Rapid Detection of Bacterial Growth in Blood Samples by a Continuous-Monitoring Electrical Impedance Apparatus

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A growth detection method utilizing an automated apparatus capable of rapidly detecting bacterial growth by measuring changes of electrical impedance in bacteriological medium was utilized with "mock" blood cultures containing various gram-negative and gram-positive bacteria. Measurement of changes of electrical impedance was found to be as accurate and comparable for time of growth detection as the radiometric method for detection of the same bacteria using mock blood cultures. In a limited clinical trial the use of the electrical impedance apparatus detected the 1 positive specimen from 40 clinical blood specimens as rapidly as by radiometric measurement. Both methods were considerably faster for detecting bacterial growth as compared with conventional culture methods. The selected species of gram-positive and -negative organisms tested were all detected by the electrical impedance method, including aerobes and anaerobes. However, addition of 5% CO₂ to the incubation atmosphere enhanced detection of gram-positive organisms.

Rapid detection of bacteremia is a major goal in clinical microbiology laboratories and of infectious disease personnel. The availability of mechanized and semiautomated equipment in recent years has greatly facilitated the monitoring of blood cultures for bacterial growth from patient specimens. For example, an automated radioisotopic detection system (Bactec, Johnson Laboratories, Cockeysville, Md.) has been developed recently which measures ¹⁴CO₂ formation by bacteria growing in radioisotope-containing medium (3, 4, 7, 8). This apparatus, however, depends upon batch-type detection; i.e., 25 bottles at a time are sampled over 20-min periods by an automated radioisotope-detecting system. Each bottle must be sampled individually by the same detector. An additional limitation is that sampling occurs in cycles of 1 to 4 h. When laboratory personnel are not present, trays of samples are not changed, and the detection cycle for more than 25 samples ceases.

An apparatus based on continuous monitoring of electrical impedance changes has been developed recently (Bactometer 32, Bactomatic, Inc., Palo Alto, Calif.). This apparatus permits continuous detection of alteration of impedance ratios in culture medium due to bacterial metabolism and growth (P. Cady and S. W. Dufour, Abstr. Annu. Meet. Am. Soc. Microbiol. 1974, E43, p. 8; W. K. Hadley, G. Senyk, R. Michaels, and J. Seman, Abstr. Annu. Meet. Am. Soc. Microbiol. 1974, M281, p. 113). Each sample bottle is equipped with detection electrodes so

that stepwise or batch-type sampling is eliminated. Furthermore, a prototype of the Bactometer has been developed which reportedly can monitor several hundred specimens simultaneously. In the present study the efficacy of the 32-sample electrical impedance apparatus for detecting bacterial growth in blood culture medium, to which were added "mock" blood samples "contained" with known numbers and species of bacteria, was determined. Preliminary evaluation was also made of the ability of the electrical impedance apparatus to detect bacteria in clinical specimens from 40 hospitalized patients.

MATERIALS AND METHODS

Bacteriological samples. Known stock cultures of gram-negative and gram-positive bacteria, including both anaerobes and aerobes, were used for mock blood cultures (Tables 1 and 2). The bacterial strains were cultured by standard techniques in appropriate medium. Washed suspensions of pure bacterial cultures were harvested in the log phase of growth by centrifugation. The bacteria were resuspended in brain heart infusion broth to a standard concentration (ca. 10⁷ organisms per ml initially) as determined by quantitative colony counts and 10-fold serial dilutions prepared in broth. An inoculum of 1.0 ml of a bacterial suspension containing approximately 100 to 200 bacteria for the gram-negative organisms or approximately 200 to 500 bacteria for the gram-positive organisms was added to 9 ml of a sterile blood sample from normal laboratory personnel or, occasionally, to human blood specimens from the blood bank or from

patients without detectable bacteremia as determined by prior culture on conventional culture medium. This resulted in a further 1:10 dilution of the bacterial suspension. For the anaerobes, the bacteria were diluted in a thioglycolate broth, and a 1.0-ml suspension containing approximately 100 to 1,000 bacteria was added to 9.0 ml of the sterile blood sample.

