Enferm Infecc Microbiol Clin. 2017;35(9):586–592

Enfermedades Infecciosas y Microbiología Clínica

www.elsevier.es/eimc

Continuing medical education: Methods of rapid diagnosis

Molecular methods for septicemia diagnosis☆

Francesc Marco[a,b]

[a] Servicio de Microbiología, Centro de Diagnóstico Biomédico, Hospital Clínic, Barcelona, Spain
[b] ISGlobal, Barcelona Institute for Global Health, Hospital Clínic-Universitat de Barcelona, Barcelona, Spain

ARTICLE INFO

Article history:
Received 3 March 2017
Accepted 6 March 2017
Available online 31 October 2017

Keywords:
Sepsis
Molecular diagnostics
Rapid diagnostics

ABSTRACT

Septicemia remains a major cause of hospital mortality. Blood culture remains the best approach to identify the etiological microorganisms when a bloodstream infection is suspected but it takes long time because it relies on bacterial or fungal growth. The introduction in clinical microbiology laboratories of the matrix-assisted laser desorption ionisation time-of-flight mass spectrometry technology, DNA hybridisation, microarrays or rapid PCR-based test significantly reduce the time to results. Tests for direct detection in whole blood samples are highly desirable because of their potential to identify bloodstream pathogens without waiting for blood cultures to become positive. Nonetheless, limitations of current molecular diagnostic methods are substantial. This article reviews these new molecular approaches (LightCycler SeptiFast, Magicplex sepsis real time, Septitest, VYOO, PCR/ESI-MS analysis, T2Candida).

Métodos moleculares para el diagnóstico de septicemia

Palabras clave:
Septicemia
Diagnóstico molecular
Diagnóstico rápido

RESUMEN

La septicemia es una de las causas más importantes de muerte en pacientes hospitalizados. El hemocultivo es el método de referencia para detectar el agente etiológico responsable, pero el resultado definitivo depende de la velocidad de crecimiento del microorganismo. En los últimos años el empleo de diversas tecnologías como la espectrometría de masas *(matrix-assisted laser desoption ionization time-of-flight)*, la hibridación del ADN, los *microarrays* o las reacciones de PCR rápidas han disminuido de forma considerable el tiempo necesario para la identificación de los microorganismos y la detección de genes de resistencia a partir de hemocultivos positivos. El diagnóstico molecular de una septicemia directamente de la sangre del paciente permite conocer el resultado en pocas horas, aunque todavía existen diversas limitaciones que dificultan su empleo. En esta revisión se exponen los diversos métodos moleculares disponibles (LightCycler SeptiFast, Magicplex sepsis real time, Septitest, VYOO, PCR/ESI-MS análisis, T2Candida) y su posible utilidad.

DOI of original article: http://dx.doi.org/10.1016/j.eimc.2017.03.002
☆ Please cite this article as: Marco F. Métodos moleculares para el diagnóstico de septicemia. Enferm Infecc Microbiol Clin. 2017;35:586–592.
E-mail address: fmarco@clinic.ub.es

Introduction

Early microbiological diagnosis of a circulatory system infection caused by bacteria (bacteraemia), fungus (fungaemia) or virus (viraemia) should be a priority objective of any microbiology laboratory. In severe clinical symptoms that evolve towards a serious situation of septicaemia with shock, adoption of appropriate therapeutic measures and administration of proper antimicrobial treatment as early as possible are essential for decreasing the high morbidity and mortality observed in these cases.[1] Septicaemia is one of the leading causes of death in hospitalised patients. According to the data collected in various publications, in which European and North American patients are included, the number of deaths attributable to this clinical condition is estimated at 400,000 per year.[2–4] From a clinical perspective, septicaemia presents as an imprecise syndrome for which the diagnosis is based on the clinical suspicion of an infection combined with signs of organ dysfunction. Confirmation of septicaemia requires identification of the aetiological agent. To date, the standard recommended methodology is based on performing haemocultures that, if positive and using traditional methods, require a minimum of 48–72 h to obtain the result identifying the microorganism responsible and its susceptibility to antibiotics. The yield of haemocultures is variable. If 2–4 haemocultures are collected (40–80 ml of blood) before starting antimicrobial treatment, the aetiological agent is detected in 80–96% of cases.[5,6] However, haemocultures are negative in a high proportion of cases (50%) when the patient has severe septicaemia.[7] This may be due to various factors, such as prior antimicrobial treatment, few microorganisms circulating in the blood or non-culturable or slow-growing microorganisms. Also, it is estimated that in patients with septic shock, each hour that elapses from the start of hypotension to the administration of active antibiotics causes a mean decrease in survival of 7.6%.[8] Since antimicrobial treatment is a critical factor in the survival of patients with septicaemia, broad-spectrum antibiotics are usually used initially to cover all possible pathogenic agents and later, based on the results of the haemocultures, the treatment is adapted. Therefore, it is clear that our goal should be to shorten the time needed to reach a microbiological diagnosis of septicaemia. In light of this challenge, two options are posited. The first one — which is, in theory, the most desirable due to the immediacy of the diagnosis — involves identifying the microorganism responsible for the septicaemia directly from the patient's blood. The second option seeks to identify the aetiological agent as soon as possible once the haemoculture has been found to be positive. In both cases, detection of resistance genes to the most common antibiotics and/or determining susceptibility to antibiotics should also be possible.

