

BLOOD CULTURE

A key investigation for the
diagnosis of bloodstream infections





INTRODUCTION

“...Positive blood culture results either establish or confirm an infectious etiology of a patient’s illness. Moreover, [they also provide] the etiologic agent for AST*, which, in turn, optimizes antimicrobial therapy.”¹

The laboratory detection of bacteremia and fungemia using blood cultures is one of the most simple and commonly used investigations to establish the etiology of bloodstream infections.

Rapid, accurate identification of the bacteria or fungi causing bloodstream infections provides vital clinical information required to diagnose and treat sepsis.

Sepsis is a complex inflammatory process that is largely under-recognized as a major cause of morbidity and mortality worldwide. There are an estimated 49 million cases and 11 million deaths worldwide each year,² meaning that sepsis causes 1 death almost every 3 seconds.³

Early diagnosis and appropriate treatment make a critical difference when it comes to improving sepsis patient outcomes. Chances of survival decline drastically the longer initiation of treatment is delayed. If a patient receives antimicrobial therapy within the first hour of diagnosis, chances of survival are close to 80%; this is reduced by 7.6% for every hour after. Yet, if a patient initially receives inappropriate antimicrobial treatment, they are five times less likely to survive.⁴

This booklet aims to:

- **answer key questions** commonly asked in relation to blood culture
- **provide practical recommendations** for routine blood culture procedures
- **offer an illustrated step-by-step guide** to best blood culture collection practices.

This booklet is intended to be a useful reference tool for physicians, nurses, clinical microbiologists, phlebotomists, laboratory personnel and all other healthcare professionals involved in the blood culture process.

* AST: Antimicrobial Susceptibility Testing

OUR SPECIAL THANKS GO TO



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for her helpful advice and comprehensive review of this booklet.

DEFINITIONS

Bacteremia: the presence of bacteria in the blood. It may be transient, intermittent or continuous.

Blood culture: blood specimen submitted for culture of microorganisms. It enables the recovery of potential pathogens from patients suspected of having bacteremia or fungemia.

Blood culture series: a group of temporally related blood cultures that are collected to determine whether a patient has bacteremia or fungemia.

Blood culture set: the combination of blood culture bottles (one aerobic and one anaerobic) into which a single blood collection is inoculated.

Bloodstream infection (BSI): an infection associated with bacteremia or fungemia.

Contaminant: a microorganism recovered from a blood culture that was introduced during specimen collection or processing and is not considered responsible for BSI (i.e. the isolates were not present in the patient’s blood when the blood was sampled for culture).

Contamination: presence of microorganisms in the bottle that entered during sampling but were not actually circulating in the patient’s bloodstream.

Fungemia: the presence of fungi in the blood.

Sepsis: life-threatening organ dysfunction caused by a dysregulated host response to infection characterized by fever, chills, malaise, tachycardia, etc. when circulating bacteria multiply at a rate that exceeds removal by phagocytosis.⁵

Septic episode: an episode of sepsis or septic shock for which a blood culture or blood culture series is drawn.

Septic shock: a subset of sepsis in which underlying circulatory and cellular metabolism abnormalities are profound enough to substantially increase mortality.⁵

Source: CLSI. Principles and Procedures for Blood Cultures. 2nd ed. CLSI guideline M47. Clinical and Laboratory Standards Institute; 2022 unless otherwise specified.

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1 BLOOD CULTURE ESSENTIALS

1 What is a blood culture?

A blood culture is a laboratory test in which blood, taken from the patient, is inoculated into bottles containing culture media to determine whether infection-causing microorganisms (bacteria or fungi) are present in the patient's bloodstream.

→ Blood cultures are intended to:

- confirm the presence of microorganisms in the bloodstream
- identify the microbial etiology of the bloodstream infection
- help determine the source of infection
- provide an organism for antimicrobial susceptibility testing and optimization of antimicrobial therapy

3 MAIN AIMS OF BLOOD CULTURE*:

- Confirm infectious etiology
- Identify the etiological agent
- Guide antimicrobial therapy

* Adapted from ESCMID (European Society of Clinical Microbiology and Infectious Diseases) guidelines, 2012.⁶

2 Why are blood cultures important?

Blood culture is the most widely used diagnostic tool for the detection of bacteremia and fungemia. It is the most important way to diagnose the etiology of bloodstream infections and sepsis and has major implications for the treatment of those patients.

A positive blood culture either establishes or confirms that there is an infectious etiology for the patient's illness.¹ A positive blood culture also provides the etiologic agent for antimicrobial susceptibility testing, enabling optimization of antibiotic therapy.¹ Sepsis is one of the most significant challenges in critical care, and early diagnosis is one of the most decisive factors in determining patient outcome. Early identification of pathogens in the blood can be a crucial step in assuring appropriate therapy, and beginning

BLOOD CULTURE ESSENTIALS

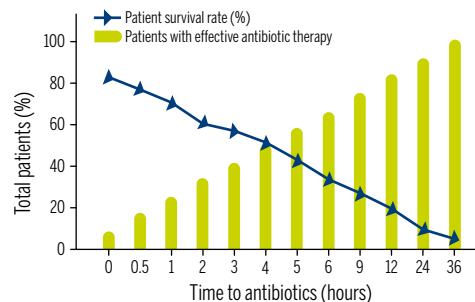
effective antibiotic therapy as early as possible can have a significant impact on the outcome of the disease.^{7,8}

→ Providing adequate antibiotic therapy within the first 24-48 hours leads to:⁹⁻¹³

- decreased infection-related mortality (20-30%)
- earlier recovery and shorter length of hospital stay
- reduced risk of adverse effects
- reduced risk of antimicrobial resistance
- cost reduction (length of stay, therapy, diagnostic testing)

Figure 1: Fast effective antimicrobial therapy increases survival chances¹⁴

Adapted from Kumar A, et al. *Crit Care Med.* 2006;34(6):1589-96. Reproduced with permission.



