Review Article

Role of the Microbiology Laboratory in the Diagnosis of Sepsis

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Management of patients with sepsis remains a challenge for clinicians despite advances in medical interventions. Sepsis is associated with high morbidity and mortality. Lack of early diagnosis of sepsis is at the core of the issue. Sepsis requires early diagnosis and prompt implementation of the treatment and the clinical microbiology lab is key in the process. Blood cultures historically represent the "gold standard" for diagnosis of septicemia. Pre-analytic factors that influence the recovery of an organism from the blood include the blood volume collected, the number of blood culture bottles collected, and avoidance of skin flora contamination during collection. Numerous methods of commercially available detection systems are available to clinical microbiology labs to choose from and they include both manual methods and automated continuous-monitoring systems. Time to detection of positive cultures varies with the method utilized and the organism recovered. Gram stain, acridine orange stain and other staining techniques can be employed to visualize organisms and the interpretation of the stain is reported immediately to the physician for targeted treatment. Although blood agar and chocolate agar culture plates are generally inoculated for organism isolation, special techniques and extended incubation time may be required for fastidious organisms. Limitations of culture-based methods for detection of sepsis include that positive results require hours to days of incubation. No one culture medium or system in use has been shown to be best suited to the detection of all potential bloodstream pathogens. Some microorganisms grow poorly, or not at all, using blood culture systems and conventional blood culture media. Questions remain for microbiology laboratorians, will culture-based systems continue to be the methods of choice or will they be replaced by molecular techniques or newer diagnostic methods?

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Introduction

Despite the progress made in the pathogenesis of sepsis and advances achieved in medical interventions, the management of sepsis remains a challenge for clinicians. The core problem that precludes the promotion in the management of sepsis is the lack of early and precise prediction. Sepsis requires early diagnosis and prompt implementation of the treatment. Bacterial infections are common and are associated with high morbidity and mortality. The detection and identification of microorganisms is necessary to confirm the diagnosis and tailor antibiotic therapy. Blood culture has been long recognized as the gold standard for definitive diagnosis of bacterial and fungal septicemia. However, this method is too slow and labor intensive to significantly influence treatment and the successful management of the patient's condition. Moreover, recent and more effective diagnostic technologies have been introduced into clinical practice.

Specimen Collection and Transport

The proper collection and transport of clinical specimens is critical for the isolation, and identification of the microorganisms that cause septicemia. Blood cultures are collected as a set, consisting of aerobic and anaerobic culture bottles. The first set is collected when the physician's order is initiated and is typically followed by a second set 30 minutes later.[1] Two blood culture sets are usually sufficient for the recovery of most clinically significant microorganisms. A request for "second site" blood cultures obtained concurrently on opposite arms is useful when the physician suspects bacteremia due to a local internal infection such as respiratory or urinary tract infections. However, a second site culture is not an effective tool for routine blood cultures orders and provides relatively little information that properly spaced, timed blood cultures cannot provide.[2] It has been reported that the cumulative yield of pathogens from three blood culture sets (2 bottles per set), with a blood volume of 20 ml in each set (10 ml per bottle), was 73.1% with the first set, 89.7% with the first two sets and 98.3% with the first three sets. However, to achieve a detection rate of >99% of bloodstream infections, as many as four blood culture sets may be needed.[3,4,5] Transport of the inoculated bottles and the blood culture request to the clinical microbiology laboratory should be as quickly as possible, preferably within 2 hours.[6] Delay in testing the inoculated bottles may potentially lead to an increased risk of false negative results.

Recommended Volume and Numbers of Blood Cultures

The optimal recovery of the etiologic agents from blood depends on culturing an adequate volume of blood. The collection of a sufficient quantity of blood improves the detection of pathogenic organisms present in low quantities.[7] Blood culture bottles are designed to accommodate the recommended blood-to-broth ratio (1:5 to 1:10) with optimal blood volume.[1] Commercial continuously monitoring blood culture systems may use a smaller blood-to-broth ratio (<1:5) due to the addition of sodium polyanethole sulfonate (SPS) which inactivates inhibitory substances which are present in blood.[8] For an adult, the recommended volume of blood to be obtained per culture is 20 to 30 ml. Since each set includes an aerobic and an anaerobic bottle, each bottle should be inoculated with approximately 10 ml of blood.[8] This volume is recommended to optimize pathogen recovery when the bacterial/fungal burden is less than 1 Colony Forming Unit (CFU) per ml of blood, which is a common finding. It is also generally recommended that two or three culture sets (two bottles per set) are used per septic episode, i.e. 40 to 60 ml of blood collected from an adult patient for the 4 to 6 bottles, with 10 ml per bottle.[5] For each additional milliliter of blood cultured, the yield of microorganisms recovered from adult blood increases in direct proportion up to 30 ml. This correlation is related to the relatively low number of CFU in a milliliter of adult blood.

