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Comparison of Improved BACTEC and Lowenstein-Jensen Media for Culture of Mycobacteria from Clinical Specimens

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A 4-month trial involving 2,563 routine clinical specimens was conducted to compare the improved BACTEC TB system (12B medium) with the conventional Lowenstein-Jensen (LJ) media for the isolation, identification, and susceptibility testing of mycobacteria. One hundred sixty-two mycobacterial isolates were recovered, 147 (91%) with BACTEC and 118 (73%) with LJ media. Of these, 62 were *Mycobacterium tuberculosis* complex strains, 59 (95%) of which were isolated with BACTEC and 54 (87%) of which were isolated with LJ media. Of the remaining 100 isolates, which were mycobacteria other than tuberculosis (MOTT), BACTEC and LJ media detected 88 and 64%, respectively. The contamination rate was significantly higher in BACTEC (5%) than in LJ media (3.3%). The mean isolation time for *M. tuberculosis* complex with BACTEC was 15.5 days, compared with 25.6 days with LJ. For MOTT, the mean isolation times were 5.8 and 21.4 days, respectively. Identification of 32 *M. tuberculosis* complex isolates and 38 isolates of MOTT by the BACTEC NAP (*p*-nitro-α-acetylamino-β-hydroxypropiophenone) inhibition test gave 100% agreement with conventional biochemical identifications. The results of susceptibility testing of 18 *M. tuberculosis* complex isolates with BACTEC agreed completely with those obtained by the resistance ratio method.

The radiometric BACTEC TB system (Johnston Laboratories, Inc., Towson, Md.) was developed for the rapid detection of mycobacteria by using Middlebrook 7H12 medium containing C^{14}-labeled palmitic acid substrate (6). This system can rapidly differentiate members of the *Mycobacterium tuberculosis* complex from mycobacteria other than tuberculosis (MOTT) by testing for inhibition of growth in the presence of *p*-nitro-α-acetylamino-β-hydroxypropiophenone (NAP) (4, 11) and can be used to determine drug susceptibilities of *M. tuberculosis* complex isolates (12).

The use of BACTEC in early investigations demonstrated equivalent or improved isolation rates as well as earlier detection times for *M. tuberculosis* complex and MOTT isolates, compared with conventional culture media (2, 8–10). These studies involved the inoculation of 0.1 ml of specimen concentrates into 2 ml of BACTEC 12A medium supplemented with PACT, an antimicrobial mixture containing polymyxin B, amphotericin, carbenicillin, and trimethoprim designed to suppress the microbial flora present in respiratory specimens (7). Improved isolation rates were also reported by Morgan et al. (M. A. Morgan, D. R. DeYoung, and G. D. Roberts, Abstr. Annu. Meet. Am. Soc. Microbiol. 1983, C 222, p. 348) and Libonati et al. (J. P. Libonati, M. E. Carter, N. M. Hopper, J. F. Baker, and S. H. Siddiqi, Abstr. Annu. Meet. Am. Soc. Microbiol. 1983, C 223, p. 348), who used a larger inoculum, 0.5 ml, and a larger medium volume, 4 ml, in their studies. These modifications resulted in higher contamination rates; consequently, a new antimicrobial supplement, PANTA, containing polymyxin B, amphotericin, nalidixic acid, trimethoprim, and azlocillin, was formulated to overcome this problem. PANTA was subsequently reported by Stager and Davis (C. E. Stager and J. R. Davis, Abstr. Annu. Meet. Am. Soc. Microbiol. 1986, U-36, p. 125) to suppress contamination significantly better than PACT did. Recently, a growth-promoting substance, polyoxyethylene stearate, has been shown to enhance the viability of poorly growing strains of *M. tuberculosis* complex isolated from treated patients with chronic infection (J. P. Libonati, M. E. Carter, N. M. Hooper, J. F. Baker, and S. H. Siddiqi, Abstr. Annu. Meet. Am. Soc. Microbiol. 1985, U 45, p. 105).

