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Automated blood culture systems

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Summary — This review compares automated systems of blood culture for the detection of positive bottles, excluding mycobacteria. The performance of different systems is influenced by several key variables, including volume of the blood sample, the use of resins, shaking to increase the recovery of aerobic microorganisms, duration of incubation and final subculture. The Bactec, BacT/Alert, BioArgos and ESP systems require further study and technical improvement. There is no single ideal system of blood culture, and combinations of two or more methods are likely to provide the best results.

automation / blood culture

Résumé — Systèmes automatisés d'hémoculture. *Nous avons comparé différents systèmes d'automatisation des hémocultures pour la détection de microorganismes (sauf les mycobactéries). Les résultats obtenus dans les différents systèmes varient selon le volume de l'échantillon, l'utilisation de résines et la durée de l'incubation et des cultures. L'existence d'une agitation augmente la récupération des microorganismes aérobies. Les systèmes Bactec, Bact-Alert, Bio Argos et ESP nécessitent d'autres études et des améliorations techniques. Il n'existe pas un système unique, idéal pour les hémocultures, et il est nécessaire d'associer deux ou même plus de deux méthodes pour obtenir les meilleurs résultats.*

hémoculture / automate

Introduction

Recent years have seen the development of increasingly rapid and sensitive methods for the detection of positive blood cultures. Several physical detection systems have been proposed to detect early growth of microorganisms, excluding mycobacteria. These systems are based on the measurement of changes in impedance between two electrodes, of ATP by bioluminiscence, the detection of carbon dioxide generated during bacterial metabolism, or the production of fluorescent metabolites. The purpose of this article is to compare some currently available automated systems in terms of their potential to replace conventional blood culture techniques.

One conventional technique comprises the so-called diphasic systems such as Septi-Chek, used to detect aerobic bacteria. A solid layer of chocolate agar, McConkey agar and malt agar is attached to the bottle; the liquid phase can consist of TSB, TSB with sucrose, BHI broth (alone or supplemented), thioglycolate, Columbia broth, Schaedler broth or BHI broth with saponin. The broth is subcultured twice on the slide in the upper chamber of the system (twice on the first two days and once daily until day 7).

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Automated methods for detecting bacterial growth

The Bactec system (Becton-Dickinson)

This system is based on the detection of CO_2 with a scintillation counter or by infrared spectroscopy. The amount of CO_2 produced by bacterial metabolism is translated into a growth index (GI). The levels of CO_2 produced are not characteristic of each species, although attempts have been made to relate these values with specific types of microorganisms. Bottles showing significant GI are subjected to subculture and staining. Each laboratory sets its own time to detection, number and sequence of subcultures. Culture for 5 to 7 days detects 90% of the microorganisms, although most of them are recovered within the first three days [1, 2]. The Bactec package uses different types of bottles depending on the assay (table I) [3-7]. Nonradiometric methods to detect microorganisms other than mycobacteria include Bactec NR-660, NR-730 and NR-860. The NR-660 and NR-730 systems differ in that the latter includes a rack incubator and a monitor for several functions, which tracks bottles inoculated with specimens from different patients and episodes of sepsis. The new NR-860 system incorporates automatic reading triggered when the first rack is loaded. These systems may also be of use in isolating mycobacteria [8] from routine blood cultures following subculture in specific medium. They detect *Legionella* in the few cases that present bacteriemia [9], but do not detect *Helicobacter pylori* [10, 11]. The recently developed Bactec 9240 system is based on the detection of CO_2 that reacts with characteristic meta-

bolites, which in turn fluoresce under ultra-violet light. This system avoids the use of gases and the risk of contamination between bottles due to deficient sterilization of the needle. Bottles for aerobes and for anaerobes, as well as resin-containing cultures, are available for use with TSB as the culture medium. Bottles without resin should be inoculated with 5–7 ml blood, bottles with resin requiring 8–10 ml. The incubator can handle up to 240 bottles, which are scanned at 10 min intervals. This system detects more aerobes in less time than Bactec NR-660 [12, 13].

The BacT/Alert system (Organon Teknika)

This automated colorimetric system detects bacterial growth as a function of CO_2 production. It uses aerobic and anaerobic bottles containing 40 ml of medium (TSB, sodium polyanetholsulfonate and CO_2), and required inoculation with 5 or 10 ml of blood. Once inoculated, the bottles are bar-coded and placed in a self-contained incubator-shaker-detector, that provides rapid access to clinical and microbiological information for each patient. Detection of CO_2 production, and the addition of gas, are noninvasive. At the bottom of each bottle is a CO_2 sensor, which is separated from the medium by a semipermeable membrane. Under alkaline conditions, the sensor is blue or dark green, but becomes light green or yellow when pH decreases.

