Growth and detection of filamentous fungi in the BacT/Alert blood culture system

A Breathnach, J Evans

Abstract
Little is known about the behaviour of filamentous fungi in most blood culture systems, despite their increasingly recognised role in infections of immunocompromised hosts. The ability of the BacT/Alert system (Organon Teknika, Durham, North Carolina, USA) to detect the growth of 19 such fungi was examined. Eleven species grew and were detected rapidly; two species did not grow. Six species grew slowly, and were generally only recovered with terminal subculture after prolonged incubation. The CO₂ production graph for some of these fungi showed a slow but steady rise, insufficient to cause the apparatus to signal positive. These results show that the BacT/Alert system may miss some fungi, either because of no growth in the medium or undetected slow growth. The latter problem could be overcome by prolonged incubation and terminal subculture when fungal infection is considered likely. Alteration of the signalling mechanism might permit earlier detection of some slow growing fungi.

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Keywords: BacT/Alert, blood cultures, filamentous fungi.

Filamentous fungi are an increasingly important cause of infection in the immunocompromised host, accounting for much morbidity and mortality in these patients. The diagnosis of systemic fungal infection still presents many difficulties and is frequently established only by histological examination of biopsy or necropsy samples. Biopsies are invasive and in many patients the presenting features are vague, with little indication of which organ, or organs, is affected. Serological tests often lack both sensitivity and specificity, each test only detects one species of fungus and they are rarely available outside reference centres. Molecular biological techniques are similarly not widely available. Blood cultures are simple and safe, are widely used and occasionally yield a diagnosis of fungal infection. However, relatively little is known about the behaviour of non-yeast (mycelial) fungi in most blood culture systems. Failure to diagnose fungi by this method may be due to either an inability of the blood culture system to detect the fungus, or the absence of fungal spores or hyphae from the bloodstream. Most blood culture systems are designed primarily to detect the growth of bacteria and yeasts, and in trials of these systems mycelial fungi have seldom been isolated. This is the case even with systems designed to optimise the isolation of fungi. The BacT/Alert system (Organon Teknika, Durham, North Carolina, USA) is a recently introduced automated blood culture system which can rapidly isolate many bacteria and yeasts. We examined its ability to support and detect the growth of a range of pathogenic filamentous fungi.

Methods
The BacT/Alert blood culture system is an automated system in which the base of each blood culture bottle contains an indicator which responds to a change in CO₂ concentration by a change in reflectance: the incubator contains a photometric apparatus which measures this reflectance. Repeated measurements are analysed electronically and the apparatus signals positive when there is either a sustained rise in reflectance of a certain minimum rate, an acceleration in the rate of reflectance increase or a high initial reflectance. There are three types of bottle: aerobic, anaerobic and paediatric. The adult aerobic and anaerobic media are based on triptic soy broth, and the paediatric bottles contain a smaller volume of medium based on brain heart infusion broth with a reduced concentration of liquid, the intention being to allow better growth of fastidious organisms.

Nineteen strains of potentially pathogenic fungi obtained from a mycology reference laboratory (table) were inoculated into paediatric and adult aerobic blood culture bottles. Anaerobic bottles were not used because the fungi examined would not be expected to grow anaerobically. The inoculum was between 10 and 130 cfu; this was obtained by making serial dilutions of a suspension of the fungus in distilled water and subculturing fixed volumes onto Sabouraud's dextrose agar (Oxoid) to establish the colony count. Whole human blood was also added to each bottle (5 ml for adult bottles, 1 ml for paediatric bottles), and the
Detection of filamentous fungi

Detection of filamentous fungi for Exophiala spinifera: Growth

I.) Growth detected by apparatus
(2) No growth detected by apparatus
(Fungi isolated from one or both bottles after prolonged incubation)
Exophiala dermatitidis*
Phialophora verrucosa*

(3) Growth detected in paediatric bottle only
(Fungi isolated from aerobic bottle after prolonged incubation)
Bipolaris haawaiensis*
Exophiala spinifera

(4) No growth
Acremonium sp.

* Visible fungal growth inside bottle.

