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Large-Scale Clinical Comparison of the Lysis-Centrifugation and Radiometric Systems for Blood Culture

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The Isolator 10 lysis-centrifugation blood culture system (E. I. du Pont de Nemours & Co., Inc., Wilmington, Del.) was compared with the BACTEC radiometric method (Johnston Laboratories, Inc., Towson, Md.) with 6B and 7D broth media for the recovery of bacteria and yeasts. From 11,000 blood cultures, 1,174 clinically significant organisms were isolated. The Isolator system recovered significantly more total organisms, members of the family *Enterobacteriaceae*, *Staphylococcus* spp., and yeasts. The BACTEC system recovered significantly more *Pseudomonas* spp., *Streptococcus* spp., and anaerobes. Of the Isolator colony counts, 87% measured less than 11 CFU/ml of blood. Organisms, on an average, were detected the same day from each of the two culture systems. Only 13 of the 975 BACTEC isolates (0.01%) were recovered by subculture of growth-index-negative bottles, and 12 of the 13 were detected in another broth blood culture taken within 24 h. Contaminants were recovered from 4.8% of the Isolator 10 and 2.3% of the BACTEC cultures.

A lysis-centrifugation (Isolator) blood culture system (E. I. du Pont de Nemours & Co., Inc., Wilmington, Del.) has been compared with nonradiometric broth-bottle systems (1–4, 7, 8, 11) and the BACTEC radiometric system (Johnston Laboratories, Inc., Towson, Md.) (6, 13). Each of these methods has shown advantages for the recovery of certain groups of bacteria and yeasts. In 1983, at Memorial Sloan-Kettering Cancer Center, the Isolator 7.5-ml system was compared with a conventional two-bottle Columbia broth system (11). The Isolator system recovered more total organisms, *Escherichia coli*, and *Candida* spp., whereas the broth system detected significantly more *Streptococcus* spp. and low-level *Pseudomonas aeruginosa* bacteremias. In a continuing effort to define optimal blood culture methods for the recovery of bacteria and yeasts, we compared the Isolator 10-ml blood culture system to the BACTEC radiometric system, a broth method not previously used in our laboratory.

MATERIALS AND METHODS

From February through November 1984, 11,000 blood cultures from adult patients at Memorial Sloan-Kettering Cancer Center were collected by the venipuncture team. Equal amounts of blood ranging from 3 to 8 ml per tube, average 6.5 ml, were collected sequentially from the same draw into an Isolator 10 tube and a VACUTAINER tube (100 by 16 mm) containing 5.95 mg of sodium polyanetholesulfonate (Becton Dickinson Vacutainer Systems, Rutherford, N.J.). The order of collection of the two tubes was changed each week. After collection, the two tubes were inverted several times for proper mixing and then transported to the microbiology laboratory where they were processed within 2 h of blood collection. Blood samples from the VACUTAINER tubes were divided equally into an aerobic 6B and an anaerobic 7D BACTEC bottle. All broth cultures were incubated at 35°C for 5 days. The 6B broth bottles were agitated for the first 2 days, and they were visually and radiometrically examined three times on days 1 and 2 and once a day for the remaining 3 days. The 7D broth bottles were examined visually and radiometrically once each day. Samples from all negative broth bottles, both 6B and 7D, were inoculated onto chocolate agar as a blind subculture procedure. The chocolate agar plate was incubated at 35°C in 5% CO_2 in air for 48 h. After lysis and centrifugation, the concentrated sediment from the Isolator 10 tube was inoculated in equal portions onto two Columbia sheep blood agar plates and two chocolate agar plates. One blood agar plate was incubated anaerobically at 35°C for 5 days, and the other three agar plates were incubated at 35°C in 5% CO_2 in air for 5 days. All Isolator plates were inspected daily for visible colonies.

When growth of an organism was detected in the radiometric broth system, the bottle in which growth was first seen and the time to first detection were recorded. When growth of an organism was detected on the plates inoculated from the lysis-centrifugation system, the time to first detection and the number of colonies per plate were recorded.

The following organisms were considered contaminants unless multiple bottles or plates within a culture set or multiple culture sets were positive or a review of other laboratory and clinical information indicated that the organisms were clinically significant: *Bacillus* spp., diphtheroids, *Micrococcus* spp., *Neisseria* spp., *Proprionibacterium* spp., *Staphylococcus* spp., and viridans group streptococci.

Statistical analysis. The asymptotic chi-square test of McNemar (14) as applied to comparative blood culture methods by Ilstrup (5) was used for statistical analysis. Chi-square values of ≥ 3.84, which defined P values of ≤ 0.05, were considered significant.

