JOURNAL OF CLINICAL MICROBIOLOGY, Sept. 1997, p. 2229–2234 Vol. 35, No. 9
0095-1137/97/$04.00+0
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Rapid Detection of Mycobacteria in Clinical Specimens by Using the Automated BACTEC 9000 MB System and Comparison with Radiometric and Solid-Culture Systems

GABY E. PFYFFER,[1]* CORNELIA CIESLAK,[2] HANS-MARTIN WELSCHER,[1] PASCALE KISSLING,[1] AND SABINE RÜSCH-GERDES[2]

Department of Medical Microbiology, Swiss National Center for Mycobacteria, University of Zurich, CH-8028 Zurich, Switzerland,[1] and Research Center of Borstel, National Reference Center for Mycobacteria, D-23845 Borstel, Germany[2]

Received 20 March 1997/Returned for modification 25 April 1997/Accepted 1 June 1997

Recovery rates of acid-fast bacilli (AFB) and the mean time to their detection from clinical specimens were determined by using the fluorescent BACTEC 9000 MB system. Data were compared to those assessed by the radiometric BACTEC 460 system and by cultivation on solid media. A total of 3,095 specimens were processed with *N*-acetyl-L-cysteine–NaOH by two laboratories. The contamination rates for the BACTEC 9000 MB system were 6.8% (center 1) and 9.8% (center 2). A total of 451 mycobacterial isolates were detected (*Mycobacterium tuberculosis* complex, $n = 296$; nontuberculous mycobacteria [NTM], $n = 155$). These isolates originated from 94 (20.8%) smear-positive and 357 (79.2%) smear-negative specimens. The BACTEC 9000 MB system was significantly better than solid media ($P < 0.05$) in detecting AFB, but it was less efficient than the radiometric system ($P < 0.01$). The BACTEC 9000 MB system plus solid media (combination A) recovered 393 (87.1%) of the isolates, while the BACTEC 460 system plus solid media (combination B) detected 430 (95.3%) of all AFB isolates. Between combination A and B there was no statistically significant difference for the detection of isolates from smear-positive specimens ($P > 0.05$), in contrast to the recovery of AFB from smear-negative specimens for *M. tuberculosis* complex, $P < 0.05$; for NTM, $P < 0.01$). The mean time to detection of *M. tuberculosis* complex was 12.2 days for smear-positive specimens and 18.1 days for smear-negative specimens with the BACTEC 9000 MB system; 9.3 and 15.6 days, respectively, with the BACTEC 460 system; and 21.2 and 28.4 days, respectively, with solid media. For NTM, the average detection times were 15.1, 17.3, and 31.3 days by the three methods, respectively. In conclusion, the BACTEC 9000 MB system is a rapid, less labor-intensive detection system which allows for higher levels of recovery of AFB than solid media. There is no risk of cross contamination, which is known to be the case for the BACTEC 460 system, and data management is greatly facilitated. As a whole, however, the BACTEC 9000 MB system should only be used in conjunction with solid media.

Recent technological advances in the clinical mycobacteriology laboratory have led to faster detection and identification of acid-fast bacilli (AFB). Although rapid progress is largely due to molecular biology, which allows for the direct diagnosis of mycobacteria from clinical specimens within a couple of hours (14), nucleic acid amplification techniques are, at present, considered merely an adjunct to conventional laboratory diagnostics (10), which still include culture of AFB as one of the time-consuming, but indispensable components.

Recent publications from the Centers for Disease Control and Prevention (Atlanta, Ga.) recommend turnaround times extending up to 21 days for the isolation and identification of *Mycobacterium tuberculosis* (2, 16). Not only should laboratories aim at achieving this goal but they should also aim at using a combination of solid and liquid media, which is the current "gold standard" for accurate cultural detection of mycobacteria (8). While smaller laboratories usually cultivate AFB in broths, larger ones often detect mycobacteria with the radiometric BACTEC 460 TB system. (In Switzerland, for instance, as many as 64% of all laboratories performing AFB culture used this technology in 1996 [10a].) However, universal application of the radiometric BACTEC 460 TB system, although it is both very rapid and sensitive, could never be achieved, mainly because of the well-known limitations associated with radiolabeled substrates, in particular, safety and regulatory principles. Several new growth-based strategies which try to circumvent these problems have therefore been recently developed; among these are the Mycobacteria Growth Indicator Tube (MGIT; Becton Dickinson [9, 12]) as well as fully automated technologies such as the ESP Culture system II (Difco [20]), the MB/BacT (Organon-Teknika [7]), and the BACTEC 9000 MB system (Becton Dickinson).

