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Comparison of Automated Difco ESP Blood Culture System with Biphasic BBL Septi-Chek System for Detection of Bloodstream Infections in Pediatric Patients

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We compared the Difco ESP 384 blood culture system with the pediatric Septi-Chek system for the detection of bloodstream infections in pediatric patients. A total of 10,762 blood culture sets included an ESP aerobic bottle and a Septi-Chek bottle. From these cultures, a total of 278 organisms classified as probable pathogens were isolated, including 237 from ESP bottles and 221 from Septi-Chek bottles. This difference was not statistically significant. More organisms classified as possible contaminants were also isolated from ESP bottles (for ESP, 480 bottles; for Septi-Chek, 418 bottles; $P < 0.01$). The time to detection was shorter for probable pathogens isolated from ESP bottles (median times for organisms isolated from both systems: ESP, 14.0 h; Septi-Chek, 34.5 h; $P < 0.001$). The proportions of all probable pathogens detected by 24 and 48 h after inoculation were 78 and 96%, respectively, for ESP compared with 31 and 74%, respectively, for Septi-Chek. The time to final identification was also shorter for organisms grown in ESP bottles (median times for organisms isolated from both systems: ESP, 48.8 h; Septi-Chek, 58.5 h; $P \leq 0.001$). A subset of 4,442 cultures also included an ESP anaerobic bottle in addition to an ESP aerobic bottle and a Septi-Chek bottle. There were no significant differences in the recovery of probable pathogens by any of the possible two bottle combinations, but five anaerobic pathogens were recovered only in the anaerobic bottle. We conclude that the ESP 384 is an excellent system for culturing pediatric blood samples and that it provides for the very rapid detection of bloodstream pathogens.

Rapid detection of bacteremia and fungemia is important in the management of patients with possible sepsis. Recently, new blood culture systems have been developed. These systems achieve rapid detection of bloodstream pathogens by frequent automated monitoring of inoculated bottles (5, 8, 10). One of those is the ESP system manufactured by Difco Laboratories (Detroit, Mich.). This system detects the presence of bacterial growth in broth bottles by monitoring for pressure changes resulting from either the consumption or the production of gas. In the present study, we compared the ESP system with a conventional system, Septi-Chek (Becton Dickinson, Cockeysville, Md.). The Septi-Chek system is a biphasic system that uses brain heart infusion broth, and it has performed well compared with other widely used pediatric blood culture systems (13, 14). A question of interest in the present study was whether rapid detection could be achieved with an 80-ml ESP bottle for low-blood-volume pediatric blood cultures.

MATERIALS AND METHODS

Blood samples for culture were collected from patients on all patient care units at St. Louis Children's Hospital. Patients at St. Louis Children's Hospital range in age from newborn to 21 years, with approximately 50% of patients being under 2 years of age. All pediatric specialties and subspecialties are represented at the hospital, resulting in a diverse patient population. Blood samples for quantitative cultures, which were usually obtained from patients suspected of catheter-related infection, were excluded from the study. These blood samples made up 29% of all blood samples submitted for culture during the time period of the study and were often from patients in the intensive care unit. For all blood samples for nonquantitative culture, blood was drawn into transport tubes containing sodium polyanesulfonate as described previously (14), and the tubes were transported to the laboratory. Inoculation of bottles was carried out in a biological safety cabinet by laboratory personnel who were available at all times to process the blood samples for culture. If the volume of blood received was less than 1.5 ml, the blood was divided equally between one 80-ml aerobic ESP bottle and one 20-ml pediatric Septi-Chek bottle (brain heart infusion broth). If the volume of blood received was equal to or greater than 1.5 ml, the blood was divided equally between one 80-ml ESP aerobic bottle, one 80-ml ESP anaerobic bottle, and one 20-ml Septi-Chek bottle. The amount of blood inoculated into each bottle ranged from 0.2 to 1.0 ml, resulting in a blood-to-broth ratio of 1:80 to 1:400 in ESP bottles and 1:20 to 1:100 in Septi-Chek bottles.

ESP system. After inoculation, a connector with a recessed needle was attached to each ESP bottle. ESP aerobic bottles were placed on the Difco ESP 384 instrument and were incubated with continuous agitation at 35°C for 7 days unless they were designated as positive by the instrument. ESP anaerobic bottles were placed on the Difco ESP 384 instrument in the anaerobic section of the instrument for stationary incubation. After 8,288 blood culture sets had been entered into the study, the time of incubation was decreased to 6 days. Until that time, no probable pathogens had been detected in the ESP system on day 7. After an additional 100 blood culture sets had been entered into the study, the time of incubation was decreased to 5 days. Until that time, one probable pathogen had been detected on day 6.

