Advantages of BACTEC Hypertonic Culture Medium for Detection of *Haemophilus influenzae* Bacteremia in Children

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The hypertonic and aerobic culture media in the BACTEC system were compared for the detection of *Haemophilus influenzae* bacteremia in children. Of 1,611 blood cultures, 30 were positive for this pathogen. The aerobic and hypertonic media gave positive results in 28 and 29 cultures, respectively. Within the first 12 h, *H. influenzae* was detected in the hypertonic medium in 48.5% of the positive cultures as compared to 35% for the aerobic medium. Importantly, after the first 12 h, the hypertonic medium yielded positive results sooner than did the aerobic medium, the difference being statistically significant (P < 0.01). The hypertonic medium yielded positive results earlier than the aerobic medium in nine cultures; the reverse was seen in only one culture. Furthermore, the aerobic medium gave negative growth index readings despite growth, as shown by microscopy and subculture, in 43% of the total cultures in contrast to only 13% for the hypertonic medium, a significant difference (P < 0.05). Thus, the present study indicates a distinct advantage of hypertonic medium compared with aerobic medium in the automated BACTEC system for earlier detection of *H. influenzae* bacteremia and is recommended for those age groups in which this pathogen plays a predominant role.

Recovery and early detection of bacteremia are of great clinical significance. The culture medium used has profound effects on the pathogen recovery and detection time (14). Several investigators have reported on the beneficial effect of hypertonic medium, with sucrose as an osmotic stabilizer, in the detection of bacteria from blood by both conventional (5, 6, 10, 12) and radiometric (1, 2) methods. However, other reports have indicated no advantage to using a hypertonic culture medium (9, 15).

The most widely used automated procedure for the diagnosis of bacteremia is the BACTEC radiometric system (Johnston Laboratories, Inc., Cockeysville, Md.). This method uses media containing 14C-labeled substrates which, when metabolized by bacteria, yield detectable levels of 14CO2, which are read as growth index (GI) readings (3, 4). A recent report by La Scolea et al. (8) identified a shortcoming of the BACTEC system with regard to a delay in the diagnosis of *Haemophilus influenzae* bacteremia. In numerous instances, growth of this pathogen occurred, as documented by positive microscopy and subculture, but GI values were negative. More recently, Crist et al. (2), using simulated blood cultures with eight fresh isolates of *H. influenzae*, reported that the hypertonic medium was superior to the aerobic medium, because it permitted earlier detection by positive GI readings. The present study was designed to compare the BACTEC hypertonic and aerobic culture media in the diagnosis of *H. influenzae* bacteremia in children.

A total of 1,611 blood samples were obtained from patients in the outpatient department of The Children's Hospital of Buffalo for a 9-month period (October 1981 through June 1982). The outpatient department was supplied with special blood culture packages consisting of one BACTEC aerobic bottle with a quantitative direct plating (QDP) heparin tube attached and one BACTEC hypertonic bottle with instructions for the inoculation of the patient's blood. Equal amounts (1 to 3 ml) of blood were injected into the BACTEC aerobic and hypertonic bottles, and 0.5 to 1 ml of blood was inoculated into the QDP heparin tube. The QDP method was used to identify the number of organisms per milliliter of blood. Specimens containing less than 2 ml of blood were not included in the study. The time between the bleeding and the arrival of specimens in the laboratory was usually 1 h or less.

The radiometric system and the QDP method were used as previously described (8). The hypertonic medium (no. 8B) used in this study contained sucrose 10% (wt/vol). GI readings of 30 and 20 or higher were considered positive for
the aerobic and hypertonic bottles, respectively.

The methods to monitor aerobic and hypertonic bottles by GI readings, microscopy, and subculture have been previously described (7, 8). Identification of all isolates was performed by conventional procedures. All data obtained were statistically examined by the chi-square method (13).

