Diagnostic Microbiology and Infectious Disease 94 (2019) 7–14

Contents lists available at ScienceDirect

Diagnostic Microbiology and Infectious Disease

journal homepage: www.elsevier.com/locate/diagmicrobio

Pathogen enrichment from human whole blood for the diagnosis of bloodstream infection: Prospects and limitations

Matthias Pilecky [a], Anita Schildberger [a], Dorothea Orth-Höller [c], Viktoria Weber [a,b,*]

[a] Center for Biomedical Technology, Department for Biomedical Research, Danube University Krems, Dr.-Karl-Dorrek-Strasse 30, 3500 Krems, Austria
[b] Christian Doppler Laboratory for Innovative Therapy Approaches in Sepsis, Department for Biomedical Research, Danube University Krems, Dr.-Karl-Dorrek-Strasse 30, 3500 Krems, Austria
[c] Division of Hygiene and Medical Microbiology, Medical University of Innsbruck, Schöpfstraße 41, A-6020 Innsbruck, Austria

ARTICLE INFO

Article history:
Received 26 September 2018
Received in revised form 20 November 2018
Accepted 20 November 2018
Available online 26 November 2018

Keywords:
Molecular diagnostics
Bloodstream infection
Pathogen enrichment
Selective lysis: Sepsis

ABSTRACT

Blood culture represents the current reference method for the detection of bacteria or fungi in the circulation. To accelerate pathogen identification, molecular diagnostic methods, mainly based on polymerase chain reaction (PCR), have been introduced to ensure early and targeted antibiotic treatment of patients suffering from bloodstream infection. Still, these approaches suffer from a lack of sensitivity and from inhibition of PCR in a number of clinical samples, leading to false negative results. To overcome these limitations, various approaches aiming at the enrichment of pathogens from larger blood volumes prior to the extraction of pathogen DNA, thereby also depleting factors interfering with PCR, have been developed. Here, we provide an overview of current systems for diagnosing bloodstream infection, with a focus on approaches for pre-analytical pathogen enrichment, and highlight emerging applications of pathogen depletion for therapeutic purposes as a potential adjunctive treatment of sepsis patients.

Contents

1. Introduction

Sepsis, a life-threatening organ dysfunction caused by a dysregulated host response to infection, afflicts 18 million people worldwide each year, with mortality rates of 30 to 50% even in state-of-the-art intensive care, and its incidence continues to increase (Fleischmann et al., 2016; Martin, 2012; Mayr et al., 2014; Mellhammar et al., 2016; Singer et al., 2016).

Over 600 pathogen species have been associated with sepsis (Vincent et al., 2009). Gram-positive bacteria account for more than half of all sepsis cases, followed by gram-negative bacteria and fungi (Martin, 2012; Vincent et al., 2006). The spread of antibiotic-resistant pathogens, evidenced by the global emergence of methicillin-resistant *Staphylococcus aureus* or by increasing resistances among gram-negative bacilli and common respiratory pathogens, poses particular challenges to the treatment of bloodstream infections.

* Corresponding author. Tel.: +43-2732-893-2632, fax: +43-2732-893-4600.
E-mail addresses: matthias.pilecky@donau-uni.ac.at (M. Pilecky), anita.schildberger@donau-uni.ac.at (A. Schildberger), dorothea.orth@i-med.ac.at (D. Orth-Höller), viktoria.weber@donau-uni.ac.at (V. Weber).

https://doi.org/10.1016/j.diagmicrobio.2018.11.015
0732-8893/

Current Guidelines for the Management of Severe Sepsis and Septic Shock (Rhodes et al., 2017) recommend immediate antibiotic therapy, which can significantly improve the survival of septic patients (Ferrer et al., 2014; Gaieski et al., 2010; Garnacho-Montero et al., 2006; Zhang et al., 2015). To ensure rapid onset of therapy, antibiotic treatment is initiated based on clinical and epidemiological information, and adjusted or de-escalated following identification of the etiologic agents. Culture-based diagnosis (blood culture) remains the reference standard to identify the causative pathogens in bloodstream infection (Lamy et al., 2016; Opota et al., 2015). Blood culture methods have been optimized to increase their sensitivity and specificity, but still, up to 50% of suspected bloodstream infections occur with negative blood culture, which can delay adequate antibiotic therapy. This can be due to the presence of slow-growing or intracellular pathogens, such as *Rickettsia spp*., *Bartonella spp*., *Coxiella spp*., *Mycoplasma spp*., or *Chlamydia spp*. (Fenollar and Raoult, 2007; Lamas and Eykyn, 2003; Lamy et al., 2016), or to antibiotic treatment initiated before blood sampling.

Molecular diagnostic methods, in particular polymerase chain reaction (PCR), are less sensitive to previously initiated antibiotic treatment than blood culture, and circumvent time-consuming and potentially pre-selecting culture steps (Dark et al., 2015; Stevenson et al., 2016), but PCR-based pathogen detection may be inhibited (Mancini et al., 2010) by sample matrix components, such as non-target DNA (Doring et al., 2008), heparin (Djordjevic et al., 2006; Garcia et al., 2002), immunoglobulins (Al-Soud and Radstrom, 2001), or iron associated with hemoglobin and lactoferrin. Efficient protocols for pre-analytical sample processing and pathogen enrichment are therefore required, as summarized in Fig. 1.

In this review, we provide an overview of current systems for the diagnosis of bloodstream infection. The most common approaches relying on pathogen identification after blood culture are summarized in Table 1 and have been reviewed extensively elsewhere (Dubourg and Raoult, 2016). Here, we focus on pathogen identification without previous blood culture, as compiled in Table 2. We particularly address methods relying on pre-analytical pathogen enrichment and compare their workflow to blood culture based approaches (Fig. 2).

2. Pathogen enrichment for diagnostic purposes

Pathogen enrichment prior to DNA extraction aims to increase the ratio of pathogens to non-target cells and to deplete factors potentially interfering with PCR (Beutler et al., 1990; Doring et al., 2008; Garcia et al., 2002; Mancini et al., 2010). Pathogen enrichment may further be required in sepsis patients, where pathogen loads of less than one CFU/mL whole blood have been reported (Arpi et al., 1989; Mermel and Maki, 1993), which is below the detection limit of current PCR methods (Ginn et al., 2017).

Pathogen enrichment may either exploit physical properties of pathogens, such as their density, size, or resistance to detergents (label-free methods), or may target pathogen-specific surface properties, such as the expression of pathogen-associated molecular patterns (label-assisted methods).

Pathogen enrichment from whole blood is frequently combined with the selective lysis of human blood cells in order to release intracellular pathogens that might otherwise remain undetected (Fraunholz and Sinha, 2012) and to deplete factors interfering with PCR (Al-Soud and Radstrom, 2001; Djordjevic et al., 2006; Doring et al., 2008; Garcia et al., 2002). Selective lysis of blood cells can be achieved by addition of hypotonic or chaotropic buffers containing apolar, zwitterionic, or polar detergents, of which only the latter also lyse the nucleus to release human DNA (Brennecke et al., 2017; Caspar et al., 2017; Katholnig et al., 2015; Trung et al., 2016). Bacteria and fungi are able to withstand the osmotic pressure during lysis due to their cell walls and can subsequently be separated from the supernatant containing the lysed blood cells. While mainly applied in combination with molecular diagnostics, selective lysis has also been used in blood culture systems, which has been reported to increase the sensitivity of blood culture for selected gram-positive bacteria and yeasts (Kiehn et al., 1983;

