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Narrative review

Diagnosis of bloodstream infections from positive blood cultures and directly from blood samples: recent developments in molecular approaches

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ABSTRACT

Background: Bloodstream infections are a major cause of death with increasing incidence and severity. Blood cultures are still the reference standard for microbiological diagnosis, but are rather slow. Molecular methods can be used as add-on complementary assays. They can be useful to speed up microbial identification and to predict antimicrobial susceptibility, applied to direct blood samples or positive blood cultures.

Aim: To review recent developments in molecular-based diagnostic platforms used for the identification of bloodstream infections, with a focus on assays performed directly on blood samples and positive blood cultures.

Sources: Peer reviewed articles, conference abstracts, and manufacturers' websites.

Content: We give an update on recent developments of molecular methods in diagnosing BSIs. We first describe the currently available molecular methods to be used for positive blood cultures including: a) *in situ* hybridization-based methods; b) DNA-microarray-based hybridization technology; c) nucleic acid amplification-based methods; and d) combined methods. Subsequently, molecular methods applied directly to whole blood samples are discussed, including the use of nucleic acid amplification-based methods, T2 magnetic resonance-based methods, and metagenomics for diagnosing BSIs.

Implications: Advances in molecular-based methods complementary to conventional blood culture diagnostics and antimicrobial stewardship programmes may optimize infection management by allowing rapid identification of pathogens and relevant antimicrobial resistance genes. Rapid diagnosis of the causing microorganism and relevant resistance determinants is important for early administration and modification of appropriate antimicrobial therapy. Ultimately, this may lead to improved quality and cost-effectiveness of health care, as well as reduced antimicrobial resistance selection. **N. Peker, Clin Microbiol Infect 2018;24:944**

Introduction

Infections caused by the presence of viable microorganisms in blood are described as bloodstream infections (BSIs). They are often associated with severe diseases with high morbidity and mortality [1–3]. The worldwide increase in incidence emerges as one of the leading causes of death [4], especially in critically ill patients [5]. Increased mortality of BSIs is often related to delayed, insufficient, or inappropriate anti-infective treatment [2,3,6].

Currently, blood cultures (BCs) are considered to be the reference standard for diagnosing BSIs and are still indispensable for the diagnosis of BSIs. The single most important factor for achieving a sensitivity of >95% is sampling sufficient volumes (i.e. at least two to three sets, filled with 10 mL per bottle), as >50% of the patients present with a bacteraemia of <1 colony-forming unit (CFU)/mL [7]. BCs are rather straightforward to perform and display a good sensitivity for cultivable pathogens [8]. However, major drawbacks remain: i) a rather long turnaround time [9]; ii) failure to identify

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slow-growing or obligate intracellular microorganisms and pathogens other than bacteria or yeast [8]; and iii) substantial delay or even failure to detect microorganisms involved in BSIs when the patient has previously received antimicrobials [9].

These drawbacks may lead to important consequences. First of all, starting appropriate antimicrobial therapy for sepsis as soon as possible, preferably within the first 1 hour after clinical recognition of sepsis, is crucial to reduce morbidity and mortality [10]. Secondly, as culture-based diagnosis is slow, physicians often have to start empiric, broad-spectrum antimicrobial treatment. Therefore, rapid detection and identification of pathogen(s) is required for optimizing the therapeutic effect of antimicrobial therapy and for de-escalation of the started therapy to limit collateral damage, such as toxicity, side effects, and selection of antimicrobial resistance [2]. Additionally, the long turnaround time may also result in delayed specific clinical diagnosis, increased length of stay, a higher complication rate, and thus ultimately increase medical costs [8].

Consequently, for more than a decade pathogen-specific assays aiming at early identification of pathogens and some resistance determinants have been developed and tested. These molecular tools have been tested as an alternative for or complementary to BCs [8]. Depending on the Gram-staining results of the positive BC, microbiology laboratories may decide on which specific molecular test panel meet their needs. Approaches, methods, and commercialized products available before 2015 have been reviewed previously [2,8,11,12]. This review gives an update on recent developments of molecular methods in diagnosing BSIs, focusing on starting from positive BCs. The data presented here were obtained from independent studies; only when these were not available was information acquired from manufacturers' websites.

Currently available molecular methods

Currently available molecular methods can be divided into four groups: a) *in situ* hybridization-based methods; b) DNA-microarray-based hybridization technology; c) nucleic acid amplification-based methods; and d) combined methods. The different methods reviewed are summarized in Tables 1 and 2. In addition, Fig. 1 provides an overview of the entire process from patient-to-patient. After incubation of BCs and Gram-staining examination, samples can be plated on agar plates or can undergo direct molecular detection (Fig. 1). We also discuss a recently developed highly promising culture-free metagenomics-based method together with other platforms based on molecular techniques used for BSIs diagnosis directly from blood. These methods could potentially reduce time to detection further by passing the first incubation step and Gram-staining (blood culture bottles).

Molecular methods from positive blood culture bottles

In situ hybridization-based methods

A hybridization assay involves specific binding of two complementary nucleic acid strands in which one, the probe, is a nucleic acid sequence of known identity and the other one, the target, is part of the genome of an unknown microorganism to be identified [13]. Fluorescent *in situ* hybridization (FISH) assays are based on this principle. In FISH, fluorescently labelled oligonucleotide probes are used to specifically bind to the ribosomal DNA (rDNA) region in the genome of targeted pathogens, that is to the 16S rDNA gene of bacteria or to the 18S rDNA gene of fungi. This specific binding between probe and complementary sequence is visualized by fluorescence microscopy [14]. Although FISH technology allows identification of most commonly found bacteria and yeast in blood, some bacteria can be identified only at the genus level because of the limited number of species-specific probes available in the assay [14].