Another feature of the system is its incubator capacity, which can process 240 bottles simultaneously. The system scans each bottle every 10 min, and transmits the signals to a microcomputer for analysis. The results are expressed as reflectance units in relation to time, and can be used to generate a bacterial growth curve. Changes in CO_2 concentration are compared with values obtained in previous runs, so that the threshold value for positive growth is determined with reference to earlier assays. This makes it possible to distinguish true positive cultures from signals produced by CO_2 dissolved in the patient's blood. When CO_2 concentration rises, a light indicating a positive bottle comes on, and the system's printer reports the bottle number, the patient identifier and the time elapsed to detection. The bottle is then removed, and the free well can be occupied by another sample. After 7 days, the bottles that fail to trigger a positive reading are listed on a print-out, and their pilot lights come on, signaling that they can be discarded. The cut-off for negative cultures can be programmed at will. This system obviates the chance of cross-contamination since it does not require gas to be aspirated off, and may provide earlier detection of bacterial growth, given its frequent scanning cycles.

The main disadvantage of the Bact/Alert system is the unavailability of bottles containing resins. In the Pedi-BacT system (14), a recently-developed pediatric blood culture bottle, the number of false positive results is lower than 1% [15]. Recent studies with the most frequently isolated microorganisms indicate a high rate of recovery during the first week of incubation, without the need for final subculture [16].

BioArgos (Sanofi Diagnostic Pasteur)

BioArgos is a self-contained, closed system. The sample loading unit includes a bar code reader, and up to 57 bar-coded vials can be loaded before transfer to the measuring and shaking unit. An infrared spectrophotometer detects CO_2 through the headspace of the glass bottles. An incubator accommodates up to 720 vials on six thermostatic trays. Four utility programs are available on the computer-controlled system: specimen processing, operating parameters, user maintenance and assistance. As Bio-Argos is a hands-off system, no aerobic or anaerobic gas is injected into the vials during processing.

The usual incubation time of 7 days can be either reduced by the microbiologist to as little as 5 days, or extended up to 14 days [17]. When vials are considered positive, the machine automatically transfers them to the incubating box until further processing. Negative blood culture vials are automatically discarded by the machine on day 8.

Three optimized blood culture media designed to enhance CO_2 production by microorganisms are available. Aerobic medium (BioArgos AER) is a brain heart infusion enriched with cysteine, hemin and vitamins B2, B5, B6 and K, suited for growth of aerobes and facultative anaerobes. Anaerobic medium (BioArgos ANAER) is prereduced Schaedler broth enriched with hemin, thiols and vitamins B6 and K. Anticoagulants used are 0.035% sodium polyanetholsulfonate in the aerobic medium, and 0.02% sodium

Table I. Bottles, media and sample volumes usable with the Bactec system [3–7].

Bottle	*Volume of blood*	*Volume of medium*	*SPS*[a]
6A/7A	5 ml	30 ml/TSB	0.035% w/v
8A	5 ml	30 ml/TSB + sucrose 10% w/v	0.025% w/v
6AX/7AX	5 ml	30 ml/TSB	———
16A/17A	5 ml	30 ml/TSB+ resin	0.025% w/v
26A/27A	10 ml	25 ml/TSB+ resin	0.05% w/v
PED Plus	3 ml	20 ml/TSB+ resin	0.025% w/v
LYTICO	5 ml	30ml/TSB+ saponin	0.035% w/v

[a] Sodium polyanetholsulfonate.

Table II. Most important differences between the systems compared (See text for further details).

	Bactec
BacT/Alert	BacT/Alert takes longer to detect yeasts. However, gram-negative rods and both aerobic and anaerobic Gram-positive cocci are detected earlier with BacT/Alert.
Septi-Chek	Bactec is superior in detecting Gram-positive aerobic microorganisms. Septi-Chek is superior in producting microorganisms in subcultures.
ESP	ESP detects growth more rapidly, although the number of false-positives is somewhat higher than with Bactec.

amylosulfate in the anaerobic medium. The atmosphere is composed of CO_2 in aerobic vials, and a reducing atmosphere in anaerobic vials. The usual volume of blood needed for inoculation is 3 to 5 ml.

All information on positive vials is automatically printed out. Moreover, a light located on the system signals the presence of positive vials.

The ESP system (Difco)

The recently introduced ESP system detects bacterial growth electronically, by sensing changes in pressure within the blood culture bottle. When bacteria begin to grow, gas pressure (O_2) initially falls, then rises (mainly due to the production of CO_2 and N_2). Aerobic (ESP-80A), and anaerobic bottles (ESP-80N) can be used, and the basic growth medium is TSB, enriched to enhance the changes in pressure. Anaerobic bottles include vitamin K_1, hemin and cysteine. Each bottle contains 80 ml of medium, and requires inoculation with 5–10 ml of blood. The incubator, which can process up to 384 bottles at a time, reads aerobic cultures at 12-min intervals, an anaerobic cultures at 24-min intervals.