The BACTEC 9000 MB system is a fluorescence-based, continuously monitoring detection system which uses a modified Middlebrook 7H9 broth in conjunction with a supplement as well as antibiotics to suppress the growth of contaminating microorganisms. For the growth and detection of AFB the BACTEC 9000 MB system differs from the BACTEC 9000 blood culture instrument in two ways: (i) It uses an oxygen-specific sensor, and (ii) environmental parameters specific to mycobacteria have been accounted for (1). At present, there is little published information about this new nonradiometric and noninvasive walk-away culture system. Boyle et al. (3) have presented preliminary results for respiratory specimens which were cultivated in parallel in the BACTEC 460 system and on solid media, and very recently van Griethuysen et al. (17) have compared the performance characteristics of the BACTEC

* Corresponding author. Mailing address: Department of Medical Microbiology, Swiss National Center for Mycobacteria, University of Zurich, Gloriastrasse 30, 8028 Zurich, Switzerland. Phone: 41 1 257 27 86. Fax: 41 1 261 87 90.

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9000 MB system versus those of Septi-Chek AFB (Becton Dickinson) and solid media.

The present two-center study was undertaken in order to evaluate the reliability of the new BACTEC 9000 MB growth technology in comparison with those of radiometry and solid media by using a broad range of specimens of both respiratory and nonrespiratory origin.

MATERIALS AND METHODS

Specimens. A total of 3,095 clinical specimens were consecutively received for culture by the two reference centers for mycobacteria participating in the study (center 1, Zurich, Switzerland; center 2, Borstel, Germany). Samples originated from patients who were admitted to hospitals or who consulted private physicians. The majority of specimens (85.4%) were from the respiratory tract (sputum samples, bronchial aspirates, and bronchoalveolar lavage specimens; n = 2,643). A total of 452 nonrespiratory specimens were processed at center 2 and included specimens of urine (99 specimens), gastric fluid (97 specimens), cerebrospinal fluid (CSF; 68 specimens), skin biopsies (64 specimens), pleural aspirates (61 specimens), feces (52 specimens), ascitic fluid (5 specimens), blood (4 specimens), and bone marrow (2 specimens).

Specimen processing. Processing was done twice a day. At both centers (center 1 and 2) respiratory specimens were liquefied and decontaminated with *N*-acetyl-L-cysteine (NALC)–NaOH (5) (center 1, 15 min; center 2, 20 min). Lymph nodes and tissue specimens were homogenized in a Ten Broeck mortar. At center 1, 100 μl of these homogenates as well as 100 μl of other, normally sterile nonrespiratory specimens (CSF, biopsy samples, pleural aspirates, etc.) was applied to Chocolate II Agar (Becton Dickinson Microbiology Systems, Cockeysville, Md.) and incubated for 48 h at 36 ± 1°C. Decontamination was performed only when growth of bacteria or fungi was observed on this medium. At center 2, all nonrespiratory specimens were pretreated with NALC-NaOH.

Use of the BACTEC 9000 MB system. (i) Medium. Each BACTEC 9000 MB MYCO/F culture vial (Becton Dickinson Diagnostic Instrument Systems, Sparks, Md.) contained 40 ml of broth supplemented with 2 ml of an antimicrobial mixture (PANTA) consisting of 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). Lyophilized PANTA was previously reconstituted with 10 ml of supplement F.

(ii) Quality control. Reference mycobacterial strains (*M. tuberculosis* ATCC 27294, *M. kansasii* ATCC 12478, and *M. fortuitum* ATCC 6841, as well as *M. bovis*, *M. avium* complex [MAC], and *M. scrofulaceum* [clinical isolates]) were initially tested in each new lot of MYCO/F medium. According to the study protocol, mycobacterial suspensions (in sterile saline) were prepared from Löwenstein-Jensen (LJ) subculture slants (up to 14 days old). Serial dilutions (1:100 and 1:500) of an inoculum with a density comparable to the density of a 0.5 McFarland nephelometer standard were made, and 0.1 ml was inoculated into the MYCO/F media.

(iii) Cultivation and reading. The BACTEC MYCO/F vials were inoculated with 0.5 ml of the processed sediment and were incubated for 8 weeks. Since fluorescence levels are measured every 10 min by the BACTEC 9000 MB system, the vials were almost continuously monitored for the growth of AFB. The acid fastness of the microorganisms was confirmed by Ziehl-Neelsen staining. A BACTEC 9000 MB signal was considered false positive if neither AFB nor contaminants were found in the smear made immediately after this signal and no mycobacteria were subcultured on LJ slants and in BACTEC 12B medium.