Direct diagnosis from blood

The application of molecular techniques directly on whole blood samples offers the possibility of identifying the aetiological agent responsible for the septicaemia in a short period of time. Also, depending on the method used, it is possible to detect the presence of certain antibiotic resistance genes, facilitating the choice of the most appropriate antimicrobial treatment. This diagnostic option has benefited in recent years from the changes made to techniques that allow for extraction of nucleic acids, their amplification methods and the possibility of using multiplex polymerase chain reactions (PCRs) to increase diagnostic options. However, and a priori, use of these molecular techniques must face various disadvantages. There is a large amount of human DNA in the patient's blood, along with contaminating DNA and persistent DNA from dead microorganisms. The presence of PCR inhibitors, such as iron ions or immunoglobulins, must also be kept in mind.[9] The amount of DNA present can be reduced by extracting leukocytes or using methods that allow it to be extracted or degraded specifically. Also, anticoagulants such as heparin should be avoided due to the risk of PCR inhibition, and EDTA will be used. The second disadvantage that should be kept in mind is the low number of microorganisms circulating in the blood during an episode of bacteraemia, which is estimated between 1 and 10 CFU/ml.[10] These values are based on quantitative studies conducted with conventional methods that perhaps do not represent the real number of circulating and viable microorganisms. Bacconi et al.[11] suggest that for methods like PCR, it is better to consider the number of genomic copies (GC) of a microorganism present in a sample. This concept would also consider the DNA of dead bacteria or those captured by circulating phagocytic cells. According to this option, it is estimated that in an episode of bacteraemia the number of circulating GCs would be between 10^3 and 10^4/ml. This value would be higher than the detection limit for most PCRs.

Various systems that can be used in direct diagnosis from whole blood have been marketed. The ideal technique should consider the following features: speed, high sensitivity and specificity, capacity to detect non-culturable microorganisms, detection of various resistance mechanisms, the highest automation possible, easy to implement in the daily routine of a microbiology laboratory and being cost-effective. It is difficult to meet all these requirements and it should also be taken into account that using this technique has certain disadvantages, such as not having the identified microorganism or contamination of the process with external genetic materials, which could hamper interpretation. All studies conducted to date with these new molecular methods raise certain questions about their actual sensitivity, since they are compared to the standard haemoculture, which is probably not sufficiently sensitive to be considered a reference method in the diagnosis of septicaemia. For the reasons commented, the most sensible option would be to use both methods and evaluate the results based on the patient's clinical situation.

LightCycler SeptiFast

The LightCycler SeptiFast system (Roche Molecular System, Switzerland) was the first to be marketed and has been evaluated in various clinical studies.[12–16] It allows for direct detection and identification from blood of 25 pathogens that make up 90% of the most common aetiological agents in septicaemia. The study panel includes the gram-negative bacilli: *Escherichia coli*, *Klebsiella (pneumoniae/oxytoca)*, *Serratia marcescens*, *Enterobacter (cloacae/aerogenes)*, *Proteus mirabilis*, *Pseudomonas aeruginosa*, *Acinetobacter baumannii* and *Stenotrophomonas maltophilia*; gram-positive cocci: *Staphylococcus aureus*, coagulase-negative staphylococci, *Streptococcus pneumoniae*, other strains of*Streptococcus*, *Enterococcus faecalis*, *E. faecium*, and *Candida* spp. (5 species) and *Aspergillus fumigatus*. It can also detect the presence of the *mecA* gene, responsible for methicillin resistance. It only requires 1.5 ml of whole blood, and the duration of the detection process is 3.5–6 h, depending on the results. The system uses the sequences located between 16S and 23S of ribosomal DNA as the target for identifying the bacteria, and, for fungi, those located between 18S and 5.8S. Once the DNA has been extracted, it is purified and 3 multiplex PCRs are conducted (gram-negative bacteria, gram-positive bacteria and fungi) which allows for identification of the microorganisms included in the panel on a genus and species level, based on the analysis of the melting points of the amplicons obtained. The method's detection limit is 3–30 CFU/ml for bacteria and 100 CFU/ml for yeasts.

Results of several clinical studies conducted on patients with severe septicaemia,[13,14] neutropaenic patients with fever,[13,15] paediatric patients[16] or patients admitted to intensive care units[13]

have been reported. The specificity and sensitivity data observed vary, probably because the population studied is different. Chang et al.[17] conducted a systematic review of the data published in the literature and conducted a meta-analysis evaluating a total of 8438 episodes of suspected septicaemia in 6012 patients. The global sensitivity in detecting septicaemia was 75% and the specificity was 92%. If only bacteraemias are analysed, the sensitivity was 80% and the specificity was 95%. For fungaemias, the results were 61% and 95%, respectively. In a recent systematic review conducted by Dark et al.[18] of 41 phase III studies evaluating diagnostic precision comparing SeptiFast with standard haemoculture, the sensitivity was 68% and the specificity was 86%.

Magicplex Sepsis Real-Time

The Magicplex Sepsis Real-Time system was developed by Seegene (South Korea) and can identify more than 90 microorganisms. The detection panel includes 73 gram-positive microorganisms, 12 gram-negative microorganisms, 6 fungi and 3 resistance-determining genes (*vanA*, *vanB* and *mecA*). Only 1 ml of blood is needed and the final result can be obtained in 3–6 h. After extracting the DNA, the sample undergoes a conventional PCR and, in a second phase, another PCR in which a dual oligonucleotide probe is used that, since it is marked with a fluorescent substance, allows for real-time detection of the amplicon obtained.

