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Comparison of the BACTEC System with Blind Subculture for the Detection of Bacteremia

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One thousand blood specimens were cultured in BACTEC vials containing modified Columbia broth in aerobic, anaerobic, and hypertonic formulations. Radiometric readings and subcultures were performed on aerobic and hypertonic vials at 24 h and 7 days, and on anaerobic vials at 48 h and 7 days. Significant numbers of false-positive BACTEC readings were obtained. Although all positive cultures were eventually detected by the BACTEC, approximately 20% of blood specimens yielding positive subcultures at 24 h did not give positive BACTEC readings until 48 h.

In recent years, studies have appeared reporting the radiometric detection of bacteremia. DeLand and Wagner (2) first reported a technique for the radiometric detection of microorganisms in blood cultures and subsequently described an automated device for this purpose (3). In a number of clinical studies, they found the radiometric method to be faster than conventional techniques and comparable in accuracy (1, 3). Other investigators have also noted slightly earlier detection of growth by using the radiometric technique (6). The importance of the 24-h subculture in the early detection of bacteremia has been clearly established. Since only 10% of all blood cultures become positive,, however, plating of the remaining cultures represents a significant expenditure of time, effort, and materials. Radiometric readings at 24 h could provide an indication of the bottles requiring subculture. The present study was undertaken to determine whether radiometric readings can be used as a reliable indication for selective subculturing of blood cultures as a substitute for the routine blind subculture.

MATERIALS AND METHODS

This study was performed using the BACTEC 225 provided by Johnston Laboratories, Inc. (Cockeysville, Md.). Details of this automated instrument have previously been described (1).

The machine was used with two different gas sources. Ten percent CO_2 in air was used with the aerobic and hypertonic vials, and a mixture of nitrogen, carbon dioxide, and hydrogen (85:10:5) was used with the anaerobic vials.

Media. All vials contained 30 ml of a basal medium consisting of Columbia broth modified by the addition of 0.05% cysteine and the ^{14}C-labeled substrate. (The ^{14}C-labeled substrate consisted of a proprietary mixture of approximately 1.5 μCi of labeled dextrose and simple alcoholic, carboxylic, and amino compounds.) Glucose (0.25%) was added to the basal medium in the anaerobic vial. Sucrose (10%) was added to the basal medium in the hypertonic vial.

All media used was supplied by Johnston Laboratories and was prepared from dehydrated material (Bioquest). Each aerobic and hypertonic vial contained a small magnetic stirring bar.

Quality control of media. All vials were tested for sterility by incubating them at 35 C for 24 h prior to inoculation. The ability of the broth to support the growth of small inocula was determined by the following procedure. Replicate sets of aerobic vials were inoculated with 0.3 ml of a broth culture of *Diplococcus pneumoniae* or *Streptococcus* viridans group. These cultures were diluted to concentrations of approximately 100, 10, or 1 organism per ml. Anaerobic vials were similarly tested by using *Bacteroides fragilis* subsp. *thetaiotaomicron*. Vials were incubated for 72 h. Growth was determined by turbidity and appropriate subculture.

A single batch of each medium was used throughout the entire study.

Test protocol. Blood specimens for culture were submitted to the Bacteriology Laboratory in vacutainer tubes (165 by 16 mm) containing sodium polyanethol sulfonate (4). A 9-ml amount of blood was withdrawn from the vacutainer tube with a sterile syringe and inoculated into the aerobic, anaerobic, and hypertonic vials in 3-ml amounts. The aerobic vials were immediately placed on the BACTEC machine where they were incubated at 35 C and continuously stirred. The anaerobic vials were placed in a conventional incubator at 35 C without agitation. Hypertonic vials were placed in an incubator-shaker and agitated at 200 rpm.

Radiometric readings (growth indices) were taken

on the aerobic vials at 3-h intervals up to 24 h. Anaerobic and hypertonic vials were placed on the BACTEC the following morning (16 to 24 h) and read. Subsequent radiometric readings on all vials were performed at 48 h and at 7 days.