RESULTS

From 11,000 cultures, 1,174 isolates were considered to be clinically significant. Table 1 lists significant organisms isolated and the blood culture system from which the organisms were isolated. Significantly more total isolates ($P < 0.025$), members of the family *Enterobacteriaceae* ($P < 0.01$),

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TABLE 1. Number of significant isolates from Isolator 10 and BACTEC systems

Organism	No. of isolates	No. of patients	No. of isolates from[a]:			P value (system)[b]
			Isolator and BACTEC	Isolator only	BACTEC only	
Enterobacteriaceae	424	222	299	80	45	<0.005 (I)
Citrobacter spp.	14	15	10	3	1	
Enterobacter spp.	52	25	36	9	7	
Escherichia coli	141	74	94	27	20	
Klebsiella spp.	154	77	120	22	12	
Morganella spp.	4	2	4	0	0	
Proteus spp.	30	15	14	13	3	<0.025 (I)
Salmonella spp.	11	2	9	2	0	
Serratia spp.	17	11	11	4	2	
Yersinia spp.	1	1	1	0	0	
Staphylococcus spp.	293	90	245	37	11	<0.001 (I)
Coagulase-negative staphylococci	128	26	113	11	4	
S. aureus	165	64	132	26	7	<0.001 (I)
Streptococcus spp.	130	67	86	15	29	<0.05 (B)
S. pneumoniae	17	11	13	0	4	<0.05 (B)
Streptococcus groups A, B, C, D, F, and G	62	26	42	12	8	
Viridans group streptococci	51	30	31	3	17	<0.005 (B)
Yeasts	112	43	57	41	14	<0.001 (I)
Candida spp.	81	32	50	19	12	
Cryptococcus spp.	14	6	3	10	1	<0.01 (I)
Rhodotorula spp.	10	2	2	7	1	<0.05 (I)
Torulopsis spp.	7	3	2	5	0	<0.05 (I)
Pseudomonas spp.	81	39	44	7	30	<0.001 (B)
P. aeruginosa	69	36	33	6	30	<0.001 (B)
Pseudomonas spp.	12	3	11	1	0	
Anaerobes	74	42	38	9	27	<0.005 (B)
Bacteroides spp.	50	21	33	5	12	
Clostridium spp.	15	14	5	3	7	
Other anaerobes	9	7	0	1	8	<0.025 (B)
Other bacteria	60	27	50	10	0	<0.005 (I)
Acinetobacter spp.	16	8	12	4	0	<0.05 (I)
Aeromonas spp.	3	2	3	0	0	
Bacillus spp.	5	3	4	1	0	
Corynebacterium spp.	14	8	13	1	0	
Listeria spp.	21	5	17	4	0	<0.05 (I)
Vibrio cholerae-non O1	1	1	1	0	0	

[a] Of the 1,174 isolates, 819 (70%) were isolated with both Isolator and BACTEC systems, 199 (17%) were isolated by the Isolator system only, and 156 (13%) were isolated with the BACTEC system only ($P < 0.025$).

[b] I, Isolator; B, BACTEC.

Staphylococcus spp. ($P < 0.001$), and yeasts ($P < 0.001$) were isolated from the Isolator 10 system than from the BACTEC system. The BACTEC system recovered significantly more isolates of *Streptococcus* spp. ($P < 0.05$), *Pseudomonas* spp. ($P < 0.001$), and anaerobic organisms ($P < 0.005$) than did the Isolator system. Of the 1,174 isolates, 281 (23.9%) were from cultures with more than one significant organism. From these polymicrobic cultures, 53 organisms were recovered only from the Isolator 10 system, 38 organisms were recovered only from the BACTEC system; this difference was not significant.

Table 2 shows the number of CFU per milliliter of blood for clinically significant organisms detected by the Isolator 10 system. Of the organisms detected only by the Isolator system, the colony counts of 133 (69%) were less than 1 CFU/ml of blood, and the colony counts of 174 (87%) were less than 11 CFU/ml of blood. Of the 25 organisms with colony counts greater than 10 CFU/ml of blood and detected only by the Isolator system, 14 (56%) were yeasts; seven of the yeasts were *Cryptococcus neoformans*, and six were *Rhodotorula rubra*.

The mean time to first detection of growth of all organisms was 2.0 days with the Isolator 10 system and 1.9 days with the BACTEC system. Organisms were usually detected the same day with each of the two culture systems, except that yeasts were detected an average of 1 day earlier with the Isolator 10 system (2 days versus 3 days), and anaerobes were detected an average of 1 day earlier with the BACTEC system (2 days versus 3 days).

Of the 975 organisms recovered from the BACTEC system, 13 were detected only by subculture of growth-index-negative bottles, and 5 of the 13 were yeasts. All of the organisms but one, a *P. aeruginosa* isolate, were recovered from at least one other broth blood culture taken within 24 h from that patient.

Contaminants were recovered from 4.8% of the Isolator 10 cultures and 2.3% of the BACTEC cultures.

