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Enhanced detection of *Candida* in blood cultures with the BACTEC 460 system by use of the aerobic-hypertonic (8B) medium

P. YAGUPSKY, F. S. NOLTE, AND M. A. MENEGUS

Clinical Microbiology Laboratories, University of Rochester Medical Center, 601 Elmwood Avenue, Box 710, Rochester, New York 14642, USA

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SUMMARY

The blood culture records during the 6-year period 1984–9 were reviewed to compare the performance of the BACTEC aerobic (6B) and aerobic-hypertonic (8B) media for the recovery of *Candida* spp. and *Torulopsis glabrata* from blood. There were 137 positive blood culture sets that contained both a 6B and an 8B bottle. Sixty-eight different yeasts were recovered from 65 patients including 35 *Candida albicans*, 19 *C. tropicalis*, 6 *C. parapsilosis*, 4 *C. krusei*, 1 *C. pseudotropicalis* and 3 *Torulopsis glabrata*. The 8B medium detected 120 of the positive cultures (87·6%) and was the only positive medium in 35 (25·6%) sets, while the 6B medium detected 102 positive cultures (74·4%) and was the only positive medium in 17 (12·4%) sets ($P < 0{\cdot}04$). For those sets in which both bottles were positive, radiometric detection occurred first in the 8B bottle in 39 sets and first in the 6B bottle in 11 sets ($P < 0{\cdot}001$). The superior performance of the 8B bottle was not related to the administration of amphotericin B. Cultures of stock strains of *C. albicans*, *C. tropicalis*, and *C. parapsilosis* in 6B and 8B media with and without added blood confirmed the finding that 8B was substantially superior to 6B for the detection of candidaemia. It is concluded that an 8B bottle should be included in the blood culture set whenever candidaemia is suspected.

INTRODUCTION

A marked increase in the incidence of disseminated candidosis and other opportunistic systemic fungal infections has been observed in recent years [1]. This may be related to the prolonged life expectancy of patients at risk and to the increased use of intravenous catheters, broad spectrum antibiotics and immunosuppressive therapy [2]. The mortality rate for these infections remains unusually high due, at least in part, to the difficulties in making the diagnosis [1]. Although blood cultures detect yeast in only half of the autopsy-proven cases of disseminated candidosis, a positive blood culture is commonly the only antemortem diagnostic test in such patients [3]. Improved methods for the recovery of yeasts from blood should result in earlier treatment of the infection and thus in a better prognosis.

In recent years, hypertonic media were developed to enhance the recovery of cell-wall damaged microorganisms from blood and other body fluids. The BACTEC

(Johnston Laboratories, Towson, MD) aerobic-hypertonic medium (8B) has been shown to enhance the recovery of *Staphylococcus aureus*, members of the family Enterobacteriaceae and *Haemophilus influenzae* type b from blood cultures containing antibiotics in both clinical and experimental studies [4–7].

An observation made by one of us that the 8B medium appeared to improve detection of candidaemia, prompted a review of the blood culture records of the University of Rochester Medical Center, New York, to determine the relative performance of the aerobic (6B) and the 8B medium for the recovery of *Candida* spp. and *Torulopsis glabrata* (*Candida glabrata*) from the blood.

MATERIALS AND METHODS

Patient blood cultures

BACTEC 6B, 8B, and anaerobic (7C or 7D) media were available at all patient locations throughout the hospital. Blood for culture was drawn by the staff physicians and various combinations of the three available media were inoculated at the patients' bedside. The 6B and 8B bottles were agitated during the first 24 h of incubation. All bottles were incubated at 35 °C for a maximum of 7 days. The head space gas of the 6B and 8B bottles was analysed radiometrically three times per day on day 1, twice per day on day 2, and once per day on days 3, 4, 5 and 7, using a BACTEC model 460 instrument equipped with an Apple II plus microcomputer (Apple Computer, Inc., California) and MicrA (Argus Inc., Tampa, Florida) data management software. The anaerobic bottles were examined radiometrically once per day on days 2, 3, 4, 5 and 7. Radiometric criteria for positivity on days 1 and 2 were: growth index $\geqslant 20$ and $\geqslant 30$ for 8B and 6B media, respectively, or an increment of the growth index by 10 or more units between two consecutive readings, and on days 3, 4, 5 and 7 a growth index $\geqslant 30$ for the 8B medium and $\geqslant 40$ for 6B medium or an increment $\geqslant 20$ units between consecutive readings for both media. Gram-stained smears were prepared from radiometrically positive bottles and the results were used to select the appropriate media for subculture. Gram-stained smears were also made from radiometrically negative bottles of the same blood culture set and subcultures were performed on all bottles in the set regardless of the Gram-stain results. Radiometric monitoring was continued for those bottles with a growth index less than the threshold value and a negative Gram stain. Terminal subcultures of radiometrically negative bottles were not performed.

