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Comparison of Fluorescent BACTEC 9000 MB System, Septi-Chek AFB System, and Lowenstein-Jensen Medium for Detection of Mycobacteria

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The newly developed fluorescent BACTEC 9000 MB system for automated culture of mycobacteria was compared with the Septi-Chek AFB system and Lowenstein-Jensen medium (LJ). A total of 2,005 clinical specimens were included in the study. Mycobacteria were isolated from 202 (10.1%) specimens, including 155 *Mycobacterium tuberculosis* complex isolates and 47 Mycobacteria other than *M. tuberculosis* isolates. Of 131 isolates detected by the BACTEC system, the Septi-Chek AFB system, or both, 120 (91.6%) were detected by the BACTEC system and 105 (80.2%) were detected by the Septi-Chek AFB system ($P < 0.02$). The recovery rate in the BACTEC system compared with that in the Septi-Chek AFB system was significantly higher for *M. tuberculosis* complex isolates ($P < 0.005$) and for isolates from acid-fast smear-negative specimens ($P < 0.01$). Of 148 isolates detected by the BACTEC system, LJ, or both, 142 (95.9%) were detected by the BACTEC system and 118 (79.9%) were detected by LJ ($P < 0.001$). The recovery rate in the BACTEC system compared with that on LJ was significantly higher for *M. tuberculosis* complex isolates ($P < 0.001$). The BACTEC system detected more mycobacteria from both smear-positive and smear-negative specimens than LJ. The mean times to detection of mycobacteria were 17.6 days for the BACTEC system, 26.0 days for the Septi-Chek AFB system, and 29.4 days for LJ. The BACTEC fluorescent 9000 MB system is a rapid, sensitive, and efficient method for the isolation of mycobacteria.

Mycobacteria are responsible for considerable human morbidity and mortality worldwide. *Mycobacterium tuberculosis* infects 1.7 billion people worldwide and causes 3 million deaths each year, the most deaths caused by any single infectious disease (8). Of major concern is the appearance of multiple drug-resistant strains of *M. tuberculosis*. The prevalence of disease caused by nontuberculous mycobacteria, especially *M. avium-M. intracellulare* complex, increased dramatically because of AIDS. Disseminated infection with the *M. avium-M. intracellulare* complex is a frequently observed opportunistic infection in patients with AIDS, occurring in as many as 43 percent of patients (9).

The definitive diagnosis of mycobacterial disease depends on the isolation and identification of the mycobacterium. Culturing mycobacteria, however, is time-consuming, and much depends on the techniques used by the laboratory. The radiometric BACTEC 460 TB system (Becton Dickinson Diagnostic Instrument Systems, Sparks, Md.) has been reported to significantly decrease the time required for detection (5, 10, 15, 16, 21, 22). The use of radioisotopically labeled substrates has, however, inhibited universal application of this system. A new automated culture system for mycobacteria has been developed: the fluorescent BACTEC 9000 MB system (Becton Dickinson). This system uses MYCO/F medium, a modified Middlebrook 7H9 broth, which can be supplemented with an antimicrobial mixture to suppress the growth of contaminating microorganisms. The system responds to changes in oxygen concentration. Each vial contains a silicon rubber disk, impregnated with a ruthenium metal complex, which serves as an oxygen-specific sensor. Oxygen quenches the fluorescent output of the sensor. Oxygen consumption by microorganisms present in the medium can be detected by the increase in fluorescence. The BACTEC 9000 MB system monitors fluorescence levels and detects the growth of microorganisms on the basis of software-based positivity algorithms.

This report summarizes a study comparing the fluorescent BACTEC 9000 MB system, the Septi-Chek AFB system (Becton Dickinson), and Lowenstein-Jensen medium (LJ) for recovery rates and the time required for detection of mycobacteria from clinical specimens.

MATERIALS AND METHODS

The study was conducted at two study sites: the Clinical Microbiology Laboratory of the St. Elisabeth Hospital, Tilburg, and the Clinical Microbiology Laboratory of PAMM, Veldhoven, The Netherlands. Both laboratories receive approximately 5,000 clinical specimens for mycobacterial culture each year. Clinical specimens submitted between February and September 1995 were included in the study. Priority was given to acid-fast smear-positive specimens and specimens that were difficult to obtain.

