Chapter 15

Blood Culture Systems: From Patient to Result

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Additional information is available at the end of the chapter

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1. Introduction

Bacteraemia and sepsis is associated with a high mortality and an increased incidence of hospital stay and associated costs (1, 2). A recent multicentre retrospective evaluation has shown that more than 80% of bacteraemias and fungaemias occur within the hospital or other heathcare settings, with indwelling catheters being the most common source (3).

Blood cultures are still considered to be the 'gold standard' for the detection of microbial pathogens related to bacteraemia and sepsis despite newer molecular techniques (4, 5). This method allows for microbial identification and susceptibility testing to be performed which is a critical component to managing sepsis, however the lack of rapid results and decreased sensitivity for fastidious pathogens has led to the development of improved systems and adjunctive molecular or proteomic testing.

Clinicians need to utilize their respective laboratories' culturing systems optimally by adhering to the correct way of submitting blood culture specimens, understanding the principle of the testing method and making informed decisions regarding the results obtained.

This chapter will focus on the use of blood culture systems in the era of modern technology and aim to highlight the best practice from collection to interpretation of results.

2. The blood culture

The term blood culture refers to a single venipuncture, either from a peripheral site or central or arterial line, with blood inoculated into one or more blood culture bottles. One bottle is considered a blood culture where two or more are considered a set. Multiple sets are from multiple venipunctures and are associated with different sites.

Bacteraemia is defined as the presence of microorganisms in the blood, compared to sepsis which is defined as bacteraemia in the presence of clinical symptoms and signs such as fever, tachycardia, tachypnea and hypotension.

The sensitivity and specificity of the test are influenced by the clinicians' ability to predict bacteraemia or sepsis prior to collection. The clinical indication will guide the timing and the numbers of cultures being send. The person taking the sample will also influence the results by adhering to aseptic technique and inoculating the correct volume.

Blood cultures are taken to establish microbial invasion of the vascular system. Common mechanisms include dissemination from a primary site after inadequate control by host defense mechanisms, intravascular device mediated or infection of the vascular system e.g. infective endocarditis. Transient bacteraemia occur due to translocation of bacteria e.g. during chewing with rapid clearance by immune mechanisms compared to intermittent bacteraemia where bacteria are periodically released into the blood from e.g. an abscess while continuous bacteraemia points to an intravascular infection.

Blood cultures should always be obtained to investigate the possibility of a bacteraemia. Various indications will lead to obtaining these samples (Table 1). Clinical parameters e.g. fever, raised inflammatory markers and suggestive imaging as well as a clinical suspicion of specific disease entities e.g. meningitis, pneumonia, osteomyelitis and pyelonephritis will prompt clinicians to investigate for bacteraemia. Part of the diagnostic criteria for infective endocarditis include obtaining positive blood cultures (6, 7). Blood cultures are taken to confirm or exclude central line associated bloodstream infection (CLABSI) as well as to follow up the response to therapy in certain conditions e.g. fungaemia and infective endocarditis.

Acute bacterial sepsis	Meningitis
	Pneumonia
	Osteomyelitis
	Pyelonephritis
Vascular	Infective endocarditis
Closed space infections	Intra-abdominal abscesses
Catheter related bacteraemia	
Follow up cultures	Fungaemia, infective endocarditis

Table 1. Indications for taking blood cultures

The yield of blood cultures will depend on the site of infection. Up to 99% of blood cultures can be positive with acute suppurative thrombophlebitis, up to 50% with acute bacterial meningitis and only 2% with acute cellulites.

3. Principles of blood culture collection

Collection of blood cultures is a critical component and can either positively affect the patient outcome by providing an accurate diagnosis or adversely affect the outcome by prolonging antimicrobial therapy and the length of hospital stay with the isolation of a contaminant.

3.1. Timing

In general, clinicians will collect blood cultures around the time of temperature elevation to increase the chance of detecting bacteraemia, however this practice can become complicated especially in patients that are hypothermic or unable to mount a temperature response with clinical sepsis. Fever can also be related to non-infectious causes e.g. drug reaction or malignancy. A multiceneter study showed no significant enhanced detection of bacteraemia when taking cultures around elevated temperatures (8).

The general rule of sending two to three blood culture sets from different sites in a period of 24 hours has also been challenged. For continuous bacteraemia e.g. infective endocarditis the first culture are likely to be positive in contrast to patients with intermittent bacteraemia where 3 or more cultures over a period of time may necessary to detect the pathogen. A study showed no difference in taking cultures simultaneously or serially at spaced intervals (9).

Current recommendation with regard to timing and interval include obtaining two sets within minutes apart from two distinct sites at the onset of the 24 hour period with two subsequent sets being taking at different time intervals over the 24 hour period if the clinical condition deteriorates (10).

