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Comparison of the BacT/Alert Pediatric Blood Culture System, Pedi-BacT, with Conventional Culture Using the 20-Milliliter Becton-Dickinson Supplemented Peptone Broth Tube

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The performance of the Pedi-BacT system, the BacT/Alert (Organon Teknika Corp., Durham, N.C.) pediatric blood culture bottle, was compared with that of a conventional 20-ml supplemented peptone broth tube (Becton-Dickinson Corp., Cockeysville, Md.) (BD system) in matched aerobic cultures. The tubes of the BD system were visually examined daily for 7 days and were subcultured during the first 24 h of incubation. Pedi-BacT cultures were mechanically agitated and continuously monitored for growth by the instrument. Of the 6,628 compliant pairs, 331 (5.0%) were positive in both systems, 220 (3.3%) were positive in the Pedi-BacT system only, and 170 (2.6%) were positive in the BD system only. One (0.02%) false-negative culture and 15 (0.2%) false-positive cultures occurred with the Pedi-BacT system while 20 (0.3%) false-negative cultures and 35 (0.5%) false-positive cultures occurred with the BD system. Of 288 clinically significant organisms detected in matched pairs from which a single isolate was recovered, 176 (61%) were recovered from both systems, 83 (29%) were recovered from the Pedi-BacT system only ($P < 0.0001$), and 29 (10%) were recovered from the BD system only. Members of the family *Enterobacteriaceae* ($P < 0.01$), miscellaneous nonfermenters ($P < 0.05$), and *Candida* spp. ($P < 0.01$) were isolated more frequently in the Pedi-BacT system than in the BD system. No significant difference in recovery of other organisms was found between the systems. The average time to detection for the Pedi-BacT system ranged from 11.5 h for streptococci to 29.7 h for enterococci, while that for the BD system ranged from 20.3 h for streptococci to 66.4 h for some nonfermenters. The BacT/Alert system is a reliable, labor-saving alternative to conventional blood culture methods.

Pediatric blood cultures are processed by clinical laboratories by using either a manual broth or a biphasic system, lysis and direct plating, or semiautomated instrumentation (2). In many instances, a combination of systems must be used to adequately detect all potential pathogens including mycobacteria and fungi. Although blood culture bottles or tubes containing broth formulations that are conducive to the growth of pediatric pathogens and that are designed to accommodate smaller pediatric specimen volumes are available for both manual and semiautomated procedures, the number of times that each bottle is inspected for growth is limited when either method is used (2, 10). Lysis and direct plating reduce the time to isolation of the organisms present in a positive blood culture, but they are labor-intensive when processing large numbers of blood cultures and are often associated with increased recovery of contaminants (3, 16).

Recently, a new and innovative, fully automated blood culture system, the BacT/Alert system (Organon Teknika Corp., Durham, N.C.), was introduced for laboratory use. The BacT/Alert system detects microbial growth through noninvasive colorimetric detection of the CO_2 produced during bacterial metabolism. The bottles, which are agitated throughout the recommended 5- to 7-day incubation period, are monitored every 10 min by the instrument, and growth is determined by an algorithm based on an initial high CO_2 level, a high rate of CO_2 production, or an acceleration in the rate of CO_2 production (14).

A pediatric blood culture bottle, the Pedi-BacT system, has been developed for use with the instrument. In the present study, the performance of the Pedi-BacT system was compared with that of a manual blood culture method, the Vacutainer 20-ml supplemented peptone broth tube system (Becton-Dickinson Corp., Cockeysville, Md.) (BD system), to assess the recovery rates and time to detection of microorganisms in suspected cases of bacteremia in our pediatric population.

