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Evaluation of a Nonradiometric System (BACTEC 9000 MB) for Detection of Mycobacteria in Human Clinical Samples

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This study was carried out to evaluate the rate of recovery and time required for detection of mycobacteria from pulmonary and extrapulmonary human clinical samples, by using a fluorescence-quenching-based oxygen sensor (BACTEC 9000 MB; Becton Dickinson Microbiology Systems, Sparks, Md.). The results were compared with those obtained by microscopy, conventional culture in Lowenstein-Jensen (LJ) medium, and a BACTEC radiometric system (BACTEC 460 TB; Becton Dickinson). Of the 779 clinical samples processed, 364 from pulmonary sites and 415 from extrapulmonary sites, 62 (7.9%) were positive for mycobacterial isolates; of the positive samples, 59 (95.1%) were detected with the fluorescent BACTEC 9000 MB system, 57 (91.9%) were detected with the radiometric system (BACTEC 460 TB), and 43 (69.3%) were detected with LJ conventional culture. The mean times to detection of all mycobacteria with BACTEC 9000 MB and BACTEC 460 TB were similar (10.3 and 10.0 days, respectively). The results obtained indicate that the nonradiometric BACTEC (BACTEC 9000 MB) system is as efficient as Bactec 460 TB and significantly more efficient than LJ for the rapid recovery of mycobacteria from both pulmonary and extrapulmonary clinical specimens. Though the BACTEC 9000 MB system is recommended for respiratory specimens, we demonstrated that it can be successfully used also for recovery of mycobacteria from clinical specimens from various extrapulmonary sites.

Tuberculosis has made a dramatic comeback in industrialized countries because of the AIDS pandemic (11) and the increase in the number of immigrants and homeless people. Today, the "gold standard" for diagnosis of tuberculosis is still detection of *Mycobacterium tuberculosis* by culture. Traditional methods for isolating *M. tuberculosis* from human specimens by culture can take up to 8 weeks. A radiometric method (BACTEC 460 TB) has reduced the culture time to less than 2 weeks (1, 2, 7, 8). More recently, a new, fully automated, nonradiometric method, fluorescent BACTEC 9000 MB (Becton Dickinson), which uses an oxygen-quenched fluorescence indicator for the rapid detection of *Mycobacterium* spp., has been introduced. The fluorescence of the sensor is a function of the oxygen depletion that results during microbial metabolism. The presence of fluorescence indicates growth of microorganisms.

In this study we evaluated the fluorescent BACTEC 9000 MB system and compared the results with those obtained by conventional culture in Lowenstein-Jensen (LJ) medium and with the BACTEC 460 radiometric TB system.

MATERIALS AND METHODS

This study was conducted at two sites: the Clinical Microbiology Laboratory at the University of Sassari and the Clinical Microbiology Laboratory of the Catholic University of the Sacred Heart in Rome, Italy.

Specimens. Specimens, entered consecutively and processed for mycobacterial culture, included 325 sputum, 39 bronchoalveolar lavage fluid (BAL), 189 urine, 120 stool, 39 cerebrospinal fluid, and 67 miscellaneous samples.

Specimen processing. Specimens were decontaminated and digested with equal volumes of *N*-acetyl-L-cysteine and sodium hydroxide (2% final concentration) for 20 min, according to standard procedures (6, 9). Aseptically collected specimens were centrifuged without decontamination. All specimens were centrifuged at 3,000 × *g* for 30 min, washed with 10 ml of phosphate-buffered saline (0.067 M, pH 6.8) and resuspended with 0.2% fatty-acid-free albumin. After being processed and concentrated, the specimens were used for making smears for acid-fast staining and for inoculation into media.

Media and culturing methods. The medium used in BACTEC 9000 MB is a modified Middlebrook broth formulation (Myco/F sputa; Becton Dickinson Microbiology Systems, Sparks, Md.). Each Myco/F vial was supplemented with 2 ml of supplement F enrichment (lactic acid [3.5 mg/ml], polyoxyethylene stearate [2.3 mg/ml], bovine serum albumin [116.0 mg/ml], dextrose [23.0 mg/ml], biotin [0.012 mg/ml] [Becton Dickinson]) and with an antimicrobial mixture of PANTA (polymyxin B [1,000 U/ml], amphotericin B [100 μg/ml], nalidixic acid [400 μg/ml], trimethoprim [100 μg/ml], azlocillin [200 μg/ml] [Becton Dickinson]). After processing, 0.5 to 1 ml (manufacturer's recommendations) of each specimen was inoculated into one Myco/F medium bottle.

