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Clinical Evaluation of Difco ESP Culture System II for Growth and Detection of Mycobacteria

GAIL L. WOODS,[1]* GEOFFREY FISH,[1] MARIANNE PLAUNT,[2] AND THERESA MURPHY[1]

Department of Pathology, University of Texas Medical Branch, Galveston, Texas 77555-0740,[1] and Difco Laboratories, Detroit, Michigan 48232[2]

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The reliability of the ESP Culture System II (ESP II; Difco Laboratories, Detroit, Mich.), a continuously monitoring mycobacterial culture system, was evaluated by comparing its performance with the BACTEC TB 460 (BACTEC TB) and Middlebrook 7H11/7H11 selective agar systems. A total of 2,283 specimens of all types (70.7% were respiratory specimens) were cultured; 149 (6.5%) yielded mycobacteria. The most common species recovered were *Mycobacterium avium* complex (MAC, 73 isolates) and *Mycobacterium tuberculosis* complex (MTBC, 53 isolates). The recovery rates by individual system were 87, 81, and 65% for ESP II, BACTEC TB, and Middlebrook agar, respectively, for all mycobacteria; the recovery rates were 89, 92, and 89%, respectively, for MTBC. For liquid plus solid medium system combinations, recovery rates for all mycobacteria and for MTBC, respectively, were 91 and 94% for ESP II plus Middlebrook agar and 85 and 96% for BACTEC TB plus Middlebrook agar. The difference between the recovery rates of all mycobacteria by ESP II and by BACTEC TB was not significant, whereas for the individual species, the only significant difference was recovery of more isolates of MAC by ESP II. For those isolates recovered in the individual systems, mean times to detection of all mycobacteria, MTBC, and MAC, respectively, were 13.1, 15.5, and 10.9 days for ESP II; 14.4, 16.6, and 12.1 days for BACTEC TB; and 17.8, 18.3, and 18.8 days for Middlebrook agar. ESP II is a reliable, nonradiometric, less labor-intensive alternative to BACTEC TB for growth and detection of mycobacteria, but as with other liquid culture methods, ESP II should be used in combination with a solid medium, not as a stand-alone system.

During the past several years, considerable effort has been directed toward the development of rapid, efficient systems for growth and detection of mycobacteria and more rapid methods of mycobacterial identification and susceptibility testing of *Mycobacterium tuberculosis* complex (MTBC). Reasons for this renewed interest include the resurgence of tuberculosis in the United States during the past decade, the appearance of multidrug-resistant strains of *M. tuberculosis*, and the increasing importance of disease caused by the *Mycobacterium avium* complex (MAC) in patients with the acquired immunodeficiency syndrome (4–7, 9–11). In regard to mycobacterial culture, experts at the Centers for Disease Control and Prevention (CDC) recommend using both a liquid and a solid medium, and they have suggested an aggressive goal of detection of mycobacterial growth within 14 days of specimen inoculation (15). For many years, the only culture system with the potential to provide this target turnaround time was the radiometric method BACTEC TB 460 (BACTEC TB; Becton Dickinson, Cockeysville, Md.), which not only decreases the time to detection of mycobacteria but also increases the rate of recovery (1, 2, 14). This system, however, is labor-intensive, and it requires laboratories to deal with the various safety and regulatory issues associated with the use of radioisotopes. For these reasons, a technically more efficient, nonradiometric mycobacterial culture system is desirable.

The ESP Culture System II (ESP II; Difco Laboratories, Detroit, Mich.) is a fully automated, continuously monitoring system for growth and detection of microorganisms, including mycobacteria, that has recently received clearance by the Food and Drug Administration for mycobacterial culture. ESP II is an adaptation of the ESP blood culture system that has been available for clinical use for over 3 years. The technology is based on detection of pressure changes within the headspace above the broth culture medium in a sealed bottle, i.e., either gas production or gas consumption due to microbial growth. A special detection algorithm has been developed for the very slowly growing mycobacteria, in addition to the current ESP detection algorithm. The purpose of this study was to evaluate the reliability of ESP II for growth and detection of mycobacteria from clinical specimens by comparing its performance with the BACTEC TB system and a solid medium.

