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Commercial Multiplex Technologies for the Microbiological Diagnosis of Sepsis

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Abstract

In patients with suspected sepsis, rapid and accurate diagnosis of the causative infectious agent is critical. Although clinicians often use empiric antimicrobial therapy until the blood cultures are available to potentially adjust treatment, this approach is often not optimum for patient care. Recently, several commercial molecular multiplex technologies have shown promise for fast and comprehensive diagnosis of microorganisms and their antimicrobial resistance signatures. While one class of multiplex technologies is directed at improving the speed and diagnostic information obtained from positive blood cultures, the other identifies the causative microorganisms directly from clinical blood samples. The goal of this review is to provide an overview of these molecular technologies and describe their performance capabilities compared to standard blood cultures and in some cases to each other. We discuss the current clinical impact, limitations, and likely future advances these multiplex technologies may have in guiding the management of patients with sepsis.

Keywords

Multiplex technology; PCR; Sepsis

1. Background

Sepsis is a disease with high morbidity and mortality that involves the inflammatory response of the body to infectious agents. It is associated with fever, tachypnea, and tachycardia with additional changes seen along the progression to severe sepsis and septic shock (1–3). Sepsis frequently afflicts patients in the intensive care unit and in the emergency room, but can occur following surgery and in those with hematological malignancies (4). In the United States, severe sepsis affects 750,000 people per year with an estimated annual cost of \$17 billion, which is expected to increase 1.5% each year (5–7). Confirmation of sepsis necessitates the identification of the offending pathogens to guide appropriate antimicrobial therapy. Currently, standard microbiological analysis includes blood cultures, single colony growth assays, and biochemical analyses (8). However, results from this conventional testing can take 72 hours or longer to receive. Inadequate or inappropriate treatment is currently seen in 20–25% of septic patients and is associated with a five-fold reduction in survival (5, 9–11). Not only can it take a long time to identify the pathogen, but since often no pathogen is identified at all, more sensitive testing is required.

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Standard of care guidelines advise broad-spectrum antibiotics while awaiting culture results. Although this pre-emptive therapy saves lives, its empiric nature often results in the overuse of precious broad-spectrum therapies yet in some cases cannot provide adequate coverage of the causative pathogens. It is also associated with an increased rate of antibiotic resistance in the community and can result in serious infections (12). A retrospective study examining culture-positive sepsis found that 24% of these patients did not receive the correct antimicrobial therapy (10).

In sepsis, 90% of the causative microbes are bacteria, in which gram-positive bacteria remain slightly more common than gram-negative bacteria (13). The presence of drug-resistant bacteria, such as methicillin-resistant *Staphylococcus aureus* (MRSA), are becoming more clinically relevant and can increase mortality rate, length of hospital stay, and total cost (14). Fungal infections also account for about 10% of cases and are responsible for high levels of mortality due to severity of infection and length of time required for diagnosis (15). Unfortunately, blood cultures can only detect bacterial and fungal agents in 20–40% of patients with severe sepsis (11, 16). These failures are often due in part to localized tissue infection with little shedding of the microorganisms into the bloodstream. Additionally, the long time-to-positivity for the microbe, especially for slow growing pathogens (e.g. fungi), and the detrimental impact from ongoing-therapy is also problematic (17, 18). Thus, new technologies are needed for rapid, sensitive, and accurate detection of the causative pathogens in the blood of septic patients.

Nucleic acid testing (NAT) has revolutionized the diagnosis of many infectious agents (19). For example, the application of molecular pathogen identification by peptide nucleic acid fluorescence in situ hybridization (PNA-FISH), has already been shown to improve sepsis outcome but is limited by its low-throughput and non-comprehensive format (20–22). A majority of NATs are also PCR-based and offer the ability to provide a diagnostic answer in only a few hours compared to several days with standard microbiological testing. However, most published laboratory-based PCR tests target only one or a few microorganisms and are impractical for sepsis diagnosis (23, 24). Due to the large and diverse spectrum of bacterial and fungal pathogens associated with sepsis, multiplex technologies are ideally suited for improving the diagnosis of this condition. The feasibility of using a broad, multiplex PCR approach in sepsis was first demonstrated in 1999, in which a conserved region of 16S bacterial ribosomal DNA was amplified from clinical samples and the resulting amplicons were used in DNA sequencing to identify the causative agent (25). Since then, several commercial multiplex technologies have been developed for two different clinical applications: to improve the diagnosis from positive blood cultures and to identify microorganisms directly from patient blood samples at the time of phlebotomy. The aim of this review is to focus on these commercial multiplex technologies for use in patients with suspected sepsis, which all have the capability to evaluate at least 10 or more different pathogens in parallel. We provide an overview of these technologies, describe their diagnostic applications, and discuss whether these multiplex assays have the potential to improve patient care. In this review, we cite articles published in PubMed based on the use of these commercial technologies with applications in sepsis.