3 When should a blood culture be performed?

Blood cultures should always be requested when a bloodstream infection or sepsis is suspected.¹⁵

→ Clinical symptoms in a patient which may lead to a suspicion of a bloodstream infection are:¹⁶

- undetermined fever ($\geq 38.3^{\circ}\text{C}$) or hypothermia ($\leq 36^{\circ}\text{C}$)
- shock, chills, rigors
- vomiting
- severe local infections (meningitis, endocarditis, pneumonia, pyelonephritis, intra-abdominal suppuration...)
- abnormally raised heart rate (>90 beats/min)
- low blood pressure (<90 mm Hg)
- raised respiratory rate (>20 breaths/min)

→ Blood cultures should be collected:

- as soon as possible after the onset of clinical symptoms;
- ideally, prior to the administration of antimicrobial therapy.¹⁷

If the patient is already on antimicrobial therapy, recovery of microorganisms may be increased by collecting the blood sample immediately before administering the next dose and by inoculating the blood into bottles containing specialized antimicrobial neutralization media.^{17,18}

4 What volume of blood should be collected?

The optimal recovery of bacteria and fungi from blood depends on culturing an adequate volume of blood. The collection of a sufficient quantity of blood improves the detection of pathogenic bacteria or fungi present in low quantities.

> The volume of blood that is obtained for each blood culture set is the most significant variable in recovering microorganisms from patients with bloodstream infections.^{19,20}

Blood culture bottles are designed to accommodate the recommended blood-to-broth ratio (1:5 to 1:10) with optimal blood volume.²¹ Some commercial blood culture systems use a blood-to-broth ratio that is less than 1:5. This ratio is acceptable because proprietary substances that bind to and inactivate the inhibitory substances are added to blood specimens.¹

→ Adults

For an adult, the recommended volume of blood to be obtained per culture is 20 to 30 mL.^{1,17}

Since each set includes an aerobic and an anaerobic bottle, each bottle should be inoculated with approximately 10 mL of blood. 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 bottle sets** (2 bottles per set) are used per septic episode, meaning, for adults, 40 to 60 mL of blood collected from the patient for the 4 to 6 bottles, with 10 mL per bottle.

For each additional milliliter of blood cultured, the yield of microorganisms recovered from adult blood increases in direct proportion up to 30 mL.²² This correlation is related to the relatively low number of CFU in a milliliter of adult blood.¹

→ Pediatric

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.^{17,23} The recommended volume of blood to collect should be **based on the weight of the patient** (see **Table 1**)²⁴, and an aerobic bottle should be used, unless an anaerobic infection is suspected.²⁵

Recent studies have shown that facultative anaerobes are detected earlier or solely in the anaerobic blood culture bottle in certain pediatric populations suggesting that they should continue to be used. For newborns, infants, and children, the volume of blood drawn should be no more than 1% of the patient's total blood volume.¹

Specially formulated blood culture bottles are commercially available for use in children <2 years of age. They are specifically designed to maintain the usual blood-to-broth ratio (1:5 to 1:10) with smaller blood volumes, and have been shown to improve microbial recovery.¹

Table 1: Recommendations for optimal blood volume for pediatric blood cultures based on self-defined age or weight classes ²⁴	
Adapted from Huber <i>et al.</i> The correct blood volume for paediatric blood cultures: a conundrum? <i>Clin Microbiol Infect.</i> 2020;26(2):168-173. Reproduced with permission.	
Patient weight (kg)	Total blood volume ^a (mL)
≤2.0	1.0 - 4.5
>2.0 - 5.0	1.0 - 6.0
>5.0 - 10.0	1.5 - 10.0
>10.0 - 20.0	6.0 - 23.0
>20.0 - 30.0	≥10.0
Patient age	Total blood volume ^a (mL)
<1 y	>0.5 - 3.0
≥1 - 3 y	1.0 - 4.0
>3 - 10 y	3.0 - 8.0
≥10 y	20.0

^a Minimum and maximum recommended total blood volume to be drawn for paediatric blood culture.

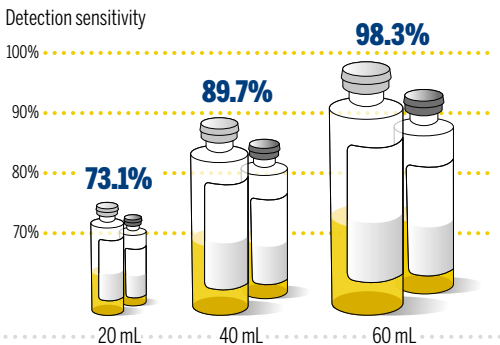
5 How many blood culture sets should be collected?