MATERIALS AND METHODS

Specimens. A total of 2,283 specimens submitted for detection of mycobacteria from January through September 1995 were evaluated, including 1,614 sputum and other respiratory specimens, 262 blood specimens, 118 other sterile body fluids, 87 tissue specimens, 96 urine specimens, 74 stool specimens, 2 gastric aspirates, and 30 wound specimens. Specimens were processed according to standard, accepted methods (13). *N*-Acetyl-L-cysteine-2% sodium hydroxide was used to decontaminate specimens that were potentially contaminated with normal flora. Specimens were concentrated by centrifugation at 3,000 × *g*, and pellets were resuspended in 1.5 ml of sterile phosphate buffer. Blood specimens for mycobacterial culture were collected in Isolator tubes (Wampole Laboratories, Cranbury, N.J.). Smears for detection of acid-fast bacilli (AFB) were stained with auramine O.

Culture and identification. A portion of the processed specimen was inoculated to each culture medium by using a needle and syringe as follows: 0.5 to 1 ml into the Difco ESP II bottle, 0.5 ml into a BACTEC 12B vial (except for blood, in which case 0.2 ml of the sediment was inoculated into the 12B vial), and 0.2 ml onto each side of a Middlebrook 7H11/7H11 selective biplate. Prior to inoculation of the liquid media with the specimen, the respective manufacturer's antibiotic supplement was added to ESP II (polymyxin B, vancomycin, nalidixic acid, and amphotericin B [PVNA]) and BACTEC 12B (polymyxin B, amphotericin B, nalidixic acid, trimethoprim, and azlocillin [PANTA]) bottles, and a growth supplement (Middlebrook OADC enrichment) was also added to ESP II bottles. Difco bottles were placed into the ESP II instrument and incubated at 35°C and monitored for bacterial growth as described above. Cultures were incubated for 6 weeks or until signaled by the ESP II instrument as positive. BACTEC 12B vials were incubated at 37°C and monitored for growth by the

* Corresponding author. Phone: 409-772-4851. Fax: 409-772-5683.

TABLE 1. Rate of recovery of mycobacteria by individual system and system combinations

Culture system	No. (%) positive for:			
	All mycobacteria	MTBC	MAC	Other[a]
ESP II	130 (87)	47 (89)	65 (90)	18 (75)
BACTEC TB	121 (81)	49 (92)	53 (74)	19 (79)
MA	97 (65)	47 (89)	37 (51)	13 (54)
ESP II and MA	135 (91)	50 (94)	66 (92)	19 (79)
BACTEC TB and MA	127 (85)	51 (96)	56 (78)	20 (83)
ESP II and BACTEC TB	148 (99)	53 (100)	72 (99)	23 (96)
Total no. of specimens positive	149	53	73	24

[a] For one specimen, MAC was recovered in the ESP II and BACTEC TB system; *M. gordonae* grew only on Middlebrook agar (MA).

BACTEC 460 instrument. The day 12B vials were read on the BACTEC 460 was determined by the Argus TB Data Program (Argus, Inc., Tampa, Fla.). In general, vials were monitored for growth every third day for 2 weeks and then weekly for 3 weeks, although the day of the last reading varied up to day 38, depending on the day of the week the culture was inoculated. If the growth index (GI) of the 12B vial was <30 on the last reading, the culture was considered negative for mycobacterial growth; vials with a GI of ≥30 were reincubated and read again at the end of 6 weeks. If no growth occurred in the ESP II or the reincubated BACTEC 12B vials by the end of week 6, the culture was considered negative for mycobacterial growth. When an ESP II bottle was signaled as positive, and for BACTEC, when the GI reached 100, a sample of the broth was removed and used to prepare a smear that subsequently was stained for AFB and for subculture to a Lowenstein-Jensen slant. If AFB were present in the smear, this was considered the time at which the specimen was positive for mycobacteria. All solid media were incubated at 37°C in 5 to 10% CO_2 and inspected weekly for 8 weeks or until mycobacterial colonies were detected.