This system detects growth rapidly, with high recovery rates for *S agalactiae*, *Enterococcus* spp, *Enterobacter* spp and anaerobes [13].

Comparison of systems

The reading systems used in the microbiological analysis of blood culture are difficult to compare, due to differences in many key variables. No single system clearly stands out as superior to the others, and combinations of two or more systems, such as those suggested below, will be required to achieve optimum results. In table II we summarize the most important differences between the systems compared here.

Bactec versus conventional techniques (Septi-Chek)

The automated Bactec system, in contrast to conventional methods, provides early detection of most microorganisms: aerobic cultures can often be read within 2 days, anaerobic cultures requiring up to 4 or 5 days. The contamination rate is low. With nonradiometric systems, if the GI on day 10 is negative, the bottles can be safely discarded. The occurrence of false negative cultures after 10 days for *Brucella*, dimorphic fungi and yeasts [18–21] makes subculturing advisable to rule out infection. Our experience with the Bactec NR system has shown that false negative GI values can appear after 10 days of culture in bottles from which *Brucella* were subsequently subcultured. It may therefore be advisable to perform routine subcultures after 5 days of primary culture to accelerate detection, and after 10 days if primary culture yields a negative GI when brucellosis, fungemia or HACEK group infection is suspected, or when the blood sample is from an immunocompromised patient. False positive cultures have been associated with hypercapnia and polyglobulia [22, 23]. Other major disadvantages are the risk of contamination between bottles due to deficient sterilization of the inoculation needle. No statistically significant differences have been found in the species isolated, or the number of microorganisms isolated after 6 days of culture, with one or the other approach. The addition of resins in the Bactec system reduces the time to detection, and increases the number of organisms detected (but not the number of species) in patients receiving antibiotics [24, 25]. These advantages justify the high initial cost of the system. However, more false positives have been obtained with Bactec [26], and like conventional systems, it is ineffective in the early detection of slow metabolizing microorganisms [27].

The Bactec system is superior to Septi-Chek, especially in the rapid detection and isolation of gram-positive aerobic microorganisms [28, 29]. If biphasic bottles are subjected to constant shaking, the speed and yield approach those of Bactec, at the expense, however, of higher rates of contamination [30]. Bactec bottles provide higher yields of staphylococci (NR 26A and 27A bottles), streptococci and *Enterobacteriaceae* (NR 26A bottle) in less time than Septi-Chek, with agitation during the first 24 h [29, 31, 32]. The main advantage of the Septi-Chek system is the production of microorganisms in subcultures, which accelerates identification and sensitivity studies. The advantages of the Bactec system include the use of bottles containing resins, and a computer-based system of access to the results.

Bactec versus BacT/Alert

Similar yields and times to detection have been reported with Bactec 660 and BacT/Alert [15], although BacT/Alert takes longer to detect fewer yeasts, grows fewer enterococci, and is slower in detecting *Staphylococcus epidermidis* than Bactec. On the other hand, gram-negative rods and both aerobic and anaerobic gram-positive cocci are detected earlier with BacT/Alert. Wilson *et al* [33] recently studied the recovery of microorganisms and the speed of detection of microbial growth by the BacT/Alert and Bactec 660/730 nonradiometric systems. Members of the family *Enterobacteriaceae* were recovered more often from BacT/Alert bottles, and more gram-positive cocci were recovered from the Bactec system. Growth of *S aureus*, coagulase-negative staphylococci, streptococci, *Escherichia coli* and *Pseudomonas aeruginosa* was detected earlier in BacT/Alert bottles. The overall recoveries of microorganisms from the two anaerobic bottles were not significantly different. The study cited above did not compare the performance of these systems in isolating *Brucella* spp. In conclusion, both systems are similar in recovering bacteria (perhaps including *Brucella*) or fungi, but the BacT/Alert system detected microbial growth earlier with fewer false-positives. Howewer, Solomon and Jackson [34] recently demonstrated that *B melitensis* was detected earlier by the BacT/Alert system in a laboratory replication study, and suggested that the system should reliably detect this organism when it is present in blood samples.

Bactec versus ESP

Both systems detect bacterial growth automatically and noninvasively (in the more recent releases of Bactec), using frequent scanning times (10 min in Bactec 9240 and 12 to 24 min in ESP). ESP seems to detect the growth of aerobes more rapidly, and recove-

ry of yeasts and *S pneumoniae* is greater, possibly because of small differences in the composition of the culture medium, or in the system of detection itself. However, the rates of contamination and false positives with ESP are somewhat higher than with Bactec [13].

Conclusion

A number of automated systems are currently available that detect many of the microorganisms that cause bacteremia or fungemia. The slight differences in the number of microorganisms isolated, the time to detection, and the contamination rate, reflect features inherent to each system (*eg*, volume of blood needed for inoculation and culture medium). Thus the choice of one system over another will be conditioned mainly by the infrastructure available in the microbiology laboratory, and on the needs of the hospital it serves.

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