Carrara et al.[19] evaluated the usefulness of this methodology in a study including 267 patients from intensive care units (ICU) and hospital emergency departments. In 98 samples it was positive (37%); of these, 63 were considered true positives, and the rest, contaminants. Of the positive samples, 23 (36%) were positive both with Magicplex and with haemocultures, 22 (35%) only with haemocultures and 18 (25%) only with Magicplex. The sensitivity and the specificity were 65% and 92%, respectively, for Magicplex, and 71% and 88%, respectively, for the haemocultures. In the study by Loonen et al.,[20] the sensitivity and specificity values observed were even lower: 33% and 77%, respectively.

SepsiTest

SepsiTest, by manufacturer Molzym (Germany), is a semiautomatic system based on the use of a PCR that uses universal primers directed at targets of ribosomal RNA of bacteria (16S) and fungi (18S). The PCR result indicates the possible presence of bacteraemia or fungaemia and, if positive, the product obtained (amplicon) is sequenced to obtain the final identification. It can identify 345 pathogenic agents, including bacteria and fungi, and the detection limit is 20–460 CFU/ml.[21] The volume of blood needed is 1 ml, although up to 10 ml can be processed, and it offers a clear advantage, such as its ability to be used in other sterile fluids. The process takes 4 h to determine whether any aetiological agent is present in the sample and 4 more hours until the result of the sequencing is known. In a multicentre study[22] SepsiTest was evaluated in 187 patients (382 samples) with various pathologies (neutropaenic patients with fever, septicaemia, SIRS). If the results are compared to positive haemocultures, the sensitivity was 87% and the specificity was 86%. It is worth nothing that SepsiTest detected 12.5% of patients with a polymicrobial infection while the percentage was 7.4% in conventional haemocultures. It also detected anaerobic bacteria that did not grow in the haemocultures, as well as other microorganisms that were considered contaminants. Of the 54 positive haemocultures, SepsiTest did not detect any microorganisms in 7 cases and in the 288 negative haemocultures, the PCR was positive in 41 samples collected from 31 patients. Of these PCR positive samples, it was deemed that in 25 patients bacteraemia was possible or probable, since the majority (17 of 25) had received antimicrobial treatment before sample collection. With this data, the diagnostic yield of SepsiTest was 25.7% and of haemocultures, 15.8% The results from various published papers indicate that the sensitivity and specificity are highly variable, between 21% and 85% and 58% and 95%, respectively.[20,22–24] To date, detection of resistance genes has not been included in the system.

VYOO

The VYOO system (Analytik Jena, Germany) uses a multiplex PCR that allows for identification of the most commonly-implicated microorganisms in septicaemia: 34 bacteria and 7 fungi. The detection of 5 resistance markers is included. Five ml of blood are needed and the final result is obtained in 7 h. Once the DNA is extracted, it is subjected to an enrichment process and then multiplex amplification with specific probes and identification via microarray technology (in the original design, electrophoresis gels were used). According to the manufacturer's data, the system has a detection limit of 3–10 CFU/ml.[25] The panel of microorganisms detected is fairly comprehensive and includes, apart from the more common bacteria, *H. influenzae*, *N. meningitis*, various anaerobics, *A. fumigatus* and *Candida* spp. (the five most common species). The resistance genes detected are: *mecA*, *vanA*, *vanB*, as well as extended-spectrum beta-lactamases (ESBLs): *blaSHV* and *blaCTX-M*, including some variants.

In an observational study[26] the yield of the VYOO system was compared to the conventional haemoculture practice in 311 samples from 245 patients with suspected septicaemia. According to the results obtained, 30.1% (94/311) of the PCRs were positive while only 14.5% (45/311) of the haemocultures conducted were positive. In 27 samples (8.7%) both methods were positive, and in 199 samples (64%) both methods were negative. In 27 samples (21.5%) only the VYOO system was positive, while the inverse situation (haemoculture positive, VYOO system negative) was observed in 18 samples (5.8%). The global sensitivity of the VYOO method in detecting positive bacterial cultures was 60% and the specificity was 75%. According to the authors, the time required to obtain the result via the VYOO method was 7.2 h, while for positive haemocultures it was 68.8 hours and 191 hours in negative haemocultures. In an observational study, Schreiber et al.[23] compared yields of 3 automated PCR systems (VYOO, SepsiTest and SeptiFast) with the practice of haemoculture. They included 50 patients with suspected septicaemia, severe sepsis or septic shock. Thirteen haemocultures (26%) were positive, but only 8 were considered evaluable. The 3 molecular methods did not identify any microorganisms in 5 of the 8 positive haemocultures. There were 32 negative cases both via haemoculture and via the 3 PCR methods. The SepsiTest method detected 6 positives (12%), whose microorganisms matched those recovered in 6 positive haemocultures. Five samples (10%) were positive according to the VYOO system, of which 4 were considered evaluable but none were consistent with the positive haemocultures. SeptiFast identified 8 microorganisms in 7 samples, but it was only consistent with the positive haemocultures in 3 cases. In some studies[26,27] the VYOO system is more sensitive than haemocultures as a reference to evaluate the results if the clinical information and markers such as procalcitonin are used. This higher sensitivity could be due to its low detection limit (3–10 CFU/ml), associated with a higher volume of blood (5 ml).

