Rapid Automated Diagnosis of Bacteremia by Impedance Detection

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Received for publication 10 August 1976

Lysis and filtration of blood culture specimens were combined with impedance detection of bacterial growth to facilitate the diagnosis of bacteremia. A blood lysis-filtration technique (Zierdt et al., 1976) was coupled to a simple, inexpensive automated detection system. The practical and technical aspects of the impedance detection system are discussed. This new blood culturing system was compared to a conventional system for 264 aerobic blood cultures. A 30-ml sample of the blood-broth mixture was withdrawn from the conventional aerobic blood culture bottle and processed in parallel. Excluding the isolation of the commonly recognized contaminants, the detection efficiency was 36% greater in the new system. A total of 53 blood cultures from 107 patients were positive by one or both methods. The new system detected 92% of the total number of positive cultures, compared with 56% detected by the routine method. The explanation of the differences is discussed.

Despite the availability of excellent antimicrobial agents, the morbidity and mortality attributed to bacteremia continues to rise (4). Prompt detection and isolation of the organism in bacteremia provides earlier initiation of specific therapy for the patient. One of the major factors that determines recovery from bacteremia is early administration of appropriate antibacterial therapy (9, 10). Standard blood culture methods can rarely supply sensitivity testing results before 48 h (2). There is also reason to believe that currently available techniques fail to grow an organism from blood in a certain proportion of real bacteremias (7). These criticisms also apply to the radiometric blood culture system (6), a semiautomated detection method for which earlier detection times are claimed (3, 13). Serious deficiencies of the radiometric method include a high rate of false positives and, of lesser incidence but more important, false negatives (3, 12, 13, 16). The use of radioactive substrates is expensive and incurs certain risks in the handling and disposal of the cultures. Therefore, there remains a need for an automated, rapid, sensitive, inexpensive,

ecologically nonpolluting blood culture technique.

The system described here combines two techniques. The lysis-filtration technique, which effectively lowers the threshold for initiating bacterial growth, thus increases overall sensitivity (19). This paper describes a sensitive, accurate detection system that automatically signals the occurrence of bacterial growth. In 1973, Ur and Brown noted that the impedance of a growing culture decreases with time in a way that follows an integral of the viable count curve (18). As bacteria grow, complex nutrients are converted to end products whose chemical composition is reflected by a change in the electrical properties of the media. There is a net increase in the number of ionic and electrically charged molecules. This results in a corresponding increase in conductivity or decrease in impedance, which can easily be measured.

A small clinical trial evaluates the efficacy of this new blood culturing system in comparison with our conventional system.

MATERIALS AND METHODS

The aerobic blood culture samples from two patient wards having high risk for bacteremia were examined by two culturing systems run in parallel. At the bedside of each patient, 5 ml of venous blood was inoculated into each of two screw-capped vacuum bottles containing 90 ml of brain heart infusion

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broth with sodium polyanethol sulfonate, *p*-amino-benzoic acid, and CO₂ (BBL). Upon receipt in the laboratory, one bottle was vented with a needle attached to a sterile Swinney filter, and 30 ml of the blood-broth mixture was aseptically removed from the aerobic bottle and transferred by syringe to the lysis-filtration unit. The original aerobic brain heart infusion bottle was then processed with the anaerobic bottle in the conventional method.

Conventional method. Both bottles were incubated at 35°C and inspected daily for evidence of growth. At 24 h Gram staining was performed, and subcultures from both bottles were made onto BYE (1) agar and incubated at 35°C anaerobically and aerobically under 10% CO₂. Subculture was repeated at 7 days.