The optimal volume of blood to be obtained from infants and children is less well prescribed, however, available data indicate that the yield of pathogens also increases in direct proportion to the volume of blood cultured.[10] The recommended volume of blood to collect should be based on the weight of the patient (see Table 1), and an aerobic bottle should be used, unless an anaerobic infection is suspected.[10] However, smaller volumes of blood should still be cultured, because some infants will have high levels of bacteremia of greater than 1,000 CFU/mL.[5] Specially formulated blood culture bottles are commercially available for use in children <2 years

of age. They are specifically designed to maintain the blood-to-broth ratio (1:5 to 1:10) with smaller blood volumes and have been shown to improve microbial recovery.[9]

Blood culture collection protocols are designed to detect bacteremia based on a suspected disease state or symptoms that the patient is exhibiting. Commonly accepted protocols for obtaining blood cultures are as follows:[1,6,9]

1. Systemic and localized infections

(a) Suspected acute sepsis, meningitis, osteomyelitis, arthritis or acute untreated bacterial pneumonia. Obtain two blood culture sets from two separate sites.

(b) Fever of unknown origin (FUO). Obtain two blood culture sets initially; 24 to 36 hours later, obtain two additional sets. More than four sets are not necessary.

(c) Suspected early typhoid fever and brucellosis (rarely isolated). Obtain three blood culture sets over 24 to 36 hours.

2. Infective endocarditis

(a) Obtain three blood culture sets at three separate venipuncture sites during the first one to two hours of evaluation and begin antimicrobial therapy; if all are negative 24 hours later, obtain two more sets.

(b) Culture negative endocarditis. Consult with the microbiology department after five negative cultures. Serum could be analyzed for *Bartonella*, *Coxiella*, and *Chlamydia* species antibodies. Additional testing such as microscopy, culture, histology, and relevant polymerase chain reaction can be performed.[4]

Table 1: Blood volumes suggested for cultures.[10]

Weight of patient		Patient's total blood volume	Recommended volume of blood for culture		Total volume for culture	% of patient's total blood volume
Kg	**Ib**	**ml**	**Culture no. 1**	**Culture no. 2**	**ml**	**%**
<1	<2.2	50-99	2	--	2	4
1.1 – 2	2.2 – 4.4	100-200	2	2	4	4
2.1 – 12.7	4.5 – 27	>200	4	2	6	3
12.8 – 36.3	28 – 80	>800	10	10	20	2.5
>36.3	>80	>2,200	20 30	20 – 30	40 – 60	1.8 – 2.7

Collection Procedures and Timing

Optimally, clinical specimens should be obtained before antimicrobial therapy commences to avoid loss of viability of the etiological agents. Treatment of the patient, however, should not be delayed while awaiting collection of specimens or results from the laboratory. A specimen should be obtained in all suspect cases as bacterial pathogens can still be detected even after antimicrobial therapy has begun.

Each laboratory will have a specific procedure for blood culture collections. Variations may include site cleansing technique and types of equipment used. A variation in procedure does not indicate the laboratory method is improper but illustrates variations in protocols and manufacturer's directions.

The most critical step in collecting a blood culture is the proper cleansing of the site. It is imperative for quality test results that contaminating bacteria are not introduced into the specimen being collected. The site selected for blood culture collection must be prepared by sterile technique, which requires different antiseptics than routine venipuncture. The 2017 Clinical Laboratory Standards Institute (CLSI) standards no longer recommend using a circular target motion method for cleansing the arm.[6] Their studies suggest that the back and forth friction is superior to the circular concentric cleansing. The CLSI standards recommend cleansing the site with friction using a clean gauze pad with 70% isopropyl alcohol solution or a commercially prepared alcohol pad. The alcohol must be allowed to air dry for approximately one minute prior to venipuncture to properly disinfect the site, prevent hemolysis of the specimen, and avoid discomfort for the patient.[6]

Contamination

Contamination of blood cultures during the collection process can produce a significant level of false-positive results, which can have a negative impact on patient outcome.[11] A false-positive is defined as growth of bacteria in the blood culture bottle that were not present in the patient's bloodstream and were most likely introduced during sample collection. Contamination can come from a number of sources: the patient's skin, the equipment used to take the sample, the hands of the person taking the blood sample, or the environment. Contamination forces clinicians to determine whether the organism represents a clinically significant infection associated with great risk of morbidity and mortality or a false-positive result of no clinical consequence.

