Enhanced Detection of Bacteremia with a New BACTEC Resin Blood Culture Medium

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In this multicenter study, 621 sets of blood culture specimens were drawn from 280 patients who were suspected of being septic and who were receiving antimicrobial therapy. Equal volumes of each specimen were inoculated into BACTEC 6B and 16B media. The 16B medium contained adsorbent and cationic resins for neutralizing the effects of the drugs. Of the 621 sets drawn, there were 72 positive cultures in 16B and 52 positive cultures in 6B. In 23 cases the organism was detected only in the 16B medium, and in 3 cases the organism was detected in 6B only. The remaining 49 positives were detected in both culture bottles. In 13 of these 49 cultures, detection in 16B was made between 1 and 5 days earlier than in 6B, whereas 3 of 49 specimens were detected 1 day earlier in 6B; the remaining 33 cultures became positive at approximately the same time in both media. There were a total of 43 patients with positive cultures in this study. Of these patients, 28 had sepsis detected in both the 16B and 6B media. The 6B medium alone detected an additional three cases of sepsis, and the 16B resin medium alone identified 12 additional cases. Supplementary culturing of samples from patients receiving antimicrobial therapy significantly increased the number of positive cultures and positive patients, as well as significantly shortening the time to positivity in these cultures.

Increasing emphasis is being placed upon rapid, accurate information on microbiological specimens (3), particularly for bacteremic patients receiving antimicrobial therapy. Patients with suspected bacteremia may receive empirical (and often unsuitable) antimicrobial therapy before cultures are taken, thus masking the bacterial etiology and complicating effective therapy. Additionally, patients receiving antimicrobial prophylaxis may become septic while on antibiotic therapy. Improvement of the yield of positive blood cultures for these patients would facilitate more rational antimicrobial therapy.

The Antibiotic Removal Device (ARD; Marion Laboratories, Inc., Kansas City, Mo.) is a bottle containing resin designed for removal of antimicrobial agents from blood cultures. Previous studies (1, 5, 7) documented the efficacy of this method in increasing the detection of bacteremia and decreasing the time required for bacterial identification in clinically septic patients receiving antimicrobial therapy. The current study evaluates a new resin medium, 16B, designed for use with the BACTEC blood culture system (Johnston Laboratories, Towson, Md.).

(Part of this work was presented at the 82nd Annual Meeting of the American Society for Microbiology [P. Appelbaum, D. Beckwith, J. DiPersio, J. Dyke, J. Salventi, and L. Stone, Abstr. Annu. Meet. Am. Soc. Microbiol. 1982, C122, p. 291].)

MATERIALS AND METHODS

Patients. Patients were included in the study if they were considered to have clinical sepsis in spite of current antimicrobial therapy. Clinical sepsis was defined as an increase in the patient's pulse and temperature ($\geq 38°C$); the presence of chills, prostration, or hypotension; mild intermittent fever in the presence of a heart murmur; leukocytosis ($>10,000/mm^3$), especially with a shift to the left; or granulocytopenia ($<1,000$ mature polymorphs per mm^3). All patients were receiving at least one but usually two or more antibiotics during the course of the study. Drugs and combinations are detailed below. Study patients were drawn from three general acute-care units, two burn units, and one oncology unit. Patient selection was

TABLE 1. Detection of positive blood cultures by BACTEC 16B and 6B media

Culture positive in:		No. of positive cultures
16B	6B	
+	+	49[a]
+	−	23
−	+	3

[a] Detected at the same time in both, 33; detected earlier in 16B, 13; detected earlier in 6B, 3.

made by infectious disease service physicians and by chart review.

Specimen collection and processing. Blood (7 to 10 ml) was collected at bedside. For each venipuncture, two BACTEC bottles were inoculated, one containing 6B and the other 16B (resin bottle). The 16B medium contained nonionic adsorbent (Rome and Haas, Philadelphia, Pa.) and cationic exchange resins (Fisher Chemical, Pittsburgh, Pa.) for neutralizing the effects of antimicrobial agents but was otherwise identical to 6B medium (2). Equal amounts of blood were distributed into both bottles, which were then incubated with agitation at 35°C for the first 24 h. Incubation was continued without agitation for up to 7 days (see below). Records were kept for each patient of drug(s) given, with dosage, route, and time of administration noted.