Impedance method. Lysing and filtration of the 30-ml blood-broth mixture removed from the conventional aerobic bottle was performed as described in the preceding paper (19). After filtration, the membrane filter pad was transferred to a culture bottle that was connected by means of electrodes to an impedance measuring device. Originally, 5-in (ca. 12.70 cm) stainless-steel electrodes were inserted directly into the standard 90-ml brain heart infusion (BBL or Scott-Robbins) blood culture bottles by thrusting them through the rubber stopper after flame sterilization. Presently, the culture bottle is a wide-mouth 2-oz (ca. 59 ml) screw-capped jar (no. B-7565-2; Scientific Products Corp.) with a specially fitted cap that incorporates stainless-steel electrodes. Leads connect the immersed electrodes to the impedance measuring device (Fig. 1).

The measuring circuit for the impedance detection consists of a generator driving a 2-cycle/s sine wave alternating current into the secondary coil of a

transformer. The secondary coil is also connected to the input of a tuned amplifier. The primary coil of the transformer is connected sequentially through relay contacts to sets of electrodes. Any decrease in impedance across the electrodes will result in a proportionate voltage drop being passed through to the amplifier. The voltage leaving the amplifier will be proportional to the impedance of the solution in the blood culture bottle. The dwell time on each electrode can be set, and a zero impedance short circuit is internally measured as a reference value to compensate for electronic drift. The absolute value of the output from the amplifier goes through a low-pass filter with a 5-s time constant and is recorded.

Recording data. Initially, the signal from each electrode was recorded on a strip chart recorder. A progressive decrease in impedance was designated as a positive culture. Decreases in impedance always occur with bacterial growths; however, two patterns are observed. There may be a rapid decrease (Fig. 2) or a gradual decrease. At this point, subculture and identification of the organism proceeded in the routine manner. When we were satisfied that the impedance decrease of a growing bacterial culture was a physical characteristic of all of a large representative sampling of clinical isolates encountered in our laboratory, we proceeded to refine the data collection system. The computer was interfaced with impedance detection so that data could be stored and positive cultures signaled automatically. A Tektronix-31 programmable calculator is used to indicate a positive decision. At the same time, the channel number, incubation time, base-line impedance, percentage of change from the base line, and the slope of the impedance growth curve are printed. A positive culture is determined by whether or not

FIG. 1. Scanner (10-channel multiple multiplexer) consists of a set of 10 relays controlled by a counter. The counter is advanced by pulses from the TeK-31 calculator that are obtained by commands in the calculator program. The impedance meter consists of a 2-Hz current source supplying the secondary winding of a transformer. The primary winding is sequentially connected to the blood culture bottles by the relays in the scanner. The secondary coil is also connected to the input of the 2-Hz tuned amplifier. The voltage output from this amplifier is proportional to the impedance of the electrodes in the blood culture bottles. The voltage is measured by the TeK-501 digital voltmeter as commanded by the TeK-31 calculator. The paper tape printout on the calculator is only actuated when the impedance value satisfies the criteria of the programmed algorithm for calling a blood culture positive.

the impedance data generated by the culture conforms to the mathematical algorithms written into the program. The computer program is in electronic memory and on magnetic tape, so that the computer can be easily reprogrammed in case of power failure.

RESULTS

Impedance measurement and electrode material. The initial step in development of the system was an investigation into the nature of the impedance decrease as a function of electrical frequency. The electrical impedance of a bacterial culture is a measurement of the opposition to the flow of an electrical current. In our system impedance includes the electrical resistance of the media and the capacitive reactance at the electrode-media interface. Both of these parameters vary inversely with the applied frequency. The impedance at the electrode-electrolyte interface is actually an inverse function of the square root of the applied frequency (11). Another consideration was that for stable, long-term data collection electrode polarization must be avoided. Using a small alternating current minimizes the possibility of electrode polarization. At low frequencies a larger impedance signal is available for the same electrode current density.

