respectively, while LJ medium detected 95.9%. The recoveries of Mycobacterium tuberculosis complex by MB/BacT, BACTEC 460 TB, and LJ medium were 99.1, 98.2, and 100%, respectively. In the case of nontuberculous mycobacteria (NTM) isolated in our study (mostly MAC and M. chelonae) the MB/BacT system (91.5%) was comparable to LJ medium (91.5%) but showed a lower recovery rate than the BACTEC 460 TB system. All the systems detected Mycobacterium tuberculosis complex or NTM with no significant differences in smear-positive specimens (Table 2); in smear-negative specimens, the recovery rates of all mycobacteria were 95.4 and 98.4% by the MB/BacT and BACTEC 460 TB systems, respectively, and 89.5% by LJ medium. MB/BacT and BACTEC 460 TB detected 100% of Mycobacterium tuberculosis isolates from smear-negative specimens, while LJ medium detected 88.8%. All methods detected 100% of NTM from smear-positive samples. Both the MB/BacT system and LJ medium detected 89.5% of NTM isolates from smear-negative specimens, whereas the BACTEC 460 TB system detected 97.9% of these isolates. MB/BacT missed a total of six isolates (one isolate of Mycobacterium tuberculosis, three isolates of MAC, one isolate of M. chelonae, and one isolate of M. gordoniae), BACTEC 460 missed one isolate (M. chelonae), and LJ medium missed seven isolates (two isolates of Mycobacterium tuberculosis, four isolates of MAC, and one isolate of M. gordoniae). By assuming that a culture which is positive or negative for mycobacteria reflected the probability that the disease is present or absent, respectively, the positive predictive value of the three culture methods was 100% and the negative predictive values of MB/BacT, BACTEC 460 TB, and LJ medium were 99.6, 99.8, and 99.5%, respectively.

The average number of days and the range of times required for the detection of mycobacteria by each culturing system are summarized in Table 3. Mycobacterial growth was detectable in liquid media after as little as 2 days of incubation, whereas in LJ medium detection required a minimum of 7 days. The mean times to detection for all isolates were 11.7, 11.2, and 26.8 days by the MB/BacT system, the BACTEC 460 TB system, and LJ medium, respectively. Mycobacterium tuberculosis complex strains were detected from smear-positive specimens after 11.8 and 8.0 days, when using the MB/BacT and BACTEC 460 TB systems, respectively (BACTEC 460 TB versus MB/BacT, P <

### Table 1. Rates of recovery of mycobacteria from clinical specimens with MB/BacT, BACTEC 460 TB, and LJ medium

<table>
<thead>
<tr>
<th>Isolate (no. of specimens)</th>
<th>No. (%) of isolates detected by:</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>MB/BacT</td>
</tr>
<tr>
<td>All mycobacteria (173)</td>
<td>167 (96.5)</td>
</tr>
<tr>
<td>M. tuberculosis complex (114)</td>
<td>113 (99.1)</td>
</tr>
<tr>
<td>All NTM (59)</td>
<td>54 (91.5)</td>
</tr>
</tbody>
</table>

### Table 2. Detection of mycobacteria from clinical specimens according to initial smear results

<table>
<thead>
<tr>
<th>Isolate (no. of specimens)</th>
<th>No. (%) of isolates detected by:</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>MB/BacT</td>
</tr>
<tr>
<td>All smear-positive specimens (107)</td>
<td>106 (99)</td>
</tr>
<tr>
<td>All smear-negative specimens (66)</td>
<td>63 (95.4)</td>
</tr>
<tr>
<td>Smear-positive M. tuberculosis (96)</td>
<td>95 (98.9)</td>
</tr>
<tr>
<td>Smear-negative M. tuberculosis (18)</td>
<td>18 (100)</td>
</tr>
<tr>
<td>Smear-positive NTM (11)</td>
<td>11 (100)</td>
</tr>
<tr>
<td>Smear-negative NTM (48)</td>
<td>43 (89.5)</td>
</tr>
</tbody>
</table>
In our study. Both MB/BacT and BACTEC 9000 MB systems detected on average (i) *M. tuberculosis* in smear-positive and -negative samples 3 days later than BACTEC 460 TB; (ii) *M. tuberculosis* in smear-positive samples and MAC in all samples within the same time period (11 days); and (iii) MAC and other NTM several days earlier than BACTEC 460 TB (MB/BacT, 7 and 5 days earlier, respectively; BACTEC 9000 MB, 5 and 1 days, respectively).

Two assessments of the performance of the ESP II system in direct comparison with that of BACTEC 460 TB have been published to date. ESP II performance was better than that of the radiometric method in one study, while the two systems performed similarly in the other. The difference was due to the higher number of MAC isolates used in the former study (49 versus 17%); as documented by both studies MAC grew better in ESP II than in BACTEC broth. In contrast, *M. tuberculosis* strains were more readily detected by the BACTEC vials. However, the performance of the BACTEC 460 TB system might have been underestimated in both studies, since the authors checked the positivity of the BACTEC vial when the GI reached ≥100 by preparing a smear, and if acid-fast bacilli were present in the smear, this was considered the time at which the specimen was positive for mycobacteria, whereas BACTEC positivity is conventionally reported when the GI is ≥10.

The nonlabor costs associated with each mycobacterial culture system vary among laboratories based on volumes and equipment purchased from the manufacturers. In our laboratory, the per test of MB/BacT (15,000 lire [US $1 = ca. 1,709 lire]) is competitive with that of BACTEC 460 TB (25,000 lire).

In conclusion, the MB/BacT system is a rapid, efficient method for the detection of mycobacteria in a clinical laboratory. In combination with a solid medium it may be considered a valid and less expensive alternative to the radiometric system for the laboratory diagnosis of mycobacteriosis.

We thank Nicoletta Tracco for her invaluable technical assistance. F.B. was supported by a grant from Organon Teknika. This study was supported by the National Tuberculosis Project (Istituto Superiore di Sanità-Ministero delle Sanità, Rome, Italy), grant 96/D/150. We thank Organon Teknika for providing us with the MB/BacT system and for assistance.

**REFERENCES**


