Current approaches to the diagnosis of bacterial and fungal bloodstream infections in the intensive care unit

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Healthcare systems spend considerable resources collecting and processing blood cultures for the detection of blood stream pathogens. The process is initiated with the collection of blood cultures that depend upon proper skin disinfection, collection of an adequate number of specimens and volume of blood, and prompt processing in a sensitive culture system. Complementing blood cultures and gaining in use are techniques such as nucleic acid amplification tests and mass spectroscopy that allow clinical laboratories to detect and identify organisms from blood cultures substantially faster than conventional systems. Furthermore, certain resistance mutations can be detected within hours of organism detection, thus providing valuable guidance to clinicians who strive to initiate the appropriate antimicrobial therapy as rapidly as possible, and who wish to discontinue unnecessary drugs expeditiously. Molecular and mass spectroscopy techniques are changing sepsis diagnosis rapidly and will provide far more specific information far more quickly, but the performance characteristics of these systems must be understood by intensivists who use such information to guide their patient management. (Crit Care Med 2012; 00:0–0)

Key Words: bacteremia; blood culture; fungemia; matrix-assisted laser desorption/ionization; molecular; sepsis

Prompt, accurate detection and identification of bloodstream pathogens are essential for optimal management of intensive care unit patients with sepsis syndromes. Healthcare facilities spend considerable resources in terms of labor and equipment drawing, processing and analyzing blood cultures. Missed opportunities to document the true cause of blood stream infections can adversely affect patient outcome if the true causative organism is not identified and treated with an active antibiotic. Conversely, the identification of organisms in blood cultures that are contaminants can lead to the unnecessary administration of antimicrobial agents that will increase cost, increase toxicity, and distract the clinical team from treating the true causative agent. Thus, techniques that are sensitive, specific, and rapid for identifying the microbial cause of sepsis are major operational tools for all critical care units, and intensivists must recognize that technology is revolutionizing the tools that are being used.

For the past 30 yrs, healthcare systems have relied on techniques in which blood is incubated in various media, semiautomated instruments are used to monitor microbial growth, and organisms are identified by Gram stain and biochemical tests. In recent years, clinical laboratories have begun to move to novel approaches: nucleic acid amplification tests and mass spectrometry are two of the most common approaches that have been introduced into clinical laboratories for routine detection and identification of organisms, and molecular tests are being used increasingly to rapidly identify microbial sequences that confer drug resistance. Clinicians must understand the sensitivities and specificities of results derived from both conventional diagnostics and newer molecular approaches. The implications of using these technologies for the assessment of septic patients will be evaluated in this review.

Evolution of Conventional Blood Culture

Historically, blood cultures were performed by inoculating a large volume of blood into one or more bottles of a nutrient medium after which the bottles were examined visually each day for evidence of microbial growth (e.g., visualization of discrete colonies, turbidity, gas production). Instruments were introduced in the early 1970s that could automatically monitor the bottles for microbial growth (e.g., production of carbon dioxide) and alert staff when growth was detected. Subsequent refinements in both culture media and detection systems have improved the overall recovery and time to detection of organisms in septic patients while reducing lab-related contamination of cultures.

The value of blood cultures for confirming the clinical diagnosis of sepsis, severe sepsis, and septic shock (i.e., disseminated infection from a localized focus such as meninges, lungs, abdomen, urinary tract, or from febrile neutropenia) is suboptimal. Although most untreated patients with bacterial meningitis have positive blood cultures, only 30% of patients with bacterial pneumonia and intra-abdominal infections have positive cultures, and positive blood cultures in patients with urologic disease are primarily restricted to those with acute pyelonephritis. Only 5% to 15% of the all cultures drawn for any reason, and only 50% of patients with septic shock, are positive. Whether the low rate for positive blood cultures is related to the sensitivity of the diagnostic techniques or the biology of the infectious process is unclear.

