

Update on Detection of Bacteremia and Fungemia

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INTRODUCTION

Physicians and microbiologists have long recognized that the presence of living microorganisms in the blood of a patient carries with it considerable morbidity and mortality. Indeed, because invasion of the bloodstream represents one of the most important sequelae of infection, blood cultures have become critically important and frequently performed tests in clinical microbiology laboratories. The problem is one of considerable magnitude; it has been estimated that approximately 200,000 patients develop bacteremia or fungemia annually in the United States and that the associated mortality ranges from 20 to 50% (94, 183, 191, 199, 199a). Many septic episodes are nosocomial and in some hospitals represent a majority; such episodes may be due to microorganisms with increased antimicrobial resistance and are associated with greater mortality than are community-acquired episodes.

This review will focus on the clinical importance of blood cultures; diagnostic strategies for the detection of bacteremia and fungemia, including a review of innovative and new methods and systems; and approaches to the detection of specific microorganisms.

CLINICAL IMPORTANCE OF BLOOD CULTURES

Pathophysiology of Bacteremia and Fungemia

Invasion of the blood by microorganisms usually occurs by one of two mechanisms: drainage from the primary focus of infection via the lymphatic system to the vascular system, or direct entry from needles (e.g., in intravenous drug users) or other contaminated intravascular devices such as catheters or graft material. The presence of bacteremia or fungemia represents either the failure of an individual's host defenses to localize an infection at its primary site or the failure of a physician to remove, drain, or otherwise sterilize that focus. Ordinarily, host defenses respond promptly to a sudden influx of microorganisms, particularly by efficient phagocytosis by macrophages of the mononuclear phagocytic system that help clear the blood within minutes to hours. Clearance may be less efficient when microorganisms are encapsulated, or it may be enhanced if the host has antibodies specific for the infecting organism. Clearance of the bloodstream is not always successful. Examples of this problem are bacteremia associated with intravascular foci and endovascular infections and episodes that occur in individuals whose host defense mechanisms either are too impaired to respond efficiently or are simply overwhelmed.

Clinical Patterns of Bacteremia and Fungemia

Conceptually, it continues to be useful to categorize bacteremia as transient, intermittent, or continuous. Transient bacteremia, lasting minutes to hours, is most common and occurs

after the manipulation of infected tissues (e.g., abscesses and furuncles); during certain surgical procedures; when procedures are undertaken that involve contaminated or colonized mucosal surfaces (e.g., dental manipulation, cystoscopy, and gastrointestinal endoscopy); and, predictably, at the onset of acute bacterial infections such as pneumonia, meningitis, septic arthritis, and acute hematogenous osteomyelitis. Intermittent bacteremia is that which occurs, clears, and then recurs in the same patient due to the same microorganism. Classically, this type of bacteremia is associated with undrained closed-space infections, such as intra-abdominal abscesses, but could also occur in patients with focal infections like pneumonia or osteomyelitis. Continuous bacteremia is characteristic of infective endocarditis as well as other endovascular infections (suppurative thrombophlebitis, infected ["mycotic"] aneurysms); it also occurs early in the course of brucellosis and typhoid fever.

Bacteremias may also be categorized as unimicrobial or polymicrobial (6 to 18% of episodes), and the term "break-through bacteremia" has been used to describe the occurrence of bacteremia in patients receiving appropriate therapy for the microorganism that is grown from the blood.

Sources of Bacteremia and Fungemia

Although almost any localized infection can disseminate to the bloodstream, systematic studies of bacteremia and fungemia have shown that the most common primary foci are intravascular devices (catheters primarily), the respiratory tract, the urinary tract, and various intra-abdominal sites (183, 199, 199a). Moreover, such studies have shown that despite the best efforts of clinicians, the source of bacteremia or fungemia cannot be determined in one-quarter to one-third of patients.

The increased importance of intravascular catheters as a source of bacteremia and fungemia has paralleled the increased use of long-term central and peripheral lines for chemotherapy access and for parenteral nutrition. Concomitantly, "sticky" organisms of low virulence, such as the coagulase-negative staphylococci, which are capable of adhering to the surfaces of catheter materials, have become more prominent as etiologic agents of bacteremia.

Clinical Manifestations

Confusion exists and is perpetuated by interchangeable use of the terms "bacteremia," "sepsis," and "septicemia." Bacteremia and fungemia are terms that simply identify the presence of bacteria or fungi, respectively, in the blood. Sepsis is the presence of clinical symptoms of infection in the presence of positive blood culture(s). Septicemia is a serious clinical syndrome associated with evidence of acute infection and organ failure related to release of mediators, like cytokines, into the circulation. Septicemia may or may not be associated with positive blood cultures. The signs and symptoms of sepsis are nonspecific. Often there is an abrupt onset of fever, associated

rigors (uncontrollable shaking chills), malaise, apprehension, and hyperventilation. Symptoms and signs referable to the primary focus of infection are present in the majority of patients, and some patients have cutaneous manifestations such as rash, septic emboli, or ecthyma gangrenosum (highly suggestive of sepsis due to *Pseudomonas aeruginosa* or other gram-negative bacilli). As in virtually all infectious syndromes, atypical presentations may confuse the clinical picture. For example, not all patients are febrile; neonates, aged patients, or patients on corticosteroids or nonsteroidal anti-inflammatory agents may fail to mount a febrile response to sepsis. Furthermore, some patients with bacteremia or fungemia may be hypothermic, a poor prognostic sign (199).

Risk Factors for Bacteremia and Fungemia

The conditions that predispose an individual to bloodstream infection include not only age and underlying diseases but also medications and procedures whose primary purposes are maintenance or restoration of health. There is increased risk at the extremes of age; premature infants are especially at risk for bacteremia. A partial list of illnesses that are associated with an increased risk of bloodstream infection would include hematologic and nonhematologic malignancies, diabetes mellitus, renal failure requiring dialysis, hepatic cirrhosis, immune deficiency syndromes, and conditions associated with the loss of normal skin barriers such as serious burns and decubitus ulcers. Therapeutic maneuvers associated with an increased risk of bacteremia include procedures as benign as placement of intravascular catheters (as mentioned above), as well as surgery of all types but especially involving the bowel and genitourinary tract and endoscopic procedures of the genitourinary and lower gastrointestinal tracts. Finally, certain medications such as corticosteroids alter cell-mediated immune function and increase the risk of bloodstream infection with obligate intracellular microorganisms, whereas other agents such as cytotoxic drugs used for chemotherapy predictably cause granulocytopenia and increase the risk for septicemia due to pyogenic bacteria and fungi.

Etiologic Microorganisms

The spectrum of microorganisms that invade the bloodstream in adults has been systematically evaluated in several large studies in recent years. Notably, two studies using identical criteria to define clinically significant isolates (as opposed to contaminants) provide perspective about changes in the microbiology of septicemia in the present era (Table 1). The most common etiologic agents, *Staphylococcus aureus* and *Escherichia coli*, have not changed over the past two decades. A key change in 1992 to 1993 compared with 1975 to 1977 is the dramatic increase in the incidence of coagulase-negative staphylococci (CNS) as clinically significant agents of bacteremia. The ascendance of this group of staphylococci has created increased interpretative difficulties for clinicians, since the great majority of CNS (85%) continue to represent contamination rather than true bacteremia (183, 199a). Other important interval changes are the proportionate increase in fungemias, enterococcal bacteremias, and AIDS-associated mycobacteremias and the reduction in anaerobic bacteremias.

In children, the microorganisms causing bacteremia and fungemia are similar to those in adults, but there are several important differences. Anaerobic bacteremias are even less common in pediatric patients than in adults. In general, non-immunosuppressed bacteremic children have an increased likelihood of being infected by staphylococci, streptococci including *Streptococcus pneumoniae*, and meningococci; gram-

TABLE 1. The 10 most frequent microorganisms causing bacteremia and fungemia in adults from 1975 to 1977^a and 1992 to 1993^b in descending order of frequency

Microorganism	
1975-1977	1992-1993
<i>E. coli</i>	<i>S. aureus</i>
<i>S. aureus</i>	<i>E. coli</i>
<i>S. pneumoniae</i>	Coagulase-negative staphylococci
<i>K. pneumoniae</i>	<i>K. pneumoniae</i>
<i>P. aeruginosa</i>	<i>Enterococcus</i> spp.
<i>B. fragilis</i>	<i>P. aeruginosa</i>
<i>Enterococcus</i> spp.	<i>S. pneumoniae</i>
<i>S. pyogenes</i>	Viridans group streptococci
<i>C. albicans</i>	<i>C. albicans</i>
<i>P. mirabilis</i>	<i>E. cloacae</i>

^a Reference 199.

^b References 183 and 199a.

negative rod bacteremia is less likely except in neonates. *Haemophilus influenzae* bacteremia, formerly common in children, has nearly vanished in many medical centers as immunization against *H. influenzae* type B has become widespread.

Prognosis of Bacteremia and Fungemia

The outcome of bacteremia depends on many factors; overall, mortality has ranged between 20 and 50% in large studies (94, 191). Weinstein et al. reported that about half of all deaths in bacteremic patients could be attributed to the septicemic episodes themselves (199), and Bryan et al. corroborated this finding (25). Recent studies suggest that the mortality associated with bacteremia may be decreasing. A report from Israel noted an overall mortality of 29% (94), and a recent multi-center evaluation of more than 800 episodes of bacteremia and fungemia in the United States showed a mortality rate of 22.5% (183, 199a).

Multivariate analysis has been used to assess the relative prognostic importance of different clinical variables. Weinstein et al. (199) found that several were independent predictors of increased mortality. Poor prognostic findings included advanced age (>40 years); nosocomial bacteremia; an enterococcal, gram-negative, or fungal etiology; underlying cirrhosis, malignancy, or the presence of multiple underlying problems; a primary focus in the respiratory tract, skin (including burns), surgical wound, abscess, or an unknown source; presence of septic shock; and lack of a febrile response to sepsis. Most of these findings were corroborated by Towns et al. (183, 199a).

Interpretation of Positive Blood Cultures

Several techniques are available to assist the clinician and microbiologist in interpreting the clinical importance of a positive blood culture. The identity of the microorganism isolated provides some predictive value. Common blood isolates that always or nearly always (>90%) represent true infection include *S. aureus*, *E. coli* and other members of the *Enterobacteriaceae*, *Pseudomonas aeruginosa*, *Streptococcus pneumoniae*, and *Candida albicans* (183, 199a). Other microorganisms such as *Corynebacterium* spp., *Bacillus* spp., and *Propionibacterium acnes* rarely (<5%) represent true bacteremia (183, 199a). More problematic are viridans group streptococci, enterococci, and CNS, which represented true bacteremia 38, 78, and 15% of the time, respectively (183, 199a).

Some microbiologists have used the number of culture bottles in one set that are positive to determine the clinical sig-

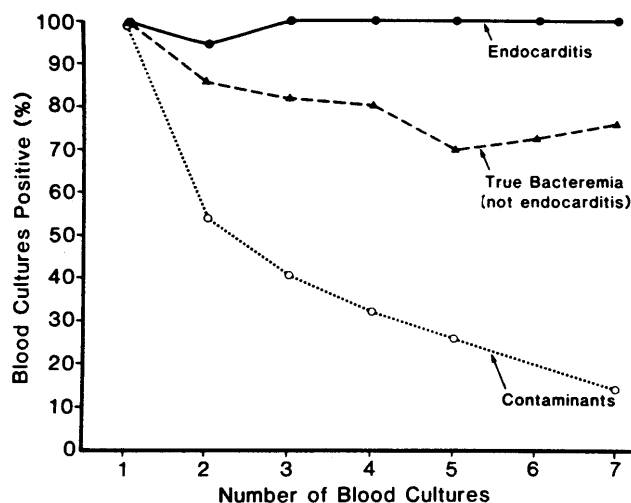


FIG. 1. Patterns of positivity in successive blood cultures, showing evidence of the diagnostic importance of separate cultures. Reprinted from reference 199 with permission of the publisher.

nificance of the isolate, but recent data suggest that this technique is flawed; in our judgment, it should be abandoned. Our group examined more than 23,000 blood cultures and focused on 1,024 that grew CNS (112). The clinical significance of each isolate was made by physician-investigators based on published criteria (199) and then correlated with the number of bottles positive from each culture set. For conventional two-bottle culture sets, 49% of pathogens and 68% of contaminants grew in one bottle whereas 51% of pathogens and 32% of contaminants grew in both bottles. Thus, the degree of overlap is so great that it is impossible to predict the clinical significance based on the number of positive bottles.

