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REVIEW ARTICLE

Blood cultures: key elements for best practices and future directions

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Abstract Bloodstream infections (BSI) cause significant morbidity and mortality among populations worldwide. Blood cultures (BCs) are regarded as the "gold standard" for diagnosis of bacteremia and are among the most important functions of the clinical microbiology laboratory. Significant changes in the methods and techniques of obtaining BCs have occurred since the first inception of BCs into clinical practice. Aside from significant improvements of established, conventional technology, new assays for diagnosis of bacteremia and fungemia, particularly those involving molecular techniques, are now available. BCs must be collected under sterile conditions and guidelines for appropriate collection, processing, and results reporting of BCs have been established. This review provides comprehensive information on optimal BC practices for laboratories, utilizing traditional approaches and emerging technology. As laboratories and clinicians must appreciate the key factors affecting the use of these techniques, improved communication between laboratory personnel and clinicians regarding such elements as duration of incubation, workup of contaminants and critical action value reporting will greatly improve the diagnostic approach to BSI.

Keywords Blood culture · Blood culture contamination · Blood culture volume · Bacteremia · Nucleic acid amplification testing · Aerobic and anaerobic blood cultures

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Introduction

Worldwide, bloodstream infections (BSI) rank among the most serious problems in patient care because they are associated with significant morbidity and mortality [1–4]. Each year in the United States, an estimated 750,000 patients develop bacteremia and/or fungemia with associated mortality of 14% (community onset BSI) to 34% (nosocomial BSI) [1, 4, 5]. In some hospitals, rates of nosocomial BSI are >50%, and data have shown that sepsis and septic shock rank as the 10th leading cause of death in the United States [5, 6]. A recent multicenter study in Germany demonstrated that sepsis ranked as the third most common cause of death in that country [3]. Finally, sepsis and related complications result in a significant economic burden to hospitals, with an estimated annual cost of $16.7 billion in the United States [1]. It is therefore important to understand not only the epidemiology, treatment, and outcome of sepsis, but to continuously review and improve methods of diagnosis of BSI. Recently published guidelines for management of sepsis and septic shock have addressed the importance of rapid detection of causative organisms and prompt initiation of broad-spectrum antimicrobial therapy [7].

Blood cultures (BCs) are regarded as the "gold standard" for the diagnosis of bacteremia [2]. Detection of bacterial and fungal organisms in blood is perhaps one of the most important functions of the clinical microbiology laboratory. From a diagnostic point of view, BCs establish the infectious etiology of a patient's illness. In addition, BCs provide the organism for further antimicrobial susceptibility testing (AST) and optimization of the antimicrobial therapy of patients. The importance of this latter aspect cannot be underestimated, and several studies have demonstrated that inappropriate antimicrobial therapy is an

independent risk factor for mortality in critically ill patients [8–11]. From a prognostic point of view, positive BC results provide evidence of failure of host defense mechanisms to contain an infection, or evidence of failure to remove, drain, or otherwise adequately treat a primary infection site. Considering the importance of BCs in the diagnosis and management of sepsis, it is only prudent to understand the clinical and technical requirements and limitations of this technology to ultimately improve patient outcome. This review will therefore focus on the key elements of "gold standard" BC technology and briefly discuss the future potential of molecular techniques for rapid detection of pathogens in BSI.

Key elements

The different forms of sepsis and related inflammatory host responses are always associated with bacteremia (or fungemia); however, bacteremia and fungemia alone, as defined by the identification of microorganisms in blood, do not designate sepsis and related syndromes. Detailed definitions of sepsis and sepsis-related syndromes are described elsewhere [12]. Bacteremia has usually been described as intermittent or continuous. Most bacterial BSIs in adults are intermittent bacteremias and are often associated with closed-spaced infections (e.g. abscesses) or distant site infections, such as pneumonia or other organ-specific infections. Continuous bacteremias in adults are commonly low-grade, persistent bacteremias associated with an intravascular site infection, for example endocarditis. As BSIs in pediatric patients differ significantly in their characteristics from BSIs in the adult patient population, we will discuss the core concepts of BC collection in pediatric patients later in this text.

Recently published guidelines regarding the management of bacteremia, sepsis, and septic shock emphasize the importance of adequate BC collection (to include optimum collection site and appropriate skin antisepsis) and early antimicrobial treatment as important steps in successful management of sepsis patients [7]. The task of collecting blood for culture by the means of a peripheral venipuncture may seem somewhat simple; in order to consistently retrieve reliable important diagnostic information from BCs, however, several key elements of BC collection and processing must be well understood not only by microbiologists but also by clinicians. The recent M47-A document, published by the Clinical and Laboratory Standards Institute (CLSI), is a comprehensive guideline to all steps and procedures involved in BC collection and processing to final results reporting [13].

A BC is defined as a specimen of blood collected for the purpose of bacterial and/or fungal culture [14]. By this definition, blood for one BC is collected from a single venipuncture, irrespective of how many bottles or tubes are inoculated with the specimen. If more than 1 bottle is inoculated, the collection of this specimen is commonly referred to as a "blood culture set". In this review, we will use the terms BC and BC set interchangeably. The following paragraphs will address important variables in BC technology that have been recognized as having an impact on organism recovery and turn-around-time (TAT) for results reporting. In general, many of these issues, and especially an appropriate specimen collection technique, have a significant impact on the sensitivity and specificity of organism recovery rates in BCs.

