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Routine Evaluation of BACTEC NR-16A and NR-17A Media

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The purpose of this study was to determine whether the BACTEC NR-16A and NR-17A media were more effective than the BACTEC NR-6A and NR-7A media in recovering organisms from the blood of patients undergoing antimicrobial therapy. A total of 986 sets of four blood culture bottles were compared, giving 141, 174, 93, and 104 isolates with BACTEC NR-6A, NR-16A, NR-7A, and NR-17A, respectively. BACTEC NR-6A and NR-7A media recovered 234 isolates, whereas BACTEC NR-16A and NR-17A media recovered 278 isolates. The recovery rate of bacteria when aerobic resin media were used was better than that with conventional aerobic media ($P < 0.001$). The mean detection times were 51.5 and 69.7 h with NR-16A and NR-6A, respectively ($P < 0.01$), whereas they were 68.2 and 71.3 h with NR-17A and NR-7A, respectively ($P > 0.05$). The small number of anaerobes recovered precluded a statistical comparison of relative recovery for that group of organisms.

The recovery of bacteria from septicemic patients is necessary to test susceptibility to antimicrobial agents with a view to monitoring treatment. Moreover, this detection must be rapid. However, the blood of patients receiving antibiotics contains these drugs, which are transferred into the blood culture bottle and thus can suppress or slow bacterial growth.

There are two methods which use resins to remove antibiotics from blood cultures. The first is the Antimicrobial Removal Device (ARD) (Marion Scientific, Kansas City, Mo.), which must be used as an intermediate step between blood sampling and inoculation of broth (4, 7, 12, 14). The second method uses resins incorporated into BACTEC medium (Johnston Laboratories, Towson, Md.) and requires no special processing. BACTEC 16B medium was previously available for the BACTEC 460 system. Since the creation of the nonradiometric BACTEC NR 660 system, aerobic and anaerobic BACTEC media with anionic and cationic resins have become available. In a comparison study, D. Jungkind, J. Bondi, and D. Woodworth-Namey (Abstr. Annu. Meet. Am. Soc. Microbiol. 1986, C42, p. 335) reported that 16B and NR-16A media were equivalent in recovery of bacteria from blood cultures. The present study follows a preliminary evaluation (R. J. Courcol, M. Roussel-Delvallez, A. Fruchart, and G. R. Martin, Abstr. Annu. Meet. Am. Soc. Microbiol. 1987, C390, p. 388) and reports the results of a clinical laboratory evaluation of the routine use of the BACTEC aerobic and anaerobic conventional media versus the BACTEC aerobic and anaerobic resin media for patients admitted to an intensive care unit and receiving antimicrobial therapy.

MATERIALS AND METHODS

Blood sampling, inoculation, and incubation. The study was carried out in the intensive care unit of the A. Calmette Hospital from July 1986 to April 1987. A total of 986 blood culture sets were collected from patients receiving antimicrobial therapy. Each set consisted of a pair of BACTEC bottles, one containing aerobic medium NR-6A and the other containing anaerobic medium NR-7A, and a pair of BACTEC bottles with resins, one containing aerobic medium NR-16A and the other containing anaerobic medium NR-17A. Blood samples were obtained at the bedsides of patients, and the 3- to 5-ml volumes of blood were put into each BACTEC bottle as recommended by the blood culture bottle manufacturer. The BACTEC NR 660 system was used as previously described (2, 6). Vials were incubated for 5 days at 37°C. The aerobic vials were placed on two rotary shakers during the first 24-h incubation. Aerobic vials were read twice per day on days 1 and 3 and once on days 4 to 5; anaerobic vials were read daily on days 1 to 5. They were both removed from the incubator on day 6.

Processing of specimens. Positive-BACTEC-vial criteria were as follows: (i) a visual inspection of all vials prior to testing on the machine to detect evidence of microbial growth, such as bulging septa, hemolysis, or turbidity; and (ii) a growth value of ≥30 or a change in the growth value of ≥15 between two readings. All information about positive vials was automatically printed out. Direct smears were prepared from putative positive vials and stained by the Gram stain method. Subculturing was done with media appropriate for the organisms observed. Microorganisms were identified by standard methods. When one vial or several vials in a set were positive, processing of the negative vial was continued and subculturing was done on day 6.

