Current Blood Culture Methods and Systems: Clinical Concepts, Technology, and Interpretation of Results

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Since the mid-1970s there has been a number of advances in blood culture practices and technology; these advances have been based largely on well-designed controlled clinical evaluations of blood culture systems and media. Thus, a sound scientific basis for the fundamental principles of blood culturing now exists. In this article, I will address issues of clinical and technical importance with regard to blood culturing; these issues include skin antisepsis, the number and timing of blood cultures, the appropriate volume of blood for culture, culture media and additives, length and atmosphere of incubation, and interpretation of positive blood culture results. Finally, I will discuss the currently available blood culture systems, with an emphasis on the newer continuous-monitoring blood culture systems.

Detection of bacteremia and fungemia traditionally has been one of the most important functions of clinical microbiology laboratories. When blood cultures yield clinically important microorganisms, it is a sign that host defenses have failed to contain an infection at its primary site or that the clinician has failed to adequately eradicate the infectious process. During the past two decades, there has been a number of changes in blood culture practices; these changes have been based on clinical investigations that have established a stronger scientific basis for this diagnostic test. Moreover, manufacturers of blood culture systems and media have improved their products, and there has been a marked trend toward the use of automated systems—most recently, continuous-monitoring blood culture devices. In this review, I will focus on important clinical and technical issues related to blood culturing, as well as on the interpretation of positive blood cultures.

Clinical Issues

Skin antisepsis. The likelihood that a positive culture represents infection rather than contamination is, at least in part, a function of skin antisepsis at the time of culture. The recommended antiseptic preparations are 70% isopropyl alcohol, followed by an iodophor or iodine tincture [1]. Iodophors require 1.5–2 minutes of contact time for maximum antiseptic effect. Iodine tincture exerts its antiseptic effect more rapidly than do iodophors [2] and may lower contamination rates in institutions where these rates are high [3], including centers where house officers and medical students, who may not wait for the iodophor to exert its effect, obtain the blood samples for culture. In one study, a commercially available skin-preparation kit for blood cultures has been shown to reduce contamination [4].

Method of obtaining blood for culture. Venipuncture remains the method of choice for obtaining blood for culture; arterial blood cultures are not associated with higher diagnostic yields than are venous blood cultures [1]. Studies comparing contamination rates for blood cultures obtained from intravascular devices vs. those obtained by venipuncture have reported conflicting results [5–8]. My observations support those of Bryant and Strand [8], who noted significantly increased rates of contamination when blood for culture was obtained from intravenous catheters (P < .001).

Furthermore, the American College of Physicians guidelines also recommend that blood for culture not be obtained from intravascular devices [9]. Although blood may occasionally be obtained from intravenous lines, a culture of blood obtained from such a device should be paired with another culture of blood obtained by peripheral venipuncture. Finally, I am unaware of published data from systematic studies that support obtaining separate blood samples from different ports of triple-lumen catheters.

Several recent studies have documented that the two-needle technique, formerly recommended for blood cultures, is not associated with significantly lower contamination rates than is the single-needle technique [10–12]. Because the handling of any needle is associated with a small but finite risk of needlestick injury, the authors of these studies have recommended that the single-needle method become the standard. However, the results of a recently published meta-analysis have suggested that the two-needle method may reduce contamination rates slightly [13]. Nevertheless, pending further data, the single-needle method probably should continue to be the standard.

Number of blood cultures. Several studies have addressed the issue of the number of blood cultures needed to detect...
bacteremia and fungemia in adults. Washington [14] reported the cumulative yield from three 20-mL blood samples obtained in sequence and cultured with use of conventional manual methods; 64 (80%) of 80 bacteremic epidoses were detected with the first culture, 70 (88%) of 80 were detected with the first two cultures, and 79 (99%) of 80 were detected with three blood cultures. My colleagues and I also used conventional manual methods and found that the first blood culture detected 257 (91%) of 282 bacteremic episodes and that the first two blood cultures detected 281 (>99%) of 282 episodes [15].

More recently my colleagues and I used the BacT/Alert continuous-monitoring blood culture system (Organon Teknika, Durham, NC) and evaluated the yield of clinically important microorganisms from three consecutive 20-mL blood samples obtained and cultured during a 24-hour period [16]. During a 15-month period, three blood cultures were performed for 218 patients; the third blood culture was the only one positive on only six occasions, and only one of these positive cultures represented true bacteremia. The conclusion that we drew from this small quality assurance study was that for the great majority of patients, two blood cultures should be sufficient to detect septicemia.