Certain microorganisms such as coagulase-negative staphylococci, viridans group streptococci, *Bacillus* spp., *Cutibacterium* spp. (previously *Propionibact -erium* spp.), diphtheroids, and *Micrococcus* spp. rarely cause bloodstream or severe bacterial infections.[8] These organisms are common skin contaminants although they are capable of causing serious infection in the susceptible host. Their detection in a single blood culture set can reasonably be considered a possible contaminant without clinical significance. However, coagulase-negative staphylococci are the primary cause of both catheter and prosthetic device-associated infections and may be clinically significant in up to 20% of cases.[12]

A contaminated blood culture can result in unnecessary antibiotic therapy, increased length of hospitalization and higher health care costs.[7,13] It has been found that each false-positive result can lead to:

- Increased length of stay – on average 1day
- 39% increase in intravenous antibiotic charges
- 20% increase in laboratory charges
- 3 days longer on antibiotics
- Other charges

Guidelines for blood culture quality recommend no more than 2 to 3% contamination rate; however rates up to 12% have been reported in the literature.[14]

Detection Systems

The selection of the most appropriate blood culture system for use in the laboratory depends on numerous factors, including costs, personnel qualifications, and patient demographics.

1. Manual Detection Systems

Manual detection systems are still available from many commercial sources. Aerobic blood and anaerobic blood culture bottles are inoculated with a patient's blood sample and usually incubated for 7 days. Each bottle is examined daily for macroscopic evidence of microbial growth (e.g., hemolysis, turbidity of the media, gas production, or formation of discrete colonies). An aliquot of the contents of the aerobic bottle is Gram stained and sub-cultured after the first overnight incubation. A terminal subculture is usually performed at the end of the

incubation period. Manual systems are flexible and require no purchase of expensive instruments but are labor intensive.

A number of systems are commercially available such as the biphasic Septi-Chek system (Roche Diagnostics, Div. Hoffmann-La Roche Inc., Nutley, N.J.), Opticult blood culture systems (Becton Dickinson Microbiology Systems, Cockeysville, MD, USA), and the Oxoid Signal broth displacement blood culture system (Oxoid, USA, Inc., Columbia, MD). The Septi-Chek and Opticult systems utilize agar-coated paddles or slants attached to the broth-containing culture bottle and allow subcultures to be performed daily or more frequently by inverting the blood-broth mixture to inoculate the agar. The Oxoid Signal system is a one-bottle system. After blood is inoculated into the bottle, a clear-plastic cylindrical signal device is attached to the top of the bottle; a long needle from the lower end of the device extends below the surface of the blood-broth mixture, creating a closed system. Gases produced as a by-product of microbial growth increase the pressure in the headspace and force some of the blood-broth mixture through the needle into the cylinder, thereby "signaling" a positive culture.

2. Automated Detection Systems

Automated blood culture systems were introduced in the 1970s. There are several manufacturers of such devices that demonstrate similar performance characteristics. Until recently, the BACTEC (Becton Dickinson, Sparks, MD, USA) automated systems were the only products commercially available in the United States; these systems were initially equipped with radiometric detection and media, followed in the mid-1980s by the nonradiometric instruments and media. Both systems (as well as in the newer BACTEC and BacT/Alert (Organon Teknika, Turnhout, Belgium) continuous-monitoring devices) are based on the utilization of carbohydrate substrates in the culture media and subsequent production of CO_2 by growing microorganisms. For the radiometric system, the instrument detects $^{14}CO_2$ in the bottle headspace, and for the nonradiometric system, CO_2 is detected by infrared spectrophotometry.[1] For both systems, bottles are loaded onto the detection portion of the instrument, where needles perforate the bottle diaphragm and sample the gas contents of the headspace once or twice daily; a bottle is flagged as positive if the amount of CO_2 in the bottle exceeds a threshold value. The flagged bottle is then removed from the instrument, and an aliquot from the bottle is Gram-stained and sub-cultured for further testing.

All commercially available continuous-monitoring blood culture systems have several features in common including self-contained modular incubation; agitation; detection units, controlled by a single computer; lack of the need for manual manipulation of culture bottles once they have been loaded into the instrument; instrument monitoring of microbial growth at constant intervals of time; and culture bottles that each accept 10 mL of blood. The BacT/Alert and BACTEC systems detect the production of CO_2 as change in pH; this is accomplished by colorimetric methods in the BacT/Alert system and by means of a fluorescent sensor in the BACTEC system. VersaTREK (VTI) (TREK Diagnostic Systems, Cleveland, OH, now ThermoFisher, Waltham, MA) is an automated continuous monitor detection system of positive cultures evolved from the original Difco ESP System. It is based on measuring pressure changes in the bottle headspace (due to consumption and production of gas by the microorganism) with an external pressure sensor.