2. Multiplex Technologies for Infectious Disease Diagnosis from Positive Blood Cultures

To diagnose sepsis, most hospitals use blood cultures and other types of microbiological tests (26). Blood cultures, the current standard of care, can take at least 1–3 days to detect bacterial growth and even longer to identify the exact microorganisms and determine the optimum course of antimicrobial therapy. To speed the time to diagnosis, three different multiplex technologies are commercially available that show promise for identifying

microbes present in positive blood cultures that have been incubated until initial growth of the organisms compared to full conventional incubation (i.e. 3 days or longer).

2.1 Prove-it™

The Prove-it™ Sepsis StripArray technology (Mobidiag, Finland) uses a combination of PCR and microarray to evaluate pathogens from incubated blood cultures (Table I). Following DNA extraction, each sample is used for broad-range PCR amplification to generate a biotinylated PCR product targeting conserved regions of the bacterial topoisomerase genes, *gyrB* and *parE* (27). Potential amplicons from this reaction are then hybridized to a DNA array of genus- and species-specific variable regions to identify the exact bacteria. This binding to the array is detected using streptavidin-conjugated peroxidase. A StripArray reader measures the colorimetric output and additional software (Prove-it™ Advisor) identifies the specific bacterial pathogens present. The Prove-it™ StripArray contains a microarray at the bottom of each well and is capable of simultaneously processing from 1 to 96 clinical samples per run. Beginning with prepared DNA from a positive blood culture, this platform can be completed in approximately 3 hours.

The current version of the Prove-it™ Sepsis test can detect over 60 types of gram-negative and gram-positive bacteria. In addition, the methicillin resistance gene found in MRSA and many coagulase-negative staphylococci (CoNS) are also amplified and detected in a similar way. Recently, a large-scale clinical study of the Prove-it™ Sepsis technology examined bacteria from culture positive samples of 2,107 patients with suspected sepsis (28). Blood cultures were incubated for six days or until they were deemed positive by conventional, automated methods. Using DNA extracted from 0.5 ml of blood, the Prove-it™ assay detected 86% of positive blood culture samples, but was unable to detect 14% of the organisms since they were not part of the screening panel. Targeting pathogens on the array, Prove-it™ showed excellent analytical performance with a sensitivity of 95% and a specificity of 99%, and showed near 100% accuracy in detecting or excluding MRSA. Compared to the standard blood cultures, the key technical advance of the Prove-it™ Sepsis test was the ability to generate clinical results 18 hours earlier than standard microbiological testing (28).

Although the Prove-it™ Sepsis test itself is rapid, prior extraction of DNA from blood cultures is required. DNA extraction must recover the small amount of microbial DNA present, but be free of environmental contaminations that could yield false positives. To extend the usability of Prove-it™, two different semi-automated DNA extraction systems, NorDiag Arrow (NorDiag, Norway) and NucliSENS® easyMAG® (bioMérieux, France) were compared (29). The results showed no significant difference between the two methods. Both automated extraction techniques required little hands-on time and could be completed within 1 hour. In this study, Prove-it™ identified pathogens in 77 of 91 blood cultures positive for bacteria and missed cases when the bacteria were not covered on the panel. Overall, these results highlight the compatibility and encouraging results found with automated DNA extraction and Prove-it™.

