Early detection of bacterial growth in blood culture by impedance monitoring with a Bactometer model 32

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SUMMARY A Bactometer model 32 was evaluated for use in early detection of bacterial growth. Experiments with simulated cultures showed that 2 ml of broth introduced into the Bactometer module wells could detect 10^2 and 10^6 CFU/ml in 6 h and 2 h respectively. Both Brain Heart Infusion (BHI) and Fastidious Anaerobic broths supported good growth. Detection of nine of 10 organisms inoculated at approximately 10^6 CFU/ml in BHI were detected within 8.5 h. A culture of *Bacteroides fragilis* failed to grow under these conditions. Of 189 blood cultures, tested by incubation of 2 ml of BHI, 18 were positive by both conventional and Bactometer methods. False-positive or false-negative specimens were not observed using the Bactometer. Use of the Bactometer enables growth detection at least 12 h earlier than culture methods.

Rapid detection of septicaemia is of primary importance in the diagnostic laboratory. Immediate Gram staining of blood specimens can detect approximately 10^5 organisms/ml. However, culture methods are necessary to detect pathogens present at lower concentrations. Conventional methods rely on culturing of blood specimens and detection of growth by subculturing and Gram staining requiring 18-24 h for the first observation, negative cultures being kept for seven days or more. More sensitive methods are therefore sought for faster detection of positive blood cultures. Newer detection methods available were reviewed by Bascomb.¹ Methods applied for detection of septicaemia include radiometric techniques² measurement of bacterial ATP³ and monitoring of impedance changes.⁴⁻⁶ A number of impedance measuring devices have been described.¹ The Bactometer⁷ is commercially available and has been used for detection of bacteriuria^{8,9} and in the food industry¹⁰ and sewage effluent monitoring.¹¹ Its use in detection of septicaemia has been limited.^{12,4-6} We have evaluated the Bactometer model 32 as to its suitability for monitoring growth of bacteria in blood cultures from the diagnostic laboratory. We also investigated the effects of type and volume of medium, concentration of inoculum, and the applicability of the instrument to the variety of organisms likely to be found in blood cultures.

Material and methods

CULTURE MEDIA

Two blood culture media were used. Brain Heart Infusion broth (BHI) + thymidine + sodium polyanethyl sulphonate (SPS) and Fastidious Anaerobic broth (FAB) + thymidine, both supplied by Lab M, Salford, Lancs.

TEST CULTURES

All strains were recent laboratory isolates identified by standard technique.¹³

BLOOD CULTURES

Five ml of patient's blood was inoculated into 75 ml of BHI and FAB bottles on the ward. The blood culture set was brought to the laboratory immediately and incubated at 37°C. β -lactamase (0.5 ml) (Genzyme Biochemicals) was added to each bottle if the patient was receiving a β -lactam antibiotic. All blood cultures were subcultured onto blood agar anaerobically and chocolate agar in 7% CO₂ after 24 h, 48 h and seven days incubation at 37°C. Any turbid bottle after 24 h incubation had a Gram film made and examined.

VIABLE COUNTS

Miles and Misra counts¹⁴ were performed on the experimental broth cultures.

IMPEDANCE MEASUREMENTS

The Bactometer consists of a thermostatically con-

trolled monitoring unit. Four plastic modules (100 × 75 mm) each having eight pairs of rectangular wells can be accommodated in the incubator. Each well contains a pair of electrodes that are connected to the monitoring unit via metal strips on the underside of the module. The metal strips converge in a tab that is plugged into the incubator.

The modules come in a sterile plastic envelope, the wells of which are covered by a plastic film, through which broth can be introduced aseptically with a syringe and needle. After filling, another sterile adhesive film supplied with each module, is placed over the wells to seal the needle holes.

Each well can hold 2.5 ml of broth but, to avoid overfilling, 2.0 ml was used. The lower row of wells, filled with sterile broth, were used as reference, the upper row of wells were filled with inoculated broth or media taken from inoculated blood cultures.

All eight wells of the module need not be filled before monitoring commences, further cultures can be added as and when they arrive in the laboratory.

Monitoring of impedance can also be achieved by inserting Bactometer electrodes into conventional growth vessels. These are reusable autoclavable electrodes that are incorporated into a screw cap that can replace the cap of the blood culture bottle. This enables the bottles to be connected to an extension socket inside an ordinary incubator which relays information back to the Bactometer. Each blood culture bottle requires a reference bottle of sterile broth with inserted electrodes. The electrodes can be reused about 30 times.

