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Controlled Evaluation of Supplemented Peptone and Bactec Blood Culture Broths for the Detection of Bacteremia and Fungemia

LARRY G. REIMER,[1,2]†* JUDY D. McDANIEL,[1] STANLEY MIRRETT,[3] L. BARTH RELLER,[3,4] AND WEN-LAN L. WANG[5,6]

Clinical Microbiology Laboratories, Medical Center,[1] and Departments of Medicine and Pathology, School of Medicine,[2] West Virginia University, Morgantown, West Virginia 26506; University of Colorado Hospital[3] and Departments of Medicine[4] and Pathology,[6] University of Colorado School of Medicine, Denver, Colorado 80262; and Veterans Administration Medical Center, Denver, Colorado 80220[5]

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Comparisons of conventional blood culture media with newer formulations of Bactec media for radiometric detection are lacking. Therefore, we compared the yield and speed of detection of clinically important microorganisms with supplemented peptone broth (SPB) and Bactec aerobic (6B) and anaerobic (7C or 7D) broths in 7,627 blood samples from adult patients. Acridine orange stains from SPB, radiometric readings from Bactec, and routine subcultures from all bottles were done at the same time intervals. Bactec grew more facultative gram-positive bacteria ($P < 0.02$), *Bacteroides* spp. ($P < 0.001$), and gram-negative anaerobes ($P < 0.001$). The two-bottle Bactec system required less time to detect *Staphylococcus aureus* ($P < 0.001$), facultative gram-positive bacteria ($P < 0.001$), *Escherichia coli* ($P < 0.02$), facultative gram-negative bacteria ($P < .001$), and fungi ($P < 0.001$). Overall, Bactec yielded 11% more microorganisms and detected bacteremia sooner in 18% of samples than did SPB. This advantage was not because of radiometric monitoring, since most positive Bactec bottles were detected macroscopically. SPB offered no advantage for any group of microorganisms. We conclude that Bactec 6B and 7C or 7D broths used as a unit are superior to a single bottle of SPB with an equal volume of blood for the detection of bacteremia and fungemia, and that Bactec's superiority is not due to the method of detection.

The Bactec radiometric blood culture system is now widely used as a method of positive blood culture detection. Few clinical studies have been done to compare Bactec with a conventional broth culture method; none has been published since 1975, although both aerobic and anaerobic Bactec media have been modified since then. In early trials, Bactec detected positive cultures earlier, and had a yield similar to conventional broth cultures (2, 5, 15, 17, 22, 24), but the volume of blood cultured in these studies was usually not the same; early Gram stains and subcultures of conventional bottles were not performed, so that slower recovery in the conventional system was predictable. Moreover, these studies were done before acridine orange staining had been recognized as a useful early screening method (12, 14, 23). Reports that radiometric culture bottles grew bacteria despite negative growth readings (1, 3, 5, 18) and that some radiometric culture bottles had high radiometric readings in the absence of bacteria (2–4, 17, 22, 25) further suggested that a controlled comparison with conventional media was needed.

The present study was done to compare the yield and speed of detection of bacteremia and fungemia in a two-bottle Bactec system (aerobic 6B and anaerobic 7C or 7D) containing 5 ml of blood each with one bottle of supplemented peptone broth (SPB) containing 10 ml of blood. A single SPB bottle was considered an adequate conventional system for comparison, since in an earlier trial SPB recovered all groups of microorganisms causing sepsis at least as well as did Trypticase soy broth (BBL Microbiology Systems, Cockeysville, Md.) (21). Monitoring of the SPB bottle included acridine orange staining at the same time intervals as radiometric monitoring of the Bactec bottles.

(This study was presented in part at the 84th Annual Meeting of the American Society for Microbiology, St. Louis, Mo. [L. G. Reimer, J. McDaniel, S. Mirrett, L. B. Reller, and W.-L. L. Wang, Abstr. Annu. Meet. Am. Soc. Microbiol. 1984, C1, p. 236].)

MATERIALS AND METHODS

Collection of samples. For a 14-month period, a 50-ml bottle of SPB with 0.03% sodium polyanetholesulfonate (BD Vacutainer Systems, Rutherford, N.J.), a 30-ml bottle of Bactec 6B aerobic medium, and a 30-ml bottle of Bactec 7C (West Virginia University) or 7D anaerobic medium (Denver Veterans Administration Medical Center) (Johnston Laboratories, Towson, Md.) were used for all blood cultures from adult patients at West Virginia University Hospital (WVU) and for 6 months at the Denver Veterans Administration Medical Center. Blood from each venipuncture was distributed by needle and syringe as follows: 10 ml of blood to a bottle with 40 ml of SPB, 5 ml of blood to a bottle with 30 ml of Bactec 6B, and 5 ml of blood to a bottle with 30 ml of either Bactec 7C or 7D medium. Thus, the volume of blood cultured for the one bottle of SPB and the two-bottle Bactec system was the same.

