JOURNAL OF CLINICAL MICROBIOLOGY, Feb. 1991, p. 359–362
0095-1137/91/020359-04$02.00/0

Vol. 29, No. 2

Performance of a BACTEC Nonradiometric Medium for Pediatric Blood Cultures

JOSEPHINE A. MORELLO,[1,2]* SCOTT M. MATUSHEK,[1] W. MICHAEL DUNNE,[3,4] AND DAVID B. HINDS[5]

Clinical Microbiology Laboratories, University of Chicago Hospitals,[1] and Departments of Pathology, Medicine, and Molecular Genetics and Cell Biology, University of Chicago Medical Center,[2] Chicago, Illinois 60637; Clinical Microbiology and Immunology Laboratories, Children's Hospital of Wisconsin,[3] and Departments of Pathology and Pediatrics, The Medical College of Wisconsin,[4] Milwaukee, Wisconsin 53233; and Microbiology Laboratory, Texas Children's Hospital, Houston, Texas 77025[5]

Received 21 September 1990/Accepted 19 November 1990

A BACTEC aerobic nonradiometric medium, PEDS Plus, designed for diagnosis of pediatric bacteremia was evaluated in three hospital centers. Equivalent blood volumes (up to 5 ml) were inoculated into and incubated in BACTEC NR-6A (6A) and PEDS Plus broths. Among 4,581 compliant sets, 289 clinically significant organisms, representing more than 20 bacterial and two *Candida* species, were isolated. One hundred eighty-one isolates were recovered in both bottles, 75 in PEDS Plus only, and 33 in 6A only ($P < 0.001$). Time to detection when both bottles were positive was the same for 129 isolates, detection with PEDS Plus was earlier for 39, and detection with 6A was earlier for 13 ($P < 0.005$). *Staphylococcus aureus* was recovered significantly more often in PEDS Plus than in 6A ($P < 0.01$), and more coagulase-negative staphylococci and pediatric pathogens (pneumococci, *Haemophilus influenzae*, and *Streptococcus agalactiae*) were recovered in PEDS Plus than in 6A. Coagulase-negative staphylococci and *H. influenzae* were detected significantly earlier in PEDS Plus ($P < 0.05$ and < 0.01, respectively). When the eight species of the family *Enterobacteriaceae* isolated were considered together, recovery in PEDS Plus was better than in 6A ($P < 0.05$). For 66 of the 143 isolates from patients known to be on antimicrobial therapy at the time blood was drawn, PEDS Plus was superior to 6A. In 45 cases, organisms were isolated from PEDS Plus only ($P < 0.001$) and in 21 cases they were isolated from PEDS Plus before 6A ($P < 0.01$). PEDS Plus broth aids diagnosis of pediatric bacteremia by increasing recovery of etiologic agents and decreasing the time required for detection.

Rapid detection of pediatric bacteremia has become increasingly important. Sophisticated medical interventions not only improve survival of infants and children with previously fatal conditions but also predispose them to the risk of blood-borne infection. These children often receive prophylactic antimicrobial therapy which may hinder diagnosis of bacteremia. In addition, the antimicrobial susceptibilities of certain common neonatal pathogens are no longer predictable, and therefore, appropriate treatment may depend on their rapid isolation and susceptibility testing in the laboratory.

Culturing blood from pediatric patients differs from performing adult blood cultures, because except with older children, only small volumes of blood are available. Although the number of microorganisms per milliliter of blood is usually higher in the pediatric population, ca. 27% of specimens may contain fewer than 10 CFU/ml (2). In addition, an important pediatric pathogen, *Neisseria meningitidis*, may be inhibited by the concentrations of sodium polyanetholesulfonate (SPS) included in broth culture media (5) and added enrichment may be needed to improve isolation of *Haemophilus influenzae* and streptococci.

We evaluated a new pediatric blood culture medium (PEDS Plus) for use with the BACTEC NR nonradiometric blood culture system (Becton Dickinson Diagnostic Instrument Systems, Towson, Md.). The medium was designed to overcome factors that might prevent isolation of pediatric pathogens. Organism recovery and speed of recovery were compared with those in BACTEC NR-6A (6A) medium, the standard aerobic broth used with the BACTEC NR system.