On the other hand, single blood cultures should be discouraged, if not forbidden altogether [9]. Not only will single blood cultures be insufficiently sensitive for detecting some bacteremias and fungemias, but they also may be difficult to interpret. For example, a single blood culture that yields a coagulase-negative staphylococcus may represent contamination or clinically important infection. However, if two samples obtained in sequence by separate venipunctures yield the same coagulase-negative staphylococcal species with the same antibiogram, the probability is high that the isolates represent true bacteremia.

On the basis of the concepts reviewed above, laboratories should recommend two blood cultures under routine circumstances. In institutions where phlebotomists or nurses obtain the blood for culture, two blood cultures should be performed automatically, even when the physician orders a single culture. On the other hand, ordering more than two blood cultures during any 24-hour period probably is unnecessary, except when the physician wishes to document the continuous bacteremia associated with an endovascular infection such as endocarditis. Therefore, it may be appropriate to require the approval of the laboratory director when more than two (or perhaps three) blood cultures are ordered during any 24-hour period.

**Timing of blood cultures.** Few studies have systematically evaluated the timing of blood cultures and the optimum interval between successive blood cultures. Strand [17] has arbitrarily recommended a 30- to 60-minute interval except for critically ill, septic patients, from whom specimens should be obtained minutes before initiating therapy. Li et al. [18] recently showed no difference in yield whether blood samples for cultures performed within a 24-hour period were drawn simultaneously or at spaced intervals [18]. However, drawing blood at intervals may be helpful if the clinician wishes to document continuous bacteremia in patients with suspected endovascular infections.

**Technical Factors.**

Many technical factors affect the sensitivity of blood culture systems. Of these factors, the most important are probably the volume of blood cultured and the broth medium used for culture. Other important factors include the ratio of blood to broth, agitation during incubation, length of incubation, atmosphere of incubation, and additives to the media (e.g., those that neutralize the effect of antimicrobial agents present in the blood at the time the sample is drawn). These and other technical issues have been reviewed in detail elsewhere [1, 19, 20], and the reader is referred to those publications for additional discussion of these matters.

**Volume of blood required for culture.** During bloodstream infections, especially those in adults, there may be relatively few microorganisms present in a given volume of blood—often in the range of <1–10 cfu/mL. Moreover, in numerous studies of adult patients, a direct relationship between the diagnostic yield of blood cultures and the volume of blood cultured has been documented [21–24]. On the basis of this information, the recommended volume of blood per culture set for an adult is 10–30 mL [1], and the preferred volume is 20–30 mL. Blood volumes of >30 mL do not enhance the diagnostic yield and contribute to nosocomial anemia; as a practical matter, blood at these volumes may clot in the syringe, thereby making it impossible to inoculate the blood culture bottles.

The optimal volume of blood that should be obtained from children has not been defined with certainty [25]. However, for infants and small children, Szmyczak et al. [26] concluded that the chance of failing to detect bacteremia was greater with blood volumes of <1 mL per culture than with blood volumes of ≥1 mL. On the basis of the available data, Paisley and Lauer [25] have recommended 1–2 mL of blood per culture for neonates, 2–3 mL for infants aged 1 month to 2 years, 3–5 mL for older children, and 10–20 mL for adolescents.

**Culture media.** All but one of the commercially available blood culture systems marketed in the United States are based on the inoculation of blood into broth culture media. No one medium or system is capable of detecting all microorganisms. Thus the results of innumerable clinical studies that evaluated different blood culture media and additives have been published. In general, most commercially marketed blood culture media perform well. However, many manufacturers supplement their base media with proprietary additives designed to enhance microbial growth, and it cannot be assumed that common generic media (e.g., supplemented soybean casein digest broth) from different manufacturers will perform in an equivalent manner. Decisions as to the choice of media formulations should be based on data from well-controlled field trials in which large numbers of cultures were assessed. The U.S. Food and Drug Administration has recently issued guidelines for
manufacturers that sponsor such clinical field trials; these guidelines have been summarized by Wilson [27].

**Blood-to-broth ratio.** Human blood contains a number of substances (e.g., complement, lysozyme, and phagocytic WBCs) capable of inhibiting microbial growth. Moreover, nearly one-third of patients are receiving antimicrobial agents at the time blood for cultures is obtained (author’s unpublished observations). Thus, to optimize the diagnostic yield, blood should be diluted adequately in the culture broth to minimize the effect of these substances. The results of studies that have addressed this issue have led to the recommendation that blood be diluted fivefold to tenfold [28–30]. Dilutions of <1:5 may result in a reduced yield; thus, filling blood culture bottles with more than the recommended amount of blood should be avoided. Dilutions of >1:10 may also be associated with a reduced yield that is not based on blood-to-broth ratio but rather on inadequate blood volume for culture.