Contaminants represent 15% to 30% of the isolated organisms in some hospitals. Overall, the success of recovering pathogens and eliminating contaminants is directly related to the techniques used

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to collect and process blood cultures, and the patient population being evaluated.

Blood Culture Contaminants. Careful attention to the blood drawing and bottle inoculation techniques are important to maximize culture specificity for the true causative organism. This begins with careful disinfection of the phlebotomy site (1, 2) with 70% ethanol followed by application of chlorhexidine (30 secs) or tincture of iodine (1 min). Betadine preparations are not recommended because they must be applied for 1.5–2 mins to be effective, and clinical staff are unlikely to wait for this long (3). Catheter access sites as well as the rubber diaphragm on blood culture bottles should also be disinfected with 70% ethanol. The contamination rate should not exceed 2% to 3% of blood culture sets (a set consists of two to three bottles inoculated with a single blood collection). Focused training for those drawing such cultures is likely to result in substantial savings in terms of reducing the prevalence and consequences of contaminated cultures.

It is no longer recommended that the needle inserted at the phlebotomy site be replaced with a second needle before the blood is transferred to the blood culture bottles. Although there is a small decrease in contaminated cultures with such needle exchange (4), this benefit does not outweigh the risk to the phlebotomist of needle-associated injury (e.g., transmission of human immunodeficiency virus, hepatitis B virus, hepatitis C virus, or rarely other pathogens), should there be a mishap in which the phlebotomist sustains a sharp injury while changing needles.

Current blood culture instruments are "closed" systems that detect microbial growth with external monitoring devices. This means that virtually all contaminants in blood cultures originate from the skin or intravenous catheter surface when the blood is collected and inoculated into bottles. Most contaminants are coagulase-negative *Staphylococci*, *Bacillus*, *Corynebacterium*, or *Propionibacterium*. When these organisms are identified by the laboratory, clinicians should be suspicious that they represent contaminants, especially if they take >48–72 hrs to grow (suggesting they were present in small numbers) and are present in only one bottle or one set of bottles. However, these organisms can occasionally be true pathogens, especially in patients with implanted hardware (prosthetic valves, implanted cardiac devices, or mechanical joints). When these pathogens grow in multiple bottles or in multiple blood culture sets, they need to be considered seriously as true pathogens, especially if they are detected in <24–48 hrs (time of blood draw to time of laboratory detection).

Cultures Drawn From Catheters. Collection of blood cultures through intravascular devices was traditionally discouraged because the prevalence of contaminated cultures is slightly higher compared with venipuncture. However, as more and more patients have indwelling intravascular devices, clinicians have recognized that blood draws through catheters can increase the likelihood of identifying the cause of sepsis because the catheter is often the infected nidus, and because patient phlebotomy can be difficult and painful when patients are in intensive care units for many days, have coagulation disorders, and are subjected to many intravascular accesses. In fact, the likelihood of obtaining contaminants by drawing blood through intravascular catheters is only slightly higher than the risk associated with venipuncture (5, 6).

An important concept for interpreting the source of bacteremia and fungemia is the recognition that the "time to positivity" (time from when the blood culture was drawn until the positive result was detected by the automated system) is meaningful. If a culture drawn through one lumen is positive at least 90–120 mins before a culture drawn from another site (percutaneously or another catheter or lumen), the results suggest that the lumen with the earlier report of positivity has a higher concentration of organisms and is the source of the infection (7).

Recent studies (8, 9) documented the need to culture all lumens in multilumen catheters to avoid missing a significant number of catheter-related septic events. However, for patients with multiple lumens and/or multiple catheters, drawing cultures from each lumen may not be feasible due to considerations of volume of blood required and cost of numerous cultures. Clinicians must then make a judgment as to which sites most merit culture. Such decisions are influenced by which lumens are accessed most often, which lumens have recently failed to function optimally, physical findings of erythema or tenderness or exudates, and knowledge about the conditions under which the catheter was placed.

