Rapid Identification of Sepsis Pathogens Using Real-Time Nested Multiplex PCR

INTRODUCTION

Sepsis is a leading cause of morbidity and mortality in the U.S. and the world. Rapid initiation of appropriate antibiotic therapy is crucial. Current methods of pathogen identification are slow, leading to delays in diagnosis. Blood cultures can take 24-48 hours to become positive. Antibiotics are often administered empirically, which can lead to the emergence of antibiotic-resistant strains.

METHODS

For bacterial and fungal (candida) detection and identification the FilmArray Sepsis System will employ a nested-multiplex PCR strategy described in Figure 3. PCR primer sets are designed using the NCBI database. Each primer set amplifies a unique product of the first-stage multiplex PCR, which can be visualized by melt curve analysis. The FilmArray Sepsis System includes a real time PCR instrument and novel thin film pouch. Together, they perform automated nucleic acid extraction, purification, and nested multiplex PCR to analyze up to 102 nucleic acid targets from a single sample in ~1 h.

For bacterial and fungal (candida) detection and identification the FilmArray Sepsis System will employ a nested-multiplex PCR strategy described in Figure 3. Oligonucleotide primers are designed to be species-specific. Assays for virulence targets are made in multiplex reactions using traditional real time PCR instruments. Inner PCR primers were tested as singleplex reactions only. Primers with high affinity were selected for the FilmArray.

Preliminary testing with 60 clinical samples from blood culture bottles demonstrated accurate identification of bacteria in 100% (60/60) of positive blood cultures when compared to standard microbiology testing. Testing of culture-negative samples showed no amplification over 24 h. The FilmArray Sepsis System is a novel tool for the detection and identification of sepsis-causing pathogens in clinical samples positive for blood culture. The FilmArray allows each sample to be evaluated for a large number of pathogens simultaneously. Preliminary testing demonstrates the utility of this system in the rapid identification of pathogens from positive blood cultures.

CONCLUSIONS

1. Nested multiplex PCR targeting of housekeeping genes and virulence factors can accurately identify bacteria from positive blood cultures.
2. Nested multiplex PCR targeting antibiotic resistance genes can accurately identify resistant bacterial isolates.
3. The FilmArray® Sepsis System will be an excellent tool for rapid identification of pathogens in positive blood cultures and will decrease the time between a positive culture and full ID.

Figure 2: Schematic of Nested Multiplex PCR

A large-scale multiplex PCR platform has been developed for the first time on this scale in the field of infectious disease. The platforms (above) allow for the detection of up to 102 nucleic acid targets from a single sample in ~1 h. The FilmArray platform uses a novel thin-film plastic pouch that contains all needed freeze-dried reagents.

Figure 3: Proposed Integration of FilmArray® into Current Blood Culture Procedures

Blood Draw Blood Culture

Positive (growth)

Gram Stain

1 Hour

FilmArray Sepsis System

PCR bacterial identification

Negative (no growth)

1-2 Days

Traditional Bacterial Identification (growing on culture plates)

Blood culture organisms are identified using FilmArray® technology, offering superior time to result compared to conventional methods. The FilmArray® technology is a rapid, high-throughput, automated, high-confidence system for detecting and identifying bloodstream pathogens. The FilmArray® technology is ILI's solution to the urgent need for faster, more accurate identification of bloodstream pathogens. The FilmArray® technology is designed to be used with blood cultures from patients with suspected sepsis.