In light of the importance of fungal infections in sepsis, the Prove-it™ panel was recently updated to include probes for seven *Candida* species and a pan-yeast taxon. This version was tested in a study of 388 fungal specimens (30). Notably, fungal cultures can frequently take days or over a week to detect growth and even longer to rule out infection. In this study, the clinical fungal samples were cultured for only 48 hours before processing and the Prove-it™ technology demonstrated high diagnostic performance of 99% sensitivity and 99% specificity (30). These results highlight the ability of Prove-it™ to accurately diagnose fungal infections much more quickly than culture. The new array system can also detect the bacterial resistance genes *mecA* and *vanA/B*, for methicillin and vancomycin resistance,

respectively. Despite the expansion of the panel, future studies are needed to evaluate the effectiveness of the test for the full range of bacterial and fungal targets in clinical samples.

2.2 Verigene® Gram Positive Blood Culture Nucleic Acid Test (BC-GP)

The Verigene® Gram Positive Blood Culture Nucleic Acid Test (Nanosphere Technology, US) has recently been reviewed in this journal (31) and only a brief synopsis is provided here (Table I). For testing, bacterial DNA is collected from blood cultures, sheared, denatured, and extracted using magnetic beads by the Verigene® processor SP, and is further hybridized to complementary DNA immobilized on a glass slide. A bifunctional oligonucleotide is then added, which is both complementary to the target and able to bind a second signal-generating gold nanoparticle. The bound nanoparticles are coated with silver and are detected by the light scattered off the array. A Verigene® Reader is then used to identify the relevant pathogens present in the sample. With a hands-on time less than 5 min, the results from the clinical samples are available in 2.5 hours. The Verigene® Gram Positive Blood Culture Nucleic Acid Test has recently been granted FDA approval (31).

The assay is designed for rapid qualitative diagnosis of 13 gram-positive bacteria including species of *Enterococcus*, *Listeria*, *Staphylococcus*, and *Streptococcus*. It can also detect *mecA* and *vanA/B* bacterial resistance genes. In a review on studies from five sites encompassing 1,642 clinical samples, the Verigene® assay showed 88% agreement with conventional microbiological methods in fresh, frozen, and contrived samples (31). In addition, Verigene® was able to detect 94% of MRSA and 98% of ceftiofur-resistant organisms. In a separate, smaller study, Verigene® showed 93% sensitivity compared to blood cultures in detecting gram-positive organisms with pathogens missed as a result of exclusion on the panel (32). In addition, Verigene® had 92% sensitivity for *mecA* and 100% sensitivity for *vanA/B*.

One advantage of the Verigene® technology is that there is a built in DNA extraction and isolation component. Overall, its speed of detection in only a few hours can prove valuable in septic patients. The current assay is limited by its narrow pathogen detection panel which currently excludes fungi and gram-negative bacteria. However, information at the Verigene website claims there is a newly developed test for gram-negative bacteria, but no clinical evaluations have been published on this assay.

2.3 FilmArray®

FilmArray® (BioFire Diagnostics, US) represents a novel all-in-one technology for microbiological analysis (Table I). In the current format for blood borne infections, 24 target microorganisms and four antibiotic resistance gene markers can be analyzed simultaneously in about 1 hour with 3 min of hands-on time using a single integrated machine (33). All of the required reagents are prepackaged in a disposal pouch, and pneumatic pumps control movement of the samples through the pouch. With the FilmArray® device, DNA from the positive blood cultures is first purified by silica magnetic beads and then amplified by a reverse transcriptase/first stage PCR reaction. Aliquots of this first amplification reaction are then dispersed into individual wells where second nested PCR reactions occur with primer pairs for each of the different pathogens. A double-stranded DNA binding dye is used to monitor fluorescence changes during amplification. A digital camera captures the result and software analysis makes a determination of the pathogens present based on post-PCR melt analysis. Although the instrument requires minimal hands-on time, only one clinical sample can be analyzed per run.