Based on microscopy, subcultures, and GI readings of the BACTEC bottles, H. influenzae was recovered from both bottles in 27 instances. The aerobic medium and hypertonic medium each gave positive results in only one and two instances, respectively. Thus, for this pathogen the total recovery rate was comparable with both culture media. Of 28 cultures positive, 12 (43%) of the aerobic bottles had negative GI readings, even though 11 cultures at that time revealed the presence of numerous bacteria as shown by positive microscopy and subculture. In contrast, only 4 of 29 (13%) cultures gave negative GI readings with the hypertonic culture medium. In fact, two of these four cultures showed a significant sequential increase in GI before reaching values of 20 and above, and all were positive 4 h later. Thus, the hypertonic medium yielded better results than the aerobic medium based on GI readings, the difference being statistically significant (P < 0.05). It is noteworthy that with BACTEC hypertonic medium, 48.5% of the positive cultures were detected within the first 12 h (Table 1), as compared with 35% detected with the aerobic medium. The superiority of the hypertonic versus the aerobic culture medium is strikingly evident also from the fact that after the first 12 h, the former yielded significant GI values before the latter in nine instances, whereas the reverse was observed only in a single instance. This difference was statistically significant (P < 0.01). In five instances the time difference was between 2 and 8 h; in two instances, between 9 and 24 h; and, in two more instances, greater than 24 h.

Data on positive GI readings with the two culture media and the relationship of the number of H. influenzae organisms present in the blood to detection times as determined by the QDP procedure are shown in Table 1. The highest percentage of positive results (60%) detected within the first 12 h was achieved by the QDP method. It is also evident that earlier detection parallels greater numbers of organisms in the blood.

In this study the hypertonic medium proved to be superior to the aerobic bottle in the early detection by GI readings of H. influenzae, particularly after 12 h, although the total number of positive cultures was comparable. Furthermore, the hypertonic medium gave a superior correlation with positive GI readings, microscopy, and subcultures in contrast to the aerobic medium. The extent to which superiority of the hypertonic culture medium is due to enhanced growth, greater production of labeled CO2, or GI values indicative of growth remains to be elucidated.

It is thought that enhanced recovery of pathogenic bacteria under hypertonic culture conditions is a result of the inhibition of normal blood phagocytic mechanisms (9, 11). Furthermore, osmotic stabilizers have also been related to a protective effect on microorganisms which have undergone partial cell wall damage and, thus, become very susceptible to lysis as a result of osmotic pressure changes (9, 11). However, it is clear that because the patients of this study were not given antibiotic treatment before the procurement of the blood cultures, the superiority of the hypertonic culture medium is not due to neutralization by antibiotics.

The present study indicates that the superiority of the hypertonic medium for the diagnosis of H. influenzae bacteremia by the BACTEC system can be anticipated in the routine laboratory, which confirms the experimental observations of Crist et al. (2). Therefore, in situations in which H. influenzae bacteremia is considered in the differential diagnosis and blood volume is a limiting factor, special consideration should be given to the selection of hypertonic medium, rather than that of the aerobic medium in the BACTEC system, as earlier detection will most likely occur with no compromise on recovery. In addition, early blind subcultures and the QDP

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**TABLE 1. Correlation of the hours required for the BACTEC aerobic and hypertonic systems and the QDP method to detect positive results for H. influenzae with the quantitation of microorganisms in blood**

<table>
<thead>
<tr>
<th>Technique</th>
<th>No. (%) of positive results detected at (h):</th>
<th>Total no. of specimens&lt;sup&gt;a&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>8</td>
<td>12</td>
</tr>
<tr>
<td>Aerobic culture (GI)</td>
<td>10 (35)</td>
<td></td>
</tr>
<tr>
<td>Hypertonic culture (GI)</td>
<td>1 (3.5)</td>
<td>13 (45)</td>
</tr>
<tr>
<td>QDP</td>
<td>2 (8)</td>
<td>13 (52)</td>
</tr>
<tr>
<td>Microscopic observation&lt;sup&gt;b&lt;/sup&gt;</td>
<td>1,000</td>
<td>100-&gt;1,000</td>
</tr>
</tbody>
</table>

<sup>a</sup> Represents a total of 30 positive specimens.

<sup>b</sup> CFU per milliliter.
method will speed the specific diagnosis of *H. influenzae* bacteremia (7, 8).

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**LITERATURE CITED**


