Extended incubation in blood cultures: necessity or nuance?

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Summary

Bloodstream Infections (BSIs) are severe conditions caused by microbial pathogens that can lead to life-threatening complications if not diagnosed and treated promptly. Blood culture remains the primary method for detecting these infections, allowing clinicians to identify causative organisms and select appropriate antimicrobial therapies. While traditional culture techniques are widely used, their long processing time has driven the adoption of automated systems such as BacT/ALERT and BACTEC, which offer faster and more accurate results. In addition, extending incubation periods has proven valuable in recovering slow-growing bacteria and fungi, particularly those that may be missed within standard time frames. This strategy supports antimicrobial stewardship by reducing reliance on empirical treatments and ensuring targeted antibiotic use. However, there are certain challenges in prolonging the incubation time, including an increased risk of contamination, higher resource demands, and workflow adjustments in laboratories. A critical balance and customisation as per the requirements of the clinicians and the resources of the laboratory are required.

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Introduction

Importance of blood cultures in diagnosing bloodstream infections

Bloodstream Infections (BSIs) are caused by various microbes, such as bacteria, fungi, or viruses, that spread throughout the body through the bloodstream. BSIs are severe diseases characterised by high morbidity and mortality, which is directly associated with the delay in administration of the first adequate anti-infectious agent (3). These infections can lead to severe complications, including a potentially lethal immune response called sepsis. Sepsis is characterised by organ dysfunction and may present with hypotension, oliguria, intestinal paralysis, impaired tissue perfusion, elevated serum creatinine, hypoxemia, hyperlactatemia, hyperbilirubinemia, coagulopathy, or metabolic acidosis. If left unattended, sepsis can escalate to septic shock, defined as refractory arterial hypotension or tissue hypoperfusion despite adequate intravascular fluid resuscitation. Hypoperfusion may be manifested as lactic acidosis, oliguria, or mental status changes, eventually leading to organ dysfunction and death (12). BSIs can originate from several sources, such as medical devices (e.g., I.V. catheters), surgical procedures, or infections at other sites in the body, like pneumonia, urinary tract infections, *etc*.

Blood cultures play a crucial role in the diagnosis and management of patients with suspected sepsis due to bacteremia or fungemia. Early detection of pathogens enables prompt optimisation of antimicrobial therapy, helping clinicians to escalate, de-escalate, or discontinue antibiotics as per the susceptibility, essential for not just patient management but also antimicrobial stewardship. Although empirical antimicrobial treatment is swiftly started after blood sampling, based on clinical and epidemiological data, its effectiveness remains doubtful until the pathogen is confirmed and susceptibility reports are obtained, particularly considering the rising prevalence of multidrug-resistant organisms. On the other hand, early targeted antibiotic administration can markedly improve clinical outcomes for patients with BSIs (3).

The isolated species, number of positive blood cultures or sets, and time to positivity, etc., are some of the factors that affect the decision of labelling the identified organism from blood as a pathogen or contaminant. The probability of blood cultures being significant increases with the presence of clinical signs of infection, whereas a high proportion of false-positive blood cultures may be seen in patients with a very low risk of bacteraemia. In emergency departments that experience a high influx of patients, individuals with severe illnesses, end-stage renal disease, and older adults tend to have a greater incidence of blood culture contamination, mainly due to the time constraints and urgency involved in collecting blood culture specimens (3).

Available methods for blood culture

Blood cultures are carried out using techniques ranging from conventional to completely automated methods. A comparison of commonly used methods is shown in Table 1. In the conventional method blood sample is added to 100 ml of brain heart infusion broth having Sodium Polyanethole Sulfonate (SPS) and incubated at 37°C for approximately 5 to 7 days. These bottles are regularly observed for indications of growth (turbidity), and subcultures are performed on solid media such as MacConkey and blood agar. Although cheaper, the conventional method has various limitations, including longer pathogen detection time, the need for manual monitoring, a higher chance of contamination due to multiple blind subcultures, etc. The arrival of continuous-reading, automated, and computerised blood culture systems marked a significant milestone in Clinical Microbiology practices. These automated systems notify the microbiologist when a culture is positive, allowing for the timely removal of relevant bottles for Gram staining and subculturing on solid media. With the advent of these automated systems, the use of manual blood culture methods has notably declined. The fully automated blood culture technique is thus regarded as superior to traditional methods with regard to speed and sensitivity (14). Important widely used automated systems include BacT/ALERT 3D System, BACTEC system, and BacT/ALERT VIRTUO.