Quantitative blood cultures have also been proposed as a method to diagnose intravenous catheter-related sepsis. An incremental increase in the quantity of bacteria obtained through the catheter with respect to that obtained from peripheral venipuncture should intuitively suggest the catheter as the source of organisms in the blood. Although numerous studies addressing this issue have been published, the value of quantitative cultures remains controversial and not well studied (125a). Not addressed in these studies is the likelihood that a catheter serves as stationary nidus for the growth of microorganisms (and hence an increase in quantitation) independent of the origin of the organisms. Moreover, as a general principle, collection of blood for culture via indwelling catheters should generally be discouraged (26).

Finally, patterns of positivity of blood cultures obtained in sequence can aid in the interpretation of clinical significance. Figure 1 depicts this concept. In endocarditis, bacteremia is continuous, and if one culture is positive, all will be so. Bacteremias not associated with endocarditis or other endovascular infections are not continuous, and host defenses clear microorganisms over time. Thus, it is not surprising that only 70 to 80% of culture sets will be positive. Finally, the presence of only a single positive culture set of several obtained in series strongly suggests that the positive represents contamination.

DIAGNOSTIC STRATEGIES FOR BLOOD CULTURES

Blood Culture Collection

Number of cultures. For adult patients, two or three blood cultures per septic episode should be obtained. This number is

based on several factors. First, studies by Washington (189) and Weinstein et al. (199) have shown that more than 95% of episodes of bacteremia and fungemia are detected when two or three blood cultures are drawn. Second, routinely obtaining more than one blood culture per septic episode has the added benefit of ensuring that adequate volumes of blood are cultured. Third, it helps physicians distinguish between clinically important and contaminant microorganisms, since the proportion of positive blood cultures is crucial in interpreting blood culture results (7). Lastly, since a finite percentage of blood cultures are contaminated (3 to 5%), routinely obtaining more than three blood cultures per septic episode usually does not help distinguish between clinically important and contaminant isolates (7). Furthermore, routinely drawing more than three blood cultures is expensive, needlessly increases laboratory work, and contributes to nosocomial (phlebotomy-induced) anemia.

Timing of cultures. Since bacteria are rapidly cleared from blood and since fever spikes follow bacteremic episodes by 30 to 90 min, the best opportunity for recovering pathogenic microorganisms should be as soon after the fever spike as possible. Interestingly, in the only published systematic study of which we are aware, Li et al. (97) showed that there was no difference in organism recovery between multiple blood cultures drawn simultaneously and those drawn serially over a 24-h period. Nonetheless, since blood cultures should be collected before antimicrobial agents are given and since newer blood culture systems can detect microbial growth within a few hours, it makes more sense to draw blood cultures simultaneously rather than serially. Moreover, it is more practical to draw multiple blood cultures simultaneously.

Collection procedure. Every clinical microbiology laboratory needs policies and procedures designed to ensure that blood cultures are collected in a timely manner, in such a way as to minimize contamination, and that proper blood volumes are inoculated into the bottles (1, 14, 110). Whenever possible, blood should be collected by venipuncture of peripheral veins and not via indwelling vascular catheters (26). Whether blood cultures are collected by medical technologists, trained phlebotomists, nurses, or other health care providers, an ongoing program to monitor compliance with these policies and procedures is necessary and should be part of the laboratory quality assurance program.

Before beginning the venipuncture procedure, the rubber septae on the blood culture bottles or tubes should be cleansed and disinfected with alcohol swabs. Iodine should not be used for this purpose since, according to the manufacturers, it can cause the rubber to deteriorate. After a likely site for venipuncture has been selected, a tourniquet is applied to the limb and the vein is palpated. The tourniquet is then released and the site of venipuncture is cleansed and disinfected with iodine or an iodophore such as povidone iodine (commercial povidone iodine swabs or pledgets are inexpensive and easy to use). Beginning over the point of anticipated venipuncture, the iodophore should be applied in a circular fashion outward for a final diameter of 5 to 6 cm. Since the antiseptic effect of povidone iodine is time-dependent, the easiest way to ensure adequate disinfection is to allow the solution to completely dry (usually 1 to 3 min) before venipuncture. Once the venipuncture sites have been disinfected, phlebotomists must not palpate the skin unless they are wearing sterile gloves. The tourniquet is then reapplied, the venipuncture is made, and blood is withdrawn.

Blood may be drawn by many different methods. The preferred method is to use a 21-gauge needle with a syringe that will hold 5 to 10 ml more blood than is needed (using syringes

with a larger volume than is needed makes drawing the desired volume of blood easier). Many phlebotomists prefer using a "butterfly" needle with the syringe, although the use of collection sets with small-bore plastic tubing increases the likelihood of clotting. The practice of drawing directly into blood culture bottles should be discouraged, since most blood culture bottles contain sufficient vacuum to aspirate more than the recommended blood volume. Concerns about "back-flushing" of broth into a patient's vein must also be taken into account before such a practice is implemented by a laboratory. Once the desired volume of blood has been drawn, the needle should be withdrawn from the skin and the culture bottles or collection transport tubes should be inoculated. If the latter are used, these tubes should be transported to the laboratory as soon as possible for laboratory inoculation of blood culture bottles. There are no studies determining the maximum allowable time during which recovery of organisms from these tubes will be maintained. Most now believe that phlebotomists should not change needles prior to inoculating bottles, as this practice increases the likelihood of needle-stick injuries. Although some studies also suggest that changing needles does not decrease contamination rates (91, 95), a recent meta-analysis disputes this claim (168). To prevent clotting, inoculated bottles should be gently inverted a few times to mix the blood in the broth medium or in the tube anticoagulant. After the blood is drawn, alcohol swabs should be used to clean the patient's skin of iodine.

Principles of Laboratory Detection

Volume of blood cultured. Culturing adequate volumes of blood improves microbial recovery for both adult and pediatric patients (8, 62, 97, 110, 129, 142, 154, 175). Indeed, the volume of blood cultured is the most important variable in optimizing microbial recovery for adult patients (142, 209). This is because the number of microorganisms present in blood in adults is small, typically fewer than 10 CFU/ml and often less than 1 CFU/ml. For adults, each additional milliliter of blood cultured increases microbial recovery by up to 3% (110). To maximize microbial recovery, 20 to 30 ml of blood should be cultured per venipuncture (191, 209). Although the number of microorganisms present per milliliter of blood drawn from infants and small children is larger, typically greater than 100 CFU/ml and often greater than 1,000 CFU/ml, increasing the volume of blood cultured still increases microbial recovery.

Although one should not reject blood cultures because of inadequate volumes of blood inoculated into bottles, bottles can be monitored for such inadequate blood volumes and prompt collection of another blood culture set (110). It is less important to monitor the volumes of blood drawn from infants and small children, since it is less practical to accurately measure the smaller blood volumes and the issue of blood volume is less critical than for adults. Information collected from such monitoring can be used as part of a laboratory quality assurance program.

Ratio of blood to broth. Diluting blood into broth to ratios of greater than 1:5 increases microbial recovery, probably by diluting antimicrobial agents and natural inhibitory factors in the blood to subinhibitory concentrations (9, 143, 153). Furthermore, diluting blood helps prevent clotting, one of the reasons that overfilling of blood culture bottles is discouraged.

Although some commercial blood culture bottles do not contain sufficient volumes of media to permit blood-to-broth ratios of 1:5 to 1:10, these media have been supplemented with materials that improve microbial recovery to the point where suboptimal blood-to-broth ratios are overcome (195, 206).

These materials presumably increase microbial recovery by lysing leukocytes and thereby releasing phagocytized microorganisms into the blood-broth mixture (77).

Media. For aerobic blood culture bottles, the most widely used medium is soybean-casein digest (SCD) broth. Other media used to recover fastidious microorganisms include brain heart infusion (BHI) and supplemented peptone broths. A variety of different media, including SCD, Columbia, and peptone broths, are used for anaerobic blood culture bottles. Most commercial media are supplemented with proprietary combinations of various nutrients and other growth factors.

Few controlled clinical comparisons of different broth media have been done to determine which medium is superior for both microbial recovery and speed of detection. In other words, most comparisons are between different bottle types and/or blood culture systems. Since sodium polyanetholsulfonate (SPS) concentrations, nutritional supplements, and head-space atmospheres vary among bottles, it is difficult to draw conclusions regarding the relative performance of broth media per se. In general, SCD is adequate for recovering most pathogenic microorganisms. BHI is probably as good as or better than SCD for recovering yeasts and some bacteria (210). Likewise, Columbia and other anaerobic broths are adequate for recovering anaerobic bacteria.

Anticoagulants. Most commercial blood culture media contain 0.025 to 0.050% SPS as an anticoagulant. SPS also inhibits lysozyme, inactivates clinically achievable concentrations of some aminoglycoside and polymyxin antibiotics, inhibits parts of the complement cascade, and inhibits phagocytosis. The last effect has little effect in improving microbial recovery, since phagocytes in blood culture bottles probably do not actively ingest microorganisms, particularly if the complement cascade is no longer functioning properly.

SPS is not without its drawbacks, however, as it has been shown to inhibit the growth of *Neisseria meningitidis*, *N. gonorrhoeae*, *Gardnerella vaginalis*, *Streptobacillus moniliformis*, and *Peptostreptococcus anaerobius* (46, 140, 169). SPS can also inhibit the growth of *Francisella tularensis* and *Moraxella catarhalis*. As a general rule, higher SPS concentrations, while promoting the recovery of gram-positive cocci, decrease the recovery of gram-negative bacteria. Supplementing blood culture media with 1.2% gelatin partially counteracts the inhibitory effect of SPS but also impairs the anticomplementary effect of SPS (46, 169).

A similar compound, sodium amylosulfate (SAS), has been evaluated as a blood culture anticoagulant. Although SAS does not inhibit the growth of microorganisms inhibited by SPS, the use of SAS results in lower recovery rates for staphylococci, members of the *Bacteroidaceae*, and *Eubacterium* (176). Detection of microbial growth is faster with SPS (176). Other common anticoagulants such as heparin, EDTA, and sodium citrate should never be used in broth blood culture media or to collect blood cultures (one notable exception being the Isolator lysis-centrifugation method).

Antimicrobial agent removal. A variety of products have been marketed for the purpose of increasing microbial recovery from the blood of patients receiving systemic antimicrobial therapy. Although bottles containing these products have been shown to increase microbial recovery rates, the preponderance of published data suggest that this is not due to inhibition or removal of antimicrobial agents. The exact mechanism(s) by which most of these products increase microbial recovery is not known, since they vary in their composition and bottles containing them differ from their standard counterparts in other ways (e.g., different volumes and types of broth). Hence, it is

not always possible to sort out the effect of the product from that of other variables.

Both BacT/Alert (Organon Teknika Corp., Durham, N.C.) and BACTEC 9000 (Becton Dickinson Microbiology Systems, Sparks, Md.) systems can be used with bottles containing products designed to increase microbial recovery. The Difco ESP system (Difco Laboratories, Detroit, Mich.) does not include such a bottle. The bottles used with the BacT/Alert and BACTEC 9000 systems recover more pathogenic microorganisms than do standard bottles (165, 194, 210). BacT/Alert FAN (an acronym no longer associated with any specific meaning) bottles contain Ecosorb, a proprietary substance composed, in part, of fuller's earth and activated charcoal particles. McDonald et al. (107a) recently studied the issue of the clinical utility of isolates recovered from FAN bottles. They found that (i) more bacteremic episodes were detected by FAN bottles; (ii) episodes detected only by FAN bottles were more often recurrent; and (iii) these episodes "more commonly occurred in patients receiving theoretically effective antibiotic therapy" (107a). A comparable study has yet to be performed on BACTEC Plus/F bottles, which contain resin-like materials similar to those present in older BACTEC Plus bottles.

Since bottles that include such products are more expensive than their standard counterparts and since the published data do not conclusively prove that use of these bottles is cost-effective, careful consideration must be given before such bottles are used routinely. For example, McDonald et al. (107a) noted that the increased benefit of using these bottles must be balanced against the higher cost of the bottles. The decision must be based on the type of system used, the patient population being served, and the types of pathogenic isolates recovered in a given laboratory. Another issue that must be considered is that of contaminants, since some of these bottles may recover more contaminants in addition to increasing the recovery of clinically important isolates (194).