Each BACTEC 460 TB culture bottle (12B medium; Becton Dickinson) contained 4 ml of modified Middlebrook 7H9 broth, casein hydrolysate, bovine serum albumin, catalase, and ^{14}C-substrate and was supplemented with PANTA. After processing, 0.5 ml of each specimen was inoculated into a BACTEC 12B medium vial and incubated at 37°C.

LJ (BioMerieux, Marcy l'Etoile, France) conventional medium was inoculated with 0.2 to 0.3 ml of each specimen on a slant, incubated at 37°C, and inspected weekly for 8 weeks. BACTEC 460 bottles were monitored every 2 days during the first week and weekly thereafter for the next 7 weeks by using a BACTEC 460 TB instrument (Becton Dickinson). BACTEC 9000 bottles were placed in a BACTEC 9000 MB instrument directly after inoculation and kept for up to 8 weeks in the instrument, which provided 37°C incubation.

Detection of growth. Growth on solid medium was detected by visual observation of colonies in radiometric BACTEC medium; a growth index of 10 or more was considered positive. Growth in BACTEC 9000 MB was detected by the instrument, based on the development of fluorescence. All positive growth was contemporaneously confirmed by making smears from the broth and staining for acid-fast bacteria.

Identification of mycobacteria. Organisms were identified by using standard biochemical methods (6) and DNA-RNA hybridization (AccuProbe; Gene Probe, Inc., San Diego, Calif.).

Statistical analysis. χ^2 values were calculated by using Epi Info (version 6.03; Centers for Disease Control and Prevention, Atlanta, Ga.).

RESULTS

Of a total of 779 clinical specimens studied, 62 (7.9%) were found to be positive for mycobacteria on all media combined; of the positive specimens 59 (95.1%) were detected by BACTEC 9000 MB, 57 (91.9%) were detected by BACTEC 460 TB, and 43 (69.3%) were detected by conventional culture

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TABLE 1. Recovery of mycobacteria from pulmonary and extrapulmonary clinical samples

Specimen type (*n*)	No. (%)[a] of cultures positive by:						
	Any method	LJ	BACTEC 460 TB	BACTEC 9000 MB	BACTEC 460 TB only	BACTEC 9000 MB only	LJ only
Sputum (325)	41 (**12.6**)	32 (78.0)	38 (92.6)	41 (100)	0 (**0**)	2 (**0.6**)	0 (**0**)
BAL (39)	4 (**10.2**)	2 (50.0)	4 (100)	4 (100)	0 (**0**)	0 (**0**)	0 (**0**)
Urine (189)	9 (**4.7**)	3 (33.3)	8 (88.8)	6 (66.6)	3 (**1.58**)	1 (**0.5**)	0 (**0**)
Stool (120)	2 (**1.6**)	1 (50.0)	1 (50)	2 (100)	0 (**0**)	0 (**0**)	0 (**0**)
CSF[b] (39)	3 (**7.7**)	2 (66.6)	3 (100)	3 (100)	0 (**0**)	0 (**0**)	0 (**0**)
Other[c] (67)	3 (**4.5**)	3 (100)	3 (100)	3 (100)	0 (**0**)	0 (**0**)	0 (**0**)
Respiratory specimens (364)	45 (**12.4**)	34 (75.5)	42 (93.3)	45 (100)	0 (**0**)	2 (**0.5**)	0 (**0**)
Nonrespiratory specimens (415)	17 (**4.1**)	9 (52.9)	15 (88.2)	14 (82.3)	3 (**0.7**)	1 (**0.2**)	0 (**0**)
Total (779)	62 (**7.9**)	43 (69.3)	57 (91.9)	59 (95.1)	3 (**4.8**)	3 (**4.8**)	0 (**0**)

[a] Values in bold are percentages of the total number of samples (*n*); all other values are percentages of the number of cultures positive by any method.
[b] Cerebrospinal fluid.
[c] Includes (*n*) pus (14), lymph node (10), gastric fluid (20), endometrium (4), ulcerative lesion (6), pleuric fluid (4), ascitic fluid (3), and others (6).

