

Evaluation of the MB/BacT System and Comparison to the BACTEC 460 System and Solid Media for Isolation of Mycobacteria from Clinical Specimens

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The MB/BacT automated system is designed for the isolation of mycobacteria from clinical specimens. It utilizes a colorimetric sensor and reflected light to continuously monitor the CO₂ concentration in the culture medium. We compared its performance to that of the BACTEC 12B media for the radiometric BACTEC 460 instrument and that of solid culture media. Respiratory specimens and urine samples were decontaminated with 4% NaOH. The vials of the two instruments were inoculated with 500 µl of sample and two solid egg-based media at 200 µl each. All vials were incubated at 37°C for 6 weeks. A total of 1,078 specimens (633 respiratory specimens, 78 cerebrospinal fluid specimens, 177 other body fluid specimens, 87 urine specimens, and 103 other types of specimens) were cultured in parallel. Mycobacteria could be identified from 73 (6.8%) specimens: 67 *M. tuberculosis*, 3 *M. kansasii*, 1 *M. xenopi*, 1 *M. terrae*, and 1 mixed *M. avium* with *M. scrofulaceum*. Of these, 63 (86.3%) specimens were positive with the MB/BacT system, 67 (91.8%) were positive with the BACTEC 460 instrument, and 58 (79.5%) were positive with the two egg-based media. MB/BacT cultures were positive on average after 17.5 (±6.4) days, BACTEC cultures with a growth index of >20 (mean, 200) were positive after 14.3 (±8.2) days, and egg-based media were positive after 24.2 (±7.5) days. Microorganisms other than mycobacteria contaminated 46 (4.3%) MB/BacT cultures and 31 (2.9%) BACTEC cultures, which had to be discarded. The MB/BacT system is a well-automated system for the detection of *M. tuberculosis* in clinical specimens without using radioactive reagents. Further trials are required to determine whether it is suitable for the culture of nontuberculous mycobacteria.

The most reliable means for the diagnosis of mycobacterial infections are cultures (3, 23). The major drawback of mycobacterial cultures is the extended incubation time needed to recognize the growth of acid-fast bacilli. More rapid laboratory alternatives are microscopy and nucleic acid amplification methods. Microscopy of samples stained with specific dyes for acid fastness can detect only 30 to 80% of all specimens containing mycobacteria (5). These results are useful to guide health-care workers as to the infectiousness of patients with pulmonary tuberculosis. Nucleic acid amplification methods have only been approved for the detection of *Mycobacterium tuberculosis* complex strains in respiratory specimens from untreated patients in conjunction with microscopy and culture (4). With mycobacterial cultures, the diagnosis can be confirmed, drug susceptibilities can be determined, the treatment response can be monitored, and strains may be typed (4). Centers for Disease Control and Prevention guidelines recommend that the laboratory identification of species of the *M. tuberculosis* complex be accomplished within 10 to 21 days after specimen collection (3, 21). Therefore, further developments of culture methods are necessary for more rapid, more reliable, cheaper, and less-labor-intensive detection of mycobacteria.

Egg-based culture media are reliable and cheap, but colonies of *M. tuberculosis* complex species are rarely visible before 18 days of incubation (5). The BACTEC 460 system (Becton Dickinson, Sparks, Md.) has been marketed since 1977 (15).

The Middlebrook 12B medium for this system contains ¹⁴C-labeled palmitic acid as the substrate. During mycobacterial growth, ¹⁴C-labeled CO₂ is produced and released into the headspace air of the vials. A ¹⁴CO₂ detection device allows early determination of mycobacterial growth. However, this system requires radioactive reagents, causing waste problems, and vials have to be handled and punctured for readings at least eight times during 6 weeks of incubation, requiring a considerable amount of work and increasing the risk of cross-contamination.

The recently developed MB/BacT system (Organon Teknika, Turnhout, Belgium) relies on a continuous colorimetric CO₂ detection device to indicate mycobacterial growth in a closed system. A solid-state sensor at the base of each vial contains the colorimetric indicator, which changes from green to yellow when CO₂ is produced in the vial. Each compartment of the instrument where the vials are incubated contains a reflectometer and a detection unit. The measured values are transmitted every 10 min to a computer, which indicates vials with mycobacterial growth based on a sophisticated algorithm. In this study, we evaluated the performance of this new system in terms of the reliability and rapidity of detection of mycobacterial growth, handling requirements, and degree of automation.