MATERIALS AND METHODS

Blood culture systems. Each Pedi-BacT blood culture bottle is prepared with an atmosphere of CO_2 in nitrogen under vacuum and contains 20 ml of supplemented brain heart infusion broth with 0.020% sodium polyenetholsulfonate (SPS). The tubes used in the BD system are also prepared with an atmosphere of CO_2 in nitrogen under vacuum and contain 20 ml of supplemented peptone broth with 0.025% SPS. Basic supplements for both systems consisted of pyridoxine, hemin, menadione, L-cysteine, vitamins, and amino acids. The Pedi-BacT bottle and the BD tube were vented if they were submitted for aerobic culture; however, venting units were removed from the Pedi-BacT bottle before placement in the instrument but were maintained in the aerobic BD tubes during the entire incubation period. BD tubes submitted for anaerobic culture were not vented prior to incubation. Inoculated Pedi-BacT bottles were placed in the instrument, which is designed to rock each bottle during incubation in ambient air at 35°C. BD tubes were placed in stationary racks and were maintained in ambient air at 35°C in a standard laboratory incubator. Both

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systems were incubated for 7 days or until the results were indicated to be positive.

Specimen collection. The protocol established at Children's Medical Center instructs individuals who collect blood for culture from inpatients to inoculate two BD tubes, one each for aerobic and anaerobic culture. Only one BD tube for aerobic culture is routinely collected from children receiving care in the Emergency Referral Center. During the evaluation period, collection packets containing both BD tubes and Pedi-BacT bottles plus collection instructions were distributed throughout the hospital. The study protocol required inoculation of blood from a single collection site into each set of BD tubes plus Pedi-BacT bottles. Individuals were instructed to inoculate the BD tube(s) first to ensure the performance of culture by the reference method. The optimum collection volume was set at 2.0 ml of blood for each tube or bottle included in the evaluation. If a minimum total of 1.0 to 1.5 ml was collected, only the BD tube was inoculated for culture. Standard recommended antisepsis procedures were used during blood specimen collection (12).

Laboratory processing. Upon receipt, each tube or bottle was visually inspected for growth, proper inoculation, and pertinent patient collection information. Only the combination of one or two BD tubes accompanied by at least one Pedi-BacT bottle containing blood from the same collection sites and times as those used for the BD tubes were included in the study. BD tubes were routinely examined twice by subculture and direct smear during the first 24 h of incubation and were visually inspected daily thereafter. Pedi-BacT bottles were monitored for the presence of bacterial growth every 10 min by the instrument and, if positive, were processed by laboratory personnel on a 24-h basis. Subcultures and direct smears from both systems were performed at the first indication of a positive culture. Any positive BD tube or Pedi-BacT bottle was processed promptly after detection, while the corresponding BD tube or Pedi-BacT bottle was not processed until the scheduled time of examination or until the bottle or tube was found to be positive by the instrument or visual examination. Cultures that were initially indicated as positive but that were not found to contain bacterial growth were reincubated and monitored for the remainder of the incubation period. Terminal subcultures were performed at the completion of the 7-day incubation period in cases in which a positive culture was accompanied by a negative BD tube or Pedi-BacT bottle result. Organisms were identified by established procedures for identification and susceptibility testing.

Data analysis. In addition to the culture collection date and time, lot numbers, and pertinent patient information, the following data were collected in conjunction with the study: (i) receipt date and time (designated for both systems as the time when the Pedi-BacT bottle was logged into the instrument); (ii) time to a positive result (designated as the time a positive signal and/or a positive smear or culture was recorded); (iii) a false-positive result (defined as a culture that was found to be positive by the instrument or visual examination but not confirmed by direct smear or subculture); and (iv) a false-negative result (defined as a culture found to contain growth only after terminal subculture). Organisms retrieved from culture were categorized as clinically significant or probable contaminants on the basis of published recommendations and examination of patient records. In those instances in which a category could not be assigned, the isolate was designated as category unknown.

Statistical methods. Unless otherwise noted, comparisons were evaluated by a modified chi-square test. The Yates' correction for small numbers of observations was used when necessary (8).

RESULTS

During the 8-month evaluation period, 6,628 matched pairs of blood culture specimens were received for comparative analysis by the BD and Pedi-BacT systems. Since the Pedi-BacT system is currently recommended exclusively for aerobic culture, the unvented BD tubes used for anaerobic blood cultures were excluded from the study. Bacterial growth was identified in both the BD and Pedi-BacT systems in 331 (5.0%) cultures, in the Pedi-BacT system only in 220 (3.3%) cultures, and in the BD system only in 170 (2.6%) cultures.