Effect of Blood Volume. The volume of blood cultured is a pivotal variable for the successful recovery of bloodstream pathogens: the more volume that is cultured, the higher the yield of the process (10–15). Cockerill et al (13) documented a 29.8% increase in positive cultures when 20 mL of blood (divided into two bottles) were cultured compared with 10 mL of blood. Additional positive cultures were observed when 30 mL (13.4% increase vs. 20 mL) and 40 mL of blood (7.2% increase vs. 30 mL) were cultured. The blood culture yield also increases with the collection of additional blood cultures (consisting of 20 mL of blood divided into two bottles). Cockerill et al (13) also reported that when a minimum of four blood culture sets were collected within a 24-hr period, the yield increased with each additional culture drawn: 61.4% of the patients with blood stream pathogens had the causative organism detected with the first collected culture, 78.2% with the first two cultures, and 93.1% with the first three cultures. Lee et al (14) reported very similar data.

Whereas patients with catheter-related sepsis, endocarditis, or other intravascular infections may be persistently bacteremic, most other infections are associated with intermittent bacteremia or fungemia. Although it is commonly believed that high-grade seeding of the blood corresponds to temperature elevations, Riedel et al (16) demonstrated in a multicenter study that timing collection of blood cultures with temperature elevations did not increase the yield of blood cultures.

Because clinical signs including fever and symptoms cannot be used to predict the optimum time for specimen collection, the Society for Critical Care Medicine, Infectious Diseases Society of America, Surviving Sepsis Campaign, and Clinical and Laboratory Standards Committee recommend that a minimum of two blood cultures consisting of 20–30 mL per culture (ideally one peripheral draw and one drawn through the catheter most suspicious of being infected if line sepsis is suspected) should be collected within a 30-min period when a septic patient is first evaluated, before antibiotics are administered or changed, and additional cultures should be collected over a 24-hr period.

Recovery of Anaerobic Bacteria. Historically it was recommended that blood should be subdivided into an aerobic bottle (supports the growth of strict aerobic and facultatively anaerobic [grows aerobically or anaerobically] bacteria as well as yeasts) and an anaerobic bottle (supports growth of strict anaerobic

and facultatively anaerobic bacteria). Retrospective studies analyzing positive blood cultures in the 1970s and 1980s documented poor recovery of anaerobic bacteria. Although laboratory recommendations have evolved as various media have been developed and assessed, most laboratories currently favor use of an aerobic bottle and an anaerobic bottle for optimum recovery of a broad spectrum of bacteria and fungi (17–20).

Recovery of Fungi and Fastidious Bacteria. The use of special medium formulations for the recovery of yeasts is generally not necessary because most grow well in conventional aerobic blood culture broths within 2–3 days. Exceptions to this rule include *Candida glabrata* and *Cryptococcus neoformans*, which typically require 3–5 days of incubation. *Fusarium* and *Paecilomyces* can be recovered in conventional blood culture broth, but most other filamentous fungi are not detected. Dimorphic molds such as *Histoplasma* and *Blastomyces* can grow in blood culture broths although incubation for >2 weeks is required which is generally impractical. Use of supplementary systems such as the lysis-centrifugation system (Isolator, Wampole Laboratories, Cranbury, NY) is recommended for isolation of slow-growing molds and fastidious bacteria.

Table 1 lists organisms that are unlikely to grow in standard blood culture sets and that require clinicians to alert the laboratory for special processing in terms of selective media, different incubation temperatures, or extended incubation times.

Antibiotic Inactivation Systems. Patients frequently receive antibiotics that suppress the growth of bacteria and fungi. Manufacturers of most blood culture systems supplement their media with proprietary formulations of antibiotic-binding resin beads or absorbent charcoal and Fuller's earth. Analysis of the performance of these compounds has demonstrated superior performance of the resins for removal of antibacterial and antifungal antibiotics, improved recovery of bacteria and fungi, and decreased time to detection of positive cultures (21–24). Thus, because these substances are now routinely used in culture systems, clinicians do not need to request "antibiotic removal systems" for cultures drawn in patients receiving antimicrobial therapy.

