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Clinical Laboratory Comparison of Lysis-Centrifugation and BACTEC Radiometric Blood Culture Techniques

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The lysis-centrifugation technique (ISOLATOR; E. I. du Pont de Nemours & Co., Wilmington, Del.) and the radiometric blood culture technique (BACTEC; Johnston Laboratories, Inc., Cockeysville, Md.) were compared on 1,000 blood cultures. A total of 16 ml of blood was distributed: 8 ml into an ISOLATOR 7.5 microbial tube and 4 ml each into BACTEC 7C and 8B bottles. The concentrate from the ISOLATOR tubes was inoculated under a laminar-flow hood onto two sheep blood agar plates (one incubated in CO_2 and one incubated anaerobically), one chocolate agar plate, and one brain heart infusion agar plate. Of 91 blood specimens obtained that yielded clinically significant organisms, 52 were positive by both systems, 27 were positive by the ISOLATOR system only, and 12 were positive by the BACTEC system only. From the positive blood specimens, 97 clinically significant organisms were isolated: 57 by both systems, 27 by the ISOLATOR system only, and 13 by the BACTEC system only. Of the 57 organisms detected by both systems, 28 were detected simultaneously, 13 were detected earlier by the ISOLATOR system, and 16 were detected earlier by the BACTEC system. Isolated colonies were obtained earlier by the ISOLATOR system in 40 cases and by the BACTEC system in 5 cases. Organisms determined to be contaminants by thorough chart review were isolated from 138 ISOLATOR tubes. In 98 instances, these were represented by one colony of *Staphylococcus epidermidis*, alpha-hemolytic streptococci, or diphtheroids. The ability to determine CFU per milliliter with the ISOLATOR system did not help differentiate clinically significant organisms from contaminants.

The ISOLATOR (E. I. du Pont de Nemours & Co., Wilmington, Del.) blood culture system involves the lysis of blood, subsequent centrifugation, aspiration of the concentrate, and inoculation directly onto appropriate media. Dorn et al. reported an increase in the isolation rate of *Staphylococcus aureus, Pseudomonas aeruginosa*, and yeasts as compared with the isolation rate in conventional supplemented peptone broth (1). In addition, the time required for isolation was shortened by the lysis-centrifugation (L-C) method. Kiehn et al. (T. E. Kiehn, F. F. Edwards, and D. Armstrong, Abstr. Annu. Meet. Am. Soc. Microbiol. 1983, C82, p. 325) reported that significantly more *Escherichia coli* and yeast isolates were recovered by the L-C technique and that more *Pseudomonas* isolates were recovered in a conventional blood culture technique. Henry et al. (N. K. Henry, C. A. McLimans, A. J. Wright, W. R. Wilson, R. L. Thompson, and J. A. Washington, Program Abstr. Intersci. Conf. Antimicrob. Agents Chemother. 22nd, Miami Beach, Fla., abstr. no. 402, 1982) reported a statistically significant increase in the frequency of isolation of *S. aureus* and *Candida* spp. and a decreased time to detection of these organisms and *Pseudomonas* spp. compared with the frequency and detection time for a conventional system. Fojtasek et al. (M. J. Fojtasek, T. M. Abbott, J. M. Matsen, and M. T. Kelly, Abstr. Annu. Meet. Am. Soc. Microbiol. 1982, C129, p. 293) also compared a conventional two-bottle system with the L-C technique and found that of 89 positive cultures, 92% were detected by the L-C technique and only 78% were detected by the conventional method and that the isolated colonies were found 24 h earlier by the L-C method. Gerlach et al. (E. H. Gerlach, R. C. Weller, and R. J. Taylor, Abstr. Annu. Meet. Am. Soc. Microbiol. 1982, C127, p. 292), however, reported that, with extensive subculturing, a three-bottle system recovered 92% of 104 positive cultures, compared with 76% recovered by the L-C system. Isenberg compared the L-C, the BACTEC radiometric, and the broth culture approaches on 996 blood samples. Significant

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organisms were isolated from 9.7% of the blood specimens by the L-C technique and from 6.4% by the radiometric technique (2).