Processing of samples. Identical methods were used for processing the blood cultures in the clinical microbiology laboratories at both hospitals. A sterile open venting unit was attached to each SPB bottle (20). Bactec bottles were not vented, but complete gas exchange occurred with each radiometric analysis. Bactec 6B bottles were shaken for the first 24 to 48 h, and all bottles were incubated at 35°C in room air for 14 days. Cultures were examined for macroscopic growth twice daily for 3 days and daily for 4 days and then

* Corresponding author.
† Current address: Department of Pathology, Veterans Administration Medical Center, Salt Lake city, UT 84148.

incubated for an additional 7 days. All SPB bottles were examined by acridine orange staining twice daily for 2 days and on day 6 and were subcultured to chocolate agar in 5% CO_2 and to brucella blood agar supplemented with hemin and vitamin K_1 in an anaerobic jar on days 1 and 14, or when positive by visual inspection or acridine orange stain. All Bactec bottles were read radiometrically twice daily for 2 days and on days 6 and 14 and were subcultured to chocolate agar in 5% CO_2 and to supplemented brucella blood agar in an anaerobic jar on day 1 or when positive by visual inspection. Bactec 6B bottles with an increase of >10 growth index units between readings or a growth index of >30 on days 1 and 2 or >45 on day 6 and Bactec 7C or 7D bottles with an increase of >10 growth index units between readings or a growth index of >15 were examined by acridine orange staining and subcultured.

Data analysis. Paired comparisons of SPB and the Bactec two-bottle unit (either or both bottles positive) were done only on adequately filled (>80% of required volume of blood) bottles that grew microorganisms causing true bacteremia or fungemia. The criteria for volume standards and clinical assessments have been described previously (19, 24). Significance testing was done with the modified chi-square test of McNemar (13).

RESULTS

A total of 7,627 culture sets were obtained during the study: 4,826 (63%) sets had all bottles adequately filled. This low compliance was due largely to inadequate or nonfilling of the larger SPB bottle. Of 4,826 adequate blood culture sets, 689 (14.2%) were positive, including 466 (9.7%) that grew microorganisms causing illness, 220 (4.6%) that grew contaminants or organisms of unknown significance, and 3 (0.02%) that grew at least one pathogen and one contaminant. A total of 505 microorganisms associated with sepsis were isolated from 4,826 adequately filled sets (Table 1). Of these 505 clinically important microorganisms, 359 (71%) grew in both the SPB and in one or both Bactec bottles, and 253 (50%) were detected on the same day in both bottles (Table 2).

Aerobic and facultative gram-positive bacteria ($P < 0.02$), anaerobic gram-negative bacteria ($P < 0.001$), clinically important bacteria ($P < 0.001$), and microorganisms overall ($P < 0.001$) were recovered more often in the Bactec set (Table 1). *Staphylococcus aureus* and gram-negative anaerobes were most notably affected. No gram-negative anaerobe grew only in SPB, whereas 14 grew only in the Bactec bottles. Even for organisms that did not grow significantly more often, the trend for all organisms was in favor of Bactec. Overall, of the 505 microorganisms recovered, Bactec media grew 458 (91%), and SPB grew 406 (80%).

All groups of microorganisms were recovered faster in one or the other Bactec bottle than in the SPB bottle (Table 2). The differences are especially remarkable for *S. aureus* (21 faster in Bactec versus 3 in SPB) and for fungi (13 in Bactec versus 0 in SPB).

Bactec 7C and 7D media were not compared directly in the same specimens, but WVU used exclusively 7C broth and the Denver Veterans Administration Medical Center used exclusively 7D broth. At the Denver Veterans Administration Medical Center 5 ml of blood each in 6B and 7D broths yielded 165 microorganisms, 26 only in 6B and 24 only in 7D (P not significant). At WVU 5 ml of blood each in 6B and 7C broths yielded 412 microorganisms, 101 only in 6B, and 55 only in 7C ($P < 0.001$).

