Review Article

Rapid Identification of Pathogens Recovered from Blood Stream Infections

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This study reviews the potential of newer rapid molecular-based methods to identify blood pathogens and/or to predict antibiotic resistance mechanisms that the organisms possess. Use of Peptide Nucleic Acid Fluorescence in Situ Hybridization (PNA-FISH) and matrix-assisted laser desorption ionization Time-of-Flight (MALDI-TOF) technologies are well-established methods for reducing turn-around-time in detecting the bacterial agent in positive blood cultures. Several manufacturers in the US, Europe and South Korea have developed methods and instrumentation to detect microorganisms directly from whole blood specimens without biological amplification on culture media. The assays utilize nucleic acid amplification of the organism DNA or RNA to a detectable level. Challenges with these methods are numerous as pathogens are present in low numbers in the circulating blood and the high background of human DNA can yield the assays less sensitive and specific. Many of the methods are not FDA-approved for use in the US although some have regulatory approval in Europe. Current rapid methods do not replace the traditional methods of culturing positive blood culture isolates to agar plates for definitive identification and antimicrobial susceptibility testing. Recent technological advances are making significant progress in the prompt detection and identification of pathogens and their antimicrobial resistance mechanisms in patients with sepsis. These new rapid methods show promise in the enhancement of patient care and positively influencing patient outcomes. Clinical and economic benefits of the rapid tests must be evaluated in conjunction with a robust antimicrobial stewardship program.

Key words: Blood Stream Infections, Pathogens, Blood cultures, Sepsis, molecular methods

Introduction

Recent technological advances provide the ability to rapidly deliver definitive organism identifications directly from blood cultures. Molecular diagnostics can identify many of the common pathogens and specific antibiotic resistant genes in microorganisms associated with bloodstream infections.

Rapid identification allows physicians to prescribe specific targeted and effective antimicrobial therapy earlier than traditional laboratory identification and susceptibility testing in patients with blood stream infections. These rapid methods enhance patient care and positively influence patient outcomes.[1,2,3]

The US Food and Drug Administration (FDA) approved several diagnostic systems for rapid identification of organisms from positive blood cultures. These methods are less labor intensive and decrease turnaround times for pathogen identification in comparison to traditional microbiological methods.[4]

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Although current technologies can be performed within minutes to a few hours, the initial steps of culturing the blood sample may take days. Similarly, determining the antibiotic susceptibility depends on additional sub-culturing from the initial positive blood culture bottles following the rapid identification method. These initial growth-based amplification methods ensure sensitive detection but do not shorten the diagnostic timeline. These methods also restrict the breadth of organisms that can be cultivated and detected by relying on a single culture medium formulation, which cannot support the growth of all organisms and may mask susceptibilities in organisms that fail to grow.[5,6]

The following discussions are intended to present brief overviews of the advances in technology that provide rapid identification and/or susceptibility testing of microorganisms in blood stream infections. However, for a full detailed discussion of specific methodologies it is important to consult the manufacturer information prior to implementing these methods in a diagnostic laboratory.

Peptide Nucleic Acid Fluorescent *In Situ* Hybridization Molecular Stains

Molecular techniques are increasingly being used to identify pathogens. Fluorescence *in situ* hybridization (FISH) is a technique whereby deoxyribonucleic acid (DNA) probes labeled with fluorophores are attached to a target sequence for identification. Peptide nucleic acid (PNA) FISH stains are used to identify selected pathogens from positive blood cultures. These constructs are more stable than traditional nucleic acid probes and are used to detect species specific ribosomal RNA (rRNA). Current available assays are able to differentiate and identify *Staphylococcus aureus* and coagulase-negative staphylococci (CoNS); *Enterococcus faecalis* and other *Enterococcus* species; *Escherichia coli*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*; and *Candida* species.[1] PNA-FISH assays have sensitivities and specificities from 96% to 100% with a turnaround time of approximately 90 minutes. A faster and less labor-intensive assay was introduced recently with a turnaround time of 20 minutes.[5,7] FISH has been shown to improve patient outcomes in terms of survival, shorten the hospital stay, and reduce the total cost associated with blood stream infections.