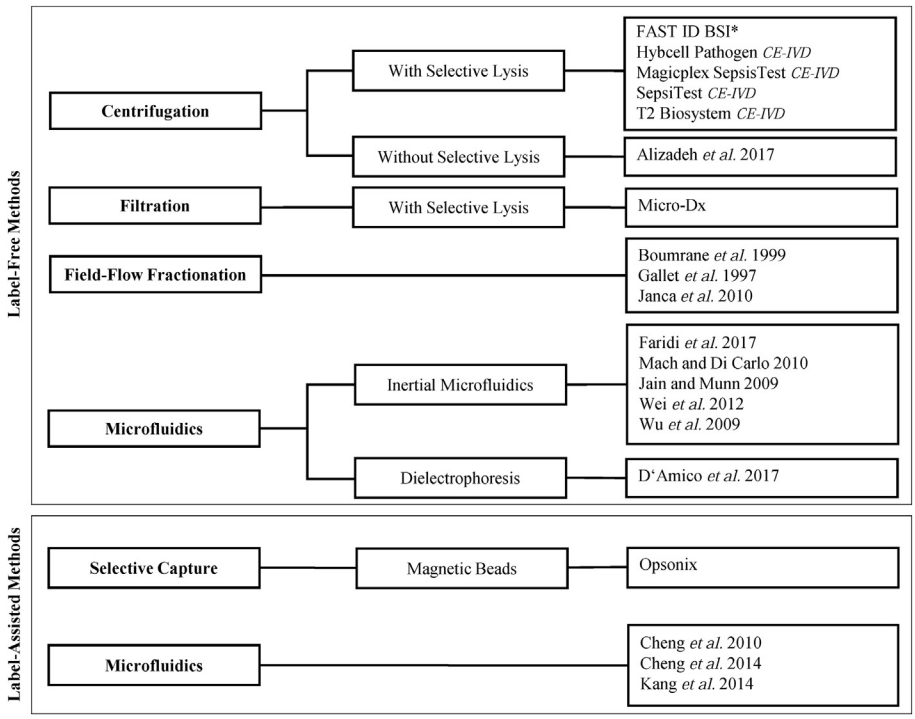

Fig. 1. Current concepts for pathogen enrichment from human whole blood. Suppliers are specified for diagnostic systems with CE-IVD certification, while references to the literature are given for approaches that are still at an experimental stage. *FAST-ID BSI is currently in clinical trials with planned CE-IVD approval in late 2019. CE, Conformité Européenne, indicating conformity with standards for products sold within the European Economic Area; IVD, *in vitro* diagnostics.

Table 1
Systems for the diagnosis of bloodstream infection relying on pathogen identification after blood culture.

System	Sample	Time-to-result [h]	Pre-analytical sample processing	Analysis	Reference
Blood culture systems					
BacT/Alert *bioMeriéux*	10 mL whole blood / bottle	24–120	Various growth media for aerobic and anaerobic organisms	Colorimetric detection of pH/CO_2	Bourbeau and Pohlman (2001), Mirrett et al. (2003)
BacTec *Becton Dickinson*		24–120	Various growth media for aerobic and anaerobic organisms; optionally with lysis of human blood cells	CO_2 detection by fluorescence	Mirrett et al. (2003), Rohner et al. (1997)
VersaTrek *ThermoScientific*		24–120	One medium for aerobic and anaerobic organisms each	Pressure sensor detecting microbial gas production or consumption	Dreyer et al. (2011)
Pathogen identification after blood culture					
QuickFISH *AdvanDx*		0.3	Fixation and hybridization with various probes	Manual assessment by fluorescence microscopy	Abdelhamed et al. (2015), Deck et al. (2012), Deck et al. (2014), Salimnia et al. (2014)
Sepsityper *Bruker Daltonics*		0.5	Gram stain (gram-positive and gram-negative bacteria are processed differently) and MALDI-TOF preparation	Mass spectrometry (MALDI-TOF) fingerprint and automated database comparison for ID	Fiori et al. (2016), Morgenthaler and Kostrzewa (2015), Oviano et al. (2017a), Oviano et al. (2017b)
FilmArray BCID *BioFire Diagnostics*		1	Mechanical lysis by beads and DNA capture by magnetic beads	PCR amplification and hybridization on a multiplex array, automated control by melting curves	Fiori et al. (2016), EvotecBiosystems (1992), McCoy et al. (2016)
ePlex *GenMark*		1.5	Magnetic solid-phase DNA extraction and amplification by PCR	Hybridization with ferrocene labeled capture probes and electrochemical detection	Babady et al. (2018), Maubon et al. (2018)
Verigene *Luminex*		2.5	Gram stain, lysis and DNA extraction using magnetic beads, hybridization to probes on a glass array	Gold labeled detection probes hybridize to captured bacterial DNA and are covered with silver once bound; Reflected light by different array positions is detected	Alby et al. (2013), Belknap et al. (2017), Scott (2013)
Unyvero Blood Culture molecular assay		4.5	Mechanical, enzymatic, chemical lysis, automated DNA isolation	PCR amplification and hybridization on a multiplex array with automated control	Burrack-Lange et al. (2018)
Automated ID and AST *(e.g. VITEK, Biomerieux; BD Phoenix, Becton Dickenson; MicroScan, Siemens)*		3 (ID) 6 (MIC)	Pre-culture on solid medium and subsequent inoculation of test medium	Automated standard biochemical testing and MIC determination	Bobenchik et al. (2015), Donay et al. (2004), Stefaniuk et al. (2003), Thomson et al. (2017)

Systems listed for automated ID and AST represent the most common devices. AST, Antibiotic susceptibility testing; ID, identification; MIC, minimal inhibitory concentration; MALDI-TOF, matrix-assisted laser desorption ionization-time of flight; PCR, polymerase chain reaction. All systems hold CE-IVD certification.

McLaughlin et al., 1983), but to result in decreased sensitivity for gram-negative bacteria and anaerobes (Henry et al., 1983; Murray, 1991).

2.1. Label-free methods

Label-free methods for pathogen enrichment rely on physical properties of pathogens, such as their size, shape, density, or their hydrodynamic characteristics. Almost all commercial systems rely on the selective lysis of blood cells followed by centrifugation. They include FAST-ID BSI (QVella, Richmond Hill, Canada), Hybcell Pathogen Array (CubeDX, St. Valentin, Austria), T2 Bacteria (T2 Biosystems, Lexington, MA) (Pfaller et al., 2016), SepsiTest (Molzym, Bremen, Germany), as well as Magicplex Sepsis Test (Seegene, Seoul, Korea) (Talebpour, 2015), as specified in Table 2. The recently introduced Micro-Dx system (Molzym) is based on a combination of selective lysis and filtration (Lustig, 2017). Additional approaches for pathogen enrichment, such as field flow fractionation and inertial microfluidics, are still at an experimental stage and have not yet reached routine application.

2.1.1. Pathogen enrichment by centrifugation

Pathogen enrichment by centrifugation relies on the quantitative sedimentation of pathogens, and microbial cells that fail to be pelleted can therefore lead to false negative results. The removal of the supernatant after centrifugation is a critical step, since larger volumes of removed supernatant efficiently reduce potentially interfering factors, while they also increase the risk of losing pelleted pathogens. Each washing step further increases the risk of pathogen loss, since the detergents contained in the washing solution may weaken the adherence of the pellet to the wall of the sample tube.

As summarized in Fig. 2, FAST-ID BSI relies on fully automated sample processing with selective lysis of the blood cells by a saponin solution, pelleting of the pathogens by centrifugation, washing, disintegration by electrical pulse pattern lysis, and amplification as well as identification of the released pathogen DNA in the sample cassette using PCR (Talebpour, 2015). Pathogen enrichment for the Hybcell Pathogen Array comprises the selective lysis of blood cells by incubation with a hypotonic detergent solution, followed by centrifugation. The resulting pellet is heated in alkaline solution, and released pathogen DNA is purified by adhesion to columns containing silica gel. Following elution, DNA is amplified by PCR for 16S or 18S rRNA for bacteria and fungi, respectively, and species are identified by array hybridization. The T2 system for candida and bacteria provides a fully automated device based on the selective hypotonic lysis of blood cells followed by centrifugation. The washed pathogen pellet is lysed mechanically using beads, the released pathogen DNA is amplified by PCR, and species are identified using sequence specific probes (Neely et al., 2013). Magicplex Sepsis Test and SepsiTest both rely on similar protocols and provide buffers for manual extraction or for semi-automated sample processing. Selective lysis is performed manually by a chaotropic buffer, followed by incubation with DNase to digest free DNA. Centrifugation and washing of the pathogen pellet are followed by automated pathogen DNA extraction, amplification by PCR, sequencing, and sequence comparison to an online database (Loonen et al., 2014; Wellinghausen et al., 2009).

