Evaluation of Detection of Positive Blood Cultures by pH Changes

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We observed that the pH of positive blood culture broths was significantly lower than that of negative broths. However, significant pH changes were generally not observed for subculture-positive, macroscopically negative blood culture broths. Therefore, although the pH of most positive blood culture broths was reduced significantly, this change was generally not useful for the early detection of positive cultures.

Modifications of conventional blood culture methods, such as using early subcultures (11) and stains (2, 6), have shortened the time required to detect bacterial growth. However, most positive cultures are only detected after 24 to 48 h of incubation. In an attempt to improve the detection of bacteria, metabolic by-products of growing organisms have been measured by methods such as radiometry (3, 5), impedance changes (4), bioluminescence (10), microcalorimetry (8), and gas-liquid chromatography (9). However, each of these methods requires expensive instruments and, except for radiometry, is not used extensively for processing blood specimens.

In preliminary studies, we observed that the pH of positive blood culture broths was significantly lower than that of negative broths. Beaman et al. (1) reported similar pH changes. Because pH changes can be easily measured with a pH meter or with indicator dyes, we assessed the value of measuring these changes as a means of early detection of bacterial growth in blood culture broths.

During a 3-month period, blood cultures were collected from patients in Barnes Hospital, St. Louis, Mo., and used in these studies. Blood was routinely inoculated into one bottle each of tryptic soy broth (TSB) and Thiol broth (Difco Laboratories, Detroit, Mich.) and processed as described previously (7).

In this first experiment, the pH of 1 to 2 ml of broth, aspirated from positive blood culture bottles, was measured with an Orion 601-A pH meter. The mean pH values of positive TSB and Thiol broth were 6.31 and 5.93, respectively, whereas the range of pH values of negative blood culture broths was 6.85 to 7.15. The pH measurements of macroscopically positive broths are summarized in Table 1. All groups of organisms except Pseudomonas spp. had pH values significantly (P < 0.01) lower than the values for negative blood culture broths; the pH was less than 6.80 for 47 of 52 (90.4%) positive TSB cultures and for 102 of 109 (93.3%) positive Thiol broth cultures. Although 12 positive cultures initially had pH values in the range observed for blood culture broths, the pH dropped below 6.8 with further incubation for all broths except those with Pseudomonas aeruginosa.

Detection of pH changes with indicator dyes was also examined. Bromthymol blue (BTB; pKw 6.6) was selected because the color change (from blue-green to yellow) was readily detected at pH values of ≤6.80. In this experiment, we measured the pH of 125 macroscopically positive broths with BTB. From samples of positive blood culture broths, 40-μl samples were mixed with 100 μl of a 0.05% (wt/vol) solution of BTB prepared in 0.9% NaCl, pH 7.0. The reactions were interpreted as positive if the indicator color was more yellow than a standard buffer at pH 6.80. The results of this experiment are summarized in Table 2. A positive BTB reaction was seen with 90 and 93% of the TSB and Thiol broth cultures, respectively. The mean pH of all positive broths was 6.04. The five BTB-negative TSB cultures included four cultures with P. aeruginosa and one with a yeast species. The four BTB-negative Thiol broth cultures included two cultures with Staphylococcus aureus and one culture each with Serratia spp. and P. aeruginosa. All pH values for the BTB-negative broths (mean pH, 7.11) were within the range observed for negative blood culture broths.

We then performed experiments to determine if BTB could be used for the early detection of positive blood culture broths. The pH values of
Eight false-positive reactions obtained with BTB were observed in cultures from a patient with an elevated leukocyte count (>240,000 cells per mm³).

In summary, we assessed the feasibility of measuring pH changes as a means of early detection of positive cultures. The pH indicator used in this study permitted us to screen a large number of broths without the delays involved in examining Gram stain smears of broth cultures or in overnight incubation of blind subcultures. However, we were not able to detect prospectively positive broth cultures with the BTB indicator. Although a significant pH change can be detected with BTB (Table 2), this change does not occur before the broths are macroscopically positive. Therefore, the measurement of pH changes, either manually or by use of indicator dyes, is not useful for the rapid detection of positive blood culture broths.

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LITERATURE CITED

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**Table 1. pH measurements of macroscopically positive blood culture broths**

<table>
<thead>
<tr>
<th>Organism</th>
<th>TSB</th>
<th></th>
<th>Thiol broth</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No. tested</td>
<td>Mean pH</td>
<td>No. tested</td>
<td>Mean pH</td>
</tr>
<tr>
<td><em>Staphylococcus spp.</em></td>
<td>10</td>
<td>6.29</td>
<td>41</td>
<td>6.30</td>
</tr>
<tr>
<td><em>Streptococcus spp.</em></td>
<td>7</td>
<td>6.25</td>
<td>20</td>
<td>5.77</td>
</tr>
<tr>
<td><em>Enterobacteriaceae</em></td>
<td>20</td>
<td>6.22</td>
<td>38</td>
<td>5.64</td>
</tr>
<tr>
<td><em>Pseudomonas spp.</em></td>
<td>4</td>
<td>7.26</td>
<td>1</td>
<td>7.20</td>
</tr>
<tr>
<td><em>Bacteroides spp.</em></td>
<td>6</td>
<td>5.96</td>
<td>4</td>
<td>5.31</td>
</tr>
<tr>
<td>Anaerobic cocci</td>
<td>1</td>
<td>6.60</td>
<td>4</td>
<td>6.08</td>
</tr>
<tr>
<td>Other*</td>
<td>4</td>
<td>6.35</td>
<td>1</td>
<td>5.90</td>
</tr>
</tbody>
</table>

*Includes one *Corynebacterium* sp., one *Bacillus* sp. (one Thiol broth, one TSB), one yeast species, and one *Haemophilus* sp.

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**Table 2. BTB reactions for positive blood culture broths**

<table>
<thead>
<tr>
<th>BTB reaction</th>
<th>TSB No. % Total</th>
<th>Thiol No. % Total</th>
<th>Mean pH*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Positive</td>
<td>47</td>
<td>90.4</td>
<td>53</td>
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<tr>
<td>Negative</td>
<td>5</td>
<td>9.6</td>
<td>4</td>
</tr>
</tbody>
</table>

*a ± Standard deviation.

800 macroscopically negative blood culture broths were measured with BTB. A volume of 100 μl of a 0.05% solution of BTB was distributed into wells of a microtiter tray. When the first routine subculture of the broths was made (at 8 to 24 h), 1 drop of broth was mixed with BTB. An immediate color change to yellow was considered to be a positive reaction. Positive reactions were confirmed by Gram stain and pH measurements. If no organisms were seen in or cultured from BTB-positive broth, the reaction was considered a false-negative. If organisms grew on subculture from a BTB-negative broth, the reaction was considered a false-negative.

Of the 800 broths screened for bacterial growth by routine blind subculturing and by pH changes with BTB, 16 were positive by subculturing. However, only two of these (both with *Escherichia coli*) were initially detected by positive BTB reactions. The pH values of the 16 culture-positive broths were the same as those of negative broths (i.e., pH ≥ 6.85) and included values for *S. aureus* (four cultures), *E. coli* (two cultures), *Klebsiella* spp. (two cultures), *Serratia* spp. (one culture), *P. aeruginosa* (six cultures), and a yeast species (one culture).