Optimal Testing Parameters for Blood Cultures


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The effects of volume of blood, number of consecutive cultures, and incubation time on pathogen recovery were evaluated for 37,568 blood cultures tested with the automated BACTEC 9240 instrument (Becton Dickinson Diagnostic Instrument Systems) at a tertiary care center over the period of 12 June 1996 through 12 October 1997. When the results for this study were compared with previous data published for manual broth-based blood culture systems and patient samples obtained in the 1970s and 1980s, the following were found: (1) the percentage increase in pathogen recovery per milliliter of blood is less, (2) more consecutive blood culture sets over a 24-h period are required to detect bloodstream pathogens, and (3) a shorter duration of incubation is required to diagnose bloodstream infections. Guidelines developed in the 1970s and 1980s for processing and culturing blood may require revision.

In the past decade, developments in blood culturing techniques have resulted in improved detection of bloodstream infections. These developments have included refinements in both blood culture media and detection methods [1, 2].

A blood culture is defined as culture of blood obtained from a single venipuncture, whether that blood is inoculated into 1 or into multiple bottles or tubes; if >1 receptacle is inoculated with each venipuncture, this is frequently referred to as a “blood culture set” [3]. (Throughout this article, we use the terms “blood culture” and “blood culture set” interchangeably.) In the late 1970s and early 1980s, studies were published that defined the optimal volume of blood per blood culture (set) [3–6], number of consecutive blood cultures (sets) [7, 8], and incubation time [7] for the detection of bloodstream pathogens in humans. These parameters were established for manual blood culture systems that used basal culture media, and except for incubation time [9–15], they have not been studied for recently developed automated blood culturing systems that use specialized media.

The objective of the current study was to determine whether parameters established for manual systems are still valid for a frequently used automated blood culture system, the BACTEC 9240 blood culture system (Becton Dickinson Diagnostic Instrument Systems). We hypothesized that, as a result of the reported increased sensitivity of the BACTEC 9240 blood culture method [16, 17], smaller volumes of blood, fewer consecutive blood cultures, and shorter incubation times would be required to detect bloodstream pathogens than had previously been reported for manual systems.

MATERIALS AND METHODS

Study design. The study included blood culture samples obtained from adult patients during the time period of 12 June 1996 through 12 October 1997 at the Mayo Medical Center in Rochester, Minnesota. Twenty milliliters of blood was obtained aseptically, equally distributed to 2 study receptacles (the BACTEC Plus Aerobic/F resin bottle and the BACTEC Lytic/10 Anaero-
For this analysis, 2 assumptions were made. First, any blood samples obtained within the same 30-min period should yield equal quantities of the causative organism responsible for a bloodstream infection. Therefore, 20-mL blood specimens, each obtained within 30 min of one another, were considered to be a single 40-mL blood culture sample (collection). Second, recovery of microorganisms from blood specimens is optimized if blood is cultured both aerobically and anaerobically [1, 2, 4, 18]. Therefore, the 40 mL of blood obtained within a 30-min period was inoculated in sequence into BACTEC bottles as follows: Plus Aerobic/F resin bottle (10 mL), Lytic/10 Anaerobic/F bottle (10 mL), Plus Aerobic/F resin bottle (10 mL), and Lytic/10 Anaerobic/F bottle (10 mL). The results for recovery of pathogenic microorganisms (detection of bloodstream infection) were determined for each 10-mL increment of blood.

Relationship between the volume of blood cultured and the detection of bloodstream infection. For this analysis, 2 assumptions were made. First, any blood samples obtained within the same 30-min period should yield equal quantities of the causative organism responsible for a bloodstream infection. Therefore, 20-mL blood specimens, each obtained within 30 min of one another, were considered to be a single 40-mL blood culture sample (collection). Second, recovery of microorganisms from blood specimens is optimized if blood is cultured both aerobically and anaerobically [1, 2, 4, 18]. Therefore, the 40 mL of blood obtained within a 30-min period was inoculated in sequence into BACTEC bottles as follows: Plus Aerobic/F resin bottle (10 mL), Lytic/10 Anaerobic/F bottle (10 mL), Plus Aerobic/F resin bottle (10 mL), and Lytic/10 Anaerobic/F bottle (10 mL). The results for recovery of pathogenic microorganisms (detection of bloodstream infection) were determined for each 10-mL increment of blood.

Relationship between the number of blood cultures and the detection of bloodstream infections. For this evaluation, 1 blood culture was defined as a 20-mL blood sample obtained from a single phlebotomy and divided equally into the BACTEC Aerobic/F resin and the BACTEC Lytic/10 anaerobic/F bottles. The total number of consecutive blood culture specimens obtained over a 24-h time period required to diagnose bloodstream infection was assessed.