The present study was undertaken to compare the yield of microorganisms from 1,000 blood specimens by the L-C technique (ISOLATOR) and the radiometric blood culture technique (BACTEC; Johnston Laboratories, Inc., Cockeysville, Md.). The study was also designed to compare the time to detect and isolate organisms by the two systems and the contamination rate of each system.

MATERIALS AND METHODS

Blood collection, inoculation, and incubation. Blood specimens included in this study were collected only by members of the phlebotomy team after appropriate disinfection of the venipuncture site. Children under the age of 6 years were not included. A total of 16 ml of blood was drawn: 8 ml was inoculated into an ISOLATOR 7.5 microbial tube, and 4 ml each was inoculated into two BACTEC vials (8B hypertonic aerobic and 7C anaerobic). BACTEC bottles were incubated at 35°C within 2 h of blood collection. Bottles were examined visually daily, and aerobic bottles were sampled on the BACTEC 460 on days 1, 2, 3, and 7, and anaerobic bottles were sampled on days 2, 4, and 7. For the purposes of this study, all BACTEC bottles were subcultured at 24 h. Broth from the 8B bottles was subcultured to a chocolate agar plate incubated aerobically, and broth from the 7C bottles was subcultured to a chocolate agar plate incubated aerobically and to a blood agar plate incubated anaerobically. These plates were held for 48 h.

All ISOLATOR tubes were processed within 2 h of blood collection. After a thorough mixing of blood with tube ingredients, the tube was centrifuged (3,000 × *g*) for 30 min. The tube was vented, and the supernatant was removed and discarded. The tube was placed on a Super-Mixer (Lab-Line Instruments, Inc., Melrose Park, Ill.), the yellow stopper was disinfected, and the 1.5 ml of concentrate was aspirated. Approximately 0.4 ml of concentrate was used to inoculate each of four plates: 2 sheep blood agar plates, one incubated for 4 days aerobically in 5% CO_2 and one incubated for 6 days anaerobically in Gas-Pak jars (BBL Microbiology Systems, Cockeysville, Md.); one chocolate agar plate incubated for 4 days aerobically in 5% CO_2; and one brain heart infusion agar plate incubated for 8 days aerobically. Plates were inoculated by the drop, tilt, and streak technique. Plates were examined daily to determine the time for isolation and the number of CFU per milliliter.

Chart review. A thorough chart review was performed on patients whose blood yielded low numbers of apparent pathogens or organisms normally considered contaminants. All but five charts were available for review. Information from laboratory records and communication with physicians treating the patients provided some data in the cases for which complete records were unavailable. Specific data obtained from each chart and systematically recorded included: (i) other positive cultures of blood or other sites; (ii) the presence of predisposing factors and sources of infections; (iii) antibiotic therapy, especially at the time of culture; (iv) the presence of fever greater than 100°F (37.8°C) or chills; (v) leukocyte count elevation; (vi) type and time of surgical procedures; and (vii) evidence of pneumonia, urinary tract infection, wound infection, endocarditis, abdominal sources of infection, or other infection based on clinical and laboratory data, including cultures.

Based on these data and on the record of other isolates from the patient, each isolate was classified as a contaminant, transient bacteremia, or septicemia. For purposes of this analysis, the term nonpathogens refers to organisms usually considered to be nonpathogenic except in patients with predisposing factors, such as immunosuppression or indwelling foreign bodies. Isolates considered to represent contaminants included: (i) nonpathogens which could not be correlated with either a source for the organism or clinical signs of infection or sepsis, (ii) nonpathogens without a source in patients with infection due to pathogens, and (iii) pathogens without a source in patients with no clinical signs of infection or sepsis consistent with that organism.

