

Guideline for the optimal use of blood cultures

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The incidence of sepsis is increasing globally, with high morbidity and mortality. Prompt, accurate detection of bacteraemia and fungaemia is imperative for improving patient care, yet health care professionals lack training in correct blood culture techniques. These guidelines discuss the clinical importance of blood cultures,

the indications for their use and the correct technique for optimal yield of pathogenic micro-organisms that cause sepsis.

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Detection of bacteraemia or fungaemia by blood culture is critical in managing patients with infection, and directs the appropriate selection of antimicrobials. Blood culture is a common laboratory investigation where blood is inoculated into culture medium and incubated. Media used in blood culture bottles support the growth of most medically important bacteria and fungi, including anaerobes, which grow adequately in the aerobic blood culture bottle, hence separate anaerobic bottles are infrequently used.^{1,2} The goal of culturing blood is to determine whether a pathogenic micro-organism(s) is responsible for the patient's clinical presentation. 'False positive' results occur when a skin commensal(s) rather than a true pathogenic organism is grown. Proper cleaning of the skin is a vital component of venesection to reduce the false-positive rate, yet poor technique is common when collecting blood for culture. This lapse results in inappropriate use of antibiotics, increased antibiotic resistance and prolonged hospital stay. A further barrier to correct laboratory interpretation of blood culture results is inadequate relevant clinical details on the accompanying request form. These guidelines contextualise the place of blood cultures in the management of sepsis and detail the appropriate method for acquiring blood for culture, to reduce the false-positive rate.

Methods

We performed a literature review of the diagnosis and management of sepsis; identified a number of international best practice standards, including recommendations of the United Kingdom Department of Health, International Sepsis Forum, Society of Critical Care Medicine,

Surgical Infection Society, American Association of Critical Care Nurses, American Association of Chest Physicians, American College of Emergency Physicians, American Thoracic Society, Australian and New Zealand Intensive Care Society, European Society of Intensive Care Medicine, Indian Society of Critical Care Medicine, European Respiratory Society, Infectious Diseases Society of America, and the Clinical and Laboratory Standards Institute; and studied consensus expert opinion documents.

Definitions

Blood culture. Collection and inoculation of blood into culture medium with the aim of growing pathogenic bacteria or fungi for diagnostic purposes.

Bacteraemia. The presence of viable bacteria in the bloodstream which may be transient (e.g. following dental procedures), intermittent (e.g. undrained abscesses), or continuous (e.g. endovascular infection).

Fungaemia. The presence of viable fungi in the bloodstream.

Infection. An inflammatory response to one or more micro-organisms, or the invasion of normally sterile sites by those organisms.

Systemic inflammatory response syndrome (SIRS). A consequence of the host inflammatory response that can follow infection or other injury, defined as the clinical cluster of two or more of:

- temperature $>38^{\circ}\text{C}$ or $<36^{\circ}\text{C}$
- heart rate >90 beats/minute
- respiratory rate >20 breaths/minute or $\text{paCO}_2 <4.3$ kPa (32 mmHg)
- white cell count $>12\ 000$ cells/ mm^3 , $<4\ 000$ cells/ mm^3 , or $>10\%$ immature white blood cells (band forms).³

Sepsis. SIRS resulting from documented infection.^{3,4}

Severe sepsis. Documented sepsis associated with organ dysfunction, hypoperfusion or hypotension. Perfusion abnormalities may manifest as, but are not limited to:

- lactic acidosis
- oliguria
- acute alteration in mental state
- areas of mottled skin
- capillary refilling requiring ≥ 3 seconds
- disseminated intravascular coagulation
- acute lung injury or acute respiratory distress syndrome (ARDS)
- cardiac dysfunction, as defined by echocardiography or direct measurement of the cardiac index.^{3,5}

Septic shock. The presence of severe sepsis plus one or both of the following:

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- systemic mean blood pressure <60 mmHg (or <80 mmHg if the patient has baseline hypertension) despite adequate fluid resuscitation
- maintaining systemic mean blood pressure >60 mmHg (or >80 mmHg if the patient has baseline hypertension) requires support with inotropic/vasoactive agents.^{3,4}

Multi-organ dysfunction/failure. Multi-organ failure is often a consequence of severe sepsis and septic shock, and refers to the presence of altered function affecting more than one organ in an acutely ill patient such that homeostasis cannot be maintained without intervention. Multiple organ dysfunction syndrome (MODS) is either primary (directly attributable to a well-defined insult) or secondary (as a consequence of the host response). MODS is characterised by, but not limited to, abnormalities in serum creatinine, platelet count, serum bilirubin, Glasgow coma score, pressure adjusted heart rate, and arterial oxygenation.^{3,5}

Sepsis and the significance of blood culture

Some 1.8 million cases of sepsis occur worldwide each year;⁶ however, owing to variable definitions and reporting, this is probably an underestimate. A more accurate estimate may be as high as 18 million, an incidence of 3/1 000 population.⁷ Sepsis is the most common cause of death in non-coronary intensive care units worldwide.^{8,9} The mortality rate from sepsis is generally between 30% and 70%, and is higher in persons with a pre-existing disease.^{3,8} Elderly, critically ill and immunocompromised persons are particularly vulnerable.¹⁰ Population-based studies of incidence and prevalence of sepsis in South Africa have not been undertaken.