Matrix-Assisted Laser Desorption-Ionization/Time-of-Flight Mass Spectroscopy (MALDI-TOF MS)

Because of the rapid results and accuracy associated with the identification of pathogens using MALDI-TOF MS, there has been an increased interest in the use of this technique for the detection of pathogens in blood stream infections. MALDI-TOF MS has been successful in the identification of aerobic and anaerobic bacterial pathogens encountered in the clinical microbiology laboratory. The technology has been successfully used on pure, isolated colonies and has not been widely used for the identification of organisms from primary specimens such as blood cultures in the clinical laboratory.[8] Colonies recovered from an agar plate are prepared and loaded into the specimen ionization chamber. Ionized particles produced by the laser pulse differ by size and are separated according to mass. The distribution of particle sizes is unique to each organism resulting in a protein spectrum. The results are typically available within 10–30 minutes.[9,10] The spectrum are useful for the identification of most common pathogens isolated from the vast majority of routine microbiological cultures in the clinical laboratory. However, there are some limitations associated with the identification of fastidious and slow growing organisms.

More recently, several studies have examined the identification of microorganisms directly from blood culture bottles without sub-culturing on routine laboratory media. One study investigated the efficacy of identification of a variety of microorganisms from positive blood cultures using a lysis buffer system to lyse red and white blood cells followed by a protein extraction procedure and identification using MALDI-TOF MS.[11] The study compared the lysis and extraction method to the standardized procedure for identification using a pure culture isolate and ethanol formic acid extraction. The study examined three different blood culture broth formulations as well as representative microorganisms commonly identified in positive blood cultures including *Escherichia coli*, *Pseudomonas aeruginosa*, *Klebsiella pneumoniae*, *Streptococcus pneumoniae*, *Bacteroides fragilis* and *Haemophilus influenzae*. Although the study demonstrated some promising results for the direct identification of the organisms, the ability to identify each organism varied with each broth formulation and

lysis buffer concentration.[11] Additional studies have indicated that direct detection and identification of microorganisms from positive blood cultures demonstrates a reliability of approximately 71-91% in monomicrobic infections.[12]

Overall MALDI-TOF MS provides a benefit to the patient by rapidly providing clinicians with information needed for clinical management resulting in improved patient outcomes and reduced length of hospital stay.[13,14] The major drawback of MALDI-TOF MS is instrument cost.

Rapid Methods for Detection of Microorganisms Directly in Blood Specimens

Researchers are investigating detection methods to rapidly, directly detect and identify microorganisms in blood samples from patients with suspected septicemia. Nucleic acid amplification assays (NAATs) have promise to detect specific microorganisms in blood samples.[15] These assays rapidly create copies of DNA or RNA originating from pathogen or host cells through biochemical reactions and amplify the nucleic acid sequences to a detectable level, thus, identifying the infecting agent or the status of the immune response. NAAT may be valuable in detecting pathogens that are non-cultivable or non-viable as a result of prior antibiotic treatment. Capturing and amplifying pathogenic nucleic acids from blood is extremely challenging. Pathogens are usually present at low levels in circulation or found in contaminating material such as a high background of human DNA making the assays less sensitive and specific. A number of assays are currently available for direct detection of pathogens from blood samples; however, many are not FDA-approved for use in the US, although some have regulatory approval in Europe.[16,17]

LightCycler SeptiFast

LightCycler SeptiFast (Roche Molecular System, Mannheim, Germany) is intended to rapidly detect and identify bacterial and fungal DNA which may be present in the bloodstream. The test requires 1.5 mL of whole blood without prior incubation or culturing to detect 19 bacterial and fungal pathogens using multiplexed PCR coupled with probe hybridization and DNA melt curve analysis. The test involves three distinct processes: specimen preparation by mechanical lysis and purification of DNA, real-time PCR amplification of target DNA in 3 parallel reactions (Gram positive bacteria, Gram negative bacteria, and fungi) followed by detection using fluorescently labeled probes specific to the target DNA. The test takes approximately 6 hours. Sensitivity and specificity are both reported at approximately 95%, however, studies indicate significant variability.[18]