Electrical impedance monitoring. The Bactometer 32 apparatus was utilized for this study. Three milliliters of the mock blood cultures in blood culture bottles provided by Bactomatic, Inc., was used to obtain a final 1:100 dilution of the initial diluted bacterial suspensions. In addition, 3-ml blood samples from 40 patients being tested by the radioisotopic apparatus technique (see below) were also included in the Bactometer tests. All samples were monitored for bacterial growth continually at 90-s intervals for 4 to 5 days after attachment of the electrodes to the apparatus. The samples containing aerobic bacteria were incubated at 37°C in an atmosphere of air plus 5% CO₂. Samples containing anaerobic bacteria were incubated in an atmosphere of 10% CO₂-5% H₂-85% N. In all cases, at least six to eight different mock blood cultures for each bacterial species were tested on different days in duplicate or triplicate. Detection times were measured and recorded as the time of increased ratio of impedance change as shown on the strip chart recorder, $\pm$ standard error, rounded off to the nearest 0.5 h.

Radioisotope assay. For comparative purposes many of the bacterial species tested by the electrical impedance method in the mock blood cultures were also tested in the Bactec apparatus, utilizing commercially prepared culture bottles provided by Johnson Laboratories. Media 6A and 7B with substrates incorporating ¹⁴C-labeled amino acids and glucose were utilized, with aerobic conditions being utilized for medium 6A and anaerobic conditions for medium 7B (3, 7). The aerobic samples were monitored at hourly intervals for the first 8 to 12 h and then tested once daily for 6 additional days. Anaerobic samples were tested after 24 h of incubation and once daily for 6 more days. Samples of blood from the same patients

being tested for bacteremia by the Bactometer apparatus were also assessed for bacterial growth by the Bactec method. All Bactometer- and Bactec-negative patient blood culture specimens were tested for the presence of bacteria by conventional culture methods at termination of the sampling period on days 5 to 7.

Conventional blood culture procedure. Ten milliliters of either a mock-infected blood specimen or a patient specimen was placed in 50 ml of Trypticase soy broth under both aerobic and anaerobic conditions. Subculturing of 0.1-ml samples was performed on solid media at 48 h and 7 days, using blood agar, phenylethyl alcohol, and chocolate agar plates, which were incubated both aerobically and anaerobically (5, 6, 9). Colonies of microorganisms developing upon subculture were identified by standard means. All culture bottles were examined visually daily, starting on day 2 of culture (18 to 24 h), and all bottles showing possible bacterial growth were examined by the Gram stain technique and subcultured by conventional methods (6, 9).

RESULTS

All mock blood cultures containing known strains of aerobic bacteria resulted in positive growth detection within 6 to 18 h of incubation. These detection times were observed when 10 different species of bacteria were tested, including both gram-negative and gram-positive aerobic organisms (Table 1). As determined by direct pour plate colony assays, all initial inocula of aerobic bacteria used to "contaminate" the blood samples contained approximately 80 to 150 organisms per ml of medium. Since 1.0 ml of each washed bacterial suspension was added to 9 ml of sterile blood, the inoculum sizes ranged from 8 to 15 bacteria per ml of whole blood. The subsequent 1:10 dilution in the Bactometer culture medium resulted in a further reduction of inoculum sizes to 0.8 to 1.5 bacteria per ml

TABLE 1. Aerobic organisms detected in mock blood culture specimens by the electrical impedance Bactometer procedures

Organism tested ^a	Inoculum size (bacteria/ml of medium)	Avg detection time (h)	
		CO ₂ gassed ^b	Air
Gram negative ^c			
<i>Enterobacter cloacae</i>	8 ± 2		8.5 ± 1.5
<i>Klebsiella pneumoniae</i>	9 ± 4		6.0 ± 1.5
<i>Serratia marcescens</i>	14 ± 3		9.0 ± 2.0
<i>Pseudomonas aeruginosa</i>	15 ± 6		7.5 ± 1.5
<i>Escherichia coli</i>	9 ± 4		6.0 ± 1.0
<i>Proteus vulgaris</i>	7 ± 3		8.5 ± 1.5
Gram positive ^d			
<i>Staphylococcus aureus</i>	20 ± 5	14.0 ± 2.0	18.0 ± 2.0
<i>Streptococcus</i> , α-hemolytic	39 ± 8	9.0 ± 1.5	16.0 ± 2.5
<i>Streptococcus</i> , β-hemolytic	50 ± 16	7.5 ± 1.0	14.0 ± 1.5
<i>Streptococcus pneumoniae</i>	30 ± 12	7.0 ± 1.5	12.0 ± 2.0

^a Lyophilized stock cultures maintained in this laboratory.

^b Blood bottles gassed with 5% CO₂-95% air.