(This report was presented in part at the 29th Interscience Conference on Antimicrobial Agents and Chemotherapy, Houston, Tex., 17 to 20 September 1989.)

MATERIALS AND METHODS

Blood samples. Blood was drawn from pediatric patients in three hospital centers: The University of Chicago Wyler Children's Hospital, Children's Hospital of Wisconsin, and Texas Children's Hospital. Equivalent blood volumes from a single draw were inoculated into each of two broths: conventional 6A aerobic broth and the study bottle, PEDS Plus broth (described below). Equivalent volumes were determined by either examining the fluid levels of the bottle pair or assessing the weights of bottles before and after inoculation with blood. The maximum volume of blood per bottle was stipulated as 5 ml; however, most bottles contained 1 to 1.5 ml.

PEDS Plus medium. PEDS Plus broth is composed of a conventional 6A soybean-casein digest broth base modified by inclusion of nonionic absorbing resins, cationic exchange resins, and 0.06% yeast extract. In addition, the concentration of SPS is decreased from 0.035 to 0.025% and the broth volume is 20 ml rather than the 30-ml volume in the 6A bottle.

Sample processing. Both the 6A and PEDS Plus bottles were incubated at 35 to 37°C for up to 7 days with shaking during the first 48 h. The vials were examined visually and tested twice on days 1, 2, and 3 and once daily thereafter. In accordance with the manufacturer's instructions, PEDS Plus

* Corresponding author.

cultures were considered presumptively positive when they showed visual changes, when the growth value (GV) reading reached ≥20, or when the GV increased by ≥5 between consecutive readings. For 6A cultures, visual changes and the routine GV of 30 and a GV increase of ≥10 were used.

Gram-stained smears and subcultures were prepared from positive cultures, and identification and susceptibility tests were done by standard methods (9). The negative bottle from any set in which only the companion bottle became positive received a terminal subculture. Whenever possible, when a clinically significant organism was isolated, information about whether or not the patient was receiving antimicrobial therapy at the time the blood was drawn was obtained.

Compliant sets. Compliant sets were those in which equivalent volumes of blood had been inoculated into both bottles and incubated for up to 7 days under the conditions just described. Cultures not meeting these specifications were considered noncompliant and excluded from the study.

Statistical analysis. Isolation rates were evaluated by the McNemar modification of the χ^2 test, and times to positivity were evaluated by the paired t test (7).

RESULTS

Among 4,581 compliant sets, 452 (10%) were positive. From these sets, 497 organisms were isolated, of which 289 from 159 patients were considered clinically significant. The criteria for clinical significance included one or more of the following: (i) both bottles of a set positive, (ii) multiple positive specimens from the same patient, (iii) organism identity (rare or unusual contaminant), and (iv) physician consultation. Of the clinically significant organisms, 181 (63%) were isolated in both bottles, 75 (26%) were isolated in the PEDS Plus bottle only, and 33 (11%) were isolated in the 6A bottle only ($P < 0.001$) (Table 1).

Staphylococcus aureus was isolated significantly more often ($P < 0.01$) in the PEDS Plus bottle than in the 6A bottle. More strains of coagulase-negative staphylococci and the pediatric pathogens (*Streptococcus pneumoniae*, *H. influenzae*, and *Streptococcus agalactiae*) were recovered in PEDS Plus than in 6A; however, the differences were not statistically significant. Although recoveries of individual members of the family *Enterobacteriaceae* were not different in the two media, when the eight enteric species were considered together, recovery was better in PEDS Plus than in 6A ($P < 0.05$).

Among the 181 organisms isolated from both bottles, 129 (71%) were detected at the same time, 39 (22%) were detected earlier in the PEDS Plus bottle, and 13 (7%) were detected earlier in the 6A bottle ($P < 0.005$) (Table 2). Except for coagulase-negative staphylococci ($P < 0.05$) and *H. influenzae* ($P < 0.01$), however, the differences in times to detection for individual organisms were not significant.