(These results were partially presented at the 8th European Congress of Clinical Microbiology in Lausanne, Switzerland, 1997 [19].)

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MATERIALS AND METHODS

Samples for which a mycobacterial workup was requested were processed on average 26 h (median, 19 h) after specimen collection. Clinical samples, with the

exception of blood, were evenly spread on an area of about 1 by 2 cm of a clean microscope slide. The heat-fixed slides were then stained with auramine O fluorochrome dye and examined microscopically as recommended (5). Nonsterile source specimens were digested and decontaminated by the 4% sodium hydroxide method (15). Normally sterile body fluids were centrifuged at 3,000 × g for 20 min when >2 ml was available. The final sediment of all specimens was resuspended in 2 ml of sterile distilled water, of which 500 μl was inoculated immediately into MB/BacT vials (Organon Teknika), 500 μl was inoculated into BACTEC 12B vials (Becton Dickinson), 200 μl was inoculated onto Coletsos egg-based medium slants (BioMérieux, Lyon, France), and another 200 μl was inoculated onto Stonebrink egg-based slants (Becton Dickinson).

MB/BacT vials contain 10 ml of modified Middlebrook 7H9 broth supplemented with growth factors. The MB/BacT antibiotic supplement, containing amphotericin B, azlocillin, nalidixic acid, polymyxin B, and trimethoprim, was added (500 μl) shortly before specimen inoculation. MB/BacT bottles were registered and introduced into the instrument as recommended by the manufacturer and incubated at 37°C for 6 weeks. BACTEC 12B bottles, containing 4 ml of Middlebrook 7H12 broth, were flushed with CO₂ and supplemented with the antibiotic solution PANTA (polymyxin B, azlocillin, nalidixic acid, trimethoprim, and amphotericin B) just before specimen inoculation and were incubated at 37°C for 6 weeks. BACTEC vials were read with the BACTEC 460 instrument twice weekly during the first 2 weeks of incubation, and then they were read once weekly up to the 6th week of incubation. The egg-based media were incubated at 37°C for 6 weeks and were examined for colonies on the slant once a week.

From all positive MB/BacT and BACTEC 12B media (growth index [GI], ≥100), samples were drawn for Ziehl-Neelsen staining. MB/BacT cultures contaminated with microorganisms other than mycobacteria were decontaminated a second time by the 4% NaOH procedure (15) and then were reincubated. Contaminated BACTEC cultures were not further decontaminated and were immediately rejected. From all MB/BacT cultures positive for acid-fast bacilli and all BACTEC 12B media with a GI of >600, 1 ml was drawn for hybridization with DNA probes (AccuProbe; Gen-Probe, San Diego, Calif.) for the identification of *M. tuberculosis* complex and *Mycobacterium avium* complex species (15). All positive liquid cultures were subcultured onto two Coletsos egg-based media; the species of the *M. tuberculosis* complex were subsequently identified by conventional biochemical tests (5, 15). Cultures negative for *M. tuberculosis* complex and *M. avium* complex species by hybridization were identified by amplification and sequencing of the 16S rRNA gene (2). For statistical analysis, the McNemar exact test was applied.

RESULTS

The MB/BacT system was evaluated with 1,078 specimens collected from 583 patients (1.8 cultures per patient). Respiratory samples ($n = 633$) included 318 sputum samples, 177 bronchial or tracheal aspirates, and 138 bronchoalveolar lavage specimens. Body fluid specimens ($n = 255$) included 89 pleural fluid specimens, 78 cerebrospinal fluid specimens, 40 abdominal fluid specimens, 26 synovial fluid specimens, and 22 other normally sterile fluid specimens. The remaining 190 specimens were 87 urine specimens, 24 biopsies, and 79 other types of specimens. Of these 1,078 specimens, 50 (4.6%) were microscopically positive for acid-fast bacilli, and 47 could be confirmed by at least one of the three culture methods used. The remaining three samples, microscopically positive but culture negative, were obtained from treated patients who previously had samples positive for *M. tuberculosis*. Mycobacteria could be identified from 73 cultures (26 patients): 67 *M. tuberculosis*, 3 *M. kansasii*, 1 *M. xenopi*, 1 *M. terrae*, and 1 mixed culture of *M. avium* and *M. scrofulaceum*. Of the 67 *M. tuberculosis*-containing samples, 43 (64.2%) were microscopically positive for acid-fast bacilli. The MB/BacT system yielded 63 (86.3%) specimens, the BACTEC 12B system detected 67 (91.8%) specimens, and the two egg-based media (Coletsos and Stonebrink) yielded 58 (79.5%) specimens.