Isolates classified as transient bacteremia included: (i) nonpathogens in patients with a clearly identifiable source and route into the bloodstream but without either clinical signs indicative of serious or persistent infection or signs of bloodstream infection by that organism and (ii) pathogens in patients with a clearly identifiable source but without either signs of serious or persistent infection or signs of bloodstream infection by that organism. Isolates considered to represent septicemia included: (i) nonpathogens in patients with an identifiable source and route into the bloodstream, with clinical signs of persistent or serious infection consistent with that organism, and with no other identifiable cause for the clinical manifestation of infection and (ii) pathogens in patients with clinical signs and symptoms consistent with infection of the bloodstream by that organism.

RESULTS

One thousand blood specimens were cultured by the ISOLATOR and BACTEC techniques over a 5-month period. Ninety-one yielded organisms that were clinically significant. From these positive blood specimens, 97 clinically significant organisms were isolated: 57 by both systems, 27 by the ISOLATOR system only, and 13 by the BACTEC system only. Table 1 lists the number of organisms isolated by the two systems.

Thirteen *Staphylococcus epidermidis* isolates, determined to be significant by our criteria, were cultured from 10 patients by the ISOLATOR technique only. The BACTEC media did not grow any *S. epidermidis* strains not isolated by the ISOLATOR technique. The BACTEC technique yielded five anaerobes not detected by the ISOLATOR technique: a *Bacteroides* sp., three *Clostridium perfringens* isolates (from two patients), and a *Clostridium* sp. The ISOLATOR technique yielded three yeast isolates not detected in BACTEC media: a *Candida albicans* isolate, a *Candida glabrata* isolate, and a *Crypto-*

TABLE 1. Number of clinically significant organisms isolated by one or both techniques

Organisms	No. of isolates detected by:		
	BACTEC only	ISOLATOR only	BACTEC and ISOLATOR
Staphylococcus aureus	3	3	13
Staphylococcus epidermidis	0	13	10
Group D streptococci	1	1	5
Beta-hemolytic streptococci, not group A, B, D	1	1	0
Streptococcus mutans	0	0	3
Alpha-hemolytic streptococci	0	1	0
Haemophilus parainfluenzae	1	0	0
Escherichia coli	1	1	5
Enterobacter agglomerans	0	1	0
Enterobacter aerogenes	0	1	2
Enterobacter cloacae	0	0	2
Serratia marcescens	0	0	2
Klebsiella oxytoca	0	0	1
Pseudomonas aeruginosa	1	0	4
Acinetobacter calcoaceticus	0	0	2
Aeromonas hydrophila	0	0	2
Bacteroides fragilis	0	0	4
Bacteroides spp.	1	0	0
Clostridium perfringens	3	0	0
Clostridium spp.	1	0	0
Lactobacillus casei	0	2	0
Cryptococcus neoformans	0	1	0
Candida albicans	0	1	0
Candida krusei	0	0	1
Candida parapsilosis	0	0	1
Candida glabrata	0	1	0

coccus neoformans isolate. Each of these grew initially only on the brain heart infusion agar incubated at 30°C, and in each case, only a single colony grew.

Among the 27 significant organisms detected by the ISOLATOR system only, 24 were present in the original blood specimens at concentrations of <1 CFU/ml. With the exception of five *S. epidermidis*, one *C. albicans*, one *C. neoformans*, and one *C. glabrata* isolates, the organisms detected by the ISOLATOR system only were from patients receiving antibiotics active against the isolates. Of 57 isolates detected by both the BACTEC and the ISOLATOR systems, 17 were present at <1 CFU/ml, as determined by the ISOLATOR method. Table 2 shows the number of strains of clinically significant organisms isolated by both systems and the number of CFU per milliliter as determined by the ISOLATOR system.

