A Critical Appraisal of the Role of the Clinical Microbiology Laboratory in the Diagnosis of Bloodstream Infections

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The detection of bloodstream infections is one of the most important functions of clinical microbiology laboratories. Despite advances in blood culture technology and clinical studies that have focused on the detection of bacteremia and fungemia, perfection has not been achieved and uncertainties persist. This review provides perspectives on a number of areas, including the recommended number of blood cultures, duration of incubation of blood cultures, use of anaerobic, in addition to aerobic, blood culture media, value of the lysis-centrifugation method, processing and reporting of probable blood culture contaminants, and limitations of current blood culture methods and systems. We also address the handling of blood cultures in point-of-care locations that lack full microbiology capabilities.

The detection of bacteremia and fungemia is arguably one of the most important functions of the clinical microbiology laboratory. Although much has been learned in recent decades and guidelines for blood cultures have been published (1, 2), absolute certainties remain elusive. There are numerous examples of questions that are still without clear answers. For example, why are there so many negative blood cultures? Only 10 to 15% of all blood cultures show growth, and approximately half of these grow contaminants, raising the questions of whether current methods are not sufficiently sensitive and whether clinicians use the wrong clinical triggers to initiate orders for blood cultures. What is the optimal number of blood cultures that should be obtained when sepsis is suspected? Is it 2 or 3, as has been recommended for many years (16, 18), or is it 3 or 4, as suggested in two recent publications (4, 10)? Can the duration of the routine incubation of routine blood cultures be reduced to below 5 days, and conversely, is there value in extending the time of incubation to detect certain microorganisms (e.g., HACEK bacteria)? What microorganisms are missed consistently in standard broth-based blood culture systems, and do non-broth-based methods such as lysis-centrifugation provide sufficient added value to be used more widely? Should a routine blood culture set combine aerobic and anaerobic culture vials, or should a culture set utilize only aerobic culture vials, with anaerobic vials included selectively for patients with a high probability of anaerobic bacteremia? What is the role, if any, of blood cultures drawn from intravenous catheters and other access devices? If such cultures are performed, is there any value in discarding the initial aliquot of blood? A related question is whether there is any value in culturing blood specimens from different lumens of multiple-port intravenous catheters. Does a positive result from a single port implicate that only that port is infected, or does this finding simply suggest that few microbial cells are circulating per unit volume and that the culture became positive from the port in question by chance alone? How should blood cultures that grow likely contaminants be assessed and processed, and as a corollary, how does one decide which isolates growing from blood cultures are likely contaminants? In the case of the most common contaminants, coagulase-negative staphylococci, what are the best methods to determine their clinical significance or lack thereof?

There are also several very contemporary questions for which clear answers are lacking. As some institutions have moved clinical laboratories off site and others have consolidated laboratory services at a single site within a hospital system, questions have arisen regarding initial and later processing of blood cultures. Should blood cultures be sent immediately to the central laboratory, or should they be incubated locally in a stat or point-of-care laboratory? If the cultures are incubated locally, how should they be handled if and when there is evidence of microbial growth? Should Gram stains be read locally, or should the positive vials be sent to the central laboratory site? If microscopy is performed locally, how does the laboratory ensure technical competency? If the positive vials are sent to the central microbiology laboratory, what will be the delay and how will this impact patient care and clinical outcomes? Finally, what is the role of non-culture-based methods, such as PCR and other nucleic acid amplification techniques, for the direct detection of bloodstream infections (BSIs)? If such methods become commercially available, will they be able to detect the broad range of potential bloodstream pathogens, and will they provide antimicrobial susceptibility results that will allow clinicians to optimize therapy? Not all of these questions could be addressed during the symposium. Ultimately, we explored the following eight questions that address the role of the clinical microbiology laboratory in the diagnosis of bloodstream infections.

WHAT IS THE OPTIMAL NUMBER OF BLOOD CULTURES?

Several decades ago, Washington (16) reported the cumulative yield from three 20-ml blood cultures and Weinstein et al. (18) reported the cumulative yield from three 17-ml blood cultures (Table 1). These studies formed the basis for the