PCR/ESI-MS analysis

This method combines use of a PCR that detects the DNA of the pathogenic agent with later analysis of the amplicon obtained via mass spectrometry. To do so, a technique known as electrospray ionisation (ESI-MS) is used, which consists of applying a high electrical charge to a fluid that allows for creation of aerosols that would

contain the ions generated from the molecules of the analysed fluid, and that would be detected via mass spectrometry analysis. Currently the most developed system is the IRIDICA system (Abbott),[11] but it has had several predecessors, such as TIGER,[28] Ibis T5000[29] and Ibis-Abbott PLEX-ID.[30] Multiple pairs of primers are used in the process to amplify selected regions of the bacterial or fungal genome such as DNA which encodes ribosomal RNA or that of various constituent genes. After amplification, analysis via ESI-MS allows for identification of the base composition (A,G,C,T) existing in each amplicon that is then compared with a database to determine the microorganism detected. The system allows for identification of around 800 microorganisms, including 9 species of *Candida* spp. in addition to reporting the presence of 4 resistance genes (*mec*A, *van*A, *van*B and bla_{KPC}). The volume of blood needed is 5 ml and the time required to obtain the final result is 6 hours. Studies conducted to determine the system's detection limit indicate that for bacteria it is 16 CFU/ml and for *Candida* it is 4 CFU/ml. This methodology has the capacity to detect polymicrobial bacteraemias and can also be applied in samples other than blood, such as sterile fluids, or in the diagnosis of respiratory infections, expanding the number of identifiable microorganisms to nearly 1000 between bacteria, fungi, and viruses. In a recent multicentre observational study conducted in patients admitted to intensive care units, the usefulness of this methodology was compared to conventional haemocultures in the diagnosis of bacteraemia.[31] The sensitivity of the technique was 81% and the specificity was 69%, with a negative predictive value of 97%. These sensitivity and specificity values could be considered low, but it should be noted that of the total number of samples compared (625), only 68 positive haemocultures were detected, and using PCR/ESI-MS, the number of positives was 228, i.e., 3 times more. There were only 13 positive haemocultures that were negative using PCR/ESI-MS. In the prior study by Bacconi et al.[11] the sensitivity values are similar (83%) but the specificity was higher (94%). Jordana-Lluch et al.,[32] after applying clinical criteria of infection when evaluating the results obtained, obtained a sensitivity and specificity of 90.5% and 87.2%, respectively.

T2Candida

The methodology that uses the T2Candida test system is known as T2 magnetic resonance (T2MR) and was developed by T2Biosystems (USA). This system has a new approach for detecting the presence of *Candida* species directly from a patient's blood sample. The method is based on a conveniently miniaturised methodology that, via magnetic resonance, is able to analyse how water molecules react in the presence of magnetic fields. It has the capacity to detect a wide variety of targets, such as DNA (molecular targets) and proteins that are useful in immunodiagnosis.[33] The T2Candida test allows identification of the 5 most common species of *Candida*: *C. albicans*, *C. tropicalis*, *C. glabrata*, *C. parapsilosis* and *C. krusei*. To do so, it uses specific primers complementary to the ribosomal RNA sequences 5.8S and 28S that amplify the ITS2 region of the *Candida* genome that are later bound to coated paramagnetic nanoparticles with a complementary probe. Once hybridisation of the nanoparticles occurs, they group around the detected target and the magnetic signal in the sample is modified with microscopic changes in the sample's water, which indicates the presence of the target sought.[33] Variation in the signal, known as T2 relaxation, is measured with the T2Dx instrument. This is a completely automated instrument able to perform various tests at the same time, and within a period of 3–5 h. The detection limit of the T2Candida method, according to the manufacturer, is just 1 CFU/ml. Neely et al.[34] evaluated the yield of the T2Candida method in a blind study that included 24 blood samples from various patients who presented symptoms attributable to septicaemia. Eight samples were from 3 patients with candidaemia, 8 from patients with bacteraemia (n = 8) and 8 other patients with negative haemocultures (n = 8). In all the samples from patients with candidaemia, the T2Candida method detected the species responsible. Also, 21 serial blood samples collected from 3 patients with *C. albicans* were analysed. Both the haemocultures and the T2Candida method detected the presence of *C. albicans* in the patient's blood. Twenty-four hours after starting antifungal treatment, the haemocultures were negative but the T2Candida method continued to be positive, indicating the possible detection of DNA from dead cells. In the same study, the authors studied different concentrations of the *Candida* species that the system can detect and observed that the detection limit was 3 CFU/ml for *C. albicans* and *C. tropicalis*, 2 CFU/ml for *C. glabrata* and *C. krusei* and 1 CFU/ml for *C. parapsilosis*. Beyda et al.[35] compared the T2Candida method with the practice of haemoculture. They added different concentrations (between 3 and 11 CFU/ml) of the 5 species that the T2Candida method detected in the blood samples and analysed a total of 90 samples. The sensitivity of the method was 100% and the specificity was 98%, since there were 4 false positives that the authors attributed to possible contamination when preparing the samples. The time required to obtain the results was 3–5 h for the T2Candida method, while the average haemoculture result was 63 h. In the multicentre study by Mylonakis et al.[36] the global sensitivity and specificity were 99.4 and 91.1%, respectively. Also, the authors estimate a negative predictive value between 99.5% and 99% for a study population with a candidaemia prevalence between 5% and 10%, respectively. T2Biosystems is currently developing a method to detect bacteria (T2Bacteria) that would include the majority of the pathogenic agents associated with septic symptoms and with a yield similar to that observed with the T2Candida method.

New technologies

The company DNA Electronics Ltd (www.dnae.com) has designed an instrument (Genalysis) focused on quick diagnosis of septicaemia that allows for identification of the aetiological agent and the antibiotic resistance genes in a period of 2–3 h.

This technology combines DNA sequencing on an electronic platform (semiconductors) with an immunomagnetic pathogenic agent capture system that detects it in less than 30 min. A volume of 10 ml of blood is used and it has a sensitivity of 1 CFU/ml.