Timing of blood culture collection

During the past 70 years, numerous studies have investigated the possibility of predicting bacteremia in hospitalized patients. Bacteremia and its sequelae are serious disorders with significant impact on treatment and survival rates and physicians may therefore have a low threshold for obtaining BCs. On the other hand, physicians' intuitive abilities to accurately predict bacteremia have been less than optimal [15, 16]. In 1954, Bennett and Beeson [17] published their observations that bacteremia actually precedes temperature elevations (i.e. fever spikes) by 1 or 2 h. Considering the almost impossible task of anticipating the exact timing of such events, the current conventional approach is to collect blood specimens at or around the time of a spike in a patient's body temperature as a means of enhancing the likelihood of detecting bacteremia [8, 18, 19]. The most comprehensive evaluations of models for prediction of bacteremia have been published by Bates and colleagues [19–21]. In these systematic prospective evaluations, a temperature of 38.3°C and higher, presence of a rapidly or ultimately fatal disease, presence of shaking chills, acute abdominal examination, major comorbidity, and intravenous drug abuse have been identified as independent predictors of true bacteremia. As a general rule, the collection of two, simultaneously obtained BC sets in the setting of the above mentioned criteria has become an established and widely accepted practice. However, in some clinical situations, perhaps considered to be less urgent (e.g. no immediate start of antimicrobial therapy), the timing of BC collection might be less stringent and more protracted over time. However, in at least one study, no difference in the ability to detect bacteremia was found, when comparing simultaneously collected BC sets with those collected at different times over a 24-h time period [22]. In yet another multicenter study, the authors investigated the association between maximum fever spikes and BC collection in patients with bacteremia. In this study, the investigators concluded that collection of BCs exactly at

the time of the fever spike is not necessary to optimize the detection of bacteremia in adult patients [23].

Furthermore, the presence of commonly accepted signs and predictors of bacteremia are even more irregular and unpredictable in the geriatric patient population [24]. Elderly patients with bacteremia often present with fewer symptoms in the early stages, however, they demonstrate a more rapidly progressive course with higher mortality compared with younger patients [25, 26].

Despite all of these uncertainties of accurate timing of BC collection as stated above, the authors believe that the timing of BCs is ultimately a clinical decision and must take into consideration the acuity of illness and the need for rapid antimicrobial therapy. Therefore, it is reasonable to simultaneously collect at least two sets of BCs at the time of a fever spike in patients suspected to have a BSI. The drawing of BCs at intervals is only indicated for patients suspected of having endocarditis or other endovascular infections and when the pace of the illness is not acute.

Number and volume of blood cultures

Despite the fact that newer BC media and improved automated, continuous-monitoring blood culture systems (CMBCS) detect organisms faster and more frequently, common consensus remains that the volume of blood collected is the single most important variable predicting one's ability to detect bacteremia. Most bacteremias in adults have a low density of microorganisms (often $\leq$1 CFU/ml) [27]. Studies in the 1970s and 1980s investigated this relationship between blood volume and rate of organism recovery, supporting the conclusions that 20–30 ml blood should be collected from a venipuncture to ensure a high likelihood of detecting bacteremia [28–30]. Collections of volumes >30 ml have not been shown to significantly increase the yield [31]. Weinstein et al. [32] demonstrated that 91% of bacteremia episodes were detected by the collection of one BC and that >99% were detected by the first two BCs. With further evolving continuous-monitoring BC technology, interest in investigations of the relationship between blood volume and culture yield has recently re-emerged. Bouza et al. [33] concluded in their study that the volume of blood collected is still an important variable for the ability to detect a BSI, even when using automated CMBCS. Several other recent studies have demonstrated that as many as four BC sets collected over a 24-h period are necessary to achieve >99% sensitivity for the detection of a BSI [34, 35]. In 2004, Cockerill et al. [34] reported percentage yields for consecutive BCs in patients without endocarditis as 65.1% for the first BC, 80.4% for the second BC, and 95.7% for the third BC using the BACTEC 9240 system. In this study, the first BC was more frequently positive for patients with endocarditis when compared with the first BC for non-endocarditis patients.

The authors conclude that blood volume remains the most important variable predicting organism recovery for BCs in adult patients. Based on clinical suspicion, two BCs should be obtained by two venipunctures from different sites. For adults 20–30 ml from each venipuncture should be collected and equally distributed for inoculation of aerobic and anaerobic culture bottles. A third BC set may be collected some 4–6 h after the initial collection. For most bacteremic episodes in adults, collection of two to three BC sets within a 24 h time period will suffice for diagnostic purposes. However, collection of just one BC is considered insufficient for diagnosis of a BSI and with new evidence could possibly miss approximately 35–40% of bacteremic episodes [34].

Unfortunately, fewer data are available for pediatric BCs. BSI in young children are presumed to have a much higher magnitude (often >100 CFU/ml) than bacteremia in adults [36]. Earlier expert recommendations for BCs in infants and children stated a need to collect 1–2 ml for neonates, 2–3 ml for infants (age 1–24 months), 3–5 ml for older children, and 10–20 ml for adolescents [37]. However, more recent recommendations, including those based on the results of the study by Kellogg et al., suggest that the volume for pediatric BCs should be based on body weight and the estimated total blood volume in each individual child, suggesting the collection of no more than 1% of the total blood volume for neonates, infants, and young children [13, 38, 39].

Considerations for current blood-culture systems

One of the great advances in medical microbiology was the introduction of automated CMBCS [40]. Three different systems are commercially available in the US. Two of these, the BACTEC 9000 series (Becton-Dickinson, Sparks, MD, USA) and the BacT/Alert 3D system (bioMérieux, Durham, NC, USA) rely on the colorimetric or fluorimetric detection of increased CO_2 levels as a result of microorganism growth. The third system, VersaTREK (TREK Diagnostic System, Cleveland, OH, USA) detects microbial growth by measuring the change in gas pressure in the headspace of the BC bottle. Measurement intervals vary between these systems, ranging from 10 to 24 min. All three systems utilize computer algorithms to analyze the captured data and to recognize either increasing rates or sustained production of CO_2. BC bottles matching the appropriate criteria for significant CO_2 increase are then signaled as positive and identified for removal and subculture. More detailed information about these systems and their technology is available in the CLSI M47-A document

and respective technical information bulletins provided by the manufacturers of these systems [13]. The widespread use of modern CMBCS has significantly reduced the TAT for BC results, increased the efficiency of processing BCs, and significantly affected work flow and staffing levels in microbiology laboratories. While all systems are developed on good scientific principles, several important technical variables with regard to performance standards and good clinical practice are discussed in this review.

