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Laboratory Experience with Radiometric Detection of Bacteremia with Three Culture Media

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In two long-term studies, the BACTEC radiometric system for detection of bacteremia was evaluated with three culture media each: (i) BACTEC media 6A (for aerobes) and 7B (for anaerobes) plus a thioglycolate medium and (ii) BACTEC media 6A, 7B, and 8A (hypertonic). In study 1, clinically significant isolates were identified in 1,873 (13.9%) of 13,432 blood cultures with all three media. The thioglycolate medium revealed 143 (1.1%) organisms not recovered from the 6A and 7B media. In study 2, isolates were identified in 1,135 (12.9%) of 8,759 cultures with all three media; 104 (1.2%) organisms were isolated only from the hypertonic medium. The increased yield of positive cultures in the three-medium system is likely due to the larger volume of blood cultured.

A semiautomated radiometric method (BACTEC; Johnston Laboratories, Inc., Cockeysville, Md.) in which defined media and procedures are used was introduced two decades ago (6, 7) and has been improved throughout the years. It is now used by over 1,200 laboratories. This system has been previously evaluated, gaining favorable (1, 2, 5, 14, 18, 21–23) and less favorable (3, 13, 21, 23, 25) reviews. New media have since been introduced, and a slightly higher concentration of radioactive substrate has been added. BACTEC offers the option of either a two-medium system (for aerobic and anaerobic microorganisms) or a three-medium system, including one medium with 10% sucrose (hypertonic medium). The value of the osmotically stabilized medium has been examined in both the BACTEC system (4, 14, 21) and a nonradiometric system (8, 9, 19, 24, 26), but the results are still controversial.

We report here the results of two long-term evaluations of BACTEC with three-medium systems: (i) BACTEC aerobic and anaerobic media plus a thioglycolate medium (Thio) and (ii) BACTEC aerobic, anaerobic, and hypertonic media.

MATERIALS AND METHODS

Patient population. A total of 22,191 blood cultures from 6,232 patients were examined. The patients were being treated at the Erie County Medical Center, Buffalo, N.Y., a major university teaching hospital. Most of the patients with suspected infections were seen by the staff of the Infectious Disease Unit.

BACTEC system. The BACTEC model 225 automated instrument was used for blood examination. The system measures $^{14}CO_2$ generated by multiplying organisms that utilize ^{14}C-labeled substrates incorporated in the media.

Media. Three BACTEC media were available in 30-ml bottles: an enriched tryptic soy broth for aerobes (6A), a prereduced tryptic soy broth for anaerobes (7B), and an optional hypertonic tryptic soy broth with 10% sucrose added as an osmotic stabilizer (8A). All three media contained 1.5 to 2.0 μCi of ^{14}C-labeled substrates and sodium polyanetholesulfonate. (BACTEC now uses comparable media designated as 6B 7D, and 8B, respectively). Thio without indicator 135C was obtained in 50-ml volumes from BBL Microbiology Laboratories, Cockeysville, Md.

Test protocol. In study 1, lasting 16 months, aerobic medium 6A, anaerobic medium 7B, and Thio were used. In study 2, lasting 13 months, 6A, 7B, and hypertonic medium 8A were used.

The BACTEC media were inoculated with ca. 3 ml of blood (10% [vol/vol]), and Thio was inoculated with ca. 5 ml of blood (10% [vol/vol]). All media were inoculated by the physicians or phlebotomists and then sent to the laboratory, where the distribution of blood in the media was examined visually at arrival and recorded. The BACTEC cultures were incubated at 37°C with agitation, except that blood samples taken after 11:00 p.m. were incubated at 37°C without agitation until the next morning, when incubation with shaking was begun. The Thio cultures were incubated without shaking. Approximately 15% of the blood samples were taken during the night. The bottles were flushed with a mixture of 90% air and 10% CO_2 for growth index (GI) readings. The GI is an arbitrary scale, ranging from 0 to 100, which is linearly proportional to the amount of ^{14}C detected. GI readings of ≥30 were considered positive for blood cultures. The BACTEC cultures were examined every 3 h on day 1 and then once daily for the next 5 days. On day 5, the negative BACTEC cultures were examined after being Gram stained and were either cultured onto chocolate agar and incubated under CO_2 (6A and 8A) or cultured onto blood agar enriched with vitamin K and hemin and incubated anaerobically (7B). The Thio cultures were examined visually daily, Gram staining and subculturing were done on days 1, 3, 5, and 7 with blood agar enriched with vitamin K and hemin, and the cultures were incubated anaerobically.