The current FilmArray® panel for sepsis consists of gram-positive and gram-negative bacteria and various species of *Candida*. It also screens for four bacterial resistance genes,

mecA and *vanA/B*, and *Klebsiella pneumoniae* carbapenemase (KPC). In the single publication to date for sepsis, FilmArray® was used to examine 102 archived blood cultures from patients with suspected sepsis (33). The technology showed encouraging results and detected 91% of the pathogens. Moreover, 100% of MRSA and vancomycin resistant *Enterococci* (VRE) were also detected, and all but one sample with a resistance gene were validated by phenotypic susceptibility analysis. This technology is currently able to screen for carbapenem resistance, but no such drug-resistant organisms were detected in this small-scale study. Although the FilmArray® displayed good performance, it was unable to detect pathogens in 8 of 102 cases (33). The authors speculate that the detection failures were due to multiple organisms present in the blood cultures, whereby certain microorganisms became detectable earlier than others causing slow growers to be below the limit of detection.

Although FilmArray® has only been used in one study in sepsis, there was good concordance with phenotypic and blood culture analyses for detecting bacterial and fungal pathogens and resistance genes. Additional studies are clearly needed to validate the technology. One outstanding feature of FilmArray® is the automated all-in-one device that requires little hands-on time and provides an answer in less than 1 hour.

2.4 Clinical implications for therapy

The three technologies presented here, Prove-it™, Verigene®, and FilmArray®, represent a progressive improvement from blood culture diagnosis in sepsis. All of these technologies require positive blood cultures prior to microbial analysis with results becoming available in hours, thus drastically shortening the time to diagnosis. Therefore, these technologies have the possibility of pinpointing organisms and their resistance patterns earlier in the course of a disease allowing for the escalation or de-escalation of appropriate antimicrobial therapy in a timely fashion, but in some cases may provide results only after the critical treatment window. However, these assays are all dependent upon positive blood culture results and may be constrained in their ability to detect difficult-to-culture and fastidious organisms. Even if infection is highly likely, these multiplex technologies are not used in the case of negative blood cultures.

One obvious shortcoming for reviewing these technologies and their clinical performance is the paucity of publications to date. From our review of the literature, no explicit examples were found where these technologies would have altered a patient treatment regimen, though it is feasible to consider situations in which rapid diagnosis following blood cultures could be beneficial. Validation at additional locations is still required to determine the reliability and performance of these technologies by different users. While many of the technologies displayed high diagnostic sensitivity for pathogens present in their panels, none of the technologies were able to detect 100% of the clinically relevant microorganisms in sepsis. For example, Verigene® detects only gram-positive organisms and misses clinically important gram-negative and fungal infections. Prove-it™ has high diagnostic capabilities, but its newest panel has yet to be validated in a large clinical trial. Lastly, FilmArray® tests for only 24 likely pathogen candidates. As more pathogens and resistance markers are added to their panels, these three technologies may become more clinically useful and implemented in hospital settings.

3. Multiplex Technologies for Direct Diagnosis from Clinical Samples

In contrast to the previous technologies that use positive blood cultures to amplify the number of microorganisms for detection, the technologies in this section directly test clinical patient blood samples for pathogenic DNA. All of these technologies rely on highly

sensitive PCR amplification. We discuss these technologies and their performance for the diagnosis of sepsis and tissue infections.

3.1 SeptiFast®

SeptiFast® (Roche Diagnostics, Germany) is the most widely studied multiplex technology for diagnosis of infectious agents in sepsis (Table I). SeptiFast® detects 25 pathogens including gram-positive and gram-negative bacteria plus *Aspergillus fumigatus* and several species of *Candida*. The technology is based on FRET probes directed at species-specific DNA targets within the internal transcribed spacer region between the 16S and 23S areas of ribosomal DNA of bacteria and between the 18S and 5.8S ribosomal regions of the fungal genome. The SeptiFast® assay first requires preparation of DNA from the blood sample which involves mechanical lysis and purification using a MagNA Lyser instrument. The DNA from these clinical samples is then used in real-time PCR and the amplicons are further detected in three parallel reactions which emit fluorescence signals analyzed by multiple detectors. The corresponding melting points of the amplicons are then analyzed by pathogen identification software. An optional test for the *mecA* gene is available, but requires a separate assay and a subsequent run. Using whole blood samples, this test can be completed 4.5 hours after the initial blood draw and can return quantitative results, offering a unique advantage for this technology. In the last several years, there have been many published studies examining SeptiFast® (34–60), and below we describe in detail several important studies detecting pathogens in sepsis and other serious conditions.