Normal human blood contains several components such as white blood cells, lysozyme, and antibodies that may at any given point in time affect microbial growth. More importantly, in many instances, patients may have also received antimicrobial therapy before collection of the BCs. To reduce the effects of these on organism recovery, the choice of appropriate BC media, blood-to-broth ratio, additives, and incubation conditions must be taken into consideration [13]. The optimum blood-to-broth ratio of 1:5–1:10 has been shown to support the growth of most organisms [41, 42]. Current guidelines support this earlier recommendation; however, some commercial CMBCS may allow for a <1:5 ratio, as their culture media may contain proprietary additives to inactivate inhibitory substances or to enhance growth of microorganisms. Each of the CMBCS has its own broth medium for aerobic and anaerobic BC bottles. In addition to the basic medium (commonly soybean–casein digest broth), the BC broth contains additives (commonly sodium polyanetholsulfonate, SPS) to prevent coagulation of blood, and is often enriched with various supplements and other proprietary substances to enhance microbial growth. Despite the desired effects of SPS on inhibition of blood clotting, phagocytosis, and some antimicrobials, it should be recognized that SPS also has the potential to inhibit growth of some bacterial microorganisms, especially *Neisseria* spp., *Gardnerella vaginalis*, and *Moraxella catarrhalis* [40, 41, 43, 44]. Notwithstanding these differences between various CMBCS, most of the media used for these systems perform reasonably well in clinical settings, and several studies have investigated their performance [2, 45, 46]. Furthermore, manufacturers of CMBCS, and clinical microbiology laboratories, must abide by the stringent quality assurance guidelines set forth by the CLSI [47].

Considering the high mortality associated with bacteremia, 28–63% of patients are already receiving antimicrobial therapy at the time BCs are drawn. Pathogen-directed, appropriate antimicrobial therapy is regarded as a fundamental component in the treatment of bacteremia. However, early in the course of the illness, the causative organism(s) and AST results are usually not known to physicians. The choice of initial antimicrobial therapy is therefore an empirical one based on available clinical and epidemiological data and initial clinical suspicion of the most likely pathogens. Patients are usually treated with a broad-spectrum antimicrobial agent. Data on the effects of empirical antimicrobial therapy on patients' outcome and survival remain controversial [9, 48–50]. To offset the inhibitory effects of antimicrobials administered to patients before BC collection, manufacturers of CMBCS devised various methods to increase organism recovery. Antibiotic inactivating resins are present in the BACTEC Plus Aerobic/F media (BD Diagnostics, Sparks, MD, USA), and the BacT/Alert FA media (BioMérieux) contain charcoal for antibiotic inactivation. While enhanced recovery for both bacteria and yeast has been reported in multiple studies, many of these studies demonstrated improvement of overall organism recovery with the newer, supplemented media, rather than specifically addressing organism recovery in patients receiving antimicrobial therapy [51, 52]. Therefore it remains controversial whether improved organism recovery is clearly related to the removal of antimicrobial agents and data on the efficiency of antibiotic neutralization have not been well demonstrated in the literature. Flayhart et al. [53] recently compared the BACTEC Plus to the BacT/ALERT FA BC media for their respective ability to support bacterial growth at peak, mid-level, and trough concentration of various antibiotics. In this study, BCs were seeded with bacterial inoculum at a concentration defined by the concentration of antibiotic added. In their study, the authors concluded that the BACTEC system was superior in its ability to recover Gram-positive and Gram-negative organisms, particularly in the presence of β-lactam antimicrobials, gentamicin/penicillin, and vancomycin, when compared with the BacT/ALERT system. At least two earlier studies investigating the ability of different media containing resins or charcoal support the findings of Flayhart and colleagues that the BACTEC resin bottles generally detect organisms in the presence of antimicrobials more often and more rapidly than the charcoal-containing BacT/Alert bottles [54, 55].

The authors of this review recommend that laboratories should consider the clinical and epidemiological characteristics of their patient population, the approach to empirical antimicrobial therapy employed by their physicians, and, last, that they conduct an in-house validation of the media type chosen for their CMBCS. As manufacturers of CMBCS continue to modify and improve their BC media, additional studies will be needed to continue further performance evaluation of the ability of these newer media to detect bacterial or fungal growth in a timely manner.

Duration of incubation: is greater than 5 days necessary?

Most clinical microbiology laboratories in the US and Europe that use one of the CMBCS have established a

5-day incubation procedure for all BCs. Four to 5 days of incubation for most clinical BCs have been shown to be sufficient for detection of the most commonly encountered bacterial and fungal organisms [56–58]. Other studies even suggest that an incubation period of <5 days may be sufficient for recovery of clinically significant bacterial isolates [59]. One group of investigators suggested in two separate, consecutive studies that routine incubation of 3 days may be sufficient for organism recovery in the BacT/Alert system [60, 61]. In their more recent investigation Bourbeau et al. reported that 97% of all clinically significant bacterial and fungal isolates were detected within the first 3 days of BC bottle incubation. However, the current recommendations remain for 5 days of incubation for all CMBCS [13]. The authors would like to caution the reader to consider various factors that could affect the recovery rates of organisms when incubation of <5 days is used; such factors may be geographical differences in epidemiology of bacteremia and sepsis, different technologies and BC bottles, and factors within the individual healthcare setting such as transport times for BCs to the laboratory, processing procedures, and ordering patterns of physicians.