When bottles were flagged as positive by the instrument, aliquots were removed for Gram staining and were subcultured onto chocolate and blood agars. During the first 11 months of the study (6,640 cultures), the Microbiology Laboratory was not staffed during the third shift, so bottles which were flagged during that time were processed at the beginning of the next shift. For the remainder of the study the third shift was staffed and bottles were processed as soon as they were flagged as positive. Regardless of the time of workup, the time to detection used in the study was obtained from the ESP computer's graphical display.

Septi-Chek system. After inoculation, a paddle containing blood, chocolate, and malt agars was attached to the Septi-Chek bottle and the bottle was inverted to allow the blood-broth mixture to wash over the agar pads. The Septi-Chek bottle was incubated with no agitation at 35°C with inversions twice daily for the first 3 days and once daily for the remaining 4 days. Bottles were inspected at these times for the detection of growth. If growth appeared on the agar pads or the broth was cloudy or hemolyzed, the broth was subcultured to blood and chocolate agar plates and Gram staining was performed.

Incubation and terminal subcultures. Samples were incubated in each system

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TABLE 1. Probable pathogens isolated from 10,762 blood culture sets

Organism	No. of organisms recovered by:			
	ESP aerobic only	Septi-Chek only	Both systems	Total
Staphylococcus aureus	16[a]	5[a]	28	49
Coagulase-negative staphylococci	0	1	12	13
Streptococcus pneumoniae	16	14	37	67
Beta-hemolytic streptococci[b]	2	1	15	18
Enterococcus spp.	5	4	9	18
Streptococcus mutans	1	0	0	1
Micrococcus spp.	1	1	0	2
Corynebacterium spp.	0	1	1	2
Enterobacteriaceae[c]	8	4	33	45
Pseudomonas aeruginosa	1	1	2	4
Pseudomonas putida	0	0	1	1
Acinetobacter spp.[d]	1	1	1	3
Agrobacterium tumefaciens	1	0	0	1
Haemophilus influenzae[e]	1	2	2	5
Neisseria meningitidis	0	0	5	5
Neisseria gonorrhoeae	0	0	1	1
Moraxella catarrhalis	0	0	1	1
Bifidiobacterium sp.	0	0	1	1
Candida spp.[f]	3	6	31	40
Cryptococcus neoformans	1	0	0	1
Total	57	41	180	278

[a] $P = 0.03$.
[b] Includes 4 group A, 12 group B, and 2 group C isolates.
[c] Includes 13 *Escherichia coli*, 6 *Enterobacter cloacae*, 1 *Enterobacter aerogenes*, 1 *Enterobacter amniogenus*, 1 *Enterobacter agglomerans*, 2 *Citrobacter freundii*, 1 *Citrobacter diversus*, 12 *Klebsiella pneumoniae*, 2 *Klebsiella oxytoca*, 2 *Salmonella* species, and 4 *Serratia marcescens* isolates.
[d] The isolate recovered only in the ESP aerobic bottle and the isolate recovered only in the Septi-Chek bottle were *Acinetobacter lwoffi*. The isolate recovered from both bottles was *Acinetobacter baumannii.*
[e] Includes type b ($n = 3$), type e ($n = 1$), and nontypeable ($n = 1$).
[f] Includes 12 *Candida albicans*, 25 *Candida parapsilosis*, and 3 *Candida tropicalis* isolates.

and were observed without knowledge of the results for the samples in the other system. A terminal subculture and Gram staining were performed on the companion bottle of a positive bottle if the companion bottle was still negative at the end of the planned period of incubation. If growth was detected only by the terminal subculture, the companion culture was considered negative for the purposes of analysis. Instances in which bottles were signaled as positive by the instrument but growth was not confirmed by Gram staining or subculture were defined as false signals.

Criteria for clinical significance. All isolates were designated probable pathogens or possible contaminants. Probable pathogens were defined as (i) organisms that are usually considered pathogens or (ii) organisms that are usually considered contaminants but that were isolated from more than one blood culture set with the same antimicrobial susceptibility pattern. When the classification was unclear, the case was reviewed by a physician member of the Infectious Disease team.

Statistical analysis. The significance of differences in the numbers of organisms detected by each system was evaluated by McNemar's test. The significance of differences in time to detection and time to identification was evaluated by the Wilcoxon signed-rank test.

