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Comparison of MB/BacT and BACTEC 460 TB Systems for Recovery of Mycobacteria in a Routine Diagnostic Laboratory

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MB/BacT, BACTEC 460 TB, and Löwenstein-Jensen (LJ) medium were evaluated in parallel for recovery of mycobacteria from 3,700 continuous clinical specimens in a routine laboratory. Mycobacteria were identified from 123 (3.3%) specimens. The recovery rates for all mycobacteria by the different systems were 91.0, 73.0, and 53.6% for BACTEC 460 TB, MB/BacT, and LJ medium, respectively. The recovery rates for *Mycobacterium tuberculosis* complex were 97.1, 80.2, and 67.6%, respectively. The lack of sensitivity of the MB/BacT system was more pronounced with smear-negative specimens and resulted in a failure to detect three patients with infectious tuberculosis.

Rapid and sensitive detection of *Mycobacterium tuberculosis* in clinical specimens is decisive for treatment, control, and prevention of tuberculosis. Despite new DNA techniques, unequivocal diagnosis of tuberculosis still relies on cultivation of *M. tuberculosis*. The radiometric BACTEC 460 TB system (Becton Dickinson, Heidelberg, Germany) has evolved to become the "gold standard" by which all other culture and detection systems are evaluated (3, 5, 7, 8, 10, 11). One of these is the nonradiometric MB/BacT system recently developed by Organon Teknika (Eppelheim, Germany). The results of three studies indicated that the MB/BacT system is a rapid, sensitive, and efficient method for recovery of mycobacteria from clinical specimens (1, 2, 9). However, the high percentages of culture-positive specimens (6.8, 9.0, and 9.4%), large portion of smear-positive samples (up to 65%), and short mean time of detection (e.g., 11 days) reported in these studies suggest that they were performed either with a collection of patients with a high incidence of infectious tuberculosis or with selected clinical specimens. We have evaluated the reliability of the MB/BacT system in comparison with the BACTEC 460 TB system and solid medium (Löwenstein-Jensen [LJ]) in daily routine diagnostic procedures of a nonspecialized microbiology laboratory where initial screening for the presence of mycobacteria is carried out. Approximately 5,000 clinical specimens for mycobacterial culture are sent to us each year by a central hospital of a city (one million inhabitants) in Southern Germany. Patients with confirmed diagnosis of tuberculosis are transferred from the hospital to a specialized tuberculosis center. Our laboratory adheres to the monitoring procedures laid down by INSTAND (Düsseldorf, Germany) in its voluntary quality control measures (twice-annual checks for sensitivity of culture procedures and differentiation).

From 1,503 patients, a total of 3,700 continuous clinical specimens submitted for detection of mycobacteria (sputum and bronchoalveolar lavage fluid, 65%; urine, 14%; gastric fluid, 8%; cerebrospinal fluid, 4%; biopsies, 4%; pleural aspirate, 2%; stools, 4% [blood was excluded]) were investigated over a period of 10 months. Specimens were processed according to standard protocols (6). Tissues were homogenized in a tissue grinder. Nonsterile specimens were decontaminated by *N*-acetyl-L-cysteine–NAOH and concentrated by centrifugation (4,000 × *g* for 15 min). The sediment was resuspended in 2 ml of phosphate buffer (pH 6.8). Processed specimens were stained by the Ziehl-Neelsen method. The same amount of each specimen or 0.5 ml of concentrate was inoculated into the following vials, respectively: MB/BacT, containing modified Middlebrook 7H9 with antibiotic supplement, 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]); and BACTEC 460 12B, containing (modified Middlebrook 7H12 with the antibiotic supplement 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]). In parallel, two tubes containing LJ medium without antibiotics—one with glycerol and one without (Biotest, Heidelberg, Germany)—were inoculated with 0.2 ml of the same specimen. The order of inoculation was at random. All specimens were incubated at 37°C (with the exception of specimens for *M. ulcerans* [30°C]) for 8 weeks. The BACTEC 460 TB vials were monitored by the instrument every 2 days during the first week and weekly thereafter. A growth index of >15 was considered positive. MB/BacT vials were monitored automatically every 10 min. LJ slants were inspected weekly. Nonmycobacterial overgrowth was detected by using blood agar plates and valued as culture negative (detection of mycobacteria not successful). The growth of mycobacteria was verified by microscopy (Ziehl-Neelsen method) and subcultivation (LJ medium). Mycobacteria were identified by nucleic acid probes (Gen-Probe, San Diego, Calif.) or by sequencing of the 16S rRNA gene (4). The statistical significance of differences in recovery rates was determined by the chi-square test, with a P value of ≤0.05 as significant.

