Clinical Comparison of an Agar Slide Blood Culture Bottle with Tryptic Soy Broth and a Conventional Blood Culture Bottle with Supplemented Peptone Broth

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The Roche Septi-Chek biphasic blood culture system with tryptic soy broth was compared with a conventional blood culture bottle with supplemented peptone broth in 6,956 paired blood cultures from adult patients. Both systems were inoculated with equal volumes of blood (5 ml) and incubated aerobically (vented) for 2 weeks. More clinically important bacteria and fungi, including Staphylococcus aureus, S. epidermidis, Escherichia coli and other Enterobacteriaceae, Pseudomonas aeruginosa, and Candida albicans and C. tropicalis were recovered from the biphasic system (P < 0.001). In contrast, more clinically important anaerobic bacteria (P < 0.001) and Gardnerella vaginalis (P < 0.05) were recovered in conventional supplemented peptone broth. Staphylococci (P < 0.01), Enterobacteriaceae other than E. coli (P < 0.05), and fungi (P < 0.001) were detected 1 or more days earlier in the biphasic system, whereas streptococci (P < 0.001) were detected earlier in the conventional bottle. The overall superiority of the agar slide blood culture system compared with conventional blood culture bottles was confirmed by this evaluation. For optimal detection of anaerobic bacteremia, however, the agar slide bottle should be paired with an anaerobic bottle.

Several recent reports evaluating the Roche Septi-Chek agar slide blood culture system (Roche Diagnostic Systems, Nutley, N.J.) have shown it to be an attractive alternative to conventional blood culture methodologies (1, 2, 5, 8a). In only one study was the agar slide system with tryptic soy broth (RSC-TSB) compared with a conventional system with supplemented peptone broth (C-SPB) (1). In that evaluation of 1,209 blood cultures, the RSC-TSB detected more clinically important microorganisms than did C-SPB; however, the agar slide system benefited from a 20% volume advantage (8 ml for RSC-TSB versus 6.6 ml for C-SPB). Because two variables, medium and blood volume, were present, it was not possible to determine which variable was responsible for the increased yield in the RSC-TSB system. To better assess whether or not volume alone was responsible for the increased yield in RSC-TSB, we compared the two systems in 6,956 cultures of equal volumes of blood done at three collaborating hospitals.

MATERIALS AND METHODS

Collection of samples. During the study period a 45-ml bottle of supplemented peptone broth (C-SPB) with 0.03% sodium polyanetholsulfonate (Becton-Dickinson Vacutainer Systems, Rutherford, N.J.) and a 70-ml bottle of tryptic soy broth (RSC-TSB) with 0.05% sodium polyanetholsulfonate (Roche Diagnostic Systems) were used for all blood cultures from adult patients at Middlesex General-University Hospital, the University of Colorado Hospital, and the Denver Veterans Administration Medical Center. Blood cultures were obtained at the bedsides of patients after preparation of the skin with 10% povidone iodine (1% available iodine) followed by 70% isopropyl alcohol. Blood from each separate venipuncture was distributed by needle and syringe as follows: 5 ml inoculated into the bottle with 45 ml of SPB and 5 ml of blood inoculated into the bottle with 70 ml of TSB. Thus, the volume of blood was the same for both culture bottles (7), although the ratio of blood to broth was not the same (1:10 and 1:14, respectively). Both blood culture bottles had been evacuated and backflushed with 10% carbon dioxide and nitrogen at stoppering in the manufacturing process.

Volume standards. To ensure that the culture bottles actually received the specified amounts of blood, we measured the level of fluid in each container after it was filled with blood. Although all blood-containing bottles were incubated, those with fluid levels below or above the standards were coded as inadequate and were excluded from subsequent analyses. Fluid level standards were set to ensure that at least 4 ml but no more than 6 ml of blood was added to each bottle.

Processing of specimens. Identical methods were used for processing the blood cultures in the clinical microbiology laboratories at all three hospitals. Both bottles were incubated aerobically at 35°C for 14 days. When the paired samples were received, a sterile open venting unit that allowed continuous exchange of air was placed on the C-SPB bottle (8), and an agar slide paddle was attached to the RSC-TSB bottle. These steps and all subsequent manipulations were done at the laboratory bench. Cultures were examined macroscopically twice during the first 24 h and daily thereafter for 7 days; they were then reincubated until the final subcultures were done on day 14 of incubation.