Detection Time. One significant advantage with the use of automated blood culture systems that continuously monitor microbial growth throughout the incubation period is early detection of positive cultures. More than 90% of all positive blood cultures are detected within the first 48 hrs of incubation (13, 25), and extended incubation beyond 5–7 days is rarely indicated unless the pathogens listed in Table 1 are suspected (26, 27).

One under-appreciated fact is that significant delays between collection of blood cultures and initiation of incubation will prolong detection times. These delays can occur both at the patient's bedside and in the laboratory. Kerremans et al (28, 29) and van der Velden et al (30) demonstrated in a series of elegant studies that incubation delays for almost half of all blood cultures exceeded 4 hrs (including median transport times of 3.9 hrs and 16.0 hrs for specimens from the intensive care unit and emergency department, respectively), preincubation at the collection site significantly reduced the time to detection of positive cultures, and this resulted in more rapid adjustment of antibiotic therapy. Although blood culture instruments are rarely used outside the clinical lab, the installation of such instruments proximate to intensive care units should be considered in high volume settings where transport delays are likely.

Table 1. Bacteria unlikely to grow in standard blood culture broth systems

Bacteria	Detection Method
Anaplasma species, *Ehrlichia* species	Giemsa stain of peripheral blood; PCR available in reference labs
Bartonella species	Lysis centrifugation to chocolate agar incubated in capnophilic atmosphere at 37°C for 4 wks
Borrelia species	Serology; culture methods and PCR are insensitive
Brucella species	Extended incubation in conventional culture system
Campylobacter species, *Helicobacter* species	Lysis centrifugation to nonselective blood agar incubated in microaerobic atmosphere at 37°C
Coxiella burnetii	Serology; culture insensitive
Francisella tularensis	Extended incubation in conventional culture system
Legionella pneumophila	Lysis centrifugation to buffered charcoal yeast extract agar incubated aerobically at 37°C
Leptospira species	Lysis centrifugation to EMJH broth incubated in air at 30°C; PCR available in reference labs
Mycobacterium species	Lysis centrifugation to Middlebrook agar incubated in capnophilic atmosphere at 37°C for 4–6 wks
Nocardia species	Lysis centrifugation to nonselective blood agar incubated in capnophilic atmosphere at 37°C for 2 wks
Rickettsia species	Serology

PCR, polymerase chain reaction.

Molecular-Based Techniques

Detection of Bacteremia and Fungemia. There is great interest in molecular techniques to diagnose sepsis in blood samples taken directly from patients (31, 32). The goal is diagnosis of bacteremia or fungemia with simultaneous detection of resistance genes with results available in a few hours after specimen acquisition. However, such techniques are not yet optimally developed: current techniques for amplification of microbial DNA for the detection and identification of microorganisms in blood samples such as the SeptiFast system (Roche Molecular Systems) have poor sensitivity and specificity, are technically cumbersome requiring specimen batching and a minimum of 6 hrs processing time, and provide no information about antimicrobial susceptibility results (33–36). It can be argued that these tests should be used as complementary tests to traditional culture, and economic models have been developed that purport rapid polymerase chain reaction identification of microorganisms that has the potential to be a cost-effective component for managing sepsis (37); however, these models assume the molecular tests have sufficient sensitivity to detect all significant organisms with a single test, are specific and detection of microbial DNA in blood is always clinically significant, and are performed in a hospital population where there is a high proportion of inadequate empirical therapy (38). Despite the current limitations of molecular sepsis tests, we believe it is appropriate to be optimistic that the rapid progress in technology development will make such direct sample testing feasible and useful in the near future, potentially as point of care testing in emergency departments and intensive care units.

