Interpretation of blood microbiology results – function of the clinical microbiologist

Katalin Kristóf, Júlia Pongrácz

Clinical Microbiology Laboratory, Department of Laboratory Medicine, Semmelweis University, Budapest, Hungary

ARTICLE INFO

Corresponding author:
Katalin Kristóf, MD
Clinical Microbiology Laboratory
Department of Laboratory Medicine
Nagyvárad tér 4, Floor 11, H-1089
Budapest, Hungary
E-mail:
katalin.kristof@med.semmelweis-univ.hu

ABSTRACT

The proper use and interpretation of blood microbiology results may be one of the most challenging and one of the most important functions of clinical microbiology laboratories. Effective implementation of this function requires careful consideration of specimen collection and processing, pathogen detection techniques, and prompt and precise reporting of identification and susceptibility results. The responsibility of the treating physician is proper formulation of the analytical request and to provide the laboratory with complete and precise patient information, which are inevitable prerequisites of a proper testing and interpretation. The clinical microbiologist can offer advice concerning the differential diagnosis, sampling techniques and detection methods to facilitate diagnosis. Rapid detection methods are essential, since the sooner a pathogen is detected, the better chance the patient has of getting cured. Besides the gold-standard blood culture technique, microbiologic methods that decrease the time in obtaining a relevant result are more and more utilized today. In the case of certain pathogens, the pathogen can be identified directly from the blood culture bottle after propagation with serological or automated/semi-automated systems

or molecular methods or with MALDI-TOF MS (matrix-assisted laser desorption-ionization time of flight mass spectrometry). Molecular biology methods are also suitable for the rapid detection and identification of pathogens from aseptically collected blood samples. Another important duty of the microbiology laboratory is to notify the treating physician immediately about all relevant information if a positive sample is detected. The clinical microbiologist may provide important guidance regarding the clinical significance of blood isolates, since one-third to one-half of blood culture isolates are contaminants or isolates of unknown clinical significance. To fully exploit the benefits of blood culture and other (non- culture based) diagnoses, the microbiologist and the clinician should interact directly.

❖❖❖❖❖

BLOOD CULTURE – PRINCIPLE, INTRODUCTION

Bacteraemia/fungaemia can induce a systemic inflammatory response syndrome and as a clinical continuum can fall into life-threatening severe sepsis or septic shock. Huge number of studies have inferred that clinical outcomes in severe sepsis and septic shock hinge upon the optimized selection, dosing, and delivery of highly potent antimicrobial therapy (1, 2, 3). With this in mind the recovery of the causative agent is one of the most important tasks of the microbiological laboratory. Blood cultures- as a gold standard -, in which a sample of blood is allowed to incubate with a medium that promotes bacterial growth, are used to diagnose bacteraemia or fungaemia, confirmed by isolating one or more microorganisms from the blood culture. Clinicians are supposed to collect blood cultures (BC) from patients with clinical signs and symptoms indicating sepsis, or if the laboratory or imaging results suggest an infection, and the presumed infection is known to

result in haematogenous spread, or the patient has a fever of unknown origin. For sample collection they should use national guidelines and recommendations of the local microbiological laboratory (4, 5, 6). Proper formulation of the analytical request by the treating physician is essential in order to provide the laboratory with complete and precise patient information, which are inevitable prerequisites of a proper testing and interpretation. The partner microbiological laboratory should prepare useful guidelines, which contain every important pre-analytical rule (timing and sampling of blood culture – sample collection, volume of blood required, blood-to-broth ratio, formulation of the analytical request, and transportation. The microbiologist should aim to provide the clinician with proper results as soon as possible, utilizing every available diagnostic method when evaluating culture results (7, 8, 9). The clinician and the microbiologist should cooperate during the whole test procedure, but especially during the evaluation of the results, to ensure the highest possible standard of patient care. In this short summary, as microbiologists, our goal is to provide answers to the clinicians' most frequent questions.

WHEN SHOULD THE SAMPLE BE COLLECTED FOR BLOOD CULTURE?

In case of periodical bacteraemia and fungaemia blood should be collected at the beginning of the fever episode, during the chills or at the start of the fever curve. In case of continuous bacteraemia or fungaemia (e.g. suspicion of endocarditis) sample collection time is not critical (4).

