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Clinical Comparison of the Isolator 1.5 Microbial Tube and the BACTEC Radiometric System for Detection of Bacteremia in Children

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The Isolator 1.5 microbial tube (E. I. du Pont de Nemours & Co., Inc., Wilmington, Del.) was compared with the BACTEC radiometric detection system (Johnston Laboratories, Inc., Cockeysville, Md.) for the detection of bacteremia in children. The Isolator 1.5 is a blood culture system designed for small volumes of blood (0.5 to 1.5 ml). The method involves lysis of the cells of the patient and the direct plating of the entire blood lysate on agar media appropriate for the growth of fastidious microorganisms. Of 1,500 paired samples inoculated into the two systems, 68 were positive for 73 clinically significant organisms. The Isolator 1.5 recovered 81% of the positive cultures compared with 84% recovered by the BACTEC system. When paired blood samples with disproportionate volumes were excluded, the Isolator 1.5 detected 3% more positive cultures. More isolates of *Streptococcus pneumoniae* and *Neisseria meningitidis* were recovered by the Isolator 1.5, whereas *Haemophilus influenzae* was recovered most often in the BACTEC bottles ($P > 0.1$). The contamination rates were 8.7 and 3.1% for the Isolator 1.5 and the BACTEC system, respectively. In cultures positive by both systems, the mean time to detection was 4.1 h faster with the Isolator 1.5. The mean time to obtain isolated colonies was 26.6 h faster with the Isolator 1.5. These data indicate the potential value of the Isolator 1.5 microbial tube as a simple, rapid, and sensitive method for the detection of bacteremia in children.

In 1976, Dorn et al. (3, 5) described a new technique of lysis-centrifugation to recover microorganisms from the blood more efficiently. This technique, marketed as the Isolator blood culture system (E. I. du Pont de Nemours & Co., Wilmington, Del.), has been shown to be a sensitive and rapid method to detect and isolate bacteria and fungi from adult patients, when 7.5- or 10-ml samples are cultured (2, 4, 8, 9).

Pediatric patients present a particular challenge because the volume of blood drawn is small and the bacteria that cause disease in this population have fastidious growth requirements. *Streptococcus pneumoniae*, *Haemophilus influenzae*, and *Neisseria meningitidis* are common pathogens in children, as well as the more easily cultured staphylococci, streptococci, and enteric gram-negative rods.

The Isolator 1.5 microbial tube is a device for culturing blood volumes ≤1.5 ml. Unlike the larger volume Isolator, the Isolator 1.5 does not require centrifugation to concentrate the microorganisms present. The entire blood sample is lysed and directly plated to the appropriate media.

This study describes the results of a comparative evaluation of 1,500 paired blood samples drawn from a pediatric population and cultured with the Isolator 1.5 and a two-bottle BACTEC system (Johnston Laboratories, Inc., Cockeysville, Md.). Data were collected to compare the overall recovery of microorganisms, the time for detection and isolation, and the contamination rate for each system.

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MATERIALS AND METHODS

Collection of samples. The Isolator 1.5 microbial tube and the routine blood culture set of BACTEC aerobic (6B) and anaerobic (7C/7D) bottles were available on all patient floors, the emergency room, and outpatient clinics.

House staff and medical students obtained the blood samples from pediatric patients after preparation of the skin with a 2% iodine solution and 70% isopropyl alcohol. Before use, the tops of both BACTEC bottles and the Isolator 1.5 microbial tube were disinfected.

At the time of collection, blood was distributed by needle and syringe to the aerobic and anaerobic BACTEC bottles and to the Isolator 1.5. If less than 1 ml was drawn from the patient, only the BACTEC system was inoculated. A minimum of 0.5 ml to a maximum of 1.5 ml of blood was inoculated into the Isolator 1.5, which was inverted four or five times to mix the blood with the contents of the tube.