Recording and analysis of data. The following information was recorded on a report sheet for each set of positive vials: vial identification, time of sample collection, nature of antimicrobial therapy, time of antibiotic administration, results of BACTEC vials (i.e., growth value or change in growth value, test number), subculture results, and organism identification.

A paired comparison between BACTEC conventional vials and BACTEC resin vials was performed on positive vials. The time to positivity was equal to the mean time of blood sampling in the ward plus the time until detection in the laboratory. Statistical analysis was carried out with the Student t test and chi-square test. Probable contaminants were not included in the statistical calculations.

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TABLE 1. Blood culture isolates recovered with BACTEC media

Organism	No. of isolates recovered with:					
	NR-6A only	NR-16A only	NR-6A and NR-16A	NR-7A only	NR-17A only	NR-7A and NR-17A
Aerobic and facultative bacteria (gram negative)						
Escherichia coli	3	5	2		4	1
Citrobacter freundii			1			1
Klebsiella pneumoniae		3	3		1	3
Klebsiella oxytoca	1		1		1	
Enterobacter cloacae	1	1	8		1	8
Enterobacter agglomerans	1					
Serratia marcescens	1	9	8	2	6	8
Proteus vulgaris		1		1		
Proteus mirabilis	1					
Proteus rettgeri	1					
Pseudomonas aeruginosa	6	8	8	1		4
Pseudomonas maltophilia			3			
Flavobacterium meningosepticum			1			
Acinetobacter calcoaceticus	1	4	11		1	2
Neisseria spp.		1				
Anaerobic and facultative bacteria (gram positive)						
Staphylococcus aureus	3	7	12	2	3	10
Staphylococcus epidermidis	10	20	23	10	5	24
Micrococcus spp.	3	6				
Streptococcus pyogenes	1		1			1
Streptococcus agalactiae	1				2	
Streptococcus faecalis	1	2	6	1	1	4
Streptococcus faecium	1					
Streptococcus sanguis					1	
Nonhemolytic streptococci			1			1
Streptococcus pneumoniae	2	1			1	
Corynebacterium spp.	2			1		
Bacillus licheniformis		9		1	4	1
Anaerobic bacteria (*Bacteroides fragilis*)						5
Yeasts (*Candida albicans*)	4	2	6			

RESULTS

During this study, 986 sets of blood cultures were obtained; one set was incomplete, giving 986 associations of aerobic vials and 985 associations of anaerobic vials. There were 128, 156, 82, and 91 positive culture vials, which yielded 141, 174, 93, and 104 isolates with BACTEC NR-6A, NR-16A, NR-7A, and NR-17A media, respectively. Sets containing *Staphylococcus epidermidis*, *Micrococcus* spp., and *Corynebacterium* spp. were considered to be contaminated if organisms were isolated from only one bottle of the set and if the other bottles of the set did not yield growth. This was the case in 24 positive sets (18 aerobic vials and 6 anaerobic vials).

Table 1 shows blood culture isolates detected in BACTEC conventional vials and in BACTEC resin vials. The conventional media allowed recovery of 234 isolates, whereas media with resins allowed recovery of 278 isolates. *Bacillus licheniformis* was not included in the list of contaminant organisms because an immunodeficient patient developed several episodes of septicemia caused by this bacterium. There were 12 cases of discrepancies in which organisms detected in one medium versus organisms detected in the other were different. These discrepancies were equivalent between the two aerobic and anaerobic media. The recovery rate of bacteria when aerobic resin media were used (156 isolates) was better than that with conventional aerobic media (128 isolates) ($P < 0.001$). Such a significant result was not achieved with anaerobic vials.