<table>
<thead>
<tr>
<th>Days tested</th>
<th>Reflectance units</th>
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<tbody>
<tr>
<td>0</td>
<td>1000</td>
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<tr>
<td>1</td>
<td>1500</td>
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<td>2</td>
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<td>7</td>
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<td>8</td>
<td>5000</td>
</tr>
</tbody>
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bottles were then vented. An aliquot was taken from each bottle at this stage and cultured as above to establish a baseline colony count. The bottles were loaded into the incubator and incubated for 14 days at 35°C, unless they signalled positive before this. When the bottles were removed, they were examined for obvious fungal growth, and subcultured onto Sabouraud’s agar and blood agar (Oxoid). Following incubation of these plates for up to five days in air at 30°C, colony counts were noted and the identity of the colonies confirmed.7 Those bottles which remained negative at 14 days were kept at room temperature and examined and subcultured as above at 21–35 days. A graph of the repeated measurements of reflectance, indicating metabolic activity, was obtained for each bottle.

Results

As shown in the table, 11 of the 19 fungi examined grew well and were quickly detected in both paediatric and aerobic bottles (the mean time to detection was 1–9 days). Only two of the fungi examined (Acremonium sp. and Alternaria alternata) failed to grow in either bottle, even with prolonged incubation. Of the remaining six species, two (Bipolaris haawaiensis and Exophiala spinifera) were detected in the paediatric bottle after three to five days of incubation; they did not signal positive in the aerobic bottle, but were recovered after prolonged incubation. Four species grew slowly, either in both bottles (E dermatitidis) or in the paediatric bottle only (Penicillium sp., Phialophora richardia and P verrucosa). These did not signal positive, and were recovered by subculture at 14 to 35 days.

Several of the bottles examined at the end of incubation, or after one to two weeks at room temperature, had visible growth of fungus within. In two of these cases (B haawaiensis, A niger) repeated subculture yielded very few colonies, no more than the baseline colony count.

When the graphs of the serial reflectance readings for each bottle were examined, we noted that, among those bottles which failed to signal positive, some of those which subsequently yielded colonies gave readings which rose steadily, compared with genuinely negative bottles which gave a virtually flat trace (figure).

Discussion

This study shows that, while the majority of the fungi examined were easily detected by the BacT/Alert system, a significant number grew slowly and escaped detection, and some species did not grow at all. It is important to note that the inocula we used, while small, may still be much larger than that of a clinical specimen. While many of these fungi are obviously capable of growth at 35°C (A fumigatus can grow at up to 50°C), some environmental fungi may be outside their optimum growth range at this temperature, accounting for the poor growth of some strains tested. The observation that two species of fungi gave visible growth inside the bottle despite the lack of increase in colony counts on subculture may be due to the fact that in each of these cases the fungal mass did not appear particularly friable: B haawaiensis was adherent to the glass, and A niger formed a dense floating mycelium, and it is possible that they failed to release spores or viable hyphae into the broth.

These findings confirm the general limitations of blood cultures in isolating fungi. The results also show that an automated system may fail to detect organisms with a low rate of metabolism (figure). A modification of the algorithm to detect slow but sustained rises in CO2 production might overcome this problem.

The yield of fungi was increased by a factor of 27% in this study, and by similar factors in other studies,8 by the practice of prolonged incubation and terminal subcultures. We doubt that this would be worthwhile in routine laboratory practice for several reasons: it would entail considerable effort and expense, the absolute yield would be small, and the information gained, while interesting, would often be too late to be of relevance. However, clinicians and microbiologists who consider this option when fungal infection is suspected, particularly as the tendency with the BacT/Alert system seems to be to shorten the duration of incubation.9
Longstanding proliferation of CD3 negative large granular lymphocytes preceding the development of high grade non-Hodgkin's lymphoma

S R Smith, P G Middleton, P J Birch, L Morgan, P W G Saunders

Abstract
A patient with a persistent CD3 negative large granular lymphocyte (LGL) proliferation with immunophenotypic and functional characteristics of natural killer cells is described. The LGL proliferation persisted and six years after diagnosis the patient developed a high grade B cell non-Hodgkin's lymphoma. Molecular studies demonstrated clonal B cell populations in the peripheral blood, distinct from that identified in the lymphoma, both at presentation with non-Hodgkin's lymphoma and at complete remission following combination chemotherapy. It is postulated that T cell dysregulation associated with the CD3 negative LGL proliferation may have led to B cell dysfunction and loss of normal B cell control, with the subsequent development of a clonal B cell lymphoproliferative disorder.

Keywords: CD3 negative large granular lymphocytes, non-Hodgkin's lymphoma.

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