on LJ medium. Table 1 shows analysis of clinical samples according to their origins. Of a total of 189 urine samples, 9 (4.7%) were positive for mycobacteria, of which BACTEC 460 picked up 8 (88.8%), BACTEC 9000 picked up 6 (66.6%), and LJ culturing detected only 3 (33.3%). Of a total of 325 sputum samples, 41 (100%) of all positive samples were detected by BACTEC 9000 MB, while 38 (92.6%) were positive by BACTEC 460 TB and 32 (78%) were positive by LJ. Of a total of 120 stool samples, two positive samples (*Mycobacterium avium*) were detected by BACTEC 9000 MB, while only one was positive with BACTEC 460 TB and LJ. Of a total of 39 cerebrospinal fluid samples, 3 were positive with the two BACTEC methods while 2 were positive by LJ. Of 67 miscellaneous samples, only 3 (4.5%) were positive by all methods. Among the total of 779 specimens, 364 were from respiratory sources, with 45 (12.4%) positive for mycobacteria, while 415 were extrapulmonary specimens, with 17 (4.1%) positive (Table 1). A total of 48.8% of culture-positive respiratory specimens were positive on acid-fast bacillus (AFB) smears when the initial smear from specimens was examined, while only 2 of 17 (11.8%) culture-positive extrapulmonary specimens had initial positive AFB smears. Thirty-four specimens were positive for *M. tuberculosis*, 10 for *M. avium*, 9 for *Mycobacterium gordonae*, 3 for *Mycobacterium xenopi*, and 6 for *Mycobacterium terrae*, with no significant difference in isolation rates between the two BACTEC systems ($P > 0.05$) (Table 2).

TABLE 2. Different species of mycobacteria isolated with each detection system

Organism (no. of isolates) and detection system	No. of isolates of specimen type		
	Pulmonary	Extrapulmonary	Both
M. tuberculosis (34)			
LJ	25	6	31
BACTEC 460 TB	24	8	32
BACTEC 9000 MB	25	9	34
M. avium (10)			
LJ	6	3	9
BACTEC 460 TB	8	1	9
BACTEC 9000 MB	8	2	10
Other[a] (18)			
LJ	3	0	3
BACTEC 460 TB	10	6	16
BACTEC 9000 MB	12	3	15

[a] Includes nine *M. gordonae*, six *M. terrae*, and three *M. xenopi* isolates.

Contamination rate and false-positivity rate. The contamination rate for all specimens inoculated into the BACTEC 460 TB medium was 2.0% (16 of 779); for BACTEC 9000 MB, the rate was 4.1% (32 of 779), while in LJ medium, the rate was 0.0% (Table 3). With LJ, contamination was considered only when a whole slant was contaminated. The microorganisms isolated belong to the genera *Staphylococcus*, *Streptococcus*, and *Pseudomonas.*

Most of the contaminated bottles had been inoculated with stool specimens; of these, the contamination rate was 9.1% (11 of 120) for BACTEC 9000 MB versus 5.0% (6 of 120) for BACTEC 460 TB. No false-positive specimens were recovered. This was determined by microscopic observation of AFB in the smears.

Time to detection. Table 4 shows analysis of the average and range of time to detection according to smear positivity of the specimens. Overall, the average time to detection with BACTEC 460 TB was 10.0 days; with BACTEC 9000 MB it was 10.3 days, and on LJ medium it was 27.3 days (Table 4), while some of the positive cultures on LJ medium took as long as 43 days to be detected. Cumulative percentages of recovery of *M. tuberculosis* and *M. avium* in 12B and Myco/F media are shown in Fig. 1.

TABLE 3. Contaminated cultures found by using all methods[a]

Specimen type (*n*)	No. (%) of contaminated cultures found by:		
	LJ	BACTEC 460 TB	BACTEC 9000 MB
Sputum (325)	0 (0)	5 (1.5)	9 (2.7)
BAL (39)	0 (0)	0 (0)	0 (0)
Urine (189)	0 (0)	3 (1.5)	8 (4.2)
Stool (120)	0 (0)	6 (5.0)	11 (9.1)
CSF[b] (39)	0 (0)	1 (2.5)	1 (2.5)
Other[c] (67)	0 (0)	1 (1.4)	3 (4.4)
Total (779)	0 (0)	16 (2.0)	32 (4.1)

[a] All samples that were positive but contaminated received a secondary NaOH treatment to kill the bacterial contaminants.
[b] Cerebrospinal fluid.
[c] Includes (*n*) pus (14), lymph node (10), gastric fluid (20), endometrium (4), ulcerative lesion (6), pleuric fluid (4), ascitic fluid (3), and others (6).