Since bacteria and fungi may not be constantly present in the bloodstream, **the sensitivity of a single blood culture set is limited.**

Using continuous-monitoring blood culture systems, a study investigated the cumulative sensitivity of blood cultures obtained sequentially over a 24-hour time period. It was observed 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.²⁶

Figure 2: Cumulative sensitivity of blood culture sets²⁶

Adapted from Lee et al. Detection of Bloodstream Infections in Adults: How Many Blood Cultures Are Needed? *J Clin Microbiol.* 2007; 45:3546-3548



➤ A single blood culture bottle or set should never be drawn from adult patients, since this practice will result in an inadequate volume of blood cultured and a substantial number of bacteremic episodes may be missed.^{1, 26}

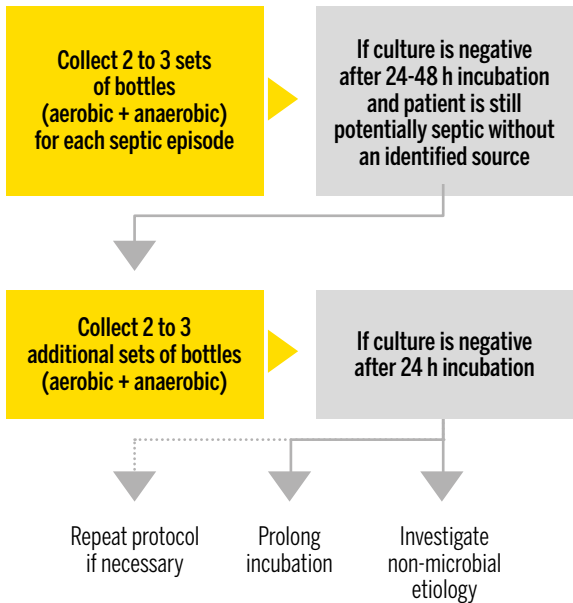
A contaminant will usually be present in only one bottle of a set of blood culture bottles, in contrast to a true bloodstream infection, in which multiple blood culture bottles/sets will be positive.

➤ Therefore, guidelines recommend to collect 2, or preferably 3, blood culture sets for each septic episode.^{1, 6, 17}

If two to three sets are taken and cultures are still negative after 24-48 hours incubation, and the patient is still potentially septic, two to three additional cultures may be collected, as indicated in the following diagram.¹⁷

Figure 3: Recommended number of blood culture sets¹⁷

Adapted from Baron, E.J., et al. Cumitech 1C, Blood Cultures IV. Coordinating ed., E.J. Baron. ASM Press, Washington, D.C. 2005



6 Which media to use?

Microorganisms causing bloodstream infections are highly varied (aerobes, anaerobes, fungi, fastidious microorganisms...) and, in addition to nutrient elements, may require specific growth factors and/or a special atmosphere.

In cases where the patient is receiving antimicrobial therapy, specialized media with antibiotic neutralization capabilities should be used. **Antibiotic neutralization media** have been shown to increase recovery and provide faster time to detection *versus* standard media.²⁷⁻³⁰

It is recommended that each adult routine blood culture set include paired aerobic and anaerobic blood culture bottles.

The blood drawn should be divided equally between the aerobic and anaerobic bottles.

If an anaerobic bottle is not used, it should always be replaced by an additional aerobic bottle to ensure that a sufficient volume of blood is cultured.³¹

→ A blood culture medium must be:

- **sensitive** enough to recover:
 - a broad range of clinically relevant microorganisms, even the most fastidious (*Neisseria*, *Haemophilus*...)
 - microorganisms releasing small amounts of CO₂ (*Brucella*, *Acinetobacter*...)
- **versatile**: able to provide a result for all types of sample collection (adults, infants, patients receiving antibiotic therapy, sterile body fluids...)

→ Which bottle should be inoculated first?

If using a **winged blood collection set**, then the **aerobic bottle should be filled first** to prevent transfer of air in the device into the anaerobic bottle.

If using a **needle and syringe**, inoculate the **anaerobic bottle first** to avoid entry of air. If the amount of blood drawn is less than the recommended volume*, then approximately 10 mL of blood should be inoculated into **the aerobic bottle first**, since most cases of bacteremia are caused by aerobic and facultative bacteria. In addition, pathogenic yeasts and strict aerobes (e.g. *Pseudomonas*) are recovered almost exclusively from aerobic bottles. Any remaining blood should then be inoculated into the anaerobic bottle.¹

* For recommended volumes, see page 6 "What volume of blood should be collected?"

7 Timing of blood cultures

Studies have shown that the time interval between collecting two blood cultures is not considered to be a critical factor as the diagnostic yield remains the same.²¹

Guidelines recommend that the **first two to three sets (2 bottles/set) of blood culture** be obtained either **over a brief time period (e.g. within 1 hour)** or as a **single sample taken at one time**.^{1,17,21} The possible impact that the blood culture collection method used (e.g. single or multiple venipunctures, winged collection set or needle and syringe) may have on contamination rates should be considered.²¹

Drawing blood at spaced intervals, such as 1 to 2 hours apart, is only recommended to monitor continuous bacteremia/fungemia in patients with suspected infective endocarditis or other endovascular (i.e. catheter-related) infections.¹⁷

Two to three additional blood culture sets can be performed if the first two to three blood cultures are negative after 24-48 hours incubation in cases of severe infection or in order to increase detection sensitivity. This also depends on the microorganisms involved: while sensitivity is relatively good for organisms like *Escherichia coli* or *Staphylococcus aureus*, it is lower for *Pseudomonas aeruginosa*, streptococci or fungi.³²

8 How to collect blood cultures

Sample collection is a crucial step in the blood culture process. Standard precautions must be taken, and strict aseptic conditions observed throughout the procedure. Compliance with blood culture collection recommendations can significantly improve the quality and clinical value of blood culture investigations and reduce the incidence of sample contamination and “false-positive” readings.

> A properly collected sample, that is free of contaminants, is key to providing accurate and reliable blood culture results.

It is recommended that blood cultures should be collected only by members of staff (medical, nursing, phlebotomist or technician) who have been fully trained and whose competence in blood culture collection has been assessed.³³

10 Key Steps to Good Sample Collection:

For an illustrated step-by-step, see page 30.