When detection times differed, PEDS Plus detected almost twice as many organisms ca. 0.5 day earlier (time between two consecutive readings on days 1 to 4) than 6A: PEDS Plus, 16 cultures; 6A, 9 cultures. Of the remaining isolates, PEDS Plus detected 23 isolates 1 to 4.5 days earlier but 6A detected only 4 isolates earlier (Table 2, footnotes *c* and *d*).

The effect of patient therapy on organism recovery is shown in Table 3. From 143 specimens drawn from patients receiving antimicrobial therapy, 82 isolates (58%) were recovered from both bottles but 45 (31%) were recovered in PEDS Plus only, compared with 16 (11%) recovered in 6A only ($P < 0.001$).

TABLE 1. Microorganisms isolated from BACTEC PEDS Plus and 6A media

Microorganism(s)	Total no. of isolates	No. of isolates positive in:		
		PEDS Plus and 6A	PEDS Plus only	6A only
Coagulase-negative staphylococci[a]	67	53	11	3
Staphylococcus aureus	56	33	18[b]	5
Candida spp.[c]	28	20	3	5
Streptococcus pneumoniae	27	12	9	6
Haemophilus influenzae	19	13	4	2
Escherichia coli	19	9	8	2
Klebsiella pneumoniae	10	7	3	0
Other enteric bacteria[d]	18	6	7	5
Enterococcus faecalis	15	11	3	1
Pseudomonas aeruginosa	9	5	3	1
Streptococcus agalactiae	6	2	3	1
Acinetobacter anitratus	5	3	1	1
Streptococcus pyogenes	3	2	0	1
Viridans group streptococci[e]	3	2	1	0
Brucella melitensis	1	1	0	0
Other[f]	3	2	1	0
Total	289	181	75[g]	33

[a] *Staphylococcus epidermidis*, $n = 54$; other coagulase-negative staphylococci or unidentified species, $n = 13$.
[b] $P < 0.01$.
[c] *Candida albicans*, $n = 14$; *Candida parapsilosis*, $n = 14$.
[d] *Enterobacter aerogenes*, $n = 6$; *Enterobacter cloacae*, $n = 5$; *Salmonella* sp. group B, $n = 3$; *Salmonella* sp. group D, $n = 1$; *Proteus mirabilis*, $n = 2$; *Serratia marcescens*, $n = 1$.
[e] *Streptococcus mitis*, $n = 2$; *Streptococcus sanguis* II, $n = 1$.
[f] Beta-hemolytic streptococcus not group A or B, $n = 1$; *Enterococcus casseliflavus*, $n = 1$; *Mycobacterium* sp. (unidentified), $n = 1$.
[g] $P < 0.001$.

A comparison of the times to detection for the 82 isolates that grew in both PEDS Plus and 6A bottles when patients were receiving antimicrobial therapy is illustrated in Table 4. Again, significantly more organisms were detected earlier in the PEDS Plus bottle than in the 6A bottle ($P < 0.01$). Among patients not on therapy at the time blood was drawn, the earlier recovery in PEDS Plus (Table 4) was significant ($P < 0.01$), although the numbers of isolates recovered from the culture media were not significantly different (Table 3).

As is usual for pediatric blood cultures (2), the contamination rate during this study was high. Contaminants were isolated from 4.2% of all specimens and 45% of positive cultures. The contamination rates among the three institutions were approximately the same regardless of whether specimens were drawn by a venipuncture team or by physicians and nurses. In addition, contaminants were not isolated more often from one bottle than from the other (51% from PEDS Plus, 49% from 6A).