The Pedi-BacT and BD systems yielded 721 positive cultures containing single isolates or polymicrobic flora. A total of 655 isolates were detected in matched pairs from which the same single isolate was recovered; 288 were classified as clinically significant, 327 were classified as probable contaminants, and 40 were classified as category unknown. Fifty-six polymicrobic cultures and 10 cultures in which different organisms were identified in both systems accounted for the remainder of the positive cultures in the Pedi-BacT or BD system. Of the clinically significant single isolates recovered from matched aerobic Pedi-BacT and BD system pairs, 176 (61%) were recovered from both systems, while 83 (29%) were recovered from the Pedi-BacT system only ($P < 0.0001$) and 29 (10%) were recovered from the BD system only (Table 1). Members of the family *Enterobacteriaceae* ($P < 0.01$), miscellaneous nonfermenters ($P < 0.01$) other than *Pseudomonas aeruginosa*, and *Candida* spp. ($P < 0.01$) were isolated more frequently in the Pedi-BacT system only than in the BD system only. The overall recovery of staphylococci, *Streptococcus pyogenes*, *Streptococcus agalactiae*, *Streptococcus pneumoniae*, alpha-hemolytic *Streptococcus* spp., enterococci, and other miscellaneous microorganisms from both systems was not significantly different. Although only one isolate each was recovered during the evaluation, fastidious organisms such as *Kingella kingae*, *Campylobacter jejuni*, and a nutritionally variant streptococcus were retrieved from the Pedi-BacT system only. The occurrence of serious *Haemophilus influenzae* infections has decreased dramatically in our institution, resulting in the recovery of only two isolates from both the Pedi-BacT and BD systems during the study period. Likewise, two isolates of *Neisseria meningitidis* were recovered from both systems during the evaluation. No significant difference was found in the recovery of microorganisms classified as probable contaminants (Table 2).

The average times to detection for both systems are presented in Table 3. The average times to detection for the BD system ranged from 20.3 h for streptococci to 66.4 h for miscellaneous nonfermenters and enterococci. In contrast, the average times to detection for the Pedi-BacT system ranged from 11.5 h for streptococci to 29.7 h for enterococci. All organism groups excluding the enterococci were detected by the Pedi-BacT system within the first 24 h of incubation. In addition to the streptococci, *Staphylococcus aureus* and members of the family *Enterobacteriaceae* were detected within 12.5 h. The overall rates of recovery for both systems are further illustrated in Fig. 1. The Pedi-BacT system detected 91.5% of positive cultures within 24 h of incubation. The cumulative proportion of positive results

TABLE 1. Comparative yields of clinically significant isolates from Pedi-BacT and BD blood culture media

Organism group	No. of isolates recovered from: Pedi-BacT and BD	Pedi-BacT only	BD only	P
Staphylococcus aureus	22	8	4	NS[a]
Coagulase-negative staphylococci	23	3	0	NS
Group A or B streptococci	7	1	2	NS
Streptococcus pneumoniae	25	15	7	NS
Alpha-hemolytic streptococci	12	3	1	NS
Nutritionally variant streptococci	0	1	0	NS
Enterococci	6	2	2	NS
Fastidious gram-negative species[b]	4	1	1	NS
Enterobacteriaceae[c]	39	18	5	<0.01
Campylobacter jejuni	0	1	0	NS
Pseudomonas aeruginosa	10	6	4	NS
Miscellaneous nonfermenters[d]	8	9	1	<0.05
Clostridium spp.	0	2	2	NS
Candida spp.	19	13	0	<0.01
Bacillus spp.	1	0	0	NS
Total	176	83	29	<0.0001