Alternating current and voltage are usually represented as sinusoidal functions of each other. When these variations are recorded simultaneously, it is easily observed graphically

whether the fluctuations of each are in phase or out of phase with each other. The impedance and phase shift were measured at 2, 10, 15, 20, 40, 80, 160, and 320 Hz to determine the frequency at which the maximum changes would occur. The results indicated that although the phase shift did not change appreciably, the impedance always decreased with bacterial growth. Detection of growth was originally dependent on visual inspection of the tracings from a strip chart recorder. Therefore, we chose the lowest frequency available, 2 Hz, that maximized the initial impedance of the culture, enabling us to see small decreases earlier. As more channels are added to the system, the decreased dwell time available for any given channel (blood culture bottle) will necessitate a higher excitation frequency. The fact that visual interpretation is no longer performed will not affect sensitivity. Mathematical algorithms evaluate the change in slope of the impedance curve as well as an absolute impedance decrease at any time as the criteria for decision making.

Electrode material. We investigated platinum versus stainless-steel electrodes, and our results agreed with those of Geddes (8), who noted that over a considerable frequency range the impedance for many metal-electrolyte interfaces are approximately equal. Cady and Dufour (Abstr. Annu. Meet. Am. Soc. Microbiol., 1974, E43, p. 8) concluded that gold and

FIG. 2. Random fluctuations in the control bottle (●) are not considered to indicate a positive culture, because there is not a progressive decrease in impedance. The open circle (○) is positive culture with a contaminant organism. The open triangle (Δ) is the impedance curve denoting continued growth of *K. pneumoniae* in the sample processed by lysis filtration 24 h after systemic antimicrobial therapy was begun. The split sample of this culture processed by the conventional method showed no growth.

stainless steel were the electrode materials that give the best response to bacterial growth. We choose stainless steel because of low cost, mechanical strength, biological inertness, and ability to withstand repeated flame sterilization. Electrodes had to be flamed to a red heat after each use. An identical culture would give a blunted impedance response if the electrodes were not flamed.

Clinical blood culture trial. A comparison of the results of the split samples of 264 blood cultures from 107 patients is seen in Table 1. There was a total of 53 positive cultures. The lysis-filtration impedance detection technique detected 49 (92%) of the positive cultures. Two of the false negatives were from a patient with *Haemophilus influenzae* bacteremia. Duplicate cultures were both positive in the conventional system. The brain heart infusion media in the impedance bottle had to be autoclaved and did not support the growth of *H. influenzae* in vitro. No explanation was found for the other two false-negative blood culture specimens which were drawn from a patient at the same time. The conventional system detected only 30 (56%) of the total number of positive cultures. In the 30 cultures that gave positive results in both systems, impedance detection identified a positive culture in 8 to 12 h. In all but three cases, the conventional blood culture bottle was recognized as positive at 26 h by either gross turbidity or Gram stain. In two of these cases the organism was an alpha-hemolytic *Streptococcus* that did not cause any visible turbidity but was positive by Gram staining. The other case was prolonged bacteremia with a very drug-resistant species of *Corynebacterium*. This organism grew quite slowly with very little visible reaction in the culture bottle. Impedance detection took 24 h, whereas the conventional bottle showed no growth until 72 h. The conventional bottle remained visually clear, but the blind 24-h routine subculture showed growth at 72 h.

Table 2 lists the 23 organisms detected only by the lysis-filtration impedance procedure. Fourteen of these organisms were isolated from patients on antimicrobial agents. It was com-

TABLE 2. Twenty-three organisms detected only by the impedance lysis-filtration procedure

Organism	No. of isolations
<i>Escherichia coli</i>	5
<i>Pseudomonas aeruginosa</i>	4
<i>Acinetobacter</i>	3
<i>Klebsiella</i>	2
<i>Neisseria</i> sp.	2
<i>Corynebacterium</i> sp.	1
<i>Achromobacter</i> group III	1
<i>Citrobacter diversus</i>	1
<i>Sarcina</i> sp.	1
<i>Streptococcus bovis</i>	1
<i>S. sanguis</i>	1
Alpha <i>Streptococcus</i> sp.	1
Total	23

