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Clinical Comparison of Aerobic, Hypertonic, and Anaerobic Culture Media for the Radiometric Detection of Bacteremia

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The BACTEC 225 was used to test 5,811 routine blood cultures over a 20-month period. Aerobic, anaerobic, and hypertonic media were employed. The BACTEC 225 detected 511 positive cultures; 407 of these were considered significant organisms, and 104 were presumed contaminants. Of the significant positive cultures, 15% were detected within the first 12 h of incubation, 52% within 24 h, 82% within 48 h, and 92% within 72 h. Aerobic, anaerobic, and hypertonic media are recommended for each venipuncture since 56 cultures were isolated from the aerobic medium only, 110 from the anaerobic medium only, and 94 from the hypertonic medium only. There were 16 patients who had multiple venipunctures from which organisms were repeatedly isolated from only one medium: two from the aerobic medium, four from the anaerobic medium, and ten from the hypertonic medium only. Detection times were not significantly different for the aerobic and hypertonic media. However, there were five patients with multiple venipunctures in which growth was detected radiometrically at least 48 h earlier in the hypertonic than in the aerobic medium. False-positive growth index readings were noted in 1,085 (19%) of the aerobic vials, 11 (0.19%) of the anaerobic vials, and 104 (1.7%) of the hypertonic vials. With some false-positive aerobic and hypertonic vials, microorganisms were isolated from at least one of the companion vials. Using 5% CO_2 to flush the aerobic vials decreased the number of false positives to about 6% of the total.

The automated BACTEC 225 instrument (Johnston Laboratories, Inc., Cockeysville, Md.) is a radiometric system that measures the $^{14}CO_2$ generated by bacteria during the metabolism of ^{14}C substrates incorporated into the culture medium. This system is a reliable, fast, and accurate system for detecting bacteremias (3, 4, 7, 12, 16).

Aerobic, anaerobic, and aerobic hypertonic media are available for use in the BACTEC 225 system. Investigators have evaluated the system using either the aerobic or hypertonic medium (3, 4, 7, 12) or the aerobic and anaerobic medium (10, 14, 16). This report stresses the importance of employing all three media, aerobic, anaerobic, and hypertonic, for routine blood cultures and relates 20 months of clinical experience with the BACTEC 225 machine at Emory University Hospital, Atlanta, Ga.

MATERIALS AND METHODS

Media. Commercially prepared aerobic, anaerobic, and hypertonic media (types 6A, 7A, and 8A, respectively, Johnston Laboratories, Inc.) were employed. Each of the blood culture vials contained a basal medium of tryptic soy broth, hemin, menadione, sodium polyanethol sulfate, distilled water, and ^{14}C-labeled substrates, which have a total radioactivity of 1.5 μCi/vial. The aerobic vials contained sodium bicarbonate and the hypertonic vials contained sodium bicarbonate and 10% sucrose. Each aerobic and hypertonic vial contained a small magnetic stirring bar. The anaerobic vials were prereduced and anaerobically sterilized and contained the basal medium with yeast extract and L-cysteine hydrochloride.

The atmosphere of the aerobic and hypertonic vials was 10% CO_2 and 90% air. The atmosphere of the anaerobic vials, when they were inoculated, was 10% CO_2 and 90% nitrogen. However, after the anaerobic vials were tested on the BACTEC machine, this initial atmosphere was replaced with 80% nitrogen, 10% CO_2, and 10% hydrogen.

Inoculation and incubation of the vials. Nine milliliters of blood was drawn by the laboratory staff from the patient using aseptic technique. At the patient's bedside, 3 ml was placed into each of three vials of medium: aerobic, anaerobic, and hypertonic. Aerobic and hypertonic vials were incubated at 35 C with agitation for the initial 24 h of incubation, as recommended by Johnston Laboratories, Inc. (2), unless the blood was collected at night. If collected at night, the vials were incubated without agitation until the next morning when agitation was begun.

About 50% of the blood cultures were collected at night. All anaerobic vials were incubated without agitation.

Test protocol. On day 1, the day of blood collection, the aerobic vials were tested during the day and night on a 3-h cycle. On days 2, 3, 4, 7, and 10, these vials were tested only once during the day. The hypertonic vials were tested during the 8-h working day of day 1 on a 3-h cycle and retested once during the day on days 2, 3, 4, 7, and 10. The hypertonic vials were not tested during the night of day 1 because of the danger to the machine when excess gas production occurred (1). The anaerobic vials were tested only once during the day on days 2, 3, 4, 7, and 10.