DISCUSSION

Bacteremia is a major cause of infant mortality; therefore, early detection of blood-borne pathogens in pediatric patients is imperative. Although a direct lysis plating method is available for pediatric blood cultures and some conventional systems can be obtained as pediatric-size tubes or broths (2), most clinical laboratories rely on a single system for both adult and pediatric blood cultures. In this study, we examined a new BACTEC NR medium, PEDS Plus, formulated for pediatric blood cultures. The medium was significantly

TABLE 2. Comparison of detection times for clinically significant microorganisms isolated from both BACTEC PEDS Plus and 6A media

Microorganism(s)	Total no. of isolates	No. of isolates detected by:		
		PEDS Plus and 6A at same time	PEDS Plus earlier	6A earlier
Coagulase-negative staphylococci	53	41	10[a]	2
Staphylococcus aureus	33	20	6	7
Candida spp.	20	13	5	2
Streptococcus pneumoniae	12	10	1	1
Haemophilus influenzae	13	6	7[b]	0
Escherichia coli	9	7	2	0
Klebsiella pneumoniae	7	4	2	1
Other enteric bacteria	6	4	2	0
Enterococcus faecalis	11	10	1	0
Pseudomonas aeruginosa	5	4	1	0
Streptococcus agalactiae	2	2	0	0
Acinetobacter anitratus	3	3	0	0
Streptococcus pyogenes	2	2	0	0
Viridans group streptococci	2	2	0	0
Brucella melitensis	1	0	1	0
Other	2	1	1	0
Total	181	129	39[c]	13[d]

[a] Significantly decreased time to detection ($P < 0.05$ by paired t test).
[b] $P < 0.01$.
[c] $P < 0.005$. Detected 0.5 day earlier, $n = 16$; 1 day earlier, $n = 12$; 1.5 days earlier, $n = 5$; 2 to 4.5 days earlier, $n = 6$.
[d] Detected 0.5 day earlier, $n = 9$; 1 day earlier, $n = 3$; 2.5 days earlier, $n = 1$.

better than routine BACTEC 6A medium in both recovery and time to detection of clinically significant microorganisms.

The advantages of the new medium were most evident when the isolates were examined in aggregate. Among individual organisms, only *S. aureus* was isolated significantly more often from PEDS Plus than from 6A, although the enteric organisms as a group also were isolated significantly more often in PEDS Plus. No significant differences were seen between PEDS Plus and 6A for recovery of the more "classic" pediatric pathogens (*S. pneumoniae, H. influenzae*, and *S. agalactiae*), but they were isolated more often from PEDS Plus than from 6A. Both coagulase-negative staphylococci and *H. influenzae* were recovered earlier in PEDS Plus significantly more often than in 6A. Thus, for the important nosocomial and iatrogenic pathogens coagulase-negative staphylococci, *S. aureus*, and enteric gram-negative bacilli, as well as the pediatric pathogen *H. influenzae*, PEDS Plus medium was superior to 6A. For

TABLE 3. Effect of antimicrobial therapy on recovery of microorganisms from BACTEC PEDS Plus and 6A media

Antimicrobial therapy	No. of microorganisms isolated from:			*P* value
	PEDS Plus and 6A	PEDS Plus only	6A only	
Yes	82	45	16	<0.001
No	89	28	17	NS[a]
Unknown	10	2	0	NS
Total	181	75	33	

[a] NS, Not significant.

TABLE 4. Effect of antimicrobial therapy on detection times for organisms from BACTEC PEDS Plus and 6A media

Antimicrobial therapy	No. of isolates detected by:		
	PEDS Plus and 6A at same time	PEDS Plus earlier	6A earlier
Yes	55	21[a]	6
No	64	18[a]	7
Unknown	10	0	0
Total	129	39	13

[a] Significantly decreased time to detection ($P < 0.01$ by paired t test).

Candida spp., the only fungal pathogens recovered during the study, the two media performed the same.

Differences in the compositions of the PEDS Plus bottle and the 6A bottle include additional yeast extract, lower broth volume, addition of resins, and a decreased SPS concentration. The effect of each difference cannot be determined separately. Inclusion of resins was important for increased recovery of bacterial pathogens from the 143 patients on antimicrobial therapy, because 127 (89%) of the organisms were recovered from PEDS Plus compared with 98 (69%) from 6A (Table 3). In addition, the times required for detection of organisms in the blood of patients not receiving therapy, as well as those on therapy, were decreased significantly in the resin-containing medium. Except for noting whether patients with candidemia were receiving an antifungal agent (amphotericin B in all instances), we did not examine whether the patients' bacterial isolates were susceptible to their antimicrobial therapy.