The impedance changes are shown as a trace on a chart recorder. Each trace is a representation of a growth curve. To fit 32 curves onto one width of a chart recorder paper, each curve is divided into segments 8 mm wide (Fig. 1a). The rate at which the trace traverses a channel width gives an indication of the rate of growth (Fig. 1b).

Bacterial growth is represented by the trace traversing a channel width from left to right whereas yeasts produce the opposite effect.

Results

RANGE OF ORGANISMS DETECTABLE BY THE BACTOMETER

Cultures of 10 organisms likely to be found in blood (Table 1) were inoculated into BHI to give an approximate concentration of 1×10^6 CFU/ml. Two ml of each broth were transferred to the wells in the top row of the module and 2.0 ml of sterile BHI into the reference wells. The modules were monitored on the Bactometer for 48 h for signs of growth.

As can be seen from Fig. 1c there is an initial period, ranging from 0.5 to 3 h, in which the traces

repeatedly traverse the channel width in either direction, they then flatten out but still tend to drift slightly. This response, seen in all the blood cultures examined, is thought not to be due to growth but rather to the erythrocytes settling to the bottom of the well.

The periods in which growth responses were observed varied from 5 to 18 h, and in two cultures, *Clostridium perfringens* and *Staphylococcus epidermidis* two growth periods were observed, with a period of several hours of no growth in between.

All but one of the organisms selected showed growth within 8.5 h. The times for appearance of detectable growth are shown in Table 1. The failure to detect growth of *Bacteroides* is probably due to the poor anaerobic conditions in the small volume of broth.

EFFECT OF INOCULUM SIZE ON SPEED OF DETECTION

Dilutions of *Escherichia coli*, *Haemophilus influenzae* and *Staphylococcus aureus* were prepared in BHI to give initial concentrations of approximately 1×10^2 , 1×10^4 and 1×10^6 CFU/ml. Viable counts¹⁴ were performed on these broths to establish the exact concentrations. All wells were monitored for 6 h. As can be seen from Fig. 2 there is a good correlation between inoculum size and detection time; 10^7 CFU/ml were detected in 1.5 h, 10^2 CFU/ml in 6 h.

EFFECT OF CULTURE MEDIA ON SPEED OF DETECTION

Eight blood cultures were chosen to show differences between the FAB and BHI media. They were monitored for 48 h and their traces examined. Six blood cultures showed no response, two showed a growth response. The duration and the shape of the growth curves were the same for both media.

DETECTION OF GROWTH IN BLOOD CULTURE SPECIMENS

One hundred and eighty nine blood cultures were examined during a period of four weeks. A 2.0 ml aliquot was drawn from the BHI bottle with a syringe and needle for examination on the Bactometer as described above. These cultures were monitored for 48 h before being discarded. Complete agreement was found between growth and impedance methods. Eighteen specimens were found to be positive by both methods. The remaining 171 were found to be negative by both. The list of species isolated is given in Table 3. The majority of cultures were detected within 3 h. One specimen containing *Streptococcus pyogenes* was detected after 4.5 h, two specimens containing *Staphylococ-*

Fig. 1 Traces of impedance monitoring obtained with Bactometer 32*. (a) A typical Bactometer chart showing the recording of 32 cultures. It is divided into four horizontal sections, each section is labelled from 0 to 7 representing the eight cultures in one module. (b) The centre trace shows a growth curve response. It demonstrates a short settling down period followed by a stationary phase and growth response. The lines get closer together as growth accelerates into the logarithmic phase. The other traces show no growth response. (c) The traces of four sterile blood cultures show how the settling down periods can vary in shape and duration. *For clarity of reproduction the Bactometer chart grid has been erased.