Cultivation of clinical specimens. Cultivation of mycobacteria was done in liquid and on solid media. These included the BACTEC 9000 MYCO/F medium and the radiometric BACTEC 460 medium (12B medium; 13A medium was used for blood and bone marrow specimens) and an LJ slant (without antibiotics at center 1 and with PACT [polymyxin B, 50,000 U/liter; amphotericin B, 5 mg/liter; carbenicillin, 25 mg/liter; and trimethoprim, 2.5 mg/liter] at center 2), Middlebrook 7H10/sel7H11 (biplate [Becton Dickinson]; center 1), and Stonebrink (center 2). All media were incubated at 36 ± 1°C. Specimens taken from superficial wounds were inoculated onto an additional set of media (BACTEC 460 12B medium and solid media) and were incubated at 30°C in order to recover mycobacteria with a lower temperature optimum (such as *M. marinum*).

Half a milliliter of sediment was cultivated in the BACTEC 9000 MB system (see above) and in the BACTEC 460 system (8). Each BACTEC 12B medium vial was supplemented with 0.1 ml of an antimicrobial mixture (PANTA). The vials were incubated for 8 weeks. The growth index (GI) was read twice a week for the first 2 weeks and weekly thereafter for an additional 6 weeks. Time to detection in the BACTEC 460 system was the interval between specimen inoculation and a vial GI of ≥50. Finally, 0.25 ml of the sediments was inoculated onto the solid media, incubated in 6% CO_2, and inspected weekly for 8 weeks. Again, the acid fastness of the cultures was verified by Ziehl-Neelsen stains.

Microscopy. Smears were stained with auramine-rhodamine fluorochrome. Positive slides were confirmed by Ziehl-Neelsen staining (5).

Identification of mycobacteria. Routine biochemical methods (5, 8) and the Accuprobe culture confirmation kits (Gen-Probe, San Diego, Calif.) were used for the identification of isolates. Some of the isolates were identified by their patterns of cellular fatty acids by gas-liquid chromatography (15) or by sequencing of the 16S rRNA gene (6).

Statistical analysis. The statistical significance of differences in recovery rates was determined by the χ^2 test (Epi Info, version 6.03; CDC).

RESULTS

Mycobacterial isolates. From the total of 3,095 specimens, 451 mycobacterial isolates were recovered: 417 isolates from respiratory specimens (n = 2,643) and 34 from nonrespiratory specimens (n = 452). Of the specimens from which AFB could be isolated, only 94 (20.8%) were smear positive and 357 (79.2%) were smear negative. The mycobacterial isolates included *M. tuberculosis* (n = 290), *M. bovis* (n = 5), *M. africanum* (n = 1), MAC (n = 72), *M. xenopi* (n = 24), *M. malmoense* (n = 15), *M. kansasii* (n = 12), *M. fortuitum* (n = 9), *M. gordonae* (n = 8), *M. chelonae* (n = 4), *M. celatum* (n = 3), *M. simiae* (n = 3), *M. haemophilum* (n = 1), *M. aurum* (n = 1), *M. scrofulaceum* (n = 1), and *M. terrae* complex (n = 1). All these species except *M. africanum* and *M. chelonae* have been isolated from respiratory specimens. Concerning nonrespiratory samples *M. tuberculosis* was isolated from skin biopsy specimens (n = 6 specimens), CSF (n = 4), feces (n = 2), gastric fluids (n = 2), and pleural aspirate (n = 1); and *M. africanum* and *M. bovis* were isolated from skin specimens (n = 1 and 4, respectively). MAC was isolated from feces (n = 5), CSF, and blood (n = 1 each), *M. chelonae* was isolated from skin biopsy specimens (n = 2), *M. gordonae* was isolated from urine (n = 2) and gastric fluid (n = 1), and finally, *M. simiae* was isolated from feces (n = 1).

Contamination and false-positivity rates. Overall contamination rates for the MYCO/F, BACTEC 460, and solid media were 6.8, 1.6, and 12.9%, respectively, for center 1; and 9.8, 9.9, and 2.9%, respectively, for center 2. Among the 2,644 negative specimens, 4 yielded a false-positive signal in the BACTEC 9000 MB system (false-positivity rate, 0.15%). In these four vials, no microorganisms could be detected, neither by smears nor by subsequent cultures.

Recovery rates by system (BACTEC 9000 MB, BACTEC 460, and solid media). The recovery rates for mycobacteria in each cultivation system are summarized in Table 1. The BACTEC 9000 MB and BACTEC 460 systems detected 75 and 86.5%, respectively, while the solid media used detected 67.6% of all isolates (BACTEC 9000 MB versus BACTEC 460, $P < 0.01$; BACTEC 9000 MB versus solid media, $P < 0.05$; BACTEC 460 versus solid media, $P < 0.01$). For the isolation of *M. tuberculosis* complex the BACTEC 9000 MB system was comparable to solid media (83.5 versus 85.5%; BACTEC 9000 MB versus solid media, $P > 0.05$), but it was inferior to the BACTEC 460 system (91.9%; $P < 0.01$). For nontuberculous mycobacteria (NTM), recovery by the BACTEC 9000 MB system was significantly higher than that on solid media (58.7 versus 33.6%; $P < 0.01$) but the fluorescent system again showed a lower recovery rate than the BACTEC 460 system (76.1%; $P < 0.01$).

