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Comparison of Recovery Rates for Mycobacteria from BACTEC 12B Vials, Middlebrook 7H11-Selective 7H11 Biplates, and Lowenstein-Jensen Slants in a Public Health Mycobacteriology Laboratory

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Recovery rates from Middlebrook 7H11-selective 7H11 biplates and Lowenstein-Jensen slants (LJ) used with BACTEC 12B vials were compared for 5,399 specimens. For 578 specimens that were inoculated onto three media, 580 mycobacteria were isolated, including 277 (48%) *Mycobacterium avium* complex isolates, 230 (40%) *Mycobacterium tuberculosis* isolates, and 73 (12%) other mycobacteria. For BACTEC 12B vials, 506 (87%) cultures were positive, 45 (8%) were negative, and 29 (5%) were lost to contamination; for 7H11–7H11-selective biplates, 469 (81%) cultures were positive, 95 (16%) were negative, and 16 (3%) were lost to contamination; for LJ, 230 (40%) cultures were positive, 111 (19%) were negative, and 239 (41%) were lost to contamination. For routine cultures, use of plate media is superior to use of LJ.

Recent recommendations for the optimal recovery of mycobacteria from clinical specimens are to inoculate a broth medium along with one or more solid media, with Lowenstein-Jensen slants (LJ) usually being recommended as the primary medium and Middlebrook medium serving as an adjunct or substitute to LJ (2–4, 9, 11). We agree with the observation of Stager et al. (10), however, who noted that use of Middlebrook agar results in the earlier detection of microbial growth, fewer contaminants, and easier observation of colonial morphology than is possible with LJ slants. We have also observed a higher contamination rate and thus a lower recovery rate with LJ than with Middlebrook 7H11-selective 7H11 biplates (BI). To determine whether routine inoculation of both LJ and BI is warranted, we prospectively collected data during a 15-month period on all specimens submitted from Denver Health and Hospitals including the Denver Metropolitan Tuberculosis Clinic.

Specimens from nonsterile sites were processed by the 2% NALC-NaOH method (4). Specimens from sterile sites (other than blood or bone marrow) were transferred to 50-ml centrifuge tubes; phosphate buffer was added to bring the total volume to 40 ml. Blood and bone marrow aspirates were submitted in SPS yellow-top Vacutainer tubes (Becton Dickinson Vacutainer Systems, Rutherford, N.J.), transferred to 50-ml centrifuge tubes, and lysed with sterile H_2O. All specimens were centrifuged at 3,000 × *g* for 30 min and were then decanted. Smears of sediment were made for fluorochrome staining. BACTEC 12B vials (12B; previously supplemented with 0.1 ml of PANTA or POES) were inoculated with up to 1.0 ml of specimen. Approximately 1 ml of 0.2% bovine serum albumin was added to the remaining sediment, which was then inoculated onto BI and LJ (both from Remel, Lenexa, Kans.). Solid media were incubated at 37°C in an atmosphere of 5% CO_2. The 12B were read twice weekly for the first 2 weeks and weekly thereafter for an additional 4 weeks. BI were examined weekly for 6 weeks; LJ were examined weekly for 8 weeks. Mycobacteria were identified by the NAP test and conventional biochemical tests (5, 7).

A total of 5,399 specimens were processed. For 578 culture-positive specimens, all three media were inoculated and culture results were available for review. These 578 specimens yielded 580 isolates (one specimen grew both *Mycobacterium fortuitum* and *Mycobacterium gordonae* and another specimen grew a *Mycobacterium tuberculosis* isolate and a rapid grower that was not an *M. fortuitum* complex isolate). The 580 isolates included 277 (48%) *Mycobacterium avium* complex (MAC) isolates, 230 (40%) *M. tuberculosis* isolates, 21 (3%) *M. gordonae* isolates, 15 (3%) *Mycobacterium kansasii* isolates, and 22 (4%) other mycobacteria.

Recovery rates from each medium are given in Table 1. For 12B, 506 (87%) cultures were positive, 45 of 578 (8%) were negative, and 29 of 578 (5%) were lost to contamination; for BI, 469 (81%) cultures were positive, 95 of 578 (16%) were negative, and 16 of 578 (3%) were lost to contamination; for LJ, 230 (40%) cultures were positive, 111 of 578 (19%) were negative, and 239 of 578 (41%) were lost to contamination. While 12B and BI were similar in recovering mycobacteria and in contamination rates, 60% of LJ were either negative or the slants could not be further processed because of microbial contamination. In fact, more LJ were lost because of contamination (n = 239) than were positive (n = 230).

Recovery rates from each medium alone or in combination with one or both of the other two media are given in Table 2. In general, most isolates were recovered from 12B in combination with one or both solid media. On the basis of these data, it can be calculated that if only two media had been used, the combination of 12B plus BI would have recovered 575 of 580 (99%) isolates, the combination of 12B plus LJ would have recovered 525 of 580 (91%) isolates, and the combination of BI plus LJ would have recovered 484 of 580 (83%) isolates. If only one medium had been used, 12B would have recovered 506 of 580 (87%) isolates, BI would have recovered 469 of 580 (81%) isolates, and LJ would have recovered 230 of 580 (40%) isolates.