Bottle headspace atmosphere. For automated systems, most commercial blood culture bottles are manufactured with a carefully controlled atmosphere in the bottle headspace (the inner part of the bottle that lies above the broth medium). Aerobic bottles generally contain ambient atmosphere to which different amounts of carbon dioxide have been added. Anaerobic bottles typically contain an atmosphere composed of carbon dioxide and nitrogen. A few modern commercial aerobic blood culture bottles require venting to room air (to increase the oxygen content) prior to inoculation or incubation.

Contrary to popular belief, maintaining adequate anaerobiosis in anaerobic bottles is less important than is maintaining adequate oxygen concentrations in aerobic bottles. This is because the incidence of anaerobic bacteremia has decreased during the past 20 years and also because aerobic microorganisms are more often recovered from aerobic than anaerobic blood culture bottles. In particular, *P. aeruginosa* and pathogenic yeasts are strictly aerobic and are unlikely to grow to detectable levels in blood culture bottles containing insufficient oxygen (18, 53, 66, 87, 146).

Commercial blood culture bottles manufactured for manual systems contain headspace atmospheres similar to those used with automated systems. One important difference is that aerobic bottles should be transiently vented to room air prior to incubation. Bottles with an external agar attachment do not have to be vented. As with automated systems, anaerobic bottles should never be vented or recovery of strictly anaerobic bacteria may be compromised.

Bottle agitation. Several clinical studies have documented that agitating bottles increases microbial recovery from aerobic

culture bottles (69, 132, 193). Agitation is thought to improve microbial recovery by increasing the oxygen concentration in the broth medium (although other mechanisms, such as mixing, may also play a role). Although different methods (including rotary, back and forth, and sinusoidal) and speeds of agitation are used (212), they do not differ significantly in their effect on microbial recovery.

If agitation does improve microbial recovery by increasing the oxygen concentration in the broth medium, it might be expected to decrease the recovery of strictly anaerobic bacteria. However, since most anaerobic blood culture bottles contain little or no oxygen in the bottle headspace atmosphere, agitation cannot increase the oxygen concentration in the broth medium. This is supported by clinical evaluations, as continuous-monitoring blood culture systems that agitate anaerobic bottles have been shown to recover anaerobic bacteria as well as automated blood culture systems that do not agitate anaerobic bottles (207, 209). Mycobacterial blood culture bottles processed on the radiometric BACTEC system (Becton Dickinson Microbiology Systems) should not be agitated per the manufacturer's recommendations, since the manufacturer suggests that mycobacterial recovery may be compromised.

Duration of incubation and testing. With manual blood culture bottles, microbial growth is detected by macroscopic examination of bottles and blind and/or terminal subcultures. Since macroscopic detection of microbial growth becomes difficult after 2 to 3 days of incubation (most inoculated blood hemolyzes by then) and since macroscopic evidence of growth may be difficult to detect or may be absent, bottles processed with manual blood culture systems should be incubated for 7 days with terminal subcultures.

In general, for recovery of common pathogenic microorganisms, automated blood culture systems yield almost as many microorganisms after 5 days of incubation and testing as after 7 days (65, 208, 210, 212). Furthermore, limiting the incubation and test period to 5 days yields fewer microorganisms ultimately judged as contaminants (208). Consequently, laboratories may realize important savings in time and effort by using a 5-day test cycle. This is particularly important with continuous-monitoring blood culture systems, since with a 5-day test period the number of modules required for purchase or lease may decrease. Lastly, the clinical impact of microorganisms recovered on days 6 and 7 of a 7-day cycle is unclear. For instance, since most clinically important microorganisms are recovered during the first 48 to 72 h of testing, it is unlikely that days 6 and 7 would represent the only time when microorganisms causing a septic episode were recovered. Even if they were, isolation of a pathogenic microorganism 6 or 7 days following a septic episode is unlikely to affect patient care.

Although this issue has not been systematically studied for every medium formulation or bottle type, there is no a priori reason to believe that 7-day test cycles significantly improve microbial recovery for any bottle type processed on commercial blood culture systems. Nonetheless, until such data are collected and verified in controlled clinical studies and the results are published, laboratory directors should use caution in immediately adopting a 5-day test cycle without performing some type of in-house study to determine the proportion of clinically important isolates recovered on days 6 and 7.

It is common practice to prolong the incubation period and/or to perform extra subcultures for blood cultures drawn from patients with suspected infective endocarditis but from whom blood cultures are negative. It should be noted, however, that there are few or no data to validate this practice when modern blood culture systems are used. Moreover, since most modern blood culture systems now accept larger blood

inocula and have more sensitive detection algorithms, there is little reason to believe that this practice increases microbial recovery. For patients with culture-negative endocarditis, a better practice is to use another blood culture method designed to improve the recovery of rare or fastidious microorganisms such as fungi or *Legionella* or *Brucella* spp. (33, 142, 205, 207).

Subcultures. In manual systems, blind subcultures are performed at an arbitrary time during the incubation cycle (usually at 24 to 48 h after the culture was drawn) and in the absence of macroscopic or other evidence of growth. Terminal subcultures, performed at the end of the incubation period in the absence of objective evidence of microbial growth, appear less important (27, 55, 85, 178, 191). Either blind or terminal subcultures are unnecessary for manual systems that include an agar paddle since subcultures are intrinsic to the system. For automated blood culture systems, neither blind nor terminal subcultures are necessary (5, 15, 96, 108, 128, 171, 211). In particular, blind subcultures are not necessary when a Gram stain of the blood-broth mixture does not reveal any microorganisms (67). For all systems, anaerobic blind subcultures are unnecessary (118, 124).

Temperature of incubation. Once inoculated, blood culture bottles should never be cooled below room temperature. Blood culture bottles can be temporarily held at room temperature without adversely affecting microbial recovery. Blood culture bottles to be processed on a continuously monitored blood culture system can be held at either room temperature or 35 to 37°C. If they are held at room temperature, the maximum delay before entry into the system has not been systematically studied. Both manual and commercial blood culture bottles should be incubated at 35 to 37°C. Subcultures should be incubated at the temperature appropriate for the suspected pathogen.

Blood Culture Systems

Manual detection systems. The simplest blood culture system consists of bottles filled with broth medium and with a partial vacuum in the headspace. To convert such bottles to aerobic bottles, the oxygen concentration is increased by transiently venting bottles to room air after they have been inoculated with blood. Unvented bottles remain relatively anaerobic. After inoculation, the bottles are incubated with or without agitation and are periodically examined for macroscopic evidence of growth. Such evidence consists of hemolysis, turbidity, gas production, "chocolatization" of the blood, and the presence of visible colonies or a layer of growth on the fluid meniscus. Blind or terminal subcultures are needed to reliably recover pathogenic microorganisms from this type of bottle.

Manual systems are too labor-intensive to be practical for most laboratories that process large numbers of blood cultures. This stems from the need to manually inspect each bottle daily for macroscopic evidence of growth and to perform blind and/or terminal subcultures. Performing blind and/or terminal subcultures also increases the likelihood that technologists will be exposed to patient blood and adds to laboratory costs.

(i) **Derivatives of "Castenada" bottles.** Manual blood culture systems that incorporate a plastic paddle containing agar media became popular during the past decade. The design of these bottles is based on the "Castenada" bottle, which included an agar slant within the bottle. Commercial versions include Septi-Chek and Opticult (Becton-Dickinson Microbiology Systems). Once aerobic bottles are inoculated, paddle devices are attached and the bottles are temporarily inverted to allow the blood-broth mixture to flow into the device and coat

the agar medium. The bottles and paddles are inspected once or twice daily for macroscopic evidence of growth and are inverted each time they are inspected. Anaerobic bottles do not have paddle attachments.

These bottles have the advantages of not requiring venting, inoculating subcultures automatically, providing an alternative means of detecting microbial growth via the agar media, and providing early isolated colonies from which final identification and susceptibility procedures can be performed (197). For laboratories that process moderately large numbers of blood cultures, the manual steps required to process these bottles make their use less practical. They are also relatively expensive for laboratories that process large numbers of blood cultures.

(ii) **Oxoid Signal blood culture system.** The Oxoid Signal (Oxoid USA, Columbia, Mo.) (193) blood culture system is no longer widely used in the United States. This manual system consists of an aerobic blood culture bottle only. After the bottle is inoculated, a "signal" device, which consists of a long needle in a plastic sleeve, is attached to the bottle. As the microorganisms grow and liberate gases into the headspace atmosphere, the pressure in the bottle increases, forcing the blood-broth mixture upward through the needle into a reservoir at the top of the plastic sleeve. Hence, in addition to other types of macroscopic evidence of growth, Signal utilizes a manometric method. The lack of an anaerobic bottle is one important disadvantage to this system.

(iii) **Isolator blood culture system.** A different type of manual system, one that is not broth based, is lysis-centrifugation. The only commercially available version is the Isolator (Wampole Laboratories, Cranbury, N.J.). Isolator tubes contain EDTA as an anticoagulant, saponin as a lysing agent, and a fluorocarbon compound that acts as a cushion during centrifugation. After blood is inoculated into the tube, cells are lysed by the saponin. The tubes are then centrifuged, and the supernatants are discarded. The pellets are removed from the tubes and are inoculated onto solid media appropriate for the type of culture being performed. The performance characteristics and advantages and disadvantages of Isolator have been well documented (209). In particular, Isolator has been found to be an excellent method for recovering pathogenic yeasts and thermal dimorphic fungi from blood. It is an acceptable method for recovering common pathogenic bacteria and fastidious pathogens such as *Bartonella* spp. Isolator is not an optimal method for recovering anaerobic bacteria. Although early reports indicated a high contamination rate with Isolator, processing specimens in a biological safety cabinet and use of the Isostat device significantly reduce the contamination rate. The main disadvantage to using Isolator is that it is labor-intensive, which, for some laboratories, precludes routine use.

The widespread use of automated blood culture systems has not totally eliminated manual systems from the market, since these systems are simple to use, require no instrumentation other than an incubator and, for some systems, an agitation mechanism, and are the least expensive method to use. For laboratories that process small numbers of blood cultures, these systems may still be a reasonable and cost-effective alternative.

Automated detection systems. To make processing blood cultures more efficient, several manufacturers have developed and marketed a number of automated blood culture systems over the past 20 years (Table 2). Since they are no longer as widely used, older systems such as the BACTEC radiometric and nonradiometric systems are discussed only briefly. New systems are presented in more detail.

(i) **BACTEC radiometric blood culture systems.** The first commercial automated blood culture system was the radiomet-

TABLE 2. Commercial automated blood culture systems^a

System	Manufacturer	Microbial growth detection	Continuous monitoring
BACTEC 460	Becton Dickinson	Radiometric CO ₂ detection	No
BACTEC 660/730/860	Becton Dickinson	Infrared CO ₂ detection	No
BacT/Alert	Organon Teknika	Colorimetric CO ₂ detection	Yes
BACTEC 9240	Becton Dickinson	Fluorescent CO ₂ detection	Yes
Vital	bioMérieux vitek	Fluorescent CO ₂ detection	Yes
ESP	Difco	Manometric	Yes
Bio Argos	Diagnostics Pasteur	Infrared CO ₂ detection	No
o.a.s.i.s.	Unipath	Manometric	Yes

^a Modified from reference 204b with permission of the publisher.

ric BACTEC system (Becton Dickinson Microbiology Systems). As with most of its successors, this system detected microbial growth by monitoring the concentration of CO₂ present in the bottle. In this system, metabolic precursors labelled with ¹⁴C are included in the broth medium; as microorganisms grow, they liberate ¹⁴CO₂ in the broth, which diffuses into the bottle headspace atmosphere. The headspace atmosphere is periodically sampled, and the amount of ¹⁴CO₂ is measured. This quantity is related to a "growth index"; a growth index exceeding a predefined threshold is considered evidence of microbial growth and prompts a Gram stain and subculture of the blood-broth mixture. Although this system has largely been replaced by other automated blood culture systems, the model 460 continues to be widely used as an automated system for processing mycobacterial cultures. In addition, it remains the only Food and Drug Administration (FDA)-approved automated method for performing antimycobacterial susceptibility testing.

(ii) **BACTEC nonradiometric blood culture systems.** In the early 1980s, Becton Dickinson introduced a new generation of automated instruments, the nonradiometric BACTEC 660, 730, and 860 systems. Conceptually and operationally similar to the radiometric systems, these instruments use infrared spectrophotometry to detect CO₂ in samples of the bottle headspace atmosphere. The newer systems hold more bottles, require shorter monitoring times, and are easier to use than the model 460.