Aerobic and hypertonic vials were routinely subcultured at 24 h by withdrawing 0.1 ml of the culture with a sterile syringe and inoculating chocolate agar plates. Plates were incubated at 35 C in 10% CO_2 for 48 h before being discarded as negative. Anaerobic vials were subcultured in a similar manner using anaerobic blood agar plates which were incubated in an anaerobic jar (5). All vials were routinely subcultured again at 7 days. Vials becoming turbid during the course of 7 days were read on the machine. Table 1 lists the growth indices which were used as threshold values.

RESULTS

Quality control of media. Only five vials of a lot of 3,000 were found to be contaminated prior to inoculation. Growth in the aerobic vials could be initiated with inocula of less than ten *Diplococcus pneumoniae* or *Streptococcus* viridans group. Similarly, growth in the anaerobic vial resulted from the inoculation of less than ten *Bacteroides fragilis* subsp. *thetaiotaomicron.*

Results of evaluation. A total of 1,000 blood cultures were tested. One hundred and four isolates were recovered from 97 cultures (9.7%). All organisms recovered on subculture were eventually detected by the BACTEC. The isolates are listed in Table 2.

The comparative results of BACTEC readings and blind subculture of all vials at 24 h are summarized in Table 3. Positive growth indices occurred with 73 of the aerobic vials. Twenty-six of these vials failed to yield organisms on subculture and were considered to be false-positives. The growth indices of these false-positive vials were noted to decline after reaching positive levels; in contrast, elevated BACTEC readings were maintained by the 47 vials that were positive on subculture. False-positive readings were only seen with one of the anaerobic and eight of the hypertonic vials. Thirteen of the 927 aerobic vials and 14 of the 935 hypertonic vials with negative BACTEC readings demonstrated growth on subculture. The organisms recovered from these vials are listed in Table 4. Three of the isolates in the aerobic vials were detected radiometrically in companion vials at 24 h. The remaining 10 cultures not detected by the BACTEC at 24 h gave positive growth indices at 48 h. The 14 isolates from BACTEC negative hypertonic vials, however, failed to show positive readings in companion vials at 24 h. Thirteen of these vials subsequently became positive at 48 h.

The results obtained with the anaerobic vials at 48 h are shown in Table 5. There were no false-positives. Bacteria were recovered on subculture from 13 vials that gave negative growth indices at 48 h. Three of these (*Bacteroides fragilis* subsp. *distasonis*, *Staphylococcus epidermidis*, and *Bacillus* sp.) grew only in the an-

TABLE 1. *Threshold values used for various vials*

Vial	Time		
	24 h	48 h	7 days
Aerobic	35	35	35
Anaerobic	30	30	30
Hypertonic	20	20	35

TABLE 2. *Isolates recovered from blood cultures*

Isolate	No.
E. coli	16
K. pneumoniae	9
S. marcescens	1
P. mirabilis	2
P. aeruginosa	8
P. maltophilia	1
B. fragilis subsp. *distasonis*	1
B. fragilis subsp. *fragilis*	2
B. fragilis subsp. *thetaiotaomicron*	1
C. perfringens	1
P. acnes	1
N. meningitidis	1
S. aureus	11
S. epidermidis	13
Micrococcus sp.	1
Streptococcus viridans group	3
S. faecalis	5
S. durans	1
S. bovis	1
S. faecium	1
S. pneumoniae	4
Beta-hemolytic *Streptococcus*	4
Yeasts	2
Diphtheroids	3
Bacillus sp.	11

TABLE 3. *Comparison of BACTEC reading and blind subculture at 24 h*

Readings	Aerobic vials	Anaerobic vials	Hypertonic vials
Positive BACTEC readings	73	20	65
Positive subculture	47	19	57
Negative subculture	26	1	8
Negative BACTEC readings	927	980	935
Positive subculture	13	5[a]	14
Negative subculture	914		921

[a] These five were detected by turbidity.

aerobic vial and did not become BACTEC positive until the third or fourth day. Growth in the remaining 10 cultures was detected radiometrically in companion vials by 48 h. The organisms included streptococcal species, *S. epidermidis*, and *Pseudomonas aeruginosa*.

