JOURNAL OF CLINICAL MICROBIOLOGY, July 1983, p. 43–48
0095-1137/83/070043-06$02.00/0

Vol. 18, No. 1

Detection of Bacteremia in Patients Receiving Antimicrobial Therapy: an Evaluation of the Antimicrobial Removal Device and 16B Medium

GARY V. DOERN[1,2]* AND NELSON M. GANTZ[2]

Department of Clinical Microbiology[1] and Division of Infectious Disease,[2] University of Massachusetts Medical Center, Worcester, Massachusetts 01605

Received 12 November 1982/Accepted 31 March 1983

A total of 1097 blood specimens obtained from patients receiving antibacterial antimicrobial agents were processed by three blood culture systems: standard aerobic and anaerobic radiometric media, resin-containing radiometric medium (16B; Johnston Laboratories, Cockeysville, Md.), and aerobic and anaerobic radiometric media inoculated with blood processed in an Antimicrobial Removal Device (Marion Laboratories, Kansas City, Mo.). A total of 73 cultures, representing 45 unique septic episodes, yielded 81 clinically significant organisms. Forty-six organisms (28 septic episodes) were recovered in standard radiometric medium. 16B medium yielded 63 organisms (37 septic episodes). Sixty-nine organisms (42 septic episodes) were isolated from radiometric blood cultures inoculated with Antimicrobial Removal Device-processed blood. Contamination rates were not significantly different among the three systems. In comparison with standard radiometric blood cultures, the length of time to detection of positive blood cultures was shorter with both 16B medium and with Antimicrobial Removal Device-processed cultures. Comparison of the latter two systems suggested enhanced recovery of clinically significant organisms in radiometric blood cultures inoculated with blood processed in the Antimicrobial Removal Device. There was no difference in the length of time to detection of positive blood cultures.

The presence of antimicrobial agents in patient blood is thought to represent a potential source of false-negative blood cultures in bacteremic patients receiving antibacterial chemotherapy. This notion has led, at least in part, to a number of blood culture practices frequently employed in clinical microbiology laboratories. These methods include dilution of blood specimens in blood culture broth, usually to a final blood concentration of 10% (3); incorporation into culture medium of chemical agents with known inhibitory effects on the activities of certain antimicrobial agents, e.g., sodium polyanethol sulfonate (3, 6); and finally, the introduction of antimicrobial inactivating enzymes, such as penicillinase, directly into blood culture broth (4, 5). These procedures, although of some benefit, are limited by the high concentrations of antimicrobial agents often achieved in patient serum, by the relatively small numbers of antimicrobial agents which are inhibited, and by the potential for contaminating blood cultures.

In 1980, Wallis et al. described a process whereby blood obtained from patients receiving antimicrobial agents was exposed, before culturing, to two antimicrobial-binding resins, a cation exchange resin, and a polymeric absorbant resin (11). Blood was inoculated into a bottle containing these resins, sodium polyanethol sulfonate, and saline; rotated for 15 min; and then transferred into suitable blood culture media. In a limited clinical study of patients receiving antimicrobial therapy, Wallis et al. noted enhanced recovery of organisms when compared with a conventional blood culture procedure. A resin-containing bottle, referred to as an Antimicrobial Removal Device (Marion Laboratories, Kansas City, Mo.), was made commercially available in 1981. Subsequently, it was shown to inactivate 13 antimicrobial agents commonly used to treat patients with bacteremia (9).

In 1982, a new radiometric blood culture medium, 16B medium (Johnston Laboratories, Cockeysville, Md.), which was based on essentially the same principle, was developed. The same two resins which are present in the Antimicrobial Removal Device were placed directly into aerobic radiometric blood culture medium, thus obviating the need for processing blood before culturing. The intent of the present study

was to compare 16B medium and the Antimicrobial Removal Device with a standard radiometric blood culture procedure as means for detecting clinically significant bacteremia in septic patients receiving antimicrobial therapy.