The purpose of this study was to evaluate the BACTEC TB system for routine use in a clinical laboratory by comparing isolation rates and times of isolation of mycobacteria from clinical specimens with the improved BACTEC system and Lowenstein-Jensen (LJ) media. The usefulness of the BACTEC NAP test as a rapid method of differentiating *M. tuberculosis* complex from MOTT was evaluated, and a comparison was made between the drug susceptibility results obtained by BACTEC and the resistance ratio method.

MATERIALS AND METHODS

Specimens and processing methods. All specimens received by the Mycobacterial Reference Laboratory at the Institute of Medical and Veterinary Science, Adelaide, Australia, between 18 July 1988 and 4 November 1988 were included in the study. These consisted of sputum and other respiratory secretions, gastric lavage specimens, urine, other body fluids, pus, and tissue biopsy specimens. All respiratory specimens were decontaminated by the *N*-acetyl-L-cysteine 2% NaOH method (3, 13) for 30 min and neutralized with 3% phosphoric acid. Urine samples were concentrated by centrifugation at 2,800 × *g* for 20 min and decontaminated with 4% H_2SO_4 for 30 min (14). Tissue and body fluids from normally sterile sites were processed and cultured without prior decontamination. Smears were made from specimen concentrates after neutralization and stained with auramine-*O*-phenol (3, 13) for the presence of acid-fast bacilli (AFB).

Media and culturing methods. (i) Samples (0.25 ml) of each specimen were inoculated onto each of two slants of glycerol-free LJ medium supplemented with sodium pyruvate (LJ-P; MEDVET Science Co. Ltd., Adelaide, Australia) and one slant of LJ medium containing nalidixic acid (35 mg/liter), vancomycin (20 mg/liter), polymyxin B (1,600 U/ml),

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and pimafucin (100 mg/liter) (LJ-NVPP; MEDVET Science Co.; P. Anargyros, Abstr. Annu. Meet. Aust. Soc. Microbiol. 1986, abstr. no. P164, p. 194). (ii) Samples (0.5 ml) of decontaminated specimens were also inoculated into Middlebrook 7H12 broth (BACTEC 12B medium; Johnston Laboratories) supplemented with PANTA (polymyxin [50 U/ml], amphotericin [5 mg/liter], nalidixic acid [20 mg/liter], trimethoprim [5 mg/liter], azlocillin [10 mg/liter]) and polyoxyethylene stearate. Specimens from sterile body sites were inoculated into antibiotic-free BACTEC 12B medium supplemented with polyoxyethylene stearate.

BACTEC vials were routinely primed before inoculation to establish an atmosphere of 4 to 5% CO_2 and were used within 1 week. All media were incubated at 36°C. Duplicate cultures of specimens taken from skin and wound sites were incubated at 30°C.

Conventional LJ media were examined for growth twice weekly for the first 2 weeks and once weekly thereafter until 8 weeks; smear-positive and tissue specimens were incubated for 12 weeks. BACTEC vials were read with the TB460 system twice weekly for the first 2 weeks and weekly thereafter until 6 weeks. Growth was expressed as a growth index value which was directly proportional to the amount of $^{14}CO_2$ liberated in the vial as a result of mycobacterial growth. The threshold for a positive growth index reading was set at a growth index of 10. Vials exceeding the threshold were read daily until a growth index $\geq$ 100 was reached, at which time smears were made and examined for the presence of AFB. The BACTEC isolation time, expressed in days, was the time at which AFB were found in smears made from positive vials.

Identification of mycobacterial isolates. All primary isolates were differentiated as *M. tuberculosis* complex or MOTT by the BACTEC NAP inhibition test (10) and identified by conventional biochemistry (3, 13). A further 12 *M. tuberculosis* complex isolates from an Australia-wide Quality Control Assurance Programme were included as reference strains.