The potential clinical utility of SeptiFast® was analyzed in a prospective, observational, multicenter trial that compared this multiplex technology against microbiological testing (34). While blood cultures detected 25% (117/467) of septic episodes, SeptiFast® detected significantly more infectious agents in 43% (201/467). Further analysis of the patients in this study revealed that 35% of the episodes were not adequately covered by the empiric antimicrobial therapy. The information gained from SeptiFast® screening would have affected clinical care in 10% (46/467) of patient episodes with half due to faster reporting and half due to increased sensitivity. However, SeptiFast® also missed 39 cases of bloodstream infections that were detected by traditional blood culture methods, and the results from SeptiFast® would have unnecessarily altered therapy in 18 patients since detection of bacterial resistance genes is not included in the panel (34).

In one multi-center trial comparing SeptiFast® to blood cultures, patients with severe sepsis were found to be positive by the technology nearly twice as frequently as with blood cultures (35). Moreover, a positive SeptiFast® result at enrollment correlated with a higher Sequential Organ Failure Assessment (SOFA) score and a trend toward higher mortality. In another study analyzing SeptiFast® and disease severity, patients with suspected bloodstream infections were prospectively enrolled and had blood drawn repeatedly for comparison with SeptiFast® (36). One of the major findings was that patients with a positive SeptiFast® test between days 3–7 had an 8-fold higher risk of developing complicated bloodstream infections defined as endocardial or extracardiac septic metastases. This diagnostic capability may prove valuable since complicated bloodstream infections often require longer hospital stays and more aggressive treatment (61). Highly sensitive on its own, SeptiFast® could be used alongside negative blood culture results to confidently rule out complicated bloodstream infections thus avoiding unnecessary tests.

SeptiFast® may also impact therapy decisions in patients with suspected sepsis (37). Along these lines, 110 patients with solid or hematological malignancies and suspected sepsis were analyzed by two panels of experts; one that had received all available clinical data and the other that had SeptiFast® test results as well. Since the reviewers only met once per month, clinical decisions were analyzed retrospectively and did not impact patient care. In the study,

the two panels of experts agreed on the best empirical antimicrobial treatment for 64% (70/110) of the patients. An additional third panel serving as the referee determined that SeptiFast® results would have improved the initial treatment in 11 patients, including the establishment of sepsis in four neutropenic patients who had been treated empirically with appropriate antimicrobials. One limitation of SeptiFast® was its high false positive rate seen in 10 patients. The overall agreement in this trial between blood cultures and SeptiFast® was 70%, including the detection of fungal pathogens, which is similar to other studies.

Analysis of both blood cultures and clinical samples taken before the start of antimicrobial therapy are valuable in guiding patient care. However, blood cultures taken after the initiation of antimicrobial therapy can frequently be negative due to the absence of viable organisms. In contrast, molecular multiplex tests such as SeptiFast® detect microbial DNA and can produce positive results for several days after the initiation of therapy and can be used to track the course of an infection. In one study, no significant difference in diagnostic sensitivity was found between blood cultures and SeptiFast® except in patients on antibiotic therapy for greater than 24 hours in which SeptiFast® was superior (38). Interestingly, the combination of blood cultures with SeptiFast® improved the overall diagnostic performance since 28 pathogens were missed by blood cultures alone. However, SeptiFast® could not detect pathogens in 16 patient samples in various clinical conditions including endocarditis, suggesting the bacterial load in some patients with these conditions may be below the limit of detection.

In summary, the results of these and many other studies with SeptiFast® demonstrate its efficacy in detecting many of the infectious agents at the initial blood draw in sepsis and throughout the course of a disease. At present, the spectrum of pathogens covered is useful, but SeptiFast® lacks the drug resistance markers detected by some of the other technologies. One unique advantage of this assay is the real-time quantitative detection which found in several studies that pathogen DNA levels correlated with disease severity. Perhaps its major current limitation is that it is not automated and requires significant technical hands-on time including the requirement for separate DNA extraction prior to real-time PCR analysis.