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long-standing concept that most if not all BSIs can be detected with 2 or 3 blood cultures. However, two recent studies (4, 10) have challenged this traditional teaching and suggest that 3 or 4 blood cultures may be needed for optimal detection of bloodstream infections (Table 1). The newer studies suggest that obtaining only two blood cultures will result in missing 10 to 18% of episodes, whereas obtaining three blood cultures reduces the false-negative rate to only 2 to 4%. One can reasonably ask why blood culture sensitivity using current, and presumably more-sophisticated, media and systems is lower now than it was in the 1970s. Potential explanations for the seemingly paradoxical findings noted in the recent studies include detection of lower levels of bacteremia by modern systems which may in turn require more blood cultures, greater numbers of patients on antimicrobials at the time of blood culture in the current versus the former era, or the criteria used to define the “first” blood culture when measuring cumulative yield (i.e., the first in a series of cultures, in contrast with the first in any 24-h time period). In the study by Lee et al. (10), for example, the first blood culture was defined as the initial culture in a 24-h time period; this definition may have biased the results against the sensitivity of the newer systems. Based on the recent studies, we concluded that 3 or possibly 4 blood cultures may be needed for optimal detection of bacteremia and fungemia. However, in acute episodes of suspected BSI, two blood cultures may be sufficient (10).

IS 3 DAYS OF INCUBATION SUFFICIENT WITH CMBCS?

Early studies of continuous-monitoring blood culture systems (CMBCS) (BacT/Alert [bioMérieux, Inc., Durham, NC]; Bactec [Becton Dickinson, Sparks, MD]) demonstrated that, compared with 7-day incubation protocols, 5-day protocols detected 98 to 99% of positive results. Based on these data, 5-day incubation protocols became standard. Studies of 3- and 4-day incubation protocols (5, 19) demonstrated a 3 to 5% reduction in detection compared with that for 5 days of incubation. Although laboratories could use incubation times of less than 5 days, this practice will reduce the ability of the systems to detect some bacteremias and fungemias. We concluded that reducing blood culture incubation protocols to below the current 5-day standard is not appropriate.

TABLE 1. Number of blood cultures needed to detect bacteremia and fungemia in adults

<table>
<thead>
<tr>
<th>No. of cultures</th>
<th>Cumulative percent positive reported by:*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>J. A. Washington II (16)*</td>
</tr>
<tr>
<td>1</td>
<td>80</td>
</tr>
<tr>
<td>2</td>
<td>88</td>
</tr>
<tr>
<td>3</td>
<td>99</td>
</tr>
<tr>
<td>4</td>
<td>&gt;99</td>
</tr>
</tbody>
</table>

* The numbers in parentheses are reference numbers.
There is no gold standard or definitive algorithm for differentiating true pathogens from contaminants or organisms of unknown significance. However, clinical studies (12, 18) and reviews of the extant medical literature (17) have provided guidelines that aid laboratorians and clinicians in making these decisions. Among the potential tools that can assist in these decisions (Table 2), we agreed that, from a laboratory perspective, the two most valuable aids for interpreting clinical significance are the identity of the microorganism itself and the number of culture sets positive, as a function of the number of sets obtained (i.e., number positive/number obtained) (12, 18).

When a blood culture grows an organism that is likely to be a contaminant, there is no benefit in having the laboratory undertake full identification and susceptibility testing of the isolate and report this information to the clinician. Indeed, performing a complete work-up and reporting the results increases institutional costs and technical workload without providing any patient benefit. Accordingly, consensus (2) and expert (1) guidelines have recommended that laboratories limit the work-up and reporting of such isolates. It was our strong opinion that the work-up of presumed contaminants should be limited, and we specifically noted the published work of Richter et al. (15). A sample algorithm based in part on that publication is shown in Table 3.

### HOW CAN THE NUMBER OF NEGATIVE BLOOD CULTURES BE REDUCED?

This is an excellent question without a good answer. We are not aware of any published studies that shed light on this issue. Papers on clinical prediction rules are not sufficiently precise, and these rules are not widely used by physicians. Moreover, a recent survey at a university medical center found that physicians would require that a bacteremia prediction rule have >99% sensitivity to be considered useful clinically (9). Currently, there is no prediction rule that meets this standard.

### SHOULD BLOOD CULTURES BE DONE IN A STAT LAB OR POINT-OF-CARE LOCATION?

We are not aware of any published data upon which to address this issue. In certain settings, for example those in which specimens are obtained locally but then sent to a central microbiology laboratory for further processing, there may be value in placing CMBCS incubator units at the local sites rather than sending all of the culture sets (vials) to the central laboratory for incubation. However, we strongly felt that it would be best to have Gram stains and other direct tests from positive-culture vials interpreted by fully trained microbiology technologists and that all work-up of positive cultures be done in the central microbiology laboratory.