PNA-FISH® and QuickFISH®

The first commercialized FISH-based technology was PNA-FISH (OpGen®, Maryland, USA, previously AdvanDx), which uses peptide nucleic acid probes (PNA) to detect *Staphylococcus aureus*, coagulase-negative staphylococci (*S. aureus*/CNS PNA-FISH®), *Enterococcus faecalis*, *E. faecium*, and other *Enterococcus* species (*E. faecalis*/OE PNA-FISH®), *Escherichia coli*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa* (Gram-Negative PNA-FISH®) and *Candida* species (*Candida* PNA-FISH® (*C. glabrata* and *C. albicans*); Yeast Traffic Light® PNA-FISH (5 *Candida* species)). The weaknesses of PNA-FISH include the limit of detection (LOD) of 10^5 CFU/mL, the limited number of PNA-FISH probes, and the lack of the antimicrobial susceptibility profiles of the identified microorganisms [12]. PNA-FISH provides results within 2.5 hours, which is reduced to less than 30 min by a faster and less labour-intensive version called *Quick*FISH®, launched in 2013 [15]. The *Quick*FISH® has a self-reporting probe design and the following panels are available: *Staphylococcus Quick*FISH®, *Enterococcus Quick*FISH®, Gram-Negative *Quick*FISH®, and *Candida Quick*FISH® (not FDA-approved, but CE marked). Results of the *Quick*FISH® can be obtained within the same timeframe as the results of Gram stain. However, to be cost-effective it has been suggested that *Quick*FISH® is performed after initial Gram staining [16]. PNA-FISH® and *Quick*FISH® exhibit a sensitivity and specificity of 97–100% and 90–100%, respectively [15,17].

AccuProbe®

The AccuProbe® system (Hologic®, previously Gen-Probe, USA) is also based on hybridization of DNA probes. Previously, Gen-Probe commercialized tests to identify *S. pneumoniae*, *S. aureus*, *Enterococcus* spp., and group A and B streptococci [18]. These tests were developed for identification of pathogens from cultures; however, Lindholm and colleagues modified the manufacturer's instructions for specimen preparation to use a pellet of bacteria made directly from positive blood culture broth [18]. Sensitivity and specificity was shown to be above 97.9% for detection of most pathogens with a turnaround time of 1 hour. For *S. aureus*, cut-off values had been modified to increase sensitivity from 72.4% to 80.8%, while only marginally decreasing specificity from 99.8% to 98.7% [18]. Currently, according to the Hologic® website, only the tests to identify *S. pneumoniae* and *S. aureus* are available.

Accelerate Pheno™

Recently, Accelerate Diagnostics™ (Arizona, USA) developed a fully automated hybridization system called Accelerate Pheno™, which identifies pathogens and performs antimicrobial susceptibility testing (AST) in approximately 7 hours [19]. This system integrates gel filtration for sample preparation and FISH technology for identification, combined with digital microscopy for subsequent AST [20]. The system performed well for the identification of Gram-positive and Gram-negative organisms with an overall sensitivity and specificity of 95.6% and 99.5%, respectively [21]. AST results were analysed for essential agreement, defined as minimum inhibitory concentrations within plus or minus 1 doubling dilution of the reference method, and for categorical agreement, defined as susceptible, intermediate, and resistant results matching to those in reference methods [21]. Accordingly, an essential agreement of 95.1% and a categorical agreement of 95.5% were reported compared with the routine methods, which included for

Table 1
Commercially available methods for identification of microorganisms and detection of antimicrobial-resistant genes directly from positive blood cultures

Assay	Gram staining need	Pathogens covered (Panel/kit)	Resistance markers	Sensitivity/specificity	Complexity -Personnel experience level -Equipment requirement	Hands-on time	Times to result	References
***In situ* hybridization-based methods**								
PNA-FISH® (OpGen®, USA)	Yes	4 Gram-positive bacteria (*S. aureus*/CNS PNA-FISH® and *E. faecalis*/OE PNA-FISH®) 3 Gram-negative bacteria (Gram-Negative PNA-FISH®) 5 *Candida* spp. (*Candida* PNA-FISH®, Yeast Traffic Light® PNA-FISH)	None	97-100%/90-100%	Not automated -Trained personnel -Proprietary equipment	10 min	2.5 hours	[12,15,17] www.opgen.com
*Quick*FISH® (OpGen®, USA)	Yes	4 Gram-positive bacteria (*Staphylococcus Quick*FISH®, *Enterococcus Quick*FISH®) 3 Gram-negative bacteria (Gram-Negative *Quick*FISH®) 3 *Candida* spp.[a] (*Candida Quick*FISH®)	None	97–100%/90–100%	Not automated -Trained personnel -Generic equipment	5 min	30 min	[15–17] www.opgen.com
AccuProbe® (Hologic®, USA)	Yes	*S. pneumoniae* (*S. pneumoniae* culture identification test) *S. aureus* (*S. aureus* culture identification test)	None	>97%/81–100%	Not automated -Trained personnel -Generic equipment	5 min	1 hour	[18] www.hologic.com
Accelerate PhenoTest™ BC (Accelerate Diagnostics™, USA)	No	15 Gram-positive bacteria 11 Gram-negative bacteria 2 *Candida* spp. (Accelerate PhenoTest™ BC Kit)	20 antimicrobial MICs determined	96%/99%	Fully automated -No trained personnel -Proprietary equipment	2 min	1 hour ID 6 hours AST	[19–21] www.acceleratediagnostics.com
DNA-Microarray based methods								
Verigene® (Luminex® Corporation, USA)	Yes	12 Gram-positive bacteria (VERIGENE® Gram-Positive Blood Culture Test) 9 Gram-negative bacteria (VERIGENE® Gram-Negative Blood Culture Test)	*mec*A, *van*A, *van*B, bla_{NDM}, bla_{VIM}, bla_{KPC}, bla_{OXA}, bla_{CTXM}	81–100%/> 98%	Fully automated -No trained personnel -Proprietary equipment	5 min	2.5 hours	[22,23] www.luminexcorp.com
Nucleic-acid amplification-based methods								
FilmArray® (bioMérieux, France)	No	8 Gram-positive bacteria 11 Gram-negative bacteria 5 *Candida* spp. (FILMARRAY® BCID Panel)	*mec*A, *van*A, *van*B, bla_{KPC}	>96%/98–100%	Fully automated -No trained personnel -Proprietary equipment	2 min	1 hour	[29,30] www.biomerieux.com
Xpert® MRSA/SA BC (Cepheid®, USA)	Yes	*S. aureus*, MRSA	*mec*A	98–100%/99–100%	Fully automated -No trained personnel -Proprietary equipment	1 min	1 hour	[32,33] www.cepheid.com
BD Max™ StaphSR Assay (BD Diagnostics, Canada)	Yes	*S. aureus*, MRSA, CoNS	*mec*A, and *mec*C	98–100%/98–100%	Fully automated -Trained personnel -Proprietary equipment	1 min/specimen	2.5 hours	[34–36] www.bd.com
Eazyplex® MRSA (Amplex Diagnostics GmbH, Germany)[a]	Yes	*S. aureus*, *S. epidermidis* (eazyplex ® MRSA)	*mec*A, and *mec*C	100%/98%	Fully automated -Trained personnel -Proprietary equipment	2 min sample preparation	30 min	[37,38] www.eazyplex.com