RESULTS

A total of 10,762 blood culture sets were included in the study. Of these, 4,442 sets included all three bottles and 6,320 sets included only the ESP aerobic bottle and the Septi-Chek bottle. All 10,762 sets were used to compare the ESP aerobic bottle and the Septi-Chek bottle. Two hundred seventy-eight probable pathogens were isolated from aerobic bottles during the study, of which 237 (85.3%) were detected by the ESP bottle and 221 (79.5%) were detected by the Septi-Chek bottle ($P = 0.16$). As shown in Table 1, there were no statistically significant differences in the detection of individual organisms by either system except for *Staphylococcus aureus*, which was detected significantly more often by ESP bottles ($P = 0.03$). The total number of possible contaminants isolated from aerobic bottles was 680, of which 480 (70.6%) and 418 (61.5%) were detected by ESP and Septi-Chek, respectively ($P = 0.01$).

At 24 and 48 h after inoculation, 78 and 96% of probable pathogens were detected by the ESP system, respectively, whereas 31 and 74% of probable pathogens were detected by the Septi-Chek system, respectively. The times for detection of 90% of probable pathogens were 34.8 h for the ESP system and 78.5 h for the Septi-Chek system. Table 2 shows the median time to detection for probable pathogens isolated by both systems. Most species were detected significantly faster in the ESP system. The median times to detection for all organisms isolated from both systems were 14.0 h for the ESP system and 34.5 h for the Septi-Chek system ($P \leq 0.001$).

The median time to final identification of probable pathogens isolated in both systems was 48.8 h for the ESP system compared with 58.5 h for the Septi-Chek system ($P < 0.001$). For most organisms, these times also reflected the times when susceptibility test results were available. Most species were identified earlier in the ESP system, but the differences were smaller than those for the time to initial detection because of the early growth on solid media in the Septi-Chek system, which facilitated final identification procedures.

The number of false signals (0.02%) by the ESP system was minimal. Terminal subcultures revealed that five organisms were present in ESP cultures but were not signaled by the ESP instrument (*Candida parapsilosis* [$n = 1$], coagulase-negative staphylococcus [$n = 1$], *Corynebacterium* species [$n = 1$], and *Propionibacterium acnes* [$n = 2$]), whereas four organisms were detected only by terminal subculture of the Septi-Chek bottle (*Klebsiella pneumoniae* [$n = 1$], *Staphylococcus aureus* [$n = 1$], group B streptococcus [$n = 1$], and *Propionibacterium acnes* [$n = 1$]).

A total of 4,442 blood culture sets included an ESP anaerobic bottle in addition to the ESP aerobic and Septi-Chek bottles (Table 3). A total of 162 probable pathogens were

TABLE 2. Time to detection of probable pathogens isolated from both systems

Organism	No. of isolates	Time to detection (h)				P^a
		ESP Aerobic		Septi-Chek		
		Median	Range	Median	Range	
Staphylococcus aureus	28	14.7	10.8–34.0	37.8	11.0–165.0	<0.001
Coagulase-negative staphylococci	12	20.2	12.8–26.0	39.8	18.0–67.0	0.002
Streptococcus pneumoniae	37	13.8	10.0–16.4	22.5	12.5–80.0	<0.001
Beta-hemolytic streptococci[b]	15	11.8	8.8–107.8	36.0	10.0–165.0	0.001
Enterococcus spp.	9	12.2	10.4–15.6	21.5	15.5–61.0	0.008
Corynebacterium sp.	1	15.2		18.5		
Enterobacteriaceae[c]	33	10.7	6.4–95.6	23.0	11.0–110.5	<0.001
Pseudomonas aeruginosa	2	12.6	9.2–16.0	48.0	38.5–57.5	
Pseudomonas putida	1	11.8		17.0		
Haemophilus influenzae	2	40.1	30.4–49.8	32.5	29.5–35.5	
Neisseria meningitidis	5	15.8	13.4–27.0	37.0	34.0–58.0	0.04
Neisseria gonorrhoeae	1	42.6		132.5		
Acinetobacter baumannii	1	10.4		72.0		
Moraxella catarrhalis	1	23.0		54.5		
Bifidiobacterium sp.	1	128.8		173.0		
Candida[d]	31	29.4	12.0–46.2	70.5	24.5–113.0	<0.001
Total	180	14.0	6.4–128.8	34.5	10.0–173	<0.001