Positive Blood Culture

A positive blood culture is a critical result and must be reported as soon as available, due to the immediate impact on patient care decisions. Once identified as a positive blood culture, a Gram stain and subculture is performed on the positive blood culture bottle. Gram stain is used to differentiate between different types of bacteria based on the biochemical properties of their cell walls. The Gram stain reaction and the morphology of the organism should be reported immediately to the physician. If a sample is Gram stain negative, no organisms are visible, no report is made to the clinician unless there is growth on subculture. The Danish clinical microbiology society recommends the use of wet mounts in association with Gram staining for positive blood cultures to determine the morphology of organisms, gross structure, and motility.[15]

Alternative staining techniques may be employed, including the use of acridine orange (to stain bacterial nucleic acids) or the use of carbol fuschin as an alternative to safranin as a counterstain in the Gram stain protocol to enhance the staining of *Campylobacter*, *Helicobacter* and *Brucella*. Acridine

orange (AO) stain has been shown to be a very sensitive, rapid and simple method in finding bacteria in blood cultures although a fluorescent microscope is needed. [16] The stain is inexpensive, easy to perform and effective in detecting bacteria in the first hours of incubation when growth is light.[16]

Media for Sub-cultivation

Subcultures of positive blood cultures should be initiated immediately in order to provide further organism identification and performing antibiotic susceptibility testing. An overnight agar medium subculture is the initial step in the microbial identification of pathogens causing bacteremia. This conventional culture method is time-consuming, and several days are usually required for microbial recovery, biochemical identification of the bacterial isolate, and determination of antimicrobial susceptibility.

With the standard method for identification and susceptibility testing, a small volume of blood culture fluid is inoculated onto blood agar and chocolate agar plates. The inoculated plates are incubated at 35°C in 5% CO_2 to enable bacterial colonies to develop. After overnight incubation, organism identification and antimicrobial susceptibility can be performed.

In most instances, organisms can be isolated with standard blood culture techniques without the need for special protocols or procedures but is not true for all fastidious bacteria. *Abiotrophia* and *Granulicatella* are usually detected with automated blood culture instruments, but do not grow well on standard media, as they require pyridoxal or cysteine for growth. This can be accomplished by co-cultivation with staphylococci, by the use of pyridoxal impregnated disks placed on the surface of standard blood agar plates, or by the use of specially supplemented or enriched media. Special techniques will be needed for the cultivation of *Bartonella* spp., including lysis centrifugation methods and/or serological investigations.[17] Similarly, *Legionella* spp. require buffered charcoal yeast extract (BCYE) for optimal growth. Recovery of *Legionella* can be achieved by sub-culturing standard blood culture medium that has been incubated according to the standard protocol for 5 days into BCYE, or by utilizing BCYE in conjunction with lysis centrifugation methods.

Fastidious Organisms

Fastidious microorganisms are rarely implicated in septicemia, but when they are isolated from blood cultures, they often represent serious infection. In routine circumstances, blood cultures need not be incubated for >7 days.[18] Incubation periods longer than 7 days may be useful when fungemia or bacteremia due to fastidious organisms is suspected. Longer incubation periods may also be useful for patients with suspected endocarditis who have been treated with antimicrobial agents before blood cultures are performed. Studies have noted that such extended incubation periods rarely increase yield.[3,4,7] However, if all blood culture bottles are negative after 5 days, and infectious endocarditis is still suspected, all bottles should be sub-cultured to chocolate agar. Mycobacterial blood cultures should be incubated for 4 weeks.

Nonculturable Organisms

Sepsis is typically presumed to be bacterial in origin until proven otherwise, but frequently bacterial cultures ultimately remain negative. Although viruses may be important causative agents of culture-negative sepsis worldwide, the incidence, disease burden and mortality of viral-induced sepsis is poorly elucidated. Consideration of viral sepsis is critical as its recognition carries implications on appropriate use of antibacterial agents, infection control measures, and, in some cases, specific, time-sensitive antiviral therapies.