MATERIALS AND METHODS

Blood cultures. This study was performed during the 7-month period March 17, 1982 through October 22, 1982. By an aseptic technique, 20 ml of blood were obtained by trained phlebotomists from patients in whom there existed some clinical indication for performing blood cultures. Blood specimens were collected directly into sterile Vacutainer tubes containing a final concentration of 0.025% sodium polyanethol sulfonate and transported to the laboratory within 15 min of collection. The following procedure was implemented with specimens obtained from patients receiving at least one antibacterial antimicrobial agent at the time specimens were collected. With a sterile plastic syringe equipped with a 20-gauge needle, 3.3 ml of blood was transferred into individual radiometric blood culture bottles (Johnston Laboratories) containing 30 ml of hypertonic 8B aerobic medium, 7C anaerobic medium, and 16B resin-containing aerobic medium. The remaining 10 ml of blood was transferred into an Antimicrobial Removal Device, which was rotated on its vertical axis at room temperature for 15 min at 70 rpm with an Antimicrobial Rotator Removal Device (Marion Laboratories). The contents of the Antimicrobial Removal Device were withdrawn with a second syringe and needle, and 5.0-ml samples were transferred into individual radiometric blood culture bottles containing 8B and 7C media. In this manner, because of dilution of the specimen in the 5.0-ml fluid contents of the Antimicrobial Removal Device, 3.3 ml of actual patient blood was transferred into the individual blood culture bottles. The rubber septa of all blood culture bottles, the Antimicrobial Removal Device, and the Vacutainer tube were thoroughly disinfected with 70% ethanol before entry with the syringe and needle.

The following abbreviations are used in this study: STD, aerobic (8B) medium or anaerobic (7C) radiometric blood culture medium or both; STD-aero, aerobic (8B) radiometric blood culture medium; STD-ana, anaerobic (7C) radiometric blood culture medium; ARD, aerobic (8B) medium or anaerobic (7C) radiometric blood culture medium or both inoculated with blood processed through the Antimicrobial Removal Device; ARD-aero, aerobic (8B) radiometric blood culture medium inoculated with blood processed through the Antimicrobial Removal Device; ARD-ana, anaerobic (7C) radiometric blood culture medium inoculated with blood processed through the Antimicrobial Removal Device; and 16B medium, radiometric resin-containing medium.

Both aerobic blood culture bottles containing 8B medium and the bottle containing 16B medium were incubated on a shaker at 35°C for the first 48 h and thereafter on a stationary rack for a total of 7 days. These cultures were examined for macroscopic evidence of growth, and if absent, their radiometric growth index was determined by a BACTEC 460 radiometric blood culture device (Johnston Laboratories) at 8-h intervals during the first 48 h of incubation and once daily thereafter. Both anaerobic blood culture bottles containing 7C medium were incubated on a stationary rack at 35°C. These cultures were examined macroscopically, and if negative, their growth index was determined on the BACTEC device once daily for a total of 7 days.

The following indicators of positivity prompted a Gram smear of uncentrifuged blood culture fluid: macroscopic evidence of growth, a growth index of $\geq$20 U, or an incremental increase of $\geq$5 growth index units over the previous reading. If Gram smears of macroscopically or radiometrically positive bottles were negative, blind subcultures of uncentrifuged blood culture fluid were performed with enriched chocolate agar incubated at 35°C in 5 to 7% CO_2 for 48 h and brucella agar base containing 5% sheep blood, vitamin K, and hemin incubated anaerobically at 35°C for 48 h.

The length of time to detection (LTD) was defined as the length of time (in hours) from inoculation of blood culture media until the first indication of positivity, i.e., macroscopic evidence of growth, radiometric evidence of growth, or recovery of organisms on blind subculture.

Organism identification. Aerobic and facultative anaerobic blood culture isolates were identified according to criteria described in the *Manual of Clinical Microbiology* (8). Anaerobic organisms were identified by using criteria described in the *Anaerobe Laboratory Manual* (7).

Clinical significance of blood culture isolates and definition of unique septic episode. The clinical significance of blood culture isolates was ascertained by patient evaluation and chart review, both conducted by one of us (N.M.G.) within 24 h of the time a blood culture was first detected as positive. *Staphylococcus epidermidis* isolates were judged to be clinically significant only when recovered from septic patients with a condition known to predispose to *S. epidermidis* bacteremia (e.g., prosthetic valve, central nervous system shunt, vascular graft, indwelling vascular catheter, etc.) and when organisms with identical antibiograms were recovered from at least two consecutive blood cultures. A unique septic episode was defined as a period during which consecutive blood cultures were obtained from a given patient at intervals of no greater than 24 h.

