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Evaluation of the BACTEC MGIT 960 and the MB/BacT Systems for Recovery of Mycobacteria from Clinical Specimens and for Species Identification by DNA AccuProbe

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A total of 120 mycobacterial isolates were recovered from 1,068 clinical specimens. Of these, 82.5% were in MGIT 960, 83.3% were in MB/BacT, 80% were in BACTEC 460, and 70% were on Löwenstein-Jensen medium. Mean times to detection of *Mycobacterium tuberculosis* ($n = 96$) were significantly shorter with MGIT 960 (12.6 days, $P = 0.003$) and BACTEC 460 (11.8 days, $P < 0.001$) than with MB/BacT (15.9 days). Although, MGIT 960 showed the lowest rate of recovery of *M. kansasii* genotype I (64.3%), the earliest growth was detected with this system (8.9 days). Low and similar rates of contamination were obtained with MGIT 960 (3.3%) and MB/BacT (3%). The AccuProbe test for identification showed excellent sensitivities with MGIT 960 (96.8%) and MB/BacT (100%) cultures. In addition to being nonradiometric, both MGIT 960 and MB/BacT are accurate, rapid, and labor-saving detection systems which could replace the radiometric method.

Today the conventional procedures of microscopy and culture are irreplaceable diagnostic tools for mycobacterial infections (19, 24). A combination of solid and liquid media is currently the "gold standard" for primary isolation of mycobacteria (13, 19, 24). The implementation of the radiometric BACTEC 460 system (Becton Dickinson, Sparks, Md.) in the last two decades has been a major step forward and, together with a DNA probe (AccuProbe; GenProbe, San Diego, Calif.), has dramatically reduced the time to diagnosis of mycobacterial infections (13, 17, 19). However, BACTEC 460 TB has a number of well-known disadvantages: (i) it requires the use of radioactive reagents ([^{14}C]palmitic acid), (ii) it is labor-intensive in the handling of vials and maintainence of the instrument, and (iii) there is a potential risk of cross-contamination of the cultures (6). Recently, several nonradiometric methods for continuous monitoring of growth have been introduced in order to resolve some of these problems (3, 5, 6, 10, 12, 15, 18, 21, 23, 25, 27). The aim of this study was to evaluate in parallel the BACTEC MGIT 960 system (Becton Dickinson) and the MB/BacT system (Organon Teknika, Boxtel, The Netherlands) for recovery of mycobacteria and also to determine the feasibility of the direct application of AccuProbe to both media for rapid mycobacterial identification. Results of simultaneous cultures in the BACTEC 460 system and on Löwenstein-Jensen (LJ) medium were used as the standard for comparison.

A total of 1,066 clinical specimens collected between April and September 1998 were studied. The specimens from the respiratory tract (76%) included 605 sputum specimens, 172 bronchial- or tracheal-aspirate specimens, 24 bronchoalveolar-lavage specimens, and 1 bronchial-brushing specimen. The nonrespiratory specimens (23.8%) comprised 64 pleural fluid, 64 urine, 30 stool, 36 tissue, 19 gastric fluid, 14 synovial fluid, 13 cerebrospinal fluid, 12 ascitic fluid, and 2 pericardic fluid specimens. In addition, two environmental samples (from tap water) suspected of having mycobacterial contamination were included. All samples were processed within 24 h of specimen collection. The specimens from nonsterile body sites were processed according to the conventional *N*-acetyl-L-cysteine-NaOH digestion-decontamination procedure (9, 13). Sterile specimens were centrifuged without decontamination. After being processed and concentrated, the final sediment of specimens was suspended in 2 ml of phosphate buffer (pH 6.8). This suspension was then used for making smears for acid-fast staining with auramine-rhodamine fluorochrome (13, 19) and for inoculation into containers with four media: an MGIT tube (Becton Dickinson), an MB/BacT bottle (Organon Teknika), a BACTEC 12B vial (Becton Dickinson), and an LJ slant (MAIM, Barcelona, Spain) (solid medium).