On an experimental level, there have also been attempts to enrich pathogens by centrifugation without selective lysis, using a hollow spin disk to separate pathogens and plasma from human blood cells, followed by separation of the pathogens by vacuum filtration. Analysis of whole blood from 98 donors spiked with *E.coli* (6 and 200 CFU/mL) achieved recovery rates of about 70% (Alizadeh et al., 2017; Buchanan et al., 2017), but intracellular pathogens are not recovered with this protocol.

2.1.2. Pathogen enrichment by filtration

Pathogen enrichment by filtration is based on the retention of pathogens, while other sample components pass the filter. Blood samples are subjected to selective lysis prior to filtration to avoid clogging of

Table 2
Systems for diagnosis of bloodstream infection relying on molecular diagnostics without previous blood culture.

System	Sample volume [mL whole blood]	Time-to-result [h]	Pre-analytical sample processing	Analysis	Reference
Molecular diagnostics using DNA extraction directly from whole blood					
SeptiFast *Roche*	1.5	6	Mechanical cell lysis by glass beads followed by automated DNA extraction	PCR amplification and identification by melting curve analyses	Stevenson et al. (2016)
IRIDICA BAC BSI *Abbott discontinued*	5	7	Mechanical cell lysis by ceramic beads followed by automated DNA extraction	PCR amplification and desalting; identification by ESI-MS	Metzgar et al. (2016), Stevenson et al. (2016)
Molecular diagnostics using pathogen enrichment prior to DNA extraction					
FAST-ID BSI *QVella*	9	1	Automated pathogen enrichment by selective lysis by hypotonic detergent buffer, centrifugation and washing, followed by electrical lysis	PCR amplification and hybridization on a multiplex array	Qvella (2015)
Hybcell Pathogen CubeDx	0.5–5	3	Manual pathogen enrichment by selective lysis by hypotonic detergent buffer and centrifugation followed by alkaline thermal lysis and DNA purification on MiniSpin columns	PCR amplification of bacterial 16S rDNA and compact sequencing	Knabl et al. (2016)
T2Bacteria *T2 Biosystems*	2	3–5	Automated selective lysis in hypotonic detergent buffer, followed by debris concentration and pathogen lysis by bead beating	PCR amplification and NMR detection and identification	De Angelis et al. (2018), Hamula et al. (2016), Munoz et al. (2018), Neely et al. (2015), Patch et al. (2018), Pfaller et al. (2016)
Micro-Dx *Molzym*	1–5	4	Automated selective lysis, followed by filtration, chemical lysis of pathogens and extraction of DNA	PCR of hypervariable 16S/18S regions, followed by sequencing and BLAST for species identification	Lustig (2017)
Magicplex Sepsis Test *Seegene*	1	6	Manual selective lysis by chaotropic buffer and detergents and enrichment by centrifugation followed by automated DNA extraction using chemicals and magnetic beads	2x PCR amplification (gram-positive and fungi; gram-negative and resistance markers), Identification by additional PCR cycles with specific detection probes	Carrara et al. (2013), Loonen et al. (2014)
SepsiTest *Molzym*	1–5	4–8	Manual selective lysis by chaotropic buffer and detergents and enrichment by centrifugation followed by an automated DNA extraction using chemicals and magnetic beads	PCR of hypervariable 16S/18S regions, followed by sequencing and BLAST for species identification	Loonen et al. (2014), Stevenson et al. (2016), Wellinghausen et al. (2009)

BLAST, basic local alignment search tool; ESI-MS, electrospray-ionization mass spectrometry; NMR, nuclear magnetic resonance; PCR, polymerase chain reaction. All systems hold CE-IVD certification with the exception of FAST-ID BSI (certification foreseen in 2019) and Micro-DX (approved for research use only).

the filter. Micro-Dx is currently the only commercial system relying on pathogen enrichment by filtration for the analysis of whole blood samples, and it has been validated for a number of matrices, including tissue samples, joint aspirates, pleural, peritoneal and cerebrospinal fluid, wound swabs, bone marrow, and broncho-alveolar lavage. After pathogen retention on the filter membrane, chemical agents are used to release the pathogen DNA, which is subsequently eluted from the filter and amplified by PCR (Lustig, 2017).

2.1.3. Pathogen enrichment by microfluidic systems and emerging label-free approaches

In addition to centrifugation and filtration, various approaches, in particular microfluidic systems, have been exploited for label-free pathogen enrichment. A major advantage of microfluidic systems is their high surface-to-volume ratio, allowing for the controlled handling of the liquid and for an efficient transduction of external parameters, such as temperature. Microfluidic systems are easily adaptable for automation, reducing hands-on time (Henzler et al., 2018; Mou and Jiang, 2017). The microfluidic approach is well established in flow cytometry for the separation and characterization of biological particles, yet its throughput is insufficient to detect pathogens in whole blood, given the required sensitivity of approximately 1 CFU per milliliter. Still, there are efforts to develop devices based on inertial microfluidics to separate pathogens from blood cells based on their size and elasticity (Faridi et al., 2017; Mach and Di Carlo, 2010; Wei Hou et al., 2012; Wu et al., 2009). This approach relies on a phenomenon known as margination, which is observed *in vivo* in blood vessels with diameters of less than 300 μm, where red blood cells migrate to the center of the vessel under constant flow, while collisions of red blood cells and leukocytes result in a radial displacement of the larger leukocytes towards the vessel wall (Jain and Munn, 2009). Similar effects have also been found *in vitro* for platelets and spiked pathogens (Wei Hou et al., 2012). A more recent experimental approach relying on inertial microfluidics employs a non-Newtonian liquid as sheath fluid to select for large particles, such as red blood cells, whose net elastic and inertial forces allow for their transition to the sheath fluid, while small particles remain in the sample and can be separated after passage through the microfluidic channel (Faridi et al., 2017). Set-ups without sheath fluids with potential applications in the therapeutic field have been proposed alike (Mach and Di Carlo, 2010; Wu et al., 2009), with average pathogen yields of 60–80%. The initial bacterial load in these set-ups, however, was several log stages higher than reported in septic patients, while results for lower pathogen concentrations have not been published yet. Currently, a limiting step common to all microfluidic approaches for pathogen enrichment is their low flow rate of about 15 μL/h, which greatly restricts the sample volume that can be processed, and it remains to be investigated whether this restriction can be circumvented in future set-ups by parallelization of microfluidic devices.

Finally, field-flow fractionation and dielectrophoresis have been exploited for pathogen enrichment from human whole blood at an experimental level. During field-flow fractionation, drag, sedimentation, or thermophoretic, electric, and magnetic forces are applied to induce differential migration of pathogens and blood components (Bouamrane et al., 1999, Gallet et al., 1997, Janca et al., 2010, Reschiglian et al., 2005). Dielectrophoretic separation has also been applied to separate polarized particles in fluids based on their differences in size, shape, density, and dielectric properties (Cheng et al., 2010; Cheng et al., 2014; D'Amico et al., 2017), and field-flow fractionation and dielectrophoresis have been combined to separate platelets from other blood cells, a principle which may be adaptable for pathogen enrichment (Piacentini et al., 2011).