[a] NS, not significant ($P > 0.05$).
[b] Includes one *Kingella kingae*, two *Neisseria meningitidis*, two *Haemophilus influenzae*, and one unidentified gram-negative rod.
[c] Includes 1 *Citrobacter freundii*, 1 *Enterobacter agglomerans*, 16 *Enterobacter cloacae*, 2 *Enterobacter* spp., 10 *Escherichia coli*, 3 *Klebsiella oxytoca*, 18 *Klebsiella pneumoniae*, 6 *Salmonella* spp., 3 *Salmonella typhi*, and 2 *Serratia marcescens*.
[d] Includes nine *Acinetobacter* spp., seven *Pseudomonas cepacia*, one *Pseudomonas putida*, and one *Pseudomonas* spp.
[e] Includes 14 *Candida albicans*, 4 *Candida parapsilosis*, 11 *Candida tropicalis*, and 3 *Candida* (*Torulopsis*) *glabrata*.

recovered from the BD system rose from approximately 44.9% by 24 h to 86.9% by 48 h.

A total of 17 terminal subcultures were performed to investigate discrepancies between the BD and the Pedi-BacT systems. Only one (0.02%) Pedi-BacT blood culture previously categorized as negative during the 7-day incubation period was found to contain growth after terminal subculture. The isolate was identified as *Acinetobacter calcoaceticus* and was classified as a probable contaminant. Of the 16 BD tubes undergoing terminal subculture, eight coagulase-negative staphylococci, two alpha-hemolytic streptococci, one *Streptococcus pneumoniae*, one *Pseudomonas aeruginosa*, and four *Candida albicans* were recovered from individual tubes. Fifteen (0.23%) Pedi-BacT cultures were flagged as positive by the instrument but remained negative by direct smear or subculture, whereas 35 (0.5%) BD cultures were falsely identified as positive by visual examination when performing the manual method.

TABLE 2. Comparative yields of probable contaminants from Pedi-BacT and BD blood culture media

Organism group	No. of organisms recovered from: Pedi-BacT and BD	Pedi-BacT only	BD only	P
Miscellaneous nonfermenters[b]	0	0	3	NS[a]
Aerobic gram-positive rods[c]	2	10	4	NS
Alpha-hemolytic streptococci	1	5	7	NS
Staphylococci and *Micrococcus* spp.	84	94	98	NS
Anaerobes[d]	1	5	7	NS

[a] NS, not significant ($P > 0.05$).
[b] Includes one *Acinetobacter* spp., one *Pseudomonas* spp., and one *Moraxella* spp.
[c] Includes nine *Corynebacterium* spp., one gram-positive rod, and six *Bacillus* spp.
[d] Includes 1 *Clostridium* spp., 1 *Peptococcus* spp., and 11 *Propionibacterium acnes*.

TABLE 3. Comparison of time to detection for clinically significant isolates by microorganism group

Organism group[a]	No. of isolates	Time to detection (h)[b] Pedi-BacT system	BD system	P[c]
Staphylococcus aureus	22	12.3	36.3	0.0001
Coagulase-negative staphylococci	23	24.5	53.2	0.0001
Enterococci	6	29.7	34.2	NS
Streptococci	44	11.5	20.3	0.0001
Enterobacteriaceae	39	12.4	31.8	0.0001
Pseudomonas aeruginosa	10	17.0	38.8	0.0039
Miscellaneous nonfermenters	8	22.8	66.4	0.0078
Candida spp.	19	19.1	37.1	0.0001
Fastidious gram-negative rods[d]	4	21.8	38.8	NS

[a] See Table 1 footnotes *b* to *e* for the isolates included in each category.
[b] Mean time to detection.
[c] Signed rank test; NS, not significant ($P > 0.05$).
[d] Excluding the unidentified gram-negative rod.