Single blood cultures containing coagulase-negative *Staphylococcus*, *Bacillus*, or *Corynebacterium* spp. or *Propionibacterium acnes* were considered contaminated, even though under certain circumstances these organisms may be considered pathogens. When multiple blood samples from a single patient contained any of these organisms, their clinical significance was evaluated on the basis of the patient's condition.

RESULTS

We examined 13,432 blood cultures from 3,746 patients in study 1 and 8,759 blood cultures from 2,486 patients in study

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TABLE 1. BACTEC results for blood cultures with a three-medium system

Parameter	No. (%) in study:	
	1	2
Cultures	13,432	8,759
Patients	3,746	2,486
Positives[a]		
Cultures	1,739 (12.9)	1,063 (12.1)
Patients	754 (20.0)	473 (19.0)
Isolates recovered[b]		
Gram-positive	870 (46.4)	511 (45.0)
Gram-negative	902 (48.2)	541 (47.7)
Fungi	101 (5.4)	83 (7.3)
Total	1,873 (13.9)	1,135 (12.9)
Cultures with contaminants	173 (1.3)	153 (1.7)
False results		
False-positives	477 (3.6)	692 (7.9)
False-negatives	36 (0.3)	18 (0.2)

[a] Positive culture in at least one medium.
[b] Some cultures contained multiple isolates.

2 (Table 1). The percentages of positive cultures were similar in both studies (12.9 and 12.1%), as were the percentages of positive patients (20 and 19%). When polymicrobial bacteremia was taken into account and the results were expressed in numbers of individual isolates recovered, the percentages were again similar (13.9 and 12.9%). Finally, the percentages of contaminants recognized were similar (1.3 and 1.7%). These gross data reflect the uniformity of the conditions used in both experiments.

In an earlier publication (23), it was reported that close to 80% of positive aerobic cultures were detected by the BACTEC system within 24 h after blood cultures were submitted to the laboratory. The present studies confirm this earlier observation. In addition, ca. 70% of positive anaerobic cultures were detected within 48 h, and 75% of fungi were detected within 72 h.

The BACTEC system is not free from false-positive or false-negative results (Table 1). False-positive results, defined as having GI readings of ≥30 but having no demonstrable microorganism upon subculturing at the time of the positive reading and after day 5 of incubation, were more prevalent in study 2 than in study 1 (7.9 versus 3.6%, respectively). False-negative results, defined as having GI readings of <30 but having microorganisms recovered after terminal (day-5) subculturing, were similar in both studies (0.3 and 0.2%). These data do not include microorganisms which were considered to be contaminants. Among the cultures which were false-negatives in both studies were those containing nonhemolytic streptococci (22 cultures), *Staphylococcus aureus* (10 cultures), hemolytic streptococci (5 cultures), *Pseudomonas aeruginosa* (5 cultures), *Candida parapsilosis* (2 cultures), *Streptococcus pneumoniae* (1 culture), and a number of gram-negative bacilli (1 culture). Only a score of patients would remain undiagnosed if subcultures were not performed.

In both studies, slightly more gram-negative than gram-positive organisms were isolated (Table 1). Systemic fungi were isolated from 5.4 and 7.3% of the total positive cultures in studies 1 and 2, respectively.

TABLE 2. Effect of a third medium on BACTEC results

Study	No. of isolates recovered[a]		
	Media	Gram-positive	Gram-negative
1	6A and 7B only	822	808
	6A, 7B, and Thio	870	903
2	6A and 7B only	459	489
	6A, 7B, and 8A	511	541

[a] Fungi not included.

Comparison of the two-medium system versus the three-medium system (Table 2) indicates the advantage of the latter approach. However, the volume of blood used for culturing in both systems is of basic importance. In study 1, the inclusion of Thio allowed the isolation of an additional 143 microorganisms, or 7.6% of all 1,873 organisms isolated. In study 2, the inclusion of 8A allowed the isolation of an additional 104 microorganisms, or 9.2% of all 1,135 organisms recovered. Some of the disease-causing organisms isolated solely from the third medium are listed in Table 3.

Very similar numbers of anaerobic microorganisms were isolated from 7B and from Thio (study 1), even though Thio was inoculated with a larger volume of blood (three more isolates were found in Thio). In study 2, the hypertonic medium (8A) allowed the isolation of 3.3% more organisms than the isotonic medium (6A) (data not shown). Fungi grew as well in 8A as in 6A.