2.2. Label-assisted methods

Affinity capture for pathogen enrichment commonly employs biomolecules that recognize and bind pathogen-associated molecular

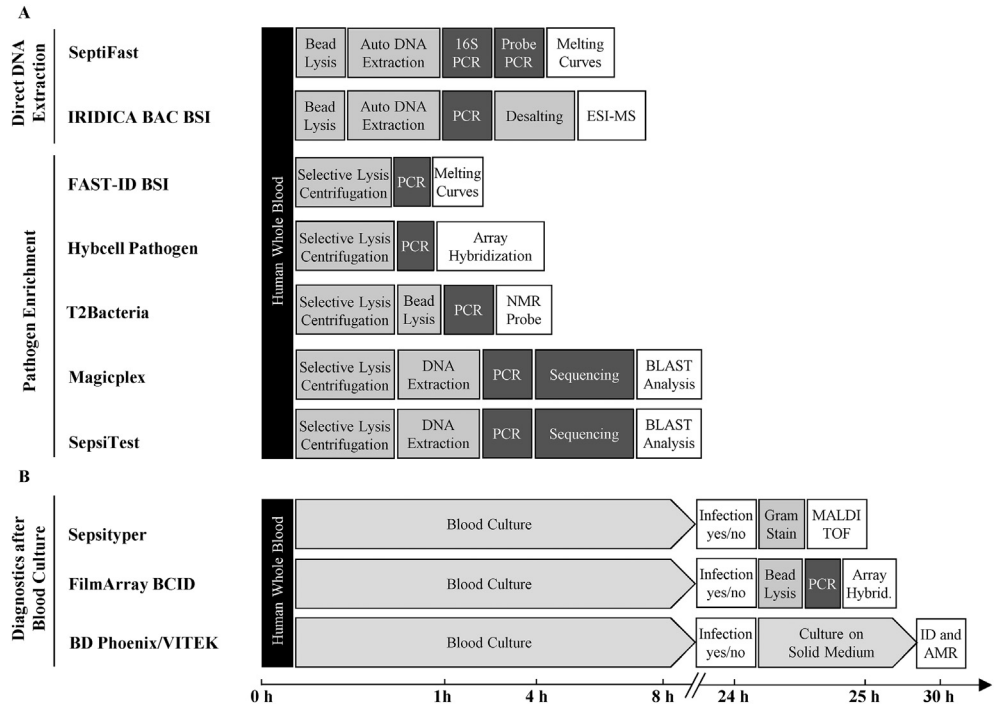

Fig. 2. Timelines for the diagnosis of bloodstream infection. (A) Systems for pathogen identification directly from whole blood (direct DNA extraction) or after pathogen enrichment. (B) The most common systems for pathogen identification following blood culture are shown for comparison. Steps depicted in light gray refer to pre-analytical sample processing, dark gray indicates DNA amplification steps, and white boxes refer to pathogen identification. Times depicted for individual steps represent approximations; note that the x-axis is not linear. AMR, antimicrobial resistance testing; BLAST, basic local alignment search tool; ESI-MS, electrospray ionization-mass spectrometry; ID, identification; MALDI-TOF, matrix-assisted laser desorption ionization-time of flight; NMR, nuclear magnetic resonance; PCR, polymerase chain reaction.

patterns (PAMPs), conserved surface motifs shared by a broad range of pathogen species, which are recognized as foreign by the human innate immune system. Examples include lipopolysaccharide for gram-negative bacteria, lipoteichoic acid for gram-positive bacteria (Messner et al., 2013), or chitin for fungi (Ruiz-Herrera and Ortiz-Castellanos, 2010). Ligands such as antibodies (Kim et al., 2017; Vutukuru et al., 2016; Yung et al., 2009), aptamers (Shen et al., 2016), lipoproteins (Almand et al., 2016; Vutukuru et al., 2016), components of the extracellular matrix (Henderson et al., 2011, Singh et al., 2012b), bacteriophages (Singh et al., 2012a), zinc-coordinated bis(dipicolylamine) (Lee et al., 2014), polymyxin B (Raman et al., 2017), or cell wall active antibiotics (Wang et al., 2014) have been immobilized on biomaterial surfaces to capture pathogens and PAMPS from blood samples (Didar et al., 2015; Herrmann et al., 2015; Kang et al., 2014).

While ligands with high specificity and affinity for their targets enable efficient binding of specific pathogens, a high degree of specificity narrows the spectrum of pathogens that can be depleted, thereby increasing the probability of false negative results and limiting the suitability of selective pathogen capture for diagnostic purposes.

The only diagnostic system using affinity capture that is close to clinical evaluation to date is an enzyme-linked lectin-sorbent assay using magnetic microbeads coated with the human opsonin mannose binding lectin (MBL) linked to an Fc domain (FcMBL; Opsonix, Wakefield, MA). The assay, which has recently been introduced to quantify PAMPs in whole blood from sepsis patients, showed a sensitivity of 85% (29/34 patients) as compared to 18% (12/67 patients) for blood culture (Cartwright et al., 2016).

3. Pathogen depletion from whole blood for therapeutic purposes

The increasing emergence of multi-drug resistant microbial pathogens and the threat of pan-resistant bacteria require a consideration of non-antibiotic approaches as adjuvant treatments or as alternative therapies. Potential non-antibiotic options to treat serious bacterial infection include inhibitors of bacterial quorum sensing, lytic bacteriophages, liposome-based cytotoxin inhibitors, advanced immunotherapies, as well as extracorporeal therapies, such as hemoadsorption, which aims at broad-range pathogen depletion from the circulation (Opal, 2016). Two adsorption systems based on affinity capture are presently under evaluation for therapeutic application (Seraph Microbind Affinity Blood Filter, ExThera Medical, Martinez, CA, as well as FcMBL hemoadsorption, Opsonix) (Didar et al., 2015, Herrmann et al., 2015, Kang et al., 2014). The hemoadsorption device developed by Opsonix employs polysulfone or polyethersulfone hollow fiber filters covalently coated with the aforementioned ligand FcMBL. When tested *in vitro*, the filter efficiently depleted gram-negative (*E. coli*) and gram-positive (*S. aureus*) bacteria, as well as fungi (*Candida albicans*) and lipopolysaccharide from human whole blood. These findings were confirmed in rat bacteremia models, where FcMBL-hemoadsorption resulted in efficient depletion of pathogens and of PAMPs released during antibiotic treatment, in decreased engraftment of living pathogens, as well as in diminished recruitment of inflammatory cells to major organs (Didar et al., 2015; Kang et al., 2014).

The Seraph Microbind Affinity Blood Filter developed by ExThera Medical is based on cartridges containing beads with endpoint-attached heparin, which binds to a broad spectrum of pathogens. It was shown to deplete cytomegalovirus (LaRosa et al., 2014), Enterobacteriaceae (McCrea et al., 2014), *Staphylococcus aureus* (Mattsby-Baltzer et al., 2011), as well as herpes simplex virus from whole blood with an efficiency of over 90%. A reduction of cytokine levels has also been proposed (Axelsson et al., 2010; Dileo and Federspiel, 2010). The safety and clinical efficiency of this device are currently evaluated in renal replacement patients with bloodstream infection.

4. Summary

A number of molecular diagnostic systems for bloodstream infection have been developed that exploit selective lysis of blood cells and centrifugation to enrich pathogens and deplete factors inhibiting or

interfering with PCR prior to analysis. The clinical performance of these systems is currently evaluated in comparison to blood culture or to first generation molecular diagnostics, such as the SeptiFast system (Roche, Basel, Switzerland). Beyond diagnostics, the capture and depletion of pathogens and PAMPs from the bloodstream with filters or beads functionalized with broad-spectrum ligands such as FcMBL or heparin represents a promising approach to lower the pathogen load in the bloodstream in sepsis patients and to reduce the spread of pathogens to distal sites.

