An automated blood culture system:  
the detection of anaerobic bacteria using a  
Malthus Microbiological Growth Analyser  

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SUMMARY

The Malthus Microbiological Growth Analyser has proved to be sensitive in  
detecting conductivity changes due to anaerobic metabolism in a number of  
widely used blood culture media. Freshly prepared cooked meat media and Thiol  
medium yielded the greatest gross conductivity changes, and were more  
sensitive of anaerobic metabolism than other media. Failure of the instrument to  
detect anaerobic metabolism was a problem particularly associated with growth  
in the thioglycollate medium. False positive detections of growth were  
attributed to a number of factors including electrode instability (6.0%) and bacterial  
contamination (8.75%).

INTRODUCTION

The early detection of microbial growth in blood culture media, and antibiotic  
sensitivity testing of the detected organism, is of great value in the management of  
septicaemic patients. However, conventional methods by frequent subculture  
are slow and time-consuming, and place a heavy workload on laboratory staff.  
Frequent subculture of conventional bottles also increases the risk of medium  
contamination and may render results difficult to interpret. These factors suggest  
the potential value of an automated system for detecting microbial growth in  
blood cultures.

A number of different approaches have been developed to accelerate the  
detection of microbial growth in blood cultures. These techniques have been  
based on a number of different physical detection methods including radiometry  
(Bactec), impedance 2 (Bactometer), microcalorimetry, 3 lysis filtration and centri-  
fugation methods. 4 Among those instruments introduced recently which detect  
microbial metabolism is the Malthus 112L. Boynes, Comrie and Prain 5 reported  
the use of the system and have suggested that the type of medium used is most  
important when detecting changes in electrical conductivity.
Anaerobic bacteria contribute a small but significant percentage of blood culture isolates. This study aims to show the conductance changes in different blood culture media developed for the isolation of obligate anaerobic bacteria. Although anaerobic bacteria comprise only 6–8% of blood culture isolates, in most series, the media required for their isolation are often adopted with the aim of combining good rapid isolation of obligate anaerobes, facultative organisms and obligate aerobes. Thus the investigation reported here has studied those media which will enhance isolation of anaerobic bacteria, and are often incorporated in a 2- or 3-bottle blood culture media isolation regimen.

MATERIALS AND METHODS

Organisms
The following stock cultures were used: *Clostridium perfringens* NCTC 8237, *Clostridium difficile* NCTC 11206, *Bacteroides fragilis* NCTC 9343, *Bacteroides fragilis* NCTC 10584, *Fusobacterium necrogenes* NCTC 10723 and three anaerobic cocci, NCTC 9803, 9811 and 9814. In addition, three current clinical isolates were examined in the system: *Propionobacterium spp.*, an anaerobic coccus, and a non-toxigenic strain of *Clostridium perfringens*.

Media
The media used in this study were thioglycollate broth (THG, Oxoid), thiol broth (THIOL; Difco), brain heart infusion broth (BHI; Gibco-Biocult), rehydrated cooked meat medium (BBL; Becton Dickinson); fresh cooked meat medium (FCMM) and fresh cooked meat medium plus agar (0.1% w/v) (FCMM + A). The media were prepared according to the manufacturers' recommendations with supplements as previously described. BBL was reconstituted using 3 gm per 80 ml distilled water instead of the recommended 10 gm. FCMM and FCMM + A were prepared with 14 gm moist weight of meat made up to 80 ml with broth. All blood culture media were dispensed in 80 ml volumes into 100 ml Malthus blood culture bottles. After autoclaving at 121°C for 15 minutes with the bottle tops loose, the tops were tightened during cooling.

Solid culture media
Tryptone Soya Agar (TSA, Oxoid Ltd) was supplemented with L-cysteine hydrochloride (0.05% w/v), yeast extract (0.01% w/v) and 5% horse blood. All media were prepared just prior to use and autoclaved for 15 minutes at 121°C.

Inoculum preparation and enumeration of bacteria
To obtain a small enough inoculum similar to that commonly found in infected blood, a 48-hour growth on TSA plates was harvested into 9 ml of sterile saline to give a slightly turbid suspension; further dilutions were made to yield 1 – 100 colony-forming units per ml (cfu/ml). Five different inoculum dilutions for each organism were used.

Each of five volumes of transfusion blood were inoculated with one of five different dilutions of the organism. One ml of diluted inoculum was added to 49 ml of blood.

The numbers of cfu/ml in the original inoculum suspensions were counted using a spread plate method.