Susceptibility testing of mycobacterial isolates. All primary *M. tuberculosis* complex isolates were tested with BACTEC by using the 1% proportion method (12) to determine their susceptibilities to critical concentrations of the following agents: streptomycin (6.0 mg/liter), isoniazid (Ciba-Geigy; 0.2 mg/liter), rifampin (2.0 mg/liter), and ethambutol (7.5 mg/liter). Isolates were also tested by the resistance ratio method (1) for susceptibility to only three drugs, as streptomycin was unavailable during the trial. Isoniazid (0.05 to 1.6 mg/liter) and ethambutol (1 to 16 mg/liter) were tested with LJ media, whereas rifampin (0.5 to 8 mg/liter) was tested with Middlebrook 7H10 agar.

Statistical analysis. Fisher's exact test was used to determine the statistical significance of differences in isolation and contamination rates between the BACTEC and LJ systems. The statistical differences in isolation times of *M. tuberculosis* complex and MOTT by both methods were determined by Student's *t* test.

RESULTS

A total of 162 (6.4%) mycobacterial isolates were obtained from 2,563 specimens cultured by both methods during the trial period. Of these, 62 were *M. tuberculosis* complex and 80 were *Mycobacterium avium-M. intracellulare* complex, accounting for 87.6% of the total (Table 1). The highest isolation rate was obtained with BACTEC, with 147 (91%) isolations, compared with 85 (52%) on LJ-P and 97 (60%) on LJ-NVPP (Table 2). The combined yield from LJ media was 73%, significantly lower than that obtained by BACTEC (P = 0.00003). A significantly higher number of contaminated cultures was obtained with BACTEC: 129 (5.0%) compared with 84 (3.3%) on the combined LJ media (P = 0.001).

TABLE 1. Distribution of 162 *Mycobacterium* spp. isolates recovered from 2,563 specimens

Species	No. of isolates
M. tuberculosis complex	62[a]
M. avium-M. intracellulare complex	80
M. chelonae	13
M. fortuitum	2
M. marinum	2
M. gordonae	1
M. terrae	1
M. nonchromogenicum	1

[a] Includes one *M. bovis* isolate and one *M. bovis* BCG isolate.

There was no statistical difference in the isolation rates of *M. tuberculosis* complex obtained with BACTEC and with LJ media (P = 0.08). BACTEC detected 98% of *M. tuberculosis* complex isolates from 46 smear-positive specimens and 88% from 16 smear-negative specimens (Table 2). The combined LJ media detected 91 and 75% of these, respectively. BACTEC, however, was significantly better at isolating MOTT, recovering 100% of these isolates from 35 smear-positive specimens and 80% from 65 smear-negative specimens (Table 2). The combined LJ media detected only 83 and 54% of these, respectively (P = 0.001). Both systems together isolated more organisms than either alone. BACTEC failed to isolate 3 *M. tuberculosis* and 12 MOTT isolates that grew on LJ media. LJ media did not grow 8 *M. tuberculosis* and 36 MOTT isolates that grew in the BACTEC system. Most of these were from patients from whom the organism had been isolated before. Only a few (two *M. tuberculosis* isolates and one MOTT isolate in BACTEC; three *M. tuberculosis* isolates and eight MOTT isolates on LJ media) were significant organisms that were isolated for the first time.

A comparison of the isolation times for 103 mycobacteria recovered by both types of media from the same specimens showed that BACTEC detected growth significantly earlier than LJ media did (Table 3). The mean detection time for *M. tuberculosis* complex was 15.5 days with BACTEC compared with 25.6 days with LJ media (P = 0.001). The mean detection time for MOTT was 5.8 days for BACTEC and 21.4 days for LJ media (P = 0.001). BACTEC consistently detected growth earlier than LJ media, regardless of the specimen type and smear positivity (Tables 4 and 5).