Detection of positive cultures. Bottles were examined visually for turbidity and radiometrically for growth index (GI) on BACTEC model 460 or 225 instruments (Johnston Laboratories) at least once within the first 24 and 48 h of receipt (days 1 and 2, respectively) and then on days 3, 4, 5, 6, and 7. When time permitted, turbidometric and radiometric examinations were carried out after 6 and 12 h on both days 1 and 2. For logistical reasons, no attempt was made to standardize the number of turbidometric and radiometric examinations performed on these days. When bottles became visually or radiometrically positive (GI, ≥30 for 6B, ≥20 for 16B), Gram smear and subculture were performed and further incubation of bottles discontinued. Blind smear and subculture were performed on day 5, and, in most cases, terminally on day 7. Aerobic, microaerophilic, and anaerobic subcultures were done by standard methods (4). Incubation was at 35°C. Organisms were identified by previously described methods (4).

Statistical methods. The χ^2 technique was used for statistical analysis.

RESULTS

Two hundred and eighty patients were enrolled in the study, yielding a total of 621 sets of bottles. Of the 280 patients, 43 had positive blood specimens, yielding 75 positive cultures.

Of the 75 positive cultures (Table 1), 72 (49 plus 23, 96.0%) occurred in the 16B bottle, and 52 (49 plus 3, 69.3%) in the 6B bottle (highly significant difference: $P < 0.005$). Forty-nine positive cultures (65.3%) occurred in both 16B and 6B. Twenty-three positive cultures (30.7%)

were seen in 16B only, and three (4.0%) in 6B only (highly significant difference: $P < 0.005$). Thirty-three cultures (44.0%) were detected at approximately the same time in both 16B and 6B. Thirteen cultures (17.3%) were detected earlier in 16B, and three (4.0%) were detected earlier in 6B (significant difference: $P < 0.01$).

Of the 43 patients with positive blood cultures (Table 2), 40 (28 plus 12, 93.0%) had positive cultures in 16B, and 31 (28 plus 3, 72.1%) had positive cultures in 6B (significant difference: $P < 0.025$). Twenty-eight patients (65.1%) had positive cultures in both 16B and 6B. Twelve patients (27.9%) had positive cultures in 16B only, and three (7.0%) in 6B only (significant difference: $P < 0.025$). In fourteen patients (32.6%), detection was made at approximately the same time in both 16B and 6B. Eleven cases (25.6%) were detected earlier with 16B and three (7.0%) with 6B (significant difference: $P < 0.01$).

In the majority of cases, the time interval between inoculation and turbidometric/radiometric positivity was 48 h. GI readings of negative bottles ranged between 2 and 15, whereas those of positive bottles were ≥20. GIs for 64 of 72 positive 16B cultures were ≥30, and for 8 of 72 they were >20 but <30. In all of these eight instances, smear and culture results were positive. With a ≥20 GI threshold, no false-positive or false-negative results were found with 16B. The time differences to positivity between 16B and 6B for the 13 cultures detected earlier by 16B were as follows: ≤24 h, six specimens; ≤48 h, three specimens; 72 h, two specimens; 4 days, one specimen; and 5 days, one specimen. The corresponding time interval for the three cultures detected earlier with 6B was ≤24 h.

Staphylococci, streptococci, and *Enterobacteriaceae* comprised the majority of organisms isolated (Table 3). There were 80 isolates from 16B bottles and 58 from 6B bottles. All species except *Escherichia coli* and *Proteus mirabilis* were isolated from both bottles. Although several organisms were isolated more commonly in 16B than in 6B, numbers were not statistically significant.

Two organisms (one *Staphylococcus aureus*, one *Corynebacterium* sp.), both isolated from

TABLE 2. Detection of patients with positive blood cultures by BACTEC 16B and 6B media

Culture positive in:		No. of patients with positive cultures
16B	6B	
+	+	28[a]
+	−	12
−	+	3

[a] Detected at the same time in both, 14; detected earlier in 16B, 11; detected earlier in 6B, 3.