With regard to microscopy (Table 2), the BACTEC 9000 MB and BACTEC 460 systems detected 93.6 and 92.6% of the isolates grown from smear-positive specimens (n = 94), respectively, whereas solid media recovered 91.5% of all those isolates (no statistically significant differences). In smear-negative specimens (n = 357), the recovery rates for AFB were 70.0 and 84.9% with the BACTEC 9000 MB and BACTEC 460 systems, respectively, compared to 61.3% with solid media (BACTEC 9000 MB versus BACTEC 460, $P < 0.01$; BACTEC 9000 MB versus solid media, $P < 0.05$; BACTEC 460 versus solid media, $P < 0.01$). For smear-positive specimens containing *M. tuberculosis* complex (n = 84) there was no statistically

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TABLE 1. Rates of recovery of mycobacteria from clinical specimens with liquid and solid culture media[a]

Isolate (no.)	No. (%) of isolates detected by the following:					
	BACTEC 9000 MB	BACTEC 460	Solid media[b]	BACTEC 9000 MB plus solid media (combination A)	BACTEC 460 plus solid media (combination B)	BACTEC 9000 MB plus BACTEC 460 (combination C)
All mycobacteria (451)	338 (75.0)	390 (86.5)	305 (67.6)	393 (87.1)	430 (95.3)	425 (94.2)
M. tuberculosis complex (296)	247 (83.5)	272 (91.9)	253 (85.5)	282 (95.3)	293 (99.0)	286 (96.6)
All NTM (155)	91 (58.7)	118 (76.1)	52 (33.6)	111 (71.6)	137 (88.4)	139 (89.7)

[a] A total of 3,095 clinical specimens were tested.
[b] The values given are means. Center 1 used LJ medium and Middlebrook 7H10/sel7H11 (biplate); center 2 used LJ medium (with PACT) and Stonebrink.

significant difference between the performance of the media. The same held true for smear-negative specimens ($n = 212$) when the BACTEC 9000 MB system was compared to solid media ($P > 0.05$). Significant differences were, however, obtained between the BACTEC 9000 MB and BACTEC 460 systems in detecting tubercle bacilli from smear-negative specimens (recovering 79.9 and 91.0%, respectively; $P < 0.01$) and between the BACTEC 460 system and solid media (91.0 and 81.6%; $P < 0.01$). As for NTM, the BACTEC 9000 MB system detected all 10 smear-positive specimens, while the BACTEC 460 system missed 2 isolates and solid media missed 4 isolates (no statistically significant difference for the different cultivation systems with this small number of organisms). For smear-negative specimens containing NTM ($n = 145$) the yields were 55.9 and 75.9% for the BACTEC 9000 MB and BACTEC 460 systems, respectively, versus 31.7% for solid media (BACTEC 9000 MB versus BACTEC 460; $P < 0.01$; BACTEC 9000 MB versus solid media, $P > 0.05$; BACTEC 460 versus solid media, $P < 0.01$).

As expected, many of the isolates were recovered only from a single medium. The BACTEC 9000 MB system alone detected 19 isolates which were missed by the BACTEC 460 system and solid media as well, the BACTEC 460 system recovered 57 additional isolates, and solid media grew 27 additional isolates (Table 3).

Recovery rates by system combination (liquid and solid media). When comparing the recovery rates of AFB on liquid and solid media in combination, the BACTEC 9000 MB system plus solid media (combination A) detected 393 (87.1%) of all isolates ($n = 451$), while the BACTEC 460 system plus solid media (combination B) yielded 430 isolates (95.3%; Table 1). Combination A detected 282 of 296 *M. tuberculosis* complex isolates (95.3%) and 111 of 155 NTM (71.6%), and combination B recovered 293 of 296 *M. tuberculosis* complex isolates (99.0%) and 137 of 155 NTM (88.4%; Table 1). Between combination A and combination B, a statistically significant difference was found for all mycobacteria ($P < 0.01$) as well as for *M. tuberculosis* complex ($P < 0.05$) and NTM ($P < 0.01$). When combining the two broths (BACTEC 9000 MB plus BACTEC 460; combination C) 425 of 451 (94.2%) of all mycobacteria, 286 of 296 *M. tuberculosis* complex isolates (96.6%), and 139 of 155 NTM isolates (89.7%) could be recovered (Table 1). A statistically significant difference was found between combination A and C for the recovery of all mycobacteria ($P < 0.01$) and of NTM ($P < 0.01$) but not for the *M. tuberculosis* complex ($P > 0.05$). Finally, in a comparison of combination B with combination C, no statistically significant differences could be found with respect to the recovery of all mycobacterial isolates, *M. tuberculosis* complex isolates, or NTM isolates ($P > 0.05$).

