Radiometric Detection of Bacteremia: Requirement for Terminal Subcultures

ROBERT R. STRAUSS,* RICHARD THROM, AND HERMAN FRIEDMAN

Department of Microbiology, Albert Einstein Medical Center, Philadelphia, Pennsylvania 19141

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A radiometric procedure for rapid detection of bacteria in clinical blood specimens was utilized over a period of 1 year in this laboratory. Although in initial studies it was felt that all positive bacteremias would be detected by radiometric examination of cultures for $^{14}\text{CO}_2$ evolution over a 7-day period, we found in the present study that a significant number of bacteria were not detected, except by blind subculturing on day 7 before discarding the culture sample. Microorganisms were detected in 490 individual specimens from 348 patients after examination of 6,200 individual blood specimens, both anaerobically and aerobically. All but 30 of the positive specimens were detected by the radiometric procedure, with an average detection time of 30.5 h. Thirty organisms, representing 6% of the total organisms isolated, were not detected by the BACTEC apparatus. The predominating organisms missed by the radiometric method were group D streptococci, both enterococcal and non-enterococcal species. These isolates represented two-thirds of the total number of organisms not detected by the radiometric procedure. A majority of bacteria detected only upon terminal subculture were isolates from a very small number of patients, suggesting that these organisms might have specific properties that preclude their detection by radiometric assay. Nevertheless, our study results indicate that it is essential that all radiometrically examined blood culture specimens be subcultured prior to discard in order to lessen the likelihood of missing a microbial pathogen.

Radiometric procedures for rapid detection of bacteremia are now relatively well established (1, 2, 5, 8). For example, the BACTEC 225 apparatus (Johnston Laboratories, Cockeysville, Md.), an automated instrument that can measure $^{14}\text{CO}_2$ produced from ^{14}C -labeled substrates in bacteriological culture medium as an indicator of bacterial metabolism, has been utilized in clinical microbiology laboratories for blood culture studies (1-9). In this laboratory, routine use of the BACTEC apparatus over a 2-year period permitted detection of bacteremia approximately three times more rapidly than by conventional culture methods. In initial studies, it was observed that all positive bacteremias were detected by the radiometric procedure within 7 days of culture initiation (R. Throm, R. Strauss, and H. Friedman, *Abstr. Annu. Meet. Am. Soc. Microbiol.* 1975, C 30, p. 32). Terminal blind (7 day) subculture of nearly 1,000 patient blood specimens, radiometrically negative during this initial study, failed to reveal any positive cultures (i.e., false-negative results). However, we subsequently observed that in some instances blind subculture on the 7th day after culture initiation re-

vealed a number of positive isolates from specimens that were considered negative by the radiometric analysis during the preceding 7 days of daily testing. This observation is contrary to some previous reports in the literature (3, 4). In the present study, 6,200 consecutive blood culture specimens were examined by the radiometric method with the BACTEC 225 apparatus. All were routinely examined by terminal blind subculturing prior to discarding the culture bottles. Nearly 500 culture specimens were positive by the radiometric assay, representing specimens from about 350 individual patients. However, an additional 30 positive cultures were obtained from 15 patients upon terminal subculturing. This occurred despite the negative results obtained by the radiometric procedure, indicating that the automated apparatus failed to detect approximately 6% of the total number of positive cultures. Group D streptococci accounted for most of the organisms undetected by the radiometric procedure.

MATERIALS AND METHODS

Medium. Commercial blood culture bottles, containing ^{14}C -labeled substrates, were purchased from

Johnson Laboratories. Aerobic bottles were designated type 6A, and anaerobic bottles were designated type 7A. Basal medium for each type of culture bottle consisted of tryptic soy, hemin, menadione, sodium polyanethol sulfonate, ^{14}C -labeled substrates (approximately $1.75 \mu\text{Ci}$ per bottle), and distilled water to 30 ml. The aerobic bottles contained sodium bicarbonate, whereas the anaerobic bottles were prereduced and contained yeast extract and L-cysteine-HCl in addition to the basal medium. The atmosphere for the aerobic bottles consisted of 5% CO_2 and 95% air, and that for the anaerobic bottles consisted of 10% CO_2 and 90% nitrogen.