- 1 Prior to use, **examine the bottles** for evidence of damage, deterioration or contamination. Do not use a bottle containing media which exhibits turbidity or excess gas pressure, as these are signs of possible contamination.
- 2 **Check the expiry date** printed on each bottle. Discard bottles that have expired.
- 3 **Strictly follow the collection protocol** in use in the healthcare setting, including standard precautions for handling blood at the bedside.
- 4 Blood culture bottles should be **clearly and correctly labelled**, including patient identification, date and collection time, puncture site (venipuncture or intravascular device).
- 5 For adults, each blood culture set should include **an aerobic and an anaerobic bottle**.
- 6 For adults and older children, blood should be drawn from veins in the antecubital fossae.^{1,21}

- 7 It is recommended to **avoid drawing blood from a venous or arterial catheter**, since these devices can be colonized with either bacteria or fungi.^{1,21}
- 8 **Carefully disinfect the skin** prior to collection of the sample using an appropriate disinfectant, including chlorhexidine in 70% isopropyl alcohol, tincture of iodine, povidone-iodine in swab or applicator form.^{1,21}
- 9 **Transport the inoculated bottles at room temperature** and the completed blood culture request to the clinical microbiology laboratory as quickly as possible, preferably within 2 hours per CLSI.¹

Delays beyond 2 hours could result in a delay in positive bottle detection or could cause a false negative result. If delays are expected, it is important to refer to the manufacturer’s Instructions For Use (IFU) for guidance.
- 10 **All blood cultures should be documented** in the patient’s notes, including date, time, collection site and indications.

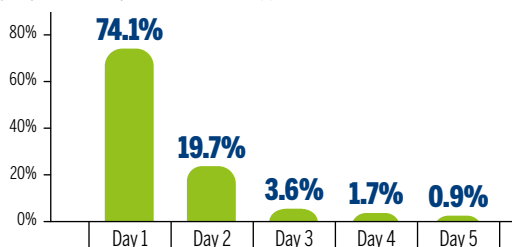
9 How many days of incubation are recommended?

➤ The current recommendation, and standard incubation period, for routine blood cultures performed by continuous-monitoring blood systems is 5 days.³⁴

A study by Bourbeau, *et al.* (JCM, 2005) demonstrated that 97.4% of clinically significant isolates were recovered within the first 3 days of incubation and 93.8% within 2 days of incubation.³⁵

Figure 4: Clinically significant isolates per day³⁵

Adapted from Bourbeau PP *et al.* Routine incubation of BacT/ALERT™ FA and FN blood culture bottles for more than 3 days may not be necessary. *J Clin Microbiol.* 2005;43(5):2506-2509



➔ Incubation of fastidious microorganisms

Another study by Cockerill, *et al.* (CID, 2004) demonstrated that, when using a continuous-monitoring blood culture system, 99.5% of non-endocarditis bloodstream infections and 100% of endocarditis episodes were detected within 5 days of incubation.²² This data suggests that extended incubation periods previously recommended for detection of the fastidious microorganisms* that sometimes cause endocarditis, are no longer necessary when using continuous-monitoring blood culture systems.¹⁷

With the exception of *Bartonella* spp., most rare or fastidious bacteria are still cultivable by traditional bacterial culture systems and can be recovered using blood culture protocols currently in use by most medical laboratories.¹

* including *Brucella*, *Capnocytophaga* and *Campylobacter* spp., and the HACEK group (*Haemophilus* species, *Aggregatibacter* species, *Cardiobacterium hominis*, *Eikenella corrodens* and *Kingella* species)³⁶

10 Is it a contaminant or a true pathogen?

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.

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.

➤ Collecting a contaminant-free blood sample is critical to providing a blood culture result that has clinical value.

Certain microorganisms such as coagulase-negative staphylococci, *Bacillus* spp., *Cutibacterium* spp., diphtheroids, *Micrococcus* spp. rarely cause severe bacterial infections or bloodstream infections. These are **common skin contaminants**, and although they are capable of causing serious infection in the appropriate setting, their detection in a single blood culture set can reasonably be identified as a possible contaminant without clinical significance. However, it is important to consider that 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.³⁷

The most difficult interpretation problem for the physician is whether the organism recovered from a blood culture is a **true pathogen causing bloodstream infection**, or a **contaminant**. If it is a contaminant, the patient may be treated unnecessarily with antibiotics, leading to additional patient risks. Interpretation of true pathogen *versus* contaminant should be based on whether the blood has been collected with a venipuncture or an intra-vascular device, and whether the same organism was recovered from multiple blood culture sets. This illustrates the crucial nature of having **collection site information included with the blood culture request sent to the laboratory**.

In contrast to patients with infective endocarditis or other true positive bloodstream infections, patients whose blood cultures grow contaminants usually have only a single blood culture that is positive. This information is of great practical value for physicians, and underlines the importance of taking two to three blood culture sets from different anatomical sites.¹⁷



Contamination rates can be most effectively reduced by strict compliance with hand hygiene rules and best practices for blood collection, particularly during the stages of skin antisepsis, venipuncture and sample transfer to blood culture bottles.