With regard to isolates from smear-positive specimens, no statistically significant differences were found between combination A and B, combination A and C, and combination B and C, no matter whether all mycobacteria, *M. tuberculosis* complex isolates, or NTM were counted. For isolates from smear-negative specimens, significant differences were, however, observed when comparing combination A with combination B (all mycobacteria, $P < 0.01$; *M. tuberculosis* complex, $P < 0.05$; NTM, $P < 0.01$) and combination A with combination C (all mycobacteria, $P < 0.01$; NTM, $P < 0.01$), but not when comparing combination A versus combination C with regard to *M. tuberculosis* complex ($P > 0.05$) and combination B versus combination C concerning all mycobacteria ($P > 0.05$ throughout).

Mean time to detection of AFB. The average number of days required for the detection of AFB by each culture system is summarized in Table 4. In contrast to solid media, mycobacteria grew in liquid media after as little as 1 day of incubation. The mean times to detection for all isolates were 15.9, 15.4, and 27.2 days by the BACTEC 9000 MB system, the BACTEC 460 system, and solid media, respectively. *M. tuberculosis* complex isolates were detected from smear-positive specimens af-

TABLE 2. Detection of mycobacteria from clinical specimens according to initial smear result[a]

Isolate (no.)	No. (%) of isolates detected by the following:					
	BACTEC 9000 MB	BACTEC 460	Solid media[b]	BACTEC 9000 MB plus solid media (combination A)	BACTEC 460 plus solid media (combination B)	BACTEC 9000 MB plus BACTEC 460 (combination C)
All smear-positive specimens (94)	88 (93.6)	87 (92.6)	86 (91.5)	94 (100)	93 (94.1)	93 (94.1)
All smear-negative specimens (357)	250 (70.0)	303 (84.9)	219 (61.3)	299 (83.8)	337 (94.4)	332 (93.0)
Smear-positive *M. tuberculosis* (84)	78 (92.9)	79 (94.1)	80 (95.2)	84 (100)	84 (100)	83 (98.8)
Smear-negative *M. tuberculosis* complex (212)	169 (79.7)	193 (91.0)	173 (81.6)	198 (93.4)	209 (98.6)	203 (95.8)
Smear-positive NTM (10)	10 (100)	8 (80.0)	6 (60.0)	10 (100)	9 (90.0)	10 (100)
Smear-negative NTM (145)	81 (55.9)	110 (75.9)	46 (31.7)	101 (69.7)	128 (88.3)	129 (89.0)

[a] A total of 3,095 clinical specimens were tested.
[b] See footnote *b* of Table 1.

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TABLE 3. Number of isolates detected by a single medium only

Species	No. of isolates exclusively detected by the following medium or media:		
	BACTEC 9000 MB	BACTEC 460	Solid
M. tuberculosis	3	13	11
M. bovis		1	
M. aurum			1
MAC	8	24	4
M. chelonae	2		2
M. fortuitum	1	4	1
M. gordonae		2	6
M. kansasii	1	2	
M. malmoense	4		1
M. simiae		1	1
M. xenopi		10	
Total	19	57	27

ter 12.2 and 9.3 days when using the BACTEC 9000 MB and BACTEC 460 systems, respectively, and after 21.2 days when using solid media. For smear-negative specimens, the values were 18.1, 15.6, and 28.4 days, respectively. NTM were recovered on average after 15.1 days (BACTEC 9000 MB system), 17.3 days (BACTEC 460 system), and 31.3 days (solid media). The mean time to detection of MAC was considerably shorter in the BACTEC 9000 MB system (10.8 days) than in any of the other media and was well above the average for the meantime to detection of NTM.

DISCUSSION

The present study compared the efficacy of detecting AFB by the continuously monitoring fluorescent BACTEC 9000 MB system with that of traditional culture procedures (BACTEC 460 system and solid media). Recovery of mycobacteria from more than 3,000 clinical specimens was assessed not only by each system alone but also by system combinations. Conceivably, studies evaluating different types of cultivation systems are fraught with multiple biases which cannot be overcome as long as these studies are performed in a routine clinical laboratory where standardized working algorithms must be strictly followed. Thus, the major drawbacks of our evaluation were (i) measurement of fluorescence levels in the BACTEC 9000 MB system every 10 min, i.e., almost immediate detection of growth, in contrast to the BACTEC 460 system (with readings twice weekly for the first 2 weeks and once weekly thereafter) and solid media (with one weekly reading); (ii) unequal inoculum size per medium (0.5 ml of sediment for both liquid media and $\leq$0.25 ml for solid media); and (iii) a 10-fold volume of broth in the BACTEC 9000 MB system (40 ml) compared to that in the BACTEC 460 system (4 ml), while the inoculum size remained the same (0.5 ml).