WHAT AMOUNT OF BLOOD SHOULD BE COLLECTED, AND FROM WHERE?

In adults, if local infection is present or suspected, or the patient presents with fever of unknown origin, an amount of 20-30 ml of blood

collected from 2 venipuncture sights (the total amount of blood should be at least 40 ml) is sufficient. The samples should be collected strictly aseptically. If endocarditis is suspected, at least 3 samples are necessary because of the low bacterial count in the blood. In case of children, an amount decreased proportionally with body mass should be collected (see guidelines). Venipuncture should be performed on intact peripheral veins; except if catheter-associated infection is suspected, which case will be addressed separately. Theoretically, it is possible to distribute a sufficient amount of blood sampled from one sight to four different bottles, but in this case, the microbiology laboratory findings cannot aid in the evaluation of the clinical relevance of certain potential pathogens which colonize the skin (and may contaminate the sample), but can also cause infection in case of certain risk factors. Since the number of pathogens in the blood during bacteraemia/fungaemia is very low (0.1-300/ml depending on the patient's age and the pathogen), the sensitivity of BC is mostly determined by the amount of blood collected. Usually, BC containing samples from two or three venipuncture sights is sufficient to support or rule out sepsis; however, a single sample is insufficient (4, 10, 11).

WHAT IF CATHETER-ASSOCIATED INFECTION IS SUSPECTED?

In this case, blood samples should be taken through the catheter and a peripheral vein at the same time. Two pairs of BC is not necessary (one sample is enough), but if the catheter has multiple lumens, a sample should be taken through each lumen. If the time to positivity (TTP) of the sample taken through the catheter is at least 2 hours shorter than that of the sample from the peripheral vein, and the cultured microbe and its antimicrobial susceptibility is the same, catheter-associated

infection can be diagnosed (12). If the catheter is removed because of suspected catheter-associated infection, the catheter end should be sent for culture as well.

WHAT TYPE OF BLOOD CULTURE BOTTLE SHOULD BE USED?
IS THE ANAEROBIC BOTTLE OR THE SPECIALIZED FUNGI BOTTLE NECESSARY?

The blood collected from one sampling is usually distributed into two (an aerobic and an anaerobic) commercially available blood culture bottles in the amount specified by the manufacturer. An anaerobic bottle is recommended in patients with neutropenia, in case of complications following abdominal surgery, patients with diabetes and in patients with complicated wound infections. Small amounts of blood samples (1-5 ml) collected from children should be distributed into special childrens' bottles containing a smaller amount of media. Fungi usually grow well in aerobic BC media prepared for the culture of bacteria, but certain studies showed that the TTP is shorter when special fungi bottles are used. In case of patients under antibiotic treatment, bottles containing agents that inactivate antibiotics (activated carbon, resin) are recommended (4, 13).

HOW SHOULD INOCULATED BOTTLES BE STORED?

The inoculated BC bottles – if it is possible – should be sent to the microbiology laboratory immediately, otherwise the bottles should be stored at room temperature. Several studies have demonstrated (and it is included in the references of the commercially available bottles) that a certain time (12-16 h) of storage at room temperature, otherwise called delayed time vial entry, does not impact the BC result significantly (4, 14).

HOW LONG UNTIL A RESULT IS AVAILABLE?

Continuous monitoring systems have revolutionized blood culture practices, because the time to detection of microbial growth is significantly shorter by continuously agitating the bottles and checking them every 10 minutes. Depending on the system, the detection method can be an indirect measurement of the CO_2 produced by the microorganism in the bottles (at the bottom of the bottles there is an integrated CO_2 sensor containing a pH indicator or the level of fluorescence change because of the reduction in pH). If the system signals a positive bottle, the microbiology laboratory should initiate analytical tests immediately, according to laboratory protocols based on international and national guidelines, and all relevant information is documented and communicated to the clinician as soon as possible. The result of a Gram stained mount is available in 30 minutes, the result of presumptive or definite identification in 4-48 h (depending on the type of microbe), and the preliminary or final antimicrobial susceptibility report is available in 16-48 h (depending on the type of microbe).