Statistical analyses. Statistical analyses were performed by using a nonparametric test for matched pair discrete data analyses (McNemar test) with 1 df.

RESULTS

During the course of this evaluation, a total of 1,097 blood cultures were obtained from patients receiving antibiotics at the time blood specimens were drawn and were thus included in the study. These 1,097 blood cultures represented 594 unique septic episodes. A total of 93 blood cultures, representing 63 unique septic episodes, were found to be culture positive in at least one blood culture bottle. A single organism was recovered in 85 cases, two organisms in 7 cases, and three organisms in 1 case, for a total of 102 blood culture isolates.

Of the 102 blood culture isolates, 81 were determined to be clinically significant (Table 1).

They had been recovered from a total of 73 blood cultures, representing 45 unique septic episodes. A total of 37 organisms were recovered from STD, 16B medium, and ARD. Two organisms were recovered only from STD. 16B medium was culture positive alone in six cases. A total of 13 organisms were recovered from only ARD. In four cases, organisms were recovered from STD and 16B medium but not ARD. In three cases, organisms were not recovered from 16B medium but were found in STD and ARD. Finally, 16 organisms were recovered from 16B medium and ARD but not STD.

Collectively then, a total of 46 clinically significant organisms were recovered from STD, 63 organisms from 16B medium, and 69 organisms from ARD. The rates of clinically significant positive blood cultures for the three systems were determined to be 4.2, 5.7, and 6.3%, respectively.

Of the 102 blood culture isolates, 21 were determined to be contaminants. These included 15 isolates of *S. epidermidis*, 2 isolates each of aerobic diphtheroids and *Bacillus* species, and 1 isolate each of *Aerococcus viridans* and *Streptococcal salivarius*. They had been recovered from a total of 20 different blood cultures. Collectively, a total of 6 contaminants were recovered from STD, 9 contaminants from 16B medium, and 10 contaminants from ARD. Contamination rates were determined to be 0.6, 0.8, and 0.9%, respectively.

Because of the manner in which this study was conducted, several comparisons of the relative recovery of clinically significant microorganisms with the three different blood culture systems were possible (Table 2). A total of 35 organisms which were not recovered from STD were recovered from either 16B medium or ARD. By contrast, only two organisms were isolated exclusively from STD.

Comparison of the results of Antimicrobial Removal Device-processed cultures with the standard radiometric blood culture procedure revealed that 29 organisms which were recovered from ARD were not recovered from STD (Table 2). Conversely, six organisms found in STD were not isolated from ARD. Similarly, 22 organisms were recovered from 16B medium but not from STD. Five organisms isolated from STD were not found in 16B medium. This comparison of 16B medium with the standard radiometric blood culture procedure is biased in favor of the standard procedure, since 16B medium is intended solely for aerobic cultivation of blood specimens. The standard radiometric blood culture system includes cultures incubated both aerobically and anaerobically. Furthermore, the total volume of blood specimen cultured in 16B medium (i.e., 3.3 ml into one bottle) was precisely one-half of that cultured by the standard procedure (i.e., 3.3 ml into each of two bottles). When this comparison was restricted to those organisms recovered from 16B medium versus those recovered only from STD-aero, the results did not change substantially. In this case, a single additional organism, for a total of 23, was recovered only from 16B medium.

This investigation also permitted comparison of 16B medium versus Antimicrobial Removal Device-processed cultures. A total of 16 clinically significant organisms were recovered from

TABLE 1. Recovery of clinically significant microorganisms in STD, 16B medium, and ARD

Organism (no.)	No. of isolates recovered in:						
	STD, 16B, and ARD	STD	16B	ARD	STD and 16B	STD and ARD	16B and ARD
Staphylococcus aureus (12)	2		3	2			5
Staphylococcus epidermidis (13)	5			1	1		6
Staphylococcus fecalis (4)	1	1	1	1			
Staphylococcus bovis (1)	1						
Group A *Streptococcus* (2)				1			1
Corynebacterium J-K (1)		1					
Escherichia coli (22)	14		2	3			3
Klebsiella pneumoniae (4)	1			1	1		1
Morganella morganii (2)	2						
Enterobacter cloacae (3)	2			1			
Enterobacter agglomerans (1)	1						
Pseudomonas aeruginosa (2)	1				1		
Haemophilus aphrophilus (1)	1						
Bacteroides fragilis (2)				2			
Bacteroides oralis (1)				1			
Candida albicans (6)	4				1	1	
Candida tropicalis (2)	2						
Candida neoformans (2)						2	