For aerobic and anaerobic cultures, the times required for the detection of positivity by using the two kinds of medium were compared in terms of the cumulative positive cultures per test and the cumulative percentage of paired positive cultures during the period of observation (Tables 2 and 3). With aerobic vials, 27.2 and 28.4% of cultures were positive on day 1 with NR-6A and NR-16A, respectively, and 88.6 and 92.0% were positive on day 2 with NR-6A and NR-16A, respectively. With anaerobic vials, 4.6% of cultures were positive on day 1 whatever the medium, and 46.1 and 50.7% were positive on day 2 with NR-7A and NR-17A, respectively. All anaerobes were recovered at test 5 (day 3). Under anaerobic conditions, most of the isolates detected were facultative bacteria. Five anaerobes, which were *Bacteroides fragilis*, were detected only with anaerobic media.

The times to recovery of organisms from positive aerobic and anaerobic cultures in both systems were compared. Thus, the mean detection time was significantly shorter with NR-16A medium (51.5 h; standard deviation, ±43.4) than with NR-6A medium (69.7 h; standard deviation, ±53.0). This difference (18.2 h) was statistically significant ($P < 0.01$). With anaerobic cultures, the mean detection times were 71.3 (standard deviation, ±42.3) and 68.2 h (standard deviation, ±41.1) with NR-7A and NR-17A, respectively. These times were not statistically different between the two anaerobic media ($P > 0.05$). The detection of aerobic and facultative bacteria required more time when anaerobic medium was used than when aerobic medium was used

TABLE 2. Cumulative positive cultures per test detected by BACTEC NR-16A and BACTEC NR-6A

Organism or parameter	Medium[a]	No. or % of cumulative positive cultures detected on test no.:						
		1	2	3	4	5	7	8
Gram-positive bacteria								
Staphylococcus aureus	6A		3	8	11			
	16A		4	11				
Staphylococcus epidermidis	6A		1	17	21	22		
	16A		1	17	22			
Streptococci	6A		3	5		6		
	16A		3	6				
Gram-negative bacteria								
Enterobacteriaceae	6A	1	8	21			22	
	16A	1	8	21	22			
Pseudomonas aeruginosa	6A		3	8	9	10		
	16A		4	9	10			
Other	6A		6	10	11			
	16A		5	10		11		
Yeasts (*Candida albicans*)	6A			3		6		
	16A					5		6
Cumulative %	6A	1.1	27.2	81.8	88.6	98.8	100	100
	16A	1.1	28.4	84.1	92.0	98.8		100

[a] 6A, BACTEC NR-6A; 16A, BACTEC NR-16A.

because culture conditions were more suitable for anaerobes.

DISCUSSION

Some studies demonstrated that the BACTEC NR 660 system provided quicker detection of positive blood cultures and produced a more acceptable level of reliability than did conventional systems (2, 6). However, this system does not improve the detection of bacteria in the presence of antimicrobial agents to a significant degree. This is generally the situation in patients receiving antibiotics and becoming septic. Thus, the availability of resins which removed antibiotics is of potential value. We have used the BACTEC resin media and compared the yield to that obtained by using conventional BACTEC media over a period of 10 months. We have found that the BACTEC resin media are superior.

TABLE 3. Cumulative positive cultures per day detected by BACTEC NR-7A and BACTEC NR-17A

Organism	Medium[a]	No. or % of cumulative positive cultures detected on day:			
		1	2	3	4
Gram-positive bacteria					
Staphylococcus aureus	7A	1	4	9	10
	17A	1	5	9	10
Staphylococcus epidermidis	7A		3	16	19
	17A		1	15	19
Streptococci	7A		3	5	
	17A		4	5	
Gram-negative bacteria					
Enterobacteriaceae	7A	2	15	18	19
	17A	2	18		19
Pseudomonas aeruginosa	7A		2	5	
	17A		3	5	
Other	7A		1	2	
	17A		2		
Anaerobes	7A		2	5	
	17A			5	
Cumulative %	7A	4.6	46.1	92.3	100
	17A	4.6	50.7	90.7	100

[a] 7A, BACTEC NR-7A; 17A, BACTEC NR-17A.