Extended time periods for BC incubation are often requested for patients with presumed endocarditis. The second most common indication for prolonged incubation times of broth-based BC bottles is the clinical suspicion of bacteremia caused by fastidious organisms. Bacteria included in this group are *Brucella* species, nutritionally variant streptococci, *Francisella* species, and the HACEK group organisms. The recovery of an organism of the HACEK group is also considered one of the major criteria for diagnosis of infective endocarditis [62]. However, only limited data are available on the utility of such extension of the incubation procedure when using CMBCS. The recommendation to incubate BC bottles for up to 2 weeks was originally based on a study by Washington et al. in 1982 using older BC systems and technology [63, 64]. More recent data from studies at the Mayo Clinic and from the Stanford University "Endocarditis/FUO protocol" support the recommendation that most organisms in endocarditis patients can be identified by using the routine 5-day incubation procedure [34, 65]. In this study, the investigators implemented a special "endocarditis protocol", whereby three sets of BCs from patients suspected of having endocarditis were incubated for 21 days in the BACTEC 9240 system. Blind subcultures were performed on days 3 and 10 from aerobic and anaerobic bottles. Four additional lysis centrifugation tubes were also collected. The investigators found only three clinically significant isolates by this method from a total of 215 endocarditis cultures. The cost for the extended incubation procedure and the additional cultures was substantial. During the same time period, 24 fastidious organisms (HACEK group) were identified by conventional, automated CMBCS. On the basis of these results, the authors of the study concluded that a standard extended BC incubation procedure for endocarditis patients is not necessary. These findings were also supported by the results of yet another study investigating the recovery of HACEK organisms from BCs during a 5 versus 21-day incubation period [66]. Other organisms that are difficult to cultivate (e.g. *Legionella*, *Bartonella*), or those that cannot be cultured (e.g. *Coxiella*, *Rickettsia*, *Chlamydophila*) are better diagnosed by molecular and/or immunological methods [67].

The authors of this review support the current recommendation of a routine 5-day incubation procedure for all BCs, including those in patients suspected of having infective endocarditis or bacteremia due to fastidious organisms. Following CLSI M47-A guidelines, we recommend that all BC sets negative at day 5 should be subcultured if diagnosis of endocarditis is still considered by the treating physician. Laboratories may develop their own algorithms for these cases based on establishing a procedure for communication and appropriate dissemination of the pertinent information in such cases.

Aerobic vs. anaerobic BC collection

Anaerobes have been described as a cause of infection at almost all body sites, including BSI [5, 68]. Early recognition and appropriate antimicrobial treatment are critically important in the care of these patients. Until the 1990s, anaerobic bacteria accounted for up to 20% of all BSIs; however, during the 1990s studies reported a decline in the rates of anaerobic bacteremia [69, 70]. Reasons for this decline in the rate of anaerobic bacteremia were not immediately evident, but several factors such as earlier recognition of infection, improved antimicrobial treatment, and changes in the patient population have been proposed. As a result, several studies investigated the role of routine anaerobic BCs. Considering the epidemiology of anaerobic bacteremia at that time, some investigators suggested that empirical antimicrobial therapy should be instituted on the basis of sufficient clinical suspicion, and that it was not necessary to identify anaerobic bacteremia by identifying the organisms in BCs [71, 72]. Rather than eliminating anaerobic BCs completely, Murray et al. [73], suggested the collection of one BC set with two aerobic bottles and an additional second paired aerobic/anaerobic BC set during the time of the initial BC collection. This suggestion was predicated on the fact that significantly more bacteremias were caused by aerobic organisms than anaerobic organisms, and the proposed method of BC collection would therefore enhance the chance of organism recovery for aerobes. Recent studies, however, documented a

re-emergence of anaerobic BSI. In 2003, Riley et al. [74] compared the recovery rate for organisms in two BacT/Alert FAN aerobic bottles to that in one FAN aerobic bottle plus one FAN anaerobic bottle. 86 isolates were recovered only in aerobic pairs (12.2%), but 131 isolates were recovered from aerobic/anaerobic pairs (18.6%). Overall more organisms (aerobic Gram-positive and Gram-negative, and anaerobes) were recovered using paired aerobic/anaerobic BC bottles.

Finally, in 2007, Lassmann et al. [75] reported that the mean incidence of anaerobic bacteremia at the Mayo Clinic increased overall by 74% from 1993 to 2004. The authors in this study concluded that anaerobic bacteremia had reemerged as a significant clinical problem, most likely attributable to the increase in patients with complex underlying diseases such as malignancies and associated chemotherapeutic treatment regimens. The ability to accurately detect anaerobic bacteremia has become even more important, as antimicrobial resistance among anaerobic organisms has emerged during the last decade [76, 77]. Because susceptibility to various antibiotics is now less predictable among anaerobes, it has become more important to perform AST on isolates recovered from BCs in order to enable selection of the most appropriate therapy.

Considering the observations of changes in the epidemiology of anaerobic bacteremia and the emergence of antimicrobial resistance among anaerobes during the past 20 years, the authors in this review recommend that both aerobic and anaerobic BCs should be routinely collected for incubation. Furthermore, as volume is the most important predictor of organism recovery, at least two BC sets should be collected at the time of initial specimen collection when patients are considered to be bacteremic. Last, we consider a 5-day incubation procedure sufficient for recovery of most bacterial and fungal organisms, including fastidious bacteria, implicated in bacteremia.

Aspects of quality assurance

Results of BCs, whether positive or negative for growth, are critical to physicians in the management of their patients. The report of results to the healthcare provider must be concise and include all pertinent information available at the time of reporting. Healthcare providers must be able to identify the status of BCs and reports in a timely manner, because the results affect the direction and choice of antimicrobial treatment and other necessary diagnostic and therapeutic intervention. Because any positive BC results may have an important effect on patient care, these results, considered to be critical action values (CAV), must be immediately reported to healthcare providers according to established laboratory policies. Such policies must be established in conjunction with the hospital's quality assurance board and healthcare providers to assure compliance with standards of medical care and legislative requirements. Monitoring of a laboratory's compliance with CAV reporting is recommended [13]. Final reports for BC results must include the Gram stain result, final organism identification, and (if applicable) the final antimicrobial susceptibility results.