### WHAT MICROORGANISMS ARE MISSED CONSISTENTLY WITH CONVENTIONAL BLOOD CULTURE APPROACHES?

There is no true gold standard for the detection of bloodstream infections, and no single commercially available blood culture system or combination of media provides optimal detection for all bloodstream pathogens (1). Some microorgan-

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**Table 2.** Tools for interpreting the clinical significance of positive blood cultures

<table>
<thead>
<tr>
<th>Item</th>
<th>Recommendation or comment</th>
</tr>
</thead>
<tbody>
<tr>
<td>Identification of microorganisms</td>
<td>Recommended based on published data.</td>
</tr>
<tr>
<td>Clinical findings in the patient (e.g., fever, leukocytosis, imaging studies, etc.)</td>
<td>Recommended; however, some of these data are not available to the laboratory.</td>
</tr>
<tr>
<td>No. of blood culture sets positive vs no. of sets obtained</td>
<td>Recommended based on published data.</td>
</tr>
<tr>
<td>Time to detection of the positive result</td>
<td>Not recommended; too much overlap between true pathogens and contaminants.</td>
</tr>
<tr>
<td>No. of bottles positive in a blood culture set</td>
<td>Not recommended; too much overlap between true pathogens and contaminants.</td>
</tr>
</tbody>
</table>

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**Table 3.** Sample laboratory algorithm for work-up and reporting of probable blood culture contaminants

<table>
<thead>
<tr>
<th>Situation</th>
<th>Action&lt;sup&gt;a,b&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>≥2 blood cultures obtained; 1 blood culture growing coagulase-negative staphylococci, <em>Micrococcus</em> spp., <em>Corynebacterium</em> spp., <em>Bacillus</em> spp. not <em>anthracis</em>, or viridans group streptococci</td>
<td>No species ID or susceptibility test; report as probable contaminant.</td>
</tr>
<tr>
<td>1 blood culture obtained growing coagulase-negative staphylococcus, <em>Corynebacterium</em> sp., <em>Micrococcus</em> sp., <em>Bacillus</em> sp. not <em>anthracis</em>, or viridans group streptococcus</td>
<td>No species ID or susceptibility test; report isolate of indeterminate significance.</td>
</tr>
<tr>
<td>≥2 blood cultures obtained within 48 h growing coagulase-negative staphylococci, <em>Corynebacterium</em> spp., <em>Micrococcus</em> spp., <em>Bacillus</em> spp. not <em>anthracis</em>, or viridans group streptococci</td>
<td>Perform full ID and susceptibility test. (i) If isolates are same species and have identical antibiograms, report ID and susceptibility. (ii) If isolates are different,* no species ID or susceptibility. Report as different strains, probable contaminant.</td>
</tr>
</tbody>
</table>

<sup>a</sup> Isolates differ by ≥2 biochemical reactions and antimicrobial susceptibilities (sensitive [S] vs resistant [R]) or differ by molecular testing.

<sup>b</sup> ID, identification.
isms grow poorly or not at all in conventional blood culture broth media. The following microorganisms are likely to be missed using routine blood culture vials and systems: dimorphic fungi (most notably Histoplasma), some yeasts, Bartonella spp., Mycobacterium spp., Mycoplasma spp., Streptobacillus spp., viruses, and rickettsiae. Special media and techniques can be used to detect uncommon or fastidious pathogens (1, 2).

OTHER ISSUES
Our discussion focused on selected technical and clinical aspects of blood cultures that have either generated controversy or not been addressed systematically in published studies. One issue that was not discussed is that of blood culture contamination associated with cultures obtained from intravenous access devices (e.g., peripherally inserted central catheter [PICC] lines and multilumen catheters) in comparison with those obtained by peripheral venipuncture. We think this is an important issue that merits additional comment in this review. A recent study has highlighted the problem of contamination associated with catheter-drawn blood cultures (7). This report documented contamination rates for catheter-drawn blood cultures of approximately 11%, regardless of whether or not the initial aliquot of blood obtained from the catheter was discarded. In contrast, contamination rates for blood cultures obtained by peripheral venipuncture are generally 3% or less (1, 2). Thus, reducing blood culture contamination overall will depend on either discontinuing the practice of obtaining blood for culture from catheters or developing devices that better lend themselves to obtaining blood specimens aseptically.

SUMMARY
One of the most important functions of the clinical microbiology laboratory continues to be the detection of bloodstream infections. During the past 4 decades, there have been important advances in both blood culture methods and culture-based technology, with continuous-monitoring blood culture systems now dominating the market in many developed countries. In addition, clinical studies have broadened our knowledge about culture media, the volume of blood needed for blood cultures, the atmosphere of incubation, the duration of incubation, the value of agitation during incubation, and the use of additives, such as lysing agents, antibiotic-binding resins, and activated charcoal. Despite these advances, important questions still remain, and clinical and technical uncertainties persist. We have tried in this report to present the current state of the art, addressing some of the questions and uncertainties. Going forward, we hope investigators will focus on these issues as well as new ones that may arise with the advent of novel techniques for detection of pathogens from blood.


REFERENCES