Identification tests were performed on colonies growing on a solid medium or, for cultures of respiratory specimens, a sample of broth removed from a positive BACTEC 12B vial (GI, >999). Methods used for identification included nucleic acid probes (Gen-Probe Inc., San Diego, Calif.), for MTBC, MAC, *Mycobacterium kansasii*, and *Mycobacterium gordonae*, and conventional biochemicals, used according to standard procedures, for *Mycobacterium fortuitum-M. chelonae* complex (13). Isolates of all other mycobacteria were sent to the Texas Department of Health laboratory for identification by high-performance liquid chromatography with or without additional biochemical tests (13).

Statistical analysis. The isolation rates of the three systems were compared by using the McNemar modification of the chi-square test (12).

RESULTS

Of the 2,283 specimens evaluated, 149 were positive for mycobacteria, including 99 respiratory specimens, 19 blood specimens, 12 other sterile body fluids, 12 stool specimens, 5 tissues, and 1 each urine and wound specimen. The mycobacteria isolated included 53 MTBC, 73 MAC, 7 *M. kansasii*, 4 *M. fortuitum*, 3 *M. chelonae*, 8 *M. gordonae*, and 2 *Mycobacterium* spp. (not further identified). Two different mycobacteria were recovered by different systems from one specimen; MAC was isolated by ESP II and BACTEC TB, whereas only *M. gordonae* grew on Middlebrook agar.

The rate of recovery of mycobacteria is shown in Table 1 by system and system combinations. Overall, there was no significant difference between the rates of recovery of mycobacteria by ESP II and by BACTEC TB (Table 2). However, when recovery of the different mycobacterial species are compared, significantly more MAC were isolated in the ESP II system than in the BACTEC TB system ($P < 0.05$). In regard to specimen type, this difference in recovery of MAC was significant only for respiratory specimens. For all other species isolated from all types of specimens, the differences in recovery rates between ESP II and BACTEC TB were not significant. Compared with solid media, significantly more mycobacteria were recovered by ESP II ($P < 0.05$), and in regard to the different mycobacterial species, ESP II recovered significantly more MAC ($P < 0.05$). The difference in recovery rates of all mycobacteria or of MTBC by ESP II plus Middlebrook agar and by BACTEC TB plus Middlebrook agar was not significant.

TABLE 2. ESP II versus BACTEC TB for recovery of mycobacteria

Organism	Total no. of isolates	No. detected by:			*P* value[a]
		ESP II and BACTEC TB	ESP II	BACTEC TB	
MTBC	53	43	4	6	NS
MAC	72	46	19	7	<0.05
Other mycobacteria	23	14	4	5	NS
Total	148	103	27	18	NS

[a] NS, not significant.

Times to detection of mycobacterial growth by each system are summarized for all mycobacteria, MTBC, and MAC in Tables 3 to 5. Results for AFB smear-positive and smear-negative specimens are shown separately in Table 4 only for respiratory specimens from which MTBC was recovered. For all mycobacteria, ESP II had the shortest mean time to detection: 13.1 days compared with 14.4 days for BACTEC TB and 17.8 days for Middlebrook agar. Mean times to detection of all MTBC were 15.5 days for ESP II, 16.6 days for BACTEC TB, and 18.3 days for Middlebrook agar. For all three systems, isolates of MTBC from respiratory sites were detected faster (by 3 to 4 days) when the smear was positive for AFB. Mean times to detection of MAC were 10.9 days for ESP, 12.1 days for BACTEC TB, and 18.8 days for Middlebrook agar.