Earlier and more rapid identification of the cause of BSI enables rapid optimization of antimicrobial treatment and may reduce length of hospitalization and cost of patient care. The current CMBCS afford laboratories more rapid detection of most BSI compared with older, non-automated methods [2, 31, 40]. Although such reduction in the length of time to detection seems intuitively important, few studies have investigated the effects of cost and length of patient stay. One study reported that the time to notification of a BSI was independently associated with increased length of stay [78]. The investigators concluded that laboratories should identify opportunities to reduce time to notification by reducing transit time of BCs from patient-care areas to the laboratory, prioritizing the processing of BCs and Gram stain results, and improving methods of communicating BC results to healthcare providers. Two other studies investigated the effects of delayed incubation of BC bottles with reference to the specifications on this issue made by the manufacturers of CMBCS. Kerremans et al. [79] demonstrated in their study that immediate incubation of BCs significantly reduced turnaround times and led to earlier changes in antibiotic therapy. The authors judged this intervention to be inexpensive and easy to implement in microbiology laboratories, including those laboratories currently not offering a 24–7 service. Sautter et al. [80] specifically investigated the effects of delayed incubation on organism recovery using the BacT/Alert and BACTEC CMBCS systems. The investigators demonstrated significant differences in organism recovery depending on time and temperature of storage before loading of BC bottles on to CMBCS. These findings are particularly important to laboratories using or serving satellite laboratories with reduced services, core laboratories, or reference laboratories, when laboratory and BC techniques are not offered on a 24–7 basis at the site of the occurrence of the actual patient care.

Other studies demonstrated that the reporting of a BC Gram stain result has much greater effect on antimicrobial treatment than culture results and antimicrobial susceptibility results [81, 82]. Some investigators found that rapid reporting of BC results substantially improves antimicrobial therapy in patients and may increase chances of survival [83, 84]. Hautala et al. [82] recently reported that a combination of the Gram stain result with epidemiological information (hospital-acquired vs. community-acquired

infection) could significantly affect the appropriateness of antimicrobial treatment.

The importance of Gram stain reports has been well described in the literature, and a variety of other promising tests for rapid identification have emerged during recent years. Some of these newer methods will be discussed in the section on current and future adjunctive tests in this review. Although these newer tests are very promising, the authors emphasize that Gram stains are important for BC results, and when performed and interpreted by experienced medical technologists are not only an inexpensive, but also fast and highly accurate methods.

Blood culture contamination

As the technology for BCs has improved over time, it has become evident during recent years that contamination of BCs is a common problem which is growing in frequency [8]. False-positive blood cultures (FPBC) may account for up to 50% of all positive BCs in adult patients, as demonstrated in a recent study by Archibald et al. [85]. Contaminated, i.e. FPBC, have a significant effect on patient care, hospital and laboratory staff, and healthcare costs [86–88]. For example, several decades ago coagulase-negative staphylococci (CoNS) were almost always regarded as BC contaminants [20, 89]; however, more recent studies demonstrated that these organisms are now more frequently regarded as pathogens and a complete workup, identification, and AST may be required [8, 90–93]. Many clinical and laboratory studies during the last 30 years have provided information and guidelines on the ways of differentiating between BC contaminants and organisms that are considered to be "true" pathogens [8, 20, 32, 89, 93]. However, a consensus on a "gold standard" definition was never achieved [89, 90]. As contamination of BCs continues to be a significant problem in many clinical microbiology laboratories, the search continues for the "perfect approach" to determine the significance of specific organisms, for example CoNS. Although some investigators search for better correlation between clinical information and laboratory results as a means of deciding which organism is a potential contaminant [94], many more studies focus on possible laboratory algorithms to eliminate unnecessary laboratory testing on contaminant isolates [90, 95]. At the University of Iowa, Richter and colleagues developed, tested, and implemented a laboratory-based algorithm to minimize the work-up of BC contaminants [90]. Aerobic and anaerobic diphtheroids, *Micrococcus* spp., and *Bacillus* spp. are almost always regarded as BC contaminants. However, CoNS and viridans group streptococci (VGS) are regarded as potential BC contaminants, to which the algorithm is applied: if two or more BC sets are obtained from a patient, and only one BC is positive for CoNS or VGS, this BC isolate is reported as a "probable contaminant". No further AST will be performed unless specifically requested by the patient-care provider. If two or more BC sets are collected and at least two sets are positive for VGS, the result is considered clinically significant and susceptibility testing will be performed. If the isolate in such a scenario is CoNS, or if only one BC is collected and positive for either CoNS or VGS, a pathology resident will conduct a patient chart review and the further laboratory work-up of such isolate will proceed according to the resident's judgment in conjunction with consultation from the patient-care provider.

As far as we are aware, this algorithm is, so far, the only comprehensively structured approach, from a laboratory's perspective, which can be used to investigate and give guidance of the work-up of potential BC contaminants. One of the authors (SR) has successfully implemented this algorithm, with a minor modification with regard to chart review responsibility (residents may not be available at weekends), at his hospital and reduced the work-up of potential contaminants by 30%.

Infection control, sample site, and skin antisepsis

Despite the improvements of CMBCS and the implementation of clinical and laboratory-based algorithms, contamination of BCs remains a significant problem for many hospitals and laboratories. As previously mentioned, BSI are associated with significant morbidity and mortality [8]. Therefore, continued review of the epidemiology and outcome of BSI is important. Such a review must include evaluation of all variables affecting not only the clinical outcome (e.g. antimicrobial choices, prevention of nosocomial infections) but also a review of laboratory practices in collection and processing of BCs [5, 13, 26]. Consumer and legislative demands for healthcare information, including information on nosocomial infection rates, have increased during the past decade [96–100]. Microbiology laboratories therefore assist the hospital infection-control practitioner in monitoring BSI for select patient-care areas, e.g. intensive care units and central venous catheter infections. Infection rates must be adjusted by eliminating BC contaminants from the counts of BSI. Commonly, reports are issued on a weekly basis to ICU physicians and infection rates are regularly reported at hospital infection-control committee meetings.