Specimens. Specimens processed for mycobacterial culture included respiratory specimens (sputum, bronchial washing, tracheal aspirate, and bronchoscopy specimens), gastric fluid, urine, stool, pus, tissue, bone marrow, and normally sterile body fluids (pleural fluid, pericardial fluid, cerebrospinal fluid, synovial fluid, and ascites) including blood.

Specimen processing. From each specimen a smear was prepared and was stained by the auramine-rhodamine fluorochrome method. Smears from liquid specimens were prepared after centrifugation (2,000 × *g* for 20 min). If a smear was found to be positive, another smear was prepared and stained by the Ziehl-Neelsen (ZN) method. Specimens collected from contaminated sites were digested and decontaminated with an equal volume of *N*-acetyl-L-cysteine–4% NaOH. After 15 min at room temperature this mixture was neutralized with phosphate buffer (0.067 M; pH 6.8) and concentrated by centrifugation at 2,000 × *g* for 20 min. Specimens collected from sterile sites were concentrated by centrifugation and were cultured without prior decontamination. Tissue specimens were ground in sterile 0.9% NaCl before centrifugation. Blood specimens were processed by lysis-centrifugation (Isostat Microbial System; Merck, Darmstadt, Germany). The sediment obtained after centrifugation was resuspended in approximately 1.5 ml of phosphate buffer (0.067 M; pH 6.8) and was used for inoculation of the media.

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Media and culturing methods. Each BACTEC MYCO/F culture vial contained 40 ml of modified Middlebrook 7H9 broth supplemented with 2 ml of an antimicrobial mixture (PANTA) containing polymyxin B (400,000 U/liter), amphotericin B (70 mg/liter), nalidixic acid (280 mg/liter), trimethoprim (70 mg/liter), and azlocillin (80 mg/liter). The Septi-Chek AFB system consisted of a bottle containing medium, linked with a plastic cylinder containing a plastic dipslide supporting three different agar slides. The Septi-Chek AFB culture bottle contained 20 ml of modified Middlebrook 7H9 broth supplemented with 1 ml of supplement containing, apart from enrichment substances, polymyxin B sulfate (130 mg/liter), amphotericin B (130 mg/liter), nalidixic acid (400 mg/liter), trimethoprim (100 mg/liter), and azlocillin (210 mg/liter). The Septi-Chek AFB slide consisted of Middlebrook 7H11 agar, egg-containing Middlebrook 7H11 agar, and chocolate agar. Three LJ solid medium slants were used: two slants with glycerol (10 ml/liter) and without pyruvate and one slant without glycerol and with pyruvate (4.6 g/liter).

Each of the BACTEC MYCO/F and Septi-Chek AFB vials was inoculated with 0.5 ml of the processed sediment. Another 0.5 ml of the sediment was divided over the three LJ slants. BACTEC MYCO/F vials were incubated for 6 weeks. LJ and Septi-Chek AFB vials were incubated at 37°C for 10 weeks. The BACTEC system almost continuously monitors the vials for growth, because fluorescence levels are measured every 10 min. LJ and the Septi-Chek AFB system were inspected at least once a week. For the Septi-Chek AFB system both the broth and the slide were inspected for signs of growth. When growth was observed in any of the media (MYCO/F, Septi-Chek AFB, or LJ), an acid-fast smear was made to determine whether they contained mycobacteria or contaminants. From BACTEC vials with a positive signal a sample of broth was subcultured onto LJ slants. A BACTEC signal was called false positive if neither mycobacteria nor contaminants were found either in the acid-fast smear made after this signal or in a smear made after a possible next signal, and no mycobacteria were subcultured onto LJ.

Identification of mycobacteria. Isolates were identified with Accuprobe culture identification tests for *M. tuberculosis* complex and *M. avium-M. intracellulare* complex (GenProbe, Inc., San Diego, Calif.). The test was performed with a sediment of centrifuged broth (at 15,000 × *g* for 10 min), without prior subculturing onto solid media, or with a colony from solid medium. All isolates were subcultured onto LJ and were sent to the National Institute of Public Health and Environmental Protection (Rijksinstituut voor Volksgezondheid en Milieuhygiëne) in Bilthoven, The Netherlands, which serves as the referral laboratory for mycobacterial cultures in The Netherlands, for confirmation. This laboratory performed further identification of all *M. tuberculosis* complex isolates and, depending on clinical importance, of isolates found to be negative with both probes (mycobacteria other than *M. tuberculosis* [MOTT] isolates).

