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Comparison of the BACTEC MGIT 960 and ESP Culture System II for Growth and Detection of Mycobacteria

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The performances of two continuously monitoring mycobacterial culture systems—ESP Culture System II (ESP II; Trek Diagnostics, Inc., Westlake, Ohio) and BACTEC MGIT 960 (BD Biosciences, Sparks, Md.)—were compared. In addition to both liquid media, all specimens were plated onto Middlebrook 7H11/7H11 selective agar. A total of 3,151 specimens of all types (56.3% were respiratory specimens) were cultured; 231 (7.3%) yielded mycobacteria. The most common species recovered were *Mycobacterium avium* complex (69 isolates) and *Mycobacterium tuberculosis* complex (MTBC; 65 isolates). The recovery rates for ESP II, BACTEC MGIT 960, and Middlebrook agar, respectively, were 71.2, 63.9, and 61.8% for all mycobacteria; 70.2, 72.6, and 66.3% for all mycobacteria except *Mycobacterium gordonae*; and 73.8, 84.6, and 87.7% for MTBC. For liquid plus solid medium combinations, recovery rates for all mycobacteria and for MTBC, respectively, were 84.1 and 92.3% for ESP II plus Middlebrook agar and 81.5 and 98.5% for BACTEC MGIT 960 plus Middlebrook agar. The differences in recovery of all mycobacteria by ESP II and by BACTEC MGIT 960 were not significant; for the individual species, the only significant difference was recovery of more isolates of *M. gordonae* by ESP II. For those isolates recovered in both automated systems, mean times to detection of all mycobacteria and MTBC, respectively, were 15.8 and 17.4 days for ESP II and 12.5 and 11.9 days for BACTEC MGIT 960 ($P < 0.05$). False-positive signals occurred with 23 (0.7%) BACTEC MGIT 960 cultures and 84 (2.7%) ESP II cultures ($P < 0.01$). Overall contamination rates were 17.1% for BACTEC MGIT 960, 18.9% for ESP II, and 11.0% for Middlebrook agar. In summary, the ESP II and BACTEC MGIT 960 systems performed comparably with regard to growth and detection of mycobacteria, and the contamination rates were similar. However, with ESP II, times to detection of all mycobacteria and of MTBC were significantly longer, the recovery rate of *M. gordonae* was significantly higher, and the number of false-positive signals was greater than with BACTEC MGIT 960.

During the past several years, several manufacturers have directed considerable effort toward the development of rapid, efficient systems for growth and detection of mycobacteria. With regard to mycobacterial culture, experts at the Centers for Disease Control and Prevention recommend using both a liquid and a solid medium, and they have suggested an aggressive goal of detecting mycobacterial growth within 14 days of specimen inoculation (7). For many years, the only culture system with the potential to provide this target turnaround time was the radiometric method (BACTEC 460 TB; BD Biosciences, Sparks, Md.), which not only decreases the time to detection of mycobacteria but also increases the rate of recovery (1, 3). This system, however, is labor-intensive, and it requires that laboratories deal with the various safety and regulatory issues associated with the use of radioisotopes. More recently, various fully automated, continuously monitoring, nonradiometric systems for growth and detection of mycobacteria have become commercially available. Two such systems are the ESP Culture System II (ESP II; Trek Diagnostics, Inc., Westlake, Ohio) and the BACTEC MGIT 960 (BD Biosciences). In most clinical evaluations, the performance of these two systems has been compared with that of the BACTEC 460 TB (2, 4, 9–11). In general, investigators have concluded that either automated system, when used in combination with a solid medium, is reliable for mycobacterial culture. To our knowledge, however, the performances of ESP II and BACTEC MGIT 960 have not been compared. The purpose of this study was to compare the performances of ESP II, BACTEC MGIT 960, and Middlebrook 7H11/7H11 selective agar for growth and detection of mycobacteria.

