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# The microbiology of sepsis is more than the application of new technologies in diagnosis

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## ABSTRACT

Adequate and rapid microbiological diagnosis of sepsis is essential for correct treatment, having a direct impact on patient prognosis. Clinical Microbiology Services must adapt fast circuits that allow prioritizing and individualizing the diagnosis of these patients. The measures adopted should not be based solely on the incorporation of new technologies but, to a large extent, on ensuring accurately collection and processing of samples, avoiding unnecessary losses of time in processing and ensuring that the information derived from this process adequately reaches the prescribing physician.

**Keywords: Sepsis, Sepsis Code, Sepsis microbiological diagnosis**

## INTRODUCTION

According to the World Health Organization, sepsis is currently considered a global health priority and the leading infectious cause of death**.** Despite the lack of a single definition, adequate epidemiological records and underestimation of the data available, a study published in *The Lancet* in January 2020 estimated the global burden of sepsis in 2017 to be 48.9 million incident cases and 11.0 million deaths worldwide [1].

There are two fundamental aspects to consider in sepsis: anyone can suffer an infection and almost any infection can lead to sepsis. When, this occurs every second counts (https://www.cdc.gov/patientsafety/features/get-ahead-of-sepsis.html), since the associated mortality must be fought with a diagnosis and proper management within the first hours. Currently, application of the measures recommended by the Surviving Sepsis Campaign reduces morbidity and mortality to around 25% [2]. In Spain, the key points of this intervention are focused on the Sepsis Code, implemented in Catalonia and other autonomous communities since 2015. The main goal of this code is the early detection of patients at risk and the rapid application of a set of measures to establish an etiological diagnosis, monitoring the different organs susceptible to failure, and starting empirical antibiotic treatment, resuscitation with fluids and life support.

Regarding microbiological diagnosis, although these recommendations are individually adapted in each centre, it is recommended to take at least 2-3 sets of blood cultures (BC) early, preferably before starting antimicrobial treatment, in addition to collecting other clinical samples of the probable source of infection. Rapid diagnostic laboratory techniques must be applied to these samples to report preliminary results quickly. Therefore, the microbiology laboratory must use all the available resources to help differentiate whether a patient really has sepsis or another condition which could appear with the same non-specific symptoms. In the case of considering that it is a septic condition, the source of infection must be established, as well as determination of the causative agents and how to direct the treatment adequately, all within the shortest possible time (ideally in less than 24h from symptom onset, if possible). Several studies have reported that the initial antibiotic therapy in sepsis needs to be not only timely but also appropriate [3]. Despite the publication of therapeutic guidelines and protocols, around 1 in 5 patients with bloodstream infection (BSI) in the United States receive susceptibility-discordant empirical antibiotic therapy [4] and this number may be even higher if the choice of the drug, the dose and method of administration are considered.

According to Brigitte Lamy and the European Society of Clinical Microbiology and Infectious Diseases (ESCMID) Study Group for Bloodstream Infections, Endocarditis and Sepsis (ESGBIES) [5], to achieve progress in bloodstream infections, aetiological diagnoses should be based on a bundle approach. This approach is based on optimizing pre-analytical measures

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(skin preparation, volume of blood sampled, sample transportation to laboratory and rapid start of incubation), improving the analytical process (fast processing of positive flagged bottles and use of quick identification and antimicrobial susceptibility testing methods) and post-analytical actions, especially close collaboration with the sepsis team. By combining all of these actions, the diagnosis of sepsis can be significantly improved.