The desire to significantly reduce BC contamination rates has been repeatedly stated for many reasons, such as improvement in acute patient care, antimicrobial utilization, and improved assessments of true infection rates for epidemiological purposes. While it is impossible to achieve

a 0% contamination rate, there are certainly many approaches in the literature used to reduce BC contamination. Contamination rates of ≤3% are reasonable and accepted rates in most hospitals and laboratories [8, 13]. Various laboratory and clinical algorithms have already been mentioned in this review. There is also evidence that various approaches to skin antisepsis during the collection of BCs can significantly affect the extent of contamination. Povidone iodine preparation requires approximately 2 min contact time to produce an antiseptic effect, whereas iodine tincture requires approximately 30 s. Little et al. [101] demonstrated that iodine tincture was superior to povidone iodine for venipuncture site antisepsis before BC collection. In another study investigating the use of 0.5% chlorhexidine gluconate for skin antisepsis the investigators found that chlorhexidine was associated with significantly lower BC contamination than povidone iodine [102]. In a more recent investigation by Barenfanger et al. [103] no statistically significant difference was identified between the use of chlorhexidine and tincture of iodine. A study comparing four different antiseptic preparations found alcohol-containing antiseptics to be most efficient [104]. Commercially available BC skin-preparation kits are also available. Studies investigating the utility of these skin-preparation kits in reduction of contamination led to variable results, ranging from reduction of contamination to no differences when compared with commonly established, older methods of skin antisepsis [105, 106]. The results of these studies comparing various methods for skin antisepsis are summarized in Table 1. Finally, studies have investigated the effects on contamination of using dedicated phlebotomy teams for BC collection [105, 107]. Although additional resources for dedicated BC collection teams may be required, the results of these studies demonstrated a significant reduction in BC contamination. At least one of the studies suggested that the additional cost of establishing dedicated BC collection services would be clearly off-set by cost savings associated with improved patient care and reduced length of stay because of reduced BC contamination [107].

Considering the evidence surrounding greater BC contamination, the authors recommend that laboratories, in conjunction with patient-care providers and infection-control practitioners, develop a method to reduce and/or maintain BC contamination rates at or below the 3% level. An efficient approach to achieving contamination at the desired level is to combine the most effective skin antisepsis with dedicated and trained phlebotomists. Providing contamination rates for each individual procuring the BCs and retraining those individuals whose rates exceed the threshold, have also been shown to be effective in maintaining low rates. Complex laboratory algorithms require more extensive personnel resources and training, and may

Table 1 Comparison of various studies investigating antiseptic preparations for skin antisepsis before blood culture (BC) collection

Study	Number of BC drawn	Number of BC contaminated (%)	Contamination rate for specific antiseptic preparations				
			Chlorhexidine	Tincture of iodine (%)	Povidone-iodine (%)	70% Isopropyl alcohol (%)	Povidone-iodine plus70% ethyl alcohol (%)
Little et al. [101]	TI: 1,904 Pov-I: 1,947	TI: 46 Pov-I: 74	Not included in this study	3.8	2.4	Not included in this study	Not included in this study
Calfee et al. [104]	TI: 3,138 Pov-I: 3,378 IPA: 3,125 Pov-I-A: 3,051	TI: 81 Pov-I: 99 IPA: 78 Pov-I-A: 75	Not included in this study	2.58	2.93	2.5	2.46
Barenfanger et al. [103]	TI (January–June 2002): 5,802 CHX: (January–June 2003): 5,936	TI (2002): 158 CHX (2003): 186	2.9	2.7	Not included in this study	Not included in this study	Not included in this study
Mimoz et al. [102]	CHX: 1,019 Pov-I: 1,022	CHX: 14 Pov-I: 34	1.4	Not included in this study	3.3	Not included in this study	Not included in this study

BC blood culture, *TI* tincture of iodine, *Pov-I* povidone-iodine solution, *IPA* 70% isopropyl alcohol, *Pov-I-A* povidone-iodine plus 70% ethyl alcohol, *CHX* chlorhexidine

Table 2 Adjunctive tests performed on blood cultures or directly on whole blood for detection of bacteremia

Assay	Manufacturer	Principles of the assay	Pathogens detected	Turnaround time (h)
Assays performed on positive blood cultures				
Non-molecular				
Tube coagulase	Various	Detects the free extracellular enzyme coagulase produced by *Staphylococcus aureus*	*Staphylococcus aureus* (does not differentiate MSSA from MRSA)	2–4
Thermonuclease	Various	Detects a heat stable DNAse specific for *Staphylococcus aureus*	*Staphylococcus aureus* (does not differentiate MSSA from MRSA)	2–4
MicroPhage assay	MicroPhage, Longmont, CO, USA	Lytic phage specific for *Staphylococcus aureus* is detected by an immunoassay	Differentiates MSSA, MRSA from CoNS	5
MALDI-TOF MS		Proteomic profiles are detected by matrix-assisted laser desorption-ionization time-of-flight mass spectrometry	N/A	N/A
Molecular non-amplified				
PNA-FISH	AdvanDx, Woburn, MA, USA	Fluorescence-based hybridization with peptide nucleic acid probes	10+ pathogens	1.5–3
AccuProbe	Gen-Probe	Chemiluminescent DNA probes that detect rRNA	*Staphylococcus aureus*	2.5
Molecular amplified				
StaphSR	BD GeneOhm, San Diego, CA, USA	A multiplex real time PCR test assay that amplifies specific target sequences of *S. aureus* and a specific target near the *SCCmec* insertion site (*orfX* junction) in MRSA	*Staphylococcus aureus*—differentiates MSSA from MRSA	2.5–3
Xpert MRSA/SA	Cepheid Diagnostics	Real-time PCR assay that detects sequences in the staphylococcal protein A (*spa*) gene, the SCC*mec* inserted into the *S. aureus* chromosomal *attB* insertion site and the *mecA* gene	*Staphylococcus aureus*—differentiates MSSA from MRSA	1
Prove-it sepsis	Mobidiag, Helsinki, Finland	Multiplex PCR combined with microarray	50 different pathogens	3
Ibis	Ibis Biosciences	Broad range PCR combined with electrospray ionization mass spectrometry.	Theoretically hundreds of pathogens	8
Assays performed directly on whole blood				
SepsiTest	Molzyn, Bremen, Germany	Broad range PCR followed by sequencing	300 different pathogens	8–12
LightCycler SeptiFast	Roche Molecular Systems, Branchburg, NJ, USA	A multiplex real-time PCR assay that uses dual-fluorescent resonance energy transfer (FRET) probes targeting the species specific internal transcribed spacer (ITS) regions of bacteria and fungi	25 different pathogens	3–30
Vyoo	SIRS Lab, Jena, Germany	Multiplex PCR with gel electrophoresis	40 pathogens along with some resistance genes	8

Data obtained from Refs. [107–130]

N/A not available

be more applicable to larger university-associated and teaching hospitals.

