

Value of Terminal Subcultures for Blood Cultures Monitored by BACTEC 9240

JANET T. SHIGEI, JULIE A. SHIMABUKURO, MARIE T. PEZZLO,
LUIS M. DE LA MAZA, AND ELLENA M. PETERSON*

*Division of Medical Microbiology, Department of Pathology, University of California
Irvine Medical Center, Orange, California 92668*

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Blood cultures collected in BACTEC Plus Aerobic/F bottles and BACTEC Plus Anaerobic/F bottles were monitored for 5 days by BACTEC 9240 and subsequent terminal subcultures. Of the 13,471 bottles subcultured, 11.0% (1,477 of 13,471) were culture positive. Of these, 94.0% (1,388 of 1,477) were detected by BACTEC 9240; the additional 6.0% (89 of 1,477) were considered to be false negatives by BACTEC 9240 since they were detected by terminal subculture only. The false-negative bottles consisted of 17 BACTEC Plus Aerobic/F and 72 BACTEC Plus Anaerobic/F bottles, accounting for 2.2 (17 of 786) and 10.4% (72 of 691) of the total positive aerobic and anaerobic bottles, respectively. The positive blood culture bottles most frequently not detected by BACTEC 9240 grew *Pseudomonas* spp. (24), *Staphylococcus* spp. (21), and yeasts (24). Of the 86 blood cultures represented by the 89 false-negative bottles, 41 would not have been identified as positive since the other bottle in the blood culture set was either a false negative or a true negative. In general, terminal subcultures of false-negative BACTEC bottles had heavy growth, indicating that BACTEC Plus media were able to support the growth of microorganisms, but the BACTEC 9240 instrument was unable to detect this growth.

Rapid, reliable detection of microorganisms from blood is one of the most critical functions of a diagnostic microbiology laboratory. Noninvasive, automated blood culture systems with continuous monitoring have introduced technology that reduces the time needed to detect positive blood cultures as well as decreases specimen handling (4, 6-8). One such system, BACTEC 9240 (Becton Dickinson Diagnostic Instrument Systems, Sparks, Md.), monitors blood cultures by using a fluorescent sensor incorporated into a blood culture bottle that detects CO₂ production as an indicator of microbial growth. Computer algorithms analyze the rate and amount of CO₂ increase, which corresponds to an increase in fluorescence, thereby enabling the instrument to recognize a positive culture. When inoculated blood culture bottles are incubated and tested in the BACTEC instrument, microorganisms are detected as they enter the logarithmic phase of growth.

Different media have been developed to enhance recovery and detection of positive cultures with this instrument. BACTEC Plus Aerobic/F and Plus Anaerobic/F media, which contain mixed resins, have been shown to be superior in their ability to support the growth of positive blood cultures (1, 4, 6-8). Jungkind et al. (3) have suggested that enhanced recovery in resin-containing BACTEC media is due to lysis of leukocytes and subsequent release of viable, phagocytized microorganisms. The manufacturer of Plus media states that to maximize the number of positive blood cultures detected, it is recommended that blind Gram stains and/or subcultures be performed. The purpose of this study was to determine the value of terminal subcultures in a large clinical evaluation and to determine the significance of positive blood cultures not detected by BACTEC 9240 with BACTEC Plus Aerobic/F and Anaerobic/F media.

A total of 6,857 aerobic and 6,614 anaerobic blood culture

bottles were collected from adult patients at the University of California Irvine Medical Center. The majority of blood culture sets (6,524) consisted of one BACTEC Plus Aerobic/F bottle and one Anaerobic/F bottle; however, 423 blood cultures consisted of a single bottle (333 aerobic and 90 anaerobic). All bottles were inoculated with 5 to 10 ml of blood. To prevent compromising the detection of blood cultures because of delays in entering the BACTEC 9240 instrument (thus possibly beyond the logarithmic phase of growth), bottles incubated for ≥ 8 h or held at room temperature for ≥ 16 h were subcultured according to the manufacturer's instructions prior to entry into the instrument. With a 1-ml syringe, 0.2 ml was withdrawn and inoculated onto a chocolate agar plate (Becton Dickinson Microbiology Systems, Cockeysville, Md.). Bottles that did not exceed delay criteria were placed into the instrument shortly after arrival in the laboratory. Then each bottle was incubated and continuously monitored by the instrument every 10 min for 5 days.