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MATERIALS AND METHODS

Specimens. A total of 3,151 specimens (collected from 1,688 patients) submitted for detection of mycobacteria from 1 December 1998 through 30 December 1999 were evaluated. This included 1,775 sputum and other respiratory specimens, 457 sterile body fluids, 178 tissue specimens, 99 urine samples, 195 stool specimens, 217 wound samples, 209 aspirates, and 21 samples from miscellaneous sources. Specimens were processed according to standard, accepted methods (6). *N*-Acetyl-L-cysteine–1% sodium hydroxide (final concentration after mixing with the specimen) 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. Smears for detection of acid-fast bacilli (AFB) were stained with auramine O.

Culture and identification. A portion of the processed specimen was inoculated into each culture medium by using a needle and syringe as follows: 0.5 ml into an ESP II bottle, 0.5 ml into a BACTEC MGIT tube, 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 growth supplement and antibiotic mixture (polymyxin B, vancomycin, nalidixic acid, and amphotericin B for ESP II and polymyxin B, amphotericin B, nalidixic acid, trimethoprim, and azlocillin [PANTA] for MGIT) were added. ESP bottles were placed into the ESP II instrument, in which they were incubated at 35°C. MGIT tubes were placed into the BACTEC MGIT 960 instrument, where they were incubated at 37°C. Liquid cultures were continuously monitored for bacterial growth for 6 weeks or until signaled by the respective instrument as positive. All solid media were incubated at 37°C in 5 to 10% CO_2 and inspected weekly for 6 weeks or until mycobacterial colonies were detected. If no growth occurred in broth or on agar by the end of week 6, the culture was considered negative for mycobacterial growth, with two exceptions. Cultures of specimens that were AFB smear positive were held for a total of 8 weeks before being considered negative for mycobacterial growth, and occasionally a physician would specifically request that a culture be incubated for 8 rather than 6 weeks.

When either liquid medium was signaled as positive, a sample of the broth was removed and used to prepare a smear that was stained for AFB. If AFB were

TABLE 1. Rates of recovery of mycobacteria by individual systems and system combinations

Specimens	Culture system	No. (%) of specimens positive for:			
		Any mycobacteria[a]	Any mycobacteria excluding *M. gordonae*	MTBC	Other[b]
All	MGIT 960	149 (63.9)	138 (72.6)	55 (84.6)	83 (66.4)
	ESP II	166 (71.2)	133 (70.0)	48 (73.8)	85 (68.0)
	MA[c]	144 (61.8)	126 (66.3)	57 (87.7)	69 (55.2)
	MGIT 960 and MA	190 (81.5)	166 (87.4)	64 (98.5)	102 (81.6)
	ESP II and MA	196 (84.1)	153 (80.5)	60 (92.3)	93 (74.7)
	MGIT 960 and ESP II	212 (91.0)	175 (92.1)	62 (95.4)	113 (90.4)
Total		233	190	65	125
Respiratory	MGIT 960	105 (62.5)	97 (72.9)	47 (83.9)	50 (64.9)
	ESP II	108 (64.3)	86 (64.7)	40 (71.4)	46 (59.7)
	MA	94 (56.0)	78 (58.6)	49 (87.5)	29 (37.7)
	MGIT 960 and MA	134 (79.8)	118 (88.7)	55 (98.2)	63 (81.8)
	ESP II and MA	139 (82.7)	101 (75.9)	50 (89.3)	53 (68.8)
	MGIT 960 and ESP II	142 (84.5)	122 (91.7)	53 (94.6)	69 (89.6)
Total		168	133	56	77

[a] Includes 43 total *M. gordonae* isolates from all specimens (11 recovered by BACTEC MGIT 960, 33 recovered by ESP II, and 18 recovered by Middlebrook agar) and 35 total *M. gordonae* isolates from respiratory specimens (8 recovered by BACTEC MGIT 960, 22 recovered by ESP II, and 16 recovered by MA).
[b] Mycobacteria other than MTBC and *M. gordonae*.
[c] MA, Middlebrook agar.