Overall, the automated BACTEC 9000 MB system was a rapid and simple way to detect AFB. From a technical point of view, the system offers several advantages over the BACTEC 460 system. Apart from being a nonradiometric system, it is less labor-intensive since vials constantly remain in the cabinet until they become positive, nor are there needles to be cleaned or replaced or CO_2 tanks to be exchanged. Cross contamination from vial to vial, as is known to be the case for the BACTEC 460 system (4, 18), is not an issue either, and computerized data management greatly facilitates tracking of the results. At 0.15%, the false-positivity rate was negligible and lower than those reported by Boyle et al. (3) (1.5%) and van Griethuysen et al. (17) (0.8%). While at center 1 a contamination rate of 6.8% was found for the BACTEC 9000 MB system (which compares well with that reported by van Griethuysen et al. [17] [6%]) and a contamination rate of 1.6% was found for the BACTEC 460 system, the contamination rate at center 2 was higher (9.8% for the BACTEC 9000 MB system and 9.9% for the BACTEC 460 system), although pretreatment was extended to 20 min at center 2. Most likely, a higher contamination rate occurred at center 2 because of the commonly observed delays in sample processing due to the late arrival of specimens. Although the contamination rate seen on solid medium at center 1 was high (12.9%) and was largely due to the absence of antibiotics, it is still in the range of what has commonly been reported (up to 41% for LJ [19]).

Recovery rates of AFB from the BACTEC 9000 MB and BACTEC 460 systems were significantly higher than those from solid media ($P < 0.05$ and $P < 0.01$, respectively), similar to previous studies which are based on comparisons of different liquid with solid media (the BACTEC 460 system [19], the BACTEC 460 system and the Septi Chek system [13], and the BACTEC 460 system and MGIT [12]). AFB from smear-positive specimens were excellently detected by both liquid and solid media (93.6%, BACTEC 9000 MB system; 92.6%, BACTEC 460 system; 91.5%, solid media; no statistically significant differences in the respective *P* values). The same held true for smear-positive specimens containing *M. tuberculosis* complex and NTM. For recovery of the latter, the BACTEC 9000 MB system detected all isolates, while the BACTEC 460 system missed two isolates and solid media missed four isolates. A lower sensitivity of the fluorescence system cannot be denied, however, when focusing on the detection of mycobacteria from smear-negative specimens. Whether all mycobacte-

TABLE 4. Mean time to detection of mycobacteria in clinical specimens[a]

Culture method	Average no. of days (range) to detection of the following:				
	All isolates	*M. tuberculosis* complex		All NTM[b]	MAC[c]
		Smear positive	Smear negative		
BACTEC 9000 MB	15.9 (1–42)	12.2 (2–34)	18.1 (1–42)	15.1 (3–39)	10.8 (3–39)
BACTEC 460	15.4 (1–51)	9.3 (1–47)	15.6 (3–42)	17.3 (2–51)	16.0 (3–47)
Solid media[d]	27.2 (9–57)	21.2 (9–51)	28.4 (16–57)	31.3 (15–44)	28.5 (11–51)

[a] A total of 3,095 clinical specimens were tested.
[b] Smear-positive specimens contained 10 isolates (4 MAC, 2 *M. haemophilum*, 2 *M. malmoense*, 1 *M. kansasii*, and 1 *M. celatum* isolate); smear-negative specimens contained 145 isolates.
[c] Smear-positive specimens contained 4 isolates; smear-negative specimens contained 68 isolates.
[d] See footnote *b* of Table 1.

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rial isolates, *M. tuberculosis* complex isolates, or NTM are considered, there were statistically significant differences throughout in the detection of AFB when the BACTEC 9000 MB system was compared to the radiometric system (all mycobacteria, 70.0 versus 84.9% [$P < 0.01$]; *M. tuberculosis* complex, 79.9 versus 91.0% [$P < 0.01$]; NTM, 55.9 versus 75.9% [$P < 0.01$]). Comparing the BACTEC 9000 MB system versus solid media, there was, on the contrary, no statistically significant difference in recoveries (all mycobacteria, 70.0 versus 61.3% [$P > 0.05$]; *M. tuberculosis* complex, 79.9 versus 81.6% [$P > 0.05$]; NTM, 55.9 versus 31.7% [$P > 0.05$]).