Table 1 *Taxa detected by Bactometer*

	Time for initial detection (h)
<i>Staphylococcus aureus</i>	1
<i>Staphylococcus epidermidis</i>	1½
<i>Streptococcus pneumoniae</i>	½
<i>Streptococcus pyogenes</i>	2¾
<i>Streptococcus mitior</i>	8½
<i>Escherichia coli</i>	1½
<i>Haemophilus influenzae</i>	3½
<i>Candida albicans</i>	5
<i>Clostridium perfringens</i>	3½
<i>Bacteroides fragilis</i>	(failed to grow)

Table 2 *Effect of broth volume on time for initial detection of growth (h)*

	Volume of broth (ml)				
	0.5	1.0	1.5	2.0	2.5
<i>Staphylococcus aureus</i>	4½	4½	4	3¾	3¾
<i>Streptococcus pyogenes</i>	4½	3¾	3¾	3	2¾
<i>Haemophilus influenzae</i>	4	4	4	3½	3½

cus epidermidis and *Staphylococcus aureus* were detected after 8 h, and two specimens containing *Staphylococcus epidermidis* and *Streptococcus mitior* were detected after 8.5 h.

Discussion

Monitoring of growth by the Bactometer can be done in the special 2.5 ml capacity wells of the growth modules, or by inserting reusable sterile electrodes into conventional culture containers. To avoid any danger of contaminating patients' specimens we used the growth modules only, in this preliminary study. Reading of the Bactometer charts is easily acquired though the initial period of settling of blood cells can sometimes confuse the interpretation. It might be useful to investigate further the use of non-ionic detergent for lysis of the blood cells prior to incubation as suggested by Makin and Cor-kill¹⁵ to avoid such problems.

Both media used for blood cultures in our laboratory were suitable for use in the Bactometer. In simulated cultures, presence of 10² CFU/ml could be detected in about 6 h, that of 10⁶ in 2 h.

Table 3 *Bactometer 32 detection time of organisms found in blood cultures*

Isolates	Detection time (h)								Total number of cultures
	0.5	1	1.5	2	2.5	4.5	8	8.5	
<i>Staphylococcus epidermidis</i>	2	2	1	1	2	—	2	1	11
<i>Streptococcus aureus</i>	—	2	—	1	—	—	—	—	3
<i>Streptococcus pneumoniae</i>	1	—	—	—	—	—	—	—	1
<i>Streptococcus pyogenes</i>	—	—	—	—	—	1	—	—	1
<i>Streptococcus mitior</i>	—	—	—	—	—	—	—	1	1
<i>Escherichia coli</i>	—	—	1	—	—	—	—	—	1

Fig. 2 *Relation between initial microbial concentration and impedance response detection time. Two ml of suspensions of E coli (▲), Staph aureus (●) and Haemophilus influenzae (■) in BHI were inoculated into Bactometer 32 wells and monitored for impedance change. Microbial concentrations determined by viable counts. Fitted line $y = 8.157 - 1.0148x$ and 95% confidence limits; $r^2 = 0.87908$.*

Moreover, complete agreement was found between conventional and impedance detection of septicemia with 189 blood specimens. Most infected cultures being detected by the Bactometer method within 8.5 h, this being at least 10–36 h earlier than conventional methods, thereby providing a valuable rapid service to the patient.

In simulated cultures nine of 10 different organisms tested were detected within 10 h of incubation. The culture of *Bacteroides fragilis* failed to show

change in impedance during 48 h of monitoring, probably owing to insufficient anaerobiosis in the growth well. Over-laying with liquid paraffin might produce better conditions for anaerobic growth, but would probably prove difficult to introduce into the wells through the plastic strip. A better solution might be to insert the Bactomatic electrodes into an anaerobic culture bottle. Detection of growth of *Eubacterium limosum* and of *Fusobacterium nucleatum* has been reported.⁷

In preliminary work with the Bactometer before the study began, a problem was encountered with a blood culture that had been incubated over the weekend before being monitored. It yielded large numbers of bacteria on subculture but showed no change of impedance. This was probably due to the fact that nutrients in the culture media were exhausted during the weekend and metabolism could not therefore be detected by the Bactometer. However, the bacteria present could grow when supplied with new nutrients by subculturing. The Bactometer procedure is therefore not suitable for such specimens. These heavily infected cultures can be quickly detected by Gram staining those bottles which show turbidity. Alternatively, such specimens should be subcultured into fresh broth before Bactometer monitoring.

The model 32 Bactometer system has one serious limitation, the number of cultures it can deal with at one time (32) is too small. As impedance is much affected by temperature it is essential to incubate a reference cell for each culture. If each specimen is tested in both aerobic and anaerobic broth, this reduces the number of specimens to 16. Moreover, if they need to be kept for up to two days, it allows for only eight blood specimen investigations per day. In our laboratory serving a group of hospitals with a total of 547 beds, an average of 14 blood cultures are processed per day. A machine with greater capacity would be necessary in busy laboratories.