The blood culture set was labeled appropriately, including the time that the culture was performed. Blood cultures drawn between the hours of 7 a.m. and 10 p.m. were processed as soon as possible in the microbiology laboratory. Specimens submitted after 10 p.m. were held at 35°C until the next morning.

Laboratory processing. Only those paired samples that met the criteria of volume (≥0.5 ml in the Isolator 1.5) and a maximum hold time (≤12 h) were processed for this study. The aerobic BACTEC bottles were analyzed for microbial growth by radiometric detection twice on day 1 and once on days 2, 3, and 7. The anaerobic bottles were read on days 2, 3, 4, and 7. After a 24-h incubation, all aerobic bottles were routinely subcultured to chocolate agar plates, which were held at 35°C in 5% CO_2 for 72 h. When a positive culture was detected by either system, its paired sample was automatically examined for the presence of microorganisms, regardless of the routine reading schedule.

The contents of the Isolator 1.5 were mixed vigorously on a Vortex Genie (Scientific Products Div., McGaw Park, Ill.).

The stopper was disinfected with povidone-iodine and was allowed to dry. At least two plates were inoculated for each culture: one chocolate agar plate and one Schaedler agar plate. Additional chocolate agar plates were added for culture volumes greater than 0.6 ml.

No centrifugation was required before plating the lysate. A needle (20 gauge by 1.5 in. [ca. 3.81 cm]) with a 3-ml syringe was inserted into the center of the stopper at an angle. The contents of the tube were removed by gradually tilting the tube until as much fluid as possible was withdrawn. The lysate was dispensed in a straight line across the surface of each plate, avoiding the edge of the agar. A maximum of 0.3 ml was cultured on each plate.

With a sterile wire loop, the inoculum was distributed evenly on the plate, streaking in three directions. Plates were incubated upright for several hours and then inverted for the remaining time. Chocolate agar plates were incubated at 35°C in 5% CO_2 for 4 days. Schaedler blood agar plates were incubated at 35°C in anaerobic GasPak jars (BBL Microbiology Systems, Cockeysville, Md.) for 4 days.

Aerobic plates were examined twice a day for the first 48 h and once a day thereafter. Anaerobic plates were incubated 48 h before their first daily inspection. The presence of bacterial growth was noted each time. If colonies were observed within the area of streaking, their number was recorded and a gram strain was made. Biochemical identification was done according to conventional techniques.

Definition of contaminants. Only those colonies that grew within the lines of streaking were considered as possible pathogens or as contaminants. Colonies outside the streaked area of inoculation were considered to be handling contaminants and not associated with the blood specimens. Colonies outside the area of streaking were noted but not further identified.

A contaminant was defined as an organism that was not considered to be responsible for the illness of the patient. The relevance of the microbial isolate was based on the number of times it was recovered from multiple blood cultures, the identification of the microorganism, the clinical condition of the patient, the response of the primary care physicians to the positive blood culture report, and the response of the patient to antimicrobial therapy.

RESULTS

During the 5-month study, 1,500 paired blood samples were submitted that met the criteria of appropriate volume (0.5 to 1.5 ml) and hold time (≤12 h) in the Isolator 1.5 microbial tube. Sixty-eight cultures were positive for microorganisms considered to be clinically significant. Because the volume of blood drawn significantly influences the recovery of organisms (11), the data were examined in two ways. The first was the calculation of the total number of cultures with significant isolates regardless of the volume in the paired samples. By this analysis, the BACTEC system detected 57 of the 68 cultures (84%), and the Isolator 1.5 detected 55 (81%). In the second method, cultures which were positive only by one system were excluded if the volume in either system was more than twice that cultured in its paired sample. Analysis of these data showed that the BACTEC system recovered clinically significant isolates in 51 of 60 cultures (85%), whereas the Isolator 1.5 detected 53 positive cultures (88%).