The BACTEC NAP inhibition test gave 100% agreement with conventional biochemistry in differentiating 32 strains of *M. tuberculosis* complex and 38 MOTT isolates. One strain of *M. marinum* could have been misidentified as *M. tuberculosis* complex by BACTEC. As the isolate was obtained from a cutaneous specimen, NAP testing was also performed at 30°C and identified it as a MOTT. The average reporting time of identifying MOTT with BACTEC was 2.6 days. Susceptibility testing of 18 *M. tuberculosis* complex isolates by the BACTEC system gave 100% correlation with the resistance ratio method for susceptibility to isoniazid, rifampin, and ethambutol. As streptomycin was tested for only by the BACTEC system, such a comparison was not possible for that drug. The average reporting time of susceptibilities with BACTEC was 6.1 days, compared with 21 days by the resistance ratio method.

TABLE 2. Isolation of *M. tuberculosis* complex and MOTT by BACTEC and LJ media

Species	Smear result	No. (%) of specimens	No. (%) of isolates recovered on:			
			BACTEC medium	LJ-P	LJ-NVPP	Combined LJ media
M. tuberculosis complex	Positive	46	45 (98)	31 (67)	37 (81)	42 (91)
	Negative	16	14 (88)	6 (38)	11 (69)	12 (75)
MOTT	Positive	35	35 (100)	22 (63)	25 (71)	29 (83)
	Negative	65	53 (80)	26 (40)	24 (37)	35 (54)
Total		162 (100)	147 (91)	85 (52)	97 (60)	118 (73)

DISCUSSION

The improved BACTEC 12B medium with PANTA supplement proved to be significantly better than LJ media for the isolation of mycobacteria from clinical specimens. Isolation rates, although comparable to those reported by Stager and Davis (Abstr. Annu. Meet. Am. Soc. Microbiol. 1986), failed to demonstrate any statistical differences in the isolation of *M. tuberculosis* complex by both systems. BACTEC isolated 59 of 62 *M. tuberculosis* complex isolates, compared with 54 obtained on LJ media. These results were not unexpected, as Middlebrook broth and LJ media were designed primarily for the growth of *M. tuberculosis* from clinical specimens.

Isolation of MOTT, however, was significantly better with 12B medium. Of the 100 MOTT isolates recovered, BACTEC isolated 88, compared with 64 isolated with LJ media. This difference was most marked with smear-negative specimens and contrasted with the work of Kirihara et al. (2) and Morgan et al. (8), who could not demonstrate any increase in MOTT isolations with 12A medium with PACT supplement. The use of a larger inoculum and medium volume in our study may account for this difference. Furthermore, Siddiqi and Hwangbo (S. H. Siddiqi and C. C. Hwangbo, Abstr. Annu. Meet. Am. Soc. Microbiol. 1986, U-35, p. 125) reported that PANTA used in their study was less inhibitory to MOTT than PACT was. The improved isolation of MOTT by the BACTEC system is clinically important, as the incidence of mycobacterial infection is increasing, especially in immunosuppressed patients (15).

The main advantage of the BACTEC system is earlier isolation times than those obtainable with other systems. The isolation of *M. tuberculosis* complex by BACTEC from smear-positive and smear-negative specimens occurred 9.8 and 11.5 days earlier, respectively, than isolation with LJ media. These figures were similar to those reported by Morgan et al. (8) and Roberts et al. (10), who also used 12A medium and PACT supplement. The earlier isolation times with BACTEC were observed for all specimen types. Factors such as inoculum volume and degree of smear positivity may affect isolation times. We found that an average of only 5.3 days was required to isolate *M. tuberculosis* complex with BACTEC from specimens containing numerous AFB on smears compared with 17.2 days for specimens containing few AFB. The more rapid isolation of *M. tuberculosis* by BACTEC from smear-negative decontaminated specimens in this study contrasts with the findings of Kirihara et al. (2). Since similar decontamination procedures were used, it is most likely that the larger inoculum and larger volume of 12B medium used in our protocol accounted for this difference. For MOTT isolates, greater differences in isolation times were observed. BACTEC isolated these 15.9 and 15.1 days earlier than LJ media from smear-positive and smear-negative specimens, respectively. These figures correlated well with those reported by Morgan et al. (8). Anargyros and Lim (P. Anargyros and I. S. L. Lim, Abstr. Australas. Soc. Infect. Dis. 1989, p. 28) reported that it required 7 weeks for LJ media to isolate 97% of all mycobacteria from smear-negative specimens. Patients suspected of having mycobacterial disease and whose initial smears for AFB were negative were usually commenced on antituberculosis therapy or subjected to invasive procedures to obtain a diagnosis. The earlier isolation times achieved with BACTEC should help to reduce the frequency of occasions when these procedures become necessary.