Using an arbitrary scale of 0 to 100, the positive growth index (GI) on the BACTEC machine is ≥30 for the aerobic and anaerobic vials and ≥20 for the hypertonic vials. Detection time was calculated from the time of venipuncture to the time a positive GI was indicated. The vials were observed visually for evidence of growth before being placed on the machine and also on days 5, 6, 8, and 9, which were days the vials were not tested on the BACTEC 225 machine. All negative blood cultures were Gram stained on the final day of incubation. Routine subculturing of negative vials was not done. Caslow et al. (5) have shown that all organisms recovered on subculture were detected by the BACTEC machine within 7 days.

All vials which were either radiometrically and/or visually positive were immediately Gram stained and subcultured. Aerobic and hypertonic vials were subcultured on blood agar, chocolate agar, MacConkey agar, and thioglycollate broth. Anaerobic vials were subcultured on the same media as the aerobic and hypertonic vials, with the addition of anaerobic blood agar, anaerobic laked blood agar, and a prereduced chopped meat carbohydrate broth (Scott Laboratories, Fiskeville, R. I.). Standard biochemical tests were used for the identification of all aerobic organisms that were isolated. Identification schemes described by Holdeman and Moore (8) were used to characterize anaerobic bacteria.

Single blood cultures containing diphtheroids, coagulase-negative staphylococci, *Micrococcus*, or *Bacillus* sp. were considered contaminants. This follows the generally accepted arbitrary criterion for defining presumed contaminants (4, 14, 15).

RESULTS

Growth of microorganisms was detected radiometrically in 511 of the 5,811 blood cultures tested. Microorganisms that were regarded as contaminants were isolated from 104 of these radiometrically positive cultures. The most frequently isolated contaminant was *Propionibacterium* sp., 82 isolates. Johnson and Kaye (9) have shown that diptheroids, which can include *Propionibacterium* sp., can cause bacterial endocarditis. None of the cultures with *Propionibacterium* sp. in the present study was isolated from patients with bacterial endocarditis. Clinically significant organisms were isolated from 407 radiometrically positive cultures from 255 patients.

The detection times of various organisms isolated from blood cultures are shown in Table 1. Gram-negative rods were isolated from 237 cultures. Of these, *Escherichia coli* was the most frequent isolate (73 isolates), followed by *Pseudomonas* sp. (47 isolates) and *Klebsiella pneumoniae* (30 isolates). Gram-positive cocci were isolated from 110 cultures; 48 of these were staphylococci and 62 were streptococci. Anaerobes were isolated from 31 positive cultures. *Bacteroides fragilis* (15 isolates of *B. fragilis* subsp. *fragilis* and 3 of *B. fragilis* subsp. *thetaiotaomicron*) was the most frequent anaerobe. Yeast was isolated from eight cultures and *Listeria monocytogenes* was isolated from two cultures. In addition, there were 19 cultures from which multiple organisms were isolated. Of the 407 clinically significant organisms isolated, 15% were detected radiometrically within 12 h, 52% within 24 h, 82% within 48 h, and 92% within 72 h. In contrast, only 21% of the contaminants were detected radiometrically within 72 h of incubation. These overall detection times for the significant organisms obtained with the BACTEC 225 machine agree with other published reports (3, 4, 5, 16).

There were four cultures that were detected by visual examination but were radiometrically negative at that time. Two of these four cultures yielded *P. aeruginosa*, one culture yielded *B. fragilis* subsp. *fragilis*, and one culture yielded *Streptococcus pneumoniae*. Two additional cultures were detected only by Gram

TABLE 1. *Number of organisms isolated from positive blood cultures and radiometric detection times*

Organisms isolated	Total no.	% of cultures positive in any vial by detection time (h):			
		12	24	48	72
Significant isolates					
Gram-negative rods	237	17	56	79	90
Gram-positive cocci	110	13	53	89	97
Yeast	8	0	13	63	63
Gram-positive rods	2	0	100	100	100
Anaerobes	31	0	3	71	84
Multiple organisms	19	21	84	100	100
Total	407	15	52	83	92
Presumed contaminants	104	0	11	18	21

stain on the final incubation day. One of these cultures yielded an alpha-hemolytic streptococcus, and one yielded a beta-hemolytic streptococcus.