Seventy-three clinically significant microorganisms were recovered during the study. Their identification and method of recovery are listed in Table 1. Statistical analysis with the McNemar chi-square test (12) indicated that both blood culture systems were comparable for detecting bacteremia. Neither method of data analysis had a P value ≤0.1

TABLE 1. Comparative recovery of clinically significant isolates from the Isolator 1.5 microbial tube and the BACTEC system

Microorganism	No. of isolates from:				
	All cultures[a]			Vol B:I 1.5 ≤ 2:1[ab]	
	Both systems	I 1.5 only	B only	I 1.5 only	B only
Staphylococcus aureus	7	0	0	0	0
Staphylococcus epidermidis	1	0	0	0	0
Staphylococcus hominis	3	0	0	0	0
Staphylococcus simulans	2	0	1	0	0
Streptococcus pyogenes	0	0	2	0	0
Streptococcus agalactiae	2	0	0	0	0
Streptococcus faecalis	2	0	1	0	0
Streptococcus pneumoniae	9	8	4	6	3
Escherichia coli	7	0	1	0	1
Salmonella enteritidis	4	1	1	1	0
Pseudomonas aeruginosa	3	0	1	0	1
Haemophilus influenzae	4	0	2	0	2
Neisseria meningitidis	2	2	0	2	0
Bacteroides fragilis	1	0	0	0	0
Clostridium septicum	2	0	0	0	0

[a] All differences in organism isolation were not statistically significant by the McNemar chi-square test ($P > 0.1$).

[b] I 1.5, Isolator 1.5 microbial tube; B, BACTEC blood culture broths.

Both blood culture systems positive. A total of 44 blood specimens were culture positive by both systems, and 49 clinically significant microorganisms were recovered. Three specimens were from patients with polymicrobic bacteremia. *Escherichia coli*, *Pseudomonas aeruginosa*, *Streptococcus faecalis*, and *Staphylococcus epidermidis* were recovered from one patient with a ruptured bowel. Although the Isolator 1.5 plates and the BACTEC broths were both positive at 17 h, the multiplicity of organisms was readily apparent by the Isolator 1.5 system. The colonies of the four organisms were available for biochemical identification 24 h before those of the BACTEC system.

The second patient with polymicrobic bacteremia had Burkitt's lymphoma and was neutropenic at the time that blood specimens were drawn. *Staphylococcus aureus* was recovered in the BACTEC system several days after the anaerobic bacterium *Clostridium septicum* was detected. Both organisms were isolated from two successive blood cultures.

In 24 cultures, the pathogens were detected by both systems simultaneously, and in 15 cultures the Isolator 1.5 detected the organism before the BACTEC system did. In five cultures microorganisms were detected earlier by BACTEC; in all five cases bacterial growth was present on the Isolator plates at the same time as, or earlier than, on the BACTEC subculture plates. When both systems were positive, the Isolator 1.5 was 4.1 h faster to detect a clinically significant positive culture, and isolated colonies were available 26.6 h sooner than with the BACTEC system.

Isolator 1.5 blood culture positive only. A total of 11 cultures from 11 patients were positive by the Isolator 1.5 only. Of these 11 cultures, 8 grew *Streptococcus pneumoniae*, two grew *N. meningitidis*, and one grew a *Salmonella enteritidis*. These bacteria commonly cause bacteremia in children. Table 2 presents the culture conditions when only

TABLE 2. Comparison of conditions when only the Isolator 1.5 microbial tube detected a clinically significant microorganism

Organism	CFU/ml	BACTEC vol	I 1.5[a] vol	I 1.5 hold time (h)	Time to detect positive (h)
N. meningitidis group C	1	1.0	1.0	5.0	23
N. meningitidis group B	36	2.0	1.0	1.2	17
S. pneumoniae type 9	187	1.8	1.0	3.6	27
S. pneumoniae type 9	>1,000	2.5	1.0	9.5	34
S. pneumoniae type 6	475	3.0	0.8	8.0	36
S. pneumoniae type 14	37	1.0	0.9	0.8	24
S. pneumoniae type 14	921	1.1	1.0	6.8	17
S. pneumoniae type 19	>1,000	1.5	1.0	8.0	18
S. pneumoniae type 14	>1,000	0.55	0.6	10.0	34
S. pneumoniae type 19	>1,000	1.0	0.6	8.3	31
Salmonella group B	132	0.9	0.5	6.5	35

[a] I 1.5, Isolator 1.5 microbial tube.

the lysis-direct plating system was positive. None of the patients in this category were receiving antibiotics at the time that the blood was drawn.