Combined methods (Multiplex PCR and hybridization based methods)								
Sepsis Flow Chip (Master Diagnóstica, Spain)[a]	No	>36 bacteria *Candida* spp (non-albicans). *Candida albicans* (Sepsis Flow Chip Kit)	20 antimicrobial resistance markers	93–94 %/100%	Fully automated -Trained personnel -Proprietary equipment	Not provided	3 hours	[39,40] www.masterdiagnostica.com
ePlex® BCID (GenMarkDx®, USA)[a]	Yes	20 Gram-positive bacteria (BCID-GP Panel) 21 Gram-negative bacteria (BCID-GN) 16 yeasts (including 10 *Candida* spp.) (BCID-FP Panel)	BCID-GP: *mec*A, *mec*C, *van*A, *van*B BCID-GN: bla_{CTXM}, bla_{KPC}, bla_{NDM}, bla_{VIM}, bla_{IMP}, bla_{OXA}	Not provided	Fully automated -No trained personnel -Proprietary equipment	2 min	1.5 hours	[41,42] www.genmarkdx.com

Specifications of the assays were collected from the literature and manufacturer's information.
[a] Not available in the USA.

Table 2
Commercially available methods for identification of microorganisms and detection of antimicrobial-resistant genes directly from whole blood

Assay	Pathogens covered	Resistance markers	Sensitivity/ specificity	Complexity -Personnel experience level -Equipment requirement	Hands-on time	Times to result	References
Nucleic-acid amplification-based methods							
The LightCycler® SeptiFast (Roche Molecular System, Switzerland)	19 Bacteria 5 Candida spp. and *Aspergillus fumigatus*	*mec*A	83–90%/73%	Automated -Trained personnel -Proprietary equipment	3 hours	6 hours	[47,49,50] www.molecular.roche.com
Magicplex™ Sepsis Real-time Test (SeeGene, Korea)[a]	>90 pathogens 27 pathogens at species level	*mec*A, *van*A, *van*B	37–65/66–92%	Multi-step automated -Trained personnel -Proprietary equipment	Not provided	3–6 hours	[51–53] http://www.seegene.com
SepsiTest™ (Molzym, Germany)[a]	345 bacteria, *Candida*, *Cryptococcus* and *Aspergillus* species	None	87%/86%	Partially automated -Trained personnel -Generic equipment	75 min (PCR) 70 min (sequence analysis)	8–12 hours	[54–57] www.sepsitest.com
T2 Magnetic resonance-based methods							
T2Candida Panel (T2Biosystems, USA)	5 *Candida* species	None	99%/91%	Fully automated -No trained personnel -Proprietary equipment	5 min	3–5 hours	[58–61] www.t2biosystems.com
Metagenomics							
iDTECT™ Dx Blood (PathoQuest SAS, France)[a]	>1200 bacteria and viruses	None	Not provided	Not provided -Trained personnel -Generic equipment but proprietary software	Not provided	Not provided	[69] (www.pathoquest.com)

Specifications of the assays were collected from the literature and manufacturer's information.
[a] Not available in the USA.