IRIDICA BAC BSI assay

The IRIDICA BAC BSI assay (Abbott Diagnostics) is an *in vitro* diagnostic test for detecting and identifying bacteria and *Candida* DNA in 5ml EDTA-treated whole blood. The test can also detect the *mecA*, *vanA* and *vanB*, and KPC (*Klebsiella pneumoniae* carbapenemase) genes which are associated with antibiotic resistance. The test combines broad range PCR with electrospray ionization time of flight mass spectrometry to amplify and detect pathogens. The IRIDICA analysis computer consists of a proprietary database and software which identifies the organism present in the sample by comparing the sequence of the sample with a library of known sequences. The BAC BSI assay is able to identify over 780 bacteria and *Candida*, with the exception of *Aspergillus fumigatus* and *Candida krusei*. The estimated time to result is 5 hours and 55 minutes. There are several published studies that compare the performance of the IRIDICA to traditional blood culture and identification. Reports suggest that the specificity is higher than sensitivity for the IRIDICA, but is similar to the Roche SeptiFast assay. However, a current review indicates that the published studies should be viewed with caution due to the limitations associated with each.[18]

MagicPlex Sepsis

Magicplex™ Sepsis Real-time Test (Seegene, Seoul, South Korea) screens for more than 90 pathogens from whole blood samples within 3 hours (excluding nucleic acid extraction time). The assay can detection more than 90% of the sepsis-causing pathogens. The test can detect 73 Gram-positive bacteria, 12 Gram-negative bacteria and 6 fungi at the genus level, 27 organisms at the species level and 3 drug-resistant genes (*mecA, vanA,* and *vanB)*. The assay relies on a

two- step preparation and extraction method. Human cells are lysed and then the DNA is degrading using a selective degradation process (MolDNaseB), followed by bacterial cell lysis and DNA extraction. Similar to other molecular assays, the test requires specialized equipment and technical experience. The Magicplex test also has several manual steps as indicated, that make it laborious and increases the risk of possible contamination. In comparison with traditional blood cultures, the assay sensitivity is reported to vary between 29 to 95% depending on the type of isolate and whether or not the sample is mono or polymicrobic.[19] The reported low sensitivity makes its implementation as a routine test difficult in clinical microbiology laboratories.

YVOO

YVOO- Sepsis pathogen identification test (SirsLab, Jena, Germany) combines the separation of specific binding prokaryotic DNA from initially extracted whole DNA from human blood samples coupled with a multiplex PCR amplification and detection on Gel Electrophoresis. The assay is capable of detecting 34 bacteria, 7 fungi and 5 different resistance markers using a 5 mL whole blood sample.[20]

SepsiTest

The SepsiTest™ Blood test (Molzym Molecular Diagnostics, Bremen, Germany) consists of three major steps that includes the extraction of microbial DNA followed by universal PCR amplification and nucleic acid sequencing. The assay utilizes universal primers to amplify bacterial 16S rRNA and fungal 18S rRNA, which enables the detection of over 200 bacteria and 65 genera of fungi.[18] This is followed by Sanger sequencing and using the SepsiTest-BLAST online tool for the analysis of the nucleic acid amplicons for identification. The test includes several steps, requires 1 mL of potassium-EDTA or citrated whole blood, and can take 6–12 h. The platform does not include detection of any resistance markers.[3,18,21,22] Published studies indicate that the platform has a higher specificity than sensitivity and is comparable to traditional blood culture identification. However, the review cautions the reader regarding the liability of published data based on deficiencies in the quality of the available studies.[18]