The salmonella bacteremia was initially detected by only the Isolator 1.5, but subsequent cultures were positive by both systems. None of the other 11 patients had repeat cultures that were positive.

The pneumococccal isolates not recovered by the BACTEC system were a variety of serotypes, and the CFU per milliliter ranged from 37 to >1,000. In four of these cultures, the aerobic BACTEC broth was radiometrically positive, but the gram stain revealed gram-negative cocci, and the pneumococcal organisms were nonviable on subculture. However, pneumococcal antigen could be detected in these BACTEC broths by latex or coagglutination.

BACTEC blood culture positive only. A total of 13 cultures from 11 patients were positive for microorganisms detected by the BACTEC system only. The isolates included seven streptococci, one staphylococcus, and five gram-negative rods, of which two were *H. influenzae* type b, an important pediatric pathogen. Culture conditions when only the BACTEC system detected the pathogen are listed in Table 3. Of the 11 patients, 7 (64%) were receiving appropriate antimicrobial therapy at the time of culture. Of the four pneumococcal isolates missed by the Isolator 1.5, three originated from the same patient, who was taking cefoxitin and gentamicin when the cultures were taken.

Hold time and volume. The duration of time in the Isolator 1.5 tube before plating did not appear to adversely affect the recovery of microorganisms. The mean hold time in the Isolator 1.5 was 4 h, whether microorganisms were recovered or missed by this system.

The volume of blood in the BACTEC system was approximately twice that inoculated into the Isolator 1.5. The ratio of blood in the BACTEC set compared with that in the Isolator 1.5 tube averaged more than 2:1 when only BACTEC or both systems detected pathogenic microorganisms. When only the Isolator 1.5 detected the microbial pathogens, the ratio of BACTEC volume to Isolator volume was 1.7:1.

Detection of nonpathogens. The contamination rate was 8.7% for the Isolator 1.5 microbial tube compared with 3.1% for the BACTEC bottles. In general, contaminants detected by both the Isolator 1.5 and the BACTEC system were the same genus and species (Table 4). The majority of nonpathogenic isolates were coagulase-negative staphylococci with common skin commensals and normal respiratory flora comprising the remaining isolates. Environmental contaminants, such as *Bacillus* spp. and molds, were recovered more often from cultures processed by the lysis-direct plating technique than from the closed BACTEC bottles.

DISCUSSION

In this clinical evaluation of 1,500 paired samples, the Isolator 1.5 microbial tube and the BACTEC radiometric system were comparable in their ability to detect bacteremia in children. There was no statistically significant difference in the overall number or type of organisms recovered by either system alone. Twice the volume of blood was cultured in a BACTEC two-bottle set compared with the Isolator 1.5 microbial tube. It is not unexpected, therefore, that 3% more clinically significant positive cultures were detected by the BACTEC system. When paired samples with disproportionate volumes (>2:1) were excluded, the recovery of clinically significant isolates was greater in the Isolator 1.5 microbial tube (88 versus 85%).

McLaughlin et al. (9) compared the Isolator lysis-centrifugation technique and the BACTEC radiometric technique. Eighty-seven percent of the clinically significant cultures were detected by the Isolator compared with 70% by the BACTEC system. In that study, none of the patients sampled was less than 6 years of age, and none of the important pediatric bacteria, *Streptococcus pneumoniae*, *H. influenzae*, *N. meningitidis*, and *Salmonella enteritidis*, were isolated.