Microbial Identification. New approaches are having impact on the

identification of organisms once the organism is growing in the blood culture broth. The traditional approach for processing a positive blood culture is to remove a portion of the broth, subculture it to agar media, and after overnight incubation select isolated colonies for identification and antimicrobial susceptibility tests. This process requires 1–3 days before definitive results are available. The use of fluorescence *in situ* hybridization using peptide nucleic acid probes (PNA-FISH) has been used for direct identification of *Staphylococcus*, *Enterococcus*, *Klebsiella*, and *Candida* species in positive blood cultures (39–42) in <2 hrs. This identification approach is laborious because individual probes have to be created for each species.

More promising is mass spectrometry that can identify organisms (but not detect antibiotic susceptibility) within an hour or less from the culture broth, that is, from the time the system alarm alerts the technician of growth. Mass spectrometry, specifically matrix-assisted laser desorption/ionization time-of-flight (MALDI-TOF) mass spectrometry, has been used for identification of isolated colonies of bacteria and fungi and is rapidly replacing biochemical and gene sequencing methods for organism identification because it is highly accurate, inexpensive, and results are available in <1 hr. MALDI-TOF can also be used for the direct identification of bacteria and yeasts isolated in blood culture broths (43–49). Processing these specimens is more complex because the nonmicrobial cells, serum proteins, and broth culture nutrients must be removed before the microbial cells are evaluated. However, definitive identification results from positive blood culture broths are generally available in <1 hr after the technician is alerted to growth. Approximately 15% to 20% of the isolates are not initially identified primarily because insufficient numbers of cells are in the positive blood culture broth (e.g., skin bacteria such as coagulase-negative *Staphylococci* and *Corynebacteria*). However, modification of the extraction procedures is improving the test sensitivity (50, 51). It should be noted that not all blood culture broth formulations produce adequate results, particularly media supplemented with charcoal (52–54). This technique has not been used reliably on direct patient specimens, for example, blood samples.

Antimicrobial Susceptibility Tests. The ability to obtain a definitive identification of a positive blood culture isolate within 1 hr of detection can be used to guide empiric therapy. Although the results of most antimicrobial susceptibility tests are not available for 8–24 hrs, 1- to 2-hr polymerase chain reaction tests for the presence of genes that encode resistance to oxacillin, vancomycin, and the carbapenemases are commercially available and used in many clinical laboratories. These tests are useful; however, the tests may provide misleading results because they do not measure if the resistance gene is expressed. For example, presence of the mecA gene is associated with methicillin resistance in *Staphylococcus aureus*, and the vanA gene is associated with vancomycin resistance in *Enterococcus faecium*. If the regulatory genes that control expression of these resistance markers are inoperable, then the bacteria will remain drug susceptible, and the gene detection assay would mislead the clinician (55, 56). Likewise, the absence of mecA or vanA cannot be used to predict susceptibility to methicillin or vancomycin because resistance to the antibiotic may be the result of another mechanism. For this reason, assessment of antibiotic susceptibility by genomic techniques should be considered a presumptive test that must be confirmed by the current phenotypic tests that assess the growth of bacteria in the presence of the test antibiotic. It is likely that rapid susceptibility tests will continue to evolve beyond the detection of resistance gene sequences or detection of growing organisms by visual methods and ultimately rely on early detection of the response to antibiotics by measuring gene expression or metabolic activity without the need for cell division.

CONCLUSIONS

Molecular and mass spectroscopy techniques are changing sepsis diagnosis rapidly. These techniques provide substantially more rapid and more specific information on organism identification and on the presence of resistance mechanisms than conventional broth-based techniques. Critical care physicians will have to expeditiously learn what information to expect from such systems, and how such information can be used to assure that initial antimicrobial regimens are appropriate, and the unnecessary drugs are discontinued. These techniques are expected to contribute substantially to improving antibiotic stewardship and to improving "time to appropriate antibiotics," one of the most pivotal parameters in improving the prognosis of patients with life-threatening infections.

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