Radiometrically false-positive culture vials were encountered. These vials gave GI readings in the low 30s, which might be due to unusually high metabolic activity of blood cells (3, 14). During the period of study in our laboratory, 1,085 of the 5,811 aerobic vials (19%) gave a GI of greater than 30, although no organisms could be demonstrated by subculture or Gram stain. During this time period, 10% CO_2 was used to flush the aerobic vials. Following the recommendation of Johnston Laboratories, Inc., we changed from 10% CO_2 to 5% CO_2, and the number of false positives was reduced by more than a factor of 3.

False-positive GI readings were also noted in 104 (1.78%) of the hypertonic vials and 11 (0.19%) of the anaerobic vials. Some of the vials (39 aerobic vials and two hypertonic vials) that had a false-positive GI had significant organisms isolated from one of the companion vials. The radiometrically false-positive vials, aerobic, anaerobic, and hypertonic, were associated primarily with blood cultures of patients with either leukemia or carcinoma or patients who had recently undergone cardiovascular surgery.

All three media were employed throughout this study. Table 2 shows the distribution of positives in each of the three media and all possible combinations of the three media. Using the Cochran chi-square test (6), there is a statistically significant difference ($P < 0.001$) in the number of positive cultures in the aerobic medium only (56 cultures) versus the hypertonic medium only (94 cultures).

The detection times for the aerobic and hypertonic medium were equal in 185 of the 229 cultures that were positive in both media. Twenty-six cultures were detected first in the hypertonic medium and 18 were detected first in the aerobic medium. This difference is not statistically significant ($P > 0.05$). However, there were 5 patients (Table 3) of the 255 patients with multiple cultures that were radiometrically positive in the hypertonic medium at least 48 h earlier than in the aerobic medium. The difference in detection times of growth in the two media for these five patients was statistically significant ($P < 0.001$). Three of the four cultures on patient J.J., as well as all the cultures on patients J.B., A.C., and B.E., were not detected visually or radiometrically in the aerobic medium. Either the organisms were seen on the Gram stain on the final incubation day and were subsequently isolated (patient J.J.), or they were isolated from a blind

TABLE 2. *Distribution of positive cultures isolated from the three media employed*

Organisms isolated	Total no.	No. positive in a single medium			No. positive in combinations of media			
		Aerobic	Anaerobic	Hypertonic	Aerobic, anaerobic	Aerobic, hypertonic	Anaerobic, hypertonic	Aerobic, Anaerobic, Hypertonic
Gram-negative rods	237	34	13	44	5	47	10	84
Gram-positive cocci	110	6	2	30	0	45	3	24
Yeast	8	1	0	3	0	4	0	0
Gram-positive rods	2	2	0	0	0	0	0	0
Anaerobes	31	0	25	3	1	2	0	0
Multiple organisms	19	0	0	0	2	2	0	15
Contaminants	104	13	70	14	0	2	1	4

TABLE 3. *Earlier detection times with hypertonic medium in blood cultures from five patients*

Patient	Diagnosis	Organism isolated	No. of venipunctures with detection time of:	
			Hypertonic ≥ 48 h before aerobic	Hypertonic equal to aerobic
D.G.	SBE[a]	*Actinobacillus actinomycetemcomitans*	8	1
J.J.	SBE	Alpha-hemolytic streptococci	4	0
J.B.	Chronic hepatitis	Alpha-hemolytic streptococci	2	0
A.C.	Carcinoma	Beta-hemolytic streptococci	2	0
B.E.	Acquired hemophilia	*Candida albicans*	2	0

[a] SBE, Subacute bacterial endocarditis.

subculture after the hypertonic vial became radiometrically positive (patients J.B., A.C., and B. E.).

The number of patients with positive blood cultures from which organisms were isolated from one medium only is listed in Table 4. For the gram-negative rods, gram-positive rods, yeasts, and anaerobes, the difference between the number of patients with positive cultures in the aerobic medium only and the number positive in the hypertonic medium only was not statistically significant. For the gram-positive cocci, however, the difference in the number of patients who had organisms isolated from the hypertonic medium only compared with the number of patients who had organisms isolated from the aerobic medium only was statistically significant ($P < 0.001$). Streptococci were isolated from blood cultures of 13 of these patients and *Staphylococcus aureus* was isolated from blood cultures of 8 patients.