Blood collection, inoculation, and incubation procedures. Blood was collected at the patient's bedside by aseptic technique with a BD Vacutainer containing saline and sodium polyanethol sulfonate. Approximately 8 to 9 ml of venous blood was drawn by the blood-collecting team and delivered to the laboratory as rapidly as possible. Laboratory personnel aseptically transferred 3 ml of blood each to an aerobic and an anaerobic bottle. All specimens collected from 9:00 a.m. through 12 midnight were cultured in aerobic vials and placed on the BACTEC apparatus for examination on a 1-h cycle until 4:00 p.m., after which a 3-h cycle was run. These bottles were removed from the machine at 8:00 a.m. the next morning. The blood cultures were then tested once daily over a 7-day period. Specimens inoculated into anaerobic bottles were incubated at 35°C overnight and tested once daily for 7 days on the BACTEC apparatus. After 7 days, all culture bottles that were negative radiometrically were subcultured onto blood agar plates (Trypticase soy agar with 5% sheep blood) aerobically and anaerobically (GasP), chocolate agar plates (10% CO_2), and thioglycolate broth.

Culture bottles were subcultured for bacterial isolation if suspected positive by visual examination (i.e., turbidity and/or hemolysis evident) or by radiometric analysis if the BACTEC growth index was 30 or greater. All suspected positive culture bottles were immediately examined by Gram stain technique and subcultured onto blood agar and thioglycolate broth. Standard laboratory procedures were used for identification of all positive isolates.

Group D streptococci were identified by their reaction on bile-esculin plates (without azide). Enterococci were further identified by their ability to grow in the presence of 6.5% NaCl and at 42°C . Bile-esculin-positive streptococci that were unable to grow under these conditions were considered to be non-enterococcal group D organisms.

RESULTS

During a 12-month period ending 31 October 1975, a total of 6,200 blood specimens were submitted to the laboratory for bacteriological examinations. The specimens were obtained from over 5,000 patients admitted for various medical and surgical procedures. Microorganisms were detected in 490 individual specimens obtained from 348 patients. All but 30 of the posi-

tive specimens were first detected by the radiometric procedure. Although each specimen was observed daily for 7 days with the BACTEC 225 apparatus, all specimens found positive radiometrically showed significant $^{14}\text{CO}_2$ evolution within the first 5 days. Table 1 indicates the total number of specimens cultured, the number and percentage of specimens found positive for pathogens or presumed contaminants, and the number and percentage of positive specimens that were not detected by the BACTEC apparatus but showed positive bacterial growth on the day-7 subculture. As shown in Table 1, approximately 8% of the specimens examined were positive. Seventeen of these positive cultures, or 0.27% of the total number, appeared to be contaminants by the usual criteria. Most significant, however, was the observation that 30 positive specimens, i.e., approximately 6% of all positives, were not detected by the radiometric-assay procedure.

A compilation of the different classes of organisms isolated from all positive blood culture specimens detected by the BACTEC 225 is presented in Table 2. These results are generally similar to those from other laboratories that use the radiometric procedure for detecting microorganisms on blood culture specimens. Approximately 94% of all organisms were detected by the BACTEC apparatus, with an average detection time of 30.5 h. For those 30 specimens in which organisms were not detected by the

TABLE 1. Number and percentage of positive blood culture isolates detected by radiometric procedure with the BACTEC 225 apparatus

Determinations	No.	%
Specimens cultured	6,200	
Positive microbial isolates	490	7.9 ^a
Isolates presumed contaminants	17	0.27 ^a
Isolates not detected by BACTEC apparatus	30	6.1 ^b

^a Percentage of total specimens cultured.

^b Percentage of total number of isolates.

TABLE 2. Types of microorganisms isolated from positive blood specimens by radiometric assay with the BACTEC 225 apparatus

Type of organism	No. of isolates	Percent of total isolates
Gram-positive cocci	214	43.67
Gram-negative cocci	2	0.42
Gram-positive bacilli	16	3.06
Gram-positive bacilli	211	43.06
Anaerobic bacteria	37	7.55
Yeasts	11	2.24

BACTEC apparatus, the detection time took 8 days or longer. None of these bottles was positive by visual examination after 7 days of radiometric testing, thus necessitating subculture by conventional methods.

The types of organisms detected by subculture on day 7 and missed by the BACTEC apparatus, as well as the numbers of such organisms, are presented in Table 3. Gram-positive and gram-negative microorganisms, including both aerobic and anaerobic bacteria as well as yeasts, are represented in this group of organisms. Marked differences in colonial morphology or staining characteristics were not noted at the time of isolation. However, the predominating organisms were group D streptococci, both enterococcal and non-enterococcal varieties. This group comprised 67% of the total number of the organisms not detected by the BACTEC apparatus.

A list of the various streptococci isolated from blood specimens submitted for culture is shown in Table 4. These data show that, although many different species of streptococci were isolated from blood culture specimens during this study, the ones that were not detected by the radiometric procedure were those classified in the Lancefield group D category. Over 50% of the enterococci and 25% of the non-enterococcal group D isolates were missed by the BACTEC apparatus and were only detected because of the 7-day routine subculture procedure used in this study.