DISCUSSION

The expedient laboratory diagnosis of bacteremia is fundamental to quality pediatric care. However, the selection of a blood culture system that is both cost-effective and reliable for the rapid detection of important pediatric pathogens often poses a dilemma for many laboratories. Parameters such as medium composition and volume, incubation conditions, and method of detection of a positive culture must be evaluated to determine the technology best suited to the needs of a particular institution. Recent expansion of services at the Children's Medical Center of Dallas prompted a reassessment of our blood culture protocol. A conventional method that uses the 20-ml supplemented peptone broth tube system (BD system) performed satisfactorily for a number of years but could not efficiently accommodate the approximately 20,000 blood cultures per year currently received by the laboratory. In the present study, the recovery and time to detection of microorganisms by the Pedi-BacT system, a new pediatric bottle designed for the BacT/Alert automated instrument, and the BD system were evaluated in 6,628 matched aerobic cultures obtained from our patient population.

Both systems were comparable for the recovery of the majority of clinically significant isolates. The Pedi-BacT system was superior to the BD system in recovery and time to detection of several organism groups such as members of the family *Enterobacteriaceae*, *Candida* spp., and miscellaneous nonfermenters. Important pediatric pathogens such as *Haemophilus influenzae*, *Neisseria meningitidis*, and *Strep-*

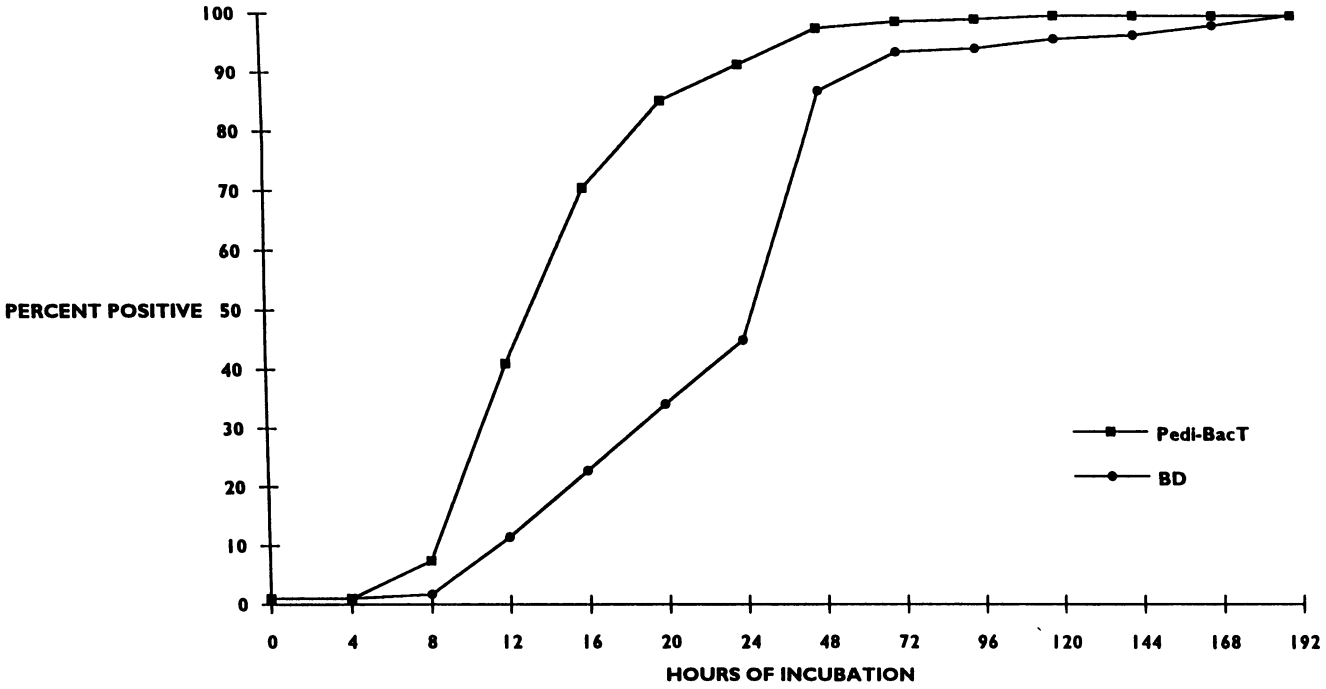

FIG. 1. Cumulative yields of 176 clinically significant microorganisms recovered as single isolates from both the Pedi-BacT and BD systems during the 7-day incubation period.

tococcus pneumoniae were recovered equally well by both systems; however, a greater number of *Streptococcus pneumoniae* was recovered by the Pedi-BacT system only.