Next Generation Sequencing Technology

The next generation sequencing (NGS) technology has the potential to identify pathogens and their resistance genes in blood samples from patients with sepsis within 8 hours. NGS identification is very challenging due to the amount of human DNA present compared with pathogen DNA. A variety of protocols are available for the analysis of cell-free DNA (cfDNA). Increased levels of cfDNA have been reported in patients with inflammatory diseases, trauma, cancer, and surgery.[23] NGS sequencing platforms offers the ability to quantitatively assess the level of bacterial cfDNA in plasma that may allow the differentiation of microbial pathogens, bacterial contaminants and the identification of antimicrobial resistance markers. NGS technology requires the development of a genomic library followed by sequencing of the sample. These methods require a substantial investment in instrumentation, personnel and cost. Although newer sequencing instrumentation and supplies are becoming more cost effective, these methodologies are only available in large laboratories with extensive resources and qualified personnel. The instrument employed is a portable DNA/RNA sequencer with relatively short library prep times and relatively low cost. The MinION (Oxford Nanopore Technologies, Oxford, UK) is such an example of a portable DNA/RNA sequencer with relatively short library prep times and low cost.[20]

FDA Approved Platforms

T2 Biosystems

The first FDA-approved platform for the direct detection of a bacterial or fungal pathogen from the bloodstream was the T2Candida (T2C) assay (T2 Biosystems, Lexington, MA, USA). T2C detects the five *Candida* spp. most commonly implicated in invasive candidiasis. The assay reports a sensitivity of 91% and specificity of 98% in the detection of candidemia, further studies are warranted in cases of invasive candidiasis.[20,24] The assay can also be used to detect and quantitate beta-D-glucan (BDG), a normal constituent of fungal cell walls. Monitoring the decreasing level of BDG in the patient's bloodstream during treatment of fungemia provides direct indications of successful patient management and can serve as a predictor for patient recovery.[24] More recently, the FDA approved T2Bacteria (T2B)

for direct detection of 5 different organisms directly from whole blood. The organisms include *Enterococcus faecium, Staphylococcus aureus, Klebsiella pneumoniae, Pseudomonas aeruginosa,* and *Escherichia coli.*[20]

T2 Biosystems is a fully automated, tabletop T2Dx platform combining magnetic resonance (MR) with nanotechnology. T2MR identification does not rely on growth-dependent blood cultures like other identification systems currently available and FDA approved. The assay uses approximately 4 mL of whole blood to provide a result in less than 5 hours. During processing on the T2Dx instrument, pathogens are concentrated directly in whole blood, then lysed to release the target DNA. Bacterial DNA is amplified with target-specific primers and amplicons are hybridized to target-specific probes attached to superparamagnetic particles causing clustering of the particles. A signal is detected by T2MR indicating the presence of the target organism. Whereas the pathway to identification of a positive blood culture can take approximately 1 to 3 days, T2MR results are available in approximately 6 hours. The results are interpreted by the device software as valid or invalid (based on the result of the internal control or target detections), and if valid, results are reported as "Positive" or "Target not Detected" for each specific target. For *E. coli*, results are reported as Positive, Indeterminate or "Target not Detected". An Indeterminate result is a valid result, but the presence or absence of *E. coli* in the specimen cannot be definitively assessed.[24] Comparative analysis of the T2B assay performance to traditional blood culture identification indicates that the T2B assay demonstrates a sensitivity of approximately 83% and specificity of 98%. Sensitivity increased to 90% when the patient's condition, signs and symptoms were clinically suspect of true sepsis.[24]

The Accelerate Pheno™

The Accelerate Pheno™ system (Accelerate Diagnostics, Inc., Tucson, Arizona) delivers phenotypic antibiotic susceptibility results along with microbial identification directly from positive blood cultures 40 hours faster than current methods used in most labs today. It is a multiplexed in vitro diagnostic test utilizing both qualitative nucleic acid fluorescence in situ hybridization (FISH) identification and quantitative, antimicrobial susceptibility testing (AST) methods. Susceptibility data is available 24-64 hours faster than traditional methods.[25] The test is capable of simultaneous detection and identification of multiple microbial targets. Results are intended to be interpreted in conjunction with Gram stain results.