The American Society for Microbiology and CLSI recommend targeting contamination rates of 1% of the total of collected sets.^{1,17}

→ Impact of contamination rates

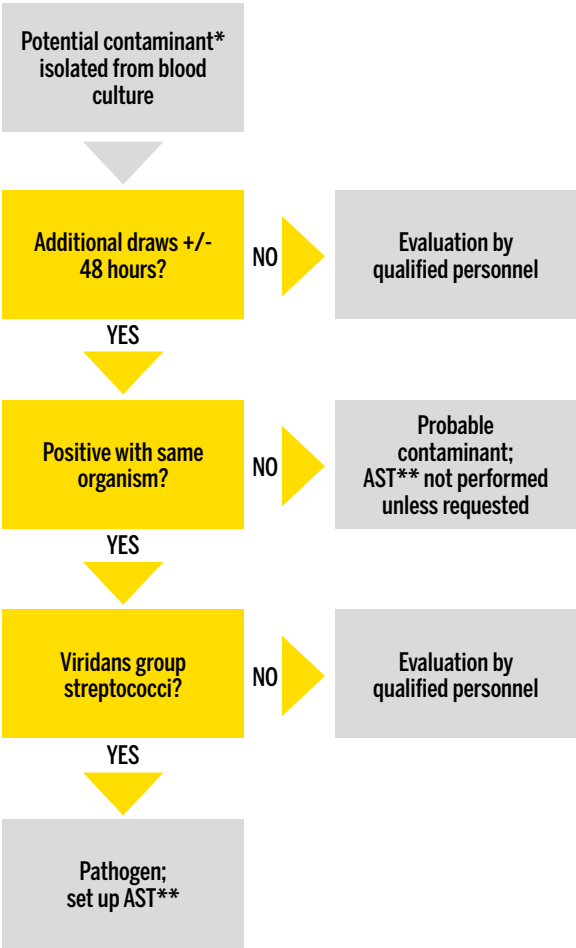
A contaminated blood culture can result in unnecessary antibiotic therapy, increased length of hospitalization and higher costs.

It has been found that each false positive result can lead to:

- increased length of stay - on average 1 day³⁷
- 39% increase in intravenous antibiotic charges³⁷
- \$5,000 to \$8,720 additional charges^{38, 39}
- 20% increase in laboratory charges³⁷
- 3 days longer on antibiotics³⁷

Figure 5: Example of a laboratory-based algorithm to determine blood culture contamination⁴⁰

Adapted from Richter *et al.* Minimizing the workup of blood culture contaminants: implementation and evaluation of a laboratory-based algorithm. *J Clin Microbiol.* 2002;40(7):2437-2444. Reproduced with permission.



* Microorganisms such as coagulase-negative staphylococci, viridans group streptococci, *Bacillus* spp., *Cutibacterium* spp., diphtheroids, *Micrococcus* spp.

** AST: Antimicrobial Susceptibility Testing

2 SPECIAL TOPIC: INFECTIVE ENDOCARDITIS

Blood culture is essential in the diagnosis of infective endocarditis (infection of the heart valves). In this elusive disease, blood cultures may need to be taken repeatedly during febrile episodes, when bacteria are shed from the heart valves into the bloodstream. For patients with infective endocarditis, positive blood cultures will be obtained in over 90% of cases, if optimal culture conditions are respected.⁴¹

→ Acute Infective Endocarditis

This is a fulminant illness progressing rapidly over days to weeks, which may be caused by highly virulent pathogens, such as *Staphylococcus aureus*. When suspected, the severity of this disease requires blood cultures to be drawn immediately to avoid unnecessary delays in appropriate treatment.

- Multiple blood culture sets should be drawn during a 30-minute period prior to administration of empiric antimicrobial therapy.⁴²

→ Subacute Infective Endocarditis

If subacute infection is suspected, although it is important to attempt to establish the microbiological diagnosis, antimicrobial therapy should nevertheless be initiated as soon as possible.

- Multiple blood culture sets should be obtained prior to initiation of antimicrobial therapy, with sets spaced 30 minutes to one hour apart. This may help document a continuous bacteremia, and could be of additional clinical value.¹

→ Fungal Infective Endocarditis

Fungal endocarditis is a relatively rare disease, with fungi responsible for <10% of infective endocarditis cases.⁴³ However, it is the most severe form of infective endocarditis, and is associated with high mortality and morbidity.^{44,45} *Candida* species are the most common fungal pathogens involved in infective endocarditis.⁴⁰ Diagnosis of fungal endocarditis can be very challenging, as blood cultures may take a long time to yield growth, with a yield for positive blood cultures of around 50%.⁴³

→ How many cultures?

In order to distinguish between contamination and true bacteremia, a total of three to five blood culture sets should be sufficient.

- Initially, two to three blood culture sets should be obtained from patients with suspected infective endocarditis. If the results of these sets are negative at 24 hours, two more sets of cultures should be obtained, for a total of five sets overall.¹

Often patients with suspected infective endocarditis have been put on antibiotics prior to blood collection. This is the most common reason for **“culture-negative” infective endocarditis**. It is therefore important to use a blood culture medium that has antimicrobial neutralization capacity in order to sustain microbial growth in the presence of antibiotics (see page 10 “Which media to use?”).²⁴⁻²⁸

However, “culture-negative” endocarditis may also be due to fastidious microorganisms, such as *Aspergillus* spp., *Brucella* spp., *Coxiella burnetii*, *Chlamydia* spp. and HACEK* microorganisms.

- Since current continuous-monitoring blood culture systems can recover all HACEK and other fastidious organisms within a 5-day period, extending incubation beyond this period is no longer considered to be necessary. However, if all blood culture bottles are negative after 5 days, and infectious endocarditis is still suspected, all bottles should be subcultured to chocolate agar.⁴⁶

*HACEK = *Haemophilus* species, *Aggregatibacter* species, *Cardiobacterium hominis*, *Eikenella corrodens* and *Kingella* species³⁶

3 PROCESSING POSITIVE BLOOD CULTURES

Today, continuously-monitored blood culture systems provide the optimum solution for blood sample processing with a recommended incubation period of 5 days.¹ The study discussed in **Figure 4** shows that >97% of all positive specimens were detected within the first 3 days (see page 14).³⁵

> Patients who progress to septic shock have a 7.6% increase in mortality every hour while not on appropriate therapy.¹⁴

Following an instrument-flagged positive event, the bottle is removed from the system and a Gram stain and subculture is performed.