present in the smear (considered a true positive), a portion of the broth was subcultured onto a 7H11/7H11 selective biplate. If both AFB and other microorganisms were present, the broth was subcultured and then redecontaminated and cultured again. The time to detection was based on the earliest time of positivity recorded by the instrument that correlated with the positive AFB smear result. If the smear was negative for AFB, a smear of broth was stained with the Gram stain. If bacteria or yeast was present, the culture was considered contaminated and was discontinued. If no organisms were seen (considered a false-positive result), the culture was returned to its instrument for further incubation. If a result of "no organisms seen" occurred for a culture on two (for MGIT) or three (for ESP) successive occasions, a portion of the broth was subcultured onto a 7H11/7H11 selective biplate, and the culture was removed from the instrument. If, by the end of 6 weeks, the solid medium alone or only one of the liquid media was positive for AFB, terminal subculture of the negative liquid medium or media onto a 7H11/7H11 selective biplate was performed. The result for the system(s) that did not signal positive was considered a false negative if the mycobacterium was recovered from the terminal subculture after incubation for up to 6 weeks.

Identification tests were performed on colonies growing on a solid medium. For identification, nucleic acid probes (Gen-Probe Inc., San Diego, Calif.) were used for *Mycobacterium tuberculosis* complex (MTBC), *Mycobacterium avium* complex (MAC), *Mycobacterium kansasii*, and *Mycobacterium gordonae* and conventional biochemicals were used in standard procedures for rapidly growing mycobacteria (6). Isolates not identified by one of these methods were sent to the Texas Department of Health laboratory for identification by high-performance liquid chromatography and/or biochemical tests (6).

Statistical analysis. The isolation rates of the three systems were compared by the McNemar modification of the chi-square test (5). Times to detection of mycobacterial growth by BACTEC MGIT 960 and ESP II were compared by the Student *t* test. False-positive rates were compared using Pearson's chi-square test with Yates' continuity correction.

RESULTS

Of the 3,151 specimens evaluated, 231 (7.3%) from 165 patients were positive for mycobacteria, including 158 respiratory specimens, 4 sterile body fluids, 32 stool specimens, 10 tissues, 17 aspirates, 8 wound specimens, 3 urine specimens, and 1 specimen from a miscellaneous source. Among the mycobacteria recovered were 65 MTBC, 69 MAC, 4 *M. kansasii*, 6 *M. fortuitum*, 15 *M. fortuitum-chelonae* complex, 43 *M. gordonae*, 11 *M. abscessus*, 3 *M. mucogenicum*, 5 *M. xenopi*, 3 *M. szulgai*, 3 *M. marinum*, 2 *M. terrae*, and 1 *M. simiae* isolate and 3 *Mycobacterium* spp. (not further identified). Two cultures were positive for two different mycobacteria; MTBC and *M. gordonae* were recovered from one MTBC and MAC were recovered from the other. With BACTEC MGIT 960, there were 10 false-negative results (defined in Materials and Methods)—5 for MTBC and 5 for MAC. ESP II had 13 false-negative results (8 for MTBC, 3 for MAC, 1 for *M. fortuitum*, and 1 for *M. kansasii*). A false-positive signal occurred with 23 (0.7%) BACTEC MGIT 960 cultures and 84 (2.7%) ESP II cultures ($P < 0.01$).

The number of mycobacteria recovered from all specimens and from respiratory specimens only is shown in Table 1 for each system and system combination. Recovery rates by ESP II and BACTEC MGIT 960 are compared in Table 2. Overall, there was no significant difference between the rates of recovery of all mycobacteria or of MTBC by ESP II and by BACTEC MGIT 960. Likewise, the difference in rates of recovery of all mycobacteria or of MTBC by ESP II plus Middlebrook agar and by BACTEC MGIT 960 plus Middlebrook agar was not significant. Although neither ESP II plus Middlebrook agar nor BACTEC MGIT 960 plus Middlebrook agar recovered all isolates of MTBC, BACTEC 960 plus Middlebrook agar detected all patients with tuberculosis and ESP II plus Middlebrook agar detected all except one. With