Blood cultures taken while on antimicrobial therapy will prevent detection of some bacteraemias, therefore sampling should precede administration of antibiotics at all costs. If patients are on antimicrobial therapy, specific resin containing bottles must be used to neutralize antimicrobials and enhance pathogen detection. Antimicrobial therapy should however never be withheld to obtain subsequent cultures at different intervals.

Key points!

1. Blood cultures must be taken on suspicion of bacteraemia and not only around fever spikes.
2. Two sets must be taken at the onset of a 24 hour period within minutes apart.
3. Two more sets can be obtained during the 24 hour period if the clinical condition deteriorates.
4. Blood cultures must be obtained before administration of antimicrobial agents.

3.2. Site

The recommended practice is to obtain a blood culture from a peripheral venipuncture, however patients who are critically ill and where venous access is a problem will have a central venous catheter (CVC) or an arterial line from which sampling can be performed. This practice has been discouraged due to the concerns of possible contamination (11, 12). Other studies have shown the benefit of using sampling from CVCs to detect bacteraemia (13, 14). A recent study by Beutz et al in 2003 evaluated the clinical utility of using blood cultures taken through central venous catheters and peripheral venipunctures in critically ill medical patients and found an overall good negative predictive value, however they

warned that in a setting with a high incidence of true bacteraemia the use of taking cultures either through CVCs or peripheral venipunctures should be interpreted with caution, as many critically ill patients who are receiving antimicrobials through their central lines can have negative cultures despite true bacteraemia. They recommend that in patients with a CVC, blood cultures from both the CVC and peripheral venipuncture should be obtained to increase sensitivity but that additional samples may be necessary to trouble shoot discordant results (15). A recent meta-analysis also showed better sensitivity and negative predictive value with cultures taken from an intravascular site and therefore recommends that at least one culture should be from the CVC (16).

The practice of using the two needle technique, removing the needle after drawing the blood and attaching another sterile needle to inoculate the blood into the bottle, is currently discouraged due to the risk of acquiring needlestick injuries although a meta-analysis showed a slight reduction in contamination rates (17).

Key points!

1. Peripheral venipuncture is preferred.
2. If an invasive line is present, do both and correlate.
3. Keep in mind that the culture from the CVC may be negative if antimicrobials are administered through the line, despite true bacteraemia.
4. Although the negative predictive value of one culture is good, the sensitivity of taking a culture either peripherally or through a CVC is not adequate.

3.3. Skin antisepsis

Blood culture contamination can lead to significant increase in healthcare related costs (12). Skin antisepsis therefore plays a critical role in reducing these contaminants.

Various antisepsis agents are commercially available and these agents differ by onset of action, mechanism of action and cost. A comparison between povidine – iodine, 70% isopropyl – alcohol, tincture of iodine and povidine – iodine with 70% alcohol detected no significant difference with regard to blood culture contamination rates (18).

Current infection control bundles for insertion of central line catheters and best practice guidelines for taking of blood cultures recommend using 2% chlorhexidine – gluconate in 70% isopropyl – alcohol as skin antisepsis due to the enhanced activity compared to other formulations (19).

In addition to alcohol containing antiseptics, the use of a prepackage antiseptic may play a role in reducing contamination rates (20). ChloraPrep (Enturia Limited) is a commercial antisepsis system that uses a plastic applicator to release 2% chlorhexidine – gluconate and 70% isopropyl – alcohol into a sponge thereby reducing cross contamination from the care givers' hands. A study by Tepus et al has shown a reduction in culture contamination rates (21) whereas another showed no significant decrease compared to 70% alcohol impregnated wipes (22).

Key points!

1. Various skin antisepsis agents are commercially available.
2. These agents are equally effective in reducing blood culture contamination rates.
3. The current recommended agent for skin antisepsis when performing venipuncture is 2% chlorhexidine – gluconate in 70% isopropyl – alcohol.

3.4. Volume and number of cultures

Adequate sample volume remains a critical factor to detect bacteraemia and have been evaluated extensively over last few years. Clinical and Laboratory Standards Institute (CLSI) recommend four 10 ml bottles to detect 90 – 95% of bacteraemias (23). In order to detect up to 99% of organisms a total of 60 ml of blood will need to be cultured (11).

In the early 1980s Washington proposed that culturing of a higher volume will result in a higher detection rate of blood stream infections (24, 25, 26), however the question arose whether this dictum still holds true for the newer continuous monitoring blood culture systems. Weinstein answered the question by comparing the speed and yield of detection of microorganisms from aerobic bottles inoculated with both 5 ml and 10 ml using a continuous monitoring blood culture system by showing the overall recovery of microorganisms to be higher with the 10 ml inoculated bottles ($P < 0.001$) (27).