[a] Wilcoxon signed rank test.
[b] Includes 3 group A, 10 group B, and 2 group C isolates.
[c] Includes 9 *Escherichia coli*, 5 *Enterobacter cloacae*, 1 *Enterobacter aerogenes*, 1 *Enterobacter amnigenus*, 1 *Enterobacter agglomerans*, 2 *Citrobacter freundii*, 1 *Citrobacter diversus*, 9 *Klebsiella pneumoniae*, 1 *Klebsiella oxytoca*, 1 *Salmonella enteritidis*, and 2 *Serratia marcescens* isolates.
[d] Includes 8 *Candida albicans*, 20 *Candida parapsilosis*, and 3 *Candida tropicalis* isolates.

isolated from these sets, of which 122 (75.3%) were detected by the ESP aerobic bottle, 111 (68.5%) were detected by the Septi-Chek bottle, and 101 (62.3%) were detected by the ESP anaerobic bottle ($P = 0.17$ for the Septi-Chek bottle versus the ESP aerobic bottle; $P = 0.02$ for the ESP aerobic bottle versus the ESP anaerobic bottle; $P = 0.3$ for the Septi-Chek bottle versus the ESP anaerobic bottle). A total of six anaerobic pathogens were detected (one each of *Bifidobacterium* species, *Bacteroides fragilis*, *Prevotella intermedia*, *Fusobacterium necrophorum*, *Veillonella* species, *Peptostreptococcus asaccharolyticus*). The isolate of *Bifidobacterium* species was the only anaerobe detected by the ESP aerobic bottle or the Septi-Chek bottle. Culture results from the 4,442 sets which included all three bottles were also examined to determine the yield from each possible combination of two bottles. The total yield of pathogens from each of the three possible two-bottle sets was equivalent, but the set consisting of ESP aerobic and Septi-Chek bottles failed to detect five anaerobic pathogens.

TABLE 3. Probable pathogens isolated from 4,442 blood culture sets including ESP aerobic and anaerobic bottles and a Septi-Chek Bottle

Organism	No. of isolates recovered by the following blood culture bottles:							
	All systems	ESP Aerobic and Septi-Chek	ESP aerobic and ESP anaerobic	ESP anaerobic and Septi-Chek	ESP aerobic only	Septi-Chek only	ESP anaerobic only	Total
Staphylococcus aureus	15	1	9	1	3	3	1	33
Coagulase-negative staphylococci	9	1				1		11
Streptococcus pneumoniae	12	2		2	5	3	6	30
Beta-hemolytic streptococci	5	2			1	1		9
Enterococcus spp.	7				2	1	4	7
Streptococcus mutans			1					1
Corynebacterium spp.		1				1		2
Enterobacteriaceae	17		3	1	2	1	3	27
Pseudomonas aeruginosa					1	1		2
Pseudomonas putida		1						1
Agrobacterium tumefaciens					1			1
Haemophilus influenzae						1		1
Neisseria meningitidis		2						2
Neisseria gonorrhoeae		1						1
Bifidiobacterium spp.	1							1
Bacteroides fragilis							1	1
Prevotella intermedius							1	1
Fusobacterium necrophorum							1	1
Veillonella spp.							1	1
Peptostreptococcus asaccharolyticus							1	1
Candida spp.	5	16			2	3	1	27
Cryptococcus neoformans							1	1
Total	64	27	13	4	18	16	20	162

DISCUSSION

We have compared the performance in a pediatric setting of a frequently monitored blood culture system, the Difco ESP system, with that of a widely used conventional pediatric system, the Becton-Dickinson Septi-Chek system. Our previous studies showed that the Septi-Chek system (marketed by Roche Laboratories at the time of those studies) was comparable in performance to two other pediatric systems, the Becton-Dickinson Vacutainer (13) and the Bactec Peds Plus (14) systems. In the present study we found that a Difco ESP aerobic bottle provided slightly better recovery of probable pathogens than a Septi-Chek bottle. Not surprisingly, since the ESP system monitors aerobic bottles every 12 min, the initial detection of organism growth was dramatically faster with the ESP system. The earlier detection in the ESP system also allowed for more rapid final identification and susceptibility testing of pathogens, even though the biphasic Septi-Chek system provided early growth on solid media. It should also be noted that the shorter time to final identification of pathogens detected by the ESP system occurred even though 61% of the cultures were performed during a period when third-shift personnel were not available to process positive cultures, so that notification of physicians and laboratory identification procedures for cultures detected as positive by the ESP system late at night were not begun until the next morning.