The rapid and reliable differentiation of MOTT from *M. tuberculosis* complex in only 2.6 days is useful. Patients with suspected tuberculosis whose smears are positive are hospitalized and commenced on therapy. If the infection is subsequently shown to be caused by MOTT, treatment is continued on an outpatient basis. The rapid isolation and identification of these isolates can significantly reduce health care costs. The rapid identification of MOTT is also useful for discarding nonsignificant isolates; however, NAP testing may give erroneous results. Several strains of *M. tuberculosis* required repeated testing to demonstrate reliable inhibition. The manufacturers recommend that an organism be tested for NAP inhibition at the optimal growth temperature for that organism. It is important to test isolates from lymph nodes and skin at 35 and 30°C. With the addition of 60 μM hemin to the 12B medium and testing at 30°C, we were recently able to demonstrate NAP resistance in *M. haemophilum* isolated from a hand ulcer. Laszlo and Siddiqi (5)

TABLE 3. Comparison of isolation times of 103 mycobacterial specimens

Specimens	Isolation time in days (mean ± SD) for:			
	M. tuberculosis complex (n = 51)		MOTT (n = 52)	
	BACTEC	LJ media	BACTEC	LJ media
All	15.5 ± 11.3	25.6 ± 10.2	5.8 ± 3.2	21.4 ± 10.5
Smear positive	14.1 ± 11.7	23.9 ± 10	4.3 ± 2.1	20.2 ± 8.4
Smear negative	21.0 ± 6.4	32.5 ± 7.6	7.7 ± 3.4	22.8 ± 12.6

TABLE 4. Comparison of isolation times for 51 *M. tuberculosis* isolates according to specimen type

Medium	Mean isolation time (days) for indicated smear result and specimen source			
	Positive		Negative	
	Respiratory system and urine (n = 39)	Tissues and body fluids (n = 2)	Respiratory system and urine (n = 8)	Tissues and body fluids (n = 2)
BACTEC 12B	14.1	13.5	20.5	23.0
Combined LJ	24.0	21.0	31.5	41.5

have highlighted problems associated with NAP testing with mixed cultures of *M. tuberculosis* and MOTT. The routine use of Middlebrook agar to screen for mixed populations would help in this regard. Susceptibility testing by the BACTEC method correlated well with the results obtained by the resistance ratio method. Results were easier to interpret and were available significantly earlier.

The improved BACTEC TB system is a rapid, sensitive, and efficient method for the isolation, differentiation, and susceptibility testing of mycobacteria in a clinical laboratory. The highest isolation rates were obtained when BACTEC and LJ media were used together. However, the routine use of both media would be time consuming and labor intensive and may not be cost effective. Since only a few significant cultures failed to grow in BACTEC, this medium can be used as the sole isolation medium for respiratory and urine specimens. For specimens which are difficult to obtain, such as tissue biopsies and body fluids, the use of LJ media and BACTEC may be justified to maximize isolation of mycobacteria.

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TABLE 5. Comparison of isolation times for 52 MOTT isolates according to specimen type

Medium	Mean isolation time (days) for indicated smear result and specimen source			
	Positive		Negative	
	Respiratory system and urine (n = 28)	Tissues and body fluids (n = 1)	Respiratory system and urine (n = 20)	Tissues and body fluids (n = 3)
BACTEC 12B	4.2	6.0	7.0	11.0
Combined LJ	20.5	14.0	21.3	34.0

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