The MB/BacT system in conjunction with the two egg-based media recovered 67 (91.8%) positive specimens; the BACTEC system with the egg-based media recovered 69 (94.5%) positive specimens. When it was the only medium positive, the MB/BacT medium detected three *M. tuberculosis* isolates, the BACTEC medium alone detected four *M. tuberculosis* isolates and one *M. xenopi* isolate, and the egg-based media were the only media to recover one *M. tuberculosis* isolate. In a comparison of the MB/BacT system directly with the BACTEC 460

TABLE 1. Comparison of the number of specimens from which mycobacteria could be isolated with the MB/BacT and BACTEC 12B media

Species identified	Total no. of specimens	No. of specimens positive in:			P
		MB/BacT and BACTEC 12B media	MB/BacT medium only	BACTEC 12B medium only	
<i>M. tuberculosis</i>	66	55	4	7	0.55 (NS) ^a
<i>M. kansasii</i>	3	3	0	0	
<i>M. xenopi</i>	1	0	0	1	
<i>M. terrae</i>	1	0	1	0	
<i>M. avium</i> with <i>M. scrofulaceum</i>	1	0	0	1	
Total	72	58	5	9	

^a NS, not significant ($P > 0.05$).

system, 58 cultures were positive for both, 5 isolates (4 *M. tuberculosis*, 1 *M. terrae*) grew only in the MB/BacT system, and 9 (7 *M. tuberculosis*, 1 *M. xenopi*, and 1 *M. avium* with *M. scrofulaceum*) isolates grew only in the BACTEC system (Table 1). The MB/BacT system recovered more mycobacteria than the two egg-based Coletsos and Stonebrink media without reaching statistical significance (Table 2). The two egg-based media were particularly complementary for the isolation of mycobacteria from 58 specimens: 25 were isolated from both media, 16 were isolated from the Coletsos medium only, and 17 were isolated from the Stonebrink medium only.

The 67 samples containing viable *M. tuberculosis* cells originated from 22 patients (average of 3 positive specimens per patient), of whom 11 had 1 sample positive. Of the 67 samples, 33 (49.3%) were collected from 7 (31.8%) patients receiving specific antituberculous treatment at the time of specimen collection, which results in a mean of 4.7 samples per treated patient.

Microorganisms other than mycobacteria contaminated 101 (9.4%) MB/BacT cultures. Of these, 46 (4.3%) cultures remained contaminated after the subsequent decontamination and were discarded; 31 (2.9%) contaminated BACTEC cultures were also discarded. Together, eight MB/BacT and BACTEC cultures were contaminated, 38 cultures were contaminated only in the MB/BacT system, and 23 cultures were contaminated only in the BACTEC 460 system ($P = 0.07$ [not significant]).

TABLE 2. Comparison of the number of specimens from which mycobacteria could be isolated with the MB/BacT system and the egg-based Coletsos and Stonebrink media

Species identified	Total no. of specimens	No. of specimens positive in:			P	
		MB/BacT system and egg media	MB/BacT system only	Egg-based media only		
<i>M. tuberculosis</i>	63	49	10	4	0.18 (NS) ^a	
<i>M. kansasii</i>	3	3	0	0		
<i>M. terrae</i>	1	1	0	0		
<i>M. avium</i> with <i>M. scrofulaceum</i>	1	0	0	1		
Total	68	53	10	5		0.30 (NS)

^a NS, not significant ($P > 0.05$).

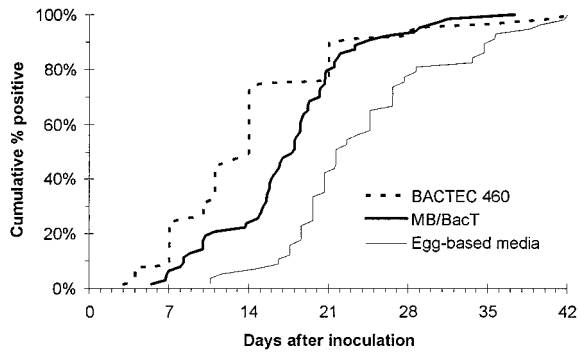


FIG. 1. Cumulative detection time for 63 specimens from which mycobacteria could be detected with the MB/BacT, 67 specimens detected with the BACTEC 460 system, and 58 specimens detected with the two egg-based Coletsos and Stonebrink media.