It remains to be seen whether improved molecular diagnostics will replace classical blood culture, or whether it will rather be established as a complementary tool to detect non-cultivable or slow-growing pathogens and to identify resistance genes. Furthermore, currently ongoing clinical trials will evaluate the concept of pathogen depletion as supportive therapy for sepsis patients.

Declaration

Availability of data and materials

All data included in this article are publicly available, as this review summarizes published data.

Authors' contributions

MP compiled the review and wrote the first draft. AS supported with writing and with the preparation of the figures. DOH reviewed the draft and provided input from a medical perspective. VW extensively reviewed and critically revised the manuscript. All authors read and approved the final manuscript.

Competing interests

This work was supported by the project “SmartDiagnos” [Project ID:687697] funded by the European Commission under Horizon2020.

Consent for publication

All authors agreed to submit to Diagnostic Microbiology and Infectious Diseases.

Ethics approval and consent to participate

Not applicable.

References

Abdelhamed AM, Zhang SX, Watkins T, Morgan MA, Wu F, Buckner RJ, et al. Multicenter evaluation of Candida QuickFISH BC for identification of Candida species directly from blood culture bottles. J Clin Microbiol 2015;53(5):1672–6. https://doi.org/10.1128/JCM.00549-15.

Alby K, Daniels LM, Weber DJ, Miller MB. Development of a treatment algorithm for streptococci and enterococci from positive blood cultures identified with the Verigene gram-positive blood culture assay. J Clin Microbiol 2013;51(11):3869–71. https://doi.org/10.1128/JCM.01587-13.

Alizadeh M, Wood RL, Buchanan CM, Bledsoe CG, Wood ME, McClellan DS, et al. Rapid separation of bacteria from blood - chemical aspects. Colloids Surf B Biointerfaces 2017;154:365–72. https://doi.org/10.1016/j.colsurfb.2017.03.027.

Almand EA, Goulter RM, Jaykus LA. Capture and concentration of viral and bacterial foodborne pathogens using apolipoprotein H. J Microbiol Methods 2016;128:88–95. https://doi.org/10.1016/j.mimet.2016.07.014.

Al-Soud WA, Radstrom P. Purification and characterization of PCR-inhibitory components in blood cells. J Clin Microbiol 2001;39(2):485–93. https://doi.org/10.1128/JCM.39.2.485-493.2001.

Arpi M, Bentzon MW, Jensen J, Frederiksen W. Importance of blood volume cultured in the detection of bacteremia. Eur J Clin Microbiol Infect Dis 1989;8(9):838–42.

Axelsson J, Ferreira M, Adolfsson L, McCrea K, Ward R, Larm O. Cytokines in blood from septic patients interact with surface-immobilized heparin. ASAIO J 2010;56(1):48–51. https://doi.org/10.1097/MAT.0b013e3181c3fec8.

Babady NE, England MR, Jurcic Smith KL, He T, Wijetunge DS, Tang YW, et al. Multicenter Evaluation of the ePlex Respiratory Pathogen Panel for the Detection of Viral and Bacterial Respiratory Tract Pathogens in Nasopharyngeal Swabs. J Clin Microbiol 2018;56(2). https://doi.org/10.1128/JCM.01658-17.

Belknap A, Grosser DS, Hale DA, Lang BJ, Colley P, Benavides R, et al. Clinical uptake of antimicrobial stewardship recommendations following Nanosphere Verigene blood culture gram-negative reporting. Proc (Bayl Univ Med Cent) 2017;30(4):395–9.

Beutler E, Gelbart T, Kuhl W. Interference of heparin with the polymerase chain reaction. Biotechniques 1990;9(2):166.

Bobenchik AM, Deak E, Hindler JA, Charlton CL, Humphries RM. Performance of Vitek 2 for antimicrobial susceptibility testing of Enterobacteriaceae with Vitek 2 (2009 FDA) and 2014 CLSI breakpoints. J Clin Microbiol 2015;53(3):816–23. https://doi.org/10.1128/JCM.02697-14.

Bouamrane F, Assidjo NE, Bouteille B, Dreyfuss MF, Darde ML, Cardot PJ. Sedimentation field-flow fractionation application to toxoplasma gondii separation and purification. J Pharm Biomed Anal 1999;20(3):503–12.

Bourbeau PP, Pohlman JK. Three days of incubation may be sufficient for routine blood cultures with BacT/Alert FAN blood culture bottles. J Clin Microbiol 2001;39(6):2079–82. https://doi.org/10.1128/JCM.39.6.2079-2082.2001.

Brennecke J, Kraut S, Zwadlo K, Gandi SK, Pritchard D, Templeton K, et al. High-yield extraction of Escherichia coli RNA from human whole blood. J Med Microbiol 2017;66(3):301–11. https://doi.org/10.1099/jmm.0.000439.

Buchanan CM, Wood RL, Hoj TR, Alizadeh M, Bledsoe CG, Wood ME, et al. Rapid separation of very low concentrations of bacteria from blood. J Microbiol Methods 2017;139:48–53. https://doi.org/10.1016/j.mimet.2017.05.004.

Burrack-Lange SC, Personne Y, Huber M, Winkler E, Weile J, Knabbe C, et al. Multicenter assessment of the rapid Unyvero blood culture molecular assay. J Med Microbiol 2018. https://doi.org/10.1099/jmm.0.000804.

Carrara L, Navarro F, Turbau M, Seres M, Moran 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(Pt 11):1673–9. https://doi.org/10.1099/jmm.0.064758-0.

Cartwright M, Rottman M, Shapiro NI, Seiler B, Lombardo P, Gamini N, et al. A broad-Spectrum infection diagnostic that detects pathogen-associated molecular patterns (PAMPs) in whole blood. EBioMedicine 2016;9:217–27. https://doi.org/10.1016/j.ebiom.2016.06.014.

Caspar Y, Garnaud C, Raykova M, Bailly S, Bidart M, Maubon D. Superiority of SDS lysis over saponin lysis for direct bacterial identification from positive blood culture bottle by MALDI-TOF MS. Proteomics Clin Appl 2017;11(5–6). https://doi.org/10.1002/prca.201600131.

Cheng IF, Lin CC, Lin DY, Chang HC. A dielectrophoretic chip with a roughened metal surface for on-chip surface-enhanced Raman scattering analysis of bacteria. Biomicrofluidics 2010;4(3). https://doi.org/10.1063/1.3474638.

Cheng IF, Chen TY, Lu RJ, Wu HW. Rapid identification of bacteria utilizing amplified dielectrophoretic force-assisted nanoparticle-induced surface-enhanced Raman spectroscopy. Nanoscale Res Lett 2014;9(1):324. https://doi.org/10.1186/1556-276X-9-324.

D'Amico L, Ajami NJ, Adachi JA, Gascoyne PR, Petrosino JF. Isolation and concentration of bacteria from blood using microfluidic membraneless dialysis and dielectrophoresis. Lab Chip 2017;17(7):1340–8. https://doi.org/10.1039/c6lc01277a.

Dark P, Blackwood B, Gates S, McAuley D, Perkins GD, McMullan R, et al. Accuracy of LightCycler((R)) 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 2015;41(1):21–33. https://doi.org/10.1007/s00134-014-3553-8.

De Angelis G, Posteraro B, De Carolis E, Menchinelli G, Franceschi F, Tumbarello M, et al. T2Bacteria magnetic resonance assay for the rapid detection of ESKAPEc pathogens directly in whole blood. J Antimicrob Chemother 2018;73(suppl_4):iv20–6. https://doi.org/10.1093/jac/dky049.

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(6):1994–8. https://doi.org/10.1128/JCM.00225-12.

Deck MK, Anderson ES, Buckner RJ, Colasante G, Davis TE, Coull JM, et al. Rapid detection of enterococcus spp. direct from blood culture bottles using enterococcus QuickFISH method: a multicenter investigation. Diagn Microbiol Infect Dis 2014;78(4):338–42. https://doi.org/10.1016/j.diagmicrobio.2013.12.004.