3.2 SepsiT_{est}®

SepsiT_{est}® (Molz_{ym}, Germany) is a multiplex PCR-based approach utilizing universal pathogen amplification (Table I). This technology can detect more than 345 bacteria and fungi and incorporates separate DNA extraction and purification of pathogen DNA with PCR and sequencing. For amplification of the pathogenic DNA, PCR primers target the 16S rRNA gene and the amplicons are detected by gel electrophoresis and sequenced. Although DNA sequencing requires additional time and resources, it allows for exact identification at the species level. Although the technology is reported to take 2.5 hours of hands on time and the results are available in 4 hours, the additional requirement of DNA sequencing extend this beyond 10 hours for most laboratories.

In one multicenter, prospective study, 187 patients with suspected sepsis were tested with SepsiT_{est}® (62). The assay demonstrated 87% sensitivity and 86% specificity compared with blood cultures. In 31 of 342 clinical samples, SepsiT_{est}® was positive while blood cultures were negative. Additional follow up showed that the SepsiT_{est}® was accurate in a majority of these cases. SepsiT_{est}® has also been compared with SeptiFast® in samples from 57 critically ill septic patients (63). SepsiT_{est}® showed 29% sensitivity and 85% specificity compared to 7 day blood cultures; however, the SeptiFast® technology had a higher sensitivity of 43% compared to blood cultures. While blood cultures were more sensitive than the multiplex technologies, the results suggest that SeptiFast® performed better than SepsiT_{est}® and also had a shorter turnaround time of 6 hours compared to 9 hours. It is important to point out that this published study is from an academic hospital with

expertise in DNA sequencing, and in most other clinical institutions, DNA sequencing would likely take longer than 10 hours.

Multiplex technologies have also been used in other types of microbial infections including bone and joint infections (64) and infectious endocarditis (65–67). In infectious endocarditis, patients can have negative blood culture results due to low pathogenic load in the blood, prior antimicrobial therapy, or the presence of fastidious microorganisms. These factors make sensitive multiplex technologies capable of analyzing clinical patient samples ideal for use in this disease. In one study, SepsiT[®] detected organisms in the blood of 57% (17/30) of patients with suspected infectious endocarditis, while blood cultures detected only 10% (3/30) (65). The assay was able to identify mixed infections involving bacteria and *Candida* and proved to be valuable for rapid diagnosis. In a study of 51 febrile intravenous drug users (IDUs), SepsiT[®] was found to have 87% sensitivity and specificity for detecting microorganisms compared to blood culture, but showed perfect diagnostic performance in detecting pathogens in patients with infective endocarditis (66). In another similar study, SeptiFast[®] was found to be useful in the diagnosis of infective endocarditis, particularly in patients who had been pre-treated with antibiotics (67).

At present, there are only a few studies on the diagnostic performance of the SepsiT[®]. In one study comparing the diagnostic performance of SepsiT[®] to SeptiFast[®], both technologies were found to be equivalent, but the results from SepsiT[®] took longer to receive. Although SepsiT[®] has the ability to profile the broadest spectrum of pathogens, it also requires significant time and labor directed at obtaining DNA sequencing information. In particular, SepsiT[®] requires several pieces of equipment including ones for DNA isolation, amplification, electrophoresis, and DNA sequencing. As the speed and cost of DNA sequencing decreases, it is possible that this approach will become more clinically attractive.

3.3 VYOO[®]

VYOO[®] (SIRS Lab, Germany) also uses PCR to detect a large number of pathogens in sepsis (Table I). This technology first employs a novel step to enrich pathogen DNA via a unique affinity resin. This pathogen-enriched DNA is then amplified using proprietary products for 33 bacteria, 7 fungi, and 5 antibiotic resistance genes including *mecA*, *vanA/B*, and probes for the β -lactamase resistance genes *blaSHV* and *blaCTX-M*. The generated amplicons for different agents are then analyzed by gel electrophoresis.