(iii) **Bio Argos blood culture system.** The Bio Argos System (Sanofi Diagnostics Pasteur, Marnes-la-Coquette, France) is similar to the BACTEC nonradiometric blood culture systems in that it does not monitor continuously and uses infrared spectrophotometry to detect CO₂ production (38). The system is more fully automated, however, as robotic devices move bottles to and from the incubator into the testing device. Unlike the BACTEC systems, the infrared spectrophotometer reads through the glass wall of each vial. The system, therefore, is noninvasive. No data from clinical evaluations have been published. The system is not available in the United States.

Continuous-monitoring blood culture systems. The most important technological advance in blood cultures during the past 20 years has been the development of continuous-monitoring blood culture systems (CMBCS). CMBCS differ from other automated blood culture systems in several ways. First, these instruments electronically monitor bottles for evidence of microbial growth on a nearly continuous basis, typically once every 10 min. Data collected from this monitoring are transmitted to a microcomputer, where they are stored and analyzed. Second, since many data points are collected per bottle per day, sufficient data are available to permit the use of sophisticated computer algorithms to determine when microbial growth has occurred. In combination with the more fre-

quent test cycle, such computer algorithms allow for earlier detection of microbial growth. The algorithms are also thought to minimize the number of false-positive instrument signals generated by continuous-monitoring instruments. Third, all CMBCS incorporate the detection system, incubator, and agitation mechanism into a single unit. To do so, the manufacturers have created instruments that can test each bottle individually, obviating the need for manual manipulation of bottles after they have been placed in the instrument and eliminating the chance of cross-contamination between bottles. Lastly, all CMBCS have bottles that accept blood inocula of up to 10 ml, a feature not always available with earlier commercial blood culture systems.

CMBCS have been described in detail elsewhere (204, 212); Tables 2 through 4 provide updated information on instrument, bottle, and systems characteristics, respectively. The relative advantages and disadvantages of these systems are summarized in Table 5. The information that follows provides a general description of each system along with the results of clinical evaluations designed either to establish system performance characteristics or to compare one system with another.

(i) **BacT/Alert blood culture system.** BacT/Alert (Organon Teknika Corp.) was the first commercial CMBCS (180). Within the base of each bottle is a solid sensor that undergoes a color change as the CO₂ concentration in the broth medium increases. Light-emitting and -sensing diodes are incorporated in the base of each cell in which the bottles reside. As the sensor changes color, the amount of light reflected off the sensor increases and is quantified as an increasing voltage signal. These signals are recorded by a microcomputer and are analyzed by an algorithm. The algorithm recognizes three criteria as evidence of microbial growth: an initial reading that exceeds a predefined threshold (analogous to the BACTEC growth indices), a sustained linear increase in CO₂ concentration (production), or an increase in the rate of CO₂ production (dx/dt). Currently, standard aerobic and anaerobic (accepting blood inocula of up to 10 ml), pediatric, and FAN aerobic bottles are available.

The first evaluation of BacT/Alert (180) included both in vitro seeding experiments and a limited clinical trial. The former demonstrated that the broth media were capable of supporting the growth of a wide range of microorganisms and that the instrument and computer algorithm could reliably detect microbial growth. The clinical trial compared a prototype BacT/Alert instrument with the BACTEC 460 blood culture system. BacT/Alert was found to be sufficiently comparable to BACTEC to warrant full-scale controlled clinical trials.

In 1992, Wilson et al. (211) published results from the first controlled clinical evaluation of BacT/Alert. BacT/Alert was found to be comparable to the BACTEC 660/730 systems for microbial recovery with earlier detection of microbial growth

TABLE 3. CMBCS instrument characteristics^a

System	Detection mechanism	Bottle capacity	Max no. of units (bottles)/system	Test cycle (min)	Agitation type/speed	Instrument dimensions ^b
BacT/Alert 240 BacT/Alert 120	Colorimetric	240/unit 120/unit	6 (1,440)	10	Rocking/34 ^c	175 × 87 × 66
BACTEC 9240 BACTEC 9120	Fluorescent	240/unit 120/unit	5 (1,200) ^d 5 (600) ^d	10	Rocking/30 ^c	93 × 128 × 55
ESP 128 ESP 256 ESP 384	Pressure	128/unit 256/unit 384/unit	5 (640) ^d 5 (1,280) 5 (1,290) ^d	12/24 ^c	Rotary/160 ^f	90 × 86 × 65 Not available 199 × 86 × 65
Vital 400 Vital 300 Vital 200	Fluorescent ^g	400/unit 300/unit 200/unit	3 (1,200) ^d 3 (900) ^d 3 (600) ^d	15	Sinusoidal/150 ^h	108 × 78 × 114 108 × 78 × 114 108 × 78 × 114
o.a.s.i.s.	Pressure	100/unit ⁱ	NA ⁱ	5	Magnetic stirrer/240	NA ⁱ

^a Modified from reference 204 with permission of the publisher. All information is current as of May 1995. The information shown is based on verbal interviews with representatives of each manufacturer and is subject to change without notice. The authors and ASCP assume no responsibility for the information presented, nor do they guarantee the accuracy of the information.

^b Does not include computer or recommended clearance; rounded to nearest centimeter; height by width by depth.

^c Cycles per minute.

^d The different sizes of instruments can be used together in any combination.

^e 12 min for aerobic bottles and 24 min for anaerobic bottles.

^f Revolutions per minute.

^g Unlike BacT/Alert and BACTEC, which use sensors bonded to the bottom interior surface of bottles, Vital uses a fluorescent compound that is in solution in the broth medium (see the text).

^h Vital bottles are agitated in a "sinusoidal and symmetrical" manner.

ⁱ NA, not applicable; information from reference (51) and therefore for prototype system only.

and fewer false-positive instrument signals. For recovery of specific microorganisms, only members of the family *Enterobacteriaceae* were recovered more often from BacT/Alert aerobic bottles than from BACTEC NR 6A bottles. The time to detection of microbial growth was significantly shorter in BacT/Alert aerobic bottles for *S. aureus*, CNS, streptococci, *E. coli*, other members of the family *Enterobacteriaceae* combined (excluding *K. pneumoniae*), *P. aeruginosa*, and all microorganisms combined. Similarly, the time to detection of microbial growth was significantly shorter in BacT/Alert anaerobic bottles for *S. aureus*, CNS, enterococci, *Streptococcus pneumoniae*, viridans group streptococci, *E. coli*, *K. pneumoniae*, other members of the family *Enterobacteriaceae* combined, and all microorganisms combined.

In a subsequent publication based on a separate analysis of the same data set (208), Wilson et al. showed that BacT/Alert bottles can be processed in a 5-day test cycle without an important reduction in the recovery of pathogens but with a significant reduction in the number of contaminants recovered. Hardy et al. (65) also showed that a 5-day test cycle is adequate for the recovery of microbial pathogens and that subcultures of instrument-negative bottles are unnecessary.

In these initial evaluations, the blood culture bottles against which BacT/Alert bottles were compared accepted only 5-ml blood inocula. Since standard aerobic and anaerobic BacT/Alert bottles were designed to accept 10-ml blood inocula, Weinstein et al. (196) evaluated the effect of inoculating either 10 or 5 ml of blood into standard aerobic BacT/Alert bottles. Bottles inoculated with 10 ml of blood recovered significantly more *E. coli*, other members of the *Enterobacteriaceae*, and all microorganisms combined. The time to detection of microbial growth was significantly shorter for CNS, streptococci, *E. coli*, other members of the *Enterobacteriaceae*, and all microorganisms combined. The mean times to detection of growth for clinically important microorganisms were very close, although for some microorganisms the time was shorter in bottles inoc-

ulated with 5 ml of blood. The reason for this phenomenon is unknown. Possible explanations include the lower blood-to-broth ratio in bottles inoculated with 10 ml of blood and the presence of greater amounts of antimicrobial agents in bottles containing more blood (196).

Results from the first clinical evaluation of the pediatric BacT/Alert bottle, Pedi-BacT, were published by Krisher et al. in 1993 (90). When compared with a 20-ml supplemented peptone broth tube (Becton Dickinson Microbiology Systems), Pedi-BacT recovered significantly more members of the *Enterobacteriaceae*, nonfermenters other than *P. aeruginosa*, *Candida* spp., and all microorganisms combined. In 1995, Pickett and Welch (126) published the results of a clinical comparison of the Pedi-BacT bottle with the "pediatric" Isolator 1.5-ml tube. There were no significant differences in the ability of the two systems to recover pathogenic microorganisms or in the time required to detect microbial growth.

In 1994, Zwadyk et al. (218) published the results of a comparison between BacT/Alert and the Difco ESP blood culture system. These results are discussed below.

In 1995, Rohner et al. (149) published the results of a clinical comparison of BacT/Alert with the Signal blood culture system. To control for the volume of blood cultured in each system, 20 ml of blood was drawn from each patient and was divided by inoculating 5 ml into each BacT/Alert bottle and 10 ml into the Signal bottle (10 ml for each system). BacT/Alert recovered significantly more *E. coli*, other gram-negative bacteria, yeasts, and all microorganisms combined. Significantly more septic episodes caused by all microorganisms combined were detected by the BacT/Alert system. The time to detection of microbial growth was significantly shorter in BacT/Alert bottles for *S. aureus*, *Streptococcus pneumoniae*, other streptococci, *E. coli*, other members of the *Enterobacteriaceae*, *Pseudomonas* spp., and all microorganisms combined. The results of this evaluation might have been predicted, since although the volume of blood used with each system was con-

TABLE 4. CMBCS bottle characteristics^a

System and bottle	Broth medium	Broth vol (ml)	Inoculum vol ^b	Blood: ratio ^c	Headspace atmosphere	Venting required	Anticoagulant and concn
BacT/Alert							
Aerobic	SCD	40	10	1:4	CO ₂ + air	Yes	0.035% SPS ^d
Anaerobic	SCD	40	10	1:4	CO ₂ + N ₂	No	0.035% SPS
Pedi-BacT	BHI	20	4	1:5	CO ₂ + N ₂	Yes	0.020% SPS
FAN aerobic	BHI	40	10	1:4	CO ₂ + air	Yes	0.050% SPS
FAN anaerobic	BHI	40	10	1:4	CO ₂ + N ₂	No	0.050% SPS
BACTEC							
Standard aerobic/F	SCD	40	5	1:8	CO ₂ + air	No	0.025% SPS
Standard anaerobic/F	SCD	40	5	1:8	CO ₂ + N ₂	No	0.025% SPS
Plus aerobic/F	SCD	25	10	1:2.5	CO ₂ + air	No	0.050% SPS
Plus anaerobic/F	SCD	25	10	1:2.5	CO ₂ + N ₂	No	0.050% SPS
PEDS PLUS/F	SCD	40	2	1:20	CO ₂ + air	No	0.020% SPS
Lytic/10 Anaerobic/F	SCD	40	10	1:4	CO ₂ + N ₂	No	0.035% SPS
ESP							
80A (aerobic)	SCP ^d	80	10	1:8	CO ₂ ^e	No ^f	0.006% SPS
80N (anaerobic)	PP ^g	80	10	1:8	CO ₂ + N ₂	No ^f	0.070% TSC ^h
EZ DRAW 40A	SCP	40	5	1:8	CO ₂ ^e	No ^f	0.006% SPS
EZ DRAW 40N	PP	40	5	1:8	CO ₂ ^e	No ^f	0.070% TSC
Vital							
Aerobic	SCD	40	10	1:4	CO ₂ + O ₂ mixture	No	0.025% SPS
Anaerobic	SCD	40	10	1:4	CO ₂ + N ₂ mixture	No	0.025% SPS
o.a.s.i.s. ⁱ	SCD	90	10	1:9	Not specified	No	Not specified

^a Modified from reference 204 with permission of the publisher. All information is current as of May 1995. The information shown is based on verbal interviews with representatives of each manufacturer and is subject to change without notice. The authors and ASCP assume no responsibility for the information presented, nor do they guarantee the accuracy of the information.

^b Recommended blood volume.

^c Minimum volume:volume rate.

^d Soy-casein peptone broth.

^e Other atmospheric components may be added by the manufacturer.

^f ESP bottles, which are transiently vented as adapters are placed.

^g Proteose peptone broth.

^h Trisodium citrate.

ⁱ Information from reference 51 and therefore for the prototype system only.

trolled, the blood-to-broth ratios differed and there is no anaerobic Signal bottle.