All MGIT tubes and BACTEC 12B vials were supplemented with the specific antibiotic solution PANTA, while the MB/BacT antibiotic supplement was added only to the MB/BacT bottles for culture of nonsterile specimens, as recommended by the manufacturer. Equal volumes (0.5 ml) of the processed specimens were inoculated in each liquid medium, while LJ medium was inoculated with 0.4 ml of these treated specimens. The order of the inoculation was randomized. All cultures were incubated at 35 to 37°C for up to 6 weeks. The MGIT tubes were placed in the BACTEC MGIT 960 system, which is a fluorescence-based detection system, and the MB/BacT bottles were introduced into the MB/BacT instrument, which uses a colorimetric CO_2 detection system. The specimens in LJ medium were incubated in a 5% CO_2 incubator and were examined once a week. BACTEC 12B vials were read with the BACTEC 460 instrument twice a week for the first two weeks and then weekly up to the end of incubation. Any BACTEC 12B vial with a growth index (GI) of ≥30 was read daily until the GI reached 500. When positive cultures were detected and a GI of a specimen in BACTEC 12B was ≥500, such specimens were removed for confirmation by microscopy and identification of the microorganism. All positive MGIT tubes, MB/BacT bottles, and BACTEC 12B vials, which were acid-fast-stain

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TABLE 1. Detection of mycobacteria from 1,068 smear-positive and smear-negative specimens with each culture system

Organisms	Culture system	No. of isolates (%)		
		Total	Smear positive	Smear negative
All isolates		120	56	64
	MGIT 960	99 (82.5)	55 (98.2)	44 (68.8)
	MB/BacT	100 (83.3)	55 (98.2)	45 (70.3)
	BACTEC 460	96 (80)	55 (98.2)	41 (64.1)
	LJ medium	84 (70)	53 (94.6)	31 (48.4)
M. tuberculosis complex		96	51	45
	MGIT 960	84 (87.5)	50 (98)	34 (75.6)
	MB/BacT	85 (88.5)	50 (98)	35 (77.8)
	BACTEC 460	78 (81.3)	50 (98)	28 (62.2)
	LJ medium	70 (72.9)	48 (94.1)	22 (48.9)
Nontuberculous mycobacteria		24	5	19
	MGIT 960	15 (62.5)	5 (100)	10 (52.6)
	MB/BacT	15 (62.5)	5 (100)	10 (52.6)
	BACTEC 460	18 (75)	5 (100)	13 (68.4)
	LJ medium	14 (58.3)	5 (100)	9 (47.4)

negative, were reincubated up to the sixth week of incubation. Whenever growth was detected in only the MGIT 960 or MB/BacT system, the negative culture of the another system was subcultured in the LJ medium at the end of the incubation period.

All isolates were identified by PCR-restriction fragment length polymorphism analysis of the *hsp65* gene (20) and conventional biochemical and cultural tests (13, 19). The AccuProbe assay was performed within 72 h of a positive result to identify *Mycobacterium tuberculosis* complex, *M. avium* complex, and *M. kansasii*. These gene probes were selectively applied to each positive culture on the basis of the pigmentation of the pellet and morphological characteristics by microscopic examination (14, 26). For all liquid media, 1.5 ml was centrifuged (10 min at 13,000 $\times$ *g*) and the pellet was used in the hybridization test. Samples producing signals greater than or equal to 30,000 relative light units (RLUs) were considered positive (11). Statistical analyses were performed by the chi-square test, Fisher's exact test, one-way analysis of variance, and the Kruskal-Wallis one-way analysis of variance, when appropriate. *P* values of $\leq$0.05 were considered to be statistically significant.

A total of 120 mycobacterial isolates were recovered in at least one of the media from 1,068 specimens studied and comprised 106 isolates from respiratory specimens, 13 isolates from nonrespiratory specimens, and 1 isolate from environmental specimens.