In the largest published study on VYOO[®], Bloos et al. examined 245 patients with presumed sepsis and obtained 311 sets of blood cultures (68). Compared to blood cultures, the VYOO[®] assay had a sensitivity of approximately 60% and a specificity of 75%. The VYOO[®] results were available significantly faster (24 hours) compared to blood cultures, which required 68 hours to detect positive results and even longer to rule out infection (~190 hours). This study also used the results from multiplex molecular testing to affect patient therapy and subsequent outcome in a prospective manner. The VYOO[®] technology identified three cases of multi-resistant *Staphylococci*, four cases of fungi, and five cases of VRE. Consequently, 34% of patients with positive PCR results were found by a panel of experts to have been placed on inadequate antimicrobial therapy. In addition, therapy in 24% of patients was recommended to be de-escalated. Altering the antimicrobial therapy after detection with VYOO[®] resulted in improved clinical biomarkers in these patients including a drop in serum C-reactive protein and serum procalcitonin within four days. In a second interventional study, VYOO[®] was evaluated for the impact on treatment in patients with suspected *candidemia* (69). Importantly, of the samples that were found to be positive, the treating physician added antimycotic therapies much more quickly (31 vs. 68 hours) in those detected by VYOO[®] compared to those discovered via blood cultures. Although the sample

size was low, this study demonstrates the potential utility of multiplex molecular diagnostics to initiate early therapy (69).

VYOO® has been used in another study in sepsis, also examining its ability to predict patient outcome. Although 70% of patients with suspected sepsis showed positive concordant results between blood cultures and VYOO®, the DNA detected by VYOO® did not correlate with disease severity or clinical outcome (70). This is in contrast to several studies using SeptiFast® which have showed correlations between assay results and clinical course (34–37).

In summary, the VYOO® technology shows excellent diagnostic potential and is comparable to SeptiFast®, but also has the capacity to detect antibiotic resistance genes. While there are only three studies currently published with VYOO®, two of them are unique because the clinicians demonstrated that the results could be used to improve patient care. One study showed its utility in the escalation and de-escalation of antibiotic therapy (68), and the other demonstrated shorter time to initiation of anti-fungal treatment (69). One potential barrier to clinical acceptance is time and technical training required for DNA purification and for running and analyzing the gel electrophoresis.

3.4 Clinical Implications for Therapy

The three multiplex technologies presented here, SeptiFast®, SepsisTest®, and VYOO®, can produce valuable clinical information quickly. Since they do not require growth of the microorganisms via blood culture, they can provide results several days sooner than conventional cultures allowing the correct anti-microbial drug to be administered earlier in sepsis. This includes the potential escalation or de-escalation of appropriate antimicrobial therapy, as well as the possibility of pinpointing resistance organisms, thus allowing for guiding alternative therapy regimes. These technologies are also useful for understanding the organisms involved in polymicrobial infections in sepsis (71). It is important to mention that all three technologies require technical expertise, which make them less accessible in small hospital settings with limited technical staffing in the evening and on weekends. Two of these molecular multiplex technologies use pre-processing of the samples for removal of human genomic DNA to improve the detection of low levels of pathogenic DNA.

Many of the published studies for these technologies suggest that they can be quite valuable in a setting of suspected sepsis. Their high sensitivity is also likely to be valuable in conditions demonstrating culture-negative results such as infective endocarditis. Compared to blood cultures, these technologies are minimally confounded by antimicrobial therapy, and may be useful in tracking the course of the disease. Total microbial DNA detected by SepsisFast® in the absence of positive blood cultures was found to correlate with disease activity in a multicenter analysis of patients with sepsis (35). However, further studies are needed to explore this relationship.

Despite encouraging results with these multiplex technologies, none of them have been approved in the US. Clearly, multicenter validation trials are necessary to determine their reliability and performance. A major issue with these and any future trials is the comparison to blood cultures as the gold standard; however, additional interventional trials may produce compelling clinical results independent of these metrics with the results of these technologies compared directly to patient outcome. Lastly, blood cultures will still remain essential in the near future, but we foresee the potential integration of these technologies into standard clinical practice.

6. Conclusions

In this review, six commercial molecular multiplex technologies are discussed that show promise for the rapid and comprehensive diagnosis of microorganisms involved in sepsis. Despite their diagnostic potential, there are two major barriers to implementing these technologies into clinical medicine. The first is the lack of randomized controlled clinical studies demonstrating that the molecular diagnostic information gained from these technologies can be used to directly improve patient care. While each study has merits, none have evaluated the full potential of these technologies in a prospective manner. The second barrier, not addressed in this review, is the cost for implementing these technologies, including expenses for equipment, reagents, and personnel available 24 hours per day for adequate utilization of these technologies in a clinical setting.