TABLE 2. BACTEC MGIT 960 versus ESP II for recovery of mycobacteria

Organism	Total no. of isolates	No. detected by[a]:			*P* value[b]
		BACTEC MGIT 960 and ESP II	BACTEC MGIT 960 only	ESP II only	
MTBC	65	40	15	8	NS
M. gordonae	43	7	4	26	<0.05
Other mycobacteria	125	50	33	28	NS
Total	233	97	52	62	NS

[a] Additional mycobacteria recovered by Middlebrook agar only included MTBC (2 isolates); *M. gordonae* (6 isolates), and other mycobacteria (14 isolates).
[b] NS, not significant.

TABLE 3. Times to detection of mycobacteria[a]

Organisms	System (no. of isolates detected)	Mean no. of days to detection (range)[b]			% Detected by day:					
		All	Smear positive[c]	Smear negative[d]	7	14	21	28	35	42
All mycobacteria	BACTEC MGIT 960 (149)	13.1 (1–43)	10.5 (1–37)	14.8 (1–43)	31	67	83	90	95	99
	ESP II (166)	16.9 (2–45)	13.1 (2–33)	18.3 (2–45)	15	44	65	75	90	99
	Middlebrook agar (144)	19.2 (5–56)	16.6 (6–56)	21.0 (5–41)	7	28	71	87	93	99
MTBC	BACTEC MGIT 960 (55)	12.8 (5–26)	11.1 (5–26)	14.1 (5–22)	15	69	95	100		
	ESP II (48)	18.7 (3–39)	15.1 (3–31)	24.4 (3–39)	6	27	69	83	98	100
	Middlebrook agar (57)	19.2 (6–56)	16.5 (6–56)	22.3 (8–40)	4	35	74	91	93	98

[a] Media were routinely incubated for 6 weeks; some cultures were incubated for up to 8 weeks due to special circumstances.
[b] Difference between BACTEC MGIT 960 and ESP II times to detection for those specimens positive for any mycobacteria and for MTBC by both systems was statistically significant ($P < 0.05$, Student's t test).
[c] Numbers of MTBC smear-positive specimens for BACTEC MGIT 960, ESP II, and Middlebrook agar were 27, 26, and 31, respectively.
[d] Numbers of MTBC smear-negative specimens for BACTEC MGIT 960, ESP II, and Middlebrook agar were 28, 22, and 26, respectively.

regard to rates of recovery of mycobacteria other than MTBC, the only significant difference between ESP II and BACTEC MGIT 960 was recovery of more *M. gordonae* isolates in the ESP II system ($P < 0.05$).

Times to detection of mycobacterial growth by each system are summarized for all mycobacteria and for MTBC in Table 3. For all mycobacteria, BACTEC MGIT 960 had the shortest mean time to detection of growth overall (13.1 compared to 16.9 days for ESP II and 19.2 days for Middlebrook agar) as well as for AFB smear-positive specimens (10.5 compared to 13.1 days for ESP II and 16.6 days for Middlebrook agar) and smear-negative specimens (14.8 compared to 18.3 days for ESP II and 21.0 days for Middlebrook agar). For the 97 total specimens positive by both BACTEC MGIT 960 and ESP II, the mean times to detection were 12.5 days (range, 1 to 43 days) for BACTEC MGIT 960 and 15.8 days (range, 2 to 45 days) for ESP II ($P < 0.05$). Likewise, the mean time to detection for the 92 specimens positive by both BACTEC MGIT 960 and Middlebrook agar was significantly shorter for MGIT (11.4 days) than for Middlebrook agar (18.5 days) ($P < 0.05$). However, for the 94 specimens positive by both ESP II and Middlebrook agar, the difference between mean times to detection (ESP II, 15.8 days; Middlebrook agar, 18.4 days) was not significant. The mean times to detection of all MTBC isolates were 12.8 days for BACTEC MGIT 960, 18.7 days for ESP II, and 19.2 days for Middlebrook agar. For the 40 MTBC isolates recovered by both BACTEC MGIT 960 and ESP II, the mean times to detection were 11.9 days (range, 5 to 20 days) for MGIT and 17.4 days (range, 3 to 32 days) for ESP II ($P < 0.05$).

