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Overview of blood culture: Key elements in CLSI guideline and rapid detection methods

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Blood cultures are the gold standard for the diagnosis of bacteremia. Detection of bacterial and fungal organisms in blood is perhaps one of the most important functions of the clinical microbiology laboratory [1]. In this education track, the key elements of blood culture from CLSI document 47-A and the potential methods for rapid detection of pathogens in bloodstream infection will be introduced.

1. Timing of blood culture

Blood culture should be obtained simultaneously (or over a short timeframe). And drawing blood at intervals is only indicated when it is necessary to document continuous bacteremia in patients with infective endocarditis or catheter-related infections.

2. Number of blood culture

The CLSI guideline[2] is to collect two three sets per episode. Single blood culture results in an inadequate volume of blood cultured, and the results of single blood cultures are more difficult to interpret. Most patients with bacteremia or fungemia can be followed clinically and do not need follow up blood culture except 1) infective endocarditis and 2) *Staphylococcus aureus* bacteremia.

3. Volume of blood for culture

The volume of blood drawn for culture is the most important variable in detecting bacteremia or fungemia. For adult patient, the yield of pathogen increases in direct proportion to the volume of blood that is cultured from 2 to 30 mL. And the recommended volumes of adult are 20 to 30 mL per culture. For infants and younger children, the volume drawn should be no more than 1% of the patient's total blood volume.

4. Aerobic vs anaerobic BC collection

The CLIS guideline[2] recommended that routine blood cultures include paired aerobic/anaerobic blood culture bottles. When less than the recommended volume of blood is drawn for culture, the blood should be inoculated into the aerobic vial first; any remaining blood should then be inoculated into the anaerobic vial. Most bacteremia is caused by aerobic and facultative bacteria, which will be recovered better from aerobic bottles. In addition, pathogenic yeasts are recovered almost exclusively from aerobic bottles, as are strict aerobes, such as *Pseudomonas* and *Stenotrophomonas*.

5. Duration of incubation

For conventional manual methods, incubation for seven days is recommended and the standard incubation period for routine blood cultures performed by automated systems is five days, including cultures for HACEK and nutritionally variant streptococci. Prolonged incubation and testing periods to recover bacteria from patients suspected of having infective endocarditis also appear unnecessary.

6. Prevention of blood culture contamination

Tincture of iodine, chloride peroxide, and chlorhexidine gluconate are superior to povidone-iodine preparations. Iodine-containing preparations require sufficient time to disinfect surfaces (30 sec for tincture of iodine and 1.5 to 2 min for iodophors). Microorganism commonly associated

with contaminated blood cultures are included *Bacillus* spp., *Corynebacterium* spp., *Propionibacterium* spp., coagulase-negative staphylococci, *Aerococcus* spp., *Micrococcus* spp. Regardless of the method used to collect blood cultures, laboratories should validate that their process is effective in minimizing contamination rate to an acceptable range, typically ≤3%.

7. Others

1) Blood-to broth ratio

Normal human blood contains substances that inhibit microbial growth, which are complement, lysozyme, phagocytes, antibodies, and antimicrobial agents. To reduce the concentration of these inhibitory factors, and there by minimize their inhibitory activity, blood should be diluted in broth media at a blood-to-broth ratio of 1:5 to 1:10.

2) Additives (anticoagulants, resins, charcoal)

SPS remains the most common anticoagulant and neutralize lysozyme, inhibits phagocytosis, several bacteria, including *Neisseria* species, *Peptostreptococcus anaerobius*, *Moraxellla catarrhalis* and *Gardnerella vaginalis*. However, SPS has been shown to increase the rate and speed of recovery of both gram-positive and gram-negative microorganisms.

In addition to diluting blood in broth, and adding SPS to reduce the inhibitory effects of antimicrobial in blood culture, commercial blood culture bottles have antimicrobial-binding or adsorbing agents to their systems to enhance the recovery of microorganism from patient receiving antimicrobial therapy.

3) Agitation

Bottles agitation during the first 24hr of incubation increases yields and improves the speed of detecting microorganisms in aerobic bottles due to increased oxygenation of the broth medium.

4) Monitoring frequency/subcultures

CMBCSs monitor aerobic and anaerobic vials at regular 10- to 24-min intervals for evidence of growth. Routine, blind, or terminal subcultures from negative blood cultures performed on automated systems are unnecessary when cultures have been monitored for at least five days.

8. Blood Culture Methods

1) Manual blood culture (Conventional blood culture)

After inoculation with blood, bottles are incubated at 35℃ and examined visually for evidence of bacterial growth. Changes that suggest microbial growth include turbidity of the blood-broth mixture, growth of microcolonies, hemolysis, and gas production. Initial inspection occurs after 12 to 24 hours of incubation with twice daily inspection for two days followed daily inspection for 7 day. Gram staining of the broth along with subculture during the initial examination can hasten early detection of growth, and terminal subcultures of negative bottles should be discouraged.