Although commercially available blood culture bottles have a partial vacuum in the headspace, direct-draw techniques may result in overfilling and reduced blood-to-broth ratios. Collection of specimens with a needle and syringe provides greater control for inoculating blood culture bottles with the correct amount of blood and thus is preferred.

**Neutralization and inactivation of antimicrobials.** Given the frequency with which blood for cultures is obtained from patients already receiving antimicrobial agents, several manufacturers have marketed products designed to counteract the potential inhibitory effect on growth. These include the antimicrobial removal device (ARD; Becton Dickinson Microbiology Systems, Cockeysville, MD), BACTEC resin-containing media (Becton Dickinson Diagnostic Instrument Systems, Sparks, MD), and BacT/Alert FAN media (Organon Teknika).

The ARD, which was first introduced in 1980, has been the subject of many studies that have not defined a clear mandate for its use. By contrast, the results of studies of BACTEC resin media used with the semiautomated radiometric (BACTEC 460) and nonradiometric (BACTEC 660 and 730) systems generally were favorable [20]. In one study in which aerobic nonradiometric resin medium was used, the authors speculated that the improved yield of microorganisms might be due, at least in part, to factors other than the binding of antibiotics [31].

The resin medium is available for use in BACTEC continuous-monitoring blood culture devices (9240 and 9120). The manufacturer of the BacT/Alert continuous-monitoring blood culture instrument has recently marketed an aerobic medium, FAN, that uses activated charcoal in a proprietary substance (Ecosorb) to counter the potential inhibitory effects of antibiotics. Use of both the aerobic and the as yet unmarketed anaerobic formulations of the FAN media was associated with improved yields when they were compared with the standard BacT/Alert media [32–33]. The results of a comparative study of the FAN and BACTEC resin media suggest that the performance of these media is equivalent in the respective continuous-monitoring blood culture instruments [34].

**Atmosphere of incubation.** Traditional two-bottle blood culture systems have included one aerobic bottle and one anaerobic bottle, with the blood distributed equally between the two bottles. However, during the past two decades, the proportion of bacteremias due to obligate anaerobes has decreased substantially [35–37], leading some authorities to question the need for routine use of the anaerobic bottle. In fact, several recent studies of adults [35, 38, 39] and children [40] have concluded that the routine use of anaerobic blood culture bottles is not necessary and have recommended that these bottles be used only selectively for patients who are at high risk for bacteremia due to anaerobes.

Whether the data from these studies can be generalized to all institutions remains a subject of controversy for several reasons. Only a limited number of culture media and systems have been evaluated in these studies; for example, reports that assess the relative value of the BACTEC anaerobic resin or lytic media, the BacT/Alert anaerobic FAN medium, or the ESP anaerobic (80N) medium (Difco, Detroit) have not been published. Moreover, selective use of anaerobic bottles requires that certain logistic issues be addressed within each institution.

These issues include notifying physicians and staff of the change in procedure and the reasons the change was implemented; determining which, if any, nursing units (e.g., the gynecologic unit and the colon and rectal surgery unit) should be stocked routinely with anaerobic bottles; and, possibly, developing algorithms for the physicians ordering blood cultures or for the individuals obtaining the blood for culture (i.e., the phlebotomists, nurses, clinical care technicians, house officers, and medical students).

Indeed, an orderly and stepwise plan that includes both inservice education and postimplementation quality assurance evaluations to confirm that the recommended blood culture protocols are being followed should be developed at institutions where these changes are implemented.

**Length of incubation of blood cultures.** In routine circumstances, blood cultures need not be incubated for >7 days. Indeed, several recent studies in which instrumented blood culture systems were used have shown that 5-day incubation periods are sufficient for detecting the majority of pathogens [41–44]. Moreover, after 5 days most newly positive cultures represent contamination [44]. Incubation periods longer than 7 days may be useful when fungemia or bacteremia due to fastidious organisms such as the HACEK group of bacteria or species of Legionella or Brucella are suspected. Longer incubation periods may also be useful for patients with suspected endocarditis who have been treated with antimicrobial agents before blood cultures are performed. However, Washington [45] has noted that such extended incubation periods rarely increase yield. Mycobacterial blood cultures should be incubated for ≥4 weeks.