TABLE 1. Comparison of yield of clinically important bacteria and fungi from 10-ml samples of blood cultured in SPB and the Bactec set

Microorganism	No. of isolates recovered from:			P value
	SPB and Bactec	SPB only	Bactec only	
Aerobic and facultative bacteria	322	41	71	<0.005
Gram positive	139	14	30	<0.02
S. aureus	76	4	13	<0.05
S. epidermidis	6	0	0	NS[a]
Streptococci[b]	49	8	15	NS
Other[c]	8	2	2	NS
Gram negative	183	27	41	NS
E. coli	67	15	18	NS
Other *Enterobacteriaceae*	94	8	15	NS
P. aeruginosa	20	2	7	NS
Other[d]	2	2	1	NS
Anaerobic bacteria	18	2	21	<0.001
Gram positive[e]	4	2	7	NS
Gram negative[f]	14	0	14	<0.001
All bacteria	340	43	92	<0.001
Fungi[g]	19	4	7	NS
All microorganisms	359	47	99	<0.001

[a] NS, Not significant ($P \geq 0.05$).
[b] Four group A streptococci, 12 group B streptococci, 15 enterococci, 25 *Streptococcus pneumoniae*, 7 viridans streptococci, and 9 other streptococci.
[c] Two *Listeria* spp. and 10 diphtheroids.
[d] Three *Haemophilus influenzae* and two *Flavobacterium* spp.
[e] Four *Clostridium* spp., four peptococci, four peptostreptococci, and one *Propionibacterium* sp.
[f] Twenty-four *Bacteroides* spp., two *Fusobacterium* spp., and two *Veillonella* spp.
[g] Eleven *Candida albicans*, 5 *Candida parapsilosis*, 11 *Candida tropicalis*, 1 other *Candida* sp., *Cryptococcus neoformans*, and 1 other yeast.

The methods that first detected the clinically significant microorganisms from each broth are shown in Table 3. Most organisms were found by macroscopic inspection of bottles, but one-third of the isolates in SPB and Bactec 6B and half of the isolates in Bactec 7C or 7D were recognized first by another method. Microscopic examination with either acridine orange, Gram stain, or both detected 24% in SPB, 22% in Bactec 6B, and 31% in Bactec 7C or 7D. Radiometric readings with or without a positive acridine orange stain detected 31% in Bactec 6B and 44% in Bactec 7C or 7D. The radiometric reading alone, however, was positive first in only 9% of Bactec 6B and 13% of Bactec 7C or 7D bottles. Blind subcultures also accounted for a small number of earliest detections.

DISCUSSION

In this controlled evaluation, the Bactec two-bottle set recovered 11% more bacteria and fungi and did so faster in 18% of samples than a single bottle of SPB with equal volumes of blood in both. This result confirms the early results obtained with the Bactec system (2, 5, 15, 18, 22, 25). However, one might have expected that acridine orange staining would have matched the speed of radiometric detection, since other studies have shown the utility of early acridine orange screening in the rapid detection of bacteremia (9, 10, 12, 14, 23).

Two variables not controlled in this study may have helped favor the Bactec set. First, the aerobic Bactec bottle

alone was shaken for 24 h. Shaking improved the yield from vented Columbia broth (6) and has recently been suggested to increase the yield and decrease the time to detection of positive cultures in tryptic soy broth (B. Hawkins and E. M. Peterson, Abstr. Annu. Meet. Am. Soc. Microbiol. 1983, C90, p. 326). The effect of agitation on the performance of Bactec aerobic media and SPB have not been reported, but if the effect is similar, shaking would have provided an advantage for the aerobic Bactec bottle. Second, the ratio of blood to broth was 1:5 for the SPB bottle and 1:7 for the Bactec bottles. A greater dilution of blood in Bactec media could provide an advantage, although a previous study showed similar recovery of organisms at a 1:5 and 1:10 ratio of blood to broth with SPB for all organisms except staphylococci from patients receiving antibiotics (L. B. Reller, K. A. Lichtenstein, S. Mirrett, and W.-L. L. Wang, Abstr. Ann. Meet. Am. Soc. Microbiol. 1978, C177, p. 306).

Positive Bactec bottles were most often detected by macroscopic inspection, just as were SPB bottles. Only 9% of aerobic Bactec bottles and 13% of anaerobic Bactec bottles were detected only by radiometric monitoring, and most positive Bactec bottles that had positive radiometric readings also had positive acridine orange stains. These results suggest that the Bactec set performed better because of the ability of these media to support growth of bacteria and fungi and not because of the detection method used.

For both Bactec and SPB media, a combination of macroscopic inspection, subculture, and microscopic examination or radiometric reading was necessary to detect all microorganisms quickly. We did not routinely test already positive bottles by alternative methods because of the time already required to monitor two unique culture systems.