During the present study, 21 isolates of *Streptococcus pneumoniae*, 6 of *H. influenzae* type b, 4 of *N. meningitidis*, and 6 of *Salmonella enteritidis* were recovered. *Streptococcus pneumoniae* was recovered by the Isolator 1.5 from eight patients whose paired BACTEC sample was culture negative. In four of these cases, the aerobic BACTEC bottle was radiometrically positive, but the organism was nonviable on subculture. It was demonstrated previously (1) that *Streptococcus pneumoniae* rapidly undergoes autolysis in the aerobic BACTEC medium.

Comparing only culture-positive samples, the Isolator 1.5 performed better than the BACTEC broths in the recovery of *Streptococcus pneumoniae*. This difference was diminished, however, if radiometric detection was used as the criterion for a positive culture. Radiometrically positive but culture-negative BACTEC broths were tested for the presence of pneumococcal antigen by latex or coagglutination. This was a reliable and sensitive technique to identify the organism present. Confirmation of a nonviable culture by antigen detection methods is important because other streptococci can hemolyze the blood of the patient and give the characteristic chocolatized appearance that occurs with the growth and autolysis of *Streptococcus pneumoniae*.

TABLE 3. Comparison of conditions when only BACTEC detected a positive culture

Organism	Therapy	BACTEC vol	I 1.5[a] vol	I 1.5 hold time (h)	No. of BACTEC bottles positive
Escherichia coli	Ampicillin and gentamicin	0.7	0.5	12.0	1
Pseudomonas aeruginosa	Nafcillin, tobramycin, and cefsulodin	1.0	0.8	6.5	2
Streptococcus faecalis	None	2.0	0.5	7.2	2
Salmonella group C_1	Ampicillin	2.0	0.5	1.6	1
Staphylococcus simulans	Cephalothin	1.5	0.5	2.0	2
Streptococcus pneumoniae type 9	None	2.0	1.1	0.6	1
Streptococcus pneumoniae type 23[b]	Cefoxitin and gentamicin	3.0	1.2	0.5	1
Streptococcus pneumoniae type 23[b]	Cefoxitin and gentamicin	2.3	1.4	3.5	1
Streptococcus pneumoniae type 23[b]	Cefoxitin and gentamicin	2.0	1.0	5.5	2
Haemophilus influenzae type b	None	2.0	1.1	2.3	2
Haemophilus influenzae type b	None	1.0	0.5	1.8	1
Group A streptococcus	None	2.0	0.5	2.0	1
Group A streptococcus	None	1.5	0.5	4.0	1

[a] I 1.5, Isolator 1.5 microbial tube.
[b] Multiple positive cultures taken from the same patient.

Four cultures of *Streptococcus pneumoniae* were missed by the Isolator 1.5. Three of the four were drawn from one patient who was receiving antibiotics at the time. In three of the four samples negative by the lysis-direct plating technique, only one BACTEC bottle in each set was positive, suggesting a low number of CFU per milliliter. This is known to occur in pneumococcal bacteremia (10). The presence of a low number of organisms per milliliter may explain why neither blood culture system detected all the patients with pneumococcal sepsis. This phenomenon may well occur with other microorganisms that cause pediatric bacteremia. In 8 of the 13 cultures, when the BACTEC system alone detected a clinically significant microorganism, only one bottle of the two-bottle set was positive. Of these 13 cultures, 3 were positive within the first 24 h of collection, however, the average time to detect a positive for this group of samples was 50.4 h. These data again suggest that the number of CFU per milliliter of blood may have been low, and therefore, the recovery of these pathogens in the Isolator 1.5 would have been less likely due to the smaller sample of blood inoculated.

TABLE 4. Comparison of isolates considered to be contaminants

Microorganism	No. of isolates		
	Both positive	Only I 1.5[a] positive	Only BACTEC positive
Coagulase-negative staphylococci	10	75	14
Bacillus spp.	0	14	6
Propionibacterium spp. or diphtheroids	0	9	1
Mixed respiratory flora	7	7	1
Fungal isolates	0	5	0
Viridans streptococci	0	2	4
Acinetobacter spp.	0	1	0
Mixed fecal flora	1	0	0
Micrococcus spp.	0	0	1
Coagulase-negative staphylococci and *Acinetobacter* spp.	0	0	1

[a] I 1.5, Isolator 1.5 microbial tube.