The vivoDx system, developed by the company GeneWEAVE, which has been acquired by the Roche group, is focused on direct microorganism detection in various pathological samples and, at the same time, determining its antibiotic susceptibility in less than 4 h, although there are no data about its possible application on whole blood (www.rochemicrobiolytests.com). It uses Smarticles™ technology, an innovative tool for molecular diagnosis that combines bioparticles containing specific DNA designed to detect the microorganism by means of luciferase expression.

Direct identification from positive haemocultures

After determining the result of the Gram stain of a positive haemoculture, the microbiology laboratory must consider which diagnostic options can be used to identify the microorganism visualised as soon as possible without waiting for the results of the conventional cultures. The possibility of using techniques that, while identifying the microorganism, also detect the presence of certain antibiotic resistance genes, should also be considered. Currently, there are various systems available to quickly identify the microorganisms detected in positive haemocultures. Without a doubt, the use of mass spectrometry (matrix-assisted laser

desorption-ionisation time of flight [MALDI-TOF]) is the option selected by most laboratories, since, in less than an hour, the aetiological agent can be identified. The functioning and usefulness of MALDI-TOF are extensively commented in a specific article of this journal.[37]

Kaleta et al.[38] used the PCR/ESI-MS methodology to identify the microorganisms isolated in a positive haemoculture, obtaining high consistency with the phenotypic identification methods. Nevertheless, the complexity of this system does not justify its use in the field if compared to the simplicity of MALDI-TOF.

Fluorescence in situ hybridisation (FISH) allows for identification of a certain number of pathogenic agents. This system is based on the specific binding of fluorescent nucleic acid probes to complementary DNA sequences in the microorganism (16S rRNA for bacteria and 18S rRNA for fungi). The choice of a specific probe depends on the result of the gram staining: staphylococcus (*S. aureus*/coagulase-negative staphylococci), enterococci (*E. faecalis*, *E. faecium*), gram-negative bacilli (*E. coli*, *K. pneumoniae*, *P. aeruginosa*) or *Candida* spp. The result is obtained in less than 3 h.[39] Since 2013, a faster version of this methodology is available called Quick-FISH, which reduces the time to around 30 min.[40] The sensitivity and specificity of both techniques varies from 97% to 100% and 90% to 100%, respectively.[39,40] The company Accelerate Diagnostics INC (USA) has developed an automated system (Accelerate ID/AST) that uses FISH technology to identify the microorganism isolated in the positive haemoculture in approximately 1 hour and can determine antibiotic susceptibility in 6 h.[41] It allows for identification of the most common species and genera of gram-positive and gram-negative bacteria, although more studies are needed to validate its usefulness.

Bacteraemia due to *S. aureus* is a serious condition with significant mortality and a high risk of complications.[42] Early detection of these microorganisms and, at the same time, its sensitivity or oxacillin resistance can be carried out in approximately 1 h using various fast PCR techniques such as GeneXpert MRSA/SA BC (Cepheid) or GenomEra MRSA/SA (Abacus). The sensitivity and specificity values of both methodologies are high (>95%) and its automation facilitates its use in the daily routine of a microbiology laboratory.[43,44] Other systems, such as BD GeneOhm MRSA or BD Max MRSA,[45,46] have also demonstrated their usefulness, but require more time to be conducted: 120–140 min. Both *S. aureus* and coagulase-negative staphylococci (CoNS) are common aetiological agents of bacteraemia associated with intravenous catheters. Zboromyrska et al.[47] have shown the usefulness of the GeneXpert MRSA/SA BC system in detecting *S. aureus* (susceptible and oxacillin-resistant) and oxacillin-resistant CoNS directly in the blood drawn from an infected catheter without having to wait for the haemoculture result.

Eazyplex MRSA (Amplex ByoSistems GmbH) is a recently marketed assay that uses loop-mediated isothermal amplification (LAMP) methodology to detect *S. aureus* and CoNS, as well as the genes *mec*A and *mec*C[48] in less than 30 min. In the work by Rödel et al.,[48] the sensitivity and specificity in detecting *S. aureus* was 100% and 98.2%, respectively, and 100% in both parameters in the oxacillin-resistant strains. Use of the LAMP methodology has also shown its usefulness in detecting genes for resistance to extended-spectrum beta-lactamases (ESBLs) and carbapenemases (KPC, VIM, NDM, IMP) directly from positive haemocultures[49] with 100% sensitivity and specificity. It also offers the possibility for each laboratory to be able to design specific probes to detect the microorganisms that it deems to be of interest.

The AccuProbe system (Gen-Probe, USA) directly identifies various gram-positive microorganisms directly from positive haemocultures by using a specific DNA probe: *S. pneumoniae*, *S. aureus*, *Enterococcus* spp., *S. pyogenes* and *S. agalactiae*. The sensitivity and specificity exceed 97%, except for *S. aureus*, whose specificity is 80.8% and sensitivity is 99.8%.[50]

In recent years, molecular methods based on the use of multiplex PCRs and subsequent hybridisation in microarray panels have been developed, allowing for identification of the majority of microorganisms responsible for septicaemia from positive haemocultures. The Verigene system (Nanosphere, USA) has created a panel for gram-positive microorganisms (n = 13: 9 species and 4 genera) and another for gram-negative bacilli (n = 9: 5 species and 4 genera) with a sensitivity of 81–100% and a specificity exceeding 98%.[51] The system can also detect various resistance genes: *mec*A, *van*A, *van*B, bla_{NDM}, bla_{VIM}, bla_{KPC}, bla_{KPC}, bla_{OXA} and bla_{CTX-M}, and the time needed is 2.5 h.