At the end of 5 days, negative bottles were removed from the instrument and subcultured. With a 1-ml syringe, 3 to 5 drops of blood culture were inoculated on *Haemophilus* test medium (HTM; Becton Dickinson Microbiology Systems). HTM was used instead of chocolate agar because it is comparable in supporting microbial growth and, unlike chocolate agar, it is transparent. This allowed the use of a grid under each 150-mm-diameter culture plate on top of which 20 specimens were subcultured (Fig. 1). The grid aided in efficient subculturing and subsequent identification of positive bottles. Subcultures were incubated for 4 days at 35°C in 5 to 10% CO₂ and for an additional 3 days at room temperature. They were inspected daily for growth, which was quantitated as scant (≤ 10 colonies), moderate (>10 colonies), or heavy (confluent). When growth was observed on an HTM plate, a Gram stain was performed on the blood culture bottle, and it was subcultured to appropriate media.

For data analysis, the following criteria were established. An isolate was defined as an organism recovered from a blood culture bottle. For example, if the same organism was isolated

* Corresponding author. Mailing address: Division of Medical Microbiology, Building 14, Rte. 84, Department of Pathology, University of California Irvine Medical Center, 101 City Dr., Orange, CA 92668. Phone: (714) 824-4169. Fax: (714) 824-2160.

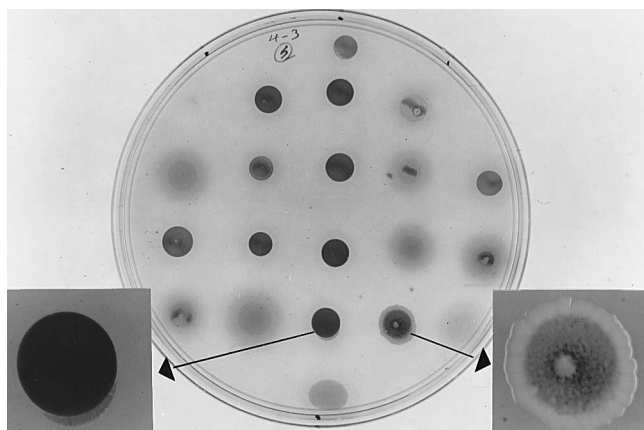


FIG. 1. Terminal blind subculture of 20 blood culture bottles on a 150-mm-diameter plate of HTM. The insert on the right is an enlargement of a negative subculture, and the insert on the left is a positive subculture with heavy growth.

from both bottles of a set, two isolates were recovered. A culture that was detected by BACTEC 9240 and confirmed to be positive by both Gram stain and subculture was considered to be a true positive. A culture that was instrument negative and negative upon terminal subculture was considered to be a true negative. Any culture that was instrument negative but positive upon terminal subculture was considered to be a false negative. Since chart review of each patient was not performed, diphtheroid-like organisms, coagulase-negative staphylococci, *Propionibacterium acnes*, *Bacillus* spp., and pleomorphic anaerobic gram-positive bacilli were considered to be probable skin contaminants and not potential pathogens. To differentiate among positive bottles and/or blood culture sets not detected by BACTEC 9240, false negatives were categorized into minor and major errors. A minor error was defined as a positive blood culture set in which one bottle was a true positive and the other bottle was a false negative. A major error occurred when a blood culture set was false negative because either one bottle or both bottles in that set yielded a false-negative result. Statistical analysis was performed by using chi-square analysis with a correction factor for a 2×2 contingency table (2).

Of the 6,857 aerobic and 6,614 anaerobic blood culture bottles included in this study, there were 1,477 positive bottles, 786 aerobic and 691 anaerobic BACTEC Plus bottles, which represented 1,000 positive blood culture sets. BACTEC 9240 detected 94.0% (1,388 of 1,477) of all positive bottles. The remaining 6.0% (89 of 1,477) of positive bottles, 17 aerobic and 72 anaerobic bottles, were detected only by terminal subculture. All of the positive bottles detected only by terminal subculture were from two-bottle sets.