The number of patients with multiple blood cultures with the repeated isolation of the same organism from a single culture medium is shown in Table 5. The blood culture of one patient with a diagnosis of lymphoma consistently yielded *Edwardsiella tarda* in the aerobic medium only, and the blood culture of another patient with a diagnosis of heart failure consistently yielded *S. aureus* in the aerobic medium only. Blood cultures from four patients with anaerobic septicemias consistently yielded organisms from the anaerobic medium only; three of these were *B. fragilis* subsp. *fragilis* and one was *Clostridium septicum*.

Multiple blood cultures from 10 patients were culturally positive from the hypertonic medium only. These included one each of *E. coli, Citrobacter freundii, Salmonella typhimurium, Actinobacillus actinomycetemcomitans, S. aureus, S. sangius,* group A streptococci, *S. intermedius,* and two isolates of *P. aeruginosa*. The difference between the number of patients with organisms isolated from the hypertonic medium only (10 patients) or from the aerobic medium only (2 patients) was statistically significant ($P < 0.025$). Therefore, this difference in positive cultures was not a random distribution of organisms in the three media.

DISCUSSION

A comparison of the results obtained with an aerobic medium and with an identical medium made hypertonic by the addition of 10% sucrose revealed several important points. The number of false positives was significantly lower in the hypertonic than in the aerobic medium. Bannatyne and Harnett (3) reported that the hypertonic medium successfully eliminated false-positive GI readings by suppressing blood background reactivity. False-positive GI readings have been a problem for many investigators (4, 5, 10, 14). We found false-positive GI readings in only 1.76% of the hypertonic vials compared with 19% of the aerobic vials. Flushing the aerobic vials with 5% CO_2 in place of 10% CO_2 decreased this figure to about 6% of the total aerobic vials.

TABLE 4. *Number of patients with significant organisms isolated from only one culture medium*

Organisms isolated	No. of patients with positive cultures from only one medium			P (χ^2)[a]
	Aerobic	Anaerobic	Hypertonic	
Gram-negative rods	31	13	32	NS[b]
Gram-positive cocci	4	2	21	<0.001
Gram-positive rods	2	0	0	NS
Anaerobes	0	12	1	NS
Yeast	0	0	3	NS
Total	37	27	57	<0.025

[a] Aerobic versus hypertonic medium.
[b] NS, Not significant.

TABLE 5. *Patients with repeated isolation of the same organism from the same medium*

Culture medium positive	Total no. of patients	No. of patients with repeated isolation of:			
		Gram-negative rods	Gram-positive rods	Anaerobes	Yeast
Aerobic only	2	1	1	0	0
Anaerobic only	4	0	0	4	0
Hypertonic only	10	6	3	1	0

Organisms were often recovered from one medium only (Table 2). The difference in the number of cultures isolated from the hypertonic medium only (94 cultures) compared with the number from the aerobic medium only (56 cultures) was statistically significant. Hypertonic medium was shown to be particularly valuable for the isolation of gram-positive cocci.

Caslow et al. (5) previously reported that 20% of their total isolates was recovered from the hypertonic medium only. Their hypertonic medium was made with 10% sucrose in a basal medium of Columbia broth. Their report did not indicate whether the same organism was repeatedly isolated from the hypertonic medium only. In the present study, the repeated isolation of the same organism from the same patient from different venipunctures from the hypertonic medium only indicated that the hypertonicity of the medium is important and not merely random variation in recovery using a third vial.

Rosner (11) and Sullivan et al. (13) suggested that the isolation of organisms from the hypertonic medium only could be correlated to the diagnosis of the patient or the effect of antibiotic therapy. However, we were unable to demonstrate this correlation. Two patients had a diagnosis of leukemia, three patients had a diagnosis of cardiovascular problems, and one patient each had a diagnosis of carcinoma, fever, hepatic coma, or renal failure. Adequate information to evaluate the effect of antibiotic therapy on the isolation of organisms from only the aerobic medium or only the hypertonic medium was not available.

The difference of detection times for all organisms recovered from both the aerobic and hypertonic medium was not statistically significant. However, there were five patients with multiple cultures that were radiometrically positive in the hypertonic medium at least 48 h earlier than in the aerobic medium, which was statistically significant. Organisms were never detected radiometrically in the aerobic vial with multiple venipunctures from four of these five patients.

The detection of 52% positives within 24 h for all positive cultures with significant organisms in our study is lower than that reported by other workers. DeBlanc et al. (7) reported that 65% of their positive cultures were detected within 24 h. Rosner (12) reported a detection rate of 93% positive within 24 h. These discrepancies may be attributed to the medium employed and, consequently, the types of organisms isolated. DeBlanc et al. (7) used only aerobic vials in their evaluation and Rosner (12) used only hypertonic vials. Therefore, these workers did not isolate any anaerobes. Anaerobes are known to have a longer detection time with the BACTEC 225 machine than aerobic organisms (10, 14).