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The Malthus system

The Malthus Microbiological Growth Analyser 112L consists of four waterbaths, each housing 28 blood culture bottles linked to an Exorcet 100 computer. The computer is programmed to detect changes in electrical conductivity. Each bottle contains two electrodes on a ceramic strip which can be connected to the analyser. The analyser automatically measures the electrical conductivity of the contents of each bottle, scanning each individual cell every 30 minutes. The data from each cell is stored on a floppy disk in the Exorcet computer, the capacity of which is sufficient to hold information from 128 cells for a maximum of nine days. Once the computer detects a significant change in electrical conductivity, it displays this as a detection time on a visual display unit.

Inoculation of Malthus cell

One ml of 2.5% sterile saponin was added by syringe to each Malthus culture bottle before addition of blood. The bottles, with added saponin, were all pre-incubated at 37°C in the water baths. Five ml of inoculated blood were added into each bottle. After inoculation the bottles were immediately placed back in the water bath and connected to the Exorcet computer.

Detection of change in conductivity: routine procedure

All detection times for each cell were noted and subcultures were made on TSA plates as soon as possible after detection by the instrument. Subcultures were made by passing a sterile Pasteur pipette through the inoculation port and removing a drop of the liquid media. All bottles were subcultured on to TSA at the completion of each experiment. Duplicate plates were incubated aerobically and anaerobically for 48–72 hours and examined for bacterial growth.

RESULTS

Conductivity change

(i) Uninoculated blood culture media. All six media evaluated were within the range of conductivity (10,000–1,000 micro-siemens) set by Malthus Instruments. Detection times appeared in four out of 32 bottles containing medium only. Electrode instability accounted for two of the four detections; these erratic changes in conductance could not be confused with changes induced by growth. The remaining two cells produced false detections because of a gradual downward drift in the base line; in both cases drifts occurred in FCCM + A medium.

(ii) Blood culture media with added lysed blood. Altogether 56 bottles were tested with media and lysed blood. Twenty-three on incubation yielded changes in conductivity which resulted in an instrument indication of detection. Five of the total were contaminated with Staphylococcus spp. The remaining 18 bottles were false-positive detections of bacterial growth.

Eight (44%) of the false detection times were due to an upward or downward drift in the base lines, three were due to electrode instability and seven (39%) were attributed to various factors which were likely to be excluded with experience. Only five false detections could possibly be misinterpreted as microbial growth when the graphical representation was examined.

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(iii) *Inoculated blood culture media.* The detection times of eleven stock cultures using minimal inocula in the six media are shown in the Table. From this data FCMM + A appeared to give the best performance; all 11 organisms were recovered. FCMM produced similar results, but one of the anaerobic cocci was not detected; in BBL the *Propionobacterium* was not detected; in THIOL, two anaerobic cocci were not detected, and in BHI four were not detected. THG gave the worst performance and only three bacteria were detected.

**Table**  
Detection times of 11 obligate anaerobic bacteria in six culture media using simulated blood cultures

| Organisms                      | Inoculum (cfu/ml) | THIOL | BBL | THG | BHI | FCMM | FCMM + A |
|--------------------------------|-------------------|-------|-----|-----|-----|------|----------|
| *C. perfringens* NCTC 8237     | 1                 | 36    | 11  | 14  | 27.5| 8.0  | 16.0     |
| *C. perfringens* clinical isolate | 2              | 17    | 12.5| ND/NG| 27.0| 18.0 | 10.5     |
| *C. difficile* NCTC 11206      | 10                | 33.5  | 42.5| ND/NG| 16.5 (2) | 17.0 | 18.0     |
| *B. fragilis* NCTC 10584       | 18                | 30.0  | 39.0| (1) | 36.5| 21.5 | 19.0     |
| *B. fragilis* NCTC 9343        | 100               | 20.5  | 6.5 | ND/NG| 13.0| 19.5 | 59.0     |
| Anaerobic cocci                 |                   |       |     |     |     |      |          |
| NCTC 9811                       | 1                 | ND/NG | 55.5| ND/NG| ND/NG| 13.7 | 32.0     |
| NCTC 9803                       | 2                 | 30.0  | 21.0| 45.0| (1) | (1)  | 86.5     |
| NCTC 9814                       | 6                 | ND/NG | 54.0| (1) | (1) | 25.5 | 26.5     |
| Anaerobic coccus clinical isolate | 100              | 91.0  | 40.5| ND/NG| 26.0| 23.5 | 43.0     |
| *Fusobacterium necrophorum*     |                   |       |     |     |     |      |          |
| NCTC 10723                      | 6                 | 18.5  | 13.5| 28.0| 33.0| 13.0 | 17.5     |
| *Propionobacterium* spp         | 113               | 110.5 | ND/NG| ND/NG| ND/NG| 82.0 | 81.5     |

**NOTES**  
(1) No Malthus detection despite growth on subculture.  
(2) Malthus detection unsubstantiated by subculture.

