INTRODUCTION
Sepsis is a leading cause of morbidity and mortality in the U.S. and the world. Sepsis in the U.S. has been estimated to be responsible for healthcare costs of $17.6 billion per year, and is the 10th leading cause of death, with as many as 225,000 deaths per year. While sepsis can be effectively treated with antimicrobial agents, rapid response is crucial. For example, after onset of hypotension, survival drops by 5% per hour after the first 6 hours. Current diagnosis of sepsis is often delayed. A patient can take 24-48 hours to become positive, which are followed by Gram staining, then culture for the isolate on solid media followed by biochemical testing. Overall, it can take up to 3-7 days to fully identify a sepsis-causing organism. Molecular diagnostics involving PCR for the detection of multiple pathogens provides more rapid results; however, current systems have not been FDA cleared and are difficult and costly to operate.

In response to the need for a diagnostic system that is rapid, multiplexed, and streamlined, FilmArray has developed the FilmArray Sepsis system. The FilmArray will have several advantages over conventional culture-based detection. First and foremost, it will allow rapid detection and identification of pathogens that are associated with sepsis and other systemic inflammatory response syndrome (SIRS) illnesses. These results suggest that the FilmArray system is a promising tool for the identification and demography of pathogens causing infection in blood streams.

METHODS

For bacterial detection and identification the FilmArray Sepsis System will employ a nested-multiplex PCR strategy described in Figure 2. Outer primers are designed to be broad-range and extend beyond the species-specific targets. These primers are used for the detection and identification of 13 organisms and the staphylococcal resistance gene, mecA. These targets have been tested in the FilmArray, and show specific and sensitive detection of pathogen nucleic acid templates. In addition, blood culture samples positive for a variety of pathogens included in