TABLE 4. Time to positive cultures

Organism[a] (total no. of positive cultures)	Avg (range) of days to positive cultures		
	LJ	BACTEC 460 TB	BACTEC 9000 MB
Smear-positive *M. tuberculosis* (15)	24 (13–43)	7 (4–10)	9 (4–12)
Smear-negative *M. tuberculosis* (22)	28 (19–36)	15 (3–28)	14 (3–26)
Smear-positive MOTT (25)	30 (25–35)	8 (5–11)	8 (5–11)
Smear-negative MOTT (0)	0	0	0
Avg for all positive cultures (62)	27.3 (13–43)	10.0 (4–28)	10.3 (3–26)

[a] MOTT, mycobacteria other than *M. tuberculosis.*

DISCUSSION

There is very little information in the literature about the efficacy of BACTEC 9000 MB for routine use in clinical laboratories (3, 10). van Griethuysen recently compared this system with Septi-Chek plus LJ medium but did not compare it with the well established BACTEC 460 radiometric system (10).

The present study evaluated the newly introduced fluorescent BACTEC 9000 MB system with the radiometric BACTEC 460 TB system and conventional culture in LJ medium for the detection of mycobacteria. Several earlier studies have demonstrated markedly reduced times to detection of mycobacteria with the BACTEC radiometric technique (1, 2, 7, 8). In this study, all *M. tuberculosis* strains were detected within 4 weeks with both BACTEC systems, with a range of 3 to 28 days. Similarly, *M. avium* was detected in 5 to 11 days in both systems. On the other hand, detection on conventional media generally took 13 to 43 days for *M. tuberculosis* and 25 to 35 days for *M. avium*. BACTEC 9000 is a continuous monitoring system, which is not possible for BACTEC 460 TB and LJ culturing because of the large number of processed samples. Therefore, while the time-to-detection data may be biased toward BACTEC 9000 MB, its continuous monitoring is an unequivocal advantage currently not enjoyed by the other methods. The times to detection reported here do not include the additional time required for conventional identification. The probe procedures can be performed in approximately 2 h (4). Moreover, the inoculum size for each medium could be different. We followed the standard recommended procedure for each medium; as BACTEC 9000 MB contains a higher volume of medium, it can take up to a 1-ml inoculum, while the recommendation for BACTEC 460 is 0.5 ml and that for LJ is 0.2 to 0.3 ml. This may have affected the positivity and introduced a bias for 9000 MB compared to LJ medium.

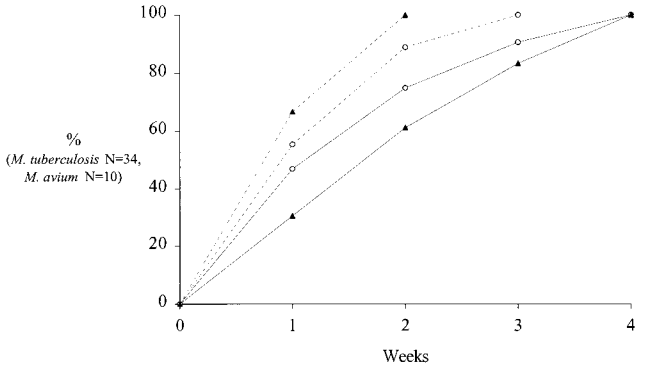

FIG. 1. Total recovery of *M. tuberculosis* (solid lines) and *M. avium* (broken lines) isolates by BACTEC 460 TB (12B medium) (circles) and BACTEC 9000 MB (Myco/F medium) (triangles).

As far as the recovery rate is concerned, there was no significant difference between BACTEC 460 TB and BACTEC 9000 MB. BACTEC 460 TB recovered 57 of 62 (91.9%) positive specimens, while BACTEC 9000 MB recovered 59 (95.1%). However, BACTEC 9000 MB recovered more than 37% more positive cultures than did LJ medium.

The BACTEC 9000 MB system has been recommended for pulmonary clinical samples only, but in our study we analyzed a remarkable number of extrapulmonary specimens to evaluate its usefulness for these samples. The results showed that the BACTEC 9000 MB system is better than conventional LJ medium (3.4 versus 2.1% recovery rates) and comparable to BACTEC 460 TB (3.4 versus 3.6%). Thus it can be successfully used to recover mycobacteria from extrapulmonary clinical samples, as BACTEC 460 TB has been reported capable of doing (5).

