Optimized Pathogen Detection with 30- Compared to 20-Milliliter Blood Culture Draws

Robin Patel,1,2* Emily A. Vetter,1 W. Scott Harmsen,3 Cathy D. Schleck,3 Hind J. Fadel,2 and Franklin R. Cockerill III1,2
Division of Clinical Microbiology, Department of Laboratory Medicine and Pathology,1 Division of Infectious Diseases, Department of Medicine,2 and Division of Biomedical Statistics and Informatics, Department of Health Sciences Research,3 Mayo Clinic, Rochester, Minnesota 55905

Received 1 July 2011/Returned for modification 1 August 2011/Accepted 23 September 2011

Using data from 23,313 patients, we assessed whether two blood culture sets of three bottles per set would detect more pathogens than two sets of two bottles per set and achieve similar sensitivity to collecting three sets of two bottles per set. We also compared the yield of aerobic and anaerobic bottles. Thirty milliliters of blood was distributed to one anaerobic and two aerobic bottles. Among 26,855 collections of ≥60 ml within 30 min, 1,379 (5.1%) were positive for a pathogen not requiring detection in more than one set to be considered a pathogen, with 72 additional distinct pathogens detected using two 30-ml compared to two 20-ml sets of one aerobic and one anaerobic bottle (increased yield, 7.9%; 95% confidence interval [CI], 6.2 to 9.8%). For conditional pathogens requiring detection in at least two positive blood cultures for classification as pathogens (i.e., otherwise classified as contaminants), there were 162 positive detections with two 30-ml sets, of which 16 would not have been detected by two 20-ml sets (increased yield, 11.0% [95% CI, 6.4 to 17.2%]). Among 134 subjects who had three sets of 30 ml each within a 30-min interval, there was complete concordance between 60 ml of blood drawn in the first two sets of 30 ml and three 20-ml sets (P = 1.0). One aerobic bottle plus one anaerobic bottle yielded more pathogens than two aerobic bottles for organisms requiring a single (P < 0.001) and two (P = 0.04) positive sets to be defined as pathogens. In conclusion, we showed that collection of two aerobic and one anaerobic blood culture bottles per set results in improved yield compared to two bottles per set. We also confirmed that an anaerobic bottle should be included in blood culture sets.

An estimated quarter million patients develop bloodstream infections in the United States every year, with 14 to 38% associated mortality (1, 11). Blood cultures, the standard means of diagnosis of bloodstream infection, are one of most important tests performed in the clinical laboratory (13). Several variables influence ideal performance, including skin preparation prior to culture collection, the method and site of collection, the types of media utilized, the number of cultures collected, and the volume of blood sampled. The last is generally considered paramount. Several studies indicate that assaying increasing volumes of blood increases the likelihood of detection of bacteremia (4, 7–10, 12, 14, 16, 18, 19). It has been recommended that 20 to 30 ml of blood be collected per set and that two to three sets be collected (13). This recommendation covers a wide range of blood volumes, sampling from 40 to 90 ml of blood. For the patient and the health care facility, the difference between collection of two and three blood culture sets may be significant in terms of cost and inconvenience. Collection of two blood culture sets using 20 ml per set (total, 40 ml of blood), which is performed in many health care facilities, may, however, compromise sensitivity.

Using conventional manual blood culture and a maximum volume of 20 ml of blood per set, Washington reported that three blood culture sets obtained in a 24-h period were needed for ideal sensitivity (17). Three 20-ml culture sets (i.e., 60-ml total volume) were necessary to detect 99% of bacteremias; only 80% were detected with the first set (20-ml total volume), and 88% were detected with the first two sets (40-ml total volume) (17). Bouza et al. showed that the volume of blood collected remains an important variable with continuous-monitoring blood culture systems (2).