- **If the sample is Gram stain positive**, the morphology of the organism should be reported immediately to the physician. Subcultures and, if available, rapid techniques (e.g. molecular diagnostics) should be initiated immediately in order to provide further organism identification and antibiotic susceptibility testing should be performed as soon as possible.
- **If a sample is Gram stain negative**, additional stains, such as auramine-rhodamine stain, acid-fast bacillus (AFB) stain or modified AFB stain, should be performed to screen for the organism or to search for specific organisms, e.g. *Mycobacterium* or *Nocardia*. If all the stains are negative, the blood bottle should be put back into the instrument to complete the full 5 days incubation period.

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. When **reports are delivered rapidly**, studies have shown broadly improved outcomes and efficiencies in patient management.^{47,48}

A study by Barenfanger, *et al.* validated that Gram stains of positive blood cultures are a very important factor influencing **appropriate therapy and patient outcomes**.⁴⁹ The study documented a statistically significant increase in the mortality rate for patients who had blood cultures processed after a delay (i.e. Gram stain performed ≥ 1 hour after being detected as

positive; $P = 0.0389$). The timely removal and reporting of Gram stain results have a positive impact on patient care and this study supports the need for 24/7 coverage of blood culture instruments.⁴⁹

Recent technological advances such as **MALDI-TOF MS** (Matrix-Assisted Laser Desorption Ionization Time of Flight Mass Spectrometry) provide the ability to rapidly deliver definitive organism identification. **Molecular diagnostics** can identify the most common pathogens in positive blood cultures as well as specific antibiotic resistance genes associated with common bloodstream pathogens. Rapid identification allows physicians to prescribe more targeted and effective antimicrobial therapy earlier to positively influence outcomes.⁵⁰⁻⁵²

Additionally, **antimicrobial susceptibility testing (AST)** should be performed on positive blood cultures to provide the clinician with a complete result. Appropriate use of antibiotics is crucial in cases of bloodstream infections and sepsis. Accurately determining the antimicrobial resistance profile of the causative pathogen in order to select the most effective antibiotic therapy can have a significant impact on patient outcomes.

It is now possible for laboratories to deliver **phenotypic AST results for gram-negative bacteria directly from positive blood cultures**. CLSI has published a rapid, **direct-from-blood-culture disk diffusion (DD)** protocol, with specific direct DD breakpoints for Enterobacterales and *P. aeruginosa*.⁵³ EUCAST has also developed a DD method delivering AST results within 4-8 hours of blood culture positivity.⁵⁴ Another new **rapid AST system** provides actionable results direct from Gram-negative blood cultures in an average of 5 hours.⁵⁵ These diagnostic solutions help clinicians address the challenge of bloodstream infections, and sepsis in particular, by allowing either **timely de-escalation** to a focused, more appropriate, and lower-cost therapy, or **life-saving rapid escalation** to more effective therapy when a multidrug-resistant (MDR) infection is present.

> When processed correctly, blood cultures provide clinically relevant information that can help improve patient outcomes, decrease length of hospital stay and reduce use of antibiotics.



4 INTERPRETATION OF RESULTS

The microbiology laboratory can provide useful information to clinicians to help them determine whether a blood culture sample is a true positive or a false positive (contaminant).

INTERPRETATION OF RESULTS

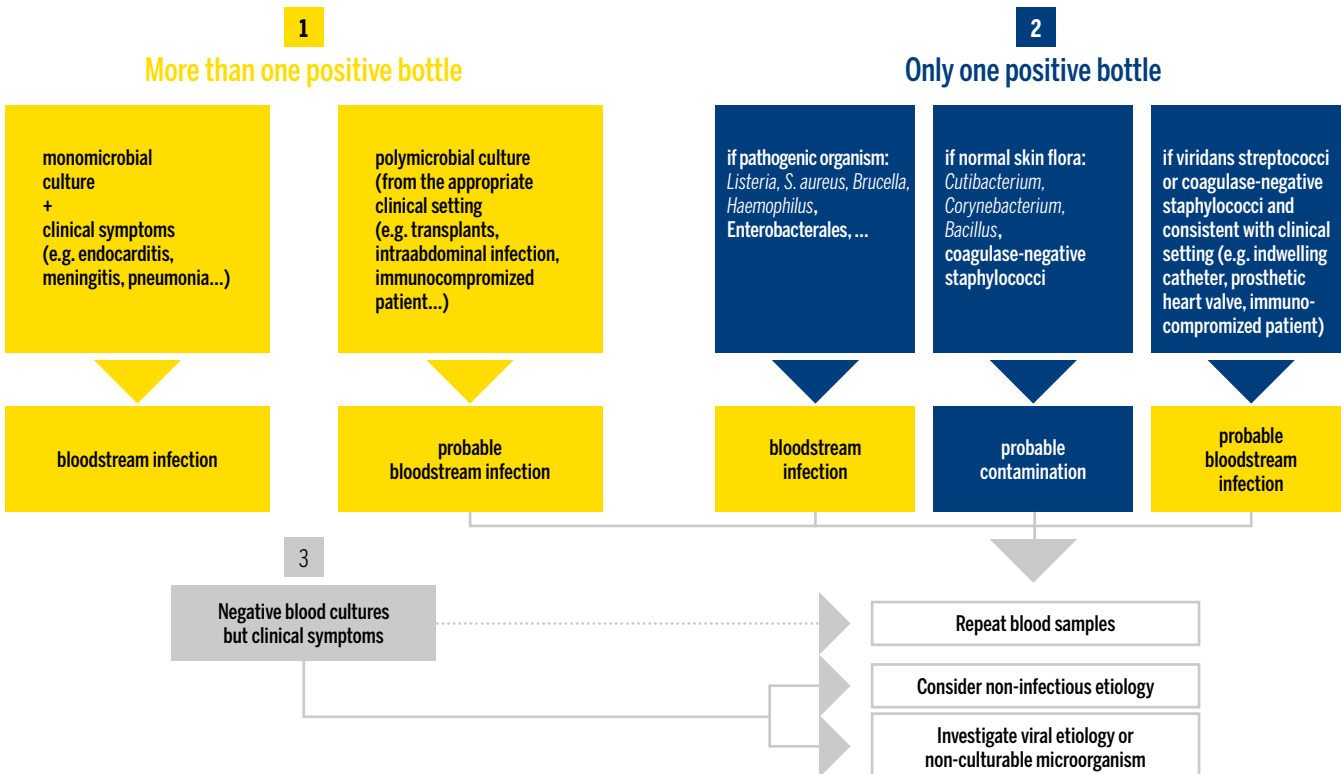
For example, the identity of the micro-organism isolated can help determine if the culture is contaminated, and the number of cultures positive with the same organism can help predict true infections.⁵⁶ Time to positivity is also a factor used to determine potential contamination as contaminants usually have a delayed (longer) time-to-detection due to a lower overall bio-load.