Current and future adjunctive tests

Currently there are numerous adjunctive tests that can be performed directly from positive BC bottles that enhance traditional methods of organism identification and resistance testing. In general, these tests lead to more rapid diagnosis, but their utility in some cases has been limited by cost. Available tests are primarily pathogen-specific and can be divided into conventional methods, molecular non-amplified tests, and nucleic acid amplification techniques. Table 2 summarizes some of these assays. A brief discussion is provided below but the reader is referred to the

review by Mancini et al. [108] for a more comprehensive discussion of this topic.

Perhaps the most commonly used conventional methods are the direct tube coagulase test and the thermonuclease test for differentiation of *Staphylococcus aureus* from coagulase-negative staphylococci. Recent literature on the 4-h tube coagulase tests cite sensitivities ranging from 80 to 96% and specificities of 99.7–100% [109–111]. Therefore a positive result can be assumed to be accurate, but a negative result needs confirmation by another method. Elaboration of a heat-stable DNase is unique to *S. aureus.* Performance of this simple test directly from positive BCs was first described by Ratner et al. [112]. Recent literature reports excellent sensitivity and specificity [113, 114]. Both tests are used together in the author's (KC) laboratory and the combined sensitivity and specificity are 88 and 100%, respectively. Limitations include the fact that these tests do not differentiate methicillin-resistant from methicillin susceptible *S. aureus.*

Several novel techniques are currently in development and/or in various phases of testing. One assay manufactured by MicroPhage (Longmont, CO, USA) discriminates MSSA, MRSA, and coagulase negative staphylococci from positive BCs by utilizing a lytic phage. The bacteriophage in this case is targeted to *Staphylococcus aureus* in the test specimen. The bacteriophage finds receptors on the surface of the target bacteria and attaches in two steps. Once the phage is irreversibly bound and the membrane is compromised the phage injects its nucleic acid into the bacterium. Replication occurs and thousands of progeny bacteriophages are produced. Lysis of the infected bacterium releases high concentrations, as much as 5-log amplification of the signal occurs in minutes. The MicroPhage test uses an immunoassay to measure the increase in concentration of bacteriophage as a surrogate to assess bacterial presence. The test requires an incubation step to facilitate lytic bacteriophage amplification. The detector is designed with antibodies specific to the test bacteriophage, and calibrated such that a known concentration will produce a visible line in the "Identification" immunoassay test device. There are no publications on this technique.

Proteomic techniques such as matrix-assisted laser desorption-ionization time-of-flight (MALDI–TOF MS) identify bacteria and fungi and some resistance and virulence factors on the basis of the organism's proteomic profiles. This technique will most likely be applied to positive BC bottles for pathogen identification and not for direct testing of whole blood [115].

Molecular non-amplified

Peptide nucleic acid fluorescence in situ hybridization (AdvanDx, Woburn, MA, USA) uses fluorescein-labeled probes that target pathogen-specific 16SrRNA of bacteria or 26SrRNA of yeast. In the USA, the following FDA approved assays are currently available: *S. aureus*/coagulase negative staphylococci dual probe; *S. aureus*; *Enterococcus faecalis*/OE (other enterococci) dual probe; *Candida albicans*; *Candida albicans*/*Candida glabrata* dual probe; *E. coli*/*P. aeruginosa* dual probe; EK/*P. aeruginosa* dual probe; and the yeast traffic light (triple probe). Several outcome studies have been performed primarily at one medical center where the PNA-FISH results were reported to a physician responsible for appropriate antibiotic utilization. The authors of those studies demonstrated reduction in vancomycin utilization and shorter length of stay when implementing the *S. aureus* PNA FISH assay [116]. In a second study the same authors demonstrated a decrease of 2.6 days in species level identification with the PNA-FISH enterococcus dual probe and lower mortality in patients with *Enterococcus faecium* [117]. In addition, a reduction in costs because of less caspofungin use when the PNA FISH *C. albicans* probe was implemented was demonstrated in studies by Forrest et al. and Alexander et al. [118, 119].

Another non-amplified probe method that has been evaluated for use directly on positive BCs is the rapid chemiluminescent non-amplified DNA probe that targets rRNA of *S. aureus* for culture confirmation (Gen-Probe). Two published studies on its use on positive BCs are available but with vastly different results [120, 121]. Both studies report specificity of 100% but one center reported an accuracy of only 80.8% [120] compared with 99.8% in the other center [120]. The reasons for the differences are not clear and neither study reported outcome data associated with assay performance in their respective centers [120, 121].

Molecular amplified

Several pathogen-specific nucleic acid amplification assays are available for differentiation of methicillin susceptible *S. aureus* from MRSA and in some cases coagulase negative staphylococci. The first of these to obtain FDA approval in the USA was the BD-GeneOhm™ StaphSR assay. This assay is a multiplex real-time PCR test that is run on the SmartCycler® instrument. The assay amplifies specific target sequences of *S. aureus* and a specific target near the *SCCmec* insertion site (*orfX* junction) in MRSA (Table 2). There are several publications on the performance of the test. While one clinical study [122] performed on 300 BCs has reported excellent performance characteristics (sensitivity for MSSA and MRSA of 98.9% and 100%, respectively), others have noted some limitations. Two studies have noted the failure to detect certain SCC*mec* types and the misidentification of revertant strains, that is, those

strains with deleted or non-functional *mecA* genes that harbor residual fragments of the SCC*mec* cassette [123, 124]. Other more practical considerations regarding this assay are the amount of time required to obtain results (2.5 h) and the expense to the laboratory, which is particularly important when small sample sizes per test run are processed. It is likely that most laboratories perform batch tests. One group has recently reported a modification of this assay in which freezing the master mix results in less reagent waste [125]. There is a single publication on the Cepheid Xpert MRSA/SA BC assay [126]. The primers and probes in this assay detect sequences in the staphylococcal protein A (*spa*) gene, the SCC*mec* inserted into the *S. aureus* chromosomal *attB* insertion site, and the *mecA* gene. In this study, sensitivity and specificity for *S. aureus* detection were 100% and 98.6%, respectively, and for MRSA detection the values were 98.3% and 99.4%, respectively [126]. Although false positives because of revertant strains in pure culture are not an issue with this assay, false positives may occur when testing both a methicillin resistant coagulase-negative staphylococcus and an isolate with a SCC*mec* empty cassette variant together in the same sample [126]. The frequency with which this situation occurs varies with geographical location, but, in general, it is expected to be low. This assay has the advantages of rapid turn around time (60 min) and random access, but the price per test and initial capital expense are prohibitive for many laboratories. Although not yet FDA-cleared in the USA, other investigators have developed real-time assays on the LightCycler instrument (Roche, Mannheim, Germany) targeting the *mecA* gene and the *S. aureus* specific *nuc* gene encoding nuclease [127].