In 1995, Weinstein et al. (194) published the results of a clinical comparison of FAN aerobic bottles with BacT/Alert standard aerobic bottles. The FAN bottles recovered signifi-

TABLE 5. Advantages and disadvantages of CMBCS^a

Advantages	Disadvantages
Continuous monitoring with earlier detection of microbial growth	Cost
10-ml blood inocula standard	Limited medium types with some systems
Decreased laboratory workload	Size of certain instruments
Blind and terminal subcultures not necessary	
No need for radioactive waste disposal	
No need for separate incubator, agitator, or gas supply	
No possibility of pseudobacteremia	
Decreased false-positive instrument signals	

^a Modified from reference 212 with permission of the publisher.

cantly more *S. aureus*, coagulase-negative staphylococci, yeasts, and all microorganisms combined. Significantly more septic episodes caused by *S. aureus* and all microorganisms combined were detected by the FAN bottles. The time to detection of microbial growth was significantly shorter in the FAN bottles for *S. aureus* and enterococci. In that same year, Wilson et al. (211) published the results of a clinical comparison of FAN anaerobic bottles with BacT/Alert standard anaerobic bottles. The FAN bottles recovered significantly more *S. aureus*, CNS, *E. coli*, and all microorganisms combined. Standard bottles recovered significantly more nonfermenters, *Torulopsis glabrata*, and other yeasts. Significantly more septic episodes caused by *S. aureus*, CNS, members of the *Enterobacteriaceae*, and all microorganisms combined were detected by the FAN bottles. In contrast, significantly more septic episodes caused by nonfermenters and yeasts were detected by the standard anaerobic bottles. The time to detection of microbial growth was significantly shorter in the FAN anaerobic bottles for *E. coli* and anaerobic bacteria. The time to detection of microbial growth was significantly shorter in standard bottles for *S. aureus*, CNS, *Enterococcus faecalis*, other streptococci, and all microorganisms combined. For 40 blood cultures for which only one of the two bottles grew a pathogenic microorganism, a modified serum bactericidal test was performed on the blood-broth mixtures from both anaerobic bottles. The results

of these studies did not support the concept that the proprietary substance in FAN bottles, EcoSorb, improves microbial recovery by inactivating antimicrobial substances present in the blood. Similar results were noted in the comparison evaluation of the two aerobic bottles, although in that study the greatest increase in the recovery of microorganisms with the FAN bottles occurred for patients who were receiving antimicrobial agents with activity against the microorganism (194). Hence, it is not clear how FAN bottles improve microbial recovery. Possible explanations include the use of brain heart infusion broth in FAN bottles and the binding of natural inhibitory substances in blood by EcoSorb (194, 210).

Two comparisons of BacT/Alert with Isolator have been performed. Hellinger et al. (71) found that significantly more staphylococci and yeasts were recovered from Isolator whereas significantly more aerobic and facultatively anaerobic gram-negative bacilli were recovered from BacT/Alert. Lyon and Woods (99) compared the two systems for recovery of fungi only; Isolator recovered significantly more *Candida* spp., *Histoplasma capsulatum*, and *Malassezia furfur*.

Two comparisons of BacT/Alert with the BACTEC 9240 were published in 1995; the results of these comparisons are discussed below.

Additional, albeit uncontrolled, observations have been published regarding the performance characteristics of BacT/Alert. The system has been used to recover both *Brucella melitensis* (167) and another *Brucella* isolate (204a) from standard aerobic bottles. The time to detection of microbial growth, less than 3 days, was similar for both isolates. Carnahan et al. (29) recovered *Campylobacter upsaliensis* from a standard aerobic bottle after 4.8 days of incubation. Shawar et al. (161) recovered *Coccidioides immitis* from a standard aerobic bottle; the time to detection of microbial growth was 82 h. Tierno et al. (181) recovered *Bartonella henselae* from the blood of five patients. Bannister and Woods (11) used BacT/Alert to evaluate the utility of routine anaerobic blood cultures and found that no microorganism or group of microorganisms was recovered more often from anaerobic bottles than from aerobic bottles but only one anaerobic organism was isolated in the whole study. Last, Kennedy et al. (82) evaluated BacT/Alert; their observations confirmed those of other studies, particularly the observations that the number of bottles positive in one set is not a reliable indicator of the clinical importance of the isolate and that nearly all pathogenic microorganisms are recovered within the first 48 h of incubation.

(ii) **BACTEC 9000 Series blood culture systems.** The BACTEC 9240 and 9120 blood culture systems received 510k FDA clearance in 1992. Of the CMBCS, these systems most closely resemble the BacT/Alert 240 and 120 systems, respectively (Tables 3 to 5). Currently, Standard Aerobic/F and Anaerobic/F, Plus Aerobic/F and Plus Anaerobic/F, PEDS PLUS/F, and Lytic/10 anaerobic/F bottles are available for use in the United States.

The first evaluation (122) of the 9240 compared standard Aerobic/F and Anaerobic/F bottles with BACTEC NR6A and NR7A bottles processed on the NR 660 instrument. Standard Aerobic/F bottles recovered significantly more *S. aureus*, CNS, and all microorganisms combined. Standard Anaerobic/F bottles recovered significantly more CNS, members of the family *Enterobacteriaceae*, and all microorganisms combined. Significantly more septic episodes were detected by the BACTEC 9240 system. The time to detection of microbial growth was significantly shorter for the BACTEC 9240 system. The two systems generated approximately the same proportion of false-positive instrument signals. Contamination rates were also similar.

In 1995, Smith et al. (165) published the results of a comparison between BACTEC 9240 and BacT/Alert. In this comparison, BACTEC Plus/F (i.e., resin-containing) bottles were compared with the standard BacT/Alert bottles. Significantly more *S. aureus*, CNS, and all microorganisms combined were recovered from the BACTEC Plus/F bottles. Although Plus/F bottles were compared with standard ones, there were no other differences in recovery. This may be explained in part by the size of the study; only 369 total isolates were recovered. There were in fact trends favoring recovery in BACTEC for nonenteric gram-negative bacilli (6 versus 0) and yeasts (16 versus 9). The time to detection of microbial growth was similar for the two systems, with BACTEC detecting the growth of CNS and nonenteric gram-negative bacilli significantly earlier than BacT/Alert.

Pohlman et al. (131) also compared BACTEC with BacT/Alert. In this study, BACTEC Plus/F aerobic bottles were compared with BacT/Alert FAN aerobic bottles. Significantly more members of the family *Enterobacteriaceae* and *P. aeruginosa* combined were recovered from the FAN bottles. Septic episodes caused by *S. aureus* were detected earlier from the FAN bottles.

Also in 1995, Schwabe et al. (156) published the results of an evaluation of BACTEC Plus/F bottles, in this instance comparing them with NR Plus 26 bottles processed on NR 660 instruments. No significant differences in microbial recovery or detection of septic episodes were observed. The times to detection were much shorter with Plus/F bottles.

Chapin and Lauderdale (31) studied the issue of microbial detection in BACTEC and Difco ESP bottles whose entry into their respective systems was delayed. Overall, delayed entry of vials into either system resulted in increased times to detection of microbial growth.

In 1996, Rohner and colleagues (150) published the results of a comparison between BACTEC aerobic Plus/F bottles and Septi-Chek release bottles (a lytic formulation not available in the United States). Significantly more "other" gram-positive cocci (not staphylococci, *Streptococcus pneumoniae*, enterococci, or group A, B, or G streptococci), *E. coli*, other members of the *Enterobacteriaceae*, *Haemophilus* spp., gram-negative anaerobes, and all microorganisms combined were recovered from the Septi-Chek Release bottles. As would be expected, the time to detection of microbial growth was significantly shorter with BACTEC.

Shigei et al. published data suggesting a possible need for terminal subcultures (162). In this study, terminal subcultures were performed on Plus aerobic/F and Plus anaerobic/F bottles incubated and monitored on a 9240 system for 5 days. A total of 1,477 of 13,471 subcultures were positive; 1,388 (94%) of these positive subcultures were also detected by the instrument. Of the 89 isolates not detected by the instrument, 17 were recovered from aerobic bottles and 72 were recovered from anaerobic bottles, representing 86 blood culture sets. Most of the isolates recovered only by terminal subculture were pseudomonads, staphylococci, and yeasts. It is of interest that many *P. aeruginosa* and yeast isolates were recovered only from the anaerobic bottles by terminal subculture. Since these microorganisms are strictly aerobic and many of them had heavy growth, it is unclear why they were not also recovered from the companion aerobic bottles.

(iii) **ESP blood culture system.** ESP (Difco Laboratories) was the third continuous-monitoring system introduced. This system differs substantially from BacT/Alert and the BACTEC 9000 systems in several ways (Tables 3 to 5). First, the instrument design differs in that the bottles are placed in drawers that pull out rather than in internal, forward-facing blocks.

Second, the drawers designated for anaerobic bottles do not include an agitation mechanism. Third, rather than monitoring the concentration of CO₂ in the broth indirectly via changes in a sensor, microbial growth is detected by manometric monitoring of gas consumption and/or production. Last, the broth medium is not soybean-casein digest; for aerobic bottles it is soy-casein peptone broth, and for anaerobic bottles it is proteose-peptone broth.

In the first clinical evaluation of ESP, Morello et al. (114) compared ESP with the BACTEC NR660 blood culture system. ESP aerobic bottles recovered significantly more *Streptococcus pneumoniae* and all microorganisms combined, whereas ESP anaerobic bottles recovered significantly more aerobic gram-positive bacteria, *Candida* spp., and all microorganisms combined. Significantly more septic episodes caused by *Staphylococcus epidermidis*, anaerobic bacteria, and all microorganisms combined were detected by the ESP bottles. The results of this study are difficult to interpret, since (i) five different BACTEC bottles were used during the study; (ii) the volume of blood inoculated into individual bottles was not controlled (although the volume per set was); (iii) some bottles were inoculated directly while others were inoculated with blood collected in yellow-top Vacutainer tubes; and (iv) standard criteria for assessing the clinical importance of isolates were not used.

The next evaluation, performed by Zwadyk et al. (218), compared ESP 384 systems with BacT/Alert 240 systems. ESP aerobic bottles recovered significantly more *S. aureus* and all microorganisms combined. ESP anaerobic bottles recovered significantly more anaerobic bacteria and all microorganisms combined. Significantly more septic episodes caused by anaerobic bacteria were detected by the ESP anaerobic bottles. Conversely, significantly more septic episodes caused by streptococci were detected by the BacT/Alert anaerobic bottles. For aerobic bottles, the time to detection of microbial growth was significantly shorter in the ESP bottles for *S. aureus*, yeasts, and all microorganisms combined. In contrast, the BacT/Alert bottles detected the growth of streptococci more quickly. The time to detection of microbial growth was significantly shorter in BacT/Alert anaerobic bottles for *S. aureus*, gram-positive cocci, streptococci, and all microorganisms combined.

Kirkley et al. (86) compared ESP 80 A aerobic bottles with the Isolator blood culture system. Isolator recovered significantly more *S. aureus*, enterococci, *Alcaligenes xylosoxidans*, *E. coli*, *Stenotrophomonas maltophilia*, *Candida albicans*, *Torulopsis glabrata*, and all microorganisms combined. Similarly, significantly more septic episodes caused by *S. aureus*, *S. maltophilia*, *C. albicans*, and all microorganisms combined were detected by Isolator. When the same microorganism was recovered from both systems, it was recovered earlier from Isolator for both *S. aureus* and for *C. albicans*.

Kellogg et al. (80) compared ESP 80 A and 80 N bottles with Isolator and a manual thiol anaerobic bottle (Difco Laboratories). In the comparison of Isolator with 80 A, Isolator recovered significantly more staphylococci combined, members of the family *Enterobacteriaceae*, and all microorganisms combined. In the comparison of Isolator with 80 N, Isolator recovered significantly more CNS, all staphylococci combined, *E. coli*, *Klebsiella pneumoniae*, members of the *Enterobacteriaceae*, *P. aeruginosa*, and all microorganisms combined. Conversely, 80 N recovered significantly more *Bacteroides* spp., other anaerobic bacteria, and all anaerobic bacteria combined. In the comparison of the thiol bottles with 80 N, the thiol bottles recovered significantly more members of the *Enterobacteriaceae*. In a system-versus-system comparison, the manual system alone (combination of Isolator plus thiol) recovered sig-

nificantly more *S. aureus*, CNS, *Streptococcus pneumoniae*, members of the *Enterobacteriaceae*, and all microorganisms combined. Significantly more septic episodes caused by enterococci, members of the family *Enterobacteriaceae*, and all microorganisms combined were detected by the conventional system alone. When the same microorganism was recovered from both systems, the time to detection of microbial growth was not significantly different.