## PRE-ANALYTICAL MEASURES

Diagnostic performance can be improved by considering some essential pre-analytical aspects. First, in addition to the BC, it is a priority to process other biological samples to determine the source of the infection. Furthermore, if possible, these samples must be collected before administering the first dose of the antimicrobial, as long as this does not delay the start of treatment by more than 45 minutes, since obtaining BCs during antibiotic therapy is associated with significant hindrance of pathogen detection [6]. Second, in all cases, proper sample collection and transport to the laboratory must be carried out [7]. In the case of BCs, as reported in the 2015 review by Snyder [8], factors such as skin antisepsis, blood volume, number of BC specimens collected, the timing of BC collection, and delays in incubation time significantly influence the sensitivity, interpretation, and clinical relevance of BCs. The volume of blood to inoculate in the BC bottles and the time needed to incubate these bottles in intelligent incubation systems are important to note. The recommendations of the Infectious Diseases Society of America (IDSA)/American Society for Microbiology (ASM) state that the volume of blood to be cultured must be related to the weight of the patient. Thus, inoculated in a single aerobic vial, between 1 and 5 ml (1:5 dilution) are required in young children, while 10-20 ml (1:10 dilution) should be collected for culture in older children and adults and, divided into two vials (anaerobic and aerobic). The positivity rate increases between 3-5% for each ml of cultured blood. A delayed entry of blood culture bottles in the automatic incubation system negatively impacts the total detection time and decrease the recovery of some pathogens. Implementing automatic loading of BC bottles with a 24h/7d strategy shortens the time to diagnosis significantly and increases the BSI diagnostic rate. Finally, the diagnosis of sepsis is based on clinical symptoms and there are no specific diagnostic criteria or a single standard diagnostic test. When a BC is positive, it is usually too late to implement the measures that would be applied to allow an early diagnosis. The laboratory should be advised before all clinical suspicion of sepsis in order to accelerate and prioritize the processing of the patient's samples. This process should ideally be supported by computer systems that facilitate the generation of alerts and control of response time. At the Vall d'Hebron University Hospital [9], a preconfigured profile has been incorporated into the request for laboratory tests, both for adult and paediatric cases, and it is adapted to the determinations that must initially be carried out in these patients, which include peripheral BC (at least two sets), complete blood count, basic biochemistry, coagulation study, acid-base balance, and fundamentally biomarkers. The request for this profile triggers a notification system by messaging (e-mail and phone SMS) that alerts the hospital and laboratory sepsis code manager and the intensive care unit and microbiology on-call teams. The entry of samples requested under this profile generates a patient label that is visible on all the samples processed or to be processed, and is deactivated after 72 hours if the BC no longer remain positive. The positivity of BCs of with this label generates an alert on the screen for the duty team to control the samples that require urgent processing. In the case of urine, a visible sign to alert the laboratory technicians is created to prioritize the sample processing. Additionally, if the sediment is positive, a direct urine disk-diffusion antibiogram is performed.

Other actions to speed up obtainment of results from additional samples are currently being studied.

## ANALYTICAL PROCESS

At present, new molecular diagnostic techniques, such as Xpert MRSA/SA BC test (Cepheid®), BD MAX StaphSR Assay (BD Diagnostics), Eazyplex MRSA (Amplex Diagnostics), PNA FISH™ rapid diagnostic tests (AdvanDx's), Bio-Fire® FilmArray® 2 panel BC identification (bioMérieux), Gram-negative and Gram-positive Verigene BC test (Luminex of Diasorin), ePlex BCID Panels (Roche Diagnostics), BC Unyvero cartridge (Curetis) and Sepsis Flow chip (Master Diagnostica of VITRO) [10] allow working from positive BCs. These tests detect the presence of the most frequent aetiological agents of bacteraemia/sepsis and, in many cases, some of the main resistance genes, in a time between 30 minutes and 5 hours. Technology applicable to direct blood is needed for real advances in time and to save the hours of pre-incubation of BC [10]. Some approaches are already available, such as the T2 magnetic resonance technique (T2MR from T2 Biosystems), which combines paramagnetic nanoparticle sensors that are detected by T2MR and allows the detection of the most relevant target bacterial and yeast species in direct blood with very high sensitivity (>95%) and at extremely low concentrations of only one cell/ml of blood. As a limitation, it is difficult to interpret some of the discrepancies found between the results of these techniques and those of traditional cultures, considering the clinical context. Thus, BC remains the gold standard for diagnosing bloodstream infection/sepsis [11].