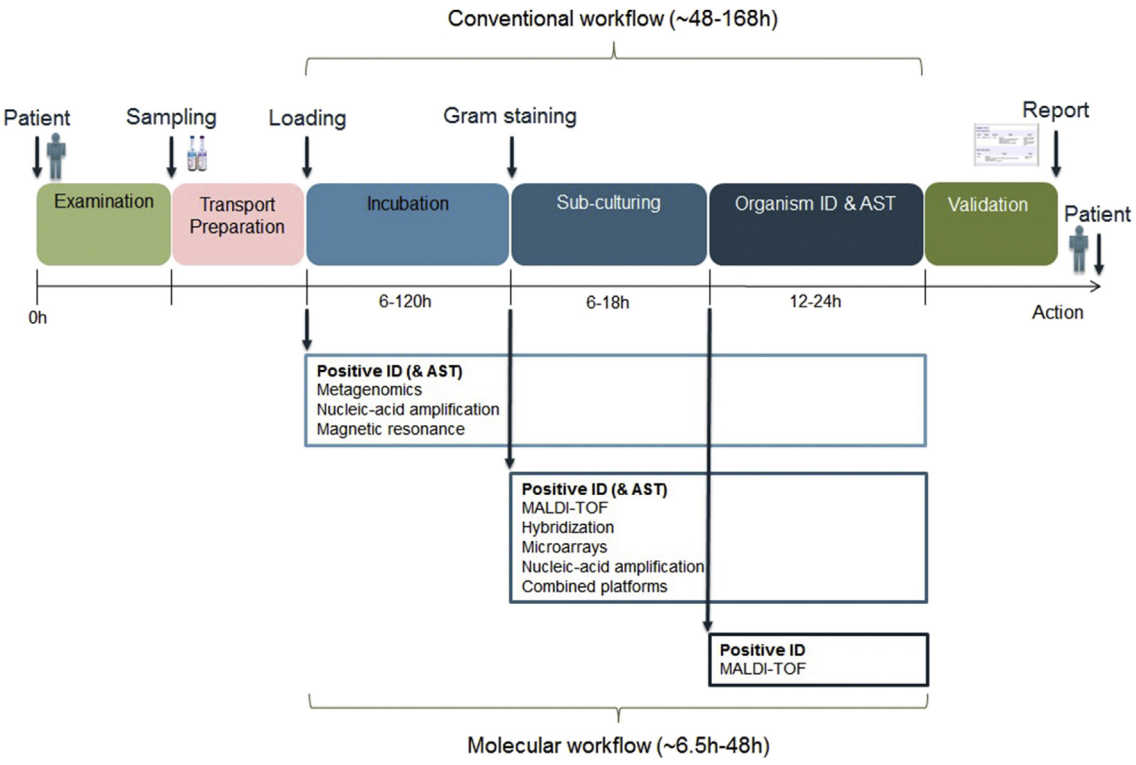

Fig. 1. Methods to identify microorganisms from positive blood cultures or blood. After physical examination of the patient the physician may decide to take a blood sample. Subsequently, the sample in a blood culture flask has to be transported to the laboratory, where it will be incubated for 6–120 hours at 35–37°C. After incubation either molecular methods can be applied, a MALDI-TOF analysis may be performed to identify the microorganism and their antimicrobial resistance genes, or sub-culturing may be started after which identification takes place by MALDI-TOF analyses and antibiotic susceptibility testing. Metagenomics can be applied directly to the blood sample without an incubation step.

identification the Vitek®2 (bioMérieux) or biochemical assays and BactiStaph (Thermo Fisher Scientific, Lenexa, USA); and for antimicrobial susceptibility testing the Vitek®2 (bioMérieux), ETEST® (bioMérieux) and disk diffusion (BD Microbiology Systems, Sparks, USA) [21].

DNA-microarray-based hybridization technology

DNA-microarrays consist of short oligonucleotides immobilized on a solid surface. They allow simultaneous, in parallel detection of many pathogens and their antimicrobial resistance genes. The species covered by DNA-microarrays are in general around 90–95% of all the pathogens known to cause BSIs with a sensitivity ranging from 10^1 to 10^5 cells/mL [2].

Verigene®

The Food and Drug Administration (FDA) approved the DNA-microarray based Verigene® system (Luminex® Corporation, previously Nanosphere, USA) that employs two distinct multiplex panels for rapid diagnosis: one for 12 Gram-positive bacteria (12 species) along with associated resistance genes (*mec*A, *van*A, *van*B), and another for nine Gram-negative bacteria (1 species not FDA approved) and their resistance markers (bla_{NDM}, bla_{VIM}, bla_{KPC}, bla_{OXA} and bla_{CTX-M}). The system can identify bacterial species in each panel along with associated resistance markers directly from positive BCs with a sensitivity of 81–100% and a specificity of higher than 98% [22,23]. However, the Verigene® panels demonstrated discordant results in mixed BCs in which no association of antimicrobial resistance marker genes to a specific organism was observed [23]. The turnaround time of the test is reported to be 2.5 hours.

Nucleic acid amplification-based methods

The polymerase chain reaction (PCR) is the most commonly used nucleic acid amplification technique for identification of pathogens directly from blood as well as from positive BCs. The PCR tests used in diagnostic laboratories are based on either broad range PCR or multiplex PCR. Broad range PCR uses universal primers targeting conserved rDNA regions of the bacterial (16S) or fungal (18S) genome and subsequent identification of the microorganism by sequencing or genus/species-specific real-time PCR assays [24,25]. Commercially available multiplex assays are designed to target different chromosomal regions of the pathogens in combination with assays to detect their antimicrobial resistance signatures.

Loop mediated isothermal amplification (LAMP) is a novel nucleic acid amplification method that amplifies DNA with high sensitivity and efficacy similar to PCR while possessing advantages over PCR [26,27], as amplification occurs under isothermal conditions and does not require sophisticated equipment. Therefore, it is suitable for detection of pathogens in field-based settings [28], or in smaller satellite laboratories.

FilmArray®

The FilmArray® (bioMérieux, France, previously Biofire Diagnostics, USA) is an automated multiplex PCR platform which combines sample preparation, PCR amplification, detection and analysis (www.biomerieux.com). The FilmArray® Blood Culture Identification Panel (BCID) can identify 19 bacterial species and five *Candida* species, as well as four resistance genes (*mec*A, *van*A, *van*B, bla_{KPC}) from positive BCs in 1 hour with 2 min hands-on time. It performs well in polymicrobial cultures [29]. In a randomized

control trial, all microbial targets were identified with a sensitivity exceeding 96% and a specificity of 99%. The resistance genes *van*A/B, bla_{KPC} were identified with 100% sensitivity and specificity [30]. However, the sensitivity and specificity observed for the *mecA* gene were reported to be 98.4% and 98.3%, respectively. In addition, false positive detection of the *mecA* gene in coagulase-negative staphylococci and *S. aureus* has been demonstrated previously [29,30]. Despite the short turnaround time of the FilmArray® system, only one sample at a time can be tested on the same instrument [31]. To overcome this latter limitation the company has now introduced the FilmArray® Torch that harbours 2–12 modules within one machine, thereby providing up to six times more sample throughput per square foot of bench top space (www.biomerieux.com).

Xpert® MRSA/SA BC

The Xpert® MRSA/SA BC assay of Cepheid® (Carlsbad, USA) is based on real-time PCRs that can identify methicillin-resistant *S. aureus* (MRSA) and methicillin-susceptible *S. aureus* (MSSA) within approximately 1 hour (www.cepheid.com). The assay detects sequences in the staphylococcal protein A (*spa*) gene, methicillin resistance (*mec*A) gene, and the junction between the staphylococcal chromosomal cassette that harbours the *mec*A gene (SCC*mec*) and the *S. aureus* genome (*orfX*) [32]. Amplification of these three targets is needed for the assay to identify MRSA strains [32]. The sensitivity and specificity for the identification were reported to be 99.6% (96.4-100%) and 99.5% (98-100%), respectively, for *S. aureus* and 98.1% and 99.6%, respectively, for MRSA [33].