The platform designed by Biofire Diagnostics (bioMérieux, France) totally automates the extraction and purification of nucleic acids and uses various nested multiplex PCRs to identify the aetiological agent in 1 h. The FilmArray Blood Culture Identification panel for haemocultures encompasses 26 microorganisms (11 species and 15 genera of gram-positive and gram-negative bacteria) in addition to the 5 most common species of *Candida* and 4 resistance genes (*mec*A, *van*A, *van*B, bla_{KPC}). The sensitivity for identifying these microorganisms is >90%[52,53] and 100% for detecting the resistance genes.[53] The Prove-it Sepsis system (Mobidiag, Finland) also combines use of a PCR with microarrays with a sensitivity and specificity of 95% and 99%, respectively, but the time it takes is 3.5 h.[54] The Sepsis Flow Chip assay (SFC, Master Diagnostica, Spain) is based on a methodology that consists of simultaneous detection of at least 36 bacterial species and various fungi by means of multiplex PCR followed by automatic hybridisation on membranes with specific DNA probes by means of DNA Flow technology (hybriSpot HS24). It also allows for detection of 20 resistance markers (*mec*A, *van*A, *van*B and various extended-spectrum beta-lactamases and class A, B and D carbapenemases) in around 4 h.[55,56]

Conclusions

The different technologies developed to directly identify the aetiological agent of septicaemia in the patient's blood have the potential to reduce the time needed to reach a definitive microbiological diagnosis. The information that they provide is of clinical relevance but does not replace that which is collected through usual haemoculture practice. Currently, they are complementary methods and studies that evaluate the possible impact that they could have on the management of patients with septicaemia are required. Other aspects, such as the number of samples to be analysed, their frequency or the interpretation of the results from a clinical and microbiological point of view, should also be assessed. It should also be remembered that the use of these technologies entails an additional economic cost that should be justified with a clear clinical benefit.

Conflicts of interest

The authors declare that they have no conflicts of interest.

References

1. Mayr F, Yende S, Angus D. Epidemiology of severe sepsis. Virulence. 2013;5:4–11.
2. Pherson D, Griffiths C, Williams M, Baker A, Klodawski E, Jacobson B, et al. Sepsis-associated mortality in England: an analysis of multiple cause of death data from 2001 to 2010. BMJ Open. 2013:3, http://dx.doi.org/10.1136/bmjopen-2013-002586.