Relationship between incubation times for blood cultures and the detection of bloodstream infections. For this analysis, the incubation time required to diagnose bloodstream infections for each blood culture (with 20 mL of blood) was assessed. The incubation time required to detect an episode of bloodstream infection was also determined. An episode of bloodstream infection was defined using criteria modified from those previously published by Kirkley et al. [19]. An episode of bloodstream infection was defined as (1) the initial recovery of a pathogen, (2) the subsequent recovery of a pathogen different from the initial pathogen, or (3) the recovery of the same pathogen after at least a 7-day interval after the recovery of the initial pathogen.

RESULTS

The results for 37,568 blood cultures obtained over the period of 12 June 1996 through 12 October 1997 were analyzed. Of these 37,568 blood cultures, 373 blood cultures were from 36 patients who had infective endocarditis. The majority of patients had samples obtained for 2 blood cultures over a 24-h period, and the second blood culture sample was frequently obtained within 30 min of the first blood culture sample.

Medical history review. Coagulase-negative staphylococci were isolated from ≥1 blood culture bottle in each of 1645 blood culture sets. The medical histories were available for the corresponding patients for 1634 (99.3%) of 1645 blood cultures. In 39 cases, the clinical significance of the coagulase-negative *Staphylococcus* isolate could not be determined, and, therefore, these isolates were not counted as pathogens. However, 482 (30%) of the remaining 1595 isolates were considered to be pathogens.

Viridans group streptococci were isolated from ≥1 blood culture bottle in each of 130 blood culture sets. The medical histories were available for all of the corresponding patients. In 6 cases, the clinical significance of the viridans *Streptococcus* isolate could not be determined. However, 87 (70%) of the remaining 124 isolates were considered to be pathogens.

Relationship between the volume of blood cultured and the detection of bloodstream infection. A total of 10,066 collections of 40 mL of blood for culture were evaluable for patients who did not have infective endocarditis. Tables 1 and 2 show that, as the volume of blood that was cultured increased, the recovery of pathogens also increased.

Not shown in tables 1 and 2 are the same volume comparisons for specific pathogens in patients without infective endocarditis.

### Table 1. Total number of all pathogens recovered related to the volume of blood cultured.

<table>
<thead>
<tr>
<th>Patient group</th>
<th>No. of patients, by volume of blood</th>
<th>10 mL</th>
<th>20 mL</th>
<th>30 mL</th>
<th>40 mL</th>
</tr>
</thead>
<tbody>
<tr>
<td>No endocarditis</td>
<td></td>
<td>235</td>
<td>305</td>
<td>346</td>
<td>371</td>
</tr>
<tr>
<td>Endocarditis</td>
<td></td>
<td>13</td>
<td>14</td>
<td>14</td>
<td>14</td>
</tr>
</tbody>
</table>

**NOTE.** A total of 40 mL of blood was obtained within a 30-min period; 20 mL was obtained separately from each of 2 phlebotomies and distributed equally between 1 aerobic (BACTEC Plus Aerobic/F resin; Becton Dickinson) and 1 anaerobic (BACTEC Lytic/10 Anaerobic/F; Becton Dickinson) bottle.
The smallest differences for recovery of organisms occurred for isolates of *Streptococcus pneumoniae*, *Enterococcus* species, and *Pseudomonas* species. Because the total number of different microorganisms recovered was low for patients with infective endocarditis, volume comparisons at the genus or species level were not possible. The specific organisms and number of isolates (>1 isolate of the same organism may be recovered from the same patient) associated with infective endocarditis included viridans group *Streptococcus* species (*n* = 50), *Enterococcus faecalis* (*n* = 35), *Staphylococcus aureus* (*n* = 29), coagulase-negative *Staphylococcus* species (*n* = 52), *Cardiobacterium hominis* (*n* = 7), group B *Streptococcus* species (*n* = 7), *Enterococcus galleranum* (*n* = 4), *Enterococcus species* (*n* = 3), *Pectostreptococcus micros* (*n* = 3), *Streptococcus mitis* species/group (*n* = 2), *Candida albicans* (*n* = 1), *Klebsiella pneumoniae* (*n* = 1), and *Pseudomonas aeruginosa* (*n* = 1).