The capital outlay for an instrument is an impor-

tant factor. In Table 4 we have shown the costs of Bactometer against that of the other instrument available for processing blood specimens, the Bactec system.² As can be seen the Bactometer is very much cheaper than the Bactec, whereas the running costs per specimen using the growth modules inoculated from the conventional broth are similar.

At present it would not be safe to replace conventional culturing methods by the Bactometer impedance monitoring, but the instrument might be used concomitantly with the conventional method, cultures being kept for 24 h only. In this way heavily infected specimens would be detected within the working day and treatment of patients could be started or amended sooner. Cultures not detected within 24 h would, for the present, have to be handled by conventional methods.

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References

- Bascomb S. Application of automation to the general and specific detection of bacteria. *Lab Practice* 1981;30:461-4.
- DeBlanc HJ Jr, Deland F, Wagner HN Jr. Automated radiometric detection of bacteria in 1967 blood cultures. *Appl Microbiol* 1981;22:846-9.
- Schrock CG, Barza MJ, Deming JW, Picciolo GL, Chapelle EW, Weinstein L. Rapid detection of bacterial growth in blood cultures by means of adenosine triphosphate determination. In: *Abstracts, American Society for Microbiology. Annual Meeting, 1976 Paper No C71:37*.
- Khan W, Friedman G, Rodriguez W, Controni G, Ross S. Rapid detection and isolation of bacteria in blood and cerebrospinal fluids in children by the electrical impedance method. In: Johnston HH, Newsom SWB, eds. *2nd International symposium on rapid methods and automation in microbiology*. Oxford: Learned Information, 1976:13-4.
- Throm R, Strauss R, Specter S, Friedman H. Automated blood culture testing using radioisotope and electrical impedance monitoring equipment. In: Johnston HH, Newsom SWB, eds. *2nd International symposium on rapid methods and automation in microbiology*. Oxford: Learned Information, 1976:21-2.
- Specter S, Throm R, Strauss R, Friedman H. Rapid detection of bacterial growth in blood samples by a continuous-monitoring electrical impedance apparatus. *J Clin Microbiol* 1977;6:489-93.
- Cady P, Dufour SW, Shaw J, Kraeger SJ. Electrical impedance measurements: rapid method for detecting and monitoring micro-organisms. *J Clin Microbiol* 1978;7:265-72.
- Zafari Y, Martin WJ. Comparison of the Bactometer® microbial monitoring system with conventional methods for detection of micro-organisms in urine specimens. *J Clin Microbiol* 1977;5:545-7.
- Cady P, Dufour SW, Lawless P, Nunke B, Kraeger SJ. Impedimetric screening for bacteriuria. *J Clin Microbiol* 1978;7:273-8.
- Martins SB, Selby MJ. Evaluation of a rapid method for the quantitative estimation of coliforms in meat by impedimetric procedures. *Appl Environ Microbiol* 1980;39:518-24.
- Silverman MP, Munoz EF. Automated electrical impedance technique for rapid enumeration of fecal coliforms in effluents from sewage treatment plants. *Appl Environ Microbiol* 1979;37:521-6.

Table 4 Comparison of purchase and running costs of Bactometer 32 and Bactec 460

	Bactometer (£)	Bactec (£)
Capital outlay	9900.00	16 604.00
Cost per blood culture bottle	0.35	0.76
Cost of module per test (2 wells)	0.45	
Cost of electrode	1.00	
Cost per patient	1.60*	1.52
Cost per patient	1.52†	

*Based on one bottle and two wells for each medium.

†Based on two bottles of each medium and 1/30th of cost of a reusable electrode.

Prices are exclusive of VAT.

¹² Hadley WK. The use of electrical impedance measurements for detection of microorganisms in blood culture. In: Johnston HH, Newsom SWB, eds. *2nd International symposium on rapid methods and automation in microbiology*. Oxford: Learned Information, 1976:14.

¹³ Cowan ST. *Cowan and Steel's manual for the identification of medical bacteria*. 2nd ed. Cambridge: Cambridge University Press, 1974.

¹⁴ Miles AA, Misra SS. The estimation of the bactericidal power of

the blood. *J Hyg* 1938;**38**:732-49.

¹⁵ Makin T, Corkill JE. The detection of bacteria in blood cultures by impedance monitoring—a pilot study. *Med Lab World* 1978:409-15.

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