3. Angus DC, Linde-Zwirble WT, Lidicker J, Clermont G, Carcillo J, Pinsky MR. Epidemiology of severe sepsis in the United States: analysis of incidence, outcome, and associated costs of care. Crit Care Med. 2001;29:1303–10.
4. Vincent JL, Sakr Y, Sprung L, Ranieri M, Reinhart K, Gerlach H, et al. Sepsis in European intensive care units: results of the SOAP study. Crit Care Med. 2006;34:344–53.
5. Cockerill F, Wilson J, Vetter E, Goodman K, Torgerson C, Harmsen W, et al. Optimal testing parameters for blood cultures. Clin Infect Dis. 2004;38:1724–30.
6. Riedel S, Bourbeau P, Swartz B, Brecher S, Carroll K, Stamper P, et al. Timing of specimen collection for blood cultures from febrile patients with bacteremia. J Clin Microbiol. 2008;46:1381–5.
7. Dellinger R, Levy M, Carlet J, Bion J, Parker M, Jaeschke R, et al. Surviving Sepsis Campaign: International guidelines for management of severe sepsis and septic shock: 2008. Intensive Care Med. 2008;34:17–60.
8. Kumar A, Roberts D, Wood K, Light B, Parrillo J, Sharma S, et al. Duration of hypotension before initiation of effective antimicrobial therapy is the critical determinant of survival in human septic shock. Crit Care Med. 2006;34:1589–96.
9. Al-Soud WA, Rådström P. Purification and characterization of PCR-inhibitory components in blood cells. J Clin Microbiol. 2001;39:485–93.
10. Wain J, Diep TS, Ho VA, Walsh AM, Nguyen TT, Parry CM, et al. Quantitation of bacteria in blood of typhoid fever patients and relationship between counts and clinical features, transmissibility, and antibiotic resistance. J Clin Microbiol. 1998;36:1683–7.
11. Bacconi A, Richmond G, Baroldi M, Laffler T, Blyn L, Carolan H, et al. Improved sensitivity for molecular detection of bacterial and *Candida* infections in blood. J Clin Microbiol. 2014;52:3164–74.
12. Bloos F, Hinder F, Becker K, Sachse S, Dessap AM, Straube E, et al. A multicenter trial to compare blood culture with polymerase chain reaction in severe human sepsis. Intensive Care Med. 2010;36:241–7.
13. Bravo D, Blanquer J, Tormo M, Aguilar G, Borrás R, Solano C, et al. Diagnostic accuracy and potential clinical value of the LightCycler SeptiFast assay in the management of bloodstream infections occurring in neutropenic and critically ill patients. Int J Infect Dis. 2011;15:e326–31.
14. Fernández-Cruz A, Marin M, Kestler M, Alcala L, Rodriguez-Creixems M, Bouza E. The value of combining blood culture and SeptiFast data for predicting complicated bloodstream infections caused by Gram-positive bacteria or *Candida* species. J Clin Microbiol. 2013;51:1130–6.
15. Lamoth F, Jaton K, Prod'hom G, Senn L, Bille J, Calandra T, et al. Multiplex blood PCR in combination with blood cultures for improvement of microbiological documentation of infection in febrile neutropenia. J Clin Microbiol. 2010;48:3510–6.
16. Lucignano B, Ranno S, Liesenfeld O, Pizzorno B, Putignani L, Bernaschi P, et al. Multiplex PCR allows rapid and accurate diagnosis of bloodstream infections in newborns and children with suspected sepsis. J Clin Microbiol. 2011;49:2252–8.
17. Chang S-S, Hsieh W-H, Liu T-S, Lee S-H, Wang C-H, Chou H-C, et al. Multiplex PCR system for rapid detection of pathogens in patients with presumed sepsis—a systemic review and meta-analysis. PLOS ONE. 2013;8:e62323.
18. Dark P, Blackwood B, Gates S, McAuley D, Perkins GD, McMullan R, et al. Accuracy of LightCycler® SeptiFast for the detection and identification of pathogens in the blood of patients with suspected sepsis: a systematic review and meta-analysis. Intensive Care Med. 2014;41:21–33.
19. Carrara L, Navarro F, Turbau M, Seres M, Morán I, Quintana I, et al. Molecular diagnosis of bloodstream infections with a new dual-priming oligonucleotide-based multiplex PCR assay. J Med Microbiol. 2013;62:1673–9.
20. Loonen A, de Jager C, Tosserams J, Kusters R, Hilbink M, Wever P, et al. Biomarkers and molecular analysis to improve bloodstream infection diagnostics in an emergency care unit. PLOS ONE. 2014;9:e87315.
21. Mühl H, Kochem A-J, Disqué C, Sakka SG. Activity and DNA contamination of commercial polymerase chain reaction reagents for the universal 16S rDNA real-time polymerase chain reaction detection of bacterial pathogens in blood. Diagn Microbiol Infect Dis. 2010;66:41–9.
22. Wellinghausen N, Kochem A-J, Disqué C, Mühl H, Gebert S, Winter J, et al. Diagnosis of bacteremia in whole-blood samples by use of a commercial universal 16S rRNA gene-based PCR and sequence analysis. J Clin Microbiol. 2009;47:2759–65.
23. Schreiber J, Nierhaus A, Braune SA, de Heer G, Kluge S. Comparison of three different commercial PCR assays for the detection of pathogens in critically ill sepsis patients. Med Klin Intensivmed Notfmed. 2013;108:311–8.
24. Orszag P, Disqué C, Keim S, Lorenz MG, Wiesner O, Hadem J, et al. Monitoring of patients supported by extracorporeal membrane oxygenation for systemic infections by broad-range rRNA gene PCR amplification and sequence analysis. J Clin Microbiol. 2014;52:307–11.
25. Sachse S, Straube E, Lehmann M, Bauer M, Russwurm S, Schmidt KH. Truncated human cytidylate-phosphate-deoxyguanylate-binding protein for improved nucleic acid amplification technique-based detection of bacterial species in human samples. J Clin Microbiol. 2009;47:1050–7.
26. Bloos F, Sachse S, Kortgen A, Pletz MW, Lehmann M, Straube E, et al. Evaluation of a polymerase chain reaction assay for pathogen detection in septic patients under routine condition: an observational study. PLoS ONE. 2012;7:e46003.
27. Fitting C, Parlato M, Adib-Conquy M, Memain N, Philippart F, Misset B, et al. DNAemia detection by multiplex PCR and biomarkers for infection in systemic inflammatory response syndrome patients. PLoS ONE. 2012;7:e38916.
28. Hofstadler SA, Sampath R, Blyn LB, Eshoo MW, Hall TA, Jiang Y, et al. TIGER: The universal biosensor. Int J Mass Spectrom. 2005;242:23–41.
29. Ecker DJ, Sampath R, Massire C, Blyn LB, Hall TA, Eshoo MW, et al. Ibis T5000: a universal biosensor approach for microbiology. Nat Rev Microbiol. 2008;6:553–8.
30. Jacob D, Sauer U, Housley R, Washington C, Sannes-Lowery K, Ecker DJ, et al. Rapid and high-throughput detection of highly pathogenic bacteria by Ibis PLEX-ID technology. PLoS ONE. 2012;7:e39928–37.