Overall contamination rates were 8.6, 4.0, and 0.8% for ESP II, BACTEC TB, and Middlebrook agar, respectively. For those cultures that became contaminated, contamination was recognized by day 5 in 68% of cases, by day 7 in 77% of cases, and by day 14 in 87% of cases. There were two MTBC isolates not detected in the ESP II system due to contamination.

DISCUSSION

Rapid diagnosis of tuberculosis is critical to control of the disease, therefore, use of the most rapid methods available for culture and identification of MTBC is advocated (3, 15). For mycobacterial culture, use of both a liquid and a solid medium is recommended, and if possible, the combination of media should allow detection of growth within 14 days of receipt of the specimen in the laboratory (15). BACTEC TB has been the most sensitive and rapid mycobacterial culture system available for several years in the United States (1, 2, 14). However, a nonradiometric culture system as reliable as BACTEC TB but less labor-intensive is desirable.

In this study, we evaluated the Difco ESP Culture System II, a fully automated, continuously monitoring instrument, by comparing its performance, when used as indicated by the

TABLE 3. Time to detection of all mycobacteria by system[a]

System (no. of isolates detected)	Mean time to detection [days (range)]	% Detected by day:					
		7	14	21	28	35	42
ESP II (130)	13.1 (2–39)	23	67	85	98	99	100
BACTEC TB (121)	14.4 (2–36)	19	63	84	91	99	100
Middlebrook agar (97)	17.8 (2–36)	3	42	79	92	99	100

[a] Media used in ESP II and BACTEC TB systems were incubated for a maximum of 6 weeks.

TABLE 4. Time to detection of *M. tuberculosis* complex by system[a]

System (no. of isolates detected)	Mean time to detection [days (range)]			% Detected by day:					
	All isolates	Smear positive[b]	Smear negative[c]	7	14	21	28	35	42
ESP II (47)	15.5 (2–34)	14.5 (2.5–34)	18.9 (6–28)	9	62	72	98	100	
BACTEC TB (49)	16.6 (4–36)	15.1 (4–36)	20.1 (8–35)	14	49	78	88	98	100
Middlebrook agar (47)	18.3 (12–36)	17.4 (12–29)	21.2 (13–36)	0	34	83	92	98	100

[a] Media used in ESP II and BACTEC TB systems were incubated for a maximum of 6 weeks.
[b] Numbers of AFB smear-positive specimens for ESP II, BACTEC TB, and Middlebrook agar were 36, 34, and 35, respectively.
[c] Numbers of AFB smear-negative specimens for ESP II, BACTEC TB, and Middlebrook agar were 11, 15, and 12, respectively.

manufacturer, with that of the BACTEC TB 460 and Middlebrook agar systems which is our usual laboratory protocol. One difference between the ESP II and BACTEC TB systems is the maximum volume of specimen that can be inoculated into the respective liquid media, i.e., 1.0 ml for the ESP II vial and 0.5 ml for the BACTEC 12B vial. Because our goal was to evaluate the ESP II as it is intended to be used, which includes a specimen volume of 0.5 to 1.0 ml, we maintained the sample volume within the specified range without compromising the volume used for the BACTEC TB system. By doing so, the ESP II vial in many cases received a larger volume of specimen than did the BACTEC 12B vial. Therefore, our results must be interpreted accordingly. A potential limitation of our design is the 5-week incubation period for the BACTEC 12B media (if the GI was <30 at the final reading). This interval, with the GI criterion, was selected based on 3 years of mycobacterial culture data (15,000 to 20,000 cultures/year) with the BACTEC TB system and Middlebrook agar from our laboratory, which showed that no clinically significant isolates were recovered after 5 weeks, if the GI at the final reading was <30.