The study described here did not address directly the clinical significance of the earlier detection of bacteremia or fungemia by the ESP system. Nevertheless, the magnitude of the differences suggests that those differences could have clinical significance (11). For example, the median times to detection of *Staphylococcus aureus* and members of the family *Enterobacteriaceae* were 14.7 and 10.7 h, respectively, with the ESP system compared with 37.8 and 23.0 h, respectively, with the Septi-Chek system. Differences of similar magnitudes were present for most other pathogens. The finding that 90% of all pathogens were detected by 34.8 h after inoculation in the ESP system compared with 78.5 h in the Septi-Chek system means that clinicians could act on negative blood culture reports approximately 1 day earlier when the ESP system is used. Given the magnitudes of these differences, it is likely that the earlier detection provided by the ESP system would be important even if a microbiology laboratory were not staffed at night and bottles that were detected as positive during the period without staffing were acted on the next morning.

The usual recommendation for the ratio of broth and blood volumes for optimal blood cultures is 5:1 to 10:1 (6, 12). This relationship has never been carefully investigated in a pediatric setting. In the present study, we used the standard 80-ml ESP aerobic bottle, resulting in a broth-to-blood ratio that ranged from 80:1 to 400:1, depending on the volume of blood inoculated. It is apparent from our findings that rapid detection can be achieved even when the broth-to-blood ratio greatly exceeds 10:1, although it is possible that a lower broth-to-blood ratio could result in even faster detection. That question could be answered by a study comparing the results obtained with different blood volumes. The same study could also address the hypothesis that a large broth-to-blood ratio has a positive effect on pathogen detection by diluting inhibitory substances including antibiotics.

In addition to a small increase in the detection of probable pathogens by the ESP system, our study also demonstrated an increase in the number of organisms classified as possible contaminants, mostly coagulase-negative staphylococci. It is likely that this finding is a reflection of the high sensitivity of the ESP system for the detection of staphylococci. It should be noted that some organisms classified as possible contaminants in the present study were probably, in fact, the causes of episodes of real bacteremia. During the study period, it was common for only one blood sample for culture to be drawn before antibiotics were started in response to an episode of possible sepsis, especially in small infants in whom coagulase-negative staphylococci might be true pathogens. Whenever only one blood sample for culture was drawn, it was consequently impossible to meet the criterion used in the present study to classify organisms such as coagulase-negative staphylococci as probable pathogens, since the criterion specified recovery from more than one culture of isolates with identical identifications and antibiotic susceptibilities. Clinical criteria were not used because of the difficulty in using such criteria to distinguish contaminants from true pathogens in episodes of coagulase-negative staphylococcal bacteremia in young infants (9). This same factor probably also contributed to the relatively low overall proportion of cultures in the study that were positive for organisms considered to be probable pathogens. It is possible that low blood volumes and the use of transport tubes containing sodium polyanesulfonate may also have accounted in part for this relatively low proportion of positive cultures.

The present study also generated data concerning the utility of an anaerobic blood culture bottle in a pediatric setting. Because of blood volume limitations, only 41% of blood culture sets included an anaerobic bottle. Similar to findings in previous studies (1–4, 7), the rate of recovery of obligate anaerobes was low (0.13% of blood culture sets which included an anaerobic bottle). In comparing the effectiveness of each possible two-bottle combination, differences in total organism recovery were not statistically significant, and thus, if real differences exist, they are likely to be small. Not surprisingly, however, the two-bottle combinations which lacked an anaerobic bottle missed five of the six anaerobic pathogens. Since the ESP anaerobic bottle was also effective in recovering most other organisms that are not obligate anaerobes, especially streptococci, it constitutes a very effective second bottle that can accompany an ESP aerobic bottle.

The present study provided strong support for the effectiveness of the ESP system for the detection of bacteremia or fungemia caused by a wide variety of pediatric pathogens. A limitation of the study was that despite the large number of blood culture sets included, the number of isolates of the important pediatric pathogens *Haemophilus influenzae* and *Neisseria meningitidis* was too small to permit evaluation of the system with regard to these pathogens. With the caveat that further study is required for *H. influenzae*, *N. meningitidis*, and other unusual organisms, it appears that a blood culture system such as ESP which incorporates frequent automated monitoring for microbial growth can be applied to pediatric blood cultures and represents an important advance in that area by virtue of its sensitive and rapid detection of bloodstream pathogens.

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