**Table 2.** Percentage increase for all pathogens recovered related to the volume of blood cultured.

<table>
<thead>
<tr>
<th>Patient group</th>
<th>20 mL vs. 10 mL</th>
<th>30 mL vs. 10 mL</th>
<th>30 mL vs. 20 mL</th>
<th>40 mL vs. 10 mL</th>
<th>40 mL vs. 20 mL</th>
<th>40 mL vs. 30 mL</th>
</tr>
</thead>
<tbody>
<tr>
<td>No endocarditis</td>
<td>29.8</td>
<td>47.2</td>
<td>13.4</td>
<td>57.9</td>
<td>21.6</td>
<td>7.2</td>
</tr>
<tr>
<td>Endocarditis</td>
<td>7.7</td>
<td>7.7</td>
<td>0</td>
<td>7.7</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

**NOTE.** A total of 40 mL of blood was obtained within a 30-min period; 20 mL was obtained separately from each of 2 phlebotomies and distributed equally between 1 aerobic (BACTEC Plus Aerobic/F resin; Becton Dickinson) and 1 anaerobic (BACTEC Lytic/10 Anaerobic/F; Becton Dickinson) bottle.

**Table 3.** Relationship between the number of consecutive blood cultures (blood culture sets) with samples obtained over a 24-h period and pathogen recovery.

<table>
<thead>
<tr>
<th>Consecutive culture draw number</th>
<th>Total no. of consecutive culturesa</th>
<th>First positive culture result when ≥1 culture(s) was performed</th>
<th>First positive culture result when ≥2 cultures were performed</th>
<th>First positive culture result when ≥3 cultures were performed</th>
<th>First positive culture result when ≥4 cultures were performed</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Patients without EC</td>
<td>Patients with EC</td>
<td>Patients without EC</td>
<td>Patients with EC</td>
<td>Patients without EC</td>
</tr>
<tr>
<td>1</td>
<td>118 (15.5)</td>
<td>6 (15.0)</td>
<td>615 (80.6)</td>
<td>37 (92.5)</td>
<td>497 (77.0)</td>
</tr>
<tr>
<td>2</td>
<td>482 (63.2)</td>
<td>16 (40.0)</td>
<td>116 (15.2)</td>
<td>2 (5.0)</td>
<td>116 (18.0)</td>
</tr>
<tr>
<td>3</td>
<td>62 (8.1)</td>
<td>7 (17.5)</td>
<td>25 (3.3)</td>
<td>0</td>
<td>25 (3.9)</td>
</tr>
<tr>
<td>4</td>
<td>98 (12.8)</td>
<td>8 (20.0)</td>
<td>7 (0.9)</td>
<td>1 (2.5)</td>
<td>7 (1.1)</td>
</tr>
<tr>
<td>5</td>
<td>0</td>
<td>2 (5.0)</td>
<td>...</td>
<td>...</td>
<td>...</td>
</tr>
<tr>
<td>6</td>
<td>3 (0.4)</td>
<td>1 (2.5)</td>
<td>...</td>
<td>...</td>
<td>...</td>
</tr>
<tr>
<td>Total</td>
<td>763 (100)</td>
<td>40 (100)</td>
<td>763 (100)</td>
<td>40 (100)</td>
<td>645 (100)</td>
</tr>
</tbody>
</table>

**NOTE.** Data are presented as no. (%) of cultures. EC, endocarditis.

a As examples, the number of patients without EC who had only 1 blood culture sample obtained over a 24-h period was 118 (15.5%) of 763, the number of patients who had only 2 blood culture samples obtained over a 24-h period was 482 (63.2%), etc.
### Table 4. Relationship between incubation times for blood cultures (blood culture sets) and the detection of all bloodstream pathogens and all contaminating microorganisms.

<table>
<thead>
<tr>
<th>Incubation time, h</th>
<th>24</th>
<th>48</th>
<th>72</th>
<th>96</th>
<th>120</th>
<th>144</th>
<th>168</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Organism group</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pathogens and contaminating microorganisms</td>
<td>Patients without EC</td>
<td>2343 (72.9)</td>
<td>144 (77.8)</td>
<td>534 (91.9)</td>
<td>175 (94.9)</td>
<td>8 (96.2)</td>
<td>92 (97.8)</td>
</tr>
<tr>
<td>Pathogens</td>
<td>Patients without EC</td>
<td>2052 (76.5)</td>
<td>144 (77.8)</td>
<td>393 (91.2)</td>
<td>123 (95.8)</td>
<td>8 (96.2)</td>
<td>61 (98.1)</td>
</tr>
<tr>
<td>Contaminating microorganisms</td>
<td>Patients without EC</td>
<td>291 (54.5)</td>
<td>...</td>
<td>141 (80.9)</td>
<td>...</td>
<td>52 (90.7)</td>
<td>...</td>
</tr>
</tbody>
</table>

**NOTE.** Data are absolute no. (cumulative %) of organisms isolated. EC, endocarditis.
pathogenic organisms, except 2 Cryptococcus neoformans isolates and 1 Candida isolate, were recovered within 6 days.