Statistical analysis. The statistical significance of differences in recovery rates was determined by the χ^2 test (7). The statistical differences in isolation time were determined by the Mann-Whitney test (19).

RESULTS

Specimens. A total of 2,005 specimens were included in the study, of which 732 specimens were inoculated in all three media, 789 specimens were inoculated in the BACTEC 9000 MB system and in the Septi-Chek AFB system, and 484 specimens were inoculated in the BACTEC system and on LJ. For comparison of the recovery rates, the results were divided into two groups, one group that compared the BACTEC system with the Septi-Chek AFB system (1,521 specimens) and one group that compared the BACTEC system with LJ (1,216 specimens). For comparison of the mean time to detection, all isolates detected by any medium were included in the analysis.

From the total of 2,005 specimens, 202 (10.1%) isolates were recovered (Table 1): 107 (52.9%) from acid-fast smear-positive specimens and 95 (47.0%) from acid-fast smear-negative specimens. The 202 culture-positive specimens were collected from 99 different patients (1 to 14 specimens per patient). Most of the isolates, 160 (79.2%), were recovered from respiratory specimens. Sixteen (7.9%) isolates were recovered from sterile body fluids (13 from pleural fluid, 2 from cerebrospinal fluid, and 1 from ascites), 10 (5.0%) were recovered from pus, 9 (4.5%) were recovered from tissue, 3 (1.5%) were recovered from feces, 2 (1.0%) were recovered from bone, 1 (0.5%) was recovered from urine, and 1 (0.5%) was recovered from blood.

During the study we evaluated which staining method, the auramine-rhodamine fluorochrome method or the ZN method, could be used best to detect mycobacteria from positive BACTEC vials. Of a total of 125 positive vials for which smears were stained by both the auramine and the ZN methods, in 93 cases both smears were positive, in 29 cases only the auramine smear was positive, and in 3 cases only the ZN smear was positive ($P < 0.001$).

TABLE 1. Distribution of 202 *Mycobacterium* isolates recovered from 2,005 specimens

Species	No. of isolates
M. tuberculosis complex	155
M. tuberculosis	153
M. bovis BCG	2
MOTT	47
M. avium-M. intracellulare complex	15
M. kansasii	7
M. malmoense	3
M. terrae	2
M. xenopi	1
Other	19

Recovery rates by BACTEC system and Septi-Chek AFB system. The recovery rate of mycobacteria by the BACTEC 9000 MB system was 91.6%, and that by the Septi-Chek AFB system was 80.2% ($P < 0.02$) (Tables 2 and 3). Four of the 11 isolates missed by the BACTEC system were lost because of contamination; of the 26 isolates missed by the Septi-Chek AFB system 8 were lost because of contamination. The BACTEC 9000 MB system was significantly better than the Septi-Chek AFB system at isolating *M. tuberculosis* complex isolates; there was no significant difference in the rate of recovery of MOTT isolates (Table 2). The BACTEC system was significantly better than the Septi-Chek AFB system at isolating mycobacteria from smear-negative specimens; there was no significant difference in the rate of recovery of mycobacteria from smear-positive specimens (Table 3).

Recovery rates by BACTEC system and LJ. The recovery rate of mycobacteria by the BACTEC 9000 MB system was 95.9%, and that by LJ was 79.9% ($P < 0.001$) (Tables 4 and 5). The BACTEC system missed six isolates. For one of these specimens the BACTEC system repeatedly gave a positive signal, but successive acid-fast smears showed no mycobacteria. Eleven of the 30 isolates not detected by LJ were lost because of contamination. The BACTEC system was significantly better than LJ at isolating *M. tuberculosis* complex isolates; there was no significant difference in recovery rates of MOTT isolates (Table 4). The BACTEC system was significantly better than LJ at recovering isolates from both smear-positive and smear-negative specimens (Table 5).

Mean time to detection. During the trial a total of 185 isolates were detected by the BACTEC system; 105 were detected by the Septi-Chek AFB system; and 118 were detected by LJ. The average number of days required for the recovery of

TABLE 2. Rates of recovery of *M. tuberculosis* and MOTT isolates by Septi-Chek AFB and BACTEC 9000 MB systems

Species (no. of isolates)	No. (%) of isolates recovered by:		*P* value
	Septi-Chek AFB system	BACTEC 9000 MB system	
M. tuberculosis complex (92)	75 (81.5)	89 (96.7)	<0.005
MOTT (39)	30 (76.9)	31 (79.5)	NS[a]
Total (131)	105 (80.2)	120 (91.6)	<0.02

[a] NS, not significant.