In the C-SPB bottles subcultures were done through the
TABLE 1. Comparison of yield of clinically important bacteria and fungi from 5-ml samples of blood cultured in C-SPB and in RSC-TSB

<table>
<thead>
<tr>
<th>Microorganisms</th>
<th>No. of isolates from:</th>
<th>C-SPB only</th>
<th>RSC-TSB only</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aerobic and facultative bacteria</td>
<td>Both C-SPB and RSC-TSB</td>
<td>339</td>
<td>78</td>
<td>164</td>
</tr>
<tr>
<td>Gram-positive</td>
<td></td>
<td>144</td>
<td>48</td>
<td>64</td>
</tr>
<tr>
<td>Staphylococcus aureus</td>
<td></td>
<td>70</td>
<td>9</td>
<td>24</td>
</tr>
<tr>
<td>Staphylococcus epidermidis</td>
<td></td>
<td>5</td>
<td>2</td>
<td>17</td>
</tr>
<tr>
<td>Streptococcus</td>
<td></td>
<td>64</td>
<td>30</td>
<td>22</td>
</tr>
<tr>
<td>Other</td>
<td></td>
<td>5</td>
<td>8</td>
<td>1</td>
</tr>
<tr>
<td>Gram-negative</td>
<td></td>
<td>195</td>
<td>30</td>
<td>100</td>
</tr>
<tr>
<td>Escherichia coli</td>
<td></td>
<td>59</td>
<td>5</td>
<td>35</td>
</tr>
<tr>
<td>Other Enterobacteriaceae</td>
<td></td>
<td>92</td>
<td>16</td>
<td>39</td>
</tr>
<tr>
<td>Pseudomonas aeruginosa</td>
<td></td>
<td>30</td>
<td>9</td>
<td>22</td>
</tr>
<tr>
<td>Other</td>
<td></td>
<td>14</td>
<td>0</td>
<td>4</td>
</tr>
<tr>
<td>Anaerobic bacteria</td>
<td></td>
<td>13</td>
<td>33</td>
<td>6</td>
</tr>
<tr>
<td>Gram-positive</td>
<td></td>
<td>1</td>
<td>16</td>
<td>2</td>
</tr>
<tr>
<td>Gram-negative</td>
<td></td>
<td>12</td>
<td>17</td>
<td>4</td>
</tr>
<tr>
<td>Bacteroides fragilis group</td>
<td></td>
<td>8</td>
<td>4</td>
<td>4</td>
</tr>
<tr>
<td>All bacteria</td>
<td></td>
<td>352</td>
<td>111</td>
<td>170</td>
</tr>
<tr>
<td>Fungi</td>
<td></td>
<td>54</td>
<td>6</td>
<td>22</td>
</tr>
<tr>
<td>Candida albicans</td>
<td></td>
<td>26</td>
<td>4</td>
<td>16</td>
</tr>
<tr>
<td>Candida tropicalis</td>
<td></td>
<td>23</td>
<td>1</td>
<td>2</td>
</tr>
</tbody>
</table>

* NS, Not significant (P > 0.05)

+ Indicates: (a) group A streptococci (6), (b) group B streptococci (11), enterococci (27), non-enterococcal group D streptococci (5), Streptococcus pneumoniae (32), viridans streptococci (25), and other streptococci (10).

+ Gram-variable Gardnerella vaginalis (7), Listeria monocyctogenes (7). Escherichia coli, (Acinetobacter spp., (7), Pseudomonas sp. (1), Cardiobacterium hominis (4), and Haemophilus influenzae (5).

+ Clostridium (6), Bifidobacterium (2), Eubacterium (2), Peptococcus (5), and Peptostreptococcus (4) spp.

+ Bacteroides (28), Fusobacterium (2), and Veillonella (3) spp.

+ Candida albicans (46), Candida tropicalis (26), Candida parapsilosis (9), and Torulopsis glabrata (1).

Results

A total of 6,956 adequately filled blood culture sets were received during the study period. Of these, 988 (14.2%) were positive including 635 (9.1%) that grew microorganisms causing illness, 269 (3.7%) that grew contaminants, 12 (0.2%) that grew one or more contaminants and pathogens, and 72 (1.0%) that grew organisms that were indeterminate as a cause of sepsis. A total of 715 isolates associated with sepsis were detected; 407 isolates grew in both systems and 220 of these were detected on the same day.

Overall, clinically important bacteria and fungi were recovered more often (P < 0.001) in the agar slide system (Table 1). This improved yield could be attributed largely to better detection of staphylococci and gram-negative aerobic and facultatively anaerobic bacteria. Specifically, Staphylococcus aureus (P < 0.05), S. epidermidis (P < 0.01), and S. aureus (P < 0.01) were favored by the agar slide system. In addition, fungi (P < 0.01) were also detected more frequently with the agar slide system. In contrast, recovery of anaerobic bacteria (P < 0.001) and Gardnerella vaginalis (P < 0.05) was greater in SPB.