The next evaluation of ESP, by Welby et al. (201), is the only one that addresses the ability of this system to recover pathogenic microorganisms from the blood of pediatric patients. When compared with the Septi-Chek blood culture system, the relative recovery rates of the two systems were not significantly different. Significantly more isolates classified as contaminants were recovered from ESP bottles. The time required to detect microbial growth and to complete microbial identification was significantly shorter for ESP.

Cockerill et al. (36) compared ESP 80A bottles with Isolator and Septi-Chek. When ESP was compared with Septi-Chek, no significant differences in microbial recovery were found; the growth of nearly all microorganisms was detected significantly earlier in the ESP bottles. ESP detected significantly more septic episodes caused by CNS than did Isolator, however, significantly more isolates of *S. aureus*, *Candida* spp., and all microorganisms combined were recovered by Isolator; the growth of CNS, *E. coli*, and all microorganisms combined was detected earlier in ESP bottles. Significantly more septic episodes caused by *S. aureus* and *Candida* spp. were detected by Isolator; more episodes caused by CNS were detected by ESP.

Boschman et al. (22) also compared ESP with Isolator and the Difco thiol bottle. When aerobic ESP bottles were compared with Isolator, Isolator recovered significantly more *Micrococcus* spp.; when compared with thiol bottles, aerobic ESP bottles recovered significantly more staphylococci and all microorganisms combined. When anaerobic ESP bottles were compared with Isolator, ESP recovered significantly more *Streptococcus pneumoniae* but Isolator recovered significantly more *P. aeruginosa*.

As mentioned previously, delayed entry of inoculated ESP vials results in delayed detection of microbial growth (31).

(iv) Vital blood culture system. Vital (bioMérieux Vitek, Hazelwood, Mo.) was developed in France and is now marketed throughout much of Europe. Vital has not received FDA clearance.

Vital differs from other CMBCS in several ways (Tables 3 to 5). First, it uses a unique method for detecting microbial growth. A fluorescent liquid compound present in the broth medium is quenched by hydrogen ions, electrons, and free radicals produced by microbial growth in the broth medium. Consequently, the computer algorithm monitors decreases rather than increases in fluorescence. Second, Vital differs mechanically in that the bottle racks are placed in large drawers. Third, Vital bottles are agitated in a "sinusoidal" manner.

To date, only preliminary results from one controlled clinical evaluation, in which Vital was compared with BACTEC 6A and 7A bottles processed on NR 660 instruments, are available (141). In the aerobic-bottle comparison, BACTEC 6A recovered significantly more yeasts. When microorganisms were recovered from both bottles, microbial growth was detected significantly earlier with Vital bottles for facultative gram-positive cocci other than staphylococci, members of the family *Enterobacteriaceae*, and all microorganisms combined. In the anaerobic-bottle comparison, Vital recovered significantly more nonfermenters and all microorganisms combined. For microorganisms recovered from both bottles, microbial growth was detected significantly earlier with Vital bottles for *S. aureus*,

CNS, other gram-positive cocci, members of the family *Enterobacteriaceae*, and all microorganisms combined.

Marchandin et al. (104) have reported that, as with other CMBCS, Vital can be used with a 5-day rather than a 7-day testing cycle without decreasing the detection of microbial pathogens but with a decrease in the recovery of contaminants.

(v) **Oxoid Automated Septicaemia Investigation System.** In 1994, Unipath (Basingstoke, United Kingdom) introduced the Oxoid Automated Septicaemia Investigation System (o.a.s.i.s.), the most recently developed of the CMBCS (170). Conceptually, it is most similar to ESP, since detection of microbial growth is manometric. Unlike ESP, however, o.a.s.i.s. detects gas production and consumption by laser monitoring of the deflection of the rubber septum that seals each bottle. A prototype instrument has been evaluated in an in vitro study and in a limited clinical trial (170). In the former, o.a.s.i.s. was found to be equivalent or superior to the BACTEC 460 system (6B and 7D bottles) for the time to detection of microbial growth. In the latter, o.a.s.i.s. was compared to the Oxoid Signal blood culture system and was found to be comparable for microbial recovery and superior for speed of detection of microbial growth. Based on these results, the authors have recommended that full-scale clinical trials be performed (170).

APPROACH TO SPECIFIC ORGANISMS

It has become clear in the recent understanding of infectious diseases processes that a variety of organisms that cause systemic illnesses may be present in the bloodstream and not be detected unless special procedures are performed. The usual approach to blood cultures—to obtain a volume of blood and inoculate it into two or three culture medium bottles to look for aerobic and anaerobic bacteria—may no longer be optimal. Based on individual patient characteristics, other pathogens, including viruses, fungi, mycobacteria, mycoplasmas, and fastidious bacteria, are more likely to be present. If the laboratory were in a position to customize specimen collection, the most useful media could be inoculated for the most likely pathogens for the individual patient. In reality, it is unlikely that we will be able to make such choices. The following discussion, however, addresses issues related to the approaches to specific pathogen groups.

Anaerobic Bacteria

Anaerobic bacteria are present in vast numbers as members of the normal bacterial flora in a variety of nonsterile body locations. The oropharynx, gastrointestinal tract, female genital tract, and skin contain numbers of anaerobic organisms that dwarf the number of aerobic bacteria by factors of 100 to 1,000. Most infectious processes that involve anaerobic bacteria occur when the integrity of these normally colonized surfaces is interrupted. The likelihood of anaerobic infection is directly related to the location of infection in continuity with one of these sites (151).

Bacteremia with anaerobic bacteria was first well documented in the 1970s, when blood culture media were improved to make their isolation routine. Anaerobic organisms typically accounted for 10 to 15% of the organisms isolated and, in one study, represented 26% of the total (151, 213).

Most centers that have analyzed the frequency of isolation of anaerobes from blood cultures have noted a declining incidence over the past several years; at the same time, other organisms such as fungi, *Pseudomonas* species, and fastidious aerobic bacteria are increasingly associated with disease (44, 98, 120). This decline may or may not represent an actual

change in the incidence of anaerobic bacteremia: in one study, even though the proportion of positive cultures with anaerobes declined, the incidence of anaerobic bacteremia per hospital admission remained constant (120). The frequency of anaerobic bacteremia in many locations is now low enough that some have suggested that routine inoculation of anaerobic media should be replaced by a second aerobic bottle; an anaerobic bottle would be used only when appropriate for the clinical situation (120). The cost of media and processing may also favor the routine inoculation of an additional aerobic medium for organisms like fungi and *Pseudomonas* spp. (10, 11, 18, 66, 120, 179, 202). Two separate clinical reviews (115, 160) have also suggested that it is not important to recognize anaerobic bacteremia microbiologically. It was suggested that these patients can be identified clinically as having probable anaerobic infection and should be treated with antimicrobial agents directed toward them without culture confirmation, especially since some patients with anaerobic infections have negative blood cultures.

Not all hospitals, however, have seen a decrease in the incidence of anaerobic bacteremia, and these centers still need to routinely inoculate anaerobic media (79). Some investigators also believe that inoculation into anaerobic media improves the recovery of some facultative organisms like *Streptococcus pneumoniae*. Finally, in settings in which clinical data are difficult to obtain by the laboratory, the use of both an aerobic bottle and an anaerobic bottle is still the easiest approach to ensuring that an adequate volume of blood is being cultured.

The spectrum of anaerobic bacteria associated with bacteremia is relatively narrow, despite the wide variety of potential anaerobic pathogens that have been identified. The genus *Bacteroides* accounts for 45 to 75% of isolates, with the *B. fragilis* group making up roughly 75% of these. *Fusobacterium*, *Prevotella*, *Porphyromonas*, *Clostridium*, *Propionibacterium*, and *Peptostreptococcus* spp. account for many of the rest. Although common as members of the normal flora of many body sites, some anaerobic bacteria recovered from blood have particular associations with specific clinical syndromes. For example, *Bacteroides* spp. are most often associated with intra-abdominal processes, and *Peptostreptococcus* spp. are most often associated with obstetrical or gynecologic infections (151).

The approach to the laboratory diagnosis of anaerobic bacteremia does not require unique processes. As long as a standard anaerobic blood culture medium is inoculated, blood collection is carried out in standard fashion (142, 173). Although blood culture media with a low redox potential should be superior for the growth of anaerobic bacteria, clinical studies have not suggested that there is a significantly improved yield for any particular medium (151).

The atmosphere of incubation is a factor in the recovery of some anaerobic bacteria in some medium preparations. An unvented bottle was statistically superior to a vented bottle for recovery of *Bacteroides* spp. and peptococci but not *Clostridium* spp. in a comparative study with tryptic soy broth (190). Recovery of anaerobic bacteria without venting is generally superior in thioglycolate media (53). Since no study has shown that venting is superior for recovery of anaerobes, it would be prudent to inoculate an unvented bottle whenever an attempt to recover anaerobes is being made.

Prolonged incubation times are not needed for recovery of anaerobes. Traditional incubation with unvented bottles yields 95 to 98% of the anaerobes isolated within 5 to 7 days of incubation (117, 124). With continuously monitored instruments, studies have not been conducted on enough anaerobic isolates to determine the length of incubation needed. How-

ever, it is unlikely that a longer time would be needed than with conventionally monitored bottles.

Anaerobes can be detected in a variety of ways. Conventional blood culture inoculation with macroscopic examination of bottles for visible growth will suffice. Microscopic examination of negative broths has not been shown to add significant additional information (61). Likewise, routine subcultures of anaerobic bottles to anaerobic agar plates have not been helpful (118, 124). Biphasic systems with a combination of broth media and an agar slide have not performed well in the recovery of anaerobes in comparative studies with unvented standard bottles containing tryptic soy or supplemented peptone broth (63, 72, 198). The lysis-centrifugation system with subculture of part of the sample to anaerobically incubated agar plates has performed variably in comparison to other systems or a variety of conventional broths. In recent studies, the number of anaerobes identified has been too small to make statistical comparisons possible. At best, it would appear that the detection of anaerobes by the lysis-centrifugation system is similar to that with conventional or automated systems (151). With 7D medium, the radiometric BACTEC system detected anaerobes at a rate similar to that with conventional media (173). The newer nonradiometric 7D medium performs similarly to radiometric 7D medium, as does the resin-containing BACTEC 27 medium (76, 103, 206). Too few anaerobic isolates have been recovered during clinical evaluations of CMBCS to adequately assess the capability of these instruments to detect anaerobes.

Fungi

With the increasing number of patients with complex clinical conditions and undergoing complex medical therapy, more unusual infectious disease processes have emerged. Fungal pathogens as agents of a variety of infections, including fungemia, are part of this trend. Fungi are now found much more frequently as the cause of both community- and hospital-acquired infections, and efforts to culture them have become increasingly sophisticated. Major predisposing factors leading to these infections include immunosuppression, use of broad-spectrum antibiotics, use of central venous catheters especially in association with the administration of hyperalimentation solutions, and more aggressive attempts to prolong the survival of patients with complicated, serious disease (125, 202). Recent data suggest that fungemias are more prevalent than anaerobic bacteremias and therefore support the routine processing of blood cultures for fungi rather than anaerobes (120).

As the culture methodology has improved, more fungal pathogens are now detected. Whereas in the past the organisms associated with invasive human infection included *Histoplasma capsulatum*, *Coccidioides immitis*, *Candida albicans*, *Cryptococcus neoformans*, *Aspergillus* spp., *Blastomyces dermatitidis*, and rarely others, there are now frequent reports of illnesses associated with a much longer list of saprophytic moulds and yeasts. Among these are dematiaceous fungi, multiple species of *Candida*, *Trichosporon beigeli*, *Fusarium* spp., *Malessezia* spp., and zygomycetes (54, 58, 125). Both because of the serious illnesses in patients who acquire these organisms and the possible resistance of the pathogens to antifungal agents, patients with systemic fungal infections often have poor clinical outcomes.

The laboratory approach to the recovery of fungi from blood cultures has evolved significantly over the past 20 years, from a time when biphasic media prepared in-house were used to the present blood culture medium formulations and processing methods, which have improved the yield of fungi and the ease

of work needed for their recovery (54). Although several variables have been evaluated in determining the adequacy of recovery of bacterial pathogens from blood cultures, the number of fungal isolates in such studies has generally been too few to determine whether the same variables are significant for fungi. There is no reason to suspect that factors critical to the detection of bacteria, such as the volume of blood cultured and ratio of blood to broth, would not also be important in the recovery of fungi. Several studies have shown that venting and aerobic incubation of conventional broth medium bottles improve the recovery of fungi (74, 146). The optimal temperature for the growth of fungi varies from one organism to another. Generally, filamentous fungi grow better at 27 to 30°C, whereas yeasts may do better at 37°C. The duration of incubation necessary for recovery is also variable, since most yeasts grow in 2 to 3 days whereas the dimorphic fungi may take as long as 3 to 6 weeks (54, 174). Unfortunately, even with the best methods available today, it is still common for patients to have severe, life-threatening disseminated fungal infections with negative blood cultures (54).