In the literature, the use of ARD and BACTEC 16B medium in patients receiving antibiotics is controversial. Some investigators have found that ARD had no advantage over the conventional processing of blood cultures (9, 10, 14). The same results were obtained by others with BACTEC 16B medium (3, 11). In these studies, neither the resin medium nor the treatment of blood with ARD enhanced the recovery of bacteria from blood cultures collected from patients receiving antibiotics. For example, Strand (10) found no difference between a three-bottle BACTEC blood culture system and the same BACTEC system using ARD-processed blood specimens. Hopfer et al. (5) detected more episodes of septicemia with BACTEC 16B medium but demonstrated no decrease in detection time with this medium. By using this processing, the recovery rate of bacteria was not statistically different. As with the results of work by other investigators using ARD processing and BACTEC 6B medium (1, 4, 8), our results showed a significantly increased detection of bacteria with BACTEC NR-16A medium as compared with NR-6A medium for patients receiving antimicrobial agents. The difference between the two media was highly significant for both recovery rate and mean detection time ($P < 0.001$ and $P < 0.01$, respectively). Indeed, in contrast to the results of Hopfer et al. (5), the mean detection time in our study was significantly shorter with BACTEC aerobic resin medium. However, this mean detection time

found with blood from patients receiving antibiotics was longer than that found in a previous study with BACTEC NR 660 with blood from patients receiving no antibiotic or with blood samples drawn from patients when the antimicrobial agent concentrations were at their lowest levels (2).

Some investigators (1, 11) noted that BACTEC 16B medium, in contrast to ARD, was not designed for anaerobic culture. However, Johnston Laboratories now supplies anaerobic resin medium for the BACTEC NR 660 system. Our study showed no differences in either the recovery rate of bacteria or mean detection times between BACTEC NR-7A and NR-17A media. However, the strict anaerobic atmosphere in BACTEC vials was not convenient for an optimum recovery of organisms such as *Pseudomonas* spp., *Acinetobacter* spp., or yeasts. This might explain their low growth in these anaerobic media. Thus, we cannot estimate the recovery rate and the mean detection time of aerobic and facultative bacteria. In our study, few anaerobes (approximately 5%) were detected. This percentage was lower than that (around 9%) found by Jungkind et al. (6) in a previous comparison between BACTEC 460 and NR-660. This discrepancy in anaerobe detection might be explained by either the type of pathology treated in the ward or the antibiotic treatment. We cannot draw any conclusions for anaerobes from such a result. An extensive study on this topic is required.

Because of the great number of manipulations with ARD, the likelihood of introducing contaminants into blood culture bottles was considerable. This drawback seems to be avoided with blood culture bottles containing resins. As in the studies by Appelbaum et al. (1) and by Doern and Gantz (4), we did not find more contaminants in comparing BACTEC conventional and resin media ($P > 0.05$). However, the number of *S. epidermidis* isolates recovered was greater with NR-16A than with NR-6A. This difference may be due to the existence of resins inactivating antibiotics in these vials and the absence of these resins in conventional media. Thus, blood sample contamination was better revealed with resin vials.

Lindsey and Riely (7) demonstrated that a resin device such as ARD removes antibiotics and some combinations of antibiotics and does not interfere with bacterial growth in ARD-treated blood specimens. However, patients with immunodeficiency or endocarditis were treated with a high-antibiotic-concentration regimen. For these patients, resins might sometimes be saturated with the antimicrobial agents present in the blood. Therefore, as recommended by Rodriguez and Lorian (9), blood samples should be always drawn when the antimicrobial concentrations are at their lowest levels. Moreover, the removal of each new antimicrobial agent should be checked with resin vials.

The practicability of the BACTEC resin vial met with a favorable reaction from physicians and technicians. No problems were experienced with testing procedures. These vials were easy to use and appeared less expensive than other antimicrobial inactivation methods. However, due to their cost, resin media should be used for selected patients with persistently septic courses, for example, patients receiving immunosuppressive drugs, patients with immunodeficiency or endocarditis, and transplant recipients (13).

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