We previously demonstrated, using a continuous-monitoring blood culture system (Bactec 9240 blood culture system; Becton Dickinson Diagnostic Instrument Systems, Franklin Lakes, NJ), that two 20-ml blood culture sets detected 80%, whereas three detected 96% of bloodstream infections (4), a finding confirmed by Lee et al. (9). Specifically, Lee et al. analyzed instances in which at least three 20-ml blood culture sets were obtained over 24 h (9). Among monomicrobial bacteremias with at least three sets collected within 24 h, 73% were detected with the first set, 90% were detected with the first two sets, 98% were detected with the first three sets, and 100% were detected with the first four sets. Among monomicrobial bacteremias with at least four cultures collected within 24 h, 73% were detected with the first set, 94% were detected with the first two sets, 97% were detected with the first three sets, and 100% were detected with the first four sets. Eighty-one percent of polymicrobial bacteremias were detected with the first set, 93% were detected with the first two sets, and 100% were detected with the first three sets.

In modern clinical practice, most laboratories use continu-
ous-monitoring blood culture systems, and many health care facilities inoculate only two bottles (~20 ml of blood) for each set of blood cultures. As noted above, two 20-ml sets detect approximately 90% of bloodstream infections; three (or more) 20-ml sets are needed for ideal sensitivity. An alternate strategy to assay a commensurate amount of blood (i.e., 60-ml total) with only two venipunctures is to inoculate three bottles (30 ml of blood) with each of two venipunctures. It has been the practice at our institution (Mayo Clinic, Rochester, MN) to routinely inoculate three blood culture bottles per adult venipuncture.

We hypothesized that collecting two sets with three bottles per set would achieve improved pathogen detection compared to collecting two sets with two bottles per set. We further hypothesized that collecting two sets with three bottles per set would achieve a similar sensitivity to collecting three sets with two bottles per set.

(Part of this research was presented at the 111th General Meeting of the American Society for Microbiology, New Orleans, LA, 21 to 24 May 2011.)

MATERIALS AND METHODS

Study design. The study included blood cultures obtained from adult patients (16 years of age or greater) from 1 January 2006 through 31 December 2008 at Mayo Clinic in Rochester, MN. Thirty milliliters of blood was obtained aseptically, equally distributed to two Bactec Plus Aerobic/F resin (hereafter referred to as aerobic) bottles and one Bactec Lytic/10 Anaerobic/F (hereafter referred to as anaerobic) bottle (Becton Dickinson) and incubated for 5 days on a Bactec 9240 instrument. The study was approved by the Mayo Clinic Institutional Review Board. Only blood cultures collected from patients who provided authorization for review of their medical records (Minnesota statute 144.335) were analyzed.

Microorganisms isolated from cultures were identified by standard techniques. In some instances, more than one pathogen was isolated (polymicrobial bacteremia); each unique pathogen was considered separately for all analyses.

Single culture isolates of coagulase-negative staphylococci (CoNS; aside from Staphylococcus lugdunensis), diphtheroids, Bacillus species, Micrococcus species, Propionibacterium species, and nonproteolytic alpha-hemolytic streptococci were classified as contaminants (15). In instances where these same organisms were isolated from two or more sets, they were classified as conditional pathogens. (Organisms not requiring two or more positive sets to be classified as pathogens are referred to as nonconditional pathogens.)

Results of the anaerobic bottle. For analysis of the role of the anaerobic bottle, the nonconditional pathogen yield of two aerobic bottles was compared with that of one aerobic plus one anaerobic bottle.

Rank order of isolated organisms. We analyzed secular trends for the organisms isolated, comparing results to those of our previous studies (3, 4).

Statistical analysis. Descriptive statistics are reported as mean ± standard deviation (SD) or as number (percentage), as appropriate. All analyses were done separately for nonconditional and conditional pathogens. By definition, only organisms in the latter group were considered contaminants.

A comparison of the 30-ml set with the mimicked 20-ml sets (either one aerobic and one anaerobic bottle or two aerobic bottles) was made by estimating the increased yield with the 30-ml set compared to that of the 20-ml set. The 20-ml set was created by randomly choosing one of the two aerobic bottles. Hereafter, we refer to this as a 20-ml set. The estimate was calculated as the number of additional detections divided by the number of detections using the 20-ml set, reported along with a 95% exact binomial confidence interval (CI) for the percent increase.

The number of detections for a 20-ml set (one aerobic and one anaerobic bottle) compared with that of a second 20-ml set (two aerobic bottles) was assessed using a McNemar test. Similarly, the same method was used to compare the yields of two three-bottle sets and three two-bottle sets. The α-level was set at 0.05 for statistical significance.