A variety of media are used for the growth of fungi from blood cultures. The relative utility of these media for recovery of fungi has not been studied in detail. Tryptic soy, Columbia, thioglycolate, and BHI broths have all been successful for growing yeasts and molds from blood. Biphasic media have traditionally been considered ideal for the growth of fungi; a commercially available biphasic system, Septi-Chek, has shown good performance in the recovery of these organisms. Septi-Chek is marketed with a variety of medium formulations, and BHI broth appears to be the best for the recovery of yeasts (119). Since molds are usually grown on solid fungal media such as inhibitory mold agar, BHI agar, or Sabouraud's dextrose agar, a system that incorporates one of these media should perform best in the recovery of molds from blood as well. The lysis-centrifugation system takes advantage of this approach because the sediment from the lysis tube can be inoculated directly onto any medium. In multiple evaluations, the lysis-centrifugation system with inoculation to a solid fungal medium has performed best in the recovery of dimorphic fungi and other molds and, in some studies, in the recovery of *Cryptococcus neoformans* (20, 21, 43, 60, 81). In automated systems with newer medium formulations, particularly the resin-containing, nonradiometric media for the BACTEC 660 instrument, *Candida* spp. have been recovered as well as in the lysis-centrifugation system, but the recovery of *Cryptococcus neoformans* and dimorphic moulds is less satisfactory than by lysis-centrifugation (205).

The importance of lysis-centrifugation should not be overstated. The majority of fungal isolates from blood cultures are *Candida* spp. Recovery rates for this genus for Septi-Chek, a number of broth formulations used in the BACTEC automated systems, and some conventional broths are comparable to those for the lysis-centrifugation system. In areas where *Histoplasma capsulatum* and *Coccidioides immitis* are endemic and for patients with severe immunocompromising conditions such as advanced human immunodeficiency virus (HIV) infection, the use of lysis-centrifugation is more important than in other circumstances (174).

A recently recognized organism in bloodstream infections, *Malessezia furfur*, is known to require lipids for growth. Approaches to the recovery of this organism have included supplementation of blood culture media with lipids for primary isolation or overlaying of solid media with olive oil when plating from the lysis-centrifugation tube or for performing subcultures. Anecdotal data suggest that this supplementation may not be necessary in all cases (54).

Finally, an important consideration in processing specimens for the recovery of fungi is the possibility of environmental contamination. *Aspergillus* spp., *Penicillium* spp., and others are ubiquitous in the environment but can also cause infections in compromised hosts. Precautions to prevent contamination are especially important with the lysis-centrifugation system, since open agar plates are used. It is recommended that processing of samples, inoculation of agar media, and observation of culture plates after inoculation be carried out in a biological safety cabinet (21, 81, 119). With any system, careful clinical evaluation of patients with positive blood cultures is necessary to determine the relevance of the isolates recovered. Multiple positive samples, a focal process typical of fungal infection, and a host likely to have a fungal infection are helpful considerations suggesting the importance of the positive sample. When positive fungal cultures are found, laboratory supervisors and clinicians should also be vigilant for positive cultures from a variety of patients who do not have evidence of disease, a finding that suggests a significant problem with laboratory procedures.

Mycobacteria

Until the mid-1980s, tuberculosis in the United States declined annually, leading to the optimistic presumption that this infection would soon be primarily of historical importance. Since that time, there has been a resurgence of infection due to *Mycobacterium tuberculosis*. At the same time, increasing numbers of patients with HIV infection are being encountered, who not only have a high risk for symptomatic tuberculosis but also have a high rate of disseminated infection due to mycobacteria other than *M. tuberculosis*. Predominant among these other mycobacteria is the *M. avium* complex (MAC).

As many as 42% of HIV-positive patients with tuberculosis have positive blood cultures (113). The true incidence of *M. tuberculosis* bacteremia is, however, poorly described since blood cultures for mycobacteria are obtained routinely from HIV-infected patients with less than 200 CD4⁺ lymphocytes per μ l (200) but rarely from HIV-positive patients with less advanced disease or from HIV-negative individuals. Bouza et al. described 50 patients with positive mycobacterial blood cultures, 81% of whom were HIV positive (24). A second study of 199 HIV-infected patients and 158 uninfected patients, all with extrapulmonary tuberculosis, identified mycobacteria in the blood of 28 HIV-infected and no uninfected patients. No denominator was provided for the total number of patients in each category who had blood cultures performed (159). Additional descriptive reports of six (13), nine (12), and nine (35) patients with *M. tuberculosis* bacteremia during HIV infection have been published. Again, these papers do not delineate the frequency with which bacteremia is seen when patients have disseminated disease.

In contrast, much more extensive information is available about the frequency of bacteremia in patients with MAC infection. Occasional patients with a variety of immunosuppressive conditions including leukemias, severe combined immunodeficiency syndrome, steroid therapy, cytotoxic chemotherapy, multiple myeloma, other nonhematologic malignancies, diabetes mellitus, anthrasicosis, asthma, and other infections appear to be at increased risk for MAC bacteremia (68, 73, 84). Despite these possible associations, the vast majority of patients with disseminated MAC infection and positive blood cultures have advanced HIV infection. Overall, 51 to 96% of patients with AIDS are infected with MAC (70, 113, 121, 216). Although not all patients who prove to have MAC infection at other sites or at autopsy have had positive blood cultures, the

majority do. Moreover, the level of bacteremia that has been identified has been very high, with colony counts typically in the range of 1,000 to 17,000 CFU/ml (84).

Whereas the majority of patients with mycobacteremia have either *M. tuberculosis* or MAC infection, occasional patients infected with *M. fortuitum* (127, 135), *M. chelonae* (50, 127, 135), and a newly described fastidious organism named *M. genavense* (23, 75, 185) have also been described.

Laboratory methods for the detection of mycobacteremia include microscopy, culture, and nonculture detection of mycobacterial products. Given the large number of organisms that are often present in the blood of patients with bacteremia, direct blood smears of buffy coat preparations have been performed as an early detection method. Positive smears were found in 13 of 14 patients with MAC infection in one study (47) and 6 of 17 patients in another (123). It does not appear, however, that the sensitivity of direct smears would be as high for infections with *M. tuberculosis* (47).

Attempts to isolate MAC from the blood of patients with AIDS began early in the HIV epidemic, when it was recognized that many patients had overwhelming numbers of mycobacteria in tissue at autopsy. Since then, blood has been collected in ordinary blood collection tubes, sterile water, and lysis-centrifugation tubes and has been inoculated into a variety of blood culture and mycobacterial media with or without special processing. The first methods for recovery included collection of 5- to 10-ml blood samples and inoculation of media including BHI, tryptic soy, brucella, and Proskauer Beck broths (100, 127). Special media supplemented for better growth of mycobacteria were formulated. These included tryptic soy agar with dextrose brain broth (127), Middlebrook 7H11 agar with BHI broth (17), and tryptic soy or Columbia broth supplemented with OADC mycobacterial enrichment (16). The BACTEC 12A mycobacterial broth has also been used for inoculation of the sediment processed by various collection methods (100, 127). Positive culture results were obtained with all of these methods, but the number of potential patients missed could not be determined. Since mycobacteria are intracellular organisms, lysis of cells prior to inoculation of media was soon incorporated into laboratory protocols. The lysis-centrifugation system soon became the most common method used, and it improved recovery in all the above media (56, 57, 84, 100, 127). Use of the Isolator system, however, required additional laboratory steps that are not otherwise necessary in inoculation of blood cultures. The BACTEC 13A radiometric medium introduced later by Becton Dickinson can be inoculated directly with 5 ml of blood. This medium lyses cells without the additional steps required for use of the Isolator (83, 152, 172, 214).

Currently, three methods are in wide use: inoculation of Middlebrook 7H11 and Lowenstein-Jensen agar with concentrated blood; inoculation of BACTEC 12B medium with lysed and concentrated blood; and direct inoculation of BACTEC 13A medium with unconcentrated blood. The simplest concentration procedure for the first two methods is to use the Isolator system. Alternatively, 5 ml of blood can be mixed with 30 ml of 0.3% sodium deoxycholate solution, incubated for 10 min at room temperature, centrifuged for 25 min at 3,500 \times g in a refrigerated centrifuge, suspended in 0.2% bovine serum albumin, and inoculated into the final medium (152).

Comparisons of these three methods have shown similar rates of recovery, with overall isolation rates of 89 to 96% (83, 152, 172). Times to positivity have varied much more. Growth may be detected as early as 3 days (152) or as late as 24 days (172) after inoculation. The most important variable in recovery appears to be the concentration of organisms in the blood.

Delay in the processing of samples inoculated into the Isolator system for up to 7 days does not appear to affect overall recovery (51).

While the methods described above are adequate for the recovery of MAC, *M. tuberculosis*, and *M. fortuitum* complex, special care is necessary for recognition of *M. genavense*. This organism grows slowly in BACTEC 12B and 13A media, with very slow rises in growth index, and does not grow at all on standard mycobacterial agars (23, 75, 185). Such slow rises in the growth index should prompt an examination of the blood-broth mixture with an acid-fast stain. If mycobacteria are present, subcultures should be performed and the isolates should be identified. The best medium to use for such subcultures has not yet been identified.

Standard methods for identification can be performed with colonies grown on solid media as part of the primary isolation procedure or with subcultures of organisms grown in broth media. Identification methods by using DNA probes, gas-liquid chromatography, and gene amplification by PCR directly on organisms in BACTEC broths are under development.

Blood cultures are a simple, relatively noninvasive method for the detection of disseminated infection caused by mycobacteria. Unfortunately, not all patients with disseminated infection have detectable bacteremia (3). Alternative methods include examination of specimens obtained by bone marrow aspirations and biopsies, liver biopsies, and tissue biopsies at other suspected sites of involvement. Examination of these specimens should include histologic staining for acid-fast organisms and granulomas as well as cultures for mycobacteria. Limited data comparing all the methods are available. In one study of eight patients with disseminated disease, six had positive blood cultures, seven had positive bone marrow studies (five by culture, four by visualization of acid-fast organisms or granulomas), and seven had positive liver biopsy studies (six positive cultures, six acid-fast organisms and granulomas seen) (134). One patient with negative blood cultures had positive liver biopsy histologic and culture results, and one had a positive bone marrow culture. The need for these additional, more invasive studies is not yet clear. With the time required for growth to occur in blood cultures, the suggestion that many patients with advanced HIV infection have disseminated MAC or *M. tuberculosis*, and the increasing availability of antimicrobial agents to treat these infections, consideration of invasive procedures will continue to be necessary.

Rare and Fastidious Organisms

Unusual organisms not typically considered to cause bacteremia are occasionally recovered from blood cultures or may be considered likely causes of infection that, if the methods used were adequate, should be recoverable from blood. This is usually true in the setting of culture-negative endocarditis, but it is also true for patients with fever of unknown origin or other focal infections when the illness is severe enough to suggest sepsis but in which the pathogens do not grow from blood cultures processed in the usual manner.

Whereas there are anecdotal reports of recovery of a variety of organisms from such patients, there are insufficient data to establish the relative merits of various approaches to isolation. In the large comparative trials of media and instruments for the recovery of organisms from blood cultures, these organisms too seldom are recovered in sufficient numbers to make statistical comparisons of various techniques possible. Therefore, meaningful conclusions about the best approach to detection, including the most appropriate media, medium supplements, best instruments, and ideal incubation conditions, are not pos-

sible. Any attempt to improve the methods for recovery of an unusual organism must always be undertaken in the context of the clinical situation and the likelihood that other, more common organisms may be present. Any alteration in conventional procedures with the intent of improving the detection of a fastidious organism should not significantly diminish the ability to detect the more typical organisms that one is likely to encounter. One can always use standard blood culture procedures with alterations that would not affect the recovery of common organisms. Prolonging the period of incubation, blind subculturing to media designed for recovery of the fastidious organism under consideration, or using additional media supplemented as recommended by the manufacturer guidelines are acceptable solutions. Other procedures that do alter the medium or instrument operating procedures should not be undertaken if inhibition of more common organisms is possible.