TABLE 3. Rates of recovery of mycobacteria from smear-positive and smear-negative specimens by Septi-Chek AFB and BACTEC 9000 MB systems

Smear result (no. of specimens)	No. of isolates recovered by:		*P* value
	Septi-Chek AFB system	BACTEC 9000 MB system	
Positive (71)	65 (91.5)	66 (93.0)	NS[a]
Negative (60)	40 (66.7)	54 (90.0)	<0.01
Total (131)	105 (80.2)	120 (91.6)	<0.02

[a] NS, not significant.

TABLE 5. Rates of recovery of mycobacteria from smear-positive and smear-negative specimens by LJ slants and BACTEC 9000 MB system

Smear result (no. of specimens)	No. (%) of isolates recovered by:		*P* value
	LJ slants	BACTEC 9000 MB system	
Positive (76)	67 (88.2)	75 (98.7)	<0.01
Negative (72)	51 (70.8)	67 (93.1)	<0.005
Total (148)	118 (79.9)	142 (95.9)	<0.001

mycobacteria by each culture system is presented in Tables 6 and 7. The overall mean times to detection were 17.6 days for the BACTEC system, 26.0 days for the Septi-Chek AFB system, and 29.4 days for LJ.

Contamination rate and false-positivity rate. The contamination rate for all specimens inoculated in the BACTEC system was 6.0% (121 of 2,005). Mycobacteria were recovered from two contaminated BACTEC vials. A total of 6.5% (79 of 1,216) of all three slants of LJ were contaminated. The contamination rate for specimens inoculated in the Septi-Chek AFB system was 7.6% (116 of 1,521). Mycobacteria were recovered from one contaminated Septi-Chek AFB vial. The false-positivity rate of the BACTEC system was very low: 0.8% (16 of 2,005 specimens).

DISCUSSION

The present study compared the fluorescent BACTEC 9000 MB system with the Septi-Chek AFB system and LJ for the detection of mycobacteria. The recovery rates by LJ and the Septi-Chek AFB system could not be compared because not all specimens were inoculated in both the Septi-Chek AFB system and LJ. All specimens, however, were inoculated in the BACTEC system and in at least one other medium, the medium of the Septi-Chek AFB system or LJ. The recovery rates by the BACTEC system were compared with the recovery rates by the other system or medium. No single medium or system recovered all of the isolated mycobacteria. There was no statistically significant difference in the rates of recovery of MOTT isolates by the BACTEC system and the Septi-Chek AFB system. The recovery rate for MOTT isolates on LJ was much lower than that by the BACTEC system, 57.0 and 80.1%, respectively. There was, however, no statistical difference, probably because only small numbers of MOTT were isolated. The mean time to detection of MOTT on LJ was longer than that of *M. tuberculosis*, 38.6 and 28.4 days, respectively. Such a difference in detection time was described before (23). It is well-known that LJ is not the best medium for the isolation of MOTT isolates, because LJ is designed primarily for the growth of *M. tuberculosis* and is not reliable for the recovery of other species (10, 12, 18).

The rate of recovery of mycobacteria from acid-fast smear-negative specimens was significantly higher by the BACTEC system than by the Septi-Chek AFB system and LJ. The radiometric BACTEC 460 TB system, the system that preceded the fluorescent BACTEC 9000 MB system, has also been reported to yield more positive cultures from clinical specimens than other media (2, 15, 16, 22). Compared with solid medium, this was also particularly significant with smear-negative specimens (1, 2, 22).

The mean time to detection by the BACTEC system was shorter than that by the Septi-Chek AFB system or LJ, regardless of the specimen type or acid-fast smear result. The reading schedule for the inoculated media, however, has a significant role in the early detection of growth. Fluorescence levels in the BACTEC system are measured every 10 min; the BACTEC system thus almost continuously monitors its vials for growth. LJ and the Septi-Chek AFB system were inspected at least once a week. More frequent reading of inoculated media would be expected to reduce the time to detection, but this would be labor-intensive and therefore expensive. The maximum time lost in the study because of less frequent reading of LJ and the Septi-Chek AFB system was 7 days. However, of the 94 isolates detected by both the BACTEC system and the Septi-Chek AFB system, the majority (51.1%) were detected at least 7 days earlier by the BACTEC system than by the Septi-Chek AFB system. Of the 112 isolates detected by both the BACTEC system and LJ, the majority (67.9%) were detected at least 7 days earlier by the BACTEC system.