The usual incubation time of BC is 5-7 days at 35-37°C. Positive bottles usually signal in the first 24-48 hours of incubation. The latest guidelines do not recommend longer incubation time in certain cases as previous recommendations did (21 days for the detection of *Brucella, Legionella,* the fastidious HACEK group bacteria that cause endocarditis (*Haemophilus, Aggregatibacter, Cardiobacterium, Eikenella, Kingella),* and in case of fever of unknown origin). The isolation of clinically relevant pathogens after 7 days of incubation is improbable (except for dimorphic/filamentous fungi) (4, 7, 8, 9).

WHY IS THE RESULT OF THE BLOOD CULTURE NEGATIVE (NO POSITIVE SIGNAL DURING INCUBATION), WHEN THE CLINICAL DIAGNOSIS OF BACTERAEMIA/FUNGAEMIA IS CERTAIN?

Blood culture is a microbiological test that is heavily dependent on the clinician's procedures (timing of sample collection, the amount of the collected sample, the number of BC bottles used), and the evaluation of the clinical symptoms (estimating the likelihood of bacteraemia/fungaemia and sepsis, the correct assumption of the probable etiological agent, and the proper evaluation of the results) (4, 7). Based on the literature. the preanalytics and analytics of blood culture testings are performed properly if 8-14% of the total number of blood cultures is positive. The assessment of this parameter is recommended in every medical institute/clinic with the help of the microbiology laboratory. If a significantly different percent is determined, the whole procedure should be revised and corrected (with the cooperation of the clinician and the microbiologist).

Sensitivity is basically determined by the type of sepsis. The BC is positive e.g. in endocarditis in 53-99%, in *S. pneumoniae* pneumonia in 25-30%, in neutropenic fever in 10-20%, in abdominal infection in 30-40%, and in disseminated fungal disease in nearly 50%.

If the symptoms of sepsis still subsist, and the BC from the day before is not positive, another 2-3 sample collections are recommended in the next 24 hours. If infective endocarditis is suspected, and the 3 pairs of BC collected on the first day are negative, another 2 pairs should be collected the next day. If an infection caused by a fastidious microorganism requiring special culture conditions is suspected despite negative BC results, consultation should be performed with the microbiologist before taking a new sample (recommendations for specialized

BC bottles, longer incubation time, alternative microbiological testing methods – e.g. serology, molecular diagnostics.) (8, 9).

WHY IS THE CULTURE RESULT NEGATIVE WHEN THE BLOOD CULTURE SYSTEM YIELDS A POSITIVE SIGNAL?

Non-conformity with preanalytical methods, namely overfilling the bottles may lead to a false positive signal in systems based on CO_2 detection, which is caused by the CO_2 contained by the excess amount of RBCs in the blood sample. A false positive signal may also be detected in BCs of ventilated patients (elevated partial CO_2 pressure), and blood samples containing high amounts of WBCs (haematology patients). The microbiologist can immediately confirm this to the clinician by assessment of BC bottle monitoring and the Gram stained mount.

In some cases, the bacterium in the blood may start to multiply, it may be seen in the mount from the positive BC bottle, but it does not grow in subculture. *Streptococcus pneumoniae*, for example, grows well in the rich BC media, but also produces a large amount of autolysin enzyme, which causes the bacteria to die. However, the antigens of the bacteria can be detected with antigen detection kits. B_6 vitamin-dependent streptococci also propagate in BC media containing pyridoxal, but may not grow on media usually applied for the culture of streptococci. Media containing pyridoxal should be used for subculturing such strains (4, 7, 8).

HOW SHOULD A POSITIVE CULTURE RESULT BE INTERPRETED? DOES EVERY MICROBE CULTURED HAVE CLINICAL RELEVANCE?

The following microorganisms are considered significant: *Staphylococcus aureus, Enterobacteriaceae* spp*., Pseudomonas aeruginosa, Streptococcus pneumoniae, Streptococcus* *β-haemolytic, Haemophilus* spp*, Neisseria meningitidis, Listeria monocytogenes, Enterococcus spp., Salmonella* spp*., Brucella* spp*., Pasteurella* spp*., Campylobacter* spp*., HACEK* group, anaerobes, *Candida* spp. These microbes are always clinically significant, even if they are cultured from only one of the (properly collected) four-six bottles.