The list of all the organisms recovered from positive blood cultures that were included in this evaluation can be seen in Table 1. From bottles detected by BACTEC 9240, there were 836 and 701 isolates recovered from the aerobic and anaerobic bottles, respectively. However, an additional 90 isolates, 17 from aerobic bottles and 73 from anaerobic bottles, were detected only upon terminal subculture. These represent 5.5% (90 of 1,627) of total isolates or 2.0 (17 of 853) and 9.4% (73 of 774) of the isolates recovered from aerobic and anaerobic bottles, respectively. When only potential pathogens were considered, the number of isolates recovered from this evaluation decreased to 1,057, with 529 isolates recovered from aerobic bottles and 528 isolates detected in anaerobic bottles. As shown in Table 1, the majority of isolates recovered from

TABLE 1. Distribution of microorganisms isolated from positive blood cultures

Microorganism	No. of isolates ^a				
	Total recovered	Aerobic		Anaerobic	
		9240 ⁺	9240 ⁻	9240 ⁺	9240 ⁻
Gram-negative aerobes					
<i>Acinetobacter</i> spp.	23	13	0	6	4
<i>Aeromonas</i> spp.	10	5	0	5	0
<i>Citrobacter</i> spp.	4	2	0	2	0
<i>Enterobacter</i> spp.	143	71	1	69	2
<i>Escherichia coli</i>	111	51	0	60	0
<i>Klebsiella</i> spp.	56	31	0	25	0
<i>Proteus</i> spp.	7	2	0	5	0
<i>P. aeruginosa</i>	59	30	0	6	23 ^b
<i>Pseudomonas</i> spp.	4	3	0	0	1
<i>Salmonella</i> spp.	16	8	0	8	0
<i>Shigella</i> spp.	1	1	0	0	0
<i>Serratia</i> spp.	9	5	0	4	0
<i>Xanthomonas</i> spp.	2	1	0	0	1
Other bacilli	5	5	0	0	0
<i>Neisseria mucosa</i>	5	2	0	3	0
Gram-positive cocci					
<i>Staphylococcus aureus</i>	282	139	0	139	4
Other staphylococci	532	292	4	223	13
<i>Enterococcus</i> spp.	81	41	0	40	0
<i>S. pneumoniae</i>	19	10	0	9	0
<i>S. pyogenes</i>	27	12	0	14	1
Other β hemolytic streptococci	9	4	0	5	0
Viridans group streptococci	75	37	0	37	1
Other	2	2	0	0	0
Gram-positive bacilli					
<i>Bacillus</i> spp.	11	7	0	4	0
Diphtheroid-like organisms	24	20	1	0	3
<i>Mycobacterium</i> spp.	4	0	4	0	0
Anaerobes					
<i>B. fragilis</i>	11	0	0	11	0
<i>B. thetaiotaomicron</i>	9	0	0	7	2
<i>Bacteroides</i> spp.	3	0	0	3	0
<i>Clostridium</i> spp.	4	0	0	4	0
<i>Propionibacterium acnes</i>	2	0	0	2	0
Gram-negative bacilli	6	0	0	6	0
Gram-positive bacilli	1	0	0	0	1
Gram-positive cocci	2	0	0	2	0
Fungi					
<i>C. albicans</i>	48	32	1	0	15 ^b
<i>C. neoformans</i>	3	0	3	0	0
Other yeasts	17	10	3	2	2
Total	1,627	836	17	701	73

^a +, positive; -, negative.

^b $P < 0.001$ (chi-square test).

false-negative bottles were *Pseudomonas* spp. (24), *Staphylococcus* spp. (21), and yeasts (24). The numbers of *Pseudomonas aeruginosa* and *Candida albicans* isolates recovered by blind subculture alone from anaerobic bottles, compared with those from aerobic bottles, were statistically significant ($P < 0.001$). *P. aeruginosa* isolates were detected by BACTEC 9240 in all 30 aerobic bottles in which it grew. This is in sharp contrast to the results for anaerobic bottles, in which only 6 positive bottles were detected by BACTEC 9240 and 23 were detected by subculture alone.