Laboratories should consult with their medical director to create an algorithm which helps determine whether or not an isolated organism is a contaminant vs. an infective agent.

Models, such as the algorithm below, can give **guidance only on the interpretation of blood culture results.**⁴⁰ These guidelines should be used in conjunction with clinical guidelines, e.g. patient's full blood count, presence of catheters, radiological findings, etc.

Figure 6: Example of interpretation algorithm for blood culture results⁵⁷

Adapted from Doern GV, et al. Clin Microbiol Rev. 2019;33(1):e00009-19



5 BLOOD CULTURE/SEPSIS GUIDELINES

■ WHO guidelines on drawing blood: best practices in Phlebotomy.

World Health Organization 2010.

https://www.euro.who.int/__data/assets/pdf_file/0005/268790/WHO-guidelines-on-drawing-blood-best-practices-in-phlebotomy-Eng.pdf

■ American Society for Microbiology: Cumitech 1C, Blood Cultures IV.

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■ Supplemental reference: Doern GV, et al. A comprehensive update on the problem of blood culture contamination and a discussion of methods for addressing the problem. *Clin Microbiol Rev.* 2020;33(1):e00009-e00021

■ CLSI. Principles and Procedures for Blood Cultures. 2nd ed. CLSI guideline M47. Clinical and Laboratory Standards Institute; 2022.

<https://clsi.org/standards/products/microbiology/documents/m47/>

■ Manual of Clinical Microbiology, 12th Edition, 2019.

<https://clinmicronow.org/doi/book/10.1128/9781683670438.MCM>

■ European Society for Clinical Microbiology and Infectious Diseases, European Manual for Clinical Microbiology, 1st Edition, 2012.

https://www.escmid.org/escmid_publications/manual_of_microbiology/

■ Surviving sepsis campaign: international guidelines for management of sepsis and septic shock 2021.

Evans, L., et al. *Crit Care Med.* 2021;49(11):e1063 - e1143.

https://journals.lww.com/ccmjournal/Fulltext/2021/11000/Surviving_Sepsis_Campaign_International.21.aspx

■ The Third International Consensus Definitions for Sepsis and Septic Shock (Sepsis-3).

Singer M., et al. *JAMA.* 2016;315(8):801-810.

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RECOMMENDATIONS FOR BLOOD CULTURE COLLECTION

A SUMMARY OF GOOD PRACTICE

A) USING WINGED BLOOD COLLECTION SET (preferred method of collection)⁵⁸⁻⁶⁰

1 PREPARE BLOOD COLLECTION KIT

Confirm the patient's identity and gather all required materials before beginning the collection process.

Do not use blood culture bottles beyond their expiration date, or bottles which show signs of damage, deterioration or contamination.



It is recommended to identify the Fill-to Mark or mark the target fill level on the blood culture bottle label about 10 mL above the media level.



2 PREPARE BOTTLES FOR INOCULATION

Wash hands with soap and water then dry, or apply an alcohol hand rub or another recognized effective hand rub solution.

Remove the plastic "flip-cap" from the blood culture bottles and disinfect the septum using an appropriate and recognized effective disinfectant, including chlorhexidine in 70% isopropyl alcohol, tincture of iodine, povidone-iodine in swab or applicator form. Use a fresh swab/applicator for each bottle.

Allow bottle tops to dry in order to fully disinfect.

Attach a winged blood collection set to a collection adapter cap*.



3 PREPARE VENIPUNCTURE SITE

If skin is visibly soiled, clean with soap and water. Apply a disposable tourniquet and palpate for a vein. **Apply clean examination gloves** (sterile gloves are not necessary).