Table 5 shows the relationship between incubation period and the detection of episodes of bloodstream infection. All episodes of bloodstream infection in patients without infective endocarditis were diagnosed within 6 days (=144 h), and only 0.5% were not detected within 5 days (=120 h). All episodes of bloodstream infection in patients with infective endocarditis were diagnosed within 5 days (=120 h).

DISCUSSION

The recommended practice for blood culturing is to culture ≥20 mL of blood divided evenly into an aerobic bottle and an anaerobic bottle [1–3]. However, a recent survey conducted by the College of American Pathology indicated that, among 649 institutions, the median estimated volume of blood obtained for culture per venipuncture for adult patients was only 10 mL [20].

Investigators have reported on the relationship of volume of blood cultured to yield of microorganisms [4–6, 21, 22]. In the present study, we also observed a relationship between the volume of blood obtained and the yield of microorganisms; however, the percentage differences in organism recovery per volume of blood cultured were less than those reported at our institution in 1983 by Ilstrup and Washington [6]. They showed that, for blood samples obtained from patients without infective endocarditis and cultured using a manual blood culture system containing standard media, yields from cultures of 20 mL and 30 mL of blood were 38% and 62% greater, respectively, than were those from 10 mL of blood. In our study, yields from 20 mL and 30 mL of blood were 29.8% and 47.2% greater, respectively, than those from 10 mL of blood. We found that, if 40-mL blood cultures were compared with 30-mL blood cultures, the increase in yield was less dramatic (7.2%). For patients with infective endocarditis, the percentage differences for pathogen recovery related to the volume of blood cultured were less pronounced. We presume that this relates to the higher sustained numbers of microorganisms in blood that occurs with infective endocarditis.

To further maximize the detection of bloodstream infection, most authorities recommend that 2–3 blood samples be obtained for culture over a 24-h period [1–3]. This recommendation is based on the results of 2 studies performed in the mid-1970s. For these studies, bloodstream infections were limited to those caused by bacteria and not fungi, and ≥3 blood samples had to be obtained for culture over a 24-h period.

In 1975, Washington [7] reported the cumulative yield from three 20-mL blood samples obtained for 80 episodes of bacteremia. A manual blood culture method and basal media were used. The blood specimen was equally distributed between 1 aerobic and 1 anaerobic bottle. All cases of infective endocarditis were excluded. Washington [7] noted that 64 (80%) of 80 cases of bacteremia were detected with the first culture, 70 (88%) of 80 were detected with the first 2 cultures, and 79 (99%) of 80 were detected with 3 blood cultures.

Weinstein et al. [8] conducted a similar study during 1975–1977. They evaluated the results for 282 bacteremic episodes but did not exclude cases of infective endocarditis. For their study, a conventional manual blood culture system and basal media were used; however, only 15 mL of blood was obtained for each culture. The blood specimen was inoculated in 5-mL aliquots into 2 aerobic bottles and 1 anaerobic bottle. They found that the first blood culture detected 257 (91%) of 282 cases of bacteremia and that the first 2 blood cultures detected 281 (>99%) of 282 cases of bacteremia.

The Weinstein et al. [8] and Washington [7] studies evaluated different amounts of blood per blood culture. Furthermore, because the study by Weinstein et al. [8] did not differentiate patients with from those without infective endocarditis, the results for that study were possibly biased in favor of fewer blood cultures. Nevertheless, these 2 studies provided the basis for the widely accepted recommendation that 2–3 consecutive blood specimens should be obtained for culture over a 24-h period from patients in whom septicemia is suspected.

The percentage yields for consecutive cultures in our study were less than those reported by Weinstein et al. [8] and Washington [7]. We observed that, for patients without infective endocarditis, 106 (65.1%) of 163 bloodstream infections were detected with the first blood culture, 131 (80.4%) were detected with the first 2 blood cultures, and 156 (95.7%) were detected with the first 3 blood cultures. When we performed the same
Pseudomonas aeruginosa P. aeruginosa Enterococcus culture set.

bacteremia or fungemia may require more blood cultures. Sec-
mETHODS. In addition, the chances of detecting such low-level
isms in blood may be more frequently detected with newer
automated systems, like the BACTEC 9240, than with manual
methods. In contrast to the period during which the studies by
Washington [7] and Weinstein et al. [8] were conducted (mid-
1970s), the current study was conducted during a time (June
1996 through October 1997) when broad-spectrum antibiotics,
especially β-lactam antibiotics, were more readily available and
frequently administered as empirical therapy to patients with
suspected bacteremia. These antimicrobics may decrease the
quantity of bacteria in the bloodstream or impair growth of
bacteria to the extent that more blood cultures are required for
detection. Third, differences in secular trends in microorgan-
isms isolated from blood have occurred over the past 2 decades.
For example, notable differences in genera of bacterial path-
ogens and increased frequencies of yeasts are noted when our
study is compared with that of Weinstein et al. [8] study (table
6). These differences may require that more consecutive blood
cultures are necessary to detect bloodstream pathogens, espe-
cially for patients without infective endocarditis.

