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Detection and Recovery of Mycobacteria by a Radiometric Procedure

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During a 6-month period, 5,375 clinical specimens were cultured on Middlebrook-Cohn 7H10 medium, on Lowenstein-Jensen medium, and in Middlebrook 7H12 medium containing [^{14}C]palmitic acid. More mycobacteria were recovered when all three media were used than when either the conventional method with 7H10 agar and Lowenstein-Jensen slants or the radiometric method with 7H12 broth was used alone.

Detection and recovery of mycobacterial species by the radiometric method have been previously reported (1, 2). Recent studies have suggested that this method is superior to conventional methods in detection time and recovery rate (J. P. Truant, B. Muller, R. Broman, G. Stroup, D. Hidalgo, and D. Zieg, Abstr. Annu. Meet. Am. Soc. Microbiol. 1979, C(H)82, p. 360; C. D. Horstmeier, D. R. DeYoung, K. A. Doerr, and G. D. Roberts, Abstr. Annu. Meet. Am. Soc. Microbiol. 1982, C187, p. 302; J. P. Libonati, S. H. Siddiqi, M. Carter, and C. Hwangbo, Abstr. Annu. Meet. Am. Soc. Microbiol. 1982, C190, p. 303). Because previous reports have emphasized the recovery of *Mycobacterium tuberculosis*, the mycobacteria recovered by the radiometric method have been categorized as either *M. tuberculosis* or mycobacteria other than tubercle (MOTT) bacilli. Owing to the heightened awareness of the role of MOTT bacilli in causing disease in humans (5), we studied the capacity of the radiometric method to recover not only *M. tuberculosis* but also MOTT bacilli from clinical specimens.

From September 1981 to February 1982, we examined 5,375 specimens for mycobacteria. In all cases, the specimen collection, digestion, and decontamination were carried out according to standard procedures (4). Two Lowenstein-Jensen (L-J) slants and one Middlebrook-Cohn 7H10 agar plate were each inoculated with 0.1 ml of processed specimen. In addition, 0.1 ml of the specimen was inoculated into a vial which contained Middlebrook 7H12 broth (Johnston Laboratories, Cockeysville, Md.) containing [^{14}C]palmitic acid. To reduce nonmycobacterial contamination, each 7H12 broth also received 0.1 ml of a modified Mitchison antimicrobial mixture (3) consisting of 100 μg of amphotericin B, 1000 U of polymyxin B, 500 μg of carbenicillin, and 50 μg of trimethoprim per ml. The L-J slants and 7H10 agar plates were incubated in 6% CO_2 at 35°C and examined for appearance of growth at weekly intervals for 8 weeks. The 7H12 vials were incubated at 35°C and screened for output of $^{14}CO_2$ from [^{14}C]palmitic acid at 4-day intervals for the first 12 days, then weekly for 4 weeks with the BACTEC 460 system (Johnston Laboratories). The Ziehl-Neelsen stain (4) was used to confirm the presence of mycobacterial growth.

Of the 5,375 specimens cultured, we recovered mycobacteria from 274 specimens (5.1%). Mycobacteria were recovered from 227 specimens (82.9%) by the radiometric method with 7H12 medium and from 215 specimens (78.5%) by the conventional method with 7H10 and L-J media. L-J medium detected 187 specimens (68.3%) and 7H10 medium detected 178 specimens (64.9%). The contamination rates in the 7H12, L-J, and 7H10 media were 6.8, 7.8, and 14.3%, respectively. The average time required to detect positive cultures in 7H12 medium was 12.8 days versus 21.0 days on 7H10 or L-J medium. The range of mycobacteria recovered during the evaluation is shown in Table 1. *Nocardia asteroides* was isolated on one occasion from the 7H12 medium only.

An analysis of the mycobacteria recovered by radiometric and conventional methods is as follows. Although neither the radiometric nor the conventional method alone was able to recover all cultures, the data suggest that improved isolation rates are possible if radiometric and conventional methods are used together. Of the 131 MOTT bacilli recovered, 106 (80.9%) were recovered by the radiometric method and 93 (71%) were recovered by the conventional method. A total of 25 MOTT bacilli (19.1%) were not recovered by the radiometric method, and 38 (29%) were not recovered by the conventional method. Of the total 143 *M. tuberculosis* iso-

TABLE 1. Mycobacteria isolated from clinical specimens by radiometric and conventional methods

Mycobacteria	No. recovered with:		Total no. recovered
	Radiometric method	Conventional method	
Mycobacterium avium complex	60	45	63
M. chelonei	1	2	3
M. fortuitum	1	1	2
M. gordonae	30	30	45
M. kansasii	10	9	10
M. marinum	0	1	1
M. phlei	1	1	1
M. simiae	1	0	1
M. terrae complex	2	4	5
M. tuberculosis	121	122	143

lates, 121 (84.6%) were recovered by the radiometric method, and 122 (85.3%) were recovered by the conventional method. A total of 22 *M. tuberculosis* isolates (15.4%) were not recovered by the radiometric method, and 21 (14.7%) were not recovered by the conventional method. When the radiometric method was used with the conventional method, the recovery of MOTT bacilli increased by 38 (29%), and the recovery of *M. tuberculosis* increased by 21 (14.7%).

In this evaluation, the question of whether the advantage gained with the use of 7H12 broth is greater than that gained with the use of another 7H10 agar plate or L-J slant was not specifically addressed. The use of 7H12 broth yielded 15 additional positive cultures which would have been missed owing to contamination if 7H10 and L-J had been the only media used. The greater recovery of the *M. avium* complex in 7H12 (95.2%) than on L-J and 7H10 (71.4%) media may reflect a difference in sensitivity for the recovery of the *M. avium* complex. Because the *M. avium* complex usually occurs in small numbers in sputum, it is also possible that the few bacilli present may end up on the edge of an L-J slant or 7H10 plate and not be detected. With the 7H12 medium, all of the inoculum is delivered into the medium.

Our experience indicates that 7H12 medium used in conjunction with conventional media appears to maximize the recovery of mycobacteria. Moreover, the detection and final identification of most cultures was more rapid when the radiometric method was used.

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