**Microorganisms that Deserve Special Consideration**

**Yeasts and filamentous fungi.** The incidence of fungemia has increased in recent decades because of the AIDS epidemic and
because patients with malignancies survive longer, the number of patients with organ transplants has increased, and long-term indwelling intravascular devices are being used more frequently. The Isolator (Wampole Laboratories, Cranbury, NJ) lysis-centrifugation system continues to be the best system for detecting filamentous fungi. The Septi-Chek biphasic blood culture system (Becton Dickinson) also has performed well. Instrumented blood culture systems in which the newer media (e.g., the BACTEC aerobic resin, nonradiometric fungal media, and the BacT/Alert aerobic FAN medium) are used have performed well for the detection of yeasts other than Cryptococcus neoformans [32, 34, 46, 47].

Mycobacteria. Before the AIDS epidemic, blood cultures for detecting mycobacteria were rarely, if ever, performed. However, mycobacterial blood cultures are now commonly performed in many laboratories. The Isolator system and the BACTEC radiometric system with 13A media have excellent sensitivity and perform comparably [48, 49]. Manufacturers of continuous-monitoring blood culture systems are in various stages of evaluating these devices in terms of their utility for mycobacterial blood cultures, but no published data are yet available.

Blood Culture Systems that Are Currently Available

Manual blood culture systems. Conventional manual systems and media are available from many commercial sources. Aerobic blood culture bottles and (usually) anaerobic blood culture bottles are inoculated with blood and usually incubated for 7 days. Each bottle is examined daily for macroscopic evidence of microbial growth (e.g., hemolysis, turbidity of the media, gas production, or formation of discrete colonies). An aliquot of the contents of the aerobic bottle is gram stained and subcultured after the first overnight incubation. A terminal subculture is usually done at the end of the incubation period. Conventional manual systems are flexible and require no purchase of expensive instruments, but they are labor intensive.

The biphasic Septi-Chek system and the Opticult blood culture systems (Becton Dickinson) and the Oxoid Signal broth displacement blood culture system (Oxoid Unipath, Basingstoke, England) are labor-saving variations of the conventional manual method.

The Septi-Chek and Opticult systems utilize agar-coated paddles or slants attached to the broth-containing culture bottle and allow subcultures to be done daily or more frequently by inverting the blood-broth mixture so that it covers the agar. Microbial growth is often identified on the agar rather than in the broth, allowing for prompt preliminary identification and susceptibility testing. As mentioned earlier, comparative studies have shown that the Septi-Chek biphasic system performs well in detecting yeasts and other microorganisms [31, 50–52].

The Oxoid Signal system is a one-bottle system. After blood is inoculated into the bottle, a clear-plastic cylindrical signal device is attached to the top of the bottle; a long needle from the lower end of the device extends below the surface of the blood-broth mixture, creating a closed system. Gases produced as a by-product of microbial growth increase the pressure in the headspace and force some of the blood-broth mixture through the needle into the cylinder, thereby "signaling" a positive culture. Comparisons of this system with other manual systems and with the BACTEC radiometric system have yielded mixed results [53–56].

The Isolator is a unique manual blood culture system that is based on lysis of blood cells, centrifugation, and direct inoculation of the centrifugate into solid media. For children, from whom only small volumes of blood may be collected, the centrifugation step is deleted. Many studies that have compared the Isolator system with other systems have been reported (as reviewed by Doern [57]). Overall, the isolator system performs well in the detection of most microorganisms—especially yeasts, filamentous fungi, mycobacteria, and opportunists such as Bartonella (Rochalimaea) henselae. Certain microorganisms, including Streptococcus pneumoniae, other streptococci, Pseudomonas aeruginosa, and anaerobic bacteria, may not be recovered optimally with this system. A potential advantage of the system is that it allows quantitation of the level of bacteremia or fungemia.

Disadvantages of the Isolator system include the following: the lysing solution is toxic for some microorganisms; specimens must be processed within 8 hours of blood collection (most microbiology laboratories are staffed for only one daily shift); substantial labor is required for the initial processing of isolator cultures; and the contamination rates for this system are higher than those for conventional broth and automated systems.

Instrumented blood culture systems. Commercially available instrumented blood culture methods were introduced in the 1970s. Until recently, the BACTEC instrumented systems were the only products commercially available in the United States; these systems were initially equipped with radiometric instruments and media, followed in the mid-1980s by the nonradiometric instruments and media. Both systems (as well as in the newer BACTEC and BacT/Alert continuous-monitoring devices) are based on the utilization of carbohydrate substrates in the culture media and subsequent production of CO₂ by growing microorganisms.