ARD but not from 16B medium (Table 2). Conversely, 10 organisms were found only in 16B medium. This difference, although suggesting enhanced recovery from ARD, was not statistically significant. Furthermore, these results are biased in favor of Antimicrobial Removal Device-processed cultures, owing to the inclusion of an anaerobic blood culture bottle in this system. When this comparison was restricted to aerobic bottles inoculated with Antimicrobial Removal Device-processed blood, 10 organisms were found to have been recovered from ARD-aero but not from 16B medium. Conversely, 13 organisms were found only in 16B medium and not in ARD-aero. This difference was also not statistically significant.

The final comparison was of organisms recovered in Antimicrobial Removal Device-processed cultures versus those recovered from 16B medium or STD-ana or both, since in most cases, use of the 16B medium, if deemed appropriate, would serve as a replacement for the standard aerobic radiometric culture and would be used in conjunction with a standard anaerobic radiometric culture (Table 2). A total of 16 organisms were recovered from ARD but not from 16B medium or STD-ana. Conversely, 11 organisms were found in 16B medium or STD-ana or both, but not in ARD. This difference was not statistically significant.

Although in most cases, the total number of clinically significant isolates of an individual organism was not large enough to permit meaningful comparisons of the relative rate of recovery of that organism in the three different blood culture systems, the following observations could be made. Only 2 of 12 isolates of *Staphylococcus aureus* were recovered from STD (Table 1). Of 12 isolates, 10 were recovered from 16B medium, and of 12 isolates, 9 were recovered from ARD. Of 22 isolates of *Escherichia coli*, 14 were recovered from STD, whereas 19 were recovered from both 16B medium and ARD. Only three anaerobic organisms were isolated during the course of this investigation. All three were recovered from ARD-ana. Finally, of a total of 10 isolates of yeast, 6 were recovered from STD (all exclusively from STD-aero), 7 were found in 16B medium, and 9 were found in ARD (all exclusively in ARD-aero).

The results of this investigation were also analyzed with respect to unique septic episodes rather than individual blood cultures. It was found that among the 45 septic episodes from which clinically significant organisms were recovered, the apparent causative organism(s) was recovered from at least one bottle of one culture with the standard radiometric procedure in 28 cases, from at least one 16B medium culture in 37 cases, and from at least one bottle of one blood culture inoculated with specimen processed in the Antimicrobial Removal Device in 42 cases. In only one instance was a septic episode documented by recovery of a clinically significant organism (*Corynebacterium* J-K) only in standard radiometric blood culture media. Similarly, in only one septic episode (*S. aureus*) was a 16B medium culture the sole source of isolation of a clinically significant organism. Blood cultures inoculated with Antimicrobial Removal Device-processed blood were the only means of documenting septic episodes in seven cases. The organisms responsible for these seven septic episodes were *S. aureus*, group A *Streptococcus, E. coli, Enterobacter cloacae, Bacteroides fragilis, Bacteroides oralis*, and in one case mixed infection with *Streptococcus fecalis* and *B. fragilis*.

For the total of 46 clinically significant organisms recovered from STD-aero, the mean LTD was 31.6 h. A total of 26 of these organisms were also recovered from STD-ana; the mean LTD was 40.8 h. For the 63 organisms recovered from 16B medium, the mean LTD was 25.0 h. The mean LTD for the 60 organisms recovered from ARD-aero and the 52 organisms recovered in ARD-ana were 26.1 and 28.3 h, respectively. If

TABLE 2. Comparison of the relative recovery of clinically significant microorganisms in three different blood culture systems

Blood culture systems compared	No. of organisms recovered from:			*P*
	A and B	A only	B only	
A. STD B. 16B or ARD or both	44	2	35	<0.005
A. STD B. ARD	40	6	29	<0.005
A. STD B. 16B	41	5	22	<0.005
A. STD-aero B. 16B	40	5	23	<0.005
A. ARD B. 16B	53	16	10	N.S.[a]
A. ARD-aero B. 16B	50	10	13	N.S.
A. ARD B. 16B or STD-ana or both	51	16	11	N.S.