During the course of this study, nine blood cultures from a total of four patients yielded mixtures of organisms. All nine of these polymicrobial bacteremic episodes were detected by the BACTEC system. Three of these episodes (in two patients) were missed by the ISOLATOR system. Organisms undetected by the ISOLATOR system were a *Clostridium* sp., a *C. perfringens* strain, an *S. aureus* strain, and a *Pseudomonas* sp. The BACTEC system did not detect one *Lactobacillus casei* strain.

No difference in the time of detection of organisms was observed between the two systems. The ISOLATOR system detected 13 organisms sooner than did the BACTEC system, the BACTEC system detected 16 sooner than did the ISOLATOR system, and 28 were detected simultaneously. Forty-six organisms yielded isolated colonies more rapidly by the ISOLATOR system, and in 36 instances, isolated colonies were obtained ≥24 h sooner by the ISOLATOR system.

A total of 154 contaminants were isolated; 138 (13.8%) were isolated by the ISOLATOR technique. Of these, 98 (71%) were single-colony isolates of *S. epidermidis*, alpha-hemolytic streptococci, or diphtheroids. A single colony on one of four plates inoculated is equivalent to a concentration of 0.125 CFU/ml. The contamination rate for the radiometric system was 1.1%.

DISCUSSION

This study confirms the study of Dorn et al. (1) that the ISOLATOR technique is a more sensitive method of detecting low-level bacteremias. Among the significant organisms isolated by the ISOLATOR technique alone, 24 of 27 had <1 CFU/ml, and only 17 of 57 (30%) of those

TABLE 2. Number of clinically significant organisms isolated in cultures positive by both the ISOLATOR and the BACTEC systems

Organisms	No. of isolates[a]			
	<1	1–10	11–100	>100
Staphylococcus aureus	4	2	5	2
Staphylococcus epidermidis	4	3	3	
Group D streptococci	2	2	1	
Streptococcus mutans			3	
Escherichia coli	1	4		
Enterobacter aerogenes		1	1	
Enterobacter cloacae	2			
Serratia marcescens				2
Klebsiella oxytoca		1		
Pseudomonas aeruginosa	4			
Acinetobacter calcoaceticus		1	1	
Aeromonas hydrophila		2		
Bacteroides fragilis		4		
Candida krusei		1		
Candida parapsilosis		1		

[a] Numbers refer to numbers of strains of each organism isolated per the indicated number of CFU per milliliter.

[b] CFU per milliliter as determined by the ISOLATOR system.

detected by both the ISOLATOR and the radiometric techniques had <1 CFU/ml. Dorn et al. (1) and Kiehn et al. (T. E. Kiehn, C. Capitolo, A. De La Cruz, and D. Armstrong, Abstr. Annu. Meet. Am. Soc. Microbiol. 1982, C128, p. 292) state that quantitation is helpful in distinguishing skin contaminants from bacteremia. Although it is true in this study that organisms isolated at >1 CFU/ml represent pathogens, those isolated at <1 CFU/ml did not necessarily represent contaminants and cannot be disregarded. The high contamination rate (13.8%) and the inability to distinguish contaminants from pathogens on the basis of quantitation limit the usefulness of the ISOLATOR technique. Contamination with the ISOLATOR technique persisted throughout the study despite measures to control it: the introduction of cotton-plugged venting needles, the inoculation of culture plates in a laminar-flow hood, the inclusion of blood specimens collected only by trained phlebotomists, and the use of the same two trained technologists for processing the Isolator tubes.

The ISOLATOR technique, as compared with conventional bottle systems, is a more sensitive means to detect yeasts. Bille et al. (J. Bille, L. Stockman, and G. D. Roberts, Program Abstr. Intersci. Conf. Antimicrob. Agents Chemother. 22nd, Miami Beach, Fla., abstr. no. 407, 1982) reported a more rapid detection time and an increased yield of yeasts and molds with L-C (93.6% by L-C versus 55.3% by biphasic brain heart infusion). In our study, three yeast isolates, including a *C. neoformans* isolate, detected by the ISOLATOR method went undetected by the BACTEC technique. The *C. neoformans* strain was isolated from a renal transplant patient seen as an outpatient for a routine postsurgical follow-up.