Didar TF, Cartwright MJ, Rottman M, Graveline AR, Gamini N, Watters AL, et al. Improved treatment of systemic blood infections using antibiotics with extracorporeal opsonin hemoadsorption. Biomaterials 2015;67:382–92. https://doi.org/10.1016/j.biomaterials.2015.07.046.

Dileo MV, Federspiel WJ. Scaling issues in the article entitled "cytokines in blood from septic patients interact with surface-immobilized heparin". ASAIO J 2010;56(4):383–4. https://doi.org/10.1097/MAT.0b013e3181e4bfc3. [author reply 4-5].

Djordjevic V, Stankovic M, Nikolic A, Antonijevic N, Rakicevic LJ, Divac A, et al. PCR amplification on whole blood samples treated with different commonly used anticoagulants. Pediatr Hematol Oncol 2006;23(6):517–21. https://doi.org/10.1080/08880010600751900.

Donay JL, Mathieu D, Fernandes P, Pregermain C, Bruel P, Wargnier A, et al. Evaluation of the automated phoenix system for potential routine use in the clinical microbiology laboratory. J Clin Microbiol 2004;42(4):1542–6.

Doring G, Unertl K, Heininger A. Validation criteria for nucleic acid amplification techniques for bacterial infections. Clin Chem Lab Med 2008;46(7):909–18. https://doi.org/10.1515/CCLM.2008.152.

Dreyer AW, Ismail NA, Nkosi D, Lindeque K, Matthews M, van Zyl DG, et al. Comparison of the VersaTREK blood culture system against the Bactec9240 system in patients with suspected bloodstream infections. Ann Clin Microbiol Antimicrob 2011;10:4. https://doi.org/10.1186/1476-0711-10-4.

Dubourg G, Raoult D. Emerging methodologies for pathogen identification in positive blood culture testing. Expert Rev Mol Diagn 2016;16(1):97–111. https://doi.org/10.1586/14737159.2016.1112274.

EvotecBiosystems. 1992. Process for the determination of in vitro amplified nucleic acids. Henco K, Eigen M, Riesner D. Feb 05, 1992. US 5,871,908

Faridi MA, Ramachandraiah H, Banerjee I, Ardabili S, Zelenin S, Russom A. Elasto-inertial microfluidics for bacteria separation from whole blood for sepsis diagnostics. J Nanobiotechnol 2017;15(1):3. https://doi.org/10.1186/s12951-016-0235-4.

Fenollar F, Raoult D. Molecular diagnosis of bloodstream infections caused by non-cultivable bacteria. Int J Antimicrob Agents 2007;30(Suppl. 1):S7–15. https://doi.org/10.1016/j.ijantimicag.2007.06.024.

Ferrer R, Martin-Loeches I, Phillips G, Osborn TM, Townsend S, Dellinger RP, et al. Empiric antibiotic treatment reduces mortality in severe sepsis and septic shock from the first hour: results from a guideline-based performance improvement program. Crit Care Med 2014;42(8):1749–55. https://doi.org/10.1097/CCM.0000000000000330.

Fiori B, D'Inzeo T, Giaquinto A, Menchinelli G, Liotti FM, de Maio F, et al. Optimized use of the MALDI BioTyper system and the FilmArray BCID panel for direct identification of microbial pathogens from positive blood cultures. J Clin Microbiol 2016;54(3):576–84. https://doi.org/10.1128/JCM.02590-15.

Fleischmann C, Thomas-Rueddel DO, Hartmann M, Hartog CS, Welte T, Heublein S, et al. Hospital incidence and mortality rates of Sepsis. Dtsch Arztebl Int 2016;113(10):159–66. https://doi.org/10.3238/arztebl.2016.0159.

Fraunholz M, Sinha B. Intracellular Staphylococcus aureus: live-in and let die. Front Cell Infect Microbiol 2012;2:43. https://doi.org/10.3389/fcimb.2012.00043.

Gaieski DF, Mikkelsen ME, Band RA, Pines JM, Massone R, Furia FF, et al. Impact of time to antibiotics on survival in patients with severe sepsis or septic shock in whom early goal-directed therapy was initiated in the emergency department. Crit Care Med 2010;38(4):1045–53. https://doi.org/10.1097/CCM.0b013e3181cc4824.

Gallet S, Métreau JM, Loiseau PM, Bories C, Cardot PJP. Isolation of bloodstream trypanosomes by sedimentation field-flow fractionation. J Microcolum Sep 1997;9(6):443–521. https://doi.org/10.1002/(SICI)1520-667X(1997)9:6<469::AID-MCS4>3.0.CO;2-#.

García ME, Blanco JL, Caballero J, Gargallo-Viola D. Anticoagulants interfere with PCR used to diagnose invasive aspergillosis. J Clin Microbiol 2002;40(4):1567–8.

Garnacho-Montero J, Aldabo-Pallas T, Garnacho-Montero C, Cayuela A, Jimenez R, Barroso S, et al. Timing of adequate antibiotic therapy is a greater determinant of outcome than are TNF and IL-10 polymorphisms in patients with sepsis. Crit Care 2006;10(4):R111. https://doi.org/10.1186/cc4995.

Ginn AN, Halliday CL, Douglas AP, Chen SC. PCR-based tests for the early diagnosis of sepsis. Where do we stand? Curr Opin Infect Dis 2017;30(6):565–72. https://doi.org/10.1097/QCO.0000000000000407.

Hamula CL, Hughes K, Fisher BT, Zaoutis TE, Singh IR, Velegraki A. T2Candida provides rapid and accurate species identification in pediatric cases of Candidemia. Am J Clin Pathol 2016;145(6):858–61. https://doi.org/10.1093/ajcp/aqw063.

Henderson B, Nair S, Pallas J, Williams MA. Fibronectin: a multidomain host adhesin targeted by bacterial fibronectin-binding proteins. FEMS Microbiol Rev 2011;35(1):147–200. https://doi.org/10.1111/j.1574-6976.2010.00243.x.

Henry NK, McLimans CA, Wright AJ, Thompson RL, Wilson WR, Washington II JA. Microbiological and clinical evaluation of the isolator lysis-centrifugation blood culture tube. J Clin Microbiol 1983;17(5):864–9.

Henzler C, Schomaker M, Yang R, Lambert AP, LaRue R, Kincaid R, et al. Optimization of a microfluidics-based next generation sequencing assay for clinical oncology diagnostics. Ann Transl Med 2018;6(9):162. https://doi.org/10.21037/atm.2018.05.07.

Herrmann IK, Schlegel AA, Graf R, Stark WJ, Beck-Schimmer B. Magnetic separation-based blood purification: a promising new approach for the removal of disease-causing compounds? J Nanobiotechnol 2015;13:49. https://doi.org/10.1186/s12951-015-0110-8.

Jain A, Munn LL. Determinants of leukocyte margination in rectangular microchannels. PLoS One 2009;4(9), e7104. https://doi.org/10.1371/journal.pone.0007104.

Janca J, Halabalova V, Ruzicka J. Role of the shape of various bacteria in their separation by microthermal field-flow fractionation. J Chromatogr A 2010;1217(51):8062–71. https://doi.org/10.1016/j.chroma.2010.10.082.

Kang JH, Super M, Yung CW, Cooper RM, Domansky K, Graveline AR, et al. An extracorporeal blood-cleansing device for sepsis therapy. Nat Med 2014;20(10):1211–6. https://doi.org/10.1038/nm.3640.

Katholnig K, Poglitsch M, Hengstschlager M, Weichhart T. Lysis gradient centrifugation: a flexible method for the isolation of nuclei from primary cells. Methods Mol Biol 2015;1228:15–23. https://doi.org/10.1007/978-1-4939-1680-1_2.