The blood culture records of the Clinical Microbiology Laboratories of Strong Memorial Hospital were retrospectively reviewed for the period 1 January 1984 to 31 December 1989 to identify cultures positive for *Candida* spp. and *Torulopsis glabrata*. Although various combinations of the three available bottles constituted a blood culture set, only sets that included both a 6B and an 8B bottle were considered in the data analysis. The recovery of fungi from the 6B and 8B media were compared. The BACTEC run in which each bottle was radiometrically positive was compared for 6B and 8B bottles for blood culture sets in which fungi were recovered by subculture from both bottles. For the comparison, a uniform growth index threshold of $\geqslant 20$ units, or an increase of $\geqslant 10$ units was used for both media. This was done to eliminate the possibility that observed differences

in the time to radiometric positivity were simply the result of differences in the positive criteria used. Blood cultures yielding bacterial as well as fungal growth were omitted from the analysis.

Simulated blood cultures

Standardized inocula of stock cultures of *C. albicans*, *C. tropicalis* and *C. parapsilosis* were prepared in Mueller–Hinton broth (BBL, Cockeyville, Maryland). One ml of the standardized inoculum was mixed with 29 ml of out-dated human donor blood and 3 ml of resultant mixture was inoculated into five 6B and five 8B bottles. The number of viable yeast per bottle at 0 time was determined by plate counts. Inoculated bottles were incubated at 35 °C, shaken during the first 24 h and radiometric growth index values for each medium was recorded over time. At the end of the experiment all positive bottles were subcultured to a blood agar plate as a purity check. In a second experiment, the growth of the three stock cultures in 6B and 8B medium without added blood was also monitored radiometrically and compared.

Yeast identification

Yeasts were identified by a combination of morphologic, physiologic, and biochemical tests according to accepted methods [8].

Patients

To evaluate the role of antifungal therapy as a confounding variable, the medical records of patients with positive blood cultures were reviewed to determine whether any were receiving systemic antifungal therapy at the time the blood cultures were drawn.

Statistical analysis

A test for matched-pair analysis of discrete data (McNemar's test with correction for continuity) was used to assess the statistical significance of the observed differences between discordant 6B-8B pairs.

RESULTS

During the 6-year period, 418 of 107177 (0·4%) blood-culture sets obtained with the BACTEC system were positive for yeasts. One hundred and thirty-seven of them included both a 6B and an 8B bottle. These positive cultures were obtained from 65 patients. The number of positive patients and cultures for each yeast are given in Table 1. Isolation of two yeast from the same blood culture occurred in three patients (*C. albicans* and *C. tropicalis*; *C. albicans* and *C. parapsilosis*; *C. tropicalis* and *C. krusei*).

Overall, 102 (74·4%) positive cultures were detected in the 6B medium compared with 120 (87·6%) positive cultures detected in the 8B medium. Yeasts from 17 (12·4%) of the positive cultures were detected in the 6B medium alone; in contrast, 35 (25·6%) were detected in the 8B medium alone ($P < 0·04$). The anaerobic medium was excluded from the data analysis because when culture sets containing any media were considered, only 11·1% of the positive cultures for yeasts were detected in the anaerobic medium (data not shown).

Table 1. *Yeasts isolated with the 6B and 8B BACTEC 460 media, 1984–9*

Yeast species	No. patients	No. positive cultures
Candida albicans	35	77
Candida tropicalis	19	36
Candida parapsilosis	6	7
Candida krusei	4	12
Candida pseudotropicalis	1	1
Torulopsis glabrata	3	4
Total	68	137

Yeasts were recovered from both the 6B and 8B bottles in 85 (62·0%) blood culture sets. A complete log of all the daily growth index readings was available for 75 (88·2%) of these cultures. Using the uniform radiometric criteria for positivity, growth was detected during the same machine run in both media for 25 (33·3%), first in the 6B medium for 11 (14·6%) cultures and first in the 8B medium for 39 (52·0%) cultures ($P < 0{\cdot}001$).

The medical records of 45 patients from whom 92 positive blood cultures were drawn were reviewed. At the time the blood cultures were drawn, two patients were receiving oral nystatin for *Candida* stomatitis and were not considered to be treated with systemic antifungal therapy. Only 13 cultures were obtained while the patients were being treated with amphotericin B. Ten were positive in both media, two were detected in the 8B medium only and one in the 6B medium only ($P > 0{\cdot}5$). Among the remaining positive cultures, obtained from amphotericin-free patients, 46 were detected in both media, 10 in the 6B only and 23 in the 8B only ($P < 0{\cdot}05$).

