Effect of Agitation and Terminal Subcultures on Yield and Speed of Detection of the Oxoid Signal Blood Culture System versus the BACTEC Radiometric System

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In an initial evaluation, we found the Oxoid Signal blood culture system inferior to the BACTEC radiometric system for detection of some microorganisms causing sepsis (M. P. Weinstein, S. Mirrett, and L. B. Reller, J. Clin. Microbiol. 26:962-964, 1988). To determine whether modified processing of the Oxoid Signal blood culture system could improve its yield and speed of detecting positive cultures relative to the BACTEC radiometric system, we agitated all Oxoid bottles during the first 24 to 48 h of incubation and performed aerobic and anaerobic subcultures of all Oxoid bottles negative after 7 days of incubation. These modifications improved the overall performance of the Oxoid system, particularly with regard to the yield of streptococci, members of the family Enterobacteriaceae, and Haemophilus, Neisseria, and Acinetobacter spp. The speed of detecting positive cultures was also improved, especially within the first 24 h of incubation. However, the BACTEC system still detected more positive cultures (P < 0.005), especially of obligate aerobes such as Pseudomonas aeruginosa (P < 0.05) and yeasts (P < 0.005). The BACTEC system also detected positive cultures earlier than the Oxoid system (e.g., at 24 h of incubation, 70.5% of BACTEC positive cultures detected versus 62.1% of Oxoid positive cultures detected). Further modifications of the Oxoid system which might include a revised medium, additional processing modifications, altered headspace atmosphere, or a complementary second broth medium should be considered, since the system is attractive in concept and is easy to use in the clinical laboratory.

Several reports now have documented the value of agitation as a means of improving the yield and speed of detection of positive blood cultures (4, 6; L. B. Reller, S. Mirrett, L. G. Reimer, M. P. Weinstein, R. Nahass, and W. L. L. Wang, Abstr. Annu. Meet. Am. Soc. Microbiol. 1987, C378, p. 386), but agitation is not recommended uniformly by manufacturers of commercial blood culture systems. In an initial comparative study of the Oxoid Signal blood culture system and the BACTEC radiometric system, the Oxoid system was inferior in the yield of certain microorganisms, notably streptococci and aerobes including Pseudomonas aeruginosa, Acinetobacter spp., Haemophilus spp., and yeasts (12). Moreover, when both systems detected positive cultures, the BACTEC system did so earlier (12). When possible reasons for these differences were reviewed, the possibility that agitation of the BACTEC aerobic medium might have improved the relative yield and speed of detection of this system was considered. We also considered the possibility that certain microorganisms might have been present in the Oxoid medium without yielding a positive signal or macroscopic evidence of positivity in the broth itself (7). To assess these issues, we revised the processing of the Oxoid system to include agitation for the first 24 to 48 h of incubation, and we performed terminal subcultures on all negative Oxoid bottles on day 7 of incubation. We report here the results of 5,156 paired comparisons of equal volumes of blood in the Oxoid Signal and BACTEC radiometric systems at three university hospitals that use identical methods of obtaining and processing specimens.

MATERIALS AND METHODS

Collection of samples. During the study period, two 30-ml BACTEC bottles (aerobic 6B and anaerobic 7D) containing tryptic soy broth with 0.025% sodium polyanetholesulfonate and one 80-ml Oxoid bottle of Oxoid special broth with 0.03% sodium polyanetholesulfonate (11) were used for all blood cultures from adult patients at Robert Wood Johnson University Hospital, the University of Colorado Hospital, and the Salt Lake City Veterans Administration Medical Center. Patient blood cultures were obtained at the bedside after preparation of the skin with 10% povidone-iodine (1% available iodine) followed by 70% isopropyl alcohol. Blood from each separate venipuncture was distributed as follows: 10 ml to the Oxoid bottle, 5 ml to BACTEC 6B, and 5 ml to BACTEC 7D. Thus, the volume of blood inoculated into both systems was the same. The blood/broth ratio in each system was not the same: BACTEC, 1:6; and Oxoid, 1:8.

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 the standards were coded as inadequate and were excluded from subsequent analyses. Fluid level standards were set to ensure that at least 8 ml of blood was added to the Oxoid bottle and at least 4 ml each was added to the BACTEC 6B and 7D bottles.

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Processing of samples. Identical methods were used for processing the blood cultures in the clinical microbiology laboratories at all hospitals. All bottles were incubated in an air incubator at 35°C for 7 days. Upon receipt in the laboratory, the Oxoid Signal device was attached to the Oxoid blood culture bottle, after which the bottle was placed in a shaker-incubator (Lab Line, model 3527) at 140 rpm for 24 to 48 h and then incubated without agitation. The BACTEC 6B bottle was placed on a BACTEC shaker in the incubator for 24 to 48 h and then incubated without agitation. The BACTEC 7D bottle was incubated without agitation for 7 days.