A total of nine anaerobes were detected in this study. Four of these were isolated by both systems. Five anaerobes were not isolated by the ISOLATOR technique but were cultured in the BACTEC media. This difference in the isolation rate of anaerobes may be attributed to the fact that the 0.4 ml of concentrate subcultured and incubated anaerobically represents only 2 ml of blood, whereas the anaerobic BACTEC medium is inoculated with 4 ml of blood. Four of these anaerobes were *Clostridium* spp. Dorn et al. (1) showed that of 10 *Clostridium* strains isolated, 6 were isolated by both the ISOLATOR technique and the bottle method they were using and 4 were detected by the bottle method only. In a study of 7,000 blood cultures, Kiehn et al. found that of 33 anaerobes detected, 9 were detected by both the ISOLATOR system and their two-bottle broth system, 13 were detected by broth only, and 11 were detected by the ISOLATOR system only (Kiehn et al., Abstr. Annu. Meet. Am. Soc. Microbiol. 1983, C82, p. 325). Of these isolates, 10 were *Clostridium* spp. Five were detected by the ISOLATOR system only, three were detected by broth only, and two were detected by both methods (T. E. Kiehn, personal communication). *S. aureus* and *Pseudomonas* strains have been reported to be detected more frequently by the ISOLATOR system, but our data show that this is not the case in comparison with the BACTEC system. Of 19 significant *S. aureus* isolates, 16 were detected by each of the two systems. Five *P. aeruginosa* strains were isolated, one by the BACTEC system only and four by both the ISOLATOR and BACTEC systems.

The large number of clinically significant *S. epidermidis* strains (13 from 10 patients) detected only by the ISOLATOR technique has not previously been reported, although Zierdt (3) did demonstrate an increased isolation of *S. epidermidis* by lysis-filtration in comparison with a conventional blood culture system. Of 2,004 patient blood cultures processed by conventional and lysis-filtration techniques, *S. epidermidis* was isolated from 2.4% in commercial brain heart infusion bottles and from 11.6% by lysis-filtration. *S. epidermidis* is usually dismissed as

a skin or laboratory contaminant, and only a thorough chart review demonstrated the significance of the 13 isolates reported here. Seven of the isolates represented bacteremia probably due to an indwelling intravenous catheter, and six (from three patients) were clearly associated with subacute endocarditis or a shunt infection. Quantitation was not helpful in differentiating isolates associated with sepsis or bacteremia, for the number of CFU per milliliter of blood for these *S. epidermidis* isolates was either 0.125 or 0.25, with only one exception. These counts are equivalent to the isolation of one or two colonies on only one of the four plates inoculated.

Our results are consistent with those of Isenberg, who also compared isolation by the ISOLATOR and BACTEC techniques (2). He reported an increased yield with the ISOLATOR technique in comparison with the radiometric technique. Our results show that 87% (84 of 97) of total significant organisms were isolated by the ISOLATOR technique and 72% (70 of 97) were isolated by the radiometric method.

The time required to process the ISOLATOR tube prohibits the use of the system for routine blood cultures at Hartford Hospital, where 19,000 blood cultures are submitted to our laboratory annually. The cost per test is also a consideration. This cost is, of course, based on test volume, the use of blind or terminal subcultures, and workload units assigned to blood cultures. The College of American Pathologists assigns 4.8 U to each BACTEC bottle and 8.8 U to each ISOLATOR blood culture. At Hartford Hospital, where 1.15 U is assigned to each BACTEC blood culture, a blind subculture is not performed, and 6.21 U is assigned to each ISOLATOR blood culture, the cost per blood culture with the BACTEC system is $4.98 and that with the ISOLATOR system is $9.03. Despite the increased cost, in cases of suspected fungemia or suspected *S. epidermidis* infections, the ISOLATOR system does offer an advantage over the the BACTEC system.

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