Overall contamination rates were 17.1, 18.9, and 11.0% for BACTEC MGIT 960, ESP II, and Middlebrook agar, respectively. Rates for stool specimens only were 41.0% for BACTEC MGIT 960, 32.3% for ESP II, and 18.5% for Middlebrook agar. Excluding the 195 stool specimens, contamination rates were 10.9% for BACTEC MGIT 960, 12.7% for ESP II, and 3.7% for Middlebrook agar. There were five MTBC isolates not detected in the ESP II system and seven not detected in the BACTEC MGIT 960 system because of contamination. However, this did not result in failure to diagnose tuberculosis for these patients because either the companion Middlebrook agar or a previous or subsequent specimen was positive.

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 (7). For mycobacterial culture, use of both a liquid and a solid medium is recommended; the combination of media should allow detection of growth within 14 days of receipt of the specimen in the laboratory (7). In this study, we compared two fully automated, continuously monitoring instruments: ESP II and BACTEC MGIT 960.

Results of our evaluation showed that the overall rates of recovery of mycobacteria by the ESP II and BACTEC MGIT 960 systems and by either automated system plus Middlebrook agar were comparable. Rates of recovery of MTBC and the different nontuberculous mycobacteria by these two systems also were comparable, with one exception. Significantly more isolates of *M. gordonae* were isolated in the ESP II than in the BACTEC MGIT 960 system. The increased rate of recovery of *M. gordonae* is a disadvantage for the laboratory and potentially for the patient. Because *M. gordonae* rarely is a true pathogen, its recovery and subsequent identification create extra laboratory work that does not benefit patient care and could have a negative impact if the patient were treated unnecessarily with antimycobacterial agents.

With regard to turnaround times, the mean times to detection of all mycobacteria and of MTBC were shorter for BACTEC MGIT 960 than for ESP II, and for those isolates recovered by both systems, the difference was significant. For BACTEC MGIT 960, these times (mean of 13.1 days for all mycobacteria and 12.8 days for MTBC) are comparable to the mean times to detection reported by other investigators (11.8 to 13.3 days for all mycobacteria and 12.6 to 14.4 days for MTBC) (2, 4, 10). For ESP II, however, the mean times to detection in the present study (16.9 days for all mycobacteria and 18.7 days for MTBC) are similar to those reported by Tortoli et al. (18.07 days for all mycobacteria and 19.06 days for MTBC) (9) but several days longer than what was reported from our institution when ESP II was initially evaluated (11). The reason for the difference between the latter and present results is not clear.

The mean times to growth and detection of mycobacteria reached the target of 14 days suggested by the Centers for Disease Control and Prevention only for the BACTEC MGIT 960 system; however, neither of the automated systems nor Middlebrook agar detected all isolates of MTBC within this time period. Of the isolates recovered in each system, 69% of MTBC isolates (27 of 30 patients diagnosed with tuberculosis based on culture results) were detected by day 14 with BACTEC MGIT 960, compared to 27% (11 of 27 patients) with ESP II and 35% (15 of 30 patients) with Middlebrook agar. For ESP II, this is considerably different from the findings of an earlier evaluation of the system performed at our institution, in which 62% of the MTBC isolates were detected by day 14 (11). The reason for the marked change is unknown.

Overall contamination rates for both automated systems in this evaluation were much higher than rates reported in other studies,

including the assessment of ESP II performed at this institution several years ago, when the rate for ESP II was 8.6% (2, 4, 9–11). Rates in this study remained high even after stool specimens were excluded. Since our initial evaluation of ESP II, we decreased the final percentage of NaOH used in the specimen decontamination procedure from 2 to 1%, which certainly could influence the contamination rate. Because the final percentage of NaOH is not specified in all of the published reports, we cannot adequately assess how this variable impacted the contamination rates in those studies. In addition to the decrease in the percentage of NaOH, we had considerable turnover in the personnel in charge of processing specimens for mycobacterial culture. Processing personnel also have changed since the end of the present study, and for the past few months the contamination rate in BACTEC MGIT 960 has been 7%.