Kiehn TE, Wong B, Edwards FF, Armstrong D. Comparative recovery of bacteria and yeasts from lysis-centrifugation and a conventional blood culture system. J Clin Microbiol 1983;18(2):300–4.

Kim G, Vinerean H, Gaitas A. A novel pathogen capturing device for removal and detection. Sci Rep 2017;7(1):5552. https://doi.org/10.1038/s41598-017-05854-4.

Knabl L, Mutschlechner W, Orth-Holler D. Evaluation of a multiplex OnSpot primer-extension PCR assay in the diagnosis of sepsis. J Microbiol Methods 2016;120:91–3. https://doi.org/10.1016/j.mimet.2015.12.001.

Lamas CC, Eykyn SJ. Blood culture negative endocarditis: analysis of 63 cases presenting over 25 years. Heart 2003;89(3):258–62.

Lamy B, Dargere S, Arendrup MC, Parienti JJ, Tattevin P. How to optimize the use of blood cultures for the diagnosis of bloodstream infections? A State-of-the Art. Front Microbiol 2016;7:697. https://doi.org/10.3389/fmicb.2016.00697.

LaRosa SP, McCrea K, Ward R. Removal of cytomegalovirus from blood by heparin-functional hemoperfusion media (Abstract). Crit Care Med 2014;42(12 (Suppl.)), A984.

Lee JJ, Jeong KJ, Hashimoto M, Kwon AH, Rwei A, Shankarappa SA, et al. Synthetic ligand-coated magnetic nanoparticles for microfluidic bacterial separation from blood. Nano Lett 2014;14(1):1–5. https://doi.org/10.1021/nl3047305.

Loonen AJ, de Jager CP, Tosserams J, Kusters R, Hilbink M, Wever PC, et al. Biomarkers and molecular analysis to improve bloodstream infection diagnostics in an emergency care unit. PLoS One 2014;9(1), e87315. https://doi.org/10.1371/journal.pone.0087315.

Lustig M. Micro-dx – automation of microbial DNA extraction in direct molecular pathogen diagnosis. Res Mol Microbiol (Molzym) 2017;1:1–2.

Mach AJ, Di Carlo D. Continuous scalable blood filtration device using inertial microfluidics. Biotechnol Bioeng 2010;107(2):302–11. https://doi.org/10.1002/bit.22833.

Mancini N, Carletti S, Ghidoli N, Cichero P, Burioni R, Clementi M. The era of molecular and other non-culture-based methods in diagnosis of sepsis. Clin Microbiol Rev 2010;23(1):235–51. https://doi.org/10.1128/CMR.00043-09.

Martin GS. Sepsis, severe sepsis and septic shock: changes in incidence, pathogens and outcomes. Expert Rev Anti Infect Ther 2012;10(6):701–6. https://doi.org/10.1586/eri.12.50.

Mattsby-Baltzer I, Bergstrom T, McCrea K, Ward R, Adolfsson L, Larm O. Affinity apheresis for treatment of bacteremia caused by Staphylococcus aureus and/or methicillin-resistant S. aureus (MRSA). J Microbiol Biotechnol 2011;21(6):659–64.

Maubon D, Dard C, Garnaud C, Cornet M. Profile of GenMark's ePlex(R) blood culture identification fungal pathogen panel. Expert Rev Mol Diagn 2018;18(2):119–32. https://doi.org/10.1080/14737159.2018.1420476.

Mayr FB, Yende S, Angus DC. Epidemiology of severe sepsis. Virulence 2014;5(1):4–11. https://doi.org/10.4161/viru.27372.

McCoy MH, Relich RF, Davis TE, Schmitt BH. Performance of the FilmArray(R) blood culture identification panel utilized by non-expert staff compared with conventional microbial identification and antimicrobial resistance gene detection from positive blood cultures. J Med Microbiol 2016;65(7):619–25. https://doi.org/10.1099/jmm.0.000277.

McCrea K, Ward R, LaRosa SP. Removal of Carbapenem-resistant Enterobacteriaceae (CRE) from blood by heparin-functional hemoperfusion media. PLoS One 2014;9(12), e114242. https://doi.org/10.1371/journal.pone.0114242.

McLaughlin JC, Hamilton P, Scholes JV, Bartlett RC. Clinical laboratory comparison of lysis-centrifugation and BACTEC radiometric blood culture techniques. J Clin Microbiol 1983;18(5):1027–31.

Mellhammar L, Wullt S, Lindberg A, Lanbeck P, Christensson B, Linder A. Sepsis incidence: a population-based study. Open Forum Infect Dis 2016;3(4), ofw207. https://doi.org/10.1093/ofid/ofw207.

Mermel LA, Maki DG. Detection of bacteremia in adults: consequences of culturing an inadequate volume of blood. Ann Intern Med 1993;119(4):270–2.

Messner P, Schaffer C, Kosma P. Bacterial cell-envelope glycoconjugates. Adv Carbohydr Chem Biochem 2013;69:209–72. https://doi.org/10.1016/B978-0-12-408093-5.00006-X.

Metzgar D, Frinder MW, Rothman RE, Peterson S, Carroll KC, Zhang SX, et al. The IRIDICA BAC BSI assay: rapid, sensitive and culture-independent identification of bacteria and *Candida* in blood. PLoS One 2016;11(7), e0158186. https://doi.org/10.1371/journal.pone.0158186.

Mirrett S, Reller LB, Petti CA, Woods CW, Vazirani B, Sivadas R, et al. Controlled clinical comparison of BacT/ALERT standard aerobic medium with BACTEC standard aerobic medium for culturing blood. J Clin Microbiol 2003;41(6):2391–4.

Morgenthaler NG, Kostrzewa M. Rapid identification of pathogens in positive blood culture of patients with sepsis: review and meta-analysis of the performance of the sepsityper kit. Int J Microbiol 2015;2015:827416. https://doi.org/10.1155/2015/827416.

Mou L, Jiang X. Materials for Microfluidic Immunoassays: A Review. Adv Healthc Mater 2017;6(15). https://doi.org/10.1002/adhm.201601403.

Munoz P, Vena A, Machado M, Gioia F, Martinez-Jimenez MC, Gomez E, et al. T2Candida MR as a predictor of outcome in patients with suspected invasive candidiasis starting empirical antifungal treatment: a prospective pilot study. J Antimicrob Chemother 2018;73(suppl_4):iv6–iv12. https://doi.org/10.1093/jac/dky047.

Murray PR. Comparison of the lysis-centrifugation and agitated biphasic blood culture systems for detection of fungemia. J Clin Microbiol 1991;29(1):96–8.

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(182), 182ra54. https://doi.org/10.1126/scitranslmed.3005377.

Neely L, Plourde D, Suchocki A, Barringer III GE, Ved U, Townsend J, Pfaller M, et al. T2Bacteria: Rapid and Sensitive Detection and Identification of Sepsis Pathogens in Whole Blood Specimens by T2MR®. T2 Biosystems - Datasheet; 2015.

Opal SM. Non-antibiotic treatments for bacterial diseases in an era of progressive antibiotic resistance. Crit Care 2016;20(1):397. https://doi.org/10.1186/s13054-016-1549-1.

Opota O, Croxatto A, Prod'hom G, Greub G. Blood culture-based diagnosis of bacteraemia: state of the art. Clin Microbiol Infect 2015;21(4):313–22. https://doi.org/10.1016/j.cmi.2015.01.003.

Oviano M, Gomara M, Barba MJ, Revillo MJ, Barbeyto LP, Bou G. Towards the early detection of beta-lactamase-producing Enterobacteriaceae by MALDI-TOF MS analysis. J Antimicrob Chemother 2017a;72(8):2259–62. https://doi.org/10.1093/jac/dkx127.