The 63 cultures positive in the MB/BacT system were detected by the instrument on average within 17.5 days (± 6.4 days; median, 18.0 days) after inoculation of the vials, while the 67 positive BACTEC cultures were detected on average 14.3 days (± 8.2 days; median, 14.0 days) after inoculation (Fig. 1). At the time of the detection of positive BACTEC cultures, the average GI was 197 (± 294 ; median, 75). For the 58 specimens positive in both instruments, 3 were detected at least 2 days earlier with the MB/BacT system, 47 were detected at least 2 days earlier with the BACTEC 460 system ($P < 0.0001$), and 8 were detected within 2 days with both instruments. Furthermore, these 58 cultures were on average positive 4.6 days (± 4.3 days; median, 4.6 days) earlier in the BACTEC 460 system than in the MB/BacT system.

Of the 59 MB/BacT cultures positive with *M. tuberculosis*, 58 (98.3%) could be identified with the *M. tuberculosis* complex DNA probe hybridization procedure, on average 20.7 days (± 5.4 days; median, 20.3 days) after inoculation. In contrast, only 49 of the 62 (79.0%) *M. tuberculosis*-containing BACTEC cultures could be identified by this hybridization method 19.8 days (± 7.5 days; median, 18.0 days) after inoculation. Of the 13 BACTEC cultures in which the GI never exceeded 500, 6 (46.2%) specimens originated from patients receiving antituberculous treatment.

The workloads for the negative MB/BacT and BACTEC vials after sample inoculation have been measured and compared. In our laboratory, we process annually approximately 5,000 nonblood specimens with requests to determine the possible presence of mycobacteria. In our hands, the handling time after sample inoculation for a negative BACTEC vial corresponded to about 40 s for eight readings during the 6 weeks of incubation. The flushing of vials prior to inoculation and instrument maintenance are not included in this handling time. For a negative MB/BacT vial, the comparative workload after sample inoculation was about 25 s, which remains independent of the incubation period.

DISCUSSION

Technical developments for the detection of mycobacteria have always received much attention. Back in 1977, Middlebrook described a radiometric detection system for mycobacterial growth (11). This BACTEC 460 system in conjunction with the Middlebrook 12B liquid medium has become a reference system, and it is therefore a widely used method for the detection of mycobacteria in industrialized countries (8). Despite all of its advantages (7, 12, 18), the BACTEC 460 system

has certain limitations, such as the inability to observe colony morphology and mixed cultures, cost, high workload, radioactive reagents, use of needles, and possible cross-contamination (15).

Certain improvements have been reported with manual systems, such as the Septi-Chek system (Becton Dickinson) (6, 9, 20), the MB Redox system (Biotest, Heidelberg, Germany) (14), and the Mycobacteria Growth Indicator Tube (MGIT; Becton Dickinson) (1, 17). However, all of these systems still require much handling. The recently developed MB/BacT system was one of the first fully automated systems for the culture of mycobacteria approved by the Food and Drug Administration since July 1996. Other automated systems for the culture of mycobacteria have been recently evaluated, including the Difco ESP culture system II (24) and the BACTEC 9000 MB system (13, 16).

The MB/BacT instrument detected the specimens with viable mycobacteria ($n = 63$; 86.3% of all isolates) as well as ($P = 0.42$, not significant) the BACTEC 460 radiometric system ($n = 67$; 91.8% of all isolates) or the two combined egg-based media Coletsos and Stonebrink ($n = 58$; 79.5% of all isolates, $P = 0.30$ [not significant]) did. We did not receive many specimens containing nontuberculous mycobacteria, representing 8.2% of all mycobacteria isolated, during the study period ($n = 6$). For the same types of specimens from the same patient population, and by identical laboratory procedures, the nontuberculous mycobacteria we identified in 1995 represented 30.5% (84 of 275 isolates) of the mycobacteria isolated, and for the entire year 1996, these represented 19.9% (53 of 267 isolates) of the mycobacteria isolated. This recent change was probably due to the introduction of combined treatment with protease inhibitors for patients with acquired immunodeficiency syndrome, who suffer less from opportunistic diseases now.