Radiometric findings and subculture results of all vials from days 2 to 7 are shown in Table 6. Significant numbers of false-positive readings were obtained with the aerobic and hypertonic vials. There were no false-negatives in the aerobic or hypertonic vials, but three *Pseudomonas* isolates and a *Streptococcus viridans* group were recovered from anaerobic vials with negative readings. These four organisms were detected radiometrically in companion vials.

DISCUSSION

Previous studies comparing the efficiency of radiometric detection of bacteremia and conventional techniques have been performed with two separate systems (6, 7). In these studies, the blood was distributed between a BACTEC vial for radiometric detection and one or more bottles of broth which were observed for turbidity and subcultured. An important feature of the present evaluation was that both radiometric readings and subculture were performed on the same vials. These vials contained modifications of Columbia broth that had previously been utilized in our laboratory. The increased cysteine concentration appeared to substitute for the glucose that was omitted for the purposes of this evaluation.

TABLE 4. *Organisms recovered from vials with negative growth indexes and positive subcultures at 24 h*

Vial	No. of isolates[a]
Aerobic	
P. aeruginosa	3
P. maltophilia	1
E. coli	2
K. pneumoniae	2
S. epidermidis	2
S. aureus	1
S. aureus and *S. faecalis*	1
S. epidermidis and *S. marcescens*	1
Hypertonic	
P. aeruginosa	3
P. maltophilia	1
E. coli	1
S. faecalis	3
S. epidermidis	1
Yeasts	1
Diphtheroids	1
Bacillus sp.	3

[a] Four of these isolates were common to both vials (*P. aeruginosa*, 2; *P. maltophilia*, 1; *S. faecalis*, 1).

TABLE 5. *BACTEC readings and blind subculture of anaerobic vial at 48 h*

Reading	No.	
Positive BACTEC readings	8	
Positive subculture		8
Negative subculture		0
Negative BACTEC readings	927	
Positive subculture		13
Negative subculture		954

TABLE 6. *Comparison of BACTEC reading and blind subculture during remaining 7 days*

Reading	Viral		
	Aerobic (2–7 days)	Anaerobic (3–7 days)	Hypertonic (2–7 days)
Positive BACTEC readings	127	4	23
Positive subculture	11	4	8
Negative subculture	116	0	15
Negative BACTEC readings	787	950	898
Positive subculture	0	4	0
Negative subculture	787	946	898

A considerable amount of laboratory time was involved in subculturing the false-positive aerobic vials. By raising the threshold to 45 at 7 days, the number of false-positive vials would be reduced by 80% without influencing the detection of positive cultures.

Although 22 isolates were recovered only from the medium containing 10% sucrose, it still remains unclear whether this was due to the hypertonicity of the medium or merely represents random variation in recovery using a third vial.

In this study, the overall recovery of organisms was 9.7%, 6% of which were anaerobes. These figures are somewhat lower than the overall recovery rate of 10.4%, 10% of which were anaerobes, normally experienced by our laboratory. These decreases may reflect the absence of glucose in the aerobic and hypertonic vials, the reduction in inoculum from 5 to 3 ml, or may merely be statistically insignificant and attributable to the number of cultures sampled.

Our results have shown that 22% of the aerobic vials and 19% of the hypertonic vials contained bacteria demonstrable on routine subculture that were not detected radiometrically at 24 h. There were also three anaerobic

vials that contained organisms which were not detected radiometrically at 48 h. One of these organisms was a *Bacteroides*. Although all positive cultures were eventually detected radiometrically, it is apparent that reliance upon BACTEC readings as a substitute for routine subculture may impose a 24-h delay in the detection of bacteremia.

The authors believe that for a laboratory report to be of clinical significance it must at least contain a description of the Gram reaction and morphology of the isolate and that early identification is essential. It has been proposed that the results of a Gram stain prepared from a vial in which a positive BACTEC reading was delayed until 48 h is equivalent to information based upon the examination of colonies derived from a 24-h subculture. However, these colonies also afford detection of mixed cultures, permit the differentiation of aerobes and anaerobes, and often provide presumptive identification based upon colonial morphology.

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