The auramine-rhodamine fluorochrome method for the detection of acid-fast microorganisms in specimens allows for rapid screening and may be more sensitive than the ZN staining method (4). Our observation that mycobacteria from positive BACTEC vials were best detected in a smear that was stained by the auramine-rhodamine fluorochrome method corresponds to that report. If only a ZN stain would have been

TABLE 4. Rates of recovery of *M. tuberculosis* and MOTT isolates by LJ slants and BACTEC 9000 MB system

Species (no. of isolates)	No. (%) of isolates recovered by:		*P* value
	LJ slants	BACTEC 9000 MB system	
M. tuberculosis complex (127)	106 (83.5)	125 (98.4)	<0.001
MOTT (21)	12 (57.0)	17 (80.1)	NS[a]
Total (148)	118 (79.9)	142 (95.9)	<0.001

[a] NS, not significant.

TABLE 6. Mean time to detection of mycobacteria by BACTEC 9000 MB system, LJ slants, and Septi-Chek AFB system

Species	Mean (SD) time (days) to detection by:		
	BACTEC 9000 MB system	LJ slants	Septi-Chek AFB system
M. tuberculosis complex	18.0 (7.5)	28.4 (10.7)	26.2 (14.2)
MOTT	15.9 (12.6)	38.6 (18.9)	25.3 (12.1)
Total	17.6 (8.7)[a]	29.4 (12.2)	26.0 (13.6)

[a] $P < 0.00001$ versus LJ slants and the Septi-Chek AFB system.

TABLE 7. Mean time to detection of mycobacteria from smear-positive and smear-negative specimens for BACTEC 9000 MB system, LJ slants, and Septi-Chek AFB system

Smear result	Mean (SD) time (days) to detection by:		
	BACTEC 9000 MB system	LJ	Septi-Chek AFB system
Positive	15.0 (7.5)	25.5 (8.8)	23.1 (13.0)
Negative	20.6 (9.1)	34.6 (14.0)	30.6 (13.2)
Total	17.6 (8.7)[a]	29.4 (12.2)	26.0 (13.6)

[a] $P < 0.00001$ versus LJ slants and the Septi-Chek AFB system.

prepared, isolates could have been missed, or at least the time to detection would have been prolonged.

One of the disadvantages often mentioned about culture in liquid medium is that it does not provide visible colonies. This could increase the time to identification and susceptibility testing of the isolate. In our study isolates could be identified with an Accuprobe culture identification test directly from a centrifuged sample of broth. No subculture on solid medium was necessary. We also observed cord formation in MYCO/F medium that grew *M. tuberculosis* complex and used this as a guide for initial probe selection. Cord formation was found to be very specific for *M. tuberculosis* complex (17, 26) and was found to be a useful criterion for initial probe selection for mycobacterial identification (14).

Published contamination rates range from 0.3 to 8.9% for the Septi-Chek AFB system (1, 5, 6, 11, 13, 20, 23) and 0.4 to 41% for LJ (5, 6, 11, 13, 15, 20, 23, 24). In our study the contamination rates were 7.6 and 6.5% for the Septi-Chek AFB system and LJ, respectively. There are not yet any reports about the contamination rate for the BACTEC 9000 MB system. The contamination rate of 6% found in our study is at least in the same range as those found for the Septi-Chek AFB system and LJ.

Our data, as far as the BACTEC system and LJ were concerned, are comparable to those reported by Boyle et al. (3). They found a significantly better rate of recovery of mycobacteria by the BACTEC system than by LJ, 93.0 and 55.0%, respectively ($P < 0.001$); in our study the rates were 95.9 and 79.9%, respectively. They found overall mean times to detection of 14.7 days by the BACTEC system and 26.2 days on LJ; in our study the times were 17.6 and 29.4 days, respectively. They also reported a very low false-positivity rate of 1.5%; in our study it was 0.8%.

The fluorescent BACTEC 9000 MB system is a rapid, sensitive, and efficient method for the isolation of mycobacteria in a clinical laboratory. Since radioisotopically labeled substrates are no longer necessary, this system provides automation for the detection of mycobacteria and so can be widely used. Since neither the BACTEC system nor any other medium recovers all detected isolates when it is used alone, the recommendation to use more than one medium for mycobacterial culture, preferably a liquid and a solid medium (25), is still valid.

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