[a] NS, Not significant ($P > 0.05$).

this comparison is restricted to the 37 clinically significant organisms which were isolated from all three systems, the mean LTDs for STD, 16B medium, and ARD were 28.3, 21.8, and 21.7 h, respectively.

DISCUSSION

Several studies in which conventional blood culture techniques were employed have addressed the utility of the Antimicrobial Removal Device as a means for recovering organisms from blood cultures obtained from septic patients receiving antimicrobial therapy (11, 12; P. Yungbluth, E. Aqui, and H. M. Sommers, Program Abstr. Intersci. Conf. Antimicrob. Agents Chemother. 22nd, Miami Beach, Fla., abstr. no. 410, 1982). At least four investigations have examined this system in conjunction with radiometric blood culture procedures (2, 10; R. P. Gruninger, M. L. Simpson, R. E. Klicker, and M. Y. Kahn, Program Abstr. Intersci. Conf. Antimicrob. Agents Chemother. 22nd, Miami Beach, Fla., abstr. no. 412, 1982; P. E. Porembski, G. W. White, M. D. Blatt, M. D. Bliss, and R. F. Lee, Program Abstr. Intersci. Conf. Antimicrob. Agents Chemother. 22nd, Miami Beach, Fla., abstr. no. 411, 1982). Although the absolute degree of enhanced recovery with the Antimicrobial Removal Device has varied in these studies, in all cases this system has led to isolation of organisms not recovered from conventional or radiometric blood cultures.

These observations are consistent with the results of the present study in which use of the Antimicrobial Removal Device clearly enhanced recovery of clinically significant organisms from blood specimens obtained from septic patients receiving antimicrobial therapy. When compared with a standard radiometric blood culture procedure, the Antimicrobial Removal Device yielded significantly greater total numbers of clinically significant organisms. In addition, detection times were shortened. These conclusions were predicated on comparisons of recovery rates from unique blood cultures and, as such, probably represent the most accurate means for comparing the ability of these two blood culture systems to detect bacteremia. However, the actual clinical utility of blood culture procedures is probably better assessed by comparing the number of septic episodes documented, since multiple blood cultures are often obtained from an individual patient during a given septic episode. Of a total of 45 septic episodes, 42 were documented in Antimicrobial Removal Device-processed blood cultures. By comparison, the standard radiometric procedure yielded at least a single positive culture in only 28 septic episodes.

A possible disadvantage of the Antimicrobial Removal Device is the potential for contamination of blood cultures, since this system necessitates extraneous manipulation of blood specimens before inoculation of culture media. However, because transfer of blood from the Antimicrobial Removal Device into blood culture media would normally be accomplished in the controlled environment of the laboratory by trained personnel, it would seem that this risk of contamination could be minimized. Indeed, contamination rates obtained in the present study with cultures inoculated with Antimicrobial Removal Device-processed blood were not significantly higher than those obtained with standard radiometric blood cultures.

Resin-containing radiometric 16B blood culture medium has recently been shown to significantly enhance recovery of bacteria from blood cultures obtained from septic patients receiving antimicrobial therapy (1). These observations are consistent with the results of the present study, in which significantly greater total numbers of clinically significant organisms were recovered in 16B medium than in standard radiometric blood cultures. Furthermore, the number of septic episodes documented with 16B medium (i.e., 37) was significantly greater than the number documented by standard radiometric blood cultures (i.e., 28), despite the fact that 16B medium represents only an aerobic culture, whereas the standard procedure includes both aerobic and anaerobic cultures. Contamination rates in these two systems were comparable.

Comparison of the numbers of organisms recovered from 16B medium versus ARD revealed that a majority of clinically significant organisms (53 of 81) were isolated from both systems. However, 16 organisms were recovered from ARD but not 16B medium. Conversely, 10 organisms recovered from 16B medium were not detected in ARD. This difference, although suggesting enhanced recovery in Antimicrobial Removal Device-processed cultures, was not statistically significant and could be explained largely on the basis of the exclusive recovery of certain organisms in the anaerobic Antimicrobial Removal Device-processed bottles. Furthermore, the total volume of blood cultured with Antimicrobial Removal Device-processed cultures (i.e., 6.6 ml) was twice that cultured in 16B medium (i.e., 3.3 ml). Indeed, when this comparison of 16B medium was restricted to ARD-aero, 13 organisms were found to have been recovered only from 16B medium, whereas 10 organisms were isolated from only ARD-aero.