Part of today's basic principles for good laboratory practice in mycobacteriology remains the use of a combination of liquid and solid media, even though it is well known that the cultural approach never reaches 100% sensitivity (12). As expected from the recovery rates for each system presented above, there was also a statistically significant difference between the efficacy of combination A (BACTEC 9000 MB system plus solid media) and that of combination B (BACTEC 460 system plus solid media), irrespective of whether all mycobacteria ($P < 0.01$), *M. tuberculosis* complex only ($P < 0.05$), or NTM only ($P < 0.01$) were concerned. Even though combination C (BACTEC 9000 MB system plus BACTEC 460 system) is not a combination that would likely be seen in a clinical mycobacteriology laboratory, we have evaluated it for the sake of the completeness of the study. Except for *M. tuberculosis* complex ($P > 0.05$), there was also a significant difference when combination A was compared to combination C, whereas between combination B and combination C, the *P* values were not significantly different.

Overall, none of the system combinations showed statistically significant differences when only smear-positive specimens were concerned. The difficulties in recovering AFB arose mainly from smear-negative specimens (Table 2) and are, in particular, accentuated by the fact that in our study smear-negative specimens amounted to almost 80%. Taking into account the recovery rate for AFB as determined by this study and assuming, in parallel, the same ratio of smear-positive to smear-negative specimens, as was the case in the study of van Griethuysen et al. (17) (53%), combination A would have, in our case, detected 417 of 457 isolates and combination B would have detected 425 of 457 isolates, which means that there would not have been a statistically significant difference between the two-system combinations A and B. Although the example is hypothetical and a ratio of smear-positive to smear-negative specimens of 53% would be extremely high and certainly does not reflect what is commonly observed in the clinical mycobacteriology laboratory, this example demonstrates that the ratio of smear-positive to smear-negative specimens is a key factor which directly influences sensitivity. Besides that, in our opinion the very large volume of broth in the BACTEC 9000 MB system (40 ml) accounts for the unsatisfactory recovery of AFB from smear-negative specimens. Our data demonstrate that too high a dilution of AFB present in 0.5 ml of sediment severely affects recovery and, thus, the performance characteristics of a system. For comparison, when the MGIT medium (4 ml/vial), whose nutrient composition is similar to that of the BACTEC 9000 MB medium, was recently evaluated, the recovery rate for all AFB isolated was 86.7% for the combination of MGIT plus solid media and 93.3% for the combination of the BACTEC 460 system plus solid media; i.e., the difference was not statistically significant ($P > 0.05$) (12). Another factor directly influencing culture sensitivity is the method of pretreatment of clinical specimens. As shown recently for the new MGIT medium, NALC-NaOH treatment is significantly superior to decontamination with sodium dodecyl (lauryl) sulfate-NaOH (11).

Mean times to detection of all mycobacteria, *M. tuberculosis* complex isolates, and NTM were very similar for the two BACTEC systems and about half of those observed on solid media (27.2 days). In particular, when aiming at approaching the target of 3 weeks for the detection of *M. tuberculosis*, as suggested by CDC (2, 16), both liquid systems complied quite well in this respect (for all isolates, 15.9 days in the BACTEC 9000 MB system and 15.4 days in the BACTEC 460 system). Our data agree, furthermore, with those of Boyle et al. (3), who reported for 382 mycobacterial isolates average detection times of 14.7 days for the BACTEC 9000 MB system, 14.0 days for the BACTEC 460 system, and 26.2 days for solid media, while van Griethuysen et al. (17) observed for 202 isolates average detection times of 17.6 days in the BACTEC 9000 MB system and 29.4 days on solid media.

The major conclusions of our study are as follows. (i) The BACTEC 9000 MB system is a rapid and efficient method for the detection of mycobacteria in a clinical laboratory. Overall, mean times to detection are comparable to those of the radiometric BACTEC system. Its potential for detecting mycobacteria makes it an attractive alternative in light of the fact that it eliminates the problems associated with radiometry. (ii) The BACTEC 9000 MB system has, in particular, excellent performance characteristics for the isolation of AFB from smear-positive specimens, while for the recovery of AFB from smear-negative specimens, the system must be improved. This could possibly be achieved by the use of a smaller amount of broth, preventing a specimen from being too diluted. (iii) Very importantly, neither of the systems tested, i.e., the BACTEC 9000 MB system, the BACTEC 460 system, and solid media alone, were capable of recovering all isolates present in the clinical specimens. Therefore, a combination of solid plus liquid media must be maintained for optimum recovery of mycobacteria from culture.

ACKNOWLEDGMENTS

We thank Becton Dickinson for providing us with the BACTEC 9000 MB system. Alexander von Graevenitz read the manuscript.