DISCUSSION

In this study, the Isolator system recovered more total isolates, members of the *Enterobacteriaceae*, *Staphylococcus* spp., and yeasts, whereas the BACTEC broth system recovered more *Streptococcus* spp., *Pseudomonas* spp., and anaerobes. Although these overall results by groups of organisms are similar to those observed when the Isolator 7.5-ml system was compared at our hospital (11), with

TABLE 2. Comparison of recovery of organisms by Isolator 10 colonies per milliliter of blood

Organism	No. of isolates when colony counts (CFU/ml) were:							
	<1		1–10		11–100		>100	
	Isolator and BACTEC	Isolator only	Isolator and BACTEC	Isolator only	Isolator and BACTEC	Isolator only	Isolator and BACTEC	Isolator only
Enterobacteriaceae	54	58	119	20	61	2	65	0
Staphylococcus spp.	48	24	63	10	37	3	97	0
Streptococcus spp.	23	12	21	1	19	1	23	1
Yeasts	14	21	21	6	10	5	12	9
Pseudomonas spp.	15	6	12	1	6	0	11	0
Anaerobes	7	7	13	1	9	0	9	1
Other bacteria	5	5	23	2	10	1	12	2

Columbia broth bottles for the recovery of bacteria and yeasts, it is important to note that the BACTEC system with 6B and 7D media is more sensitive than the Columbia broth bottles in recovering all groups of organisms except yeasts. When compared with Columbia broth, the BACTEC system increased the recovery of total organisms from 74 to 83%, members of the *Enterobacteriaceae* from 72 to 81%, *Staphylococcus* spp. from 77 to 87%, *Streptococcus* spp. from 87 to 89%, *Pseudomonas* spp. from 86 to 91%, and anaerobes from 67 to 88%. Total yeast recovery was 67 and 64%, respectively, not a significant difference, in the Columbia and BACTEC broth systems.

In the present study, increased Isolator sensitivity for the recovery of members of the *Enterobacteriaceae* and *Staphylococcus aureus* usually occurred in cultures with low colony counts; thus some cultures positive only by the Isolator system could be due to random inoculation of organisms only into the Isolator system. In addition, many of the patients in our hospital were receiving antibiotics when blood cultures were taken, and thus some organisms in low numbers may not survive and multiply in a blood-broth environment containing antibiotics. Inoculating agar media with Isolator sediment may act as a partial antimicrobial removal process. Standard antimicrobial removal systems, such as the BACTEC resin bottles and the Antimicrobial Removal Device (Marion Laboratories, Kansas City, Mo.), may increase recovery of organisms from broth-blood culture systems when our patients are receiving antibiotics.

In the Isolator-Columbia broth study, *Candida* spp., also at low colony counts, accounted for the increased recovery of yeast by Isolator, when compared with Columbia broth. In the present study, Isolator and BACTEC broth media detected *Candida* spp. fungemia equally well statistically. Agitation of the BACTEC 6B bottles and continued flushing of these bottles with a fresh aerobic gas mixture probably aided in the recovery of *Candida* spp., when compared with stationary conventional Columbia broth bottles. In the present study, increased recovery of yeasts by Isolator was partially due to isolation of *C. neoformans* and *R. rubra*, even at high colony counts. Both the presence of antifungal agents in the blood culture bottles and less than optimum media conditions in the BACTEC bottles for these yeasts may have contributed to the poor recovery from the broth bottles. However, only 50% of these fungemic patients were receiving antifungal agents at the time the blood culture was taken, and the yeasts from five of these eight patients grew in a BACTEC bottle in another culture, thus indicating that the yeast could grow in BACTEC media.

A significant number of *P. aeruginosa* isolates were detected only by the broth systems. Since these organisms did not grow on the Isolator plates, the number of organisms in these cultures was not determined. Toxicity of the Isolator lysing solution and antimicrobial agents or other factors in the blood of the patient that was possibly reduced by dilution in broth media, could have contributed to reduced detection by the Isolator system.

Most organisms were detected, on an average, at approximately the same time with both systems. We feel that that slight advantage one system may have with some organisms in this study may translate in other laboratories to different reporting times. Available culture information depends, in addition to detection times, on the hours of operation of the laboratory, the culture sampling times, and the use of rapid procedures for organism identification. Since no substantial differences in detection time were noted, a choice of system or systems based on these results should depend on other factors.

Subcultures of growth-index-negative BACTEC bottles did not significantly increase the recovery of organisms. Of the 13 isolates, 12 were recovered in another broth culture; thus, the blind subculture isolate contributed little to the clinical information. These findings are in contrast to those from a previous study (12) in which it was found that terminal subcultures of macroscopically negative Columbia broth bottles yielded clinically relevant information, particularly for yeast and *P. aeruginosa* septicemia.

Both of the blood culture systems used in this study appear useful in the detection of septicemia; however, each has advantages in the detection of specific groups of microorganisms. Use of the Isolator system also allows the laboratory to routinely report the number of CFU per milliliter of blood of the isolate. Isolator colony counts have been helpful in identifying catheter-associated septicemia (15) and assessing the treatment of *Mycobacterium avium* complex infections (9, 10, 16) and other bacterial and fungal infections (15). This study and previous reports of Isolator and broth evaluations (4, 6, 11) suggest that for optimum recovery of bacteria and yeasts, some combination of the two systems should be used. We presently use the Isolator 10 system, with all four agar plates incubated aerobically and an aerobic and anaerobic BACTEC bottle for each bottle culture.

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