The BacT/ALERT system (bioMérieux, Marcy-l'Étoile, France) detects microbial growth by measuring CO_2 production, a metabolic by-product of microorganisms. Colorimetric sensors within the culture bottles change colour in response to increasing CO_2 levels, thereby indicating microbial presence. Apart from providing real-time and earlier microbial detection, the system's Fastidious Antimicrobial Neutralisation (FAN) bottles, both aerobic and anaerobic, can neutralize antibiotics present in patient samples, thereby enhancing the recovery rates of various microorganisms (20). These features emphasise their clinical utility in diverse settings, improving the detection of pathogens that might otherwise be missed by traditional culture methods.

The BACTEC (Becton Dickinson, Franklin Lakes, USA) system employs fluorescence-based technology to detect microbial growth. It measures the amount of CO_2 produced by metabolising organisms, which correlates with fluorescence intensity. This system is known for its rapid detection capabilities and has been extensively used in clinical laboratories for its high sensitivity and specificity in identifying a wide range of pathogens (4). Earlier BACTEC systems relied on radioisotope-based detection and have been discontinued. The BACTEC™ FX system is the latest generation advanced automated blood culture platform that improves the detection of bloodstream infections by continuously observing for microbial growth via CO_2 detection (21).

BacT/ALERT VIRTUO is an advanced system that works on the same principle as BacT/ALERT 3D (colourimetric CO_2 detection), but with automation and speed enhancements, including automatic negative bottles unloading. A comparative analysis showed that the VIRTUO system surpassed the BACTEC FX400 in overall positive detection rates, especially in intensive care units, and demonstrated quicker Time-To-Detection (TTD) for both aerobic and anaerobic bacteria (15). Another study which compared BacT/ALERT Virtuo and BACTEC FX system demonstrated that BacT/ALERT Virtuo exhibited a significantly shorter TTD for 72.7% of the organisms

Table 1. Comparative features of commonly used blood culture methods.

Feature	Conventional Method (14)	BacT/ALERT System 3D (20)	BACTEC System (4,21)	BacT/ALERT VIRTUO (15)
Detection principle	Visual inspection for turbidity or blind subcultures	Colorimetric sensor detecting CO_2	Fluorescence-based detection of CO_2	Enhanced colorimetric detection and bottle volume estimation
Automation	Manual	Fully automated	Fully automated	Highly automated with workflow enhancements
Time To Detection (TTD)	Typically more than 48–72 hours	Faster than conventional methods	98% positive cultures within 96 hrs	Shortest TTD; median 2.1 hrs faster for many pathogens
Antimicrobial neutralization	Not available	FAN bottles neutralize antibiotics	Resin-containing bottles for neutralization	FAN bottles neutralize antibiotics
Sensitivity	Lower; may miss slow-growing organisms	High sensitivity for common and fastidious organisms	High sensitivity; slightly variable for some fastidious strains	Slightly superior overall; better for *E. coli*, *S. aureus*, *S. pneumoniae*
Organism recovery	Often misses fastidious or slow-growers	Good for Gram-positive, Gram-negative, and fastidious organisms	Excellent recovery for wide range of pathogens	Efficient recovery, faster detection especially in ICU settings
Limitations	Time-consuming, manual workload	Costlier, requires trained personnel	Costlier, requires trained personnel	High cost, may require infrastructure upgrades
Ideal use case	Low-resource settings or a backup method	General hospitals, OPD, ICUs	High-volume clinical labs and critical care settings	High-throughput labs, emergency departments needing rapid turnaround

OPD, Outpatient Department; ICU, Intensive Care Unit; FAN, Fastidious Antimicrobial Neutralisation.

tested, with a median TTD difference of 2.1 hours. This was particularly noted for pathogens such as *Escherichia coli*, *Staphylococcus aureus*, and *Streptococcus pneumoniae*. However, it was observed that the BACTEC FX system achieved quicker TTD for certain strains, such as *Haemophilus influenzae* and *Neisseria meningitidis*, which was attributed to varying sodium polyanethol sulfonate concentrations in the media (18).