The speed with which the two systems detected positive cultures was variable (Table 2). Although there was no difference for bacteria overall, staphylococci (P < 0.01) and members of the family Enterobacteriaceae other than E. coli (P < 0.05) were detected 1 or more days earlier in the agar slide system. In contrast, streptococci (P < 0.001) were detected earlier in SPB. The most dramatic differences, however, were noted for fungi, which were found significantly sooner (P < 0.001) with the agar slide system.
TABLE 2. Comparison of speed of detection of clinically important bacteria and fungi from 5-ml samples of blood cultured in C-SPB and in RSC-TSB

<table>
<thead>
<tr>
<th>Microorganisms</th>
<th>C-SPB + RSC-TSB positive same time</th>
<th>C-SPB positive ≧1 day earlier</th>
<th>RSC-TSB positive ≧1 day earlier</th>
<th>P</th>
</tr>
</thead>
</table>
| Aerobic and facultative bacteria | 205 | 63 | 71 | NS*
| Gram-positive | 90 | 27 | 27 | NS
| Staphylococci† | 44 | 6 | 25 | <0.01
| Streptococci‡ | 43 | 19 | 2 | <0.001
| Other§ | 3 | 2 | 0 | NS
| Gram-negative | | | |
| Escherichia coli | 115 | 36 | 44 | NS
| Other Enterobacteriaceae | 32 | 16 | 11 | NS
| Pseudomonas aeruginosa | 55 | 11 | 26 | <0.05
| Other§ | 19 | 6 | 5 | NS
| Anaerobic bacteria¶ | 9 | 3 | 2 | NS
| All bacteria | 213 | 67 | 72 | NS
| Fungi¶ | 7 | 1 | 46 | <0.001

* NS, Not significant (P > 0.05).
† Staphylococcus aureus (70), S. epidermidis (5).
‡ Group A streptococci (5), group B streptococci (5), enterococci (13), non-enterococcal group D streptococci (3), Streptococcus pneumoniae (22), viridans streptococci (14), and other streptococci (2).
¶ Gardnerella vaginalis (2), Listeria monocytogenes (3).
§ Acinetobacter spp. (4), Pseudomonas spp. (1), Clostridium hominim (4), and Haemophilus influenzae (5).
¶ Bacillus spp. (12), anaerobic gram-positive rod (1).
† Candida albicans (26), Candida tropicalis (23), and Candida parapsilosis (5).

Contaminant isolates were detected with greater frequency in the agar slide system than in the conventionally processed SPB (Table 3). In particular, S. epidermidis (P < 0.001), Bacillus spp. (P < 0.05), and Corynebacterium spp. (P < 0.001) were detected more frequently in the agar slide system. Overall, 115 contaminant isolates were detected in the SPB processed conventionally versus 241 contaminant isolates in the agar slide system.

DISCUSSION

This study has confirmed earlier reports that the agar slide blood culture system improves the detection of microorganisms in blood in comparison with conventional broth systems (1, 2, 5, 8a). In addition, the data have shown that the agar slide system with TSB detects more microorganisms than does a conventional system with SPB. Since equal volumes of blood were used in this evaluation, it would seem that the advantage of the agar slide system demonstrated by Bryan (1) was not due solely to the volume of blood cultured but rather to medium and perhaps other factors (e.g., frequency of subculture, bottle configuration, and headspace).

Although yield overall was better in the agar slide system than in conventional SPB, this finding was not true for all microorganism groups. Thus, anaerobes and G. vaginalis were detected significantly more often in conventionally processed SPB. The improved yield of anaerobic bacteria in C-SPB may have been due in part to the low redox potential of this medium (8), whereas enhanced detection of Gardnerella spp. may have been due to the presence in C-SPB of 1.2% gelatin which counteracts the inhibitory effects of SPN on this bacterium (6). Although these microorganisms are relatively less common causes of bacteremia than are staphylococci and Enterobacteriaceae, they are important pathogens in some patients (6, 9). Therefore, clinical microbiology laboratories should use blood culture systems capable of detecting these bacteria as well as more common aerobic and facultative microorganisms. Indeed, evaluations of the agar slide system with TSB (2, 8a) and SPB processed conventionally (8) suggest that a two-bottle system utilizing these media would be a reasonable approach in many laboratories.

In designing this evaluation, we attempted to use each blood culture system to maximum efficiency within the limits of practical hospital laboratory microbiology. The time and effort involved in subculturing conventional broth blood culture bottles is well known and limits the frequency with which this procedure can be done. However, the agar slide system allows subculture to be done simply by inverting the bottle, thus enabling frequent subculture with a minimum of effort. The speed advantage of the agar slide system in detecting staphylococci, Enterobacteriaceae other than E. coli, and fungi may relate in part to this fact, namely, that more frequent subculturing in the agar slide system resulted in earlier detection of positivity. Frequent subculture cannot
represent the only factor, however, since streptococci were isolated earlier in the conventional bottle with SPB.

As noted in other comparisons with conventional broth blood culture bottles (5, 8a), the agar slide system has a higher contamination rate (3.5 versus 1.7% in this study). In all probability this problem relates to the additional manipulations associated with attachment of the paddle to the bottle and occasional need to remove the agar paddle from its plastic cylinder for complete examination. It is not known whether performing all maneuvers in a laminar air flow hood would reduce contamination sufficiently to warrant the extra effort and additional cost, but this possibility deserves study.

LITERATURE CITED