^c Average for 8 to 10 cultures per group.

^d Average for 6 to 8 cultures per group.

of medium at the time of culture initiation. The mock culture specimens containing *Klebsiella* and *Escherichia coli* were generally positive within 6 to 8 h, whereas *Serratia marcescens* resulted in positive detection times of approximately 8 to 10 h. *Proteus*- and *Pseudomonas*-containing specimens also gave similar detection times. Streptococci, staphylococci, and pneumococci were detected in the mock blood cultures under both CO₂-gassed and ungassed conditions, with average detection times ranging from 7 to 9 h for streptococci and pneumococci with CO₂ gassing and 12 to 16 h for the same organisms cultured in air only. *Staphylococcus aureus*-containing blood samples resulted in average detection times of 14 and 18 h for CO₂-gassed and ungassed cultures, respectively.

Anaerobic organisms tested in the mock blood cultures resulted in detection times as short as 3 h for *Clostridium perfringens* and as long as 48 h for detection of *Bacteroides melaninogenicus* and *Propionibacterium* species (Table 2). However, it should be noted that this occurred with approximately 10 to 50 organisms per ml of blood culture (a 1:10 dilution of 10² to 10³ organisms in blood, followed by a 1:10 dilution in culture medium). Lower numbers of bacteria were not used.

In all cases the same organisms examined for growth detection by the Bactometer method have been previously detected in earlier studies by the Bactec apparatus with radioisotopically labeled medium. For comparative purposes in this study, the mock blood cultures containing these bacterial species were also tested by the radioisotopic procedure. Detection times for these organisms were essentially the same as with the Bactometer, although a 2- to 4-h batch-type sampling cycle was utilized. For example, detection times of 8 to 12 h were obtained for mock cultures containing *E. coli* or *Serratia*, *Klebsiella*, *Pseudomonas*, or *Proteus* species. Detection times for staphylococci- and streptococci-containing specimens were in the 10- to 20-h range.

Although the main purpose of this study was to determine the efficacy of an electrical impedance apparatus for detecting bacteria in artificially contaminated blood samples, a pilot study was undertaken to determine if the apparatus could detect bacteremia in clinical blood specimens. For this purpose blood from 40 individual hospitalized patients was tested by the Bactometer method and simultaneously by the Bactec radioisotope technique, as well as by the conventional two-bottle culture technique. For this purpose several Becton-Dickinson Vacutainer tubes with approximately 10 ml of blood each were obtained at one time from individual patients.

TABLE 2. *Anaerobic organisms detected in mock blood culture specimens by Bactometer procedures*

Organism tested ^a	Avg detection time (h) ^b
<i>Clostridium perfringens</i>	3.0 ± 0.5
<i>Peptostreptococcus</i> sp.	5.0 ± 1.5
<i>Propionibacter acnes</i>	7.0 ± 1.5
<i>Peptococcus</i>	8.0 ± 2.0
<i>Bacteroides fragilis</i>	9.0 ± 1.5
<i>Fusobacterium</i> sp.	12.0 ± 2.0
<i>Propionibacterium</i>	48.0 ± 8.5
<i>Bacteroides melaninogenicus</i>	48.0 ± 6.0

^a Approximately 30 to 50 bacteria per ml of blood at time of culture initiation.

^b Specimens cultured in 10% CO₂-5% H-85% N.

Informed consent had been obtained. The specimens from a single patient were pooled and used for parallel inoculation into two Bactometer blood culture bottles (3 ml each for air and air plus CO₂ incubation), plus 3-ml samples were inoculated into each of two Bactec blood culture bottles (media 6A and 7A). Ten milliliters of the remaining blood was placed into each of two 50-ml Trypticase soy broth bottles for conventional culturing. Only one of the patients showed evidence of bacterial infection during this study; a viridans group streptococcus was isolated from the blood culture of this patient with both the Bactometer and Bactec techniques, as well as by conventional procedures. The detection time was 3.5 h with the electrical impedance apparatus and 5 h by the Bactec apparatus. This was essentially an insignificant difference in time as compared with the overnight incubation necessary for turbidity to be observed in the conventional blood culture bottles examined visually.