DISCUSSION

The radiometric detection of bacteremia utilizing the automated BACTEC 225 system has been found to be both rapid and relatively efficient (1-9). In the present study, the average detection time for all microorganisms isolated from over 6,000 blood specimens over a 1-year period was 30.5 h. This is approximately three

TABLE 4. *Streptococci isolated from positive blood culture specimens*

Type	No. of isolates	No. not detected by BACTEC
Group A streptococci	1	0
Group B streptococci	6	0
Enterococci	32	17
Non-enterococcal group D streptococci	12	3
Untyped β -hemolytic streptococci	3	0
α -Hemolytic streptococci, "Viridans group"	19	0
<i>Streptococcus pneumoniae</i>	9	0
Nonhemolytic streptococci (not group A, B, or D)	1	0
Peptostreptococcus	1	0

times faster than the conventional culture procedure utilized in this laboratory with two bottles, i.e., aerobic and anaerobic, for blood culture examinations. Furthermore, none of the more than 450 bacterial isolates detected by the automated radiometric procedure took more than 5 days. Among the organisms detected were aerobic and anaerobic bacteria, both gram positive and gram negative, as well as yeasts. It is noteworthy that the majority of positive cultures were detected within 24 h after the blood culture specimen was first received in the laboratory. Indeed, many cultures were found positive within the first 4 h, providing a distinct advantage for initiating patient treatment since identification of the organism and antibiotic susceptibility testing could often be complete within an 18- to 24-h period.

Although initially it was felt that the use of the automated radiometric procedure would permit detection of all positive bacteremias within 4 to 5 days and thus eliminate the need for subculturing, the present study revealed that it is quite important to subculture all negative bottles prior to discard. Despite the increased costs involved, terminal subculture is important to prevent overlooking radiometrically undetected bacteria. Examination of over 6,000 cultures showed that 30 blood culture bottles were positive upon terminal (7 day) subculturing, although they were negative by the radiometric technique. Many species of bacteria were found among these 30 positive cultures. However, group D streptococci were the predominating organisms detected (20 of 30 specimens). Seventeen of these isolates were identified as enterococci, and three were identified as non-enterococcal group D bacteria. Furthermore, it is noteworthy that 53% of the en-

TABLE 3. *Microorganisms in blood culture specimens not detected by radiometric procedure*

Organism	No. isolated	No. not detected	Percent not detected
<i>Bacillus</i> sp.	16	2	12.50
<i>Bacteroides</i> sp.	29	1	3.45
<i>Candida albicans</i>	9	2	22.22
<i>Candida guilliermondii</i>	1	1	100.00
<i>Escherichia coli</i>	102	1	0.22
Enterococci	32	17	53.13
Non-enterococcal group D streptococci	12	3	25.00
<i>Pseudomonas aeruginosa</i>	16	1	6.25
<i>Staphylococcus aureus</i>	96	2	2.08

terococci detected in blood culture bottles during this 1-year study were not detected by the BACTEC apparatus. The undetected enterococci were isolated only after standard subculturing at the end of the 7-day incubation period prior to discarding the bottles. The reason for this discrepancy between the radiometric method and conventional subculture method is not apparent, especially since the results are based on a retrospective study and isolates of the organisms are not available for further examination.

It should be noted that of the 17 isolates of enterococci not detected by the BACTEC apparatus, 12 were obtained from a single patient. Furthermore, all of the non-enterococcal isolates not detected by the BACTEC apparatus but found on subculture were also derived from a single patient, as were the two isolates of *Candida albicans*. Thus, there may be specific strain differences between those organisms detected radiometrically and those that were not detected by this method but were isolated only upon conventional subculture. The most likely differences might be metabolic uniqueness or excessive fastidiousness, which preclude detection by the radiometric procedure. In addition, improper or incomplete inactivation of antibiotics could also be responsible for decreased bacterial metabolism in the culture medium containing the radioisotope. This factor might prevent detection of certain organisms by the radiometric method. Regardless of the underlying reason for the discrepancies observed in this study, it is important to note that 6% of the microbial isolates obtained from 6,200 blood specimens were not detected by the radiometric

procedure. This does not detract from the value of the method using the radioisotopic-detecting apparatus, since 94% of the isolates were detected, and in all cases detection occurred much sooner than could be expected by conventional blood culture procedures. However, the results of this study indicate that all radiometric-negative blood cultures should be subcultured before discarding to eliminate the possibility of missing the presence of an important pathogenic microorganism.

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