Both the ESP II and BACTEC MGIT 960 systems have limitations with regard to specimen type. Direct inoculation of blood for mycobacterial culture is not an intended use of either system. However, with ESP II, inoculation of the blood sediment from a processed Isolator tube is acceptable for mycobacterial blood culture (8). To our knowledge, this method has not been evaluated with BACTEC MGIT 960. According to the package insert, BACTEC MGIT 960 is not intended for mycobacterial culture of either urine or blood samples. The ESP II system has no such restriction. Of the 99 urine specimens cultured in the present study, 3 were positive: 2 for MAC (from two patients), both detected by BACTEC MGIT 960, ESP II, and Middlebrook agar, and 1 for *M. gordonae*, which grew in ESP II only. Because of the low number of positives, it is not possible to make firm conclusions concerning the performance of BACTEC MGIT 960 with urine. However, since liquid culture systems always should be used in conjunction with a solid medium, recovery of significant mycobacteria from urine specimens will probably not be compromised in laboratories using a combination of BACTEC MGIT 960 and Middlebrook agar or Lowenstein-Jensen agar.

Each automated system also offers certain advantages. The ESP II system includes a computer for data management, which simplifies tracking of results and can be interfaced with the laboratory's information system. Additionally, ESP II is cleared by the Food and Drug Administration for testing the susceptibility of MTBC to isoniazid, ethambutol, and rifampin. With regard to BACTEC MGIT 960, the culture tubes are plastic rather than glass, and they have screw caps, thus eliminating the need to use needles for the addition of supplements and specimen inoculation. Growth of microorganisms in MGIT tubes is clearly visible, and in most cases the pattern of growth distinguishes mycobacteria, which tend to form clumps in the bottom of the tube, from bacteria, which typically cause diffuse turbidity throughout the tube. The MGIT PANTA and enrichment supplement (consisting of oleic acid, bovine albumin, dextrose, and catalase [OADC]) are packaged in a single kit rather than individually; therefore, only one addition to the culture medium, rather than two, is required. The BACTEC MGIT 960 instrument holds many more cultures than does the largest ESP II instrument (i.e., 960 versus 384) and, therefore, is better suited for high-volume mycobacteriology laboratories. Laboratories performing up to 8,300 mycobacterial cultures per year (or an average of up to 690 per month) need only one BACTEC MGIT 960 instrument. A data management system for BACTEC 960, which can be acquired separately, also is available.

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, and the type of agreement (i.e., purchase or lease). Prices listed in the manufacturer's catalog for 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), $49.50 for the antibiotic mixture (250 tests), and $25.00 for a box of 50 connectors. Another cost that must be considered with the ESP II system is that of needles for addition of growth supplement, antibiotics, and specimen. List prices for the BACTEC MGIT 960 system components are $63,900 for the 960 instrument, $824.00 for a carton of 100 culture tubes, and $92.70 for the supplement kit (100 tests; both growth supplement and antibiotics).

In summary, the ESP II and BACTEC MGIT 960 systems performed comparably with regard to growth and detection of mycobacteria, and the contamination rates were similar. However, the ESP II system had 3.6 times as many false-positive signals as the BACTEC MGIT 960 system ($P < 0.01$) and recovered significantly more *M. gordonae* isolates. Neither system recovered all mycobacteria or all MTBC; therefore, we recommend that either system always be used in combination with another culture method, rather than as a stand-alone system. Our data showed that ESP II used in conjunction with BACTEC MGIT 960 yields the highest mycobacterial recovery rate; however, in most laboratories this combination would most likely be cost prohibitive. We believe that using an automated liquid culture system as well as a solid medium is a reasonable compromise.

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