Rationale for extended incubation in blood cultures

In routine practice, the blood culture bottles with no growth are generally discarded as negative after 5 days of incubation. Prolonged or extended incubation of blood cultures is primarily intended to enhance the recovery of slow-growing or fastidious microorganisms that may be easily missed during the standard incubation period. Extended incubation is becoming an essential practice in clinical microbiology to ensure comprehensive pathogen recovery. This practice not only supports improved patient care but also aligns with broader public health goals by contributing to antimicrobial stewardship and resource optimisation. Figure 1 shows various advantages of extended incubation of blood cultures.

Detection of slow-growing pathogens

Certain pathogens are often not detected in the first 5-day standard incubation period because they are very slow-growing. Members of the HACEK group (*Haemophilus*, *Aggregatibacter*, *Cardiobacterium*, *Eikenella*, and *Kingella*), *Brucella* species, and some fungi would need more time to grow to a level that can be detected. It has been shown that extending the incubation period beyond 7 days to a maximum of 14 days significantly improves the chances of recovering these organisms. Since these bacteria are associated with endocarditis or other complicated infections, this step is crucial for patients suffering from these conditions. A study published in the Journal of Clinical Microbiology demonstrated that extended incubation increased the detection rate of fastidious organisms by 20%, emphasising its clinical significance (11).

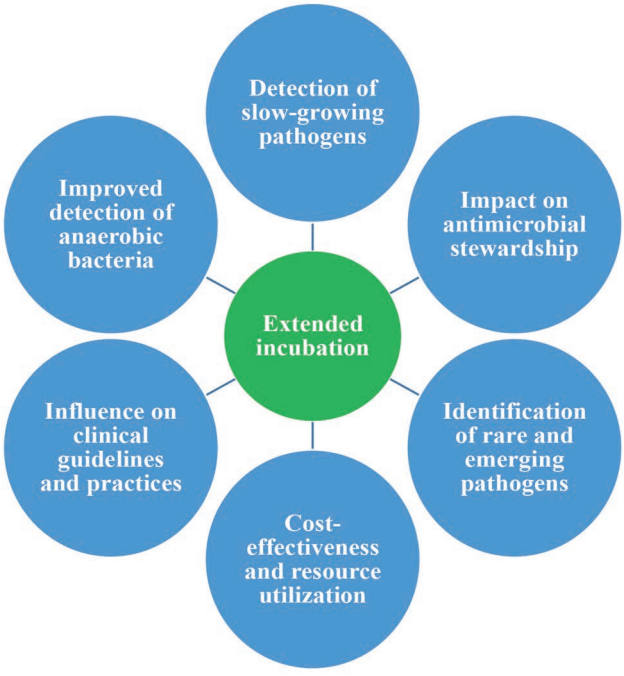

Figure 1. Advantages of extended incubation of blood cultures.

Impact on antimicrobial stewardship

Extended incubation promotes antimicrobial stewardship by increasing pathogen detection rates and guaranteeing accurate and fixed antimicrobial treatment. This minimises the development of resistance and side effects by reducing the empirical use of broad-spectrum antibiotics. Additionally, it improves patient outcomes and resource utilisation by customizing therapy according to actual pathogen profiles (11).

Identification of rare and emerging pathogens

New pathogens are constantly emerging as a result of environmental changes and globalisation; some of these pathogens may exhibit unusual growth patterns or not grow under typical incubation periods. Extended incubation allows for the detection of these rare organisms, ensuring timely and accurate diagnosis. The Centres for Disease Control and Prevention (CDC) emphasises the importance of extended incubation in identifying novel pathogens (16).

Improved detection of anaerobic bacteria

Anaerobic bacteria, often involved in systemic infections, may require longer incubation times for detection. These organisms are notoriously difficult to culture due to their oxygen sensitivity and slow growth rates. Extended incubation facilitates their recovery, aiding in accurate diagnosis and treatment. A study on antibiotic use and outcome in patients with negative blood cultures highlights the role of extended incubation in improving the detection rates of anaerobic bacteria by 15% (7).

Cost-effectiveness and resource utilisation

Although extended incubation may seem resource-heavy, it ultimately leads to long-term savings by minimising the need for repeat tests and shortening hospital stays through earlier and more precise diagnoses. It ensures laboratory resources are utilised efficiently by preventing the premature disposal of cultures.

