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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 III[1,2]

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

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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-

* Corresponding author. Mailing address: Division of Clinical Microbiology, Hilton 4-55, Mayo Clinic, 200 First St. SW, Rochester, MN 55905. Phone: (507) 538-0579. Fax: (507) 284-4272. E-mail: patel.robin@mayo.edu.

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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.

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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 nonpneumococcal 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 with 30 ml per set (including all three bottles collected) and 20 ml per set were compared. A 20-ml aerobic/anaerobic set consisted of results from one of the two aerobic bottles (randomly selected) and the anaerobic bottle. A second analysis of a 20-ml aerobic-bottle-only set was performed excluding the results of the anaerobic bottle.

Medical record review of select discrepant cases was conducted by an infectious diseases physician (R. Patel or H. J. Fadel).

Effects of blood volume. For analysis of the effects of blood volume, sets collected within a 30-min interval were analyzed. Results for nonconditional pathogens were determined for each 10-ml increment of blood. Results were also compared for 30- and 20-ml cultures. For this analysis, the first 30 ml was the result of the first set, and the second 30 ml was the result of the second set. For each set, the first 10 ml analyzed was randomly selected from the results of one of the two aerobic bottles, the second 10 ml was the result of the culture of the anaerobic bottle, and the third 10 ml was the result of the second aerobic bottle. Separate analyses were performed for nonconditional and conditional pathogens and for contaminants.

Effects of blood culture numbers. For analysis of the effects of blood culture numbers, the total number of consecutive blood culture specimens obtained over a 24-h time period required to diagnose bloodstream infection was assessed. Separate analyses were performed for nonconditional and conditional pathogens and for contaminants.

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 1. Percentage increase for nonconditional pathogens recovered related to the volume of blood cultured

Blood vol (ml)	Increase (%) in nonconditional pathogens recovered in a culture vol of:				
	20 ml	30 ml	40 ml	50 ml	60 ml
10	25.3	35.4	47.7	57.6	63.9
20		8.1	17.9	25.8	30.7
30			9.1	16.4	21.0
40				6.7	10.9
50					4.0

Role 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 volume. 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, 1,225 (4.6%) 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 2. Total number of all pathogens recovered related to the volume of blood cultured (excluding conditional pathogens) for sets collected within 30 min

Blood vol (ml)	Pathogen recovery by collection method[a]					
	30-ml collection (2 aerobic and 1 anaerobic)		20-ml collection (1 aerobic and 1 anaerobic)		20-ml collection (2 aerobic)	
	No. of patients with positive cultures	Total no. of pathogens detected	No. of patients with positive cultures	Total no. of pathogens detected	No. of patients with positive cultures	Total no. of pathogens detected
10	545	584	545	584	545	584
20	683	744	683	744	622	666
30	738	801	761	840	708	770
40	805	885	824	917	754	819
50	859	954				
60	893	989				

[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 with conditional pathogens were detected by 30- but not 20-ml cultures. Overall, therefore, 77 additional clinically significant bacteremias were detected by 30- compared to 20-ml cultures.

We compared the number of contaminated blood cultures with collection of two 30-ml blood culture sets in 30 min to the collection of one aerobic and one anaerobic bottle per set. There were 75 contaminated blood culture sets, of which 61 were detected by both culture strategies, and 14 were detected by collection of two 30-ml blood culture sets but not the two 20-ml sets, which represents an increased contaminant yield for two 30-ml blood culture sets of 23.0% (95% CI, 13.2 to 35.5%).

We analyzed the subgroup of 134 patients who had three 30-ml sets collected within a 30-min interval. A comparison of 60 ml of blood drawn in the first two sets of 30 ml with three sets of 20 ml (i.e., randomly excluding one of the two aerobic bottles) found complete concordance of pathogen detection (i.e., 10 positive patients) between the two blood culture drawing strategies ($P = 1.0$). There was a single contaminated blood culture in these patients, which was identified with both 60 ml of blood drawn in the first two sets of 30 ml and with three sets of 20 ml.

Effects of blood culture numbers. Blood culture sets collected within a 24-h period were analyzed in 23,233 patients from whom 65,702 30-ml blood culture sets were collected. Results were also analyzed excluding one of the two aerobic bottles from analysis. For nonconditional pathogens, 2,622 versus 2,419 positive cultures were detected with 30- versus 20-ml sets, respectively. This included 1,687 versus 1,559 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. Similar data are shown in Table S2 for conditional pathogens and in Table S3 for contaminants.

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

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

Rank	Isolated organism or group by study period[a]			
	2006–2008[b]	1996–1997[c]	1989–1992[d]	1984–1988[d]
1	*Staphylococcus aureus*	*Staphylococcus aureus*	*Staphylococcus aureus*	*Staphylococcus aureus*
2	*Escherichia coli*	*Escherichia coli*	*Escherichia coli*	*Escherichia coli*
3	*Klebsiella pneumoniae* complex	CoNS	CoNS	*Candida albicans*
4	*Enterococcus* species	*Enterococcus* species	*Candida albicans*	CoNS
5	CoNS	*Candida albicans*	*Enterococcus* species	*Pseudomonas aeruginosa*
6	*Pseudomonas aeruginosa*	*Pseudomonas aeruginosa*	*Pseudomonas aeruginosa*	*Enterococcus* species
7	*Streptococcus pneumoniae*	*Klebsiella pneumoniae*	*Klebsiella pneumoniae*	*Klebsiella pneumoniae*
8	*Bacteroides fragilis* group	VGS	*Streptococcus pneumoniae*	*Serratia marcescens*
9	Group B *Streptococcus*	*Enterobacter cloacae*	VGS	*Streptococcus pneumoniae*
10	*Enterobacter cloacae* complex	*Streptococcus pneumoniae*	*Enterobacter cloacae*	*Enterobacter cloacae*

[a] CoNS, coagulase-negative *Staphylococcus* species; VGS, viridans group *Streptococcus* species.
[b] This study.
[c] Reference 4.
[d] Reference 3.

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 for this study in Table 3. This likely relates to the increasing importance of non-*albicans Candida* species, especially *Candida glabrata*, as bloodstream pathogens.

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 anaerobic 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.

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