Commonly Isolated Organisms

Parameters that may be useful in interpreting results include the identity of the microorganism, the presence of more than one blood culture positive for the same microorganism, and the presence of the same microorganism as that found in the blood from another normally sterile site. Microorganisms that almost always represent true infection when isolated from the blood include *Staphylococcus aureus*, *Escherichia coli* and other *Enterobacteriaceae*, *Pseudomonas. aeruginosa*, *Streptococcus pneumoniae*, and *Candida albicans*. Isolates from

blood that rarely represent true infection include *Corynebacterium* spp., *Bacillus* spp., and *Cutibacterium acnes*.[1,13,19]

Coagulase-negative staphylococci are particularly problematic, not only because they are so ubiquitous, but also because 12%-15% of the blood isolates are pathogens rather than contaminants.[18,19] Some authorities have suggested that the number of bottles positive in a culture set is a predictor of the clinical significance of an isolate.[18,19] However, several researchers have found that this criterion is unreliable, at least for coagulase negative staphylococci.[18,19]

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

Limitations of "Gold Standard"

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

References

1. Leber AL. Clinical Microbiology Procdeures Handbook, 4th Edition. Washington DC: ASM Press, 2016.
2. Wiggers JB, Xiong W, Daneman, N. Sending repeat cultures: Is there a role in the management of bacteremic episodes? BMC Infectious Diseases 2016; 16: 286.
3. Lee A, Mirrett S, Reller LB, et al. Detection of Bloodstream Infections in Adults: How Many Blood Cultures Are Needed? J Clin Microbiol 2007; 45:3546-3548.
4. Lamy B, Dargère S, Arendrup MC, et al. How to Optimize the Use of Blood Cultures for the Diagnosis of Bloodstream Infections? A State-of-the Art. Front Microbiol 2016; 7: 697.
5. Tille PM, Bailey and Scott's Diagnostic Microbiology, ed 14. St. Louis, Missouri: Elsevier, 2017.
6. Principles and Procedures for Blood Cultures, GP41. Wayne, Pennsylvania, Clinical and Laboratory Standards Institute (CLSI), 2017.
7. Cockerill FR 3rd, Wilson JW, Vetter EA. Optimal testing parameters for blood cultures. Clin Infect Dis 2004; 38:1724-1730.
8. Henning C, Aygül N, Dinnétz P, et al. Detailed Analysis of the Characteristics of Sample Volume in Blood Culture Bottles. J Clin Microbiol 2019; 57 (8).
9. Cheruvanky A, Kirn TJ, Weinstein MP. Cost-Effectiveness of 30- Compared to 20-Milliliter Blood Cultures: a Retrospective Study. J Clin Microbiol 2015;54 (1) 64-67.
10. Kellogg JA, Manzella JP, Bankert DA. Frequency of Low-Level Bacteremia in Children from Birth to Fifteen Years of Age. Clin Microbiol 2000; 38(6): 2181–2185.
11. Mariani B, Corbella M, Seminari E, et al. Evaluation of a model to improve collection of blood cultures in patients with sepsis in the emergency room. Eur J Clin Microbiol Infect Dis 2018; 37(2):241-246.
12. Flayhart D, Borek AP, Wakefield T, et al. Comparison of BACTEC Plus blood culture media to BacT/Alert FA blood culture media for detection of bacterial pathogens in samples containing therapeutic levels of antibiotics. J Clin Microbiol 2007; 45:816-821.
13. Carroll KC, Pfaller MA, Landry ML, et al. Manual of Clinical Microbiology, ed 12. Washington DC: ASM Press, 2019.
14. Hughes JA, Cabilan CJ, Williams J, et al. The effectiveness of interventions to reduce peripheral blood culture contamination in acute

care: a systematic review protocol. Syst Rev 2018; 7: 216.

15. Strand CL. Positive Blood Cultures Can We Always Trust the Gram Stain? Am J Clin Pathol 2006; 126:671-672

16. Behera B, Mathur P, Gupta B. Blood culture gram stain, acridine orange stain and direct sensitivity-based antimicrobial therapy of bloodstream infection in patients with trauma. Indian J Med Microbiol 2010; 28(2):138-42.

17. Parikh HR, De AS, Baveja SM. Comparison of the Lysis Centrifugation Method with the Conventional Blood Culture Method in Cases of Sepsis in a Tertiary Care Hospital. J Lab Physicians 2012; 4:89-93.

18. Kirn, TJ, Weinstein, MP. Update on blood cultures: how to obtain, process, report, and interpret. Clin Microbiol Infect 2013; 19: 513–520.

19. Opota O, Croxatto A, Prod'hom G, et al. Blood culture-based diagnosis of bacteraemia: state of the art. Clin Microbiol Infect 2015; 21(4):313-22.