The individual performance of the two egg-based media (Coletsos medium recovered 56.2% and Stonebrink medium recovered 57.5% of all mycobacteria isolated) incubated for 6 weeks compared to that of the BACTEC 460 culture was similar to that of other egg-based media used in other evaluations (1, 17). The comparatively high combined recovery rate (79.5%) for these two egg-based media (22) may be due to the volume of 200 μ l we inoculated onto each slant, compared to only 100 μ l inoculated by others (1). We were able to identify an additional eight (13.8%) positive samples during the prolonged incubation of the two egg-based media Coletsos and Stonebrink from the 6th week to the 14th week. Likewise for the BACTEC 460 system, not only was *Mycobacterium genavense* detected mostly after the 6th week of incubation, but other species were detected, including some strains of *M. tuberculosis* or *M. xenopi*, as noted during this study ($n = 3$ and 1, respectively). These isolates were not considered in this evaluation (Table 1). We believe that an incubation period of more than 6 weeks for the MB/BacT system would have increased the sensitivity of the system. The 6-week incubation period recommended for the BACTEC 460 instrument (5) was also chosen for the MB/BacT system, so the evaluation could be performed with two MB/BacT incubator cabinets with a capacity of 240 vials each, since approximately 80 specimens were processed weekly during the study period. An incubation period of >6 weeks would have required a third MB/BacT cabinet, which was impossible in our laboratory because of limited space.

The relatively large number ($n = 67$; 6.2% of all samples) of *M. tuberculosis* isolates we identified during this evaluation indicates the reliability with which the three methods used can detect this species. It may also be due to our recommendation

that up to five specimens of the respiratory tract may be required for the diagnosis of respiratory tuberculosis (5). The relatively large number of specimens containing viable *M. tuberculosis* isolates (33 of 67; 49.3%) collected from patients receiving antituberculous therapy ($n = 7$ patients [i.e., 4.7 specimens per patient]) was necessary for treatment monitoring to exclude the emergence of antimicrobial resistance in patients remaining febrile. For these treated patients, the addition of antimicrobial agent inactivating substances such as charcoal, Fuller's earth, or resins to culture media for mycobacteria, as is applied in blood cultures (10), may provide more reliable and more rapid culture results.

Centers for Disease Control and Prevention recommendations for mycobacteriology performance state that reports of isolation and identification of *M. tuberculosis* complex species should be available within 10 to 14 (3) or 21 (21) days of specimen collection. Using two instruments with mean detection times of 17.5 (MB/BacT) and 14.3 (BACTEC 460) days in this study, we provided the identification of species of the *M. tuberculosis* complex within 10 days of specimen collection for only 4 specimens (6.0%), within 14 days for 14 specimens (20.9%), and within 21 days of sample collection for 42 specimens (62.7%). We did evaluate both systems in conjunction with the DNA probe method (AccuProbe) for rapid identification of *M. tuberculosis* complex species from positive cultures. Applying this procedure, we identified samples positive with *M. tuberculosis* more often from MB/BacT cultures than from BACTEC 460 cultures ($n = 57$ versus $n = 49$, respectively). Moreover, the incubation time (mean, 19.8 days) needed for these 49 BACTEC cultures to attain a GI of >600 necessary for hybridization was similar to the time (mean, 20.7 days) required for the 57 MB/BacT cultures. Thus, the initial advantage of earlier mycobacterial growth detection with the BACTEC system was reduced. The relatively large number of BACTEC cultures with GIs never exceeding 500 may have been due to the samples we received from treated patients. New nucleic acid amplification methods applied directly to samples or with early positive cultures have been successfully applied to identify *M. tuberculosis* complex species within 10 days (4).

With regard to contaminated cultures, the antibiotic supplement for the MB/BacT system we used was less active than the PANTA supplement of the BACTEC system. Just over 9% of the MB/BacT cultures were contaminated with microorganisms other than mycobacteria, compared to 2.9% of the BACTEC cultures. Only the second decontamination of MB/BacT cultures allowed a contamination rate of $<5\%$. A modified antibiotic supplement for the MB/BacT system more active against gram-positive contaminating organisms was under evaluation at other sites at the time of this study. With this new antibiotic supplement, the recommended contamination rate of 2 to 5% (5) should be achieved. Contaminated BACTEC cultures were decontaminated a second time only when acid-fast bacilli were microscopically visible and mixed with the other bacteria.