BD Max™ StaphSR assay

The BD Max™ StaphSR Assay (BD Diagnostic Systems, Québec, Canada) is a CE marked and FDA approved, fully automated diagnostic test which was developed for detection of *S. aureus* and MRSA in patients at risk for nasal colonization [34]. The test is based on a multiplex real-time PCR and differentiates *S. aureus* and MRSA by detecting the presence of the *nuc* and *mec*A/C genes, and the SCC*mec-orfX* junction within approximately 2.5 hours [34]. Although the BD Max™ StaphSR Assay was designed for rapid screening of nasal swabs, the performance of the test was also analysed for detecting *S. aureus* and MRSA from positive BCs [34–36]. The test had a sensitivity and specificity for *S. aureus of* 99.5% (99.1-100%) and 100%, respectively; for MRSA of 98.9% (97.9-100%) and 98.1%, respectively; and for CoNS of 99% and 97.9% (95.8-100%), respectively [35,36].

eazyplex®

Amplex Diagnostics GmbH (Giessen, Germany) marketed the CE approved eazyplex® test system, which combines isothermal amplification of the target and real-time photometric detection of amplified material without prior DNA extraction. Previously, one of the eazyplex® assays called eazyplex® SuperBug CRE was tested directly on positive BCs spiked with defined Gram-negative strains to detect the most common carbapenemases and ESBLs [37]. The eazyplex® system displayed a correct identification of all of the β-lactamase genes included in the assay in less than 15 min and with a simple sample processing [37]. In a recent study, investigators evaluated the potential use of LAMP methods in BC diagnosis [38]. They used the eazyplex® MRSA test, which detects *S. aureus*, *S. epidermidis*, *mec*A, and *mec*C within 30 min. The sensitivity and specificity for the identification reported was 100% and 98.2%, respectively, for *S. aureus*; and 92.3% and 98.4%, respectively, for *S. epidermidis* [38].

Combined platforms

Recently, some platforms have been developed that combine the advantages of different molecular methods.

Sepsis Flow Chip

Sepsis Flow Chip (Master Diagnóstica, Granada, Spain) is a novel CE labelled diagnostic assay, able to detect most of the pathogens causing BSIs and their resistance markers based on a multiplex PCR, followed by reverse dot blot hybridization [39]. Sepsis Flow Chip allows simultaneous detection of over 36 bacterial species, *Candida* spp. (*C. albicans* and other *Candida* spp.) and 20 antimicrobial resistance markers within 3 hours [40]. The clinical evaluation study of the Sepsis Flow Chip yielded promising results with an overall sensitivity and specificity of 93.3% and 100%, respectively, for bacterial identification, and 93.6% and 100%, respectively, for the detection of the antimicrobial resistance determinants [39]. In this validation study, a 96.2% and 89.1% concordance with standard microbiological diagnostic protocols was shown in monomicrobial and polymicrobial cultures, respectively [39].

ePlex®

The ePlex® system (GenMarkDx®, Carlsbad, USA) is a CE marked, automated, and multiplex molecular diagnostic test system. It integrates a digital microfluidics-based sample preparation and an amplification assay together with the eSensor® detection technology (GenMarkDx®, USA) for the electrochemical detection of the target DNA within the same cartridge (www.genmarkdx.com). Therefore, this fully automated system is expected to reduce sample contamination risk and it eliminates the time-consuming washing and preparation steps required in earlier multiplexing technologies [41]. In contrary to fluorescence or optical detection of the first generation multiplex assays, the ePlex® detection system is highly specific and based on competitive DNA hybridization and electrochemical detection [41]. It consists of different multiplex blood culture identification panels (BCID, Gram-positive (BCID-GP), Gram-negative (BCID-GN), and fungal pathogen (BCID-FP)), which are currently under development for the detection of common bloodstream infections. In a preliminary study, the ePlex® BCID panels were retrospectively tested with a turnaround time of 1.5 hours in clinical samples in which the discrepant results compared with routine test of the laboratory were examined by culture, sequencing, or qPCR [42]. After discrepancy analysis, 100%, 97.8%, and 97.8% concordance were reported for the detection of fungal pathogens, Gram-negative, and Gram-positive bacteria, respectively. The BCID panels were also tested on polymicrobial cultures and able to detect polymicrobial infections of bacteria and/or fungi with 95.5% concordance compared with the control methods after discordant resolution [42,43].

Molecular methods directly from blood (without previous incubation)

Molecular techniques performed directly on whole blood samples allow rapid identification of the aetiological agent of BSIs independently of a prior incubation phase. Direct diagnosis from whole blood circumvents the limitations of blood culturing in identifying slow-growing bacteria or uncultivable microorganisms, and in cases when the patient has already received antimicrobials. Also, processing of smaller volumes of blood samples compared with BCs makes these molecular methods especially useful for paediatric patients in whom it is difficult to obtain larger volumes of blood [11]. However, molecular approaches developed for the

detection of microorganisms are mostly PCR-based assays, which introduce some challenges. The presence of high amounts of human DNA in patient blood, as well as other blood components can interfere with PCR and inhibit the reaction [44,45]. Although PCR assays are usually very sensitive, they may yield false positive results because of contaminating DNA (e.g. from the environment, or PCR reagents), or cell-free DNA from dead microorganisms. Also, DNA originating from pathogens and released after an infection controlled by the immune system or an efficient antimicrobial therapy can persist in the circulation for several days [11]. Here, we discuss recently used platforms and the promising approach of metagenomics used for diagnosis of BSIs directly from whole blood.