Differences in medium composition as well as incubation conditions account for some disparities in organism retrieval between the two systems. Although a comparison of the two medium formulations for significant differences in their abilities to retrieve more fastidious organisms was not possible because of the low incidence of these isolate types, the basic Pedi-BacT medium formulation, an enriched brain heart infusion broth containing a lower concentration of SPS (0.020%), did support the growth of certain fastidious bacteria and *Candida* spp. While the higher SPS content (0.030 to 0.035% [wt/vol]) used in many commercial blood culture broths inhibits phagocytic cells and other antimicrobial factors in serum, it also may repress the growth of pathogenic neisseriae (6).

Prior to the study, anaerobic blood cultures were routinely performed by our laboratory by using the unvented BD system since the medium formulation and internal atmosphere of the tube is conducive to anaerobic bacterial growth. Application of the unvented Pedi-BacT bottle to anaerobic blood culture, however, is not approved by the manufacturer. Although pediatric facilities should provide anaerobic blood cultures when a clinically significant infection is suspected, routine culture is not warranted in most cases. Dunkle et al. (4) reported the recovery of anaerobes from only 0.75% of blood cultures acquired from 5,465 pediatric patients. Similar investigations have confirmed the low incidence of pediatric anaerobic bacteremias (1). Neonatal anaerobic sepsis, however, occurs in pediatric patients in higher numbers than in other age groups and is usually clinically significant (11).

Terminal subcultures were performed in cases when growth occurred in only one system by the completion of the 7-day incubation period. Although the majority of false-negative cultures occurred with the BD system and involved isolates that were categorized as contaminants, six false-negative BD cultures contained clinically significant isolates including *Candida albicans*, *Pseudomonas aeruginosa*, and *Streptococcus pneumoniae*. Specimens containing low blood volumes, low bacterial concentrations, or more fastidious organisms may not reach sufficient growth thresholds for detection by some manual or automated methods within the 5- to 7-day incubation period. Only one false-negative culture occurred with the Pedi-BacT system, and the isolate was categorized as a contaminant. Likewise, fewer false-positive cultures occurred with the Pedi-BacT system. Although the cause remains unresolved, the generation of a positive signal by the instrument could result from high leukocyte counts or organism growth that cannot be confirmed by routine aerobic subculture or direct smear. The greater number of false-positive cultures associated with the BD system was attributed to the difficulties involved in accurately assessing the presence of growth indicators such as turbidity, hemolysis, or gas production during visual examination of the culture.

Sustained agitation and continuous monitoring of cultures for indications of growth by the BacT/Alert instrument may account for higher yields and decreased detection times for different organism groups recovered from the Pedi-BacT system. The improved recovery and time to positivity associated with agitation of culture has been documented in previous studies (5, 7). Daily visual examination coupled with blind subculture during the first 6 to 17 h of incubation is recommended for optimum detection of bacterial growth by conventional broth systems (12). However, visual inspection of blood culture tubes for growth indicators such as turbidity, hemolysis, and gas production precludes agitation during incubation.

The need for a blood culture bottle designed specifically for pediatric specimens is controversial. Many institutions maintain only a commercial blood culture system designed primarily for adult populations. This approach, however, may impede the recovery of blood-borne pathogens from some pediatric specimens. Collection volumes in infants and children typically range from 0.5 to 1.5 ml per blood culture tube or bottle. The inoculation of such small volumes into commercial systems containing >50 ml of broth creates blood-to-broth ratios that far exceed the recommended optimum ratio of 1:10 (15). Although higher bacterial concentrations in the blood of many children during sepsis negates the concern over large blood-to-broth ratios, a percentage of cultures contain lower concentrations of organisms depending on the collection time, the infecting organism, and the source of bacteremia (9, 13). Potential loss of organism viability or decreased times to positivity in these situations may adversely affect clinical diagnosis and therapeutic decisions.

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