During the evaluation of the MB/BacT system, we appreciated the closed vials and their automated reading during the entire incubation period, which required no further handling once the samples had been inoculated and the vials had been registered in the instrument. Compared to the BACTEC 460 system, the workload per MB/BacT vial was determined to be about 40% lower. Additional handling of the MB/BacT cultures was necessary for 101 contaminated cultures. This workload should be reduced with the newly modified antibiotic supplement. Sample inoculation still required a syringe and needle, and the rubber stoppers of the bottles were rather hard

to puncture. Screw caps may be a solution, replacing the needles in the laboratory and avoiding the danger of inadvertent needle sticks. Maintenance of the MB/BacT instrument required only 10 min daily for cell calibration. With the MB/BacT instrument we used, individual vials could not be incubated at a temperature different from 37°C (e.g., 30 to 33°C for the optimal culture of mycobacterial organisms such as *M. marinum*, *M. haemophilum*, *M. chelonae*, and *M. ulcerans* [5]). As for all liquid culture media, positive MB/BacT cultures need to be subcultured onto solid media so that mixed cultures and colony morphology can be determined.

The BACTEC 460 system needs an extra step for vial flushing prior to specimen inoculation, and positive vials have to be monitored daily until the GIs have increased, allowing Ziehl-Neelsen staining, probe hybridization, and antimicrobial susceptibility testing. Furthermore, needles of the BACTEC 460 instrument have to be changed and sterilized daily. In our laboratory equipped with two BACTEC 460 instruments, quality performance tests are done weekly, HEPA filters are changed monthly, CO₂ tanks have to be changed approximately every 3 months, and the CO₂ absorber (soda lime) is changed about every 6 months. All of this additional workload is a disadvantage of the BACTEC system compared to the MB/BacT system. Our laboratory host computer system provides working lists for the series of vials to be monitored daily by the BACTEC system. Therefore, the workload per negative BACTEC bottle will exceed 40 s in laboratories in which the daily series of BACTEC bottles to be monitored has to be established manually.

For cost analysis, the most important factor to consider is the number of instruments required in the individual laboratories, which in turn depends on the maximum number of specimens processed during the chosen incubation period, which should be 6 weeks or longer. Many further variables have to be taken into account. Therefore, cost analysis should be done on an individual laboratory basis. The list price of a BACTEC 12B vial represents about 56% of the price of a MB/BacT vial in our country, Switzerland, which allows a comparison of the prices of consumable culture media. The disposal of the radioactive waste of the BACTEC 12B medium is included in the price comparison. Obviously these prices vary from country to country. The cost per MB/BacT vial is competitive with the list price of comparable culture media for other automated systems, such as the BACTEC 9000 MB system, the BACTEC MGIT 960 system, and the Difco ESP culture system II.

Each system has advantages and disadvantages compared with the other system as summarized below. The advantages of the BACTEC system over the MB/BacT system are as follows. The BACTEC system provides earlier detection of positive cultures (3 days). There is less contamination of mycobacterial cultures with microorganisms other than mycobacteria (30% of the MB/BacT system contamination rate). The BACTEC instrument occupies less space in the laboratory. Individual BACTEC vials can be incubated at temperatures other than 37°C, and the cost per vial is lower than that of the MB/BacT system (56% of the cost per MB/BacT vial in Switzerland).

The advantages of the MB/BacT system over the BACTEC system are as follows. The MB/BacT system provides a high level of automation (about 60% of the BACTEC system workload), with reduced risk of transcription or vial inversion errors. The MB/BacT system is a closed system, so that after specimen inoculation, there is no cross-contamination risk. With the MB/BacT system, more *M. tuberculosis* complex isolates were identified by the DNA probe hybridization procedures (18% more than were identified from BACTEC cul-

tures). There was no need of radioactive substrates, and therefore no radioactive waste was created. In the MB/BacT system, the incubator is included in the instrument. The MB/BacT system has data management capabilities. Finally, for the MB/BacT system, the costs of maintenance materials are low.

The MB/BacT system, with improvements under way, may be considered an alternative to radiometric systems for the culture of mycobacteria in liquid media, especially in countries where disposal of radioactive waste is restricted.

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