The following microbes are considered significant in only certain cases: *Streptococcus α-haemolyticus (40-60%), Staphylococcus coagulase-negative (20-40%).* If a coagulase-negative *Staphylococcus* or α-haemolytic *Streptococcus* is cultured from only one of the ≥2 bottles from a set of BC, the isolate is probably a contaminant. However, an α-haemolytic *Streptococcus* cannot be considered as a contaminant if there was only one bottle. In this case, repeated sampling is recommended: if ≥2 bottles are positive, the α-haemolytic *Streptococcus* is more probably a significant pathogen. Some studies say that a bacterium is more probably a contaminant if it is cultured after a longer than usual incubation time. However, this observation cannot be used in the assessment of the positive results of an individual patient, because there is significant overlapping in the growth rate of contaminants and real pathogens. Further, parallel microbiologic sampling/testing from the source of the suspected bloodstream infection (e.g. urine, lower respiratory samples, removed catheter, etc.) complements and supports the interpretation of the relevance of the microbes cultured from the blood, and aids in identifying the etiology of the infection.

The following microbes are usually considered contaminants: *Staphylococcus coagulase-negative, Micrococcus* spp*., Corynebacterium* spp*. Propionibacterium* spp*.* and *Bacillus* spp. However, there is no general rule that they are contaminants in all cases.

The specificity of BC is determined by the percentage of false positive isolates. The interpretation of contaminants depends to a certain degree on patient characteristics. The spectrum of real pathogens and contaminants can be easily determined in the case of community acquired infection. In nosocomial infections, however, bacteria that are considered contaminants in "healthy" (immunocompetent) people may be real pathogens in immunocompromised patients. Specificity can be improved primarily by strictly abiding to sample collecting guidelines, mainly the methods to ensure asepsis, and to have multiple samples collected in cases of sepsis in which potential pathogens are the same as potential contaminants (e.g. catheter or other indwelling device associated infection, neutropenic fever). The number of positive blood cultures containing a contaminant can be assessed at a certain medical institute. If the rate of these bottles is significantly more than 3%, the situation should be remedied by education and consultation (4, 5, 8, 9, 10).

WHAT DOES A POLYMICROBIAL BLOOD CULTURE RESULT SIGNIFY? WHAT IS ITS CLINICAL SIGNIFICANCE?

In about 15 % of cases multiple microorganisms are grown from blood cultures. The rate of polymicrobial blood cultures ranges from 10% to 30% in immuno-compromised patients and in nosocomial BSI of patients treated at intensive care units. Polymicrobial BSI often indicates catheter-related or intraabdominal infections (15).

SHOULD PATIENTS WITH POSITIVE BLOOD CULTURE RESULTS BE RE-SAMPLED FOR FOLLOW-UP?

The blood may not become sterile even after 2-4 days of adequate treatment; the assessment of the recovery of patients with bacteraemia/ fungaemia is the clinician's task. So-called "follow-up" BC is not necessary, except for some special cases. In infective endocarditis, it is recommended to guide treatment (the antimicrobial susceptibility of the pathogen may change after prolonged treatment). In every case of bacteraemia caused by *Staphylococcus aureus,* when isolation of the pathogen in the repeated BC taken after 2-3 days may indicate complicated sepsis caused by *S. aureus* (e.g. secondary metastatic infection), and the need for a change in therapy. Several recommendations contain "follow-up" BC in case of fungaemia to determine the necessary duration of treatment (4, 16).

ARE THERE ANY METHODS THAT SIGNIFICANTLY DECREASE THE TIME TO IDENTIFICATION OF THE PATHOGEN OF SEPSIS?

Timely initiation of adequate therapy significantly affects the patient's life expectancy; therefore microbiologic methods that decrease the time to obtaining a relevant result are more and more utilized today.

In the case of certain pathogens, the pathogen can be identified directly ***from the BC bottle after propagation*** with antigen detection or rapid identification methods (e.g. *Streptococcus pneumoniae, Neisseria meningitidis, Streptococcus agalactiae* – antigen detection by latex agglutination) (4, 7, 8).

Identification and susceptibility testing performed with automated/semi-automated systems can identify Gram-negative sepsis pathogens in 92-99% of cases, while Gram-positives are identified in 43-75% of cases. The advantage of these systems is that the most frequent pathogens in routine microbiology can be identified in 4-16 hours. Susceptibility results show 95% correlation with conventional methods (17, 18).