The BACTEC 9000 MB system had a higher contamination rate (4.1%) than did the BACTEC 460 TB system (2.0%). The reason for this contamination rate could be that the medium is very rich and/or the volume of the medium is high (40 ml). Another possible reason is that BACTEC 9000 MB measures oxygen depletion of all organisms present in the vial, whereas BACTEC 460 TB measures $^{14}CO_2$ generated from organisms which are capable of metabolizing the substrate, palmitic acid.

Because of the increase in mycobacterial infections over the past decade, there is a great need for rapid methods of detection of *Mycobacterium* spp. in clinical specimens. The results of this study indicate that the fluorescent BACTEC 9000 MB system is as rapid and sensitive for recovery of mycobacteria as the radiometric system and is much more sensitive than conventional culture in LJ medium. BACTEC 9000 MB offers several other practical advantages, such as complete automation, continuous monitoring, and data management. Most importantly, BACTEC 9000 MB is nonradiometric and based on fluorescent technology which can be used in laboratories where the use of radioactive materials is not allowed.

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REFERENCES

1. **Abe, C., S. Hosojima, Y. Fukasawa, Y. Kazumi, M. Takahashi, K. Hirano, and T. Mori.** 1992. Comparison of MB-Check, BACTEC, and egg-based media for recovery of mycobacteria. J. Clin. Microbiol. **30:**878–881.
2. **Anargyros, P., D. S. J. Astill, and I. S. L. Lim.** 1990. Comparison of improved BACTEC and Lowenstein-Jensen media for culture of mycobacteria from clinical specimens. J. Clin. Microbiol. **28:**1288–1291.
3. **Boyle, J. F., A. E. Rendo, L. B. Reller, Y. Hale, S. Dean, S. V. Juchau, and G. R. Dufour.** 1995. Comparison of the BACTEC fluorescent 9000TB detection system and the BACTEC radiometric 460TB system, abstr. U-141, p. 142. *In* Abstracts of the 95th General Meeting of the American Society for

Microbiology 1995. American Society for Microbiology, Washington, D.C.
4. **Ellner, P. D., T. E. Kiehn, R. Cammarata, and M. Hosmer.** 1988. Rapid detection and identification of pathogenic mycobacteria by combining radiometric and nucleic acid probe methods. J. Clin. Microbiol. **26:**1349–1352.
5. **Fadda, G., and S. L. Roe.** 1984. Recovery and susceptibility testing of *Mycobacterium tuberculosis* from extrapulmonary specimens by the BACTEC radiometric method. J. Clin. Microbiol. **19:**720–721.
6. **Kent, P. T., and G. P. Kubika.** 1985. Public health mycobacteriology. A guide for the level III laboratory. Centers for Disease Control, Atlanta, Ga.
7. **Kirihara, J. M., S. L. Hiller, and M. B. Coyle.** 1985. Improved detection times for *Mycobacterium avium* complex and *Mycobacterium tuberculosis* with the BACTEC radiometric system. J. Clin. Microbiol. **22:**841–845.
8. **Morgan, M. A., C. D. Horstmeier, D. R. DeYoung, and G. D. Roberts.** 1983. Comparison of a radiometric method (BACTEC) and conventional culture media for recovery of mycobacteria from smear-negative specimens. J. Clin. Microbiol. **18:**384–388.
9. **Nolte, F. S., and B. Metchock.** 1995. *Mycobacterium*, p. 400–437. *In* P. R. Murray, E. J. Baron, M. A. Pfaller, F. C. Tenover, and R. H. Yolken (ed.), Manual of clinical microbiology, 6th ed. American Society for Microbiology, Washington, D.C.
10. **van Griethuysen, A. J., A. R. Jansz, and A. G. M. Buiting.** 1996. Comparison of fluorescent BACTEC 9000 MB System, Septi-Chek AFB System, and Lowenstein-Jensen medium for detection of mycobacteria. J. Clin. Microbiol. **34:**2391–2394.
11. **World Health Organization.** 1993. Global program on AIDS. The HIV/AIDS pandemic: 1993 overview. WHO/EPA/CNP/EVA/93.1.