REFERENCES

1. **Beaty, P. S., J. Ahnell, and M. Deal.** 1994. The detection of *Mycobacterium tuberculosis* using a fluorescence quenching-based oxygen sensor, abstr. C-111, p. 510. *In* Abstracts of the 94th General Meeting of the American Society for Microbiology 1994. American Society for Microbiology, Washington, D.C.
2. **Bird, B. R., M. M. Denniston, R. E. Huebner, and R. C. Good.** 1996. Changing practices in mycobacteriology: a follow-up survey of state and territorial public health laboratories. J. Clin. Microbiol. **34:**554–559.
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 9000 TB detection system and the BACTEC radiometric 460 TB 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. **Conville, P. S., and F. G. Witebsky.** 1989. Inter-bottle transfer of mycobacteria by the BACTEC 460. Diagn. Microbiol. Infect. Dis. **12:**401–405.
5. **Kent, P. T., and G. Kubica.** 1985. Public health mycobacteriology. A guide for the level III laboratory. Centers for Disease Control, Atlanta, Ga.
6. **Kirschner, P., B. Springer, U. Vogel, A. Meier, A. Wrede, M. Kiekenbeck, F.-C. Bange, and E. C. Böttger.** 1993. Genotypic identification of mycobacteria by nucleic acid sequence determination: report of a 2-year experience in a clinical laboratory. J. Clin. Microbiol. **31:**2882–2889.
7. **Lindholm-Levy, P., and L. Heifets.** 1996. Evaluation of recovery and time to detection of mycobacterial growth in the MB/BacT system compared with three other media, abstr. U-39, p. 107. *In* Abstracts of the 96th General Meeting of the American Society for Microbiology 1996. American Society for Microbiology, Washington, D.C.
8. **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. ASM Press, Washington, D.C.

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9. **Palaci, M., S. Y. M. Ueki, D. N. Sato, M. A. Da Silva Telles, M. Curcio, and E. A. M. Silva.** 1996. Evaluation of Mycobacteria Growth Indicator Tube for recovery and drug susceptibility testing of *Mycobacterium tuberculosis* isolates from respiratory specimens. J. Clin. Microbiol. **34:**762–764.
10. **Pfyffer, G. E.** 1994. Amplification techniques: hope or illusion in the direct detection of tuberculosis? Med. Microbiol. Lett. **3:**335–347.
10a. **Pfyffer, G. E.** Unpublished data.
11. **Pfyffer, G. E., H.-M. Welscher, and P. Kissling.** 1997. Pretreatment of clinical specimens with sodium dodecyl (lauryl) sulfate is not suitable for the Mycobacteria Growth Indicator Tube cultivation method. J. Clin. Microbiol. **35:**2142–2144.
12. **Pfyffer, G. E., H.-M. Welscher, P. Kissling, C. Cieslak, M. J. Casal, J. Gutierrez, and S. Rüsch-Gerdes.** 1997. Comparison of the Mycobacteria Growth Indicator Tube (MGIT) with radiometric and solid culture for recovery of acid-fast bacilli. J. Clin. Microbiol. **35:**364–368.
13. **Sewell, D. L., A. L. Rashad, W. J. Rourke, Jr., S. L. Poor, J. A. C. McCarthy, and M. A. Pfaller.** 1993. Comparison of the Septi-Chek AFB and BACTEC systems and conventional culture for recovery of mycobacteria. J. Clin. Microbiol. **31:**2689–2691.
14. **Shinnick, T. M., and V. Jonas.** 1994. Molecular approaches to the diagnosis of tuberculosis, p. 517–530. *In* B. R. Bloom (ed.), Tuberculosis: pathogenesis, protection, and control. ASM Press, Washington, D.C.
15. **Smid, I., and M. Salfinger.** 1994. Mycobacterial identification by computer-aided gas-liquid chromatography. Diagn. Microbiol. Infect. Dis. **16:**400–402.
16. **Styrt, B. A., T. M. Shinnick, J. C. Ridderhof, J. T. Crawford, and F. C. Tenover.** 1997. Turnaround times for mycobacterial cultures. J. Clin. Microbiol. **35:**1041–1042.
17. **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.
18. **Vannier, A. M., J. J. Tarrand, and P. R. Murray.** 1988. Mycobacterial cross contamination during radiometric culturing. J. Clin. Microbiol. **26:**1867–1868.
19. **Wilson, M. L., B. L. Stone, M. V. Hildred, and R. R. Reves.** 1995. 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. J. Clin. Microbiol. **33:**2516–2518.
20. **Woods, G. L., G. Fish, M. Plaunt, and T. Murphy.** 1997. Clinical evaluation of Difco ESP Culture System II for growth and detection of mycobacteria. J. Clin. Microbiol. **35:**121–124.

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