The Oxoid system was examined for macroscopic evidence of growth in broth and for a positive signal (fluid in the upper cylinder) twice daily for the first 2 days of incubation and once daily thereafter, through day 7. Any bottle positive macroscopically or giving a positive signal was examined by Gram stain and subcultured aerobically and anaerobically. Terminal aerobic and anaerobic subcultures were performed on Oxoid bottles that were negative macroscopically and by signal after 7 days of incubation.

The BACTEC 6B bottle was examined macroscopically and radiometrically twice daily for the first 2 days of incubation and once daily thereafter, through day 7. The BACTEC 7D bottle was examined macroscopically twice daily and radiometrically once daily for the first 2 days. Thereafter, the 7D bottle was examined macroscopically and radiometrically daily through day 7. BACTEC bottles with an increase of 10 or more growth index units between readings were examined by Gram stain and subcultured. In addition, 6B and 7D bottles with a positive growth index were examined by Gram stain and subcultured. Terminal subcultures were not done on negative BACTEC bottles (10).

All microorganisms isolated were identified by standard procedures (8).

Clinical assessment. All patients with positive blood cultures were evaluated by an infectious disease specialist who defined pathogens (clinically important microorganisms causing sepsis) and contaminants by established criteria (13). An episode of bacteremia or fungemia was defined by the first positive blood culture or by a new positive blood culture result occurring more than 2 days after the preceding positive result (unless it was obvious clinically that the new positive result did not represent a new bacteremic event). Any positive culture obtained within 48 h of a previous positive culture was considered to represent the same episode (13).

Analysis of data. Paired comparisons of the two blood culture systems were done only on adequately filled bottles that grew microorganisms causing true bacteremia and fungemia. Significance testing was done with the modified chi-square test described by McNemar (9). Where appropriate, the Yates correction for small numbers of observations was utilized.

RESULTS
A total of 5,156 adequately filled blood culture sets were received during the study period. Of these, 841 (16.3%) were positive, including 601 (11.7%) that grew 660 microorganisms causing illness, 172 (3.3%) that grew contaminants, 57 (1.1%) that grew microorganisms that were indeterminate as a cause of sepsis, and 11 (0.2%) that grew both a pathogen and a contaminant. Of the 660 clinically important microorganisms, 420 (63.6%) grew in both blood culture systems, 144 (21.8%) grew only in the BACTEC system, and 96 (14.5%) grew only in the Oxoid system.

S ignificantly more microorganisms were detected by the BACTEC system (Table 1). In particular, P. aeruginosa (P < 0.05) and fungi (P < 0.005) were detected more often in the BACTEC system. Trends favoring the BACTEC system for the detection of streptococci and Acinetobacter spp. were also noted but were not significant statistically. There were no differences between the systems in the detection of members of the family Enterobacteriaceae, staphylococci, and anaerobes. Haemophilus and Neisseria species were infrequent isolates; neither system had a clear advantage.

Of the 420 microorganisms that grew in both systems, 272 (64.8%) were detected at the same time, 117 (27.9%) were detected earlier by the BACTEC system, and 31 (7.4%) were detected earlier by the Oxoid system (Table 2). Staphylococci (P < 0.001), Acinetobacter spp. (P < 0.001), other gram-negative bacteria (P < 0.05), and Candida albicans (P < 0.05) all were detected earlier by the BACTEC system. The cumulative yield time of BACTEC positive and Oxoid positive cultures is shown in Fig. 1. Figure 1 also shows the cumulative yield of Oxoid positive cultures excluding Acinetobacter spp. This uncommon blood isolate was associated with an epidemic of catheter-associated bacteremia in one of the collaborating hospitals (Robert Wood Johnson University Hospital) and often was detected only by terminal subculture at 7 days in the Oxoid system.

Of 516 isolates detected by the Oxoid system, 72 (14.0%) were detected only macroscopically, 414 (80.2%) were detected by a positive signal (many of these also were positive macroscopically in broth), and 30 (5.8%) were detected only by subculture. Excluding Acinetobacter spp., only 12 of 468 isolates (2.6%) required terminal subculture for detection. The following microorganisms were detected only by termi-
TABLE 2. Comparison of speeds of detection of clinically important bacteria and fungi from Oxoid Signal and BACTEC radiometric blood culture systems