Influence on clinical guidelines and practices and enhancing laboratory standardisation

The introduction of extended incubation has prompted revisions in clinical guidelines to ensure best practices are followed in the diagnosis of bloodstream infections. Adhering to revised guidelines enhances diagnostic precision and patient outcomes, particularly in complicated infection scenarios. The Infectious Diseases Society of America (IDSA) has revised its guidelines to include extended incubation methods, especially when slow-growing organisms are suspected (2). While the requirement for routine extended incubation is debatable, certain clinical scenarios involving fastidious organisms, such as *Brucella* or *Francisella* species, may warrant prolonged culture durations. Guidelines suggest that in such cases, clinicians should communicate with the Microbiology laboratory to ensure appropriate culture protocols are followed. Extended incubation facilitates the standardisation of laboratory techniques, ensuring consistency and comparability of results across different organisations (8).

Challenges and considerations in implementation

Increased risk of contamination

Extending the incubation period of blood cultures can inadvertently elevate the risk of contamination. Prolonged incubation establishes a conducive environment for the growth of skin flora or envi-

ronmental microorganisms introduced during sampling, leading to false-positive results. A study conducted at Osaka University Hospital over a period of 2 years investigated blood cultures that were maintained in incubation for more than 12 consecutive days. The results showed that 95.7% of positive cultures were identified during the first 5 days. Significantly, following 6 days of incubation, 80.2% of the positive results were classified as contaminants, suggesting that prolonged incubation primarily enhanced the identification of non-clinically important organisms (9). The clinical implications of such contamination are substantial. False-positive blood culture results can lead to unnecessary antibiotic administration, prolonged hospital stays, antimicrobial resistance, and increased healthcare costs. A study revealed that the rates of blood culture contamination differ significantly, spanning from 0.6% to more than 6% among various institutions (10). This variability underscores the importance of implementing stringent aseptic techniques during sample collection to minimise contamination risks.

Resource allocation, workflow integration and cost implications

Extended incubation already demands additional resources such as increased incubator space and prolonged monitoring, while the processing of clinically insignificant isolates can further strain laboratory capacity and financial resources. A study assessing blood culture contamination in a general hospital found that contamination led to significant extra laboratory costs, emphasising the need for efficient resource management and adherence to proper collection protocols to minimise financial burdens (19).

Another study evaluated the effect of a staff training intervention program on reducing blood culture contamination rates in an Intensive Care Unit (ICU), revealing that focused education significantly decreased contamination rates from 9.5% to 3.7% (1). This highlights the importance of continuous education programs and competency assessments to support the successful adoption of extended incubation practices.

Conclusions

Future directions

Instead of generally applying prolonged incubation, a focused strategy based on clinical scenarios is more efficient. Blood cultures with suspected slow-growing or fastidious pathogens may be selectively extended. Prolonged incubation for up to 14 days may help identify fastidious organisms like *Cutibacterium acnes* (13). In suspected cases of infective endocarditis, including prosthetic valve-related endocarditis caused by fastidious organisms such as *Brucella*, *Bartonella*, or *Coxiella burnetii*, extended incubation is advised, as standard incubation durations frequently fail to detect these pathogens (5,13). Organisms such as *Mycobacterium tuberculosis* and non-tuberculous mycobacteria are exceptionally slow-growing. Conventional culture techniques suggest incubation durations of as long as 60 days to support their isolation. This extended period is crucial because of the lengthy replication processes of these microorganisms (12). Dimorphic fungi, including *Histoplasma capsulatum* and *Coccidioides immitis*, also exhibit slow growth in culture. Standard incubation durations may be insufficient for their detection. While media like the BACTEC MYCO/F Lytic are effective for recovering various fungi, the time to detection for organisms such as *H. capsulatum* can be prolonged, indicating the need for extended incubation (6).

To conclude, extended incubation of blood cultures enhances the recovery of slow-growing and fastidious organisms, which are often missed during standard incubation periods. While this approach may increase resource demands and contamination risks, its selective use, guided by clinical context and local epidemiology, can significantly improve diagnostic yield. Larger, multi-centric studies are needed to better define its cost-effectiveness and clinical impact across various settings. Laboratories should tailor incubation protocols based on prevalent pathogens, clinical correlation, and available resources to strike an optimal balance between diagnostic benefit and operational feasibility.

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