As for total rates of recovery of mycobacteria, the three liquid systems were comparable, with slightly better sensitivities (not statistically significant) being shown for the MGIT 960 and MB/BacT systems (Table 1). This finding was more outstanding for recovery of *M. tuberculosis* complex from smear-negative specimens (Table 1) and suggests that the media and supplements used in these nonradiometric systems are appropriate and permit good growth of mycobacteria, even with specimens with low numbers of bacilli.

TABLE 3. Recovery of mycobacteria from 1,068 specimens with different combinations of culture media

Organism(s) (no. of isolates)	No. (%) of isolates detected with:		
	MGIT 960 plus LJ medium	MB/BacT plus LJ medium	BACTEC 460 plus LJ medium
All isolates (120)	106 (88.3)	106 (88.3)	103 (85.8)
M. tuberculosis complex (96)	88 (91.7)	88 (91.7)	83 (86.5)
All NTM species (24)	18 (75)	18 (75)	20 (83.3)
M. kansasii genotype I (14)	12 (85.7)	14 (100)	14 (100)
M. xenopi (5)	4 (80)	2 (40)	3 (60)

Interestingly, all *M. kansasii* isolates in this study were of genotype I (Table 2), and the recovery rates obtained with the BACTEC 460 system (100%) were significantly superior to those obtained with the MGIT 960 system (64.3%, $P = 0.04$) but not those obtained with the MB/BacT system (92.9%). In addition, one *M. kansasii* genotype I isolate that was not detected with the MGIT 960 instrument was recovered by subculture of the MGIT tube on LJ medium (false negative). These results are important, because *M. kansasii* (mainly genotype I) is one of the most frequent and virulent nontuberculous mycobacterium (NTM) species (1, 4). In contrast, the MB/BacT system had problems in detecting specimens yielding *M. xenopi* (Table 2). Of the five *M. xenopi* isolates recovered by all culture systems combined, four isolates (80%) were detected by the MGIT 960 system and all were missed by the MB/BacT system. These four undetected *M. xenopi* isolates grew in the MB/BacT medium, as could be demonstrated by microscopy and subculture of the bottles (false negatives). The reason for this discrepancy between detection and recovery is not exactly known. A possible explanation may be the inability of the software-based positive algorithms of the instrument to detect the slow growth of *M. xenopi* in the MB/BacT medium. After completion of this study, a new algorithm was developed for the MB/BacT system to improve the detection of some slow-growth species.

The combination of liquid and solid media improved the recovery rates by each medium alone (Table 3). Although no significant differences were found, the greatest number of *M. tuberculosis* complex isolates was recovered when LJ medium was combined with the MB/BacT or MGIT 960 system, whereas for NTM species the best sensitivity was obtained with LJ medium plus BACTEC 460.

The short time required to detect growth is one of the most important advantages of the radiometric culture method. How-

TABLE 2. Distribution of NTM species isolated with each culture system from 1,068 specimens

Organism	Total no. of isolates	No. (%) of isolates detected with:			
		MGIT 960	MB/BacT	BACTEC 460	LJ medium
Mycobacterium kansasii genotype I	14	9 (64.3)	13 (98.2)	14 (100)	11 (78.6)
Mycobacterium xenopi	5	4 (80)	0 (0)	1 (20)	2 (40)
Mycobacterium avium complex	2	1 (50)	1 (50)	0 (0)	0 (0)
Mycobacterium gordonae	2	1 (50)	1 (50)	2 (100)	1 (50)
Mycobacterium fortuitum	1	0 (0)	0 (0)	1 (100)	0 (0)

TABLE 4. Time (days) to detection of mycobacteria with each culture system and comparison of liquid systems