mon for both systems to grow the same organism when a patient became septic. After antibiotic therapy was begun, subsequent conventional blood cultures would show no growth; however, the split sample processed by lysis-filtration would continue to grow the organism. In addition to total suppression of bacterial growth in the conventional bottles, antibiotic-induced bacterial damage delayed organism identification and sensitivity testing in the conventional bottles that did not cultivate the growth of the organism. In the nine cases where an organism was detected through the use of only the new system and the patient was not on antibiotics at the time the cultures were drawn, no source could be confirmed. The isolations were assumed to be related to the sensitivity of the system, which lowers the threshold for detection, permitting isolations from sub-clinical transient bacteremias.

Three brief case histories serve to demonstrate that this increased yield is real and is related to the sensitivity of the method. The accompanying figures are graphic representations of impedance changes recorded over time from blood cultures. A 21-year-old man with aplastic anemia (Fig. 2) became septic after a bone marrow transplant. Initially, split samples were positive in both systems for *Klebsiella pneumoniae*. However, a Gram stain from the routine blood culture disclosed bizarre, filamentous, gram-negative rods. These bacteria grew poorly, showing that atypical colonial morphology and a number of extra days were required before a true antibiotic sensitivity pattern was obtained. The biochemical reactions and morphology of the organisms taken directly from the impedance bottle were typical for *K. pneumoniae*. This tracing, made from impedance readings on a blood culture drawn after antibiotic therapy was begun, was posi-

TABLE 1. Two hundred and sixty-four blood culture specimens from 107 patients examined in parallel

System	Positive culture	
	No.	%
Conventional	30	56
Automated lysis-filtration	49	92
Either or both methods ^a	53	100

^a Positive results obtained by both methods were considered as 100%.

tive only in the lysis-filtration system. The closed triangle is the impedance blood culture tracing from a severely leukopenic patient on the day he became septic. The split sample handled in the conventional manner was also positive. Blood from the same patient after 10 days of high-dose, multiple antibiotic therapy grew *Pseudomonas aeruginosa* and *K. pneumoniae* (Fig. 3). Split cultures handled in the conventional manner remained sterile, even though *Pseudomonas* was continually detected by the lysis-filtration system. Terminally, split cultures were positive in both systems.

A 44-year-old man afflicted with Darier's disease of the skin (Fig. 3) was treated with gentamicin for *Pseudomonas* skin infection after a therapeutic epiderectomy. He had a shaking chill and became febrile; blood and wounds were then cultured. Wound cultures from two separate sites grew *Achromobacter* species, as did the blood culture, signaled by a decreasing impedance curve. The conventional blood culture was negative.

DISCUSSION

The potential advantages of lysis-filtration techniques have been recognized (15). In addition to advantages that accrue by the removal of antibiotics, drugs, antibodies, complement, and opsonins, this technique also provided additional advantages to an impedance detection

system. The impedance measuring circuit devised by Ur and Brown to detect bacterial growth (18) was the same as the one Ur originally used to make whole blood clotting measurements (17). In their technique, comparative impedance rather than the absolute value is measured. A measuring cell containing the blood sample was placed in a bridge circuit balanced by an anticoagulated portion of the same blood sample. In this arrangement all events in the blood sample, except coagulation, take place in the reference well and, therefore, balance the test. Only impedance changes due to coagulation are measured and recorded. This was necessary because impedance is strongly affected by changes in the blood sample unrelated to the coagulation process, i.e., temperature changes, cell sedimentation, and particle degeneration. A commercial instrument also incorporates a reference cell for each culture being monitored and generates impedance ratios (17). Our measuring circuit is simple and inexpensive, because there is no need for a reference well and absolute impedance values can be recorded or fed into a computer for analysis.