Standard laboratory protocols for processing positive blood cultures should be followed to ensure availability of isolates for supplemental testing as needed. Additionally, subculture of positive blood culture is necessary for the identification and susceptibility testing of organisms not identified by the Accelerate PhenoTest BC kit, organisms present in polymicrobial samples, organisms for which species identification is critical for patient care (e.g., speciation of *Streptococcus* spp.), samples for which an "indeterminate" result for any probe was obtained, for testing antimicrobial agents not included on the Accelerate panel and for epidemiologic testing.

Despite well documented advantages of the system, several hurdles exist to effectively implement the system in a clinical environment. Clinical and laboratory pathways should be critically reviewed to optimize the timeliness of results. In addition, clinicians would be provided with information to escalate or de-escalate antimicrobial therapy resulting in improved patient care.[25] Depending on the laboratory microbiology staffing, considerations for automated reporting and trained personnel, as well as a sufficient investment in the cost of instrumentation, incorporating the AcceleratePheno System takes significant commitment by the facility and the providers.

Sepsis Treatment and Antibiotic Stewardship Programs

A growing body of evidence demonstrates that hospital-based programs dedicated to improving antibiotic use, commonly referred to as "Antibiotic Stewardship Programs (ASPs)," can both optimize the treatment of infections and reduce adverse events associated with antibiotic use.[26] These programs help clinicians improve the quality of patient care and improve patient safety through increased infection cure rates, reduced treatment failures, and increased frequency of prescribing the correct therapy and prophylactic treatment. They also significantly reduce hospital rates of antibiotic resistance. Moreover, these programs often achieve these benefits while reducing overall costs for the facility.

There is no single template for a program to optimize antibiotic prescriptions and stewardship in health care settings. The complexity of medical decision-making

surrounding antibiotic use and the variability in the size and types of care among health care facilities require flexibility in implementation. However, experience demonstrates that antibiotic stewardship programs can be implemented effectively in a wide variety of health care settings and that success is dependent on defined leadership and a coordinated multidisciplinary approach**.** The Centers for Disease and Control and Prevention (CDC) identified the following as the core elements for an Antibiotic Stewardship Program:[27]

- Leadership Commitment: Dedicating necessary human, financial and information technology resources.
- Accountability: Appointing a single leader responsible for program outcomes. Experience with successful programs show that a physician leader if effective.
- Drug Expertise: Appointing a single pharmacist leader responsible for working to improve antibiotic use.
- Action: Implementing at least one recommended action, such as systemic evaluation of ongoing treatment needs after a set period of initial treatment (i.e. “antibiotic time out” after 48 hours).
- Tracking: Monitoring antibiotic prescribing and resistance patterns.
- Reporting: Regular reporting information on antibiotic use and resistance to doctors, nurses and relevant staff.
- Education: Educating clinicians about resistance and optimal prescribing.

With the CDC recommended approach, patients receive only one dose of empirical broad-spectrum antibiotics before treatment can be tailored for the pathogen/patient - a true 'precision medicine' approach to antibiotic treatment.[27] According to Public Health England, this dramatic improvement to the “Start Smart - then Focus” approach to antimicrobial stewardship will lead to a reduction in the use of broad-spectrum antibiotics, mitigating selection pressure for antibiotic resistance.[28] It will also reduce the number of patients who receive inappropriate antibiotics for their infections, with contingent decreases in morbidity and mortality.[29]

Summary

The current rapid methods do not replace the traditional methods of culturing positive blood culture isolates to agar plates for definitive identification and antimicrobial susceptibility testing. However, recent technological advances are making significant progress in the prompt detection and identification of pathogens in patients with septicemia. Clinical and economic benefits of the rapid tests continue to be evaluated when combined with a robust antimicrobial stewardship program

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