Finally, since in practice laboratories contemplating the use of 16B medium would probably employ it as a replacement for aerobic radiometric cultures, it was of interest to compare recov-

ery rates obtained with Antimicrobial Removal Device-processed cultures versus those obtained with 16B medium in conjunction with STD. Although this comparison suggested enhanced recovery in ARD, the differences were not statistically significant.

Although these comparisons of 16B medium with ARD demonstrated comparable recovery rates for clinically significant organisms, it should be noted that differences were observed in the numbers of septic episodes documented by each system, i.e., 37 with 16B medium and 42 with Antimicrobial Removal Device-processed cultures. Furthermore, although the former system was the only means of documenting one septic episode, in seven septic episodes organisms were recovered exclusively in the latter system. Contamination rates between the two systems were essentially identical.

In summary, the results of this study demonstrated that both the Antimicrobial Removal Device and 16B medium had a significant positive effect on the recovery of clinically significant microorganisms in blood cultures obtained from septic patients receiving antimicrobial therapy. In this setting, both systems may be considered reliable adjuncts to, or replacements for, standard radiometric blood culture procedures. It should be recognized, however, that even when laboratory procedures aimed at detecting bacteremia in patients receiving antimicrobial agents are optimized, as was the case in this study, blood cultures will remain negative in the large majority of patients with clinical evidence of sepsis. In the present study, clinically significant bacteremia was detected in only 45 of 594 (7.6%) unique septic episodes.

ACKNOWLEDGMENT

We acknowledge the secretarial assistance of Annmarie McDonough.

LITERATURE CITED

1. **Applebaum, P. C., D. G. Beckwith, J. R. Dipersio, J. W. Dyke, J. F. Salventi, and L. L. Stone.** 1982. Enhanced detection of bacteremia with a new BACTEC resin blood culture medium. J. Clin. Microbiol. **17**:48–51.
2. **Appleman, M. D., R. S. Swinney, and P. N. R. Heseltine.** 1982. Evaluation of the Antibiotic Removal Device. J. Clin. Microb. **15**:278–281.
3. **Bartlett, R. C., P. D. Ellner, and J. A. Washington II.** 1974. Cumitech 1, Blood cultures. Coordinating ed., J. C. Sherris. American Society for Microbiology, Washington, D.C.
4. **Carleton, J., and M. Hamburger.** 1963. Unmasking of false-negative blood cultures in patients receiving new penicillins. J. Am. Med. Assoc. **186**:157–159.
5. **Dowling, H. F., and H. L. Hirsh.** 1945. The use of penicillinase in cultures of body fluids obtained from patients under treatment with penicillin. Am. J. Med. Sci. **210**:756–762.
6. **Eng, J.** 1975. Effect of sodium polyanethol sulfonate in blood cultures. J. Clin. Microbiol. **1**:119–123.
7. **Holdeman, L. V., E. P. Cato, and W. E. C. Moore.** 1977. Anaerobe laboratory manual, 4th ed. Virginia Polytechnic Institute, Blacksburg.
8. **Lennette, E. H., E. H. Spaulding, and J. P. Truant (ed.).** 1974. Manual of clinical microbiology, 2nd ed. American Society for Microbiology, Washington, D.C.
9. **Lindsey, N. J., and P. E. Riely.** 1981. In vitro antibiotic removal and bacterial recovery from blood with an antibiotic removal device. J. Clin. Microbiol. **13**:503–507.
10. **Strand, C. L.** 1982. Evaluation of the Antimicrobial Removal Device when used with the BACTEC blood culture system. Am. J. Clin. Pathol. **78**:853–856.
11. **Wallis, C., J. L. Melnick, R. D. Wende, and P. E. Riely.** 1980. Rapid isolation of bacteria from septicemia patients by use of an Antimicrobial Removal Device. J. Clin. Microbiol. **11**:462–464.
12. **Wright, A. J., R. L. Thompson, C. A. McLimans, W. R. Wilson, and J. A. Washington II.** 1982. The antimicrobial removal device. A microbiological and clinical evaluation. Am. J. Clin. Pathol. **78**:173–177.