There are several reviews of the pathophysiology of sepsis.¹¹⁻¹⁶ The direct effects of invading micro-organisms, their toxic products, or both, may contribute to pathogenesis of sepsis. Important factors include endotoxin, bacterial cell wall components (peptidoglycan, muramyl dipeptide, and lipoteichoic acid), and bacterial products such as staphylococcal enterotoxin B, toxic shock syndrome toxin-1, pseudomonas exotoxin A, and M protein of haemolytic group A streptococci.¹⁷ Cellular injury and organ dysfunction occur as a consequence of ischaemia,¹⁸ cytopathic injury¹⁹ and increased rate of apoptosis.²⁰ Panendothelial activation in sepsis also leads to widespread tissue oedema, endothelial dysfunction, hypotension,

upregulation of adhesion molecules, and impaired anticoagulant properties leading to MODS.²¹

Blood culture plays an integral role in the evaluation of sepsis.²² As bacteraemia is often associated with fever, clinicians are encouraged to obtain blood samples for culture from febrile patients.²³ The indications for blood culture are broad and ill-defined.²⁴ Other than pyrexia, clinical parameters alone are unhelpful in predicting bacteraemia and cannot be used in isolation to determine the timing of blood sampling for culture.²⁵

Clinical features of sepsis

The clinical features of sepsis relate to the causative organism, the involved site, severity, and the host response to infection. Symptoms and signs of sepsis are usually superimposed on the patient's underlying illness and primary infection. Hence, their rate of development and clinical presentation varies. Common clinical features of sepsis include lethargy, fever (although hypothermia may occur) and an elevated or reduced white blood cell count.²⁶ Sweating, rigors, tachycardia, hypotension, tachypnoea, warm peripheries, confusion, oliguria and jaundice may also occur (Table I).

Diagnosis of sepsis

Sepsis is primarily a clinical diagnosis, supported by laboratory investigation and imaging. Culture of specimens from a sterile site is the gold standard microbiological investigation and the key to successful diagnosis.^{27,28} Occasionally, serology may aid diagnosis. Culture is a slow process compared with rapid molecular diagnostic tests that detect genetic material and can be used at the 'point-of-care'.²⁹ A point-of-care test for sepsis would be an extremely useful addition to the diagnostic armory.

The indications for blood culture are shown in Table II. Although only 5 - 15% of blood cultures drawn in febrile patients are positive, approximately half of all patients with severe sepsis demonstrate bacteraemia at the time of diagnosis.³⁰ The presence or absence of a positive blood culture does not appear to influence outcome of sepsis, suggesting that prognosis is more related to severity of sepsis.³¹ Hospital-acquired infections (HAI) diagnosed from blood culture have a higher mortality than those acquired in the community.³²

Table I. Clinical and laboratory manifestations of sepsis

Symptoms	Signs	Laboratory findings
Rigors	Temperature increase or decrease	Leucocytosis or leucopenia
Sweating	Tachycardia	Hypoalbuminaemia
Nausea and vomiting	Hypotension	Increased INR, increased APTT, decreased platelets, DIC
Symptoms related to affected organ system (e.g. cough, diarrhoea, dysuria, etc.)	Tachypnoea, hyperventilation (acute lung injury)	Hyperglycaemia
	Warm pink peripheries	Hyperlactataemia
	Confusion	Increasingly negative base excess
	Oliguria	Elevated CRP, ESR,* procalcitonin, fibrinogen
	Jaundice	
	Haemorrhagic lesions, cutaneous bullae, cellulitis	

*ESR should not be used in patients with HIV, as hypergammaglobulinaemia and immune hyperactivation commonly leads to an increased ESR in the absence of sepsis.