Since the primary purpose of a blood culture set is to identify patients that have bacteremia or septicemia, in examining the data we wanted to determine whether the false-negative bottle(s) in a set resulted in a false-negative blood culture(s). Therefore, the blood culture sets represented by false-negative

TABLE 2. Culture summaries of major errors

Patient	No. of false-negative cultures	Microorganism	Bottle(s)	Quantity	Other positive culture(s) ^a
1	1	<i>P. aeruginosa</i>	Anaerobic	Heavy	Subclavian catheter
2	1	<i>P. aeruginosa</i>	Anaerobic	Heavy	Sputum
3	1	<i>P. aeruginosa</i>	Anaerobic	Heavy	Urine
4	1	<i>P. aeruginosa</i>	Anaerobic	Heavy	Sputum
5	1	<i>P. aeruginosa</i>	Anaerobic	Heavy	Sputum, urine
6	1	<i>P. aeruginosa</i>	Anaerobic	Heavy	None
7	1	<i>P. aeruginosa</i>	Anaerobic	Heavy	Blood
8	2	<i>P. aeruginosa</i>	Anaerobic	Heavy	Blood
9	1	<i>P. aeruginosa</i>	Anaerobic	Heavy	Blood
10	1	<i>P. fluorescens-P. putida</i>	Anaerobic	Heavy	None
11	1	<i>S. aureus</i>	Anaerobic	Heavy	Blood
12	1	<i>E. cloacae</i>	Anaerobic	Heavy	None
13	1	<i>E. cloacae</i>	Aerobic and anaerobic	Heavy	Blood
14	1	<i>X. maltophilia</i>	Anaerobic	Heavy	None
15	4	<i>Mycobacterium</i> spp.	Aerobic	Moderate	None
16	2	<i>B. thetaiotaomicron</i>	Anaerobic	Heavy	None
17	1	<i>C. glabrata</i>	Aerobic	Heavy	None
18	1	<i>C. glabrata</i>	Aerobic	Heavy	None
19	1	<i>C. glabrata</i>	Aerobic	Moderate	Blood
20	1	<i>C. albicans</i>	Aerobic and anaerobic	Heavy	Blood
21	3	<i>C. neoformans</i>	Aerobic	Heavy	Blood

^a Other positive cultures were obtained within 7 days of the false-negative blood culture.

bottles were classified as minor or major errors. The 90 isolates recovered from bottles by terminal subculture represented 86 of the 1,000 positive blood culture sets in this evaluation. There were 45 blood culture sets that consisted of a true-positive bottle (detected by BACTEC 9240) as well as a false-negative bottle and were therefore considered to be minor errors. With all minor errors, the aerobic bottle of each set, not the anaerobic bottle, was detected by BACTEC 9240. There were 41 sets classified as major errors because the blood culture would not have been identified as positive for a particular organism since the other bottle in the set with the false-negative bottle was either a true negative or a false negative. Of these 41 major blood culture errors, pathogens were isolated from 28 sets and contaminants were isolated from the remaining 13 false-negative sets (Table 2). The 28 false-negative sets that grew pathogens were from 21 patients. *Pseudomonas* spp. were the pathogens recovered from over half of the false-negative cultures listed in Table 2. Twice *Bacteroides thetaiotaomicron* organisms were isolated from terminal subcultures of the same patient because anaerobes had been suspected, and terminal subcultures of the anaerobic bottle were incubated aerobically and anaerobically. Major errors represented the following percentages of all positive blood culture sets that grew a particular species: 23.8% (10 of 42), *P. aeruginosa*; 22% (2 of 9), *B. thetaiotaomicron*; 2.9% (2 of 69), *Enterobacter cloacae*; 100% (4 of 4), *Mycobacterium* spp.; 50% (1 of 2), *Xanthomonas maltophilia*; 50% (1 of 2), *P. fluorescens-P. putida*; and 75% (3 of 4), *C. glabrata*.

The major false-negative cultures constituted 41% (9 of 22) of all patients with *P. aeruginosa*, 50% (1 of 2) of all patients with *B. thetaiotaomicron*, and 75% (3 of 4) of all patients with *C. glabrata*. When culture summaries of patients with major errors were reviewed, 8 of the 21 patients with potential pathogens isolated from terminal subcultures did not have another culture that was positive for the same microorganism.