For the radiometric system, the instrument detects ¹⁴CO₂ in the bottle headspace, and for the nonradiometric system, CO₂ is detected by infrared spectrophotometry. For both systems, bottles are loaded onto the detection portion of the instrument, where needles perforate the bottle diaphragm and sample the gas contents of the headspace once or twice daily; a bottle is flagged as positive if the amount of CO₂ in the bottle exceeds a threshold value. The flagged bottle is then removed from the instrument, and an aliquot of its contents is gram-stained and subcultured for further testing. Numerous controlled clinical evaluations of the BACTEC radiometric and nonradiometric
Continuous-monitoring blood culture systems. With changes in health care financing, including the recognition that labor costs need to be better controlled, manufacturers are increasingly looking to the use of automation in clinical microbiology. The early-generation BACTEC instruments had the following disadvantages: culture bottles had to be manually manipulated, gas canisters were needed for every instrument; detection needles had to be changed periodically; sterilization of the needle devices occasionally failed, resulting in a false diagnosis of bacteremia (pseudobacteremia); cultures were sometimes instrument false-positive; and bottle throughput was relatively slow (35–60 seconds per bottle). Continuous-monitoring blood culture systems were developed in an effort to address these problems.

Three of these systems are currently available in the United States: the BACTEC 9000 series (Becton Dickinson), Difco ESP, and the BacT/Alert, which was the first to be marketed in 1991. All systems have several features in common including self-contained modular incubation; agitation; detection units, of which up to five or six can be controlled by a single computer; lack of the need for manual manipulation of culture bottles once they have been loaded into the instrument; instrument monitoring of microbial growth at 10- to 24-minute intervals; and culture bottles that each accept 10 mL of blood.

The BacT/Alert and BACTEC systems detect the production of CO₂ as change in pH; this is accomplished by means of colorimetry in the BacT/Alert system and by means of a fluorescent sensor in the BACTEC system. The ESP system detects changes in pressure, either as gases are used during early microbial growth or as they are produced later during growth. The BacT/Alert and BACTEC systems monitor every bottle at 10-minute intervals and gently rock all bottles 34 and 30 times a minute, respectively. The ESP system monitors aerobic bottles every 12 minutes and agitates them at 160 cycles per minute; anaerobic bottles are monitored every 24 minutes without agitation.

All comparative studies to date have shown that the three continuous-monitoring blood culture systems have detected growth sooner than earlier-generation BACTEC instruments and manual systems [46, 59–66]; these systems have been found to be comparable in terms of performance [66, 67]. All three systems have performed reliably in my laboratory.

Interpretation of Positive Blood Cultures

Since the presence of a bloodstream infection is important in terms of both diagnosis and prognosis, correct interpretation of the positive test result is crucial. Misinterpretation of positive results can be costly both to the institution and to the patient. Bates and colleagues [68] recently calculated that the excess cost associated with each blood culture contaminant was ~$4,500 [68]. Parameters that may be useful in interpreting results include the identity of the microorganism, the presence of more than one blood culture positive for the same microorganism, and the presence of the same microorganism as that found in the blood from another normally sterile site.

Microorganisms that almost always (>90% of isolates) represent true infection when isolated from the blood include Staphylococcus aureus, Escherichia coli and other Enterobacteriaceae, P. aeruginosa, S. pneumoniae, and Candida albicans. Isolates from blood that rarely (<5% of isolates) represent true infection include Corynebacterium species, Bacillus species, and Propionibacterium acnes.

Coagulase-negative staphylococci are particularly problematic, not only because they are so ubiquitous, but also because 12%–15% of the blood isolates are pathogens rather than contaminants [69]. Some authorities have suggested that the number of bottles positive in a culture set is a predictor of the clinical significance of an isolate. However, Mirrett and colleagues [70] and Peacock et al. [71] have found that this criterion is unreliable, at least for coagulase-negative staphylococci.

A useful interpretive concept is the number of culture sets found to be positive vs. the number obtained. If most or all cultures in a series are positive, regardless of the microorganism recovered, the probability that the organism is clinically important is high [15]. Of course, it is the physician who must ultimately make the final judgment, taking into account not only the laboratory findings but also the clinical presentation of the patient.

Limitations of Blood Cultures

Blood cultures, as described herein, currently represent the "gold standard" for diagnosis of septicemia. Nonetheless, they have limitations. Positive results require hours to days of incubation. No one culture medium or system in use has been shown to be best suited to the detection of all potential bloodstream pathogens. Some microorganisms grow poorly, or not at all, in conventional blood culture media and systems. Whether culture-based systems will remain the diagnostic methods of choice into the next century or be replaced by molecular techniques or other methods remains to be determined.

References