<table>
<thead>
<tr>
<th>Microorganism</th>
<th>No. of isolates detected</th>
<th>At same time</th>
<th>BACTEC earlier</th>
<th>Oxoid earlier</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Staphylococci</td>
<td>91</td>
<td>33</td>
<td>11</td>
<td>&lt;0.001</td>
<td></td>
</tr>
<tr>
<td>Streptococci</td>
<td>58</td>
<td>11</td>
<td>5</td>
<td>NSa</td>
<td></td>
</tr>
<tr>
<td>Other gram-positive bacteria</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>NS</td>
<td></td>
</tr>
<tr>
<td>E. coli</td>
<td>37</td>
<td>5</td>
<td>1</td>
<td>NS</td>
<td></td>
</tr>
<tr>
<td>Other Enterobacteriaceae</td>
<td>66</td>
<td>16</td>
<td>12</td>
<td>NS</td>
<td></td>
</tr>
<tr>
<td>P. aeruginosa</td>
<td>9</td>
<td>2</td>
<td>0</td>
<td>NS</td>
<td></td>
</tr>
<tr>
<td>Acinetobacter spp.</td>
<td>7</td>
<td>31</td>
<td>1</td>
<td>&lt;0.001</td>
<td></td>
</tr>
<tr>
<td>Other gram-negative bacteria</td>
<td>4</td>
<td>8</td>
<td>0</td>
<td>&lt;0.05</td>
<td></td>
</tr>
<tr>
<td>Anaerobic bacteria</td>
<td>0</td>
<td>5</td>
<td>0</td>
<td>NS</td>
<td></td>
</tr>
<tr>
<td>C. albicans</td>
<td>272</td>
<td>117</td>
<td>31</td>
<td>0.001</td>
<td></td>
</tr>
<tr>
<td>All microorganisms</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

a NS, P ≥ 0.05.
b One Listeria monocytogenes isolate.
c Includes two C. violaceum, six P. maltophilia, three Haemophilus spp., and one Neisseria meningitidis isolate.
d Includes four B. fragilis and one Bacteroides sp. isolate.

FIG. 1. Cumulative yields over time of BACTEC and Oxoid blood culture systems when both systems detected sepsis. Oxoid data are shown in toto and with Acinetobacter spp. excluded (see text). Symbols: ⋄, BACTEC; □, all Oxoid; Δ, Oxoid excluding Acinetobacter spp.

Contaminant isolates occurred with equal frequencies in both systems. Overall, 161 contaminant isolates were detected with the BACTEC system and 171 with the Oxoid system.

DISCUSSION

In this reevaluation, the Oxoid Signal blood culture system demonstrated an improved performance in the detection of microorganisms causing bacteremia. Compared with our earlier evaluation (12), there was improved yield of streptococci, members of the family Enterobacteriaceae, and Haemophilus, Neisseria, and Acinetobacter spp. Overall, the Oxoid system detected 78.2% of all isolates compared with 71.1% in our initial evaluation (BACTEC, 85.5 versus 88.9% in initial evaluation). Presumably, the improved performance of the Oxoid system was related to changes in processing (agitation for a minimum of 24 h and terminal subcultures on previously negative bottles), since the medium itself was not altered. However, despite the improved performance overall, the Oxoid system still detected significantly fewer P. aeruginosa and fungi than did the BACTEC system. The reasons for the inferior detection of these obligate aerobic microorganisms are not clear but could be a function of the broth medium itself or, possibly, headspace atmosphere that is deleterious to aerobic organisms. Alternatively, the change of gas in the BACTEC bottles following each radiometric examination might enhance the detection of obligate aerobes by the BACTEC system.

As in our earlier evaluation (12), the BACTEC system detected positive cultures earlier than did the Oxoid system. However, with agitation, 57.7% of Oxoid positive cultures (62.1% excluding Acinetobacter spp.) were detected after 24 h of incubation (Fig. 1) compared with 46.4% in the earlier study in which Oxoid bottles were incubated without agitation. Thus, agitation appears to improve speed of detection as well as yield of microorganisms. This finding is not surprising in view of studies utilizing other blood culture systems that show improved yield and speed of detection with agitation (4, 6). Nevertheless, despite improved speed of detection in the Oxoid system, the BACTEC system still was faster (Fig. 1).

The need for terminal subcultures has been debated (P. A. Gross, Infect. Control 3:284, 1982; A. E. Coe, Clin. Microbiol. News 4:15, 1982; M. P. Weinstein, Infect. Control 3:284, 1982). It may be patient, institution, or system dependent (2, 3, 5). There are adequate data to show that terminal subculture is not necessary for the BACTEC radiometric system (1, 10). The data from this study suggest that it would be prudent to perform terminal subcultures on the Oxoid system. Although there were relatively few cases of bacteremia due to Haemophilus spp., anaerobes, and fungi, nearly 20% of these isolates were detected only with terminal subculture in this study.

Although this study has documented improved performance by the Oxoid system, its yield and speed of detecting positive blood cultures were not equivalent to that of the BACTEC radiometric system, and further changes should be considered. These might include other alterations in processing, a revised broth medium, altered headspace atmosphere, or a second complementary Oxoid medium that could better detect obligate aerobes. The system remains attractive in concept and is easy to use in the clinical...
laboratory. Therefore, modifications that might further improve the performance of the Oxoid Signal system should be undertaken.

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LITERATURE CITED