Nucleic acid amplification-based methods

LightCycler® SeptiFast

The LightCycler® SeptiFast (Roche Molecular System, Basel, Switzerland) was developed based on a multiplex real-time PCR assay. The assay is able to identify most commonly encountered pathogens of BSIs (19 bacterial and 6 fungal) directly from 1.5 mL of whole blood in about 6 hours. In a subsequent run, the presence of methicillin resistance in *S. aureus* positive samples is assessed. This assay uses fluorescence resonance energy transfer (FRET) probes targeting the internal transcribed spacer (ITS) regions between the 16S and 23S ribosomal DNA of bacteria and between the 18S and 5.8S ribosomal DNA of the fungal genomes for identification. After completion of the run, data analysis is performed by manual editing of the PCR data using Light Cycler 2.0 software, and bacterial and fungal identification is accomplished through automated analyses with SeptiFast Identification Software (SIS) [46]. Although the test is not available in the USA, there have been several clinical studies conducted to evaluate its performance since it was commercialized in Europe. In a study for the diagnosis of CoNS in neutropaenic haematological patients, the SeptiFast showed a sensitivity of 23.3% that was increased to 83.3% by neglecting the integrated cut off value [47]. In a recent study, 85.5% concordance between BC and SeptiFast was shown. Higher positivity rates in patients with sepsis and pneumonia, and immunocompromised patients with febrile episodes were reported than in patients with endocarditis [48]. For the diagnosis of neonatal sepsis, reported sensitivity and specificity were 90.2% and 72.9%, respectively, when using a modified multiplex PCR protocol [49]. As different patient populations were studied, variable sensitivities and specificities were reported, and variable concordances with conventional BCs were obtained. A drawback of the system is that identification is done without quantification of the pathogen. Indeed, reporting quantitative data might be useful in assessing the severity of the disease [50].

Magicplex™ Sepsis Real-time Test

Magicplex™ Sepsis Real-time Test (Seegene, Seoul, Korea) is a CE approved automated assay which makes use of conventional PCR after DNA extraction, followed by real-time PCR for the identification from 1 mL blood with a turnaround time of 3 to 6 hours. The assay has an extensive panel developed for detection of more than 90 pathogens including a further identification of 27 pathogens at species level and three resistance genes (*mec*A, *van*A, *van*B). Clinical studies show reported sensitivities and specificities of 37-65% and 66-92%, respectively [51–53]. In a later study, test performance was improved by adjusting the cut off level of the quantification cycle (Cq) values [53]. In this study, setting a lower cut off for the Cq values resulted in a decrease in sensitivity to 38%, but an increase in PPV to 59% and in specificity to 95% together with less contaminant detection. Absence of quantification and lack of identification of low number of pathogens at species level are limitations of the assay [11].

SepsiTest™

The SepsiTest™ (Molzym, Bremen, Germany) is a CE marked assay combining broad range PCR and sequencing. The test performs a broad range PCR using universal primers targeting the bacterial 16S and the fungal 18S rDNA regions, followed by sequencing of the amplicon product from positive samples and subsequent BLAST analysis for identification of the pathogens at the genus and species level. The assay processes blood volumes as low as 1 mL and can also be used on samples from other primary sterile body fluids with turnaround times of 8 to 12 hours [11,54,55] including a hands-on time of 75 min for PCR and 70 min for sequence analysis [56]. In a multicentre study of blood samples taken from patients with different pathologies, reported sensitivity and specificity of the test were 87% and 85.8%, respectively [54]. Later, the assay was evaluated for monitoring of patients for systemic infections during extracorporeal membrane oxygenation [57]. The assay was able to identify 45% of positive samples, 13 to 75 hours earlier than BC and detected blood culture-negative infections in 25% of patients [57]. Yet, detection of any resistance genes is not available in the assay.

T2 magnetic resonance-based methods

T2 Magnetic resonance is a magnetic resonance-based diagnostic method that measures changes in the water proton T2 relaxation signal in the presence of magnetic fields [14]. It usually includes a pathogen-specific PCR amplification step, followed by hybridization of the amplicons to probe-enriched superparamagnetic nanoparticles [14].

T2Candida® panel

T2Candida® Panel (T2Biosystems, Lexington, Massachusetts, USA) is an FDA approved diagnostic assay based on T2 magnetic resonance (T2MR) detection technology, which measures reacting water molecules in the presence of magnetic fields. T2Candida enables identification of five *Candida* species directly in whole blood without the need for extraction of DNA with a time to result of 3–5 hours and a LOD of 1 CFU/mL [58,59]. In a multicentre study, sensitivity and specificity of the assay were 99.4% and 91.1%, respectively [60]. In a further study, potential use of T2MR technology as a monitoring assay for candidaemia has been demonstrated [61]. Most recently, T2Biosystems has launched a CE-marked T2Bacteria® Panel, which identifies the six most common BSI-causing bacterial species (*E. coli*, *K. pneumoniae*, *P. aeruginosa*, *Acinetobacter baumannii*, *S. aureus*, and *E. faecium*) which may not respond to empiric therapy depending on the regional epidemiology.

Metagenomics

Metagenomics is the genomic analysis of specific or all genetic material present in and directly from a (clinical) sample. Sequencing of nucleic acids within a sample can be based on a specific amplicon (amplicon-based metagenomics) or on the entire genomes (shotgun metagenomics). The 16S and 16S-23S rDNA amplicon-based metagenomics methods are based on parallel deep sequencing of the prokaryote-specific 16S ribosomal RNA gene [62–65] or the entire 16S – intergenic spacer region – 23S rDNA region, respectively [66]. These methods have been applied successfully for diagnosis of bacterial BSIs from BCs [62,65,66] or

directly from blood [63–65], with equal or superior sensitivity compared with blood culture. However, no commercially available method has been developed so far. Amplicon-based metagenomics has the disadvantage of only identifying a specific set of pathogens, in this case, bacteria. Shotgun metagenomics, on the other hand, has the advantage of not being limited to certain pathogens, and provides information on all microorganisms and viruses present in a sample, even if the sample contains a complex (polymicrobial) community [67]. In addition, shotgun metagenomics can detect antimicrobial resistance and virulence genes and provide important information on molecular epidemiology [68]. Therefore, shotgun metagenomics represents an essential add-on in pathogen detection compared with conventional blood culture or amplicon-based sequencing of suspected BSI with an even higher sensitivity and reliability. So far shotgun metagenomics has only been applied to blood or blood components [67,69,70] and only one commercially available tool has been developed.

iDTECT™ Dx Blood

The iDTECT™ Dx Blood test (PathoQuest SAS, France) is a CE marked new sequencing approach for the identification of pathogens and was originally developed at the Institute Pasteur (www.pathoquest.com). To this end, blood samples are prepared (DNA/RNA extraction, library preparation) and then analysed using next-generation sequencing technology to identify genomic sequences of pathogens. In a multicentre, blinded, prospective, proof-of-concept study, the investigators compared untargeted next-generation sequencing with conventional microbiological methods for first-line diagnosis of infection in 101 immunocompromised adults [69]. The method showed a high negative predictive value (NPV) compared with conventional methods (64/65, 95% CI 0.95–1), and also detected more clinically relevant viruses and bacteria than conventional microbiological methods [69]. However, there were two discordant cases that were positive by conventional microbiological methods but negative by sequencing. In one case, this was a result of contamination of the negative control with *E. coli* DNA, which led to the *E. coli* results not being reported in the NGS analysis. Currently, the detection of yeasts/fungi is hampered by limitation of the database and extraction procedures. In addition, introduction within a clinical lab may be hampered by the high costs per sample, the batch-wise approach of testing and the highly sophisticated infrastructure needed to perform the sequencing [69,71].