Lysis-filtration processing eliminates the need for a reference well for each culture because it assures a relatively clear blood culture medium. In a conventional blood culture the patient's own erythrocytes would contribute a significant impedance at the low frequencies we

FIG. 3. This impedance curve (Δ) denotes growth of two pathogens at a time when sterile conventional blood cultures indicated adequate antimicrobial therapy. Open circles ($\circ$) represent the impedance changes caused by growth of *Achromobacter xylosoxidans* in the split sample processed with lysis-filtration. Wound cultures from two separate sites also grew out this organism, but the conventional blood culture remained sterile.

utilize to maximize the impedance change that is secondary to microbial metabolism. The increase in impedance due to erythrocytes would mask the decrease due to bacterial growth and delay detection of bacteremia. Single cells possess a significant amount of impedance (4). Cole and Curtis demonstrated the effect of frequency on the path of electrical current around and through a spherical cell (5). Paths of electrical current develop a high impedance effect at low alternating frequencies. As the frequency is increased, the impedance effect of a spherical cell is minimized until at a critical frequency the impedance remains the same. This is about 5×10^6 cycles/s for erythrocytes. At this frequency the impedance effect on the erythrocytes would approach zero; however, the impedance changes due to bacterial growth would also be minimized. Without lysis-filtration processing, a reference well is needed for each culture being monitored.

All cultures positive in both systems were first detected with the impedance detector. Earlier detection appears more related to the automated frequent-sampling system than to a significantly increased sensitivity attributed to impedance measurements. Conventional cultures are not usually observed until the day after receipt in the laboratory. At the time an impedance culture registered as positive, a faint turbidity could be visualized grossly by a trained observer. This corresponded to a colony count of approximately 5×10^5 and was obscured in the conventional bottle containing erythrocytes. For impedance detection, a critical concentration of organisms must be present to achieve the impedance change due to their microbial metabolism. In this respect, impedance detection is not different from radiometric, nephelometric, spectrophotometric, or gross visualization. Routine media are used, and detection is signaled at the earliest possible moment. Coupling the lysis-filtration technique with impedance detection is responsible for earlier real detection times, because it is possible to process a large volume of blood and concentrate organisms present on a filter pad. The filter pad is then incubated in a small volume (20 cc), giving an increased initial concentration. There is no need for the standard 1:10 dilution of blood to media. Also, clear media permit agitation of the cultures without affecting impedance measurements. By beginning incubation with a greater concentration of organisms and agitating the specimen, the critical concentration is reached sooner than with conventional detection.

The clinical trial demonstrates the increase

in sensitivity of this system. Episodes of sepsis were detected earlier and confirmed by subsequent conventional cultures. Bacteremias too small to initiate growth in conventional bottles appear to grow well in the impedance cultures. Damaged organisms revive and grow well after they are separated from drugs and other plasma constituents. Finally, subclinical bacteremias are detected by this system. This feature may be useful in studying high-risk patients for a warning of impending sepsis.

The 36% greater detection efficiency is even more impressive than it appears, as we were operating under two distinct disadvantages in the clinical trial. The conventional culture bottles were inoculated at the bedside, and the portion for lysis-filtration processing was not removed until receipt of the routine blood culture bottles in the laboratory. This delay in removing drugs and plasma constituents from bacteria does have a deleterious effect on the yield. Also, we only withdrew 30 cc of blood-broth mixture from the conventional bottles for processing. This was the equivalent of 1.5 ml of patient blood versus 3.5 ml for the conventional bottles. This could account for the one patient with sepsis who was missed by the new system.

Following these results, we are proceeding to refine this research blood culture technique for routine use. The filtering chamber will eventually be modified to produce a closed system. The physician will inoculate blood at the bedside into the filtering chamber. Lysis, filtration, addition of media, and incubation will all be accomplished in a single chamber. This self-enclosed unit will then be directly interfaced to a computer-programmed impedance detector by means of electrodes built into the disposable chamber.

ACKNOWLEDGMENTS

We wish to thank George Norris for his assistance in constructing an impedance chamber and Donald Peterson for his technical assistance.

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