RESULTS

Results of 43,158 blood cultures from 23,313 unique patients were analyzed. The mean patient age was 61 ± 18 years (range, 16 to 104 years); 55% were male.

Effects of blood culture numbers. There were 26,855 collections (in 13,358 patients) of 60 ml or more of blood for culture within a 30-min interval. Table 1 shows that, as the volume of blood cultured increased, the recovery of pathogens also increased. For example, as the volume of blood cultured increased from 20 to 30 ml, 8.1% more nonconditional pathogens were detected, and as the volume cultured increased from 40 to 60 ml, 10.9% more nonconditional pathogens were detected.

An initial analysis was performed including only nonconditional pathogens. Using this classification, 1,487 (5.5%) 30-ml sets were positive for any pathogen, with 1,606 pathogens detected. A total of 893 (6.7%) patients had positive sets for any pathogen, with a total of 989 distinct pathogens isolated (Table 2). When the results of one of the two aerobic bottles were excluded from analysis, 1,379 (5.1%) sets were positive for any pathogen, with 1,493 pathogens detected. A total of 824 (6.2%) patients had positive sets for any pathogen, with 917 distinct pathogens detected (Table 2). When the results from only the two aerobic bottles were included from analysis, 1,379 (5.1%) sets were positive for any pathogen, with 1,323 pathogens detected. A total of 754 (5.6%) patients had positive sets for any pathogen, with 819 distinct pathogens (Table 2). Overall, 72 additional distinct pathogens were detected using two 30-ml compared to two

<table>
<thead>
<tr>
<th>Blood vol (ml)</th>
<th>Increase (%) in nonconditional pathogens recovered in a culture vol of:</th>
</tr>
</thead>
<tbody>
<tr>
<td>10</td>
<td>25.3</td>
</tr>
<tr>
<td>20</td>
<td>35.4</td>
</tr>
<tr>
<td>30</td>
<td>47.7</td>
</tr>
<tr>
<td>40</td>
<td>57.6</td>
</tr>
<tr>
<td>50</td>
<td>63.9</td>
</tr>
<tr>
<td>60</td>
<td></td>
</tr>
</tbody>
</table>

The study included blood cultures obtained from adult patients at Mayo Clinic in Rochester, MN. Thirty milliliters of blood was obtained aseptically, equally distributed to two Bactec Plus Aerobic/F resin (hereafter referred to as aerobic) bottles and one Bactec Lytic/10 Anaerobic/F (hereafter referred to as anaerobic) bottle (Becton Dickinson) and incubated for 5 days on a Bactec 9240 instrument. The study was approved by the Mayo Clinic Institutional Review Board. Only blood cultures collected from patients who provided authorization for review of their medical records (Minnesota statute 144.335) were analyzed.

Effects of blood culture incubation times. For the analysis of blood culture incubation times, the incubation time required to diagnose bloodstream infections for 30-ml blood culture sets was assessed. Only nonconditional pathogens were analyzed.
TABLE 2. Total number of all pathogens recovered related to the volume of blood cultured (excluding conditional pathogens) for sets collected within 30 min

<table>
<thead>
<tr>
<th>Blood vol (ml)</th>
<th>30-ml collection (2 aerobic and 1 anaerobic)</th>
<th>20-ml collection (1 aerobic and 1 anaerobic)</th>
<th>20-ml collection (2 aerobic)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No. of patients with positive cultures</td>
<td>Total no. of pathogens detected</td>
<td>No. of patients with positive cultures</td>
</tr>
<tr>
<td>10</td>
<td>545</td>
<td>584</td>
<td>545</td>
</tr>
<tr>
<td>20</td>
<td>683</td>
<td>744</td>
<td>683</td>
</tr>
<tr>
<td>30</td>
<td>738</td>
<td>801</td>
<td>761</td>
</tr>
<tr>
<td>40</td>
<td>805</td>
<td>885</td>
<td>824</td>
</tr>
<tr>
<td>50</td>
<td>859</td>
<td>954</td>
<td></td>
</tr>
<tr>
<td>60</td>
<td>893</td>
<td>989</td>
<td></td>
</tr>
</tbody>
</table>

* a The number and type(s) of bottles are given in parentheses.