DISCUSSION

In this study a broad spectrum of gram-positive and -negative bacteria was utilized to contaminate sterile blood specimens, which were then inoculated into culture medium in bottles containing electrodes attached to an electrical impedance apparatus. Growth of the bacteria during inoculation at 37°C resulted in readily detectable changes in the impedance capacity of the broth as compared with paired control bottles without bacteria. A change in the ratio of impedance between the test and control bottles was detected by the impedance apparatus and continuously recorded on a strip chart recorder. All of the bacteria examined, including *E. coli*, *Serratia*, *Klebsiella*, and *Pseudomonas*, as well as staphylococci and streptococci, resulted in relatively rapid changes in electrical impedance; positive growth detection usually occurred within 6 to 18 h.

In all cases the bacteria were also cultured

by conventional methods in broth and on solid agar media. However, an overnight incubation of 18 to 24 h was the standard procedure for detection of the bacteria (5, 6).

Studies in other laboratories, as well as in this one, have shown the usefulness of the radioisotope technique for rapid detection of bacterial growth in blood culture specimens, as well as for other types of microbiological specimens (2-4, 7, 8; R. Throm, R. Strauss, and H. Friedman, Abstr. Annu. Meet. Am. Soc. Microbiol. 1975, C30, p. 32). Thus it was not the purpose of the present study to compare the impedance apparatus with the Bactec apparatus, which is already in widespread clinical laboratory use. The major intention of this study was to determine whether known species of bacteria readily detectable by conventional culture procedures, as well as by the radioisotope method, could also be detected by the Bactometer apparatus, and, if so, whether detection time would be rapid. It should be noted that although the radioisotope procedure detects bacterial growth quite rapidly (i.e., usually within 6 to 24 h after culture initiation), a major drawback is the batch-type operation whereby sampling occurs at fixed intervals of 1 to 4 h for a maximum of 25 culture bottles, which must be placed on a single tray. In contrast, the electrical impedance procedure is based on the continuous monitoring of culture bottles, each containing wire leads directly connected to the detection apparatus. Once the culture bottles are inoculated and the wire leads are attached to the apparatus, detection occurs automatically, as shown by changes on the strip chart recorder. The earliest detection time noted was approximately 6 to 7 h for several of the gram-negative organisms at an inoculum size of one to five bacteria per ml of medium. Gram-positive organisms, such as staphylococci and streptococci, resulted in detection times ranging from 12 to 18 h, with longer detection times occurring when aerobic conditions without 5% CO₂ were used. Some of the anaerobic bacteria tested under 10% CO₂ conditions resulted in relatively rapid detection times of 3 to 9 h. However, *Fusobacterium* species gave detection times of approximately 12 h, whereas *Propionibacterium* species and *B. melanigenicus* resulted in detection times of 48 h at an inoculum size of approximately 10 to 50 bacteria per ml of medium.

Similar analysis of the mock blood cultures in the radioisotope-containing media resulted in approximately similar detection times, indicating that except for ease of performance, the electrical impedance procedure was no more rapid than the radioisotope method. Further-

more, it should be noted that direct microscopic examination of blood culture samples by the Gram stain technique at closely spaced intervals would undoubtedly also result in rapid detection times (2). For example, microscopic examination at times earlier than 18 or 24 h after incubation of a blood culture is initiated often results in detection of bacteria. Nevertheless, the logistical and personnel difficulties encountered preclude routine microscopic examination of blood culture bottles during frequent intervals in most clinical laboratories.

Due to the developmental nature of the Bactometer apparatus now available, there are several technical problems that appear to restrict present use for routine microbiology. The model tested in this laboratory has a limitation of 32 specimens, although newer models will be able to monitor up to several hundred specimens simultaneously. With a capacity of only 32 samples per apparatus, it was not feasible to continuously monitor the several hundred blood culture samples examined weekly in a relatively large and active laboratory such as this one. Furthermore, detection electrodes are not standardized, although this is being improved. However, the rapid rate of detection time and the low cost for detection electrodes, as well as excellent reproducibility of results and ease of operation, suggest that the electrical impedance determination of microbial growth in clinical blood specimens may serve a useful place in laboratory microbiology. This was borne out by the pilot study in which duplicate samples from 40 blood specimens from hospitalized patients were examined in parallel by the electrical impedance method, the radioisotope method, and conventional culture procedures. Although all but one of the specimens were negative for microorganisms by all three techniques, the one specimen that was positive by conventional culture procedures (i.e., containing viridans group streptococci) gave relatively similar growth detection times by both electrical impedance and radioisotope methods. This difference is insignificant, especially as compared to the 18-h detection time by conventional methods.

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