Had LJ not been used, five isolates would not have been

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TABLE 1. Relative recovery of mycobacteria from each medium[a]

Organism (no. of isolates)	No. (%) of mycobacteria recovered from:		
	12B	BI	LJ
M. tuberculosis (230)	211 (92)	198 (86)	86 (37)
MAC (277)	238 (86)	223 (81)	121 (44)
M. gordonae (21)	14 (67)	12 (57)	1 (5)
M. kansasii (15)	15 (100)	14 (93)	10 (67)
Other mycobacteria (37)[b]	28 (76)	22 (59)	12 (32)
All mycobacteria	506 (87)	469 (81)	230 (40)

[a] A total of 580 isolates were recovered.
[b] Includes 10 *M. fortuitum*, 10 *M. xenopi*, 7 rapid growers that were not members of the *M. fortuitum* complex, 3 *M. flavescens*, 2 *M. haemophilum*, 3 *M. chelonae*, and 2 *M. simae.*

recovered: two MAC and one each of *M. tuberculosis*, *M. fortuitum*, and a rapid grower but not an *M. fortuitum* complex isolate. Another culture-positive specimen from the patient with *M. tuberculosis* was submitted within 5 days. Following clinical evaluation, the *M. fortuitum* isolate was determined to be not clinically important. Similarly, one of the two MAC isolates (with one colony recovered from a bronchoalveolar lavage) was determined to be not clinically important. The second MAC isolate, an isolate from blood, was clinically important but was recovered only after prolonged incubation (48 days).

Published comparisons of the BACTEC TB system with solid mycobacterial media have shown that mycobacteria are recovered more often and earlier from 12B than from solid media (Table 3) (1, 3, 5–8, 10). Excluding the study by Morgan et al. (6), which was performed soon after the BACTEC system was introduced, published recovery rates have been 87 to 95% for BACTEC bottles, 52 to 80% for Middlebrook plates, and 42 to 77% for LJs. In our study, recovery rates were 87% for 12B, 81% for BI, and 40% for LJ. Thus, although previous evaluations were performed with differing formulations and combinations of BACTEC and solid media, the results are consistent with our findings.

Stager et al. (10) reported that the combination of 12B plus 7H11 recovered 108 of 112 (96%) *M. tuberculosis* isolates, whereas 12B plus LJ recovered 111 of 112 (99%) isolates and LJ plus 7H11 recovered 96 of 112 (86%) of isolates. Kirihara et al. (5) reported that the combination of LJ plus 7H11 recovered 103 of 115 (90%) of *M. tuberculosis* and MAC isolates, but they did not report what proportions of isolates were recovered from combinations of BACTEC bottles and solid media. Thus, our recovery rates are consistent with those reported previously, indicating that the best combination of media is 12B plus a Middlebrook plate. Published contamination rates (1, 3, 5–8, 10) for various media range from 2.4 to 7.3% for BACTEC bottles, 3.2 to 5.0% for Middlebrook plates, and 2.8 to 7.9% for LJ; the highest contamination rate reported for solid media is 16.7% (8). However, some reports do not specify what proportion of specimens could not be processed further because of contamination. In our study, 5% of 12B, 3% of BI, and 41% of LJ were lost because of contamination. In contrast to the contamination rates for 12B and BI, which are identical to those reported previously, the percentage of LJ lost because of contamination was much higher in our study. The reason for the lower proportion of 12B and BI lost to contamination is because mycobacteria can more easily be recovered from those media than from LJ when they are contaminated with nonmycobacterial microorganisms.

In summary, BI were superior to LJ for the recovery of mycobacteria and were less often contaminated. BI have the additional advantages of providing simultaneous inoculation of selective and nonselective media, for the easier interpretation of colonial morphology and for the earlier detection of microbial growth. Consequently, routine use of LJ is unnecessary.

TABLE 2. Frequency of recovery of mycobacteria from each medium alone or in combination with other media

Positive media	No. (%) of mycobacteria recovered			
	M. tuberculosis	MAC	Other mycobacteria	All mycobacteria
12B only	28 (12)	46 (17)	22 (30)	96 (17)
BI only	15 (7)	29 (10)	11 (15)	55 (9)
LJ only	1 (0)	2 (1)	2 (3)	5 (1)
12B and BI	101 (44)	81 (29)	17 (23)	199 (34)
12B and LJ	3 (1)	6 (2)	1 (1)	10 (2)
BI and LJ	3 (1)	8 (3)	3 (4)	14 (2)
All three media	79 (34)	105 (38)	17 (23)	201 (35)

TABLE 3. Published data regarding relative recovery of mycobacteria from BACTEC bottles and solid media

Study (reference)	No. of mycobacteria recovered/total no. present (%)		Type of solid medium
	BACTEC (%)	Solid media (%)	
Anargyros et al. (1)	147/162 (91)	85/162 (52)	Two LJ
		97/162 (60)	One supplemented LJ
Isenberg et al. (3)	176/202 (87)	145/202 (72)	One LJ
		144/202 (71)	One Middlebrook 7H11 plate
Kirihara (5)	101/115 (88)	89/115 (77)	One LJ
		80/117 (70)	One Middlebrook 7H11 plate
Morgan et al. (6)	51/71 (72)	44/71 (62)	One LJ
		39/71 (56)	One Middlebrook 7H10 plate
		37/71 (52)	One Middlebrook S7H11 plate
Park et al. (7)	55/59 (93)	25/59 (42)	One each LJ and Gruft LJ
Roberts et al. (8)	350/370 (95)	336/370 (91)[a]	
Stager et al. (10)	127/143 (89)	100/143 (70)	First of two LJ
		104/143 (73)	Second of two LJ
		114/143 (80)	One Middlebrook 7H11 plate

[a] Five institutions collected data for this study; different combinations of one to two LJ and Middlebrook 7H10, 7H11, and/or selective 7H11 plates were used; for simplicity of tabulation, recoveries from solid media are combined.

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