Results of our evaluation showed that the overall rates of recovery of mycobacteria by the ESP II and BACTEC TB systems were comparable. Rates of recovery of MTBC and of the different nontuberculous mycobacteria by these two systems also were comparable, except for isolation of MAC. Significantly more MAC isolates were recovered by the ESP II than by the BACTEC TB system. Compared with isolation rates of mycobacteria with Middlebrook agar, both ESP II and BACTEC TB performed significantly better overall. However, neither ESP II, BACTEC TB nor Middlebrook agar recovered all isolates of MTBC or nontuberculous mycobacteria, thus lending support to the use of a combination of media. Very importantly, the rates of recovery of MTBC by the combination of ESP II plus a solid medium and by BACTEC plus a solid medium were not significantly different.

In regard to turnaround times, the mean times to detection of all mycobacteria, MTBC, and MAC were very similar for BACTEC TB and for ESP II. Although the mean times to growth and detection of mycobacteria approached the target of 14 days suggested by the CDC in both ESP II and BACTEC TB systems, neither system detected all isolates of MTBC within this time period. Of the isolates recovered in each respective system, 62% of MTBC were detected by day 14 with ESP II compared with 49% for BACTEC TB and 34% for Middlebrook agar.

ESP II has several advantages to offer. Perhaps most importantly, ESP II is less labor-intensive than BACTEC TB. Bottles are placed once in the ESP II instrument, whereas with BACTEC TB, vials are incubated off line in an incubator and then loaded and unloaded at several specified times during the total incubation period. The BACTEC TB system requires separate CO_2 tanks, which must be manually changed at regular intervals, and the needles must be manually cleaned and inspected and sterilized daily; ESP II has neither CO_2 tanks nor needles. User quality control with ESP II is minimal, the ESP II data management system considerably simplifies tracking of results, and ESP II can be interfaced with the laboratory information system. ESP II is nonradiometric, thus eliminating all of the issues associated with the use and disposal of radioactive material. Moreover, the ESP II system does not allow the possibility of cross contamination of bottles by the instrument, which has been a problem with the radiometric BACTEC TB system (8, 16). In this study, the contamination rate with ESP II was higher than the contamination rate with the BACTEC TB system. To address this issue, the manufacturer is evaluating various modifications of the supplement of antimicrobial agents that is added to each of the culture bottles, just as PANTA is added to BACTEC TB culture vials.

The nonlabor costs associated with each mycobacterial culture system will vary among laboratories based on volumes, including other supplies and equipment purchased from the manufacturer. Prices listed in the manufacturer's catalog for the ESP II and its components are $70,000 for the 384 instrument ($31,500 for the 128 instrument), $175.00 for a case of 50 culture vials, $49.95 each for the growth supplement (250 tests) and antibiotic mixture (250 tests), and $25.00 for a box of 50 connectors. List prices for the BACTEC TB components are $35,900 for the 460 instrument, $5,297 for the hood, $250.00 for a case of 100 12B vials, and $45.75 for the antibiotic supplement (500 tests). Additional costs that must be considered with the BACTEC TB system are needles, CO_2, and disposal of the radioactive waste, which at our institution is about $20,000/year. The cost of solid media is in the range of $0.50 to $1.00 per plate or tube.

In summary, the ESP II is a reliable, nonradiometric, less labor-intensive alternative to BACTEC TB for growth and detection of mycobacteria. However, as with BACTEC TB or any other liquid culture system, we recommend that ESP II be used in combination with another culture method, rather than as a stand-alone system. Our data show that ESP II plus BACTEC TB yields the highest mycobacterial recovery rate; however, in most laboratories this combination of systems probably would be cost prohibitive. We believe that a reason-

TABLE 5. Time to detection of *M. avium* complex by system[a]

System (no. of isolates detected)	Mean time to detection [days (range)]	% Detected by day:					
		7	14	21	28	35	42
ESP II (65)	10.9 (2–39)	35	74	98	99	99	100
BACTEC TB (53)	14.4 (2–32)	25	79	90	94	100	
Middlebrook agar (37)	18.8 (7–35)	3	39	72	89	100	

[a] Media used for ESP II and BACTEC TB systems were incubated for a maximum of 6 weeks.

able compromise is to use ESP II (or BACTEC TB) plus a solid medium.

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