The slightly lower percentage of positives detected within 24 h (52%) as compared with the 62% reported by Thiemke and Wicher (14) may be explained by two factors: a difference in the definition of zero time and different conditions for agitation of the cultures. In our study, zero time was the time the cultures was collected at the bedside, whereas zero time in the other study was the hour the laboratory received the specimen. About one-half of our blood cultures was received in the laboratory at night, so they were not agitated until the next morning. Lack of agitation in the first 24 h will delay the detection of positive aerobic cultures (1, 2).

There was a significant number of organisms, both gram-positive cocci (three strains) and gram-negative rods (six strains) that was isolated repeatedly from multiple venipunctures from the same patient from the hypertonic medium only. The number of patients with multiple venipunctures with organisms repeatedly isolated from the hypertonic medium only compared with the number of patients with multiple venipunctures with organisms repeatedly isolated from the aerobic medium only was statistically significant.

The value of employing all three types of media, aerobic, anaerobic, and hypertonic, for routine blood cultures was demonstrated. If the aerobic medium had not been used, 43 isolates (10%) of the 407 total significant organisms would have been missed. If the anaerobic vials had not been used, 40 isolates (10%) would have been missed. If only the aerobic and anaerobic media had been used, 80 isolates (19%) of the total organisms isolated would have been missed.

LITERATURE CITED

1. **BACTEC data.** 8A aerobic BACTEC culture vial. JLI-620A. April 1974. Johnson Laboratories, Cockeysville, Md.
2. **BACTEC data.** Necessity for agitation of aerobic cultures. JLI-623. January 1974. Johnson Laboratories, Cockeysville, Md.
3. **Bannatyne, R. M., and N. Harnett.** 1974. Radiometric detection of bacteremia in neonates. Appl. Microbiol. 27:1067-1069.
4. **Brooks, K., and T. Sodeman.** 1974. Rapid detection of bacteremia by a radiometric system. A clinical evaluation. Am. J. Clin. Pathol. 61:859-866.
5. **Caslow, M., P. D. Ellner, and T. E. Kiehn.** 1974. Comparison of the BACTEC system with blind subculture for the detection of bacteremia. Appl. Microbiol. 28:435-438.
6. **Cochran, W. G.** 1950. Comparison of percentages in

matched samples. Biometrika 37:256-266.
7. DeBlanc, H. J., Jr., F. DeLand, and H. M. Wagner, Jr. 1971. Automated radiometric detection of bacteria in 2,967 blood cultures. Appl. Microbiol. 22:846-849.
8. Holdeman, L. V., and W. E. C. Moore (ed.). 1972. Anaerobe laboratory manual. Virginia Polytechnic Institute and State University, Blacksburg, Va.
9. Johnson, W. D., and D. Kaye. 1970. Serious infections caused by diphtheroids. Ann. N.Y. Acad. Sci. 174:568-576.
10. Renner, E. D., L. A. Gatheridge, and J. A. Washington II. 1973. Evaluation of radiometric system for detecting bacteremia. Appl. Microbiol. 26:368-372.
11. Rosner, R. 1970. Comparison of a blood culture system containing Liquoid and sucrose with systems containing either reagent alone. Appl. Microbiol. 19:281-282.
12. Rosner, R. 1974. Comparison of macroscopic, microscopic, and radiometric examinations of clinical blood cultures in hypertonic medium. Appl. Microbiol. 28:644-646.
13. Sullivan, N. M., V. L. Sutter, W. T. Carter, H. R. Attenberg, and S. M. Finegold. 1972. Bacteremia after genitourinary tract manipulation: bacteriological aspects and evaluations of various blood culture systems. Appl. Microbiol. 23:1101-1106.
14. Thiemke, W. A., and K. Wicher. 1975. Laboratory experience with a radiometric method for developing bacteremia. J. Clin. Microbiol. 1:302-308.
15. Washington, J. A., II. 1971. Comparison of two commercially available media for detection of bacteremia. Appl. Microbiol. 22:604-607.
16. Waters, J. R., and A. A. Zwarun. 1973. Results of an automated radiometric sterility test as applied to clinical blood cultures. Dev. Ind. Microbiol. 14:80-89.