2) Continuous-Monitoring Blood Culture Systems (CMBCS)

Three different systems are commercially available. Two of these, BACTEC series (BD, Sparks, USA) and the BacT/Alert 3D system (bioMérieux, Durham, NC, USA) rely on the colorimetric or fluorimetric detection of increased CO2 level as a result of microorganism growth. The third system, VersaTREK (TREK Diagnostic system, Cleveland, USA) detects microbial growth by measuring the change in gas pressure.

9. Aspects of quality assurance

1) Reporting results

The results of blood cultures, whether positive or negative, are critical to patient care. The report must allow the healthcare provider to quickly and accurately identify the status and any test result for an ordered blood culture. Preliminary written results are included: Blood culture ordered, specimen not received; testing in progress, no results to date; no growth at 24 hours; no growth at 48 hours; positive blood culture. Final written results are includes; Final gram stain result; final identification; final antimicrobial susceptibility data

2) Critical value reports

The first laboratory results (stain or culture) documenting a positive blood culture should be communicated as a critical value for every positive blood culture. Critical value reports should be issued as soon as possible (within 60 min) after laboratory verification of the abnormal result.

3) Time and temperature of storage of BC bottles

The significance difference in organism recovery depending on time and temperature of storage before loading of BC bottles on to CMBCS was demonstrated.

Rapid detection methods of bloodstream infection; molecular diagnostic techniques

In the case of the most serious BSIs, septic shock, speed is of essential importance. Molecular techniques can give results more quickly than blood cultures are becoming more useful in decreasing laboratory turnaround times [3] (Table 1)[4]. Molecular techniques for identification of pathogens can be divided into two categories: detection techniques in positive blood culture (PBC) and direct detection techniques in whole blood. In methodology, there are 1) hybridization-based techniques for positive blood cultures 2) amplification-based techniques for PBC, 3) mass spectrophotometry for identifying a pathogen-specific peptide profile, 4) amplification techniques for whole blood samples. A variety molecular techniques has been developed for detection of pathogens as summarized in Table 2 [5].

Table 1. Needs and current status of method to identify bloodstream infections

Need	**Current status**		**Future**
	Culture	**Molecular methods**	**Molecular methods**
Identify all pathogen	only culturable organisms	25 spp. to panbacterial & limited fungal	panbacterial and fugal identify
High sensitivity (less 10 CFU/ml)	negative >50% of clinical sepsis	BC is more sensitive in some case as low as 3 CFU/ml	similar to culture
Rapid identification	1-5days	1day	<1h
AST	1 additional day	a few major resistance	understanding of molecular mechanisms
Quantitative measure	difficult	not	quantitative result
Low labor requirement	intensive	intensive	fully automated

Expert Rev Mol Diagn 2010;10:399-425[4]

Table 2. Commercially available molecular assays for the diagnosis of sepsis using positive blood culture or whole blood

Diagnostic technique	Detection Mechanism	No. of pathogens detected	Sensitivity (%)	Specificity (%)
A. Using positive blood cultures				
PNA-FISH	FISH	10	94-99	99-100
ACCU-PROBE	chemil. DNA probes (rRNA)	5	80.8-100	98.7-100
HYPLEX	multiplex PCR+Hybridization	10 plus *mecA*	96-100	92.5-100
PLEX-ID BAC	chemil. DNA probes (rRNA)			
PLEX-ID BAC	broad-range PCR+ESI MS	>300 diff. pathogens	95	98.8
StaphPlex	multiplex PCR + microarray	1	100	95.5-100
Staph SR	multiplex PCR	1 Plus *mecA*	50-100	86.8-98.4
MALDI-OF	matrix-assisted laser desorption ionization TOF-MS	hundreds	76-80	96-100
Probe-it-sepsis	multiplex PCR + microarray	50 plus *mecA* gene		
Verigene	nucleic-acid-based microarray	Gram-positive or negative bacteria, resistance genes	92-96	n.d.
Filmarray	PCR	Gram-positive or negative bacteria, resistance genes	91	n.d.
B. Using whole blood				
Xpert MRSA/SA	real-time PCR	2	75-100	98.4-99.4
SeptiFast	multiplex real time PCR for bacterial and fungal pathogens	25, plus *mecA* as reflex test	60-95	74-99
VYOO	multiplex PCR + electrophoresis	34 plus *mecA*, *vanA/B/C*, SHV, CTX-M	30-51	n.d.
SepsiTest	broadrange PCR with sequencing	>300 pathogens	61-88.5	83.5-85.8

Abbreviation: n.d., not determined,
Eur J Microbiol Immunol 2014, 1:1-25[5]

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