From a total of 3,700 specimens, mycobacteria were identified in 123 cultures (3.3% [58 patients]). The recovery rates for the different mycobacterial species by the different methods are summarized in Table 1. BACTEC 460 TB and MB/BacT detected 97 and 80% of *M. tuberculosis* complex isolates, respectively. For nontuberculous mycobacteria (NTM), the rates of recovery by BACTEC 460 TB and MB/BacT were 82 and 63%, respectively. The differences in recovery rates between

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TABLE 1. Isolation of mycobacteria in BACTEC 460 TB, MB/BacT, and LJ medium[a]

Mycobacteria (no. of isolates)	No. (%) of isolates recovered in:		
	BACTEC 460 TB	MB/BacT	LJ medium
Total (123)	112 (91)	90 (73)	66 (53)
M. tuberculosis complex (71)	69 (97)	57 (80)	48 (68)
M. avium (15)	14 (93)	15 (100)	9 (60)
M. intracellulare (5)	5 (100)	2 (40)	1 (20)
M. malmoense (4)	4 (100)	1 (25)	0 (0)
M. ulcerans (1)	1 (100)	0 (0)	1 (100)
M. fortuitum complex (8)	7 (87)	4 (50)	4 (50)
M. xenopi (5)	4 (80)	3 (60)	1 (20)
M. smegmatis (1)	1 (100)	0 (0)	0 (0)
M. gordonae (13)	7 (54)	8 (61)	2 (15)

[a] A total of 3,700 specimens were tested.

BACTEC 460 TB and MB/BacT are statistically significant for *M. tuberculosis* complex ($P < 0.01$) and NTM ($P < 0.05$), respectively.

Table 2 summarizes the detection of mycobacteria with regard to microscopy. The lack of sensitivity of MB/BacT was even more distinct if the recovery of *M. tuberculosis* complex in smear-negative specimens was compared to that by BACTEC 460 TB (MB/BacT, 78%; BACTEC 460 TB, 100% [$P < 0.01$]). Other studies comparing the two systems did not differentiate between smear-positive and smear-negative specimens (9), reported only a prolonged incubation time for smear-negative specimens until detection by MB/BacT (1, 2), or were performed with a large portion of smear-positive specimens (84% of specimens culture positive for *M. tuberculosis* complex [2]).

The average numbers of days required for detection of *M. tuberculosis* complex were 15.4 for BACTEC 460 TB, 17.2 for MB/BacT ($P < 0.05$), and 29.8 for LJ medium. The difference of 1 to 2 days until detection of mycobacterial growth between BACTEC 460 TB and MB/BacT has also been reported by others (1, 9). The mean times to detection of NTM were not significantly different (18.5 days for BACTEC 460 TB versus 19.3 days for MB/BacT).

The contamination rates (95% of bacterial origin) for BACTEC 460 TB), MB/BacT, and LJ medium were 3.2, 6.6, and 4.7%, respectively. In 12 specimens (*M. tuberculosis* complex [$n = 6$], *M. intracellulare* [$n = 2$], *M. fortuitum* complex [$n = 2$], *M. smegmatis* [$n = 1$], *M. gordonae* [$n = 1$]), mycobacteria were not detected by MB/BacT due to contamination, compared with 4 specimens not detected by BACTEC 460 TB (*M. tuberculosis* complex [$n = 2$], *M. fortuitum* complex [$n = 1$], *M. gordonae* [$n = 1$]).

TABLE 2. Detection of mycobacteria from clinical specimens in BACTEC 460 TB, MB/BacT, and LJ medium according to initial smear results[a]

Smear result (no. of isolates)	No. (%) of isolates recovered in:		
	BACTEC 460 TB	MB/BacT	LJ medium
M. tuberculosis complex			
Positive (25)	23 (92)[b]	21 (84)[b]	23 (92)
Negative (46)	46 (100)	36 (78)	25 (54)
NTM			
Positive (6)	6 (100)	5 (83)	5 (83)
Negative (46)	37 (80)	28 (60)	13 (28)

[a] A total of 3,700 specimens were tested.
[b] Loss of sensitivity due to bacterial contamination.

In contrast to BACTEC 460 TB, the lower sensitivity of MB/BacT failed to detect three patients with infectious tuberculosis. From each of these patients, only one respiratory specimen was submitted for cultivation (smear negative). Review of the patients' clinical charts confirmed the diagnosis of tuberculosis in all three patients. All responded to therapy and showed X-ray findings typical of tuberculosis.

Although in a routine laboratory the MB/BacT system has been shown to be a reliable culture system for mycobacteria, its lower sensitivity than that of BACTEC 460 TB, especially with smear-negative specimens, may lead to delays when identifying patients with infectious tuberculosis or may even lead to nonidentification. We recommend that new diagnostic systems, especially for tuberculosis, be evaluated under the conditions found in nonspecialized laboratories with a low incidence of *M. tuberculosis*.

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