BC media and incubators have been improved in order to detect exigent species, including anaerobic species, and reduce the time to BC positivity. When a BC is positive, it is still important to perform the Gram stain smears to determine the clinical value of the isolation and individualize the most adequate management according to the clinical context of the patient. Working with pellets undoubtedly saves significant time in both the performance of matrix-assisted laser desorption/ionization-time of flight mass spectrometry that allows microorganism identification in less than 1 hour from BC positivity, and in obtaining a direct antibiogram for which there

are already specific European Committee on Antimicrobial Susceptibility Testing (EUCAST) guidelines [12] as well as devices with different technological approaches. Some laboratories have worked with their traditional commercial systems (VITEK, MicroScan) directly from BCs for more than 20 years. Although this work method was not endorsed by any of the expert committees (EUCAST/CLSI) mainly due to the absence of standardization of the inoculum (suspension of the pellet obtained after centrifugation or supernatant drops), the excellent correlation with the antibiogram carried out from the colony and that provides results the same day of BC collection, has led to this practice continuing until now. The rapid AST (RAST) method by EUCAST and different commercial devices of accurate and fast (2-7h) susceptibility testing of positive BCs are currently available. These devices are based on automatically monitoring bacterial growth in the presence of different antibiotic concentrations using nephelometry (Alfred AST (Alifax), PNA FISH plus morphokinetic cellular analysis (Accelerate Pheno System, BD), microfluidics with live cell fluorescent microscopy for the study of cell responses in a linear antibiotic concentration gradient (QuickMIC of Gradientech), high-speed time-lapse microscopy imaging of bacteria in broth (ASTar of QLinea), inoculum standardization from liquid colony obtained by FAST System (Qvella), array detection of volatile emissions produced by microbial growth (VITEK® REVEAL™ of bioMérieux) and flow cytometry (FASTinov) [13].

The incorporation of one technique or another must be adequately assessed, with special attention given to determine the impact of the results obtained on the patient's prognosis, which is difficult to measure [14] and requires close collaboration with the clinical team.

## POST-ANALYTICAL ACTIONS

The results obtained require adequate assessment of the clinical context of the patient. The implementation of electronic records in most hospitals provides timely knowledge of patient status and antibiotic coverage. The results of diagnostic tests, especially BCs, must be clearly and immediately reported to the clinical team, especially if the patient is not receiving adequate empirical antibiotic therapy. For this, it is essential that laboratories be open 24h/7d [15]. The clinical microbiologist should not be limited to simple issuing reports by conventional means; automatic alert systems should be developed through immediate messaging or by specific Web Apps that transmit critical information and obtain return confirmation of the display of these results and, if possible, information as to whether they have generated any action on the treatment. These improvements will help improve antimicrobial stewardship and optimize patient care. Likewise, clinical microbiologists must be integrated within the multidisciplinary team that manages these patients and, even in patients who are not haemodynamically stabilized, discuss the possible need for de-escalation of antibiotic treatment according to the spectrum of the results emitted. The human and economic efforts performed to reduce the time to issuing laboratory results are useless if they are not reflected in real and immediate action in the patient that contributes to better treatment and prognosis.

## CONCLUSIONS

In order to reach a rapid and adequate sepsis microbiological diagnosis, it is essential to review all the procedures followed in the selection, collection, and processing of the different samples in order to create rapid workflows, individualized routes and automated alert systems, which allow improving diagnostic yield and avoiding unnecessary loss of time. Furthermore, in the case of positive results, reports must be available within 24 hours after the onset of sepsis. When incorporating new technologies into the diagnostic process, these must be assessed based on the expected impact on the patient and the possibility of actual incorporation, considering the technical requirements, the laboratory workflow, and the availability of staff and hours during which the laboratory is open. In centres in which a sepsis code is implemented, it is also essential that a Microbiology Service is available with a 24/7 model and a medical team capable of acting based on the results at any time of the day or night.

## CONFLICT OF INTEREST

The authors declare no conflict of interest.

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