The growth index curves obtained from simulated blood cultures of *C. albicans*, *C. tropicalis* and *C. parapsilosis* showed that all yeasts produced greater maximal growth index values in 8B medium. The times required to achieve the maximum growth index values were shorter for 8B medium with cultures of *C. albicans* and *C. tropicalis*. Maximal growth index values for 6B and 8B cultures of *C. parapsilosis* occurred at the same time. Using the criterion for radiometric positivity of $\geqslant 20$ units and/or a change $\geqslant 10$ units between two consecutive readings, 2/10 6B cultures and 10/10 8B cultures of *C. albicans* were recognized as positive after 24 h of incubation. For *C. parapsilosis* the time required for all bottles to be recognized as positive was 35 h and did not differ for medium type. For *C. tropicalis* all 8B bottles satisfied the threshold criterion after 21 h of incubation and all 6B bottles after 26 h, and all 8B bottles were positive before all 6B bottles.

The growth index curves obtained in 6B and 8B media without added blood were similar to those obtained in the media containing blood for the three stock cultures.

DISCUSSION

The BACTEC radiometric system is still one of the most widely used blood culture systems in clinical microbiology laboratories worldwide. This system has been shown to be superior to supplemented peptone broth [9] and biphasic

medium [10] for the detection of fungaemia. Although a lysis-centrifugation blood culture system (EI Dupont de Nemours & Co., Wilmington, Delaware) was shown to recover significantly more *Cryptococcus* spp., *Rhodotorula* spp., and *Torulopsis* spp., there was no statistically significant difference between the two systems for the recovery of *Candida* spp. [11]. None of these comparative studies included an aerobic-hypertonic medium as part of the BACTEC system.

Clinical experience with the use of hypertonic media for the detection of fungaemia has been limited. Ellner and colleagues, using a modified Columbia broth, with and without the addition of 10% sucrose, suggested that the hypertonic medium was inferior for the recovery of yeasts [12]. In another study using the radiometric BACTEC system, four yeasts were isolated by both the 6B and the 8B media, one by the 6B only and three by the 8B medium [13].

The present study is the only large comparison of 6B and 8B medium for the detection of candidaemia. Due to the retrospective nature of the study, the volume of blood inoculated was not controlled. Assuming that differences in the volume were distributed randomly between the 6B and the 8B media of the blood-culture set, our results show that the 8B medium recovered significantly more yeasts than the 8B medium. If the 6B bottle had been omitted, only 12·4% of the positive cultures would have been missed and if the 8B bottle had been omitted, one quarter would have been missed. The time for detection of the yeasts was also shortened by the 8B medium with the potential benefits of an earlier diagnosis of the fungaemia.

Our results are difficult to understand against the background of what is known about osmotically-stabilized media. It has been postulated that the high concentration of sucrose offers osmotic support to the cell-wall defective bacterial variants induced by β-lactam antibiotics, complement, or other components of the immune system. However, it has been demonstrated that *H. influenzae* type b showed higher growth index values in 8B than in 6B media cultured with and without the addition of blood or antibiotics, suggesting that other factors might also be involved [14]. In 1966, Rosner documented the usefulness of a hypertonic medium in the isolation of cell-wall damaged *C. tropicalis* from a case of endocarditis partially treated with amphotericin B [15]. In our study the salutary effect of the osmotically-stabilized bottle did not appear to be related to protection of amphotericin B-damaged yeasts since the 8B medium also significantly enhanced the recovery of *Candida* spp. from patients not receiving specific antifungal therapy. The results obtained in the simulated blood cultures were consistent with the clinical observations, while the blood-free experiment demonstrated that the salutary effect of the 8B medium may be unrelated to osmotic protection of the yeast from damaging blood components. Since the only significant difference in the composition of the 6B and 8B media is the sucrose concentration (0·25% and 10%, respectively) it is possible that the higher sucrose content of the 8B medium promotes the growth of the sucrose-assimilating yeasts (*C. albicans*, *C. tropicalis*, *C. pseudotropicalis*, and *C. parapsilosis*) [8].

A nonradiometric alternative to the BACTEC-460 was developed by Johnston Laboratories. The new system, the BACTEC NR-660 is similar in many aspects to the radiometric system, but identifies positive cultures by detecting increased CO_2 in the headspace gas using infrared spectroscopy rather than radiometry.

Jungkind and co-workers [16] found no significant difference between the BACTEC-460 and NR-660 systems for recovery of total isolates when the combined results from paired aerobic and anaerobic bottles were evaluated. We hope our data prompt the BACTEC NR-660 users to evaluate the role of hypertonic nonradiometric medium (NR8AO) in recovery of yeast from the blood.

We conclude that the 8B medium substantially enhances the isolation of *Candida* spp. from the blood and shortens the time required for radiometric detection. We recommend that an 8B bottle should be included in the BACTEC-460 blood culture set whenever candidaemia is suspected.

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