Polymicrobial bacteremia, represented by two to seven isolates in individual blood cultures, was identified in 3.6% of the patients in study 1 and in 2.9% of the patients in study 2. The most frequent combinations were (i) gram-negative

TABLE 3. Microorganisms recovered from the third medium only

Medium	Organism	No. recovered
Thio	*Bacteroides fragilis*	25
	Escherichia coli	24
	Staphylococcus aureus	16
	alpha-hemolytic streptococci	8
	Fusobacterium nucleatum	7
	Streptococcus pneumoniae	6
	Nonhemolytic streptococci	6
	Pseudomonas aeruginosa	6
	Clostridium perfringens	5
	Proteus mirabilis	5
	Enterobacter aerogenes	3
	Clostridium sp.	3
	Miscellaneous (one each)	29
8A	*Escherichia coli*	18
	Candida albicans	14
	Staphylococcus aureus	13
	Group D streptococci	8
	Klebsiella pneumoniae	8
	Proteus mirabilis	5
	Candida sp.	5
	Streptococcus penumoniae	3
	Pseudomonas aeruginosa	3
	Serratia marcescens	3
	Acinetobacter calcoaceticus	3
	Neisseria gonorrhoeae	2
	Enterobacter aerogenes	2
	Group B streptococci	2
	Nonhemolytic streptococci	2
	Miscellaneous (one each)	13

bacilli with gram-positive cocci or (ii) a variety of gram-negative bacilli. The patterns of the polymicrobial bacteremias and their clinical analysis are subjects of a separate report.

DISCUSSION

No one system can detect 100% of positive blood cultures, but some approaches are better than others.

A number of authors (9, 10, 15–17) have compared sets of three media for blood culturing, but because of variability in the sources, kinds, and volumes of the media (50 to 100 ml), in the volume of blood used (5 to 10 ml), and in the examination methods (nonradiometric systems), it is difficult to compare their results with ours. Although they used larger volumes of blood and media, the percentages of positive cultures obtained by these workers ranged from 7 to 10%, in comparison with our rates of 12.1 and 12.9%, respectively. Our recovery rates are among the highest reported in systems not using antibiotic-removing devices (11). It must be emphasized that laboratory results also reflect the patient's condition and hospital practices.

The protocol of Coleman et al. (4), who examined blood cultures by using the BACTEC 225 system with 6A, 7A, and 8A media, is the closest to ours and warrants comparison. Of the 5,811 blood cultures examined, only 7% of positive cultures were detected. For anaerobic cultures, however, these authors used 7A medium, which has been found inferior (23). For gram-positive cocci, our results confirm their finding that the hypertonic medium (8A) supported growth better than the isotonic medium (6A). Not only staphylococci but also *Escherichia coli* and *Enterobacter aerogenes* grew better in the hypertonic medium. Similar sucrose-containing media were found to be advantageous by some investigators (20, 21, 26) but not by others (8, 24). Henrichsen and Brunn (12) also observed selective growth in the hypertonic medium.

The ability to support selective growth might not be solely a property of the hypertonic medium. We observed higher recovery rates of group A streptococci from Thio and the anaerobic medium (7B) than from the aerobic medium (6A). In contrast, alpha-hemolytic streptococci and *S. pneumoniae* were more frequently isolated from 6A than from 7B or Thio, which also supports growth of aerobic organisms. Similarly, *E. coli*, *Haemophilus influenzae*, *Proteus mirabilis*, and *P. aeruginosa* were isolated in larger numbers from 6A than from Thio.

The volume of blood inoculated is a major factor in the recovery of microorganisms. However, selective growth conditions for certain microorganisms, especially those isolated from patients receiving medication and antibiotic treatment, must also be taken into consideration. Such a situation would definitely favor the use of media with various growth-supporting conditions.

From our gross results it is evident that whether the third medium is Thio or hypertonic medium, the percentage of recovery is similar, indicating that the increased yield in positive cultures is influenced by the increased volume of blood cultured in the three-medium system. In the first study, Thio allowed the recovery of 143 additional clinically significant isolates, or 1.1% of the total. Of these isolates, 40 (ca. 28%) were anaerobes, and the rest were aerobes. Not surprisingly, Thio itself "missed" 266 isolates which were recovered from 6A or 7B. In the second study, 104 clinically significant isolates, or 1.2% of the total, were recovered only from 8A.

In short, the results suggest that it does not make much difference whether 3 ml is cultured in 30 ml of medium or 5 ml is cultured in 50 ml of medium. However, it does make a difference whether a total of 6 to 8 ml of blood (for the two-bottle system) or 9 to 12 ml of blood (for the three-bottle system) is used. The additional expense of the third bottle is well justified by the higher percentage of positive blood cultures detected, which benefits patients, is well received by clinical staffs, and is silently absorbed by the administration.

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