20-ml (aerobic and anaerobic bottle) sets. The increased pathogen yield using 30 rather than 20 ml per set was 7.9% (72/917; 95% CI, 6.2 to 9.8%). Of the 72 additional detections, 25 were associated with urosepsis, 11 with abdominal sepsis, 9 with pneumonia, 4 with skin/soft tissue infection, 3 with bone/joint infection, and 15 with other infections; 5 (including two isolations of *Staphylococcus aureus*) were clinically insignificant.

Conditional pathogens were analyzed separately, and results were compared to those obtained when one of the two aerobic bottles in each set was excluded from analysis. There were 162 positive detections with two 30-ml sets, of which 16 would not have been detected by two 20-ml sets, resulting in an increased pathogen yield of 11.0% when 30 rather 20 ml was collected per set (95% CI, 6.4 to 17.2%). Medical record review of the 16 discrepant cases revealed one case each of endocarditis (viridans group *Streptococcus* species [VGS]), disk space infection (CoNS), and abdominal sepsis (VGS); six cases of intravascular catheter-related bacteremia (five CoNS and one VGS); one case of *Bacillus* bacteremia of undetermined source; and six cases of unclear clinical significance. Overall, 10 clinically significant bacteremias were separated by the 30- and 20-ml sets, respectively. This included 1,687 versus 1,599 unique organisms, an increased yield of 8.2% (95% CI, 6.9 to 9.7%) with the 30-ml sets over the 20-ml sets, in a total of 1,560 versus 1,444 sets, respectively. A total of 1,517 versus 1,403 patients had one or more organisms detected with the 30-ml but not with the 20-ml sets, respectively, resulting in an increased yield of 8.1% (95% CI, 6.8 to 9.7%) with the 30-ml sets. Table S1 in the supplemental material provides quantitative data regarding the relationship between the number of positive sets performed over a 24-h period and the recovery of pathogens separated by the 30- and 20-ml sets for nonconditional pathogens.

Role of the anaerobic bottle. We compared the yield of two aerobic bottles with that of one aerobic plus one anaerobic bottle for recovery of pathogenic microorganisms without restricting the number of sets per patient. For nonconditional pathogens, both strategies detected 1,186 pathogens, with the sets of two aerobic bottles detecting 190 additional pathogens and the sets of one aerobic plus one anaerobic bottle detecting 374 additional pathogens (*P* < 0.001). For conditional pathogens, both strategies detected 161 pathogens, with the sets of two aerobic bottles detecting 11 additional pathogens and the sets of one aerobic plus one anaerobic bottle detecting 24 additional pathogens (*P* = 0.04). No statistically significant detection of contaminants was identified between these two approaches (*P* = 0.77).

Rank order of isolated organisms. We analyzed secular trends for the organisms isolated (Table 3). In the current study, there were more *Bacteroides fragilis* group and group B streptococcal bacteremias and fewer CoNS and VGS bacteremias than in our previous studies (3, 4).

**DISCUSSION**

Results of this study indicate that collecting two 30-ml blood culture sets (using three bottles per set) achieves sensitivity...
similar to that of collecting three 20-ml blood culture sets (using two bottles per set) and improved pathogen detection compared to collecting two 20-ml blood culture sets (using two bottles per set). Collecting two 30-ml blood culture sets is likely to be more acceptable to patients than collecting three 20-ml blood culture sets and involves one-third less work for phlebotomists.

We observed that when two 30-ml blood cultures (using three blood culture bottles per set) were collected, 215 (75.4%) of 285 bloodstream infections were detected with the first set, and 251 (88.0%) were detected with the first two sets, whereas collecting three 20-ml blood culture sets (using two blood culture bottles per set) would have detected 201 (70.5%) of 285 bloodstream infections with the first set, 234 (82.1%) with the first two sets, and 262 (91.9%) with the first three sets (see Table S1 in the supplemental material).

In our analysis of secular trends for the organisms isolated, there were fewer CoNS and VGS bacteremias in this study than in our prior studies, probably due to the contaminant classification applied here. Candida albicans was the 11th most common pathogen in the current study and so does not appear to be more acceptable to patients than collecting three 20-ml blood culture sets and involves one-third less work for phlebotomists.