Platforms currently not available

Marketing of some molecular assays or platforms has been discontinued by the manufacturer. As their performance in diagnosis of BSIs was evaluated in recent studies and, moreover, assays as, for example IRIDICA, have been reintroduced in the past, they are briefly discussed (Table 3).

BD GeneOhmTM StaphSR

The BD GeneOhmTM StaphSR assay (Becton Dickinson Diagnostics, Franklin Lakes, USA) was a multiplexed real-time PCR-based assay, allowing detection of *S. aureus* and MRSA directly from positive BCs. This assay differentiated MSSA from MRSA by detecting two targets; one specific for *S. aureus* and another specific for the SCC*mec-orfX* junction site in MRSA [33,72]. The BD GeneOhm™ StaphSR assay displayed a turnaround time of approximately 2.5 hours [72]. Although the sensitivity and specificity of the test were 99.2% and 96.5% for MSSA and 94.3% and 97.8%, for MRSA, a poorer performance was reported in a study conducted in the USA [33]. This test has been discontinued and replaced by the newer version BD Max™ StaphSR Assay.

Prove-it™ sepsis

Prove-it™ Sepsis (Mobidiag, Espoo, Finland) was developed as a combination of a broad range PCR and a DNA-microarray. This assay identified the pathogens through the amplification of the topoisomerase gene targets (*par*E and *gyr*B) and further detection was done by microarray hybridization within 3 hours directly from BCs [8]. The first version of the test was able to identify 50 bacterial species as well as the *mec*A gene with a sensitivity and specificity of 94.7% and 98.8%, respectively [73]. In the latter version of the Prove-it Sepsis assay (Strip Array 2.0), pathogen coverage of the DNA-Microarray panel had been extended to include yeast targets as well. It was able to detect 13 yeasts (12 *Candida* species and *Saccharomyces cerevisiae*) and more than 60 bacteria, covering most of the sepsis-associated pathogens. In a recent clinical evaluation study, sensitivity and specificity of the test for the detection of yeast were 99% and 97%, respectively [74]. Although the turnaround time for the assay was said to be 3 hours, an extra hour was required for the sample preparation [74]. Although the results were promising, the product has now been discontinued.

Table 3
Methods currently not available on the market

Assay	Pathogens covered	Resistance markers	Sensitivity/specificity	(Method) Complexity -Personnel experience level -Equipment requirement	Hands-on time	Times to result	References
BD GeneOhm™ StaphSR (BD Diagnostics, USA)	*S. aureus*, MRSA	None	94–99%/97–98%	(Multiplex Real-time PCR) Not automated -Trained personnel -Proprietary equipment	Not provided	2.5 hours	[33,72] www.bd.com
Prove-it™ Sepsis: StripArray v2.0 (Mobidiag, Finland)	>60 Bacteria 12 *Candida* spp. and *Saccharomyces cerevisiae*	*mec*A	95-99%/97–99%	(Multiplex PCR and DNA-Microarray) Partially automated[a] -Trained personnel -Proprietary equipment	1.5 hour	3–4 hours	[73,74] www.mobidiag.com
IRIDICA BAC BSI Assay (Ibis Biosciences, Abbott, USA)	>600 bacteria and *Candida* species	*mec*A, *van*A, *van*B, bla_{KPC}	83–91%/87–94%	(PCR/ESI-MS) Fully automated -Trained personnel -Proprietary equipment	30 min	6 hours	[75–77] www.molecular.abbott

Specifications of the assays have been collected from the literature and manufacturer's information.
[a] Automated software for detection and analysis.

The IRIDICA™ BAC BSI assay

The IRIDICA™ BAC BSI Assay (Ibis Biosciences, (Ibis Biosciences, Abbott, Carlsbad, USA) was developed based on a PCR/electrospray ionization-mass spectrometry (PCR/ESI-MS) technology. Principally, following PCR, the mass of the PCR amplicons was determined by ESI-MS, then the nucleotide base composition was calculated based on the mass spectra and compared with a database to identify the microorganism. This assay, marketed in Europe for use in clinical research, could identify more than 600 bacteria and *Candida* species and also detect resistance genes *mec*A, *van*A, *van*B, and bla_{KPC} from 5 mL of whole blood within 6 hours [75]. An evaluation of four studies comparing the IRIDICA BAC BSI assay and blood culture found the estimated summary specificity and sensitivity of the assay to be 84% and 81%, respectively [76]. Although the results were favourable to implement the assay in routine diagnosis, the manufacturer decided to cease producing the IRIDICA assay in April 2017 [77].