Oviano M, Ramirez CL, Barbeyto LP, Bou G. Rapid direct detection of carbapenemase-producing Enterobacteriaceae in clinical urine samples by MALDI-TOF MS analysis. J Antimicrob Chemother 2017b;72(5):1350–4. https://doi.org/10.1093/jac/dkw579.

Patch ME, Weisz E, Cubillos A, Estrada SJ, Pfaller MA. Impact of rapid, culture-independent diagnosis of candidaemia and invasive candidiasis in a community health system. J Antimicrob Chemother 2018;73(suppl_4):iv27–30. https://doi.org/10.1093/jac/dky046.

Pfaller MA, Wolk DM, Lowery TJ. T2MR and T2Candida: novel technology for the rapid diagnosis of candidemia and invasive candidiasis. Future Microbiol 2016;11(1):103–17. https://doi.org/10.2217/fmb.15.111.

Piacentini N, Mernier G, Tornay R, Renaud P. Separation of platelets from other blood cells in continuous-flow by dielectrophoresis field-flow-fractionation. Biomicrofluidics 2011;5(3):34122–341228. https://doi.org/10.1063/1.3640045.

Qvella. 2015. Method for Pretreatment Of Microbial Samples. Talebpour SK, AA.; Maaskant, R.; Alavie, T. Nov 30, 2012. US 8,975,060 (US20140154687A1)

Raman R, Raper MA, Hahn E, Schilke KF. Enhanced capture of bacteria and endotoxin by antimicrobial WLBU2 peptide tethered on polyethylene oxide spacers. Biointerphases 2017;12(5), 05G603. https://doi.org/10.1116/1.4997049.

Reschiglian P, Zattoni A, Roda B, Michelini E, Roda A. Field-flow fractionation and biotechnology. Trends Biotechnol 2005;23(9):475–83. https://doi.org/10.1016/j.tibtech.2005.07.008.

Rhodes A, Evans LE, Alhazzani W, Levy MM, Antonelli M, Ferrer R, et al. Surviving Sepsis campaign: international guidelines for Management of Sepsis and Septic Shock: 2016. Crit Care Med 2017;45(3):486–552. https://doi.org/10.1097/CCM.0000000000002255.

Rohner P, Pepey B, Auckenthaler R. Advantage of combining resin with lytic BACTEC blood culture media. J Clin Microbiol 1997;35(10):2634–8.
Ruiz-Herrera J, Ortiz-Castellanos L. Analysis of the phylogenetic relationships and evolution of the cell walls from yeasts and fungi. FEMS Yeast Res 2010;10(3):225–43. https://doi.org/10.1111/j.1567-1364.2009.00589.x.
Salimnia H, Fairfax MR, Lephart P, Morgan M, Gilbreath JJ, Butler-Wu SM, et al. An international, prospective, multicenter evaluation of the combination of AdvanDx *Staphylococcus* QuickFISH BC with mecA XpressFISH for detection of methicillin-resistant Staphylococcus aureus isolates from positive blood cultures. J Clin Microbiol 2014;52(11):3928–32. https://doi.org/10.1128/JCM.01811-14.
Scott LJ. Verigene(R) gram-positive blood culture nucleic acid test. Mol Diagn Ther 2013;17(2):117–22. https://doi.org/10.1007/s40291-013-0021-z.
Shen H, Wang J, Liu H, Li Z, Jiang F, Wang FB, et al. Rapid and selective detection of pathogenic Bacteria in bloodstream infections with aptamer-based recognition. ACS Appl Mater Interfaces 2016;8(30):19371–8. https://doi.org/10.1021/acsami.6b06671.
Singer M, Deutschman CS, Seymour CW, Shankar-Hari M, Annane D, Bauer M, et al. The third international consensus definitions for Sepsis and septic shock (Sepsis-3). JAMA 2016;315(8):801–10. https://doi.org/10.1001/jama.2016.0287.
Singh A, Arutyunov D, Szymanski CM, Evoy S. Bacteriophage based probes for pathogen detection. Analyst 2012a;137(15):3405–21. https://doi.org/10.1039/c2an35371g.
Singh B, Fleury C, Jalalvand F, Riesbeck K. Human pathogens utilize host extracellular matrix proteins laminin and collagen for adhesion and invasion of the host. FEMS Microbiol Rev 2012b;36(6):1122–80. https://doi.org/10.1111/j.1574-6976.2012.00340.x.
Stefaniuk E, Baraniak A, Gniadkowski M, Hryniewicz W. Evaluation of the BD Phoenix automated identification and susceptibility testing system in clinical microbiology laboratory practice. Eur J Clin Microbiol Infect Dis 2003;22(8):479–85. https://doi.org/10.1007/s10096-003-0962-y.
Stevenson M, Pandor A, Martyn-St James M, Rafia R, Uttley L, Stevens J, et al. Sepsis: the LightCycler SeptiFast test MGRADE(R), SepsiTest and IRIDICA BAC BSI assay for rapidly identifying bloodstream bacteria and fungi - a systematic review and economic evaluation. Health Technol Assess 2016;20(46):1–246. https://doi.org/10.3310/hta20460.
Thomson G, Turner D, Brasso W, Kircher S, Guillet T, Thomson K. High-stringency evaluation of the automated BD Phoenix CPO detect and Rapidec Carba NP tests for detection and classification of Carbapenemases. J Clin Microbiol 2017;55(12):3437–43. https://doi.org/10.1128/JCM.01215-17.
Trung NT, Hien TT, Huyen TT, Quyen DT, Van Son T, Hoan PQ, et al. Enrichment of bacterial DNA for the diagnosis of blood stream infections. BMC Infect Dis 2016;16:235. https://doi.org/10.1186/s12879-016-1568-1.
Vincent JL, Sakr Y, Sprung CL, Ranieri VM, Reinhart K, Gerlach H, et al. Sepsis in European intensive care units: results of the SOAP study. Crit Care Med 2006;34(2):344–53.
Vincent JL, Rello J, Marshall J, Silva E, Anzueto A, Martin CD, et al. International study of the prevalence and outcomes of infection in intensive care units. JAMA 2009;302(21):2323–9. https://doi.org/10.1001/jama.2009.1754.
Vutukuru MR, Sharma DK, Ragavendar MS, Schmolke S, Huang Y, Gumbrecht W, et al. A rapid, highly sensitive and culture-free detection of pathogens from blood by positive enrichment. J Microbiol Methods 2016;131:105–9. https://doi.org/10.1016/j.mimet.2016.10.008.
Wang CH, Chang CJ, Wu JJ, Lee GB. An integrated microfluidic device utilizing vancomycin conjugated magnetic beads and nanogold-labeled specific nucleotide probes for rapid pathogen diagnosis. Nanomedicine 2014;10(4):809–18. https://doi.org/10.1016/j.nano.2013.10.013.
Wei Hou H, Gan HY, Bhagat AA, Li LD, Lim CT, Han J. A microfluidics approach towards high-throughput pathogen removal from blood using margination. Biomicrofluidics 2012;6(2):24115–2411513. https://doi.org/10.1063/1.4710992.
Wellinghausen N, Kochem AJ, Disque C, Muhl 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(9):2759–65. https://doi.org/10.1128/JCM.00567-09.
Wu Z, Willing B, Bjerketorp J, Jansson JK, Hjort K. Soft inertial microfluidics for high throughput separation of bacteria from human blood cells. Lab Chip 2009;9(9):1193–9. https://doi.org/10.1039/b817611f.
Yung CW, Fiering J, Mueller AJ, Ingber DE. Micromagnetic-microfluidic blood cleansing device. Lab Chip 2009;9(9):1171–7. https://doi.org/10.1039/b816986a.
Zhang D, Micek ST, Kollef MH. Time to appropriate antibiotic therapy is an independent determinant of Postinfection ICU and hospital lengths of stay in patients with Sepsis. Crit Care Med 2015;43(10):2133–40. https://doi.org/10.1097/CCM.0000000000001140.