The predominance of the B. fragilis group highlights the importance of anaerobes as bloodstream pathogens. We showed that excluding the anaerobic blood culture bottle from the blood culture set identified significantly fewer pathogens than one aerobic and one anaerobic bottle. Grohs et al. recently showed, using the Bact/Alert system with 40 ml in FAN aerobic and anaerobic bottles, that 13.5% if their positive blood cultures were positive only using the aerobic bottle (6).

There are limitations to our study. We used a different classification of contaminants versus pathogens than used in our prior study (4) (which was critiqued as possibly overcalling true bacteremia cases [20]); this definition is similar to that used in several other recently published blood culture studies (2, 5, 6). We did not analyze blood culture-related parameters such as catheter versus peripheral venipuncture blood culture collections. Ideally, all patients would have had three blood cultures collected within a 30-min interval. Although we did analyze the subgroup in which this was performed, we also expanded the collection window to 24 h. Biologic and pharmacologic changes occurring within a 24-h window may result in variability within this interval. Nevertheless, this was the only possibility for analysis of such a large number of blood culture sets and is in line with methods of other blood culture studies (9).

In conclusion, we found that collection of two aerobic and one anaerobic blood culture bottle per blood culture set results in improved pathogen detection compared to collection of two bottles per set. We also confirmed that an anaerobic bottle should be included in the blood culture set for ideal sensitivity.

ACKNOWLEDGMENTS

R.P. has received research funding from the NIH, Pfizer, Tornier, Astellas, Pradana, and Pocared and royalties for a Boxella pertussis/parapertussis assay; she has an unlicensed U.S. patent for a device and an apparatus for sonication but has foregone the right to receive royalties in the event that this patent is licensed. E.A.V., W.S.H., C.D.S., H.J.F., and F.R.C. have no conflicts of interest.

We acknowledge the outstanding contributions of the Mayo Clinic Bacteriology Laboratory staff.

REFERENCES


### TABLE 3. Rank order of microorganisms/microorganism groups most frequently isolated from blood

<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Staphylococcus aureus</td>
<td>Staphylococcus aureus</td>
<td>Staphylococcus aureus</td>
<td>Staphylococcus aureus</td>
</tr>
<tr>
<td>2</td>
<td>Escherichia coli</td>
<td>Escherichia coli</td>
<td>Escherichia coli</td>
<td>Escherichia coli</td>
</tr>
<tr>
<td>3</td>
<td>Klebsiella pneumoniae complex</td>
<td>CoNS</td>
<td>CoNS</td>
<td>CoNS</td>
</tr>
<tr>
<td>4</td>
<td>Enterococcus species</td>
<td>Enterococcus species</td>
<td>Enterococcus species</td>
<td>Enterococcus species</td>
</tr>
<tr>
<td>5</td>
<td>CoNS</td>
<td>Candida albicans</td>
<td>Enterococcus species</td>
<td>Pseudomonas aeruginosa</td>
</tr>
<tr>
<td>6</td>
<td>Pseudomonas aeruginosa</td>
<td>Pseudomonas aeruginosa</td>
<td>Pseudomonas aeruginosa</td>
<td>Enterococcus species</td>
</tr>
<tr>
<td>7</td>
<td>Streptococcus pneumoniae</td>
<td>Klebsiella pneumoniae</td>
<td>Klebsiella pneumoniae</td>
<td>Klebsiella pneumoniae</td>
</tr>
<tr>
<td>8</td>
<td>Bacteroides fragilis group</td>
<td>VGS</td>
<td>Streptococcus pneumoniae</td>
<td>Serratia marcescens</td>
</tr>
<tr>
<td>9</td>
<td>Group B Streptococcus</td>
<td>Enterobacter cloacae</td>
<td>VGS</td>
<td>Streptococcus pneumoniae</td>
</tr>
<tr>
<td>10</td>
<td>Enterobacter cloacae complex</td>
<td>Streptococcus pneumoniae</td>
<td>Enterobacter cloacae</td>
<td>Enterobacter cloacae</td>
</tr>
</tbody>
</table>

<sup>a</sup> CoNS, coagulase-negative Staphylococcus species; VGS, viridans group Streptococcus species.

<sup>b</sup> This study.

<sup>c</sup> Reference 4.

<sup>d</sup> Reference 3.