Since some blood specimens were held for several hours at 35°C before processing, no statement can be made regarding the absolute number of organisms per milliliter in cultures detected by the Isolator 1.5 only.

Two patients with meningococcal bacteremia were culture positive only in the Isolator system. The BACTEC system contains 0.025% sodium polyanetholesulfonate (SPS), which has been found to inhibit the growth of some *Neisseria* spp. (7). The Isolator 1.5 microbial tube also contains SPS, but the inhibitory effect of SPS is neutralized when a sufficient quantity of free hemoglobin is present (6). Because the erythrocytes of the patient are lysed in the Isolator 1.5, this technique may provide the concentration of hemoglobin required to protect SPS-sensitive isolates.

All aerobic BACTEC broths were routinely subcultured after 24 h to recover any organisms that had not yet become radiometrically positive. Neither of the two isolates of *N. meningitidis* was detected radiometrically or was recovered by blind subculture. Two additional cultures positive with meningococcal organisms were detected by both the BACTEC system and the Isolator 1.5. Both cultures were detected sooner (6 and 18 h) by the Isolator 1.5. The Isolator 1.5 microbial tube appears to be a better system for recovering meningococci from the blood.

Of six cultures positive for *H. influenzae* type b, two were not detected by the Isolator 1.5. There was not a prolonged hold time in the tube before plating. The volume in the Isolator 1.5 was ≤1 ml, which may explain the nonrecovery of the organism.

Although the contamination rate of the Isolator 1.5 was 8.7%, most nonpathogens were recovered as a single colony growing on only one plate of the set. The blood samples were plated on a clean laboratory bench open to the environment. The contamination rate might have been reduced with the use of a laminar flow hood for specimen processing.

Many of the organisms considered to be nonpathogens were cultured from outpatients. These children were immunocompetent and did not have intravenous lines. Therefore, in these patients it was not a difficult clinical decision to classify the coagulase-negative staphylococci as nonpathogens. In the intensive care and intermediate nurseries, coagulase-negative staphylococci must be seriously con-

sidered as pathogens. In our experience, when an infant was infected with this organism, the same species of staphylococcus was isolated from multiple blood cultures, as well as the intravenous or the central line.

Most organisms considered to be nonpathogens were recovered on day 3 or 4 of incubation, whereas 93% of the pathogens were detected within the first 48 h. The pathogens which were recovered on day 3 or 4 were usually anaerobic bacteria or from patients who had been receiving antibiotics active against the microorganism found.

In conclusion, the Isolator 1.5 microbial tube was comparable to the BACTEC system (81 versus 84%, respectively) in recovering clinically significant microorganisms found in pediatric blood cultures. When paired samples with disproportionate volumes (>2:1) were excluded, the recovery of clinically significant isolates was greater in the Isolator 1.5 microbial tube (88 versus 85%). There was no statistically significant difference in the number or type of organisms isolated by either system. Positive cultures were detected 4.1 h faster, and isolated colonies were available 26.6 h sooner with the Isolator 1.5. Although biochemical tests are available that use the bacterial pellet directly from the blood culture, most of these are for the identification of members of the family *Enterobacteriaceae*. Direct biochemical testing is less reliable for the gram-positive cocci, oxidase-positive gram-negative rods, and anaerobes. The advantage of having isolated colonies on a plate is appreciated most in cases of polymicrobic bacteremia.

The Isolator 1.5 is adaptable to most microbiological laboratories, since it does not require complicated technical skills or capital expenditures. The Isolator 1.5 should be processed as soon as possible to obtain a reliable estimate of CFU per milliliter and to avoid the deleterious action of antimicrobial agents. It appears to be a practical alternative for detecting bacteremia in pediatric patients where larger volumes of blood may not be safely or readily obtained.

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