Media examined were THIOL (Difco), BBL (BBL cooked meat medium), THG (Brewers thioglycollate medium), BHI (brain heart infusion medium), FCMM (fresh cooked meat medium) and FCMM + A (fresh cooked meat medium + agar).  
ND/NG (No instrument detection, No growth on final subculture).

**Recovery of anaerobic bacteria from six blood culture media**  
All organisms were recovered from FCMM + A and FCMM at a minimal inoculum level; 10 were recovered from THIOL, nine were recovered from both BBL and BHI; only five organisms were recovered on final subculture from THG. The relationship between growth on subculture and changes in conductivity

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resulting in detection by the system were excellent using FCMM + A. Thiol and FCMM were in agreement in all but one bottle for each media; this displayed growth on subculture without an accompanying detection by the instrument. In BBL only one detection time appeared when there was no growth; with the other 10 organisms there was a close relationship between detection time and growth on subculture. In BHI broth, with seven out of the 11 organisms tested, there was a similar close relationship. THG did not support good conductance changes when minimal inocula were used, and detection in two cells did not take place when there was evident growth on subculture.

The effect of bacterial metabolism on conductivity changes in the six media

In general the 11 different anaerobes produced the greatest conductivity changes in FCCM and FCCM + A media. Thiol produced changes that were only slightly lower. The conductivity changes generated by the 11 organisms in both BHI and BBL media were similar and generally lower than that of FCMM + A, FCCM and Thiol. THG supported little conductance change for any of the organisms.

Figure 1 (Clostridium perfringens, NCTC 8237) and Figure 2 (an anaerobic coccus, NCTC 9814) demonstrate the differing conductivity changes due to metabolism of two bacteria on a variety of media. These figures highlight the need for a medium which produces relatively large changes in conductivity. Especially with Clostridium perfringens, Thiol, BHI and THG media did not support detection by conductivity changes at minimal inocula levels. For both organisms, FCCM and FCCM + A produced large changes in conductivity.

Low initial levels of inocula prolonged detection time and decreased resulting conductivity changes. This occurred with all organisms and in all six media. In FCCM + A and FCMM the decrease in conductivity was not significantly large enough for bacterial detection to be missed with decreasing inoculum levels, but in BBL and BHI media, the effect of small inocula could cause large decreases in the changes of conductivity.

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DISCUSSION

The media selected for study are widely used in blood culture systems, and are often incorporated especially for their ability to support the growth of anaerobes. When judged by the detection of growth by routine subculture, cooked meat media and Thiol medium have been shown to have advantages in the range and speed of recovery of anaerobic bacteria. Successful use of the Malthus system depends on changes in conductivity induced by microbial metabolism of the available substrates. Boynes et al. have shown that the magnitude of conductivity change depends on the type of medium used. Our results confirm this and show that freshly prepared cooked meat media yielded the greatest gross conductivity changes. Anaerobic growth induced somewhat smaller conductivity changes in Thiol medium and substantially smaller changes in BBL, BHI and THG. In general, those media reported to be of value in anaerobic detection by conventional means seem to be the most desirable for use in anaerobic detection using the Malthus system. Cooked meat media and Thiol are the most useful and THG the least useful substrate.

The efficacy of different media in such a system should also be judged on a number of false positive and false negative alerts to detection which the instrument records. We have demonstrated with a range of anaerobic bacteria that with those media tested, excepting THG, the growth of anaerobic bacteria produces conductivity changes which are detected. Those delays in detection which we have noted in a few instances are not due to any insensitivity of the electrode detection system but to limitations of the detection programme.

False positive detection has provided a greater problem. However, at a technological level our recorded rate of false positive detections due to electrode instability of 5.4% is a confirmation of improvements made to the system since the published observations of Brown et al (1984). Bottles with electrodes should in any case be tested on the analyser or by a cell-tester before distribution to wards. This will now be a practicable proposition. Increased familiarity with the instrument over a six-month period has emphasised the importance of controlling water bath levels and temperatures, careful electrode cleaning, and rapid and efficient red cell lysis. False positive detections arising from failure in these areas can be overcome only by awareness and subsequent good management.

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