TABLE 2. Comparison of speed of detection of clinically important bacteria and fungi grown from both 10-ml samples of blood cultured in SPB and the Bactec system

Microorganism	No. of isolates recovered from:			*P* value
	SPB and Bactec (same time)	SPB ≥1 day earlier	Bactec ≥1 day earlier	
Aerobic and facultative bacteria	233	19	70	<0.001
Gram positive	102	6	31	<0.001
S. aureus	52	3	21	<0.001
S. epidermidis	4	2	0	NS[a]
Streptococci[b]	45	1	3	NS
Diphtheroids	1	0	7	<0.005
Gram negative	131	13	39	<0.001
E. coli	42	5	20	<0.02
Other *Enterobacteriaceae*	80	3	11	<0.05
P. aeruginosa	9	5	6	NS
Flavobacterium spp.	0	0	2	NS
Anaerobic bacteria	14	0	4	NS
Gram positive[c]	4	0	0	NS
Gram negative[d]	10	0	4	NS
All bacteria	247	19	74	<0.001
Fungi[e]	6	0	13	<0.001
All microorganisms	253	19	87	<0.001

[a] NS, Not significant ($P \geq 0.05$).
[b] Four group A streptococci, 9 group B streptococci, 8 enterococci, 22 *S. pneumoniae*, 1 viridans streptococcus, and 5 other streptococci.
[c] Three *Clostridium* spp. and one peptostreptococcus.
[d] Twelve *Bacteroides* spp. and two *Veillonella* spp.
[e] Seven *C. albicans*, three *C. parapsilosis*, seven *C. tropicalis*, one other *Candida* sp., and one other yeast.

TABLE 3. Methods of first detection for clinically important microorganisms recovered from SPB and Bactec broths

Detection method	No. of isolates (%) recovered from:		
	SPB	Bactec 6B	Bactec 7C or 7D
Macroscopic inspection	267 (66)	260 (67)	176 (49)
Subculture	42 (10)	8 (2)	27 (8)
Acridine orange and Gram stain	60 (15)	—[a]	—
Acridine orange only	37 (9)	—	—
Radiometric reading and acridine orange	—	85 (22)	110 (31)
Radiometric reading only	—	37 (9)	47 (13)

[a] —, Method not routinely performed.

Thus, the yield of each of these methods in overall detection was not established in this study. It does appear important to continue using a variety of techniques to assure the most rapid identification of positive cultures. The techniques appropriate for routine blood culture monitoring have been outlined elsewhere (16).

Although Bactec clearly outperformed SPB, four problems should be noted. First, as in other studies (2–4, 17, 22), false-positive radiometric readings were common and increased the work required on Bactec cultures. Despite the decreased time spent on the workup of negative bottles, the time spent on false-positive bottles resulted in a similar overall workload for Bactec and SPB. Second, cross-contamination of radiometric bottles has been previously described (7, 8) and was also a problem during our study. Third, the price of two bottles of Bactec medium compared with one of SPB, the cost of radioactive disposal, and the cost of renting the Bactec instrument at WVU resulted in $21,200 in additional expenses for the Bactec system. At WVU, Bactec detected 35 microorganisms not recovered in SPB at an additional cost of $600 each. Fourth, radioactive disposal of Bactec bottles at WVU required transport of used vials to landfill sites. We initially had to purchase containment barrels costing $4,000 per year. Recently, inexpensive containment cartons became available that substantially reduced this cost ($775 per year). Even with inexpensive containers, transport and burial of radioactive material from other states with similar regulations may make the use of radioactive blood culture media difficult (11).

Achievement of the better detection rate with Bactec clearly requires use of the two bottle set. Bactec 6B detected 12 *P. aeruginosa*, and 7C and D detected only 4; 6B detected 25 yeasts, and 7C and D detected only 2; and 7C and D detected 39 anaerobes, and 6B detected only 1. These bottles are complimentary, and neither should be used alone. Moreover, even though 10 ml of blood in the Bactec system recovered more organisms than did SPB, 9% of clinically important microorganisms were still missed by the Bactec set. Failure to detect these microorganisms is likely due to the volume of blood cultured (19, 24). In our study, at least 4 ml of blood was required for each Bactec bottle. The manufacturer's instructions only require 3 to 5 ml of blood; if the smaller amount is used, results for the Bactec system are not likely to be as good. Our results suggest that not only should a full 5 ml of blood be requested in each bottle, but also a blood culture may require four Bactec bottles or two Bactec bottles and an additional nonradiometric 10-ml draw culture vial for the optimal yield of microorganisms.

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