Organism(s) (no. of isolates)	Mean time (range)				*P* value[a]		
	MGIT 960	MB/BacT	BACTEC 460	LJ medium	MGIT 960 vs MB/BacT	MGIT 960 vs BACTEC 460	MB/BacT vs BACTEC 460
All isolates (120)	13.2 (4–40)	15.9 (6–44)	12.6 (3–40)	22.2 (13–45)	0.015	NS	0.004
M. tuberculosis complex (96)	12.6 (4–37)	15.9 (6–40)	11.8 (3–30)	22.1 (13–45)	0.003	NS	<0.001
All NTM species (24)	16.6 (5–40)	16.1 (6–44)	16.1 (3–40)	22.4 (15–44)	NS	NS	NS
M. kansasii genotype I (14)	8.9 (5–16)	14.1 (6–24)	15.3 (3–33)	19.2 (15–27)	0.012	NS	NS

[a] NS, not significant.

ever, MGIT 960's mean time of mycobacterium detection was similar to that of BACTEC 460 (Table 4). The mean times until detection of *M. tuberculosis* complex were significantly shorter in the MGIT 960 and BACTEC 460 systems than in the MB/BacT system ($P = 0.003$ and $P < 0.001$, respectively). For NTM isolates, it was surprising that MGIT 960 was the most rapid culture tested. For *M. kansasii* genotype I, the earliest growth was detected with MGIT 960, and statistically significant differences with MB/BacT ($P = 0.012$) were found. Nevertheless, MB/BacT allowed the detection of this microorganism within the same amount of time as BACTEC 460.

The contamination rates for all specimens were as follows: 3.3% for MGIT 960, 3% for MB/BacT, 1.6% for BACTEC 460, and 4.1% for LJ cultures. Nine false-positive specimens were detected for both the MGIT 960 and MB/BacT systems (false-positivity rate, 0.95%). The contamination rates obtained with MGIT 960 and MB/BacT with the different antibiotic supplements and protocols recommended by the manufactures were similar and among the lowest reported for nonradiometric culture systems (2, 3, 5, 7, 8, 10, 12, 15, 16, 18, 21–23, 25, 27).

All hybridizations by AccuProbe from MB/BacT cultures yielding *M. tuberculosis* complex, *M. avium* complex, and *M. kansasii* genotype I ($n = 99$) were positive (mean, 595,699 RLUs; range, 33,379 to >900,000 RLUs). Among the 94 positive MGIT 960 cultures for *M. tuberculosis* complex, *M. avium* complex, and *M. kansasii* genotype I, only three *M. tuberculosis* complex isolates could not hybridize with AccuProbe (mean, 445,535 RLUs; range, 30,625 to >900,000 RLUs). Therefore, the identification of the most frequent and important mycobacterium species by AccuProbe within 72 h of positive nonradiometric liquid culture is rapid and precise. However, BACTEC 460 may require additional days of incubation of positive cultures until the GI is ≥500 to achieve a positive hybridization by the AccuProbe test (18).

Finally, the MGIT 960 and MB/BacT systems have other improvements over the traditional culture techniques. (i) These nonradiometric cultures are continuously monitored, easy-to-use systems with fewer handling requirements than those of the BACTEC 460 system. (ii) The risk of cross-contamination is practically eliminated, because the tubes and bottles do not have to be handled and punctured during readings. Furthermore, the MGIT 960 tubes use screw caps and needle inoculation is not necessary. Also, the MB/BacT culture bottles have been coated with plastic to minimize the risk of shattering glass. (iii) The MB/BacT system has better computerized data management and interface capabilities, but the MGIT 960 software is enough for practical laboratory purposes. (iv) Additionally, the MB/BacT instrument occupies more space in the laboratory than the MGIT 960 instrument, though the new modular design (BacT/Alert 3D) has practically resolved this problem.

In conclusion, the nonradiometric MGIT 960 and MB/BacT systems are automated, sensitive, rapid, and less labor-intensive mycobacterial culturing systems which allow a rapid identification by DNA AccuProbe and may be considered a substitute for the radiometric BACTEC 460 system.

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