There seemed to be no correlation between collection time and placement in the instrument for those bottles detected only by terminal subculture. In addition, delayed entry of blood cultures into the instrument did not appear to be a factor in the

false-negative rate. All initial blind subcultures of bottles with delayed entries were negative, or if positive, the bottle became instrument positive within 24 h of subculture. For 70 of 89 false-negative bottles, growth was detected on terminal subculture within 24 h of incubation. Heavy growth was obtained with 49 bottles, moderate growth was obtained with 16 bottles, and scant growth was obtained with 5 bottles. Data for the quantity of growth were not available for the remaining bottles. Although BACTEC Plus media were able to support good growth in the majority of false-negative bottles, the BACTEC 9240 instrument was unable to detect these bottles as positive. Therefore, the detection system, not culture media, appears to have led to the false-negative instrument results. Since moderate to heavy growth was obtained with most of the false-negative terminal subcultures, we do not believe that extending the length of incubation would have enabled the instrument to recognize these bottles as positive.

The overall sensitivity of BACTEC 9240 for detection of culture-positive bottles with Plus Aerobic and Anaerobic/F media was 97.8 (769 of 786) and 89.6% (619 of 691), respectively. Most of the false negatives occurred in anaerobic bottles, and of these, *P. aeruginosa* and yeasts were the most common pathogens isolated from terminal subcultures. At our institution, these are important, frequently isolated pathogens in blood cultures. These microorganisms have been previously reported, in other studies with BACTEC 9240 as well as with different versions of BACTEC instrumentation and media, to be frequently detected only upon terminal subculture of an otherwise instrument-negative blood culture bottle (5, 6). Tarrand et al. (7) reported a comparison of the Isolator with BACTEC 26 Plus media incubated and monitored in the BACTEC 730 instrument. They did not perform terminal subcultures on BACTEC bottles; they found that a significant number of *Acinetobacter* spp. and *Candida* spp. that were detected with the Isolator system were not detected with the BACTEC system. From the results of our study, terminal subcultures of these false-negative BACTEC bottles probably would have revealed a significant number of positive blind subcultures.

Murray et al. (5), in examining the recovery of organisms from blood cultures over a 13-year period, concluded that bacteremia caused by anaerobic bacteria is decreasing relative to sepsis caused by fungi and aerobic organisms. They also presented data to support the statement that unvented anaerobic blood culture bottles reduce the recovery of aerobic pathogens. In our study, while yeasts were less likely to be recovered from BACTEC Plus Anaerobic/F media, this did not hold true for all other aerobic pathogenic microorganisms when a terminal subculture was performed. In fact, 480 isolates of pathogenic microorganisms were recovered from aerobic bottles and 473 isolates were recovered from anaerobic bottles. Anaerobic bottles yielded 35 pathogenic anaerobic microorganisms that were not detected in aerobic bottles. The problem is that most laboratories that use BACTEC Plus Anaerobic/F media do not perform terminal stains or subcultures, mainly because of the labor cost and increased exposure to infectious materials. A suggested alternative to the traditional blood culture set of an aerobic bottle and an anaerobic bottle is the use of two aerobic blood culture bottles with the addition of a fungal culture on the initial set drawn from a patient, with anaerobic cultures ordered on a selective basis. However, with improvements in the detection system of BACTEC 9240, it appears that anaerobic bottles will be equally valuable in supporting the growth of aerobic pathogens and providing a recovery system for anaerobic microorganisms.

In summary, at this time, the detection sensitivity of BACTEC 9240 does not parallel the capability of BACTEC Plus Anaerobic/F medium to support good growth of these aerobic organisms. We do not have data on its ability to detect the growth of anaerobic microorganisms since subcultures were not routinely incubated anaerobically. Therefore, it is likely that the numbers of false-negative bottles that grew anaerobes are underrepresented in this study. Since half of each patient sample is used to inoculate an anaerobic bottle and the medium can adequately sustain the growth of microorganisms that are not detected by BACTEC 9240, it is important that this growth be detected for the overall benefit of the patient. Hopefully, future improvements in the BACTEC 9240 detection system will enable this growth to be detected so that terminal subcultures will not be necessary. Since cost, safety, and efficient use of labor resources are important issues, each

laboratory must decide the value of terminal blind subcultures on the basis of patient population and the microorganisms that are most frequently isolated in their institution. However, it is our opinion that blood cultures, along with spinal fluids and surgical tissues, are the most important specimens received in a microbiology laboratory and that it is a laboratorian's responsibility to maximize the recovery of organisms from these specimens. The patient care costs that are incurred as a result of undetected blood cultures also need to be factored in when choosing a blood culture system.

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