An interesting study found that the higher the age of the patient and the severity of the underlying condition significantly influenced the collection and subsequent culturing of lower volumes of blood. The study also demonstrated that in critically ill patients, the higher the volume cultured, the more bacteraemias were detected and the yield of microorganism recovery increased by 3.5% per additional milliliter of blood cultured (28).

Current recommendations include collecting at least two sets of, each 20 ml of blood distributed equally between an aerobic and an anaerobic bottle from two distinct sites (10, 29). Lee et al reported that in order to detect 90% of true bacteraemias, 2 blood culture sets should be taken in a 24 hour period, however to detect > 99% up to 4 blood culture sets may be necessary (30).

Single blood cultures should be discouraged due their lack of sensitivity and difficult interpretation e.g. isolating coagulase negative *staphylococci* (CoNS) from a single blood culture may represent contamination or true bacteraemia (29).

A recent study reported yields from consecutive cultures from patients without infective endocarditis to be 65.1% after the first blood culture, 80.4% after the second blood culture and 95.7% after third blood culture. They also observed a high positivity from the first culture in patients with infective endocarditis which supports the observation of a continuous bacteraemia and fungaemia in this patient group (10).

Although paediatric bottles have been adapted to accommodate much smaller volumes of blood the optimal amount remains unknown. It is common practice to culture not more than

1 – 2 ml of blood in neonates. One study suggests that failure to detect bacteraemia was more likely when culturing < 1 ml of blood (31).

Human blood contains various factors or substances that can interfere with the detection of micro-organisms e.g. host serum factors and also antimicrobial agents. Therefore inoculated blood must be diluted to a point where these substances will have a minimal inhibitory effect. The required dilutional factor has been evaluated before and up to 10 times dilution has been recommended (32, 33), however the blood – broth ratio required for various systems will differ according to manufactures' instruction. The VersaTREK (TREK Diagnostic Systems, Cleveland, Ohio) is adapted to accommodate smaller volumes from as little as 0.1 ml – 10 ml, however the manufacture still recommends using 10 ml to achieve a 1:9 blood – broth ratio in the 80 ml bottle. Inoculating at dilutions higher than 1:10 may be associated with a lower yield due to the decreased overall volume cultured (29).

Key points!

1. The higher the volume, the higher the yield.
2. Up to 4 blood culture sets in a 24 hour period may be necessary to detect > 99% of microorganisms.
3. As least 1 ml must be cultured in neonates.
4. Adequate blood – broth ratio of 1:10 must be achieved to dilute the effects of inhibitory substances and antimicrobials present in the blood.

3.5. Training

Education and training of staff responsible for collection of blood cultures is critical. Studies have shown a decrease in contamination rates associated with combining different measures with training (34, 35) with the one study reporting contamination rates from phlebotomist vs. non-phlebotomists to be 0.8% and 4.7% respectively (35).

Key points!

1. Lower contamination rates are possible with dedicated phlebotomists.

3.6. Clinical information and labeling

Although all positive blood cultures are regarded as significant, false positive results can occur and interpretation becomes critical. Clinical information can aid the laboratory to decide whether an isolate is more likely to be significant or a contaminant (See section on Blood culture contamination).

Labeling of each bottle especially indicating the site through which the sample was taken is of critical importance to the laboratory. Sets taken through catheter lines are more likely to be contaminated and therefore correct labeling can aid interpretation.

Avoid applying the label over the barcode or the bottom of the bottle, this practice cover the sensor that is critical for detection and can result in false positive signals.

Key points!

1. Label bottle with the site where the sample was obtained from.
2. Covering the sensor at the bottom of the bottle can result in false positive signals.

3.7. Blood culture collection kits

This new strategy to decrease blood culture contamination has been implemented in some centers. The kit contains a pre-packaged antiseptic e.g. ChloraPrep sponge, a blood collection set (needle, syringe, safety lock etc), bottles and an instruction leaflet. A study evaluating the impact of these blood culture collection kits showed a reduction in blood culture contamination rates from 9.2% – 3.8%, however introduction of the kit was associated with an unintended yet sustained decrease in the amount of blood cultures collected which may have resulted in an unwanted reduction in the amount of true Gram negative bacteraemias (34). The authors recommend using the kit with ongoing training and ensuring that availability and accessibility are not compromised. Weightman et al also reported a decrease in contamination rates observed at their centre after the introduction of blood culture collection kits from 6% - 2.7% without compromising the amount of investigations performed (35).

Key points!

1. Blood culture collection kits can decrease blood culture contamination rates.

4. Selection of the correct blood culture bottle

Blood culture sets generally consist of and aerobic an anaerobic bottle. Various different bottles are available depending on the continuous monitoring system used. These bottles are specifically designed to optimize recovery of both aerobic and anaerobic organisms. This section will highlight the principles of these bottles by discussing a few examples, more detail with regard to specific bottles not mentioned should be obtained from the manufacturer.