31. Vincent JL, Brealey D, Libert N, Abidi N, O'Dwyer M, Zacharowski K, et al. Rapid diagnosis of infection in the critically ill. A multicenter study of molecular detection in bloodstream infections, pneumonia, and sterile site infections. Crit Care Med. 2015;43:2283–91.
32. Jordana-Lluch E, Giménez M, Quesada MD, Rivaya B, Marcó C, Domínguez MJ, et al. Evaluation of the broad-range PCR/ESI-MS technology in blood specimens for the molecular diagnosis of bloodstream infections. PLOS ONE. 2015;10:e0140865–140913.
33. Pfaller MA, Wolk DM, Lowery TJ. T2MR and T2Candida: novel technology for the rapid diagnosis of candidemia and invasive candidiasis. Future Microbiol. 2016;11:103–17.
34. Neely LA, Audeh M, Phung NA, Min M, Suchocki A, Plourde D, et al. T2 magnetic resonance enables nanoparticle-mediated rapid detection of candidemia in whole blood. Sci Transl Med. 2013;5:182ra54.
35. Beyda ND, Alam MJ, Garey KW. Comparison of the T2Dx instrument with T2Candida assay and automated blood culture in the detection of *Candida* species using seeded blood samples. Diag Microbiol Infect Dis. 2013;77:324–6.
36. Mylonakis E, Clancy C, Ostrosky-Zeichner L, Garey K, Alangaden G, Vazquez J, et al. T2 magnetic resonance assay for the rapid diagnosis of candidemia in whole blood: a clinical trial. Clin Infect Dis. 2015;60:892–9.
37. Siller-Ruiz M, Hernández-Egido S, Sánchez-Juanes F, González-Buitrago JM, Muñoz-Bellido JL. Métodos rápidos de identificación de bacterias y hongos. Espectrometría de masas MALDI-ToF, medios cromogénicos. Enferm Infecc Microbiol Clin. 2016, http://dx.doi.org/10.1016/j.eimc.2016.12.010.
38. Kaleta EJ, Clark AE, Johnson DR, Gamage DC, Wysocki VH, Cherkaoui A, et al. Use of PCR coupled with electrospray ionization mass spectrometry for rapid identification of bacterial and yeast bloodstream pathogens from blood culture bottles. J Clin Microbiol. 2011;49:345–53.
39. Martinez RM, Bauerle ER, Fang FC, Butler-Wu SM. Evaluation of three rapid diagnostic methods for direct identification of microorganisms in positive blood cultures. J Clin Microbiol. 2014;52:2521–9.
40. Deck MK, Anderson ES, Buckner RJ, Colasante G, Coull JM, Crystal B, et al. Multicenter evaluation of the *Staphylococcus* QuickFISH method for simultaneous identification of *Staphylococcus aureus* and coagulase-negative staphylococci directly from blood culture bottles in less than 30 minutes. J Clin Microbiol. 2012;50:1994–8.
41. Chantell C. Multiplexed automated digital microscopy for rapid identification and antimicrobial susceptibility testing of bacteria and yeast directly from clinical samples. Clin Microbiol News. 2015;37:161–7.
42. Laupland KB, Ross T, Gregson DB. *Staphylococcus aureus* bloodstream infections: risk factors, outcomes, and the influence of methicillin resistance in Calgary, Canada, 2000–2006. J Infect Dis. 2008;198:336–43.
43. Pence MA, McElvania TeKippe E, Burnham CA. Diagnostic assays for identification of microorganisms and antimicrobial resistance determinants directly from positive blood culture broth. Clin Lab Med. 2013;33:651–84.
44. Hirvonen JJ, Kaukoranta S-S. GenomEra MRSA/SA, a fully automated homogeneous PCR assay for rapid detection of *Staphylococcus aureus* and the marker of methicillin resistance in various sample matrixes. Expert Rev Mol Diag. 2014;13:655–65.
45. Stamper PD, Cai M, Howard T, Speser S, Carroll KC. Clinical validation of the molecular BD GeneOhm StaphSR assay for direct detection of *Staphylococcus aureus* and methicillin-resistant *Staphylococcus aureus* in positive blood cultures. J Clin Microbiol. 2007;45:2191–6.
46. Dalpke AH, Hofko M, Hamilton F, Mackenzie L, Zimmermann S, Templeton K. Evaluation of the BD Max StaphSR Assay for rapid identification of *Staphylococcus aureus* and methicillin-resistant *S. aureus* in positive blood culture broths. J Clin Microbiol. 2015;53:3630–2.
47. Zboromyrska Y, de la Calle C, Soto M, Sampietro-Colom L, Soriano A, Alvarez-Martínez MJ, et al. Rapid diagnosis of staphylococcal catheter-related bacteraemia in direct blood samples by real-time PCR. PLOS ONE. 2016;11:e0161684–161711.
48. Rödel J, Bohnert JA, Stoll S, Wassill L, Edel B, Karrasch M, et al. Evaluation of loop-mediated isothermal amplification for the rapid identification of bacteria and resistance determinants in positive blood cultures. Eur J Clin Microbiol Infect Dis. 2017, http://dx.doi.org/10.1007/s10096-016-2888-1.
49. Zboromyrska Y, Vergara A, Cosgaya C, Verger G, Mosqueda N, Almela M, et al. Rapid detection of β-lactamases directly from positive blood cultures using a loop-mediated isothermal amplification (LAMP)-based assay. Int J Antimicrob Agents. 2015;46:355–6.
50. Lindholm L, Sarkkinen H. Direct identification of gram-positive cocci from routine blood cultures by using AccuProbe tests. J Clin Microbiol. 2004;42:5609–13.
51. Mancini N, Infurnari L, Ghidoli N, Valzano G, Clementi N, Burioni R, et al. Potential impact of a microarray-based nucleic acid assay for rapid detection of Gram-negative bacteria and resistance markers in positive blood cultures. J Clin Microbiol. 2014;52:1242–5.
52. Altun O, Almuhayawi M, Ullberg M, Ozenci V. Clinical evaluation of the FilmArray Blood Culture Identification Panel in identification of bacteria and yeasts from positive blood culture bottles. J Clin Microbiol. 2013;51:4130–6.

53. Blaschke AJ, Heyrend C, Byington CL, Fisher MA, Barker E, Garrone NF, et al. Rapid identification of pathogens from positive blood cultures by multiplex polymerase chain reaction using the FilmArray system. Diag Microbiol Infect Dis. 2012;74:349–55.
54. Tissari P, Zumla A, Tarkka E, Mero S, Savolainen L, Vaara M, et al. Accurate and rapid identification of bacterial species from positive blood cultures with a DNA-based microarray platform: an observational study. Lancet. 2010;375:224–30.
55. Galiana A, Carrero-Lérida J, Azrak S, Olmo A, Rodríguez JC. Sepsis Flow Chip: a new molecular system for simultaneous detection of the main pathogens responsible for nosocomial infections, In: 26th ECCMID, Amsterdam, EP036.
56. Galiana A, Carrero-Lérida J, Olmo A, Azrak S, Rodríguez JC. Evaluating the Sepsis Flow Chip kit for detecting multidrug resistant bacteria. In: 26th ECCMID, Amsterdam, P0955.