The first group of three technologies, Prove-it™, Verigene®, and FilmArray®, is directed at improving the detection of microorganisms from positive blood cultures. Although there are only a few publications examining each of these technologies, all report high diagnostic performance in detecting pathogens and drug resistance markers as long as the organisms are present in their panels. It is likely that the spectrum of pathogens screened will only improve, increasing their overall sensitivity and clinical utility. A key advantage of all three technologies is that they require minimal hands-on time to produce diagnostic results quickly after positive blood cultures become available. Perhaps the best example of this is FilmArray®, which requires 3 minutes of hands-on time and produces results within 1 hour. However, because of their dependence, it is effectively impossible for any of these molecular technologies to fully supplant blood cultures. Based on their compelling potential, they could be used in conjunction to provide additional and faster diagnostic information to alter therapy in some cases.

To examine clinical blood samples directly, the second group of technologies, SeptiFast®, SepsiTtest®, and VYOO®, utilize ultrasensitive detection methodologies that provide microbiological information days earlier than blood culture results. At present, VYOO® and SepsiTtest® require gel electrophoresis and DNA sequencing, respectively, to obtain diagnostic results. Gel electrophoresis is a procedure not often performed outside a research laboratory and interpretation of bands for identification is also quite subjective. Sequencing is even more complex and will also require specialized expertise not commonly associated with most hospital settings as well as additional time for analysis which might further delay diagnosis and/or treatment.

In future, it is also likely that these technologies directly screening clinical samples for microbes will become further simplified including integration of the numerous steps currently required into a single streamlined device. In contrast to employing only a single clinical sample from one time point, future studies might involve reiterative and parallel testing, which would allow infections to be monitored in real time. One could foresee a future clinical trial in which repeat multiplex assays are used to track a patient throughout the course of their disease. This may become more practical as these technologies become simpler and less expensive. With the ability to generate large amount of molecular information, these molecular approaches have the potential to become the new gold standard for diagnosis. It is also possible that other technologies including next-generation high throughput DNA sequencing and PCR ESI-MS will be developed for routine pathogen diagnosis (72, 73). With the need to detect drug resistant microbes and the ability of single point mutations to change microbial resistance, future technologies based on next-generation-sequencing techniques may be the ultimate diagnostic technology.

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Table 1

Characteristics of the Commercial Technologies

Technology	Sample	Technology	Pathogens	Abx Resistance Genes	Pre-Analysis	Hands On Time	Total Assay Time
Prove-it®	Positive Blood culture	Array	60 bacteria + 13 fungi	<i>mecA</i> , <i>vanA/B</i>	DNA extraction	90 min	3.5 hr
Verigene®	Positive Blood culture	Array	13 grampositive bacteria	<i>mecA</i> , <i>vanA/B</i>	DNA extraction w/Verigene® Processor SP	< 5 min	2.5 hr
FilmArray®	Positive Blood culture	Real Time PCR	24 bacteria and fungi	<i>mecA</i> , <i>vanA/B</i> , <i>KPC</i>	None	5 min	1 hr
SeptiFast®	Whole blood	Real Time PCR	25 bacteria and fungi	None	DNA extraction w/MagNA Lyser	3 hr	4.5 hr
SeptiTest®	Whole blood	PCR + sequencing	345 bacteria and fungi	None	DNA extraction w/SelectNA™	2.5 hr	> 9 hrs = 4 hr + sequencing
VOO®	Whole blood	PCR + electrophoresis	39 bacteria and fungi	<i>mecA</i> , <i>vanA/B</i> , <i>KPC</i> , <i>blaSHV</i> , <i>blaCTX-M</i>	DNA extraction via Looxster®	70 min	7 hr

1. <http://www.mobidiag.com>
2. <http://www.nanosphere.us>
3. <http://www.biofire.com>
4. <http://molecular.roche.com>
5. <http://www.sepsitest.com>
6. <http://www.sirs-lab.de>