Most laboratories have access to MALDI-TOF MS (matrix-assisted laser desorption-ionization time of flight mass spectrometry) to identify cultured bacteria and fungi. Since the method is based on the mass spectrometry measurements of conserved microbial ribosomal and other proteins, the result is precise, mostly equivalent to DNA sequencing. Since a test can be performed from very little sample size ($10'^4$ - $10'^6$ CFU/ml), testing of barely visible isolated colonies after short incubation time can often be performed and the species identification result can be communicated to the treating physician.

It should be emphasized that MALDI-TOF MS can be used to identify pathogens directly from the blood culture bottles as well. Different separation and lysis protocols are available to remove proteins of human origin from the media, and concentrate the bacteria in the sample to the appropriate amount, resulting 80-96% correct identification results (compared with conventional culture and identification methods). However, the method is not always applicable (e.g. BC media containing activated carbon, polymicrobial infection) (9, 19).

Commercial and/or validated "home-made" molecular methods are also available. Another method is PNA FISH (fluorescent in-situ hybridization) which identifies microbes from positive BC bottles with 95-99% sensitivity and specificity. It is a quick method, since the whole procedure takes 90 minutes, but its disadvantage is that it is only able to identify a small number of microbial species (though the most frequent ones) (e.g. *S. aureus* and coagulase-negative *Staphylococcus* (without identification to species level), *Enterococcus faecalis, Enterococcus faecium, Escherichia coli, Klebsiella pneumoniae, Pseudomonas aeruginosa*). The identification of yeast groups is based on intrinsic azole sensitivity: *Candida*

albicans/Candida parapsilosis, Candida krusei/Candida glabrata, Candida tropicalis. The disadvantage of the method is that antimicrobial susceptibility can only be performed after conventional culture (2, 5, 9, 20).

Because of the pronounced significance of sepsis, more and more manufacturers produce complex tests based on molecular techniques (PCR), providing identification of the most frequent pathogens from positive BC bottles after a simple or more complicated protocol, with adequate sensitivity and specificity, in 1-3 h (e.g. FilmArray BCID Panel (BioFire): 19 pathogens, Hyplex BloodScreen (BAG):10 pathogens, Prove-it Sepsis (MobiDiag):50 pathogens). Molecular methods are suitable for the detection of certain resistance genes as well e.g. *mecA*, *van*-gene detection (20).

Molecular biology methods are also suitable for the rapid detection and identification of pathogens from aseptically collected **blood samples** (plasma, serum or EDTA-treated whole blood). Certain pathogens can be detected directly from blood with species-specific real-time quantitative PCR tests (e.g. *Neisseria meningitidis* DNA detection). Broad-range real-time PCR tests can be performed directly from blood samples: Gram-positive and Gram-negative bacteria and fungi can be detected (the clinically most important species in every group), along with certain resistance genes e.g. *mecA*, *van*-gene detection. Several commercially available multiplex molecular tests (e.g. Septifast Test (Roche), Sepsi Test (Molzym)) are able to detect the most frequent bacteria and fungi/the ones included in their panel, after more or less complicated test protocols in approximately 1-8 hours. The advantages of PCR testing performed directly from blood are rapid detection, it is not influenced by antibiotic treatment administered at the time of sample collection, and quantitative detection is available; its disadvantage is that it

detects bacterial DNA and not viable bacteria. A further disadvantage is that it does not detect the fastidious HACEK group. Antibiotic susceptibility/resistance detection is limited to certain resistance genes, the sample may be contaminated, and background bacterial DNA in the blood may be troublesome. After review of the currently available diagnostic palette, attention should be raised to the fact that although these methods are useful, conventional blood culture testing is still necessary. Several studies show that the two methods agree "only" in 55-85% of cases, depending on the patient population studied. In the future, if these methods become more widespread, their clinical significance should be assessed (it will be interesting to see which method will be the gold standard – how PCR positive but culture negative test results should be interpreted) (2, 9, 20).

CONCLUSION

To fully exploit the benefits of blood culture diagnoses, the microbiologist and the clinician should interact directly and discuss both the differential diagnosis as well as the treatment options. The final interpretations of the results will rest on the assessments made by the clinician and the microbiologist, taking into consideration microbiological and clinical findings.