Cleanse the skin using an appropriate disinfectant, including chlorhexidine in 70% isopropyl alcohol, tincture of iodine, povidone-iodine in swab or applicator form. **The venipuncture site is not fully clean until the disinfectant has fully evaporated.**



4 VENIPUNCTURE

To prevent contaminating the puncture site, **do not re-palpate the prepared vein before inserting the needle.** Insert the needle into the prepared vein.



5 CULTURE BOTTLE INOCULATION

Place the adapter cap over the **aerobic bottle** and **press straight down** to pierce the septum. Hold the bottle upright, below the level of the draw site, and add up to 10 mL of blood per adult bottle and up to 4 mL per pediatric bottle.** Ensure the bottle is correctly filled to the Fill-to Mark or target fill level. Once the aerobic bottle has been inoculated, repeat the procedure for the **anaerobic bottle.**



6 OTHER BLOOD TESTS

If blood is being collected for other tests, an insert placed into the adapter cap may be required. The insert is used to guide blood collection tubes onto the needle.

If other blood tests are requested, always collect the blood culture first.



7 FINISH THE PROCEDURE

Discard the winged collection set into a sharps container and cover the puncture site with an appropriate dressing. Remove gloves and wash hands before recording the procedure, including indication for culture, date, time, site of venipuncture, and any complications.

Ensure that additional labels are placed in the space provided on the bottle label and do not cover the bottle barcodes, and that the tear-off barcode labels are not removed. If additional labels contain a barcode, they should be positioned in the same manner as the bottle barcode.

Inoculated bottles should be transported to the laboratory for testing as quickly as possible, preferably within 2 hours per CLSI.¹ If delays are expected, it is important to refer to the manufacturer's Instructions for Use for guidance.



*The use of blood collection sets without blood collection adapters is not recommended.

**Avoid holding the blood culture bottle in a horizontal or upside down position or drawing blood with a needle connected directly to the adaptor cap, as fill level cannot be monitored during collection and there is a possible risk of media reflux into the bloodstream.

These recommendations illustrate the best practices for blood culture collection based on the World Health Organization recommendations (WHO guidelines on drawing blood: best practices in phlebotomy, 2010. ISBN 978 92 4 159922 1). Best practices may vary between healthcare facilities; refer to guidelines applicable in your facility.

RECOMMENDATIONS FOR BLOOD CULTURE COLLECTION

A SUMMARY OF GOOD PRACTICE

B) USING NEEDLE AND SYRINGE

Conventional needles and syringes should be replaced wherever possible with winged blood collection sets, which are safer.⁵⁸⁻⁶⁰

They should only be used if prevention measures to Accidental Blood Exposure are strictly applied*. Needles must not be recapped, purposely bent or broken by hand, removed from disposable syringes or otherwise manipulated by hand.

1 PREPARE BLOOD COLLECTION KIT

Confirm the patient's identity and gather all required materials before beginning the collection process.

Do not use blood culture bottles beyond their expiration date, or bottles which show signs of damage, deterioration or contamination. It is recommended to identify the Fill-to Mark or mark the target fill level on the blood culture bottle label about 10 mL above the media level.



2 PREPARE BOTTLES FOR INOCULATION

Wash hands with soap and water then dry, or apply an alcohol hand rub or another recognized effective hand rub solution.

Remove the plastic "flip-cap" from the blood culture bottles and disinfect the septum using an appropriate and recognized effective disinfectant, including chlorhexidine in 70% isopropyl alcohol, tincture of iodine, povidone-iodine in swab or applicator form. Use a fresh swab/applicator for each bottle.

Allow bottle tops to dry in order to fully disinfect.



3 PREPARE VENIPUNCTURE SITE

If skin is visibly soiled, clean with soap and water. Apply a disposable tourniquet and palpate for a vein. **Apply clean examination gloves** (sterile gloves are not necessary).

Cleanse the skin using an appropriate disinfectant, including chlorhexidine in 70% isopropyl alcohol, tincture of iodine, povidone-iodine in swab or applicator form.

The venipuncture site is not fully clean until the disinfectant has fully evaporated.



4 VENIPUNCTURE

Attach the needle to a syringe. **To prevent contaminating the puncture site, do not re-palpate the prepared vein before inserting the needle.**



Insert the needle into the prepared vein.

5 CULTURE BOTTLE INOCULATION

Collect the sample. Transfer the blood into the culture bottles. Hold the bottle upright, and add up to 10 mL of blood per adult bottle and up to 4 mL per pediatric bottle. Ensure the bottle is correctly filled to the Fill-to Mark or target fill level.



6 FINISH THE PROCEDURE

Discard the needle and syringe into a sharps container and cover the puncture site with an appropriate dressing. Remove gloves and wash hands before recording the procedure, including indication for culture, date, time, site of venipuncture, and any complications.

Ensure that additional labels are placed in the space provided on the bottle label and do not cover the bottle barcodes, and that the tear-off barcode labels are not removed. If additional labels contain a barcode, they should be positioned in the same manner as the bottle barcode. Inoculated bottles should be transported to the laboratory for testing as quickly as possible, preferably within 2 hours per CLSI.¹ If delays are expected, it is important to refer to the manufacturer's Instructions for Use for guidance.



* Refer to recognized guidelines such as those issued by the WHO or CDC:
<https://www.who.int/infection-prevention/tools/injections/ToolC-revised.pdf?ua=1>
<http://www.cdc.gov/niosh/docs/2000-108/pdfs/2000-108.pdf>

These recommendations illustrate the best practices for blood culture collection based on the World Health Organization recommendations (WHO guidelines on drawing blood: best practices in phlebotomy. 2010. ISBN 978 92 4 159922 1). Best practices may vary between healthcare facilities; refer to guidelines applicable in your facility.



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