Broad-based assays

Broad-based assays that target several different pathogens simultaneously are available in Europe and in some cases on a research use basis in the USA. These can be classified as those assays that are applied to positive BC bottles and those assays that test whole blood directly (Table 2). An in-depth discussion of these is beyond the scope of this manuscript and the reader is referred to the review by Mancini et al. [108]. Broad-range PCR assays that target universal genes (for example the 16SrRNA gene of bacteria) have the disadvantage that further identification procedures using sequencing, pathogen-specific PCR, or other methods must be performed after the initial amplification assay. In addition, these methods have been hampered by false-positive results that may truly reflect enhanced sensitivity of the molecular method or may reflect detectable nucleic acid from non-living or clinically insignificant microbes [128]. To address this issue, some investigators have combined PCR with mass spectrometric methods that analyze the base composition of the amplified products that identify specific pathogens after the initial PCR step. Specific assays include broad-based PCR using multiple primers followed by high-performance electrospray ionization mass spectrometry (ESI–MS) (Ibis Biosciences) and MALDI–TOF MS [129, 130]. The Prove-it sepsis assay (Mobidiag, Helsinki, Finland) combines broad-range PCR with a microarray-based platform that covers the 50 most common Gram-negative and Gram-positive bacterial species encountered in positive BCs. A recent clinical study performed in two large institutions in Europe using 2107 positive BCs demonstrated that 86% of the bottles contained a pathogen covered by the assay [131]. Overall this assay had a clinical sensitivity of 94.7% and a specificity of 98.8%. Results were also available 18 h sooner than with conventional methods [131].

Several assays are now available outside the USA for detection and identification of pathogens directly from whole-blood samples without the culture amplification step. The most published of these is the LightCycler SeptiFast test (Roche Molecular Systems, Branchburg, NJ, USA). This is a multiplex real-time PCR assay that uses dual-fluorescent resonance energy transfer (FRET) probes targeting the species-specific internal transcribed spacer (ITS) regions of bacteria and fungi. A complete description of the assay and its performance can be found elsewhere [132, 133]. The SepsiTest (Molzym, Bremen, Germany) uses broad-range PCR targeting 16SrDNA of bacteria and 18SrDNA of fungi. The samples are then run on an agarose gel and sequencing is performed, making the test vulnerable to contamination [134]. Although this enables a broad approach to pathogen detection, the TAT is longer that with the LightCycler test. The Vyoo (SIRS-Lab, Jena, Germany) is a multiplex PCR-based assay targeting 35 bacterial species and a few resistance markers [133]. The analytical sensitivities of these assays range from 3 to 40 CFU/ml [108, 133]. They are all limited by high cost and, as mentioned for some, by the long TAT and potential for contamination.

The results of these studies reviewed here are encouraging and in the future, laboratories are likely to see refinement and expansion of the techniques that will enable a broader range of organisms to be detected. Present and future ability to detect resistance markers and virulence factors by use of the assays, possibly combined with biomarkers of sepsis, will greatly improve the diagnostic and therapeutic approaches to management of patients with bacteremia and sepsis. Hopefully, reduction in size of the footprints of the instruments and reduced cost will also be realized, enabling broader use of these techniques by smaller and mid-size laboratories.

Biomarkers of sepsis

The need for early recognition of bacteremia and sepsis has been described previously [84]. Although the SIRS criteria were developed to improve physicians' ability to predict sepsis, these criteria unfortunately lack specificity for infection [135]. Some studies suggest that various laboratory markers such as interleukin (IL)-6, IL-8, IL-2, C-reactive protein, and procalcitonin, may be useful in predicting sepsis and organ failure in patients with community-acquired sepsis. [136–138] One study emphasized that the use of multiple markers might be superior to the use of a single marker only [136]. Although the data presented in these studies are promising, more recent data have demonstrated that laboratory markers of early and/or advanced inflammation may not improve the prediction of positive BCs [139].

However, the authors of this review believe that many of these markers of inflammation have indeed been shown to be of utility in the management of infections, including BSI and sepsis. Additional studies will, hopefully, provide more information on the use of these markers to predict bacteremia and sepsis, or provide guidance on the prediction and interpretation of positive BCs.

Concluding remarks

It is apparent from the foregoing discussion that the current state of the art of diagnosis of BSI remains less than optimum from a physician's perspective, considering the need for ultra-rapid prediction and/or detection of bacteremia. However, at the same time, the currently available continuous monitoring BC systems are excellent test methods for timely detection of microorganisms in BCs. With collection of an adequate volume of patient blood, most organisms causing bacteremia and/or fungemia (>90%) can be detected within the first 24–36 h of incubation. Quality assurance and avoidance of BC contamination must be emphasized when BCs are collected from critically ill patients, particularly in emergency room settings. Although molecular techniques may not yet be sensitive enough to directly detect organisms from patients' blood samples, a wide array of newer molecular techniques can rapidly identify organisms in BCs which test positive. One must, however, consider the possibility of detecting microbial DNA in negative BCs when using highly sensitive molecular tests. With regard to the ability to predict bacteremia and sepsis at the bedside, the assessment and interpretation of clinical signs and symptoms together with a variety of inflammatory markers may guide physicians in their decision making; however, the gold standard for detection and proof of a bacteremia remains the collection and preparation of a BC.

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