TABLE 3. Organism isolation in 16B and 6B bottles

Organism	No. of organisms isolated from:		
	16B only	16B and 6B	6B only
Staphylococcus aureus	6	9	0
Staphylococcus epidermidis	4	13	0
Alpha-hemolytic streptococci	1	4	0
Enterococci	4	5	0
Group B streptococci	0	4	0
Escherichia coli	4	0	1
Klebsiella pneumoniae	1	1	0
Klebsiella oxytoca	0	6	0
Citrobacter freundii	1	3	0
Proteus mirabilis	0	0	1
Pseudomonas aeruginosa	2	3	2
Salmonella enteritidis serotypes	0	2	0
Corynebacterium sp.	3	1	0
Listeria monocytogenes	0	1	0
Candida spp.	0	2	0

the 6B bottle only, were considered contaminants and excluded from the data analysis. Use of 16B bottles did not increase the rate of contaminant isolation. All 75 positive specimens were considered to be clinically significant by patient examination and chart review.

Drugs administered to patients comprised a wide variety of antimicrobial agents that included most of the antibiotics currently in use for therapy of serious microbial infections. As can be seen, therapy with two or more drugs in combination was common and reflected the serious nature of infections in these patients. The drugs in use for single-drug therapy included nafcillin, ampicillin, cephalexin, cefamandole, vancomycin, chloramphenicol, erythromycin, and amphotericin B. Drug combinations included: ampicillin and cefazolin; ampicillin and amikacin; nafcillin and amikacin; nafcillin and tobramycin; nafcillin and gentamicin; ticarcillin and amikacin; chloramphenicol and cefazolin; gentamicin and vancomycin; gentamicin and cefazolin; tobramycin and cephalexin; amikacin and vancomycin; tobramycin, vancomycin, and penicillin G; tobramycin, vancomycin, and carbenicillin; tobramycin, nafcillin, and ticarcillin; tobramycin, cefazolin, and clindamycin; and tobramycin, nafcillin, ticarcillin, and clindamycin.

DISCUSSION

The serious nature of bacteremia makes positive blood cultures among the most important results reported by the clinical microbiology laboratory. With bacteremic patients who have received inappropriate empirical antimicrobial therapy before specimens have been drawn, detection of bacteria may be prevented or delayed. Previous solutions to this problem have

included broth dilution of blood, removal of organisms by membrane filtration, and antimicrobial inactivation by penicillinase, sodium polyanethol sulfonate, or p-aminobenzoic acid (6). Recent introduction of the ARD has been a significant step toward resolving this problem (1, 5, 7).

Problems with ARD include the necessity of transferring blood from ARD bottles to BACTEC or conventional culture bottles after shaking, occasional difficulty in removal of blood from bottles due to needle blockage by resin, and high cost. In contrast, 16B bottles are easier to use and less expensive than ARD: no transfer is required, and bottles can be inserted directly into the BACTEC instrument after being inoculated. A disadvantage of the 16B medium is that, in contrast to ARD, it is not designed for anaerobic culture; additionally, it can only be used by laboratories possessing BACTEC instrumentation.

The 16B medium significantly increased detection of bacteremia as compared with 6B medium for the patients studied. Additionally, the time to detection of a positive culture was significantly shorter with 16B than with 6B. In our survey, only patients receiving antibiotics before blood samples had been taken were studied. Sonnenwirth and Weissfeld recently evaluated the 16B medium for randomly selected hospital patients, some of whom were receiving antibiotics. Results were encouraging in that organisms were recovered more often and more rapidly from patients on antimicrobial therapy with 16B than with 6B medium (A. C. Sonnenwirth and A. Weissfeld, Abstr. Annu. Meet. Am. Soc. Microbiol. 1982, C121, p. 291).

The spectrum of drugs utilized in the current study comprises those commonly used in therapy for seriously ill bacteremic patients. Results correspond with in vitro antibiotic neutralizing studies which have shown that the BACTEC resins neutralize the effects of representatives of all antibiotic groups usually used in clinical practice.

In view of the excellent sensitivity and specificity obtained with a 16B GI threshold of ≥20, we propose that as the provisional 16B threshold for positivity. Firm delineation of GI thresholds must await more extensive evaluation of the 16B medium.

Because not all bottles were read twice on days 1 and 2 (see above), the time difference to positivity between 16B and 6B could not be more accurately delineated. More precise definition of time to positivity with 16B and 6B cultures, especially on days 1 and 2, may reflect an even more rapid detection capability for 16B.

In summary, a multicenter evaluation of the 16B resin-containing BACTEC medium showed

that it significantly improved both detection and time to positivity of aerobic blood culture isolates from patients receiving antibiotics. These findings indicate an important place for this medium in laboratories possessing BACTEC instrumentation.

LITERATURE CITED

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