Most studies of BACTEC broths containing resins have been done with the radiometric system, and the results have been conflicting (1, 11). In a review of the effects of resins, Washington and Ilstrup (13) concluded that resins are most advantageous in small-volume blood culture systems, such as BACTEC, but less beneficial in large-volume blood culture systems. They noted that resins appear to improve detection of *S. aureus* but may inhibit recovery of gram-negative bacilli. In our study, the significantly better recovery of *S. aureus* from PEDS Plus broth than from 6A supports the former observation but more gram-negative enteric bacilli and *Pseudomonas aeruginosa* isolates were isolated from PEDS Plus medium than from 6A. Thus, no adverse effect on recovery of gram-negative bacilli was seen with PEDS Plus broth.

A recent evaluation of BACTEC NR-16A aerobic resin-containing medium for use with the NR-660 system was conducted with patients in an intensive care unit who were receiving antimicrobial therapy (3). Both organism recovery and mean time to detection were significantly better with NR-16A medium than with conventional 6A broth, illustrating that the benefits of resins noted with the radiometric detection system apply also to the nonradiometric system.

For blood cultures in general, the recommended blood-to-broth volume ratio is 1:10 to 1:20 (13), primarily to dilute inhibitory substances present in blood from patients. The effects of higher dilutions (8, 13) are less important than those of lower dilutions. The maximum recommended blood volume for the 20-ml-containing PEDS Plus bottle is 5 ml (1:4 dilution), but in our experience, 1 to 1.5 ml was usually inoculated (1:20 to 1:13 dilution).

In addition to its anticoagulant activity, SPS has anticomplementary and antiphagocytic activities and inactivates

some antimicrobial agents (4). Although these properties are beneficial for increasing microbial isolation from blood cultures, SPS is inhibitory to certain fastidious bacteria, including the pathogenic neisseriae, *N. gonorrhoeae* and *N. meningitidis* (5). The latter organism is recovered from ca. 3% of children with clinically significant bacteremia (2). For this reason, the concentration of SPS in PEDS Plus was decreased from the 0.035% concentration present in 6A medium to 0.025%. No meningococci were isolated during this study; therefore, it was not possible to determine the effect of this modification on their recovery. Before this study was begun, meningococci had been recovered from 6A broth in all three of our laboratories; therefore, our failure to isolate it at all was more likely related to the absence of patients with meningococcemia than to inhibition by both media. A concern with media containing low SPS concentrations is that enteric gram-negative bacilli might be inhibited by the greater availability of complement (12). This effect was not seen here because organisms of the family *Enterobacteriaceae* were isolated more often in PEDS Plus than in 6A and, generally, in a shorter time in PEDS Plus.

During this study, the positive threshold GV and the delta GV suggested by the manufacturer for detection of positive PEDS Plus cultures were lower than those for 6A. Examination of the data indicated that if the same GVs had been used, an additional five positive specimens would have been detected at the same time by both media but no additional positive cultures would have been detected earlier by 6A. The low delta GV of ≥ 5 used for PEDS Plus occasionally led to falsely positive interpretations of machine readings. This range may need to be increased as a labor-saving measure if further studies show that speed of detection would not be sacrificed.

Because two aerobic broths were examined in this study, no data about anaerobe recovery are available. PEDS Plus broth is not available as an anaerobic formulation. At one center participating in this study (Wisconsin), approximately 1,000 anaerobic NR-7A broths were run in parallel with the 6A and PEDS Plus broths but no anaerobes were recovered. During the past 4 years at another center (Chicago), among approximately 24,000 pediatric blood cultures, clinically significant anaerobes were isolated from only 17 patients (data not shown). The reported incidence of anaerobes in the pediatric population is low: 3% of isolates in one summary of three studies (2) and approximately 1.6 isolates per year over 18 years in a review of anaerobic bacteremia in a neonatal intensive care unit (10). The incidence may be as high as 6% of isolates, however, depending on the patient population (6). Considering the usually low yield of anaerobes and the increased expense of resin-containing bottles, an anaerobic PEDS Plus broth may not be warranted.