On the basis of recent studies showing superiority of the
BACTEC 9240 system over manual systems [16, 17], we antici-
pated that fewer consecutive blood cultures might be re-
quired to detect bloodstream infections with the BACTEC 9240
system than with conventional manual broth-based systems
used in the studies by Washington [7] and Weinstein et al. [8].
The fact that we observed the opposite in our study may be
explained by several factors. First, considering the fact that the
BACTEC 9240 system and the specialized media used by the
system are more sensitive than conventional manual methods,
it may be that, by using this system, bloodstream pathogens
are detected in lower quantities in the blood. Therefore, spo-
radic bacteremias or fungemias with low quantities of organ-
isms in blood may be more frequently detected with newer
automated systems, like the BACTEC 9240, than with manual
methods. In addition, the chances of detecting such low-level
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cultures are necessary to detect bloodstream pathogens, espe-
cially for patients without infective endocarditis.

Table 6. Rank order of microorganisms or microorganism groups most frequently isolated from blood samples.

<table>
<thead>
<tr>
<th></th>
<th></th>
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<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Escherichia coli</td>
<td>Staphylococcus aureus</td>
<td>Staphylococcus aureus</td>
<td>Staphylococcus aureus</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>Staphylococcus aureus</td>
<td>Escherichia coli</td>
<td>E. coli</td>
<td>E. coli</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>Streptococcus pneumoniae</td>
<td>Candida albicans</td>
<td>Coagulase-negative</td>
<td>Staphylococcus species</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>Klebsiella pneumoniae</td>
<td>Coagulase-negative</td>
<td>Staphylococcus species</td>
<td>C. albicans</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>Pseudomonas aeruginosa</td>
<td>P. aeruginosa</td>
<td>Enterococcus species</td>
<td>C. albicans</td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>Bacteroides fragilis group</td>
<td>Enterococcus species</td>
<td>P. aeruginosa</td>
<td>P. aeruginosa</td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>Enterococcus species</td>
<td>K. pneumoniae</td>
<td>K. pneumoniae</td>
<td>K. pneumoniae</td>
<td></td>
</tr>
</tbody>
</table>
| 8    | Group A Streptococcus species | Serratia marcescens         | S. pneumoniae                    | Viridans group Strepto-
ococcus species |
| 9    | C. albicans | S. pneumoniae                  | Viridans group Strepto-
ococcus species | Enterobacter cloacae |
| 10   | Candida glabrata | E. cloacae                     | E. cloacae                      | S. pneumoniae                    |

NOTE. The top 10 pathogens (microorganisms or microorganism group) are listed for each study. Data from cases of endocarditis are included.

* Coagulase-negative staphylococci and viridans group streptococci were considered to be pathogens if they were recovered from ≥2 components in a blood culture set.
of incubation; 100% of episodes of bloodstream infections were detected within 5 days (≤120 h) of incubation.

Our medical history review revealed that 30% of coagulase-negative Staphylococcus isolates were considered by the physician(s) caring for the respective patients to be pathogens. Of interest, a recent report by Mirrett et al. [23] showed similar results. For that study, the medical histories of patients from whom coagulase-negative Staphylococcus species were isolated from the blood were also reviewed, and it was concluded that, of 1024 blood cultures with coagulase-negative staphylococci, 272 (27%) were clinically significant.

In view of our findings for the current study, we recommend the following parameters for detection of bloodstream infections in adult patients when using the BACTEC 9240 system. First, 20 mL of blood should be obtained per venipuncture and distributed equally between an aerobic and an anaerobic blood culture bottle (i.e., “1 blood culture” or “1 blood culture set”). Second, two 20-mL blood samples should be obtained immediately at the time that the first blood culture is ordered for a 24-h period. Each of these 20-mL samples should be obtained from different venipunctures. Third, 2 additional 20-mL samples should be obtained at separate intervals over the remaining 24 h if signs and symptoms of septicemia persist. Finally, blood culture bottles should be incubated for 5 days.

References