Table II. Indications for blood culture

1.	Clinical features of sepsis including tachycardia, tachypnoea, increased or sub-normal temperature and change in sensorium, hypotension or prostration
2.	Suspicion of infective endocarditis
3.	Pyrexia of unknown origin
4.	Unexplained leucocytosis or leucopenia
5.	Systemic and localised infections including suspected meningitis, osteomyelitis, septic arthritis, acute untreated bacterial pneumonia or other possible bacterial infection

Principles of sample collection for blood culture

1. Blood cultures should be drawn when there is a clinical suspicion of bloodstream infection.³³
2. Blood should not be collected from indwelling arterial or venous lines unless an infected intravenous line is suspected.³⁴ Should blood be drawn for culture from an indwelling line, a second specimen should be obtained from a peripheral site.
3. Two or more blood specimens should be collected using sterile technique at separate sites, before administering antibiotics.³⁵⁻³⁷ Except in very unusual cases, no more than 3 sets of blood cultures should be collected in one 24-hour period. If all 4 sets are negative after 24 hours and sepsis is still suspected, more cultures may be collected.³⁸ A larger number of cultures may have to be collected from persons already receiving antimicrobials³⁹ although, if clinical condition allows, stopping antibiotics and re-culturing after 48 hours is preferred.
4. Ideally, a minimum of 10 ml of blood, depending on the blood culture system used, should be inoculated into a culture bottle when taking blood from adults.⁴⁰ Adequate volumes of blood improve detection of pathogenic organisms and reduce time to detection.
5. Arterial blood culture provides no advantage over venous samples.⁴¹
6. Changing needles between venepuncture and inoculation into culture bottles is controversial. Discontinuing this practice owing to lack of evidence that it decreases contamination and the increased risk of sharp injuries has been recommended.^{42,43} However, a meta-analysis challenges this recommendation, suggesting a slight overall benefit of switching needles.⁴⁴ Until more definitive studies are available, we recommend that the risk of changing needles after venepuncture does not outweigh the benefit. More important ways to decrease contamination of blood culture bottles include the use of tincture of iodine as a disinfectant,⁴⁵ avoidance of drawing blood through existing intravenous lines³⁴ and disinfecting the membrane of the blood culture bottle.⁴⁶
7. It is a medico-legal requirement that the patient's notes must always document acquiring blood for culture, including indication, site, time of taking blood culture and a legible name.

Table III details recommendations for how to correctly send blood to the laboratory for culture.

Optimal number of blood cultures

The optimal number of blood cultures that should be obtained in a febrile patient varies according to the suspected diagnosis or clinical condition, the suspicion of underlying infection, and the urgency of the need for treatment.⁴⁷

- One blood culture is rarely, if ever, sufficient or advisable.²¹ A positive result on a single culture is difficult to interpret, unless an unequivocal pathogen is isolated.
- Two blood cultures are usually adequate when continuous bacteraemia is anticipated.⁴⁸
- Three blood cultures are reasonable when intermittent bacteraemia is suspected.⁴⁹

We believe that more than 3 blood cultures is rarely justifiable in a resource-limited setting. Furthermore, the probability of obtaining a false-positive result increases steadily as more blood cultures are performed.

Timing of blood cultures

Few systematic studies have been conducted on the timing of blood cultures and the optimal interval between successive tests.³⁸ The ideal is to collect blood at time intervals ranging from one to several hours.⁵⁰ However, it is sufficient and appropriate to obtain blood from 2 separate sites within minutes of each other from patients who are acutely ill or those in whom the likelihood of continuous bacteraemia is high.⁵¹ By contrast, in patients suspected to have intermittent bacteraemia, it is advisable to obtain multiple blood cultures 6 - 36 hours apart.^{38,52} One study showed no difference in yield whether blood samples for cultures performed within a 24-hour period were drawn simultaneously or at spaced intervals.⁵³ Antibiotics should not be withheld in sick patients apart from a reasonable delay to collect more than 1 culture sample.

Volume of blood to be taken for culture

A minimum of 10 ml (and preferably 20 ml) of blood should be obtained from adults,^{38,41,53,54} and 1 - 5 ml (and preferably more) from infants and children.^{40,55,56} The yield from blood culture depends upon the volume of blood cultured,⁵⁷⁻⁵⁹ the yield in adults increasing approximately 3% per ml of blood cultured.

Maintaining the correct ratio of blood to culture medium is important. Most blood culture bottles contain the anticoagulant sodium polyanetholsulphonate (SPS). SPS can inactivate lysozyme, some antibiotics, and parts of the complement cascade. However, SPS can also be toxic to certain fastidious organisms. Adding sufficient blood to the bottle helps negate this latter effect. A dilution of blood in broth by a ratio of 1:5 has been recommended.^{60,61}

Type of blood culture bottle

Most blood culture systems are now automated, with some form of continuous monitoring to detect growth. The bottles contain broth supplemented with additives. Different bottles are designed either to be used in different patient groups, or to isolate different classes of pathogens. A few general principles apply:

- Standard aerobic bottles are suitable for the recovery of most common bacterial pathogens, including aerobes and facultative anaerobes. These are not as suitable for supporting the growth of strict anaerobes, and therefore traditional advice has been to use a paired set of aerobic and anaerobic bottles when taking blood cultures.⁶² The routine use of anaerobic bottles has been questioned, as the incidence of anaerobic bacteraemia is decreasing^{61,63,64} and most infections involving anaerobes are clinically suspected and appropriate empiric therapy is administered before blood culture results become available.^{65,66} Conversely, improved yield of microorganisms when using aerobic-anaerobic paired blood cultures has been demonstrated.^{67,68} Although firm evidence is unavailable, we believe that the routine use of anaerobic bottles is not warranted, particularly in resource-limited settings.
- Some manufacturers produce aerobic and anaerobic bottles with

Table III. Procedure for blood culture

Step	Procedure
1	Verify the patient's identity: Ask the patient for his or her name. Check the armband. Look on wall above the bed or in the patient notes to confirm identity.
2	Inform the patient of your intentions and explain the procedure. Always obtain verbal consent.
3	Assemble the correct materials required for blood culture: <ul style="list-style-type: none"> • blood culture bottle(s) • syringe (10 ml or more) • needle (22 gauge or more) • sterile gloves • tourniquet • adhesive strip • povidone iodine or alcohol solution (or other suitable skin disinfectant) • sterile pack containing cotton/gauze swabs, sterile paper x2 and waste bag • patient labels • sharps waste disposal bin.
4	Open the sterile blood culture pack onto the trolley. Remove the sterile sheet and place under the patient's arm. Pour povidone iodine or alcohol solution into fluid recess located on blood culture tray/pack. Drop needle and syringe onto sterile field.
5	Apply tourniquet and select a suitable vein. Wash hands with soap and water or disinfect with alcohol hand disinfectant. Dry your hands or rub the hand disinfectant in until dry. Apply sterile gloves.
6	Clean the puncture site with povidone or alcohol solution using aseptic technique. Allow 1 - 2 minutes for the disinfectant to dry. Place green sterile cover with opening over site for blood culture.
7	Carefully insert needle into patient's blood vessel and collect a minimum of 10 ml of blood (adults). If using the vacutainer system, the blood culture must be the first blood specimen to be collected.
8	Release tourniquet. Remove needle and syringe from puncture site. Place dry swab on puncture site and apply pressure. If blood has not been collected directly into the blood culture bottle with the vacutainer system, inoculate blood into culture bottle, having first disinfected the top of the blood culture bottle with an alcohol swab (a webcol is adequate). ⁵⁰ If blood is being collected for other tests, always inoculate blood culture bottle first. Do not change needles between blood sample collection and inoculation of blood culture bottle.
9	Gently rotate the blood culture bottle to mix the blood and culture medium (do not shake vigorously).
10	Label the blood culture bottle, making sure not to remove the 'tear-off' label on the bottle. Make sure that patient labels do not cover the blood culture bottle bar code label and are not stuck across the bottom of the blood culture bottle. Complete a laboratory request form. Remember to include the site, date and time of collection, full clinical information regarding the suspected diagnosis, and contact details for the clinician responsible for the patient.
11	Deliver the blood culture bottle to the laboratory as soon as possible. If there is a delay in getting the sample to the laboratory, do not refrigerate the bottle; rather leave it at room temperature.

additives (such as charcoal or resins) that inactivate antibiotics. These are preferable when taking blood cultures from patients already on antimicrobials, but are more expensive. There is evidence that the average time to positivity with these bottles is shorter than with normal aerobic and anaerobic bottles; however, their cost-effectiveness is unclear.⁶¹ Paediatric bottles are often supplemented with growth factors and usually have additives which will bind antibiotics, allowing improved recovery of bacteria. Together with the lower concentration of SPS, these features allow smaller volumes of blood to be inoculated.^{60,61}

- Mycobacterial blood culture bottles contain broth suitable for the isolation of mycobacteria and are valuable for the detection of disseminated mycobacterial infections, both *M. tuberculosis* and non-tuberculous mycobacteria. However, these bottles are often also used to enhance the recovery of yeasts from blood, although there is less evidence to justify this practice.^{69,70} Given the additional expense, routine blood culture bottles should be used for detection of candidaemia, and mycobacterial blood cultures should be reserved for suspected mycobacterial infections.⁷¹

Conclusion

Blood culture remains one of the most important microbiological tests available to the clinician. Members of the healthcare team who perform blood cultures must have a sound comprehension of the principles that underlie this important diagnostic tool. These guidelines provide practical and accessible guidance for medical students, nurses and doctors on the correct performance of blood collection for culture and should be applicable to all levels of healthcare in South Africa. Adoption of these guidelines and implementation of procedures to improve the quality of blood culture investigations will reduce the risk of blood sample contamination and improve the management and care of patients with sepsis.

These guidelines are endorsed by the South African Society for Clinical Microbiology (SASCM).

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