REFERENCES

1. Gonzalo M.L., Bearmana and Richard P.: Bacteremias: A leading cause of death. Arch Med Research 2005; 36: 646–659.

2. Murray, P.R., Masur, H.: Current approaches to the diagnosis of bacterial and fungal bloodstream infections in the intensive care unit. Crit Care Med 2012; 40: 3277-3282.

3. Liang S.Y., Kumar A.: Empiric antimicrobial therapy in severe sepsis and septic shock: Optimizing pathogen clearance. Curr Infect Dis Rep 2015; 17: 36

4. Clinical and Laboratory Standards Institute (CLSI). Principles and Procedures for Blood Cultures; Approved Guideline. CLSI document M47-A. Wayne, PA: Clinical and Laboratory Standards Institute 2007.

5. Caliendo A. M., Gilbert D. N., Ginocchio C. C. et al; for the Infectious Diseases Society of America (IDSA): Better Tests, Better Care: Improved Diagnostics for Infectious Diseases. CID 2013:57 (Suppl 3) S139-170.

6. Townsa M.L., Jarvisb W. R., Hsuehc PR: Guidelines on Blood Cultures. J Microbiol Immunol Infect 2010; 43(4):347–349.

7. Murray, P.R., Baron, E.J., Jorgensen, J.H., Landry M.L., Pfaller, M.A.: Manual of Clinical Microbiology. 9th Ed. ASM Press, Washington D.C., 2007.

8. Lynne S. Garcia Ed.: Clinical Microbiology Procedures Handbook (3 Vols). 3rd Edition ASM Press, Washington D.C., 2010.

9. A. van Belkum, G.Durand, M. Peyret et al.: Rapid clinical bacteriology and its future Iipact. Ann Lab Med 2013;33:14-27.

10. Riedel S., Bourbeau P., Swartz B. et al.: Timing of specimen collection for blood cultures from febrile patients with bacteremia. J Clin Microbiol 2008; 46:1381–1385.

11. Weinstein et al. Detection of bloodstream infection in adults: How many blood cultures are needed. J Clin Microbiol 2007; 45:3546-3548.

12. Guembe M., Créixems M. R., Carrillo C. S.et al.: Differential time to positivity (DTTP) for the diagnosis of catheter-related bloodstream infection: do we need to obtain one or more peripheral vein blood cultures? Eur J Clin Microbiol Infect Dis 2012; 31:1367–1372.

13. Cateau E., Cognee A.S., Tran T.C. et al.: Impact of yeast–bacteria coinfection on the detection of Candida sp. in an automated blood culture system. Diagn Microbiol Infect Dis 2012; 72:328–331.

14. Willems E., Smismans A., Cartuyvels R. et al.; The preanalytical optimization of blood cultures: a review and the clinical importance of benchmarking in 5 Belgian hospitals. Microbiol Infect Dis 2012; 73:1–8.

15. Sancho S., Artero A., Zaragoza R., et al.: Impact of nosocomial polymicrobial bloodstream infections on the outcome in critically ill patients. Eur J Clin Microbiol Infect Dis 2012; 31:1791–1796.

16. Tabriz, M.S., Riederer, K., Baran, J. Jr., Khatib, R.: Repeating blood cultures during hospital stay: practice pattern at a teaching hospital and a proposal for guidelines. Clin Microbiol Infect 2004; 10: 624-627.

17. Gherardia G., Angelettia S., Panitti M.et al.: Comparative evaluation of the Vitek-2 Compact and Phoenix systems for rapid identification and antibiotic susceptibility testing directly from blood cultures of Gram-negative and

Gram-positive isolates. Diagn Microbiol Infect Dis 2012; 72: 20–31.

18. Yonetania S., Okazakia M., Arakia K. et al.: Direct inoculation method using BacT/ALERT 3D and BD Phoenix System allows rapid and accurate identification and susceptibility testing for both Gram-positive cocci and Gram-negative rods in aerobic blood cultures. Diagn Microbiol Infect Dis 2012; 73:129–134.

19. Bizzini A, Greub G.: Matrix-assisted laser desorption ionization time-of-flight mass spectrometry, a revolution in clinical microbial identification. Clin Microbiol Infect. 2010; 16:1614-9.

20. Mancini N., Carletti S., Ghidoli N. et al.: The era of molecular and other non-culture-based methods in diagnosis of sepsis. Clin Microbiol Rev 2010; 23:235–251.