***Brucella* spp.** Brucellosis is an uncommon clinical illness in the United States although still quite common in other parts of the world. Since brucellosis usually presents as a febrile illness, and, when looked for, the organisms are usually present in blood, the need for adequate blood culture procedures is important (6, 111, 177). The usual perception is that *Brucella* spp. are quite fastidious organisms requiring special media and prolonged incubation for growth; available data suggest that this may not be so.

A number of anecdotal reports have been published for a variety of blood culture systems. Cultures of blood obtained from six patients and processed radiometrically in the BACTEC 460 instrument demonstrated bacteremia in all six patients (6). Of 19 cultures, 15 (79%) had positive growth indexes in 4 to 8 days. Not reported were the results by bottle type or the results of blind subcultures that were performed after 2 weeks of incubation. In the nonradiometric BACTEC system, 6 of 16 patients with brucellosis had positive NR6A blood culture bottles, although the time to detection and method of detection (instrument versus blind subculture) were not reported (2). Another report of a single patient showed that all five NR6A and four of five NR8A bottles were positive, although blind subcultures were positive several days before positive growth indices occurred and detection took 7 to 20 days (217). The anaerobic bottles in these studies remained negative. A comparison of BACTEC media with an unidentified biphasic medium showed that 28 isolates were recovered in the biphasic medium and 12 were recovered in the BACTEC and that 11 of the 12 BACTEC isolates required terminal subcultures for detection—growth indices were not a reliable indication of microbial growth (148). As mentioned above, brucellae have been recovered from BacT/Alert bottles. Lysis-centrifugation has recovered brucellae better than a Castaneda method (14 and 0 isolates, respectively) (48) and compares favorably to the radiometric BACTEC method (19 and 15 isolates, respectively) with shorter detection times (3.5 and 14 days, respectively) (89). In this comparison, as long as 30 days was required for detection of organisms in BACTEC media. An unidentified medium used in a manual system recovered brucellae from 11 of 15 blood cultures obtained, but 20 to 51 days was required for detection (177).

While it would appear that the majority of patients with brucellosis have positive blood cultures, the time to detection is quite variable and detection may require blind subculture even with the new automated systems.

***Campylobacter* spp.** *Campylobacter* spp. have been isolated on occasion from a variety of blood culture media. *C. upsaliensis* grew in aerobic but not anaerobic BACTEC radiometric media in 4 to 7 days, and all the samples had positive growth

indices (93). *C. cinaedi* was isolated similarly from one patient, only in aerobic BACTEC media, and by positive growth index at 6 days (34). Recovery of 11 isolates was compared in three systems; 7 were detected by lysis-centrifugation, 9 were detected by aerobic Septi-Chek, and 2 were detected in anaerobic soybean-casein digest broth, all within 4 days (78). In conventional broth media, campylobacters have been recovered but generally only by blind subculture (166, 186). Seeded blood culture studies with *C. jejuni* and *C. fetus* have shown that aerobic and anaerobic BACTEC media, and Septi-Chek broth and paddles support growth, with detection in 2 to 5 days. Septi-Chek was the faster method, and *C. fetus* grew more quickly than *C. jejuni* (188). In contrast, all blood culture systems seeded with *Helicobacter pylori* have failed to recover the organism (203).

No conclusions are possible about the relative sensitivity of various methods, or even the frequency of bacteremia with these organisms, from clinical cases. Subcultures to appropriate supplemented media are probably important.

HACEK group bacteria. The popular mnemonic for *Haemophilus aphrophilus*, *Actinobacillus actinomycetemcomitans*, *Cardiobacterium hominis*, *Eikenella corrodens*, and *Kingella kingae* is used to denote a list of fastidious organisms associated with endocarditis. Although there is a perception that these organisms are difficult to grow, there are essentially no comparative data to define ideal approaches to diagnosis. Factors considered important in recovery include prolonged incubation for up to 14 days, blind subcultures in 2 to 7 days, and terminal subcultures that include a chocolate agar plate incubated in 5 to 10% CO₂. Individual case reports have been published that support each of these methods, but reports that these measures are not required have also been published (207).

Legionella spp. There have not been many documented cases of *Legionella* bacteremia. Single isolations have been reported by using biphasic blood culture media and supplemented proteose peptone broth (45, 101, 105, 106). The BACTEC 460 instrument recovered isolates variably: in one study, aerobic 6B bottles were positive by growth index and subculture whereas the anaerobic 7D bottles were negative (32); in another study, the 7D bottles were positive by blind subculture whereas the aerobic bottles were negative (145). In this prospective study of 16 patients with Legionnaires' disease, 6 had positive BACTEC blood cultures, all determined by blind subculture of the anaerobic bottles (145). Since that report, additional positive blood cultures have been reported from four patients with endocarditis by performing subcultures of BACTEC bottles; none of these were detected by growth index (182). From these case reports, it appears that blood culture media are capable of supporting the growth of *Legionella* spp. but that recognition may require subculture to supplemented media specifically designed for the growth of members of this genus.

Mycoplasma spp. *Mycoplasma* spp. are associated with a variety of illnesses, most importantly respiratory disease caused by *Mycoplasma pneumoniae* and genital tract infections caused by *Mycoplasma hominis* and *Ureaplasma urealyticum*. Isolation of *M. hominis* in blood from women with fever postpartum or following gynecologic procedures has been described in several reports, but isolation of mycoplasmas from blood in other situations has been rare (207). There have not been large enough numbers of isolates to compare among culture methods.

The BACTEC 460 system has been the system most often used in reports of recovery of mycoplasmas. In most of these cases, there were positive growth indices but specific subcultures were necessary for detection (42, 164). Anaerobic me-

dium was more supportive of growth in one study (164). Growth has also been detected by nonradiometric BACTEC media and by the Oxoid Signal (133), but these organisms have not yet been found in the new continuous-monitoring systems. A possible caveat is recognition that mycoplasmas are inhibited by SPS, which, in turn, may be counteracted by the addition of gelatin to media (30, 39, 40, 133). In clinical comparisons of media with and without SPS and gelatin, mycoplasmas have not been recovered frequently enough to determine the relevance of either inhibition by SPS or its reversal with gelatin.

The clinical relevance of searching for or finding mycoplasmas in blood is not clear. Prolonged fever postpartum has been clearly associated with positive blood cultures (52, 92, 107, 130). The use of surveillance cultures suggests, however, that mycoplasmas may commonly cause transient asymptomatic bacteremia in as many as 8% of postpartum women (107). The frequency of isolation specifically in women with postpartum fever is not characterized. Complications from such bacteremias, especially in women with fever who do not receive antimicrobial therapy that would be effective against these organisms, has not been recognized as an important problem. Such studies would be helpful. Similarly, respiratory disease caused by mycoplasmas has not been associated with bacteremia, but there have not been surveillance studies to document the presence or absence of mycoplasmas from blood.

Patients who have suspected mycoplasma pneumonia and women with fever following gynecologic procedures or vaginal delivery may have mycoplasmaemia. Suspicion should be aroused in the laboratory when instruments signal a positive culture but routine subcultures and Gram stains are negative. In such situations, cultures should be held for more than 7 days and the use of subcultures to media supportive of mycoplasmas might be prudent (163, 164, 184, 187).

Nutritionally variant streptococci. Nutritionally variant streptococci include a number of species of viridans group streptococci that require pyridoxal for growth. Originally described as organisms that grow in blood culture media with production of visible changes in the broth and positive Gram stains but with negative subcultures on agar media, nutritionally variant streptococci continue to be seen occasionally as causes of bacteremia, endocarditis, and other focal infections. Since isolation of these organisms is unusual, no comparative study of alternative blood culture methods with clinical samples has been done. Of older broth media that were tested with either small numbers of patient samples or in laboratory inoculation studies, BHI broth was better than thioglycolate, which in turn was better than tryptic soy broth (147); none of these were supplemented with pyridoxal. Todd-Hewitt broth supplemented with 0.001% pyridoxal has also supported growth (37) but has not been compared with BHI broth. Case reports have described recovery by BACTEC 7D medium but not other media used in automated systems. Nutritionally variant streptococci should be considered whenever broth-based blood cultures appear positive with gram-positive cocci but usual subcultures are negative. When that occurs, subculture to blood agar supplemented with 0.001% pyridoxal or demonstration of satelliting on a blood agar plate around a paper disk saturated with 0.001% pyridoxal or along a staphylococcal streak should identify this group of organisms (28, 138, 139).

RAPID METHODS FOR IDENTIFICATION OF ORGANISMS IN BLOOD CULTURES

With the current availability of continuously monitored blood culture instruments, the time to detection of positive

blood cultures has decreased. Yet the time to finalization of results still requires traditional identification and susceptibility testing. There is therefore an incentive to perform rapid tests to complete this latter phase of the laboratory exercise as well (33). While it seems logical that decreasing the time to final identification is clinically relevant, there is little information to actually document that this is true (33).

Biochemical Tests

The first approach to improving the speed to final diagnosis following initial detection was to perform biochemical tests from blood culture broth either with or without centrifugation. An early attempt in the 1970s involved a battery of 14 biochemical tests including catalase, coagulase, bile solubility, bile-esculin, oxidase, lecithinase production, lactose fermentation, indole, phenylalanine deaminase, lysine decarboxylase, urease, DNase, hydrogen sulfide, and β -galactosidase. Of 114 broths tested, organisms from 112 were correctly identified including a variety of species of bacteria common then (192).

Several studies focusing on the identification of *S. aureus* followed. The susceptibility of *S. aureus* to lysostaphin was reported to be an excellent way to distinguish this organism from CNS (157, 158). Subsequently, testing with thermonuclease, a heat-stable DNase produced by *S. aureus*, was reported to be a rapid, accurate identification method (102, 137). The test could be performed from either standard blood culture broths or BACTEC bottles with a positive growth index (144). Variation in performance of thermonuclease testing was reported in the literature, however, suggesting that performance was broth medium dependent and that the products from different manufacturers were not equivalent (49). SPS was also determined to compromise the ability for identification and became a factor when small volumes of blood were inoculated into bottles (109). Finally, rapid coagulase tests directly from bottles have been reported in limited studies to have excellent specificity but questionable sensitivity (41).

Identification of *S. pneumoniae* directly from seeded blood culture bottles and small numbers of clinical blood culture broths has been tried with a modified bile solubility test (88, 116). Systematic studies on clinical specimens have not been performed. Pyrazinamidase (PYR) testing of streptococci for identification as either *Streptococcus pyogenes* or *Enterococcus* sp. has been successful for small numbers of isolates by using commercial PYR tests (59, 136).

Gram-positive cocci have also been identified in blood culture bottles by using commercial automated identification instruments. The Vitek system (bioMérieux Vitek) has been tested with some success for identifying a variety of streptococci and staphylococci. Unfortunately, the performance in one study was not ideal for *S. aureus* (60% correct) and *Streptococcus pneumoniae* (42% correct) (64).

Biochemical tests, identification kits, and automated instruments have all been studied as means of directly identifying gram-negative bacilli from blood culture broths. The results have generally been favorable, with a few caveats. Some examples of correct identifications are as follows: API 20E strips (BioMérieux Vitek), 95% correct (19); MicroID System (General Diagnostics, Morris Plains, N.J.), 96.6% correct (but none of 15 nonenterobacterial isolates) (4); and Vitek, 92.6% correct (155). All of these evaluations included a mixture of actual and seeded blood culture broths.

Immunologic Tests

A number of evaluations have been conducted with latex or coagglutination products for specific identification of beta-he-

molytic streptococci, *Streptococcus pneumoniae*, and *S. aureus*. Sensitivities, specificities, and predictive values for the streptococcal products have generally been high, but for staphylococci, the sensitivities in particular have been low (33). As with the biochemical tests described above, studies have included a mixture of actual and seeded blood cultures. The relevance of these findings to clinical practice is unclear.

Nucleic Acid Methods

Considerable research on the use of nucleic acid probes with and without amplification for the identification of infectious agents is in progress. None of these is yet available for routine use for the detection of bacteria and fungi in blood culture bottles or directly from blood, but they hold promise as alternative rapid methods for the future (215).

CONCLUSION

Numerous studies have been conducted to better define the clinical relevance and interpretation of positive blood cultures. The techniques involved in the detection of microorganisms in the bloodstream have steadily improved with the introduction of continuous-monitoring systems. None of these systems is perfect, but they all contribute to better, earlier recognition of infectious episodes. Finally, recognition that individual patient characteristics determine the list of likely pathogens can and should be used to improve the utilization of blood culture resources.

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