The cost of the PEDS Plus broth is approximately twice that of 6A ($4.20 versus $1.85 list), an important financial consideration in many laboratories. Because the advantages of this medium were most evident in patients on antimicrobial therapy, some laboratory workers may wish to substitute PEDS Plus broth for 6A only when culturing blood from patients receiving antimicrobial agents or to restrict its use to inpatients. Nevertheless, in comparison with 6A, we found faster organism recovery in PEDS Plus, as well as recovery only in PEDS Plus, even when patients were not on therapy, which may indicate its use for all aerobic pediatric blood cultures. An anaerobic culture medium (i.e., NR-7A) could be used in conjunction. From the results of this study, we conclude that PEDS Plus is an important aid in the diagnosis of pediatric bacteremia.

ACKNOWLEDGMENTS

This study was supported in part by a grant from Becton Dickinson Diagnostic Instrument Systems.

We are grateful to Ann Graziosi of Becton Dickinson Diagnostic Instrument Systems for many helpful suggestions and expert assistance throughout the course of this study. We also thank David Dennen for valuable help with statistical computations.

REFERENCES

1. **Appelbaum, P. C., D. G. Beckwith, J. R. DiPersio, J. W. Dyke, J. F. Salventi, and L. L. Stone.** 1983. Enhanced detection of bacteremia with a new BACTEC resin blood culture medium. J. Clin. Microbiol. **17**:48–51.
2. **Campos, J. M.** 1989. Detection of bloodstream infections in children. Eur. J. Clin. Microbiol. Infect. Dis. **8**:815–824.
3. **Courcol, R. J., A. V. Durocher, M. Roussel-Delvallez, A. Fruchart, and G. R. Martin.** 1988. Routine evaluation of BACTEC NR-16A and NR-17A media. J. Clin. Microbiol. **26**:1619–1622.
4. **Edberg, S. C., C. J. Bottenbley, and K. Gam.** 1976. Use of sodium polyanethol sulfonate to selectively inhibit aminoglycoside and polymyxin antibiotics in a rapid blood level antibiotic assay. Antimicrob. Agents Chemother. **9**:414–417.
5. **Eng, J., and E. Holten.** 1977. Gelatin neutralization of the inhibitory effect of sodium polyanethol sulfonate on *Neisseria meningitidis* in blood culture media. J. Clin. Microbiol. **6**:1–3.
6. **Favor, L. F., M. Tarpay, and R. Blackstock.** 1979. Septicemia in children with cancer. South. Med. J. **72**:132–135.
7. **Ilstrup, D. M.** 1978. Statistical methods employed in the study of blood culture media. p. 31–39. *In* J. A. Washington II (ed.), The detection of septicemia. CRC Press, West Palm Beach, Fla.
8. **Kennaugh, J. K., W. W. Gregory, K. R. Powell, and J. O. Hendley.** 1984. The effect of dilution during culture on detection of low concentrations of bacteria in blood. Pediatr. Infect. Dis. **3**:317–318.
9. **Lennette, E. H., A. Balows, W. J. Hausler, Jr., and H. J. Shadomy (ed.).** 1985. Manual of clinical microbiology, 4th ed. American Society for Microbiology, Washington, D.C.
10. **Noel, G. J., D. A. Laufer, and P. J. Edelson.** 1988. Anaerobic bacteremia in a neonatal intensive care unit: an eighteen-year experience. Pediatr. Infect. Dis. J. **7**:858–862.
11. **Strand, C. L., A. Hickman, J. K. Bryant, and K. Sutton.** 1983. Evaluation of the BACTEC resin blood-culture medium. Am. J. Clin. Pathol. **80**:706–710.
12. **Stratton, C. W., M. P. Weinstein, S. Mirrett, J. Paisley, B. A. Lauer, and L. B. Reller.** 1988. Controlled evaluation of blood culture medium containing gelatin and V-factor-analog for detection of septicemia in children. J. Clin. Microbiol. **26**:747–749.
13. **Washington, J. A., II, and D. M. Ilstrup.** 1986. Blood cultures: issues and controversies. Rev. Infect. Dis. **8**:792–802.