Clinical outcome and impact of molecular diagnostic tests

The specific requirements, performance, and limitations of the molecular techniques described above vary to a large extent (summarized in Tables 4 and 5). None of the molecular methods is yet able to completely replace conventional culture-based methods. This is mainly because of the importance of phenotypic antimicrobial susceptibility determination (e.g. identification of minimum inhibitory concentrations (MIC)) for tailored, directed treatment, but also because of the rather limited spectrum and sensitivity of most available assays. Thus, they need to be performed as on-top assays to standard methods for identification and AST. Furthermore, identification by these methods has now to compete with identification protocols by MALDI-TOF MS directly from positive BCs – which is ideally suited for on-demand diagnostics and has a time-to-result of 30 to 60 min (from positive blood culture bottles). Nevertheless, the molecular methods reviewed here may result in a significant reduction in the turnaround time needed for microbial identification and susceptibility testing. It is difficult to make general statements with regard to costs of each specific method, because each method has different properties and requirements. Cost-effectiveness calculations would depend to a large extent on several factors, such as the patient population served, logistics, service hours of the laboratory, etc. The globally rather high unit cost price and often additionally required infrastructure (specific assay platforms, etc.), as well as the varying on-demand availability would require a thorough analysis with regard to the specific setting (patient population served, available infrastructure, 24/7 service implemented, laboratory structure and requirements, reimbursement, epidemiology, empiric regimens and switch policies, etc.) to make useful statements. Ultimately, the use of such methods may be cost-effective if considering other costs allocated to antimicrobial use and hospital stay, as rapid identification results allow early start of (or change to) appropriate antimicrobial therapy [78]. Indeed, some recent studies have evaluated the impact of the rapid molecular tests on improved clinical outcome by means of time to optimal therapy and also association with length of stay, mortality, and costs. However, the impact of rapid BC diagnostics on clinical and economical outcomes depends on multiple aspects specific to each institution, namely the Antimicrobial Stewardship Program (ASP) [78]. Indeed, most of the observational studies suggest that rapid testing implemented with an ASP provides more favourable clinical outcomes than rapid testing alone [78]. A recent systematic review and meta-analysis investigated the clinical outcome of using rapid molecular tests for diagnosis of BSIs by analysing 31 studies [79]. The impact of

Table 4
Requirements, benefits, and limitations of molecular techniques from positive blood cultures

	Blood subculture	*In situ* hybridization-based methods	DNA-microarray-based methods	Nucleic-acid amplification-based methods	Combined methods
Duration[a]	Very slow	Fast or very fast	Fast	Fast or very fast	Fast
Complexity	Laborious	Laborious	Simple	Simple	Simple
Role	Standard diagnostics	On-top assay	On-top assay	On-top assay	On-top assay
Limit of detection	1–10 CFU/mL	10^6 CFU/mL	10^6–10^7 CFU/mL	10^2–10^6 CFU/mL	10^2–10^6 CFU/mL[c]
Suitability for therapy monitoring	Yes (bacteremia; conditionally: time to positivity)	Conditionally (with live/dead staining)	No (only conditionally indirectly via: time to positivity of culture)	No (only conditionally indirectly via: time to positivity of culture)	No (only conditionally indirectly via: time to positivity of culture)
Costs[b]	<10€ (if negative), 10–50€ (if positive)	10–50€	50–250€	30–150€	100–150€
Laboratory type (setting)	Satellite	Satellite	Centralized or academic	Satellite or standard (assay-dependent)	Centralized or academic
Limitations	Very specialized technicians	Very specialized technicians Most methods do not provide information about antimicrobial resistance	Variable sensitivity	False-positive results from artefacts	Limited clinical evaluation studies

Quantification is not addressed as these assays all start from highly enriched cultures.
[a] Four categories were considered: Very slow (6–48h); Slow (3–6h); Fast (1–3h); Very fast (<1h).
[b] Cost per test was considered, not the system cost. N.B. Cost-effectiveness analyses require more complex calculations, considering total cost and clinical impact (c.f. to ref [76], chapter 3 for an example).
[c] LOD was given for ePlex BCID-FP panel. There are a limited number of studies evaluating the analytic sensitivity of ePlex® BCID and Sepsis Flow Chip.

Table 5
Requirements, benefits, and limitations of molecular techniques from whole blood

	Blood culture	Nucleic-acid amplification based methods	T2 Magnetic resonance based methods	Metagenomics
Duration[a]	Very slow	Fast or very fast	Slow	Very slow
Complexity	Laborious	Simple	Simple	Laborious
Role	Not applicable	On-top assay	On-top assay	On-top assay
Limits of detection	1–10 CFU/mL	3–30 CFU/mL	1 CFU/mL	Possibly similar to nucleic acid amplification-based methods, depending on the depth of sequencing
Quantification possibilities	Yes (conditionally: time to positivity)	Possible	Not possible	Semi-quantitative
Suitability for therapy monitoring	Yes (bacteraemia)	Potentially (conditionally; indirectly via quantification)	No	Potentially (conditionally; indirectly via quantification)
Costs[b]	<10€ (if negative), 10–50€ (if positive)	50–250€	150–200€	150–300€
Laboratory type (setting)	Satellite	Standard	Centralized or academic	Centralized or academic
Limitations	Very specialized technicians	False-positive results from artefacts	Does not provide information on antifungal resistance	Very specialized technicians

[a] Four categories were considered: Very slow (6–48h); Slow (3–6h); Fast (1–3h); Very fast (<1h).
[b] Cost per test was considered, not the system cost. N.B. Cost-effectiveness analyses require more complex calculations, considering total cost and clinical impact (c.f. to ref [76] chapter 3 for an example).

these assays was also evaluated in the presence of an ASP intervention. Rapid molecular testing showed significant decrease in mortality risk, decrease in time to effective therapy, and shortened length of stay, when implemented with an ASP intervention [79]. In light of the evidence provided by these studies, molecular tests should be integrated as part of the diagnostic workflow in patients with BSIs.

Conclusions

In general, molecular methods available to date aim at cutting down on substantial incubation time that is still required for culture-based methods. Mainly as a result of sensitivity issues, most approaches and assays have been developed to start with positive BCs, still leaving a detection gap for some non-cultivable fastidious microorganisms. Assays starting directly from blood of affected patients have probably the highest potential with this regard, provided they perform with a sensitivity that equals at least that of current BC diagnostics. In addition, these (metagenomics) approaches have the potential to compensate for inherent shortcomings of culture-based diagnostics. Provided aspects such as on-demand availability, platform commonality, data analysis, and clinical interpretation, as well as robustness are adequately addressed, this approach could represent a quantum leap comparable with the introduction of MALDI-TOF MS in microbiological diagnostics, and thus substantially improve quality of patient care.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at https://doi.org/10.1016/j.cmi.2018.05.007.

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