Despite a decrease in the amount of anaerobic organisms isolated, the use of the anaerobic bottle as part of the routine blood culture set continuous. Various authors have questioned this practice (36, 37). Morris et al suggested that an approach of using two aerobic bottles with selective anaerobic culturing could enhance isolation with up to 6% (36), however this approach has not been adapted. Tamayose et al argued strongly to discontinue anaerobic culturing as routine practice and place attention on enhancing fungal isolation. (37). The anaerobic bottle however adds value in the fact that it allows growth of facultative organisms and thus adding to the total volume cultured and thus the sensitivity for organism recovery.

Various culturing media within one system differ with regard to constituents and performance and the choice relies heavily on controlled clinical evaluation. The BacT/ALERT FN medium (bioMérieux, Durham, N.C.) recently replaced the BacT/Alert

anerobic FAN medium. They differ in composition with the amount of activated charcoal and broth constituents where the FN bottle contains a higher concentration of activated charcoal as well as trypticase soy broth compared to brain heart infusion. BacT/ALERT also has a standard anaerobic bottle SN which does not contain activated charcoal. Mirret el al compared all three anaerobic bottles with the standard aerobic bottle, the BacT/ALERT FA medium and found better recovery of micro-organisms and a faster time to positivity (TTP) with the FN bottle (38).

Often the culturing media may differ in the structural format e.g. the BacT/ALERT 3D system (bioMérieux, Durham, N.C.) uses plastic bottles compared to the Bactec9240 system (BD Microbiology, Cockeysville,MD) that uses glass bottles. The VersaTREK system (Trek Diagnostic Systems, Cleveland, OH) offers media in two forms for both aerobic and anaerobic isolation, the 40 ml direct draw format which can accommodate 5 ml and an 80 ml format which can accommodate 10 ml. With the direct draw format the blood – broth ratio achieved is 1:8. Samuel et al compared the two media types with simulated blood cultures with clinically relevant microorganisms and found no negative impact on TTP with the smaller volume bottles (39). Caution should be applied in not compromising the total volume cultured when using small volume bottles as the recommended volume to be cultured still remains 30 ml of blood (See section on Principles of blood culture collection).

Some blood culture bottles contain the anticoagulant sodium polyanetholsulphonate (SPS) that can inactivate some of the host serum factors but also have a toxic effect on some organisms. An overall balance is achieved with the correct blood – broth ratio. The blood – broth ratio required and achieved will be different between systems (See section on Principles of blood culture collection). Bottles also differ in terms of the antibiotic binding resins used to limit the effect of inhibitory substances. A study compared the Bactec Plus media and TREK Redox media and found the former to be more efficient in recovering organisms in the presence of antimicrobial substances (40).

Paediatric bottles are specifically adapted to accommodate smaller volumes and often contain additional growth factors and binding resins to enhance organism recovery. A common misconception is that standard bottles cannot be used on paediatric patients and vice versa, however the choice of bottle will be dictated by the volume obtained. Although paediatric bottles are designed to maximize growth from smaller volumes the sensitivity to detect bacteraemia will increase with the volume of blood cultured. In neonates and infants it is assumed that you sample a much smaller pool and therefore the volume necessary to detect bacteraemia must be smaller, however as highlighted before the total volume required is not known and due to restriction in obtaining high volumes from this patient population this answer will keep eluding us. One study however has shown that the chance to fail to detect bacteraemia will increase when culturing < 1 ml in neonates (31).

Some systems provide additional culturing media optimized to detect fungal or mycobacterial pathogens more efficiently, however these will not be addressed in this chapter.

5. From collection to incubation

Over the years blood cultures has been regarded as one of the most import specimen types and microbiology laboratories take great care to process these as rapidly as possible. Blood cultures that have been collected must reach the laboratory as soon as possible and generally receive high attention for immediate incubation to allow optimal growth of organisms and rapid recovery without compromising the specimen.

Although in principle, the bottles must be inserted within the continuous monitoring blood culture systems as soon as possible, various factors can affect the time to insertion (TTI). Some laboratories do not operate a full 24 hours and bottles will then be incubated at 35^0C and can only be inserted the following day. Other factors include a delay in reaching the laboratory due to logistics. A study by Saito et al evaluated the effect of delayed insertion of blood culture bottles into continuously monitoring blood culture systems and found that although delayed insertion did not affect the sensitivity for organism recovery the mean TTP for all isolates was significantly shorter if inserted on the same day that the culture was obtained (41).

Time to removal (TTR) is defined as the time elapsed from when the system emits a positive signal until the bottle is removed for subsequent subculturing for organism recovery. A delay in removal of the bottles could be due to closure of the laboratory at night and this can result in obtaining false negative results especially when isolating more fastidious organisms. In our study when evaluating the VersaTREK system against the Bactec9240 system we found that the time to removal (TTR) for some isolates were up to 8 hours and this could have explained some false negative results where the system failed to detect *S. pneumonia* isolates (42). We know that this organism is prone to activate autolysin under stress conditions which may result in poor recovery from blood culture bottles. This phenomena has been observed with the BacT/ALERT 3D system in our laboratory where positive signals were obtained with subsequent no growth, however confirmed after positive agglutination from the bottle sediment (data not shown).

The duration of incubation is calculated from the time of insertion until the time of removal. Bottles will be removed, thus considered negative, when no positive signal is obtained after a certain amount of incubation time has elapsed. This time before removal will depend on the blood culture system used. For manual broth – based systems, 7 days are the recommended incubation time (43) in comparison to various studies that support shorter incubation times of 4 to 5 days with the continuous monitoring blood culture systems (44,45,46).

Extended incubation (up to 14 to 21 days) is still recommended to detect more fastidious organisms specifically the HACEK organisms (*Haemophilus, Actinobacillus, Cardiobacterium, Eikenella,* and *Kingella* species) that are involved in endocarditis (7). Recent reports have shown that the method of detection and not the time of incubation is critical to detect these organisms. Baron et al in 2005 reported evidence that the Bactec9240 system can detect the HACEK organisms within 5 days (47). Alternative methods e.g. a lysis centrifugation system for dimorphic fungi and molecular methods for *Bartonella henselae* may be of more value

than prolonged incubation (47, 48). In a multicentre evaluation of 15 826 positive blood cultures only 0.1% of HACEK organisms were detected across all centers with a mean time to detection of 3.4 days. They recommend based on their findings that extended incubation for HACEK organisms is unnecessary (49).

6. The choice of blood culture system

Various commercial blood culture systems are available. The choice of blood culture system will depend on various factors (Table 2). It is the responsibility of the laboratory director to liaise with clinicians on the selection of the best system to achieve the best results for their specific patient population and workload.

The various blood culture systems compete with regard to sensitivity for organism recovery, TTP, workload capacity, user interface and associated costs. Not one system is perfect and able to detect all possible micro-organisms.

These systems all require inoculation of blood into a media bottle. The media are in principle similar, however controlled clinical trials have shown some media to be superior for certain organisms (See section Selection of the correct blood culture bottle).

Sensitivity for organism recovery is the most import parameter when selecting a blood culture system. Studies have shown that the lysis centrifugation systems are more sensitive for the detection of fastidious organisms and dimorphic fungi (25). The continuous monitoring systems have shown superiority with regard to TTP compared to manual systems (50, 51, 52, 53) and are the current preferred systems. TTP has been shown to be a good predictor of clinical outcome in staphylococcal sepsis (54, 55, 56).

Sensitivity for organism recovery
Time to positivity
Workload capacity
User interface
Costs

Table 2. Factors that affect the choice of blood culture system

The workload capacity is important as many laboratories differ in the amount of blood culture they will process. The continuous monitoring systems have the capacity to be expanded to accommodate the workload. The interface must be user friendly e.g. some technologists will review the growth index of the bottle to troubleshoot possible false positive signals. Cost of implementation and maintenance may play a role as well.

6.1. Various commercial blood culture systems – Advantages and disadvantages

There are currently a wide variety of blood culture systems available. Although the continuous monitoring blood culture systems have become the preferred platform, manual systems are still available and used in some settings and will be discussed briefly.

6.1.1. Manual blood culture systems

The conventional manual method entails inoculating a commercially provided blood culture bottle, incubating the bottle at the required temperature and atmosphere with daily inspection of the bottle for macroscopic evidence of growth e.g. turbidity, haemolysis or colonies. Once growth is observed, a sample can be obtained for Gram staining and subculture for further identification. Bottles are incubated for 7 days and terminal subculture is mandatory.

Variations to the conventional manual method is combining agar in the form of paddles to the broth. These systems allow for more frequent subculturing by inverting the bottles to bring the broth into contact with the agar. These bottles can be inspected for growth and Gram staining with presumptive identification to be performed from the agar.

The Septi-Chek (BD Diagnostics) blood culture system is a biphasic-agar slide system that uses a standard blood culture bottle containing brain heart infusion or trypticase soy broth connected to a second plastic chamber with a trisurface panel consisting of chocolate, Mackonkey and malt agar. The slide chamber is screwed onto the bottle after inoculation and incubated at 35°C for 4 – 6 hours. The bottle is then inverted for the first subculture and can be inverted at various intervals thereafter to optimize isolation.

The Oxoid Signal System (Oxoid Unipath, Basingstoke,England) is unique in the sense that it is a one bottle system. After inoculation of a standard blood culture bottle a second chamber is attached with a long needle that extends below the surface of the blood – broth mixture. This closed space system uses CO_2 production to detect growth. Any gas produced will increase the pressure in the headspace and allow some of the blood – broth mixture to enter into the chamber from where sampling, Gram staining and subculturing can be performed. This system thus signals the laboratory towards possible growth without using an automated system. The advantages and disadvantages of these systems are presented in Table 3.

Advantages	**Disadvantages**
Evaluated favourably in detecting growth	More false positives
Cost effective	Lower yield of anaerobes
Useful in small laboratories with small workload	Labour intensive, need to visibly inspect for growth

Table 3. Advantages and disadvantages of manual blood culture systems

6.1.2. Lysis centrifugation systems

The principle of this test is explained in its name. The Wampole Isostat/ Isolater Microbial System (Inverness Medical) is a single tube test that uses saponin for lyses of erythrocytes and neutrophils, followed by centrifugation and subsequent inoculation of solid agar media for isolation. The system is useful for the recovery of slow growing and fastidious organisms including filamentous moulds, dimorphic fungi and *Bartonella henselae* (29). This method

also allows quantification to be performed, however limitations include a higher rate of contamination, excessive hands on time and toxic effects of the saponin that can inhibit growth.

6.1.3. Continuous monitoring blood culture systems

These systems are considered an advance in clinical microbiology and are the current preferred platform for blood culture testing worldwide. With the introduction of these systems in the 1970s they have evolved over time e.g. the Bactec series started with radiometric systems which was later replaced with non – radiometric systems. Today we face automated and computerized continuous monitoring blood culture systems.

The three main commercially available systems are the BacT/ALERT blood culture system (bioMérieux, Durham, N.C), Bactec 9000 series (BD Microbiology, Cockeysville,MD) and the VersaTREK system (Trek Diagnostic Systems, Cleveland, Ohio). All three systems have expandable detection units with self-contained incubation chambers and minimal bottle manipulation as agitation is achieved via rocking or vortexing. The principle of detection of these systems is based on the release of CO_2 in the presence of micro-organism metabolism.

The Bact/ALERT and Bactec systems both depend on a pH change due to the production of CO_2 to detect growth. The Bactec9240 systems' bottles have a sensor at the bottom that emits a fluorescent light as the CO_2 concentration increases, that will pass via an emission filter to a light sensitive diode. The system measures the voltage every 10 minutes and compares the new value with the previous value and emits a positive signal as soon as the threshold value is reached. The BacT/ALERT 3D system uses a CO_2 sensitive chemical sensor that is separated from the blood – broth mixture via a unidirectional membrane. Once the CO_2 concentration increases, the colour will change from green to yellow, this is measured with a photosensitive detector.

The VersaTREK system monitors changes in the bottle headspace every 24 minutes. Both gas consumption and production are monitored. As a result other gasses e.g. O_2 and H_2 are also detected. The system differs from the other systems in that the aerobic bottles are vortexed with a magnetic stir bar to increase oxygenation.

All three systems have been compared for both sensitivity for organism recovery and TTP. Mirret et al compared the VersaTREK system with the BacT/Alert system and found no significant difference for the detection of bacteraemia or fungaemia in clinical isolates (57). Our group compared the VersaTREK system against the Bactec9240 system and found both systems comparable to detect bacteraemia in patients with suspected sepsis however we observed a higher rate of false positive results and postulated that the threshold setting to emit a positive signal might be too low (42). Comparison of the BacT/ALERT system with the Bactec9240 systems showed slight better detection with the former (58). Advantages and disadvantages of these systems are shown in table 4.

Advantages	Disadvantages
Higher sensitivity for organism recovery	High implementation cost
Faster TTP	Equipment must be maintained
Fully automated and computerized	Need continuous power supply
Easy loading and unloading of bottles	
Expandable to accommodate larger or smaller volumes	

Table 4. Advantages and disadvantages of continuous monitoring systems

6.2. Time to positivity

Time to positivity (TTP) is a parameter provided by the automated blood culture system and is calculated from the time of incubation until a positive signal is detected. TTP can be influenced by various factors e.g. the bacterial load, the growth rate of the micro-organism, the presence of antibacterial substances in the blood as well as source of infection and clinical features.

Differential TTP has been used to diagnose CLABSI (59, 60, 61). Two sets of blood cultures are taken at the same time, one through the inserted catheter and the other peripherally. A CLABSI should be suspected if both sets yield the same micro-organisms and the set taken through the line becomes positive (TTP) 120 minutes or earlier than the peripheral set (62).

Short TTP in *S. aureus* bacteraemia can possibly predict the source of infection, specifically an endovascular source and also correlate with the attributable mortality (54).

Combining the TTP with the initial Gram stain result could predict the micro-organism as well as the source of bacteraemia, e.g. patients not on antimicrobial agents with Gram positive cocci in cluster within 14 hours was predictive of *S. aureus*, however the clinical impact of using this approach needs to be evaluated further (63).

7. Interpretation of results

Interpretation of positive blood culture results are challenging to both clinicians and microbiologists. With the background of blood culture contamination rates of up to half of all positive cultures and previously considered contaminants now more frequently implicated in disease the need for tools to assist in distinguishing contaminants from pathogens becomes eminent.

Consensus have been reached with regard to clinical and laboratory parameters that must be taken into consideration to assess positive cultures for significance or contamination. These include fever, leucocytosis, positive imaging, the identity of the organism recovered, the number of sets positive out of the number received, the number of bottles positive within a given set and the TTP (64) (Table 5).

Clinical	Laboratory
Fever	Identity of the microorganism
Leucocytosis	Number of positive sets
Positive imaging	Number of positive bottles (within set)
	Time to positivity

Table 5. Parameters as tools to distinguish contaminants from pathogens in positive blood cultures

The identity of the microorganism can aid interpretation of results (11, 65). According to an evaluation by Weinstein et al in 1997 there are organisms that will be pathogens in > 90% of cases and these include *Staphylococcus aureus*, *Streptococcus pneumoniae*, *Escherichia coli*, other *Enterobacteriaceae*, *Pseudomonas aeruginosa* and *Candida albicans* (66). Despite adequate data from large studies there are other organisms that also presents as true pathogens most of the time e.g. *Streptococcus pyogenes*, *Streptococcus agalactiae*, *Listeria monocytogenes*, *Neisseria meningitidis*, *Neisseria gonorrhoeae*, *Haemophilus influenzae*, *Bacteroides fragilis* group, *Candida* species other than *C. albicans* and *Cryptococcus neoformans* (64). Organisms that represent rarely as pathogens include *Bacillus* species, *Propionibacterium acnes, Corynebacterium* spp other than *C. jeikieum and* Viridans group streptococci (66, 67) (Table 6).

True pathogen	Probable contaminant
Staphylococcus aureus	Coagulase negative staphylococci (CoNS)*
Streptococcus pneumoniae	*Bacillis* species
Escherichia coli	*Propionibacterium acnes*
Other *Enterobacteriaceae*	*Corynebacterium* spp
Pseudomonas aeruginosa	
Candida albicans	

*Contaminant or true pathogen

Table 6. Organism identity to indicate significance or contamination

Other organisms can no longer be judged on their identity with regard to significance, these include CoNS, Viridans group streptococci and *Clostridium* species.

Isolating CoNS from positive blood culture bottles presents a challenge for clinicians in deciding the significance of the finding. Not only are CoNS the most common contaminant isolated but patients who presents with true infection often have mild symptoms which makes the interpretation very difficult (68,69). Various studies are reporting CoNS to be a true pathogen causing blood stream infections especially in patients with indwelling prosthetic devices or central venous catheters (66, 70).

The value of obtaining more than one set of blood cultures not only enhances the yield of detecting bacteraemia, but also aids interpretation when dealing with positive cultures. Contaminants are often obtained from only one set whereas with true bacteraemias multiple blood cultures will grow the same organism (66). Weinstein also reported that if an institution has a baseline contamination rate of about 3%, the chances of recovering the same organism in a two set culture and being a contaminant is less than 1 in a 1000 (64).

Using the TTP as an aid to establish significance have been debated before (64). The principle relies on the assumption that true infection will present with a much larger inoculum vs. contamination and thus result in earlier detection. One of the reasons why TTP can be misleading is the fact that continuous monitoring systems can detect micro-organisms at lower levels much faster than conventional systems.

Using the criteria that if one bottle is positive within a given set relates to contamination should be discouraged. For CoNS this has been evaluated and shown to be inadequate to predict clinical outcome (71).

8. Blood culture contamination

Culture contamination represents false positive results and are not uncommon in microbiology laboratories with rates being reported as high as around 50% of all positive cultures (66, 72). An increase in contamination rates despite advances in the field of microbiology has been observed (66) and this phenomenon could be explained by the increasing use of continuously monitoring blood culture systems and the improved culture media provided for the specific systems that may aid the detection of low numbers contaminants. The increased use of intravascular devices and the practice of taking cultures through invasive lines are also important when considering contamination rates. American Society of Microbiologists published standards that state that blood culture contamination rates must not exceed 3% (73), rates however will differ widely between institutions, but commonly exceeds 7% (74, 75).

The cost of blood culture contamination often exceeds the cost of performing the test (76). A retrospective case-control study evaluated 142 false positive blood cultures and found a significant increase in length of hospital stay as well as laboratory and pharmacy costs, they also calculated that the 254 false positives blood cultures in a year period, added 1372 extra hospital days and £1,270,381 in costs per year (77).

Various strategies have been implemented to decrease blood culture contamination rates e.g. training staff with regard to aseptic collection technique, feedback with regard to contamination rates and implementation of blood culture collection kits. Although skin antisepsis can reduce the burden of contamination, 20% of skin organisms are located deep within the dermis and are unaffected by antisepsis (78). The practice of changing needles before bottle inoculation should be abandoned as it increases the risk to acquire needle stick injuries without decreasing contamination rates (79). Also discarding the initial aliquot of blood taken from CVCs does not reduce contamination (80).

9. New technologies used in conjunction with blood culture systems in the diagnosis of sepsis

In hospital settings where resistance profiles of circulating micro-organisms are known, the use of rapid identifying methods to guide empiric antimicrobial usage is critical to improve patient outcomes. Research efforts are focused on developing molecular tests

that can be performed without prior culturing with continuous monitoring systems, however these assays are limited to date. Molecular assays performed on positive blood culture bottles has improved sensitivity compared to conventional culturing methods, and has decreased turnaround times compared to routine culture (81). Study by Karahan have shown the use of molecular methods to evaluate false positive signals for identifying microbial DNA of organisms that might have been inhibited by high leucocytes count or antimicrobials (82).

The Lightcycler® SeptiFast (Roche Diagnostics, Manheim, Germany) is a multiplex realtime PCR system that can detect up to 25 common pathogens involved in sepsis from one single blood sample within 6 hours. Various studies have evaluated the use and confirm increased detection of circulating microbial DNA compared with conventional blood culture (83, 84,85). Study by Lucignano et al evaluated the use in the paediatric population with suspected sepsis with sensitivity and specificity reported of 85% and 95% respectively. Significantly higher yields were observed from patients already on antimicrobial therapy (86). Although this method is considered culture – independent, most studies agree that this system does not replace conventional blood culturing and the clinical significance of detecting higher amounts of microbial DNA must be further evaluated.

A new strategy for the detection of blood stream pathogens include PCR/Electrospray ionization and mass spectrometry (PCR/ESI-MS). This technique in short amplifies broadly conserved regions of bacterial and fungal genomes followed by mass spectrometric analysis by weighing the PCR amplicons and comparing the product with known standards. The commercial assay is the Bac Spectrum Assay that runs on the PLEX-ID (Abbott Molecular). A study by Eshoo et al showed good sensitivity and specificity for the detection of *Erlichia* species in the blood from patients with suspected erlichiosis with the identification of additional bacterial pathogens that was determined to be clinically relevant (89). This new method will likely change the future of diagnosis of bloodstream pathogens however clinical relevant studies needs to be performed.

The Prove-it sepsis assay (Mobidiag, Helsinki, Finland) is a DNA-based microarray platform can identify more than 50 Gram positive and Gram negative bacteria that can cause sepsis (87) as well as detect the presence of the *mecA* gene that codes for methicillin resistance in *S. aureus* (88) from positive blood culture bottles. Sensitivity and specificity compared to conventional culture are reported to be 94.7% and 98.8% respectively (81). The study also showed an 18 hour faster turnaround time for identification compared to conventional culture. Due to the multiplexing capabilities this assay can also be expanded to detect pathogens involved in fungaemia.

The Xpert MRSA/SA Blood culture assay (Cepheid) was evaluated favourably for the detection of an the discrimination between methicillin resistant *staphylococcus aureus* (MRSA) and methicillin susceptible *staphylococcus aureus* (MSSA) (90) . Although this method is limited with regard to the range of pathogens it will guide initial empiric therapy towards a better clinical outcome.

Another new approach to enhance earlier specie identification from positive blood culture bottles following Gram staining include the Peptide Nucleic Acid Fluorescence *In situ* Hybridization assay (PNA-FISH). This assay uses probes that target specific conserved bacterial and fungal genomic regions and can distinguish between e.g. *S. aureus* and non – *S. aureus* as well as different *Candida* species (91, 92, 93).

Matrix-assisted Laser Desorption/Ionization–time of flight (MALDI-TOF) mass spectrometry (MS) is currently widely applied on post culture isolates for rapid identification. The system use MS signals created and compare them to standard signal patterns within a database. The use directly from positive blood culture bottles needs further evaluation but the advantage of this technology shows promise for the future.

The various new technologies appears attractive, however implementation will come at great cost and are not cost effective for routine laboratories at present. Certainly the rapidly of results being generated and the ability to detect pathogens unlikely to grew on conventional media comes as a great advantage. The clinical significance of enhanced detection of circulating microbial DNA must be established.

10. Conclusion

The use of blood culture systems still remain the gold standard for the detection of bacteraemia. It is important to understand the process from collection to obtaining a result to aid interpretation and improve the clinical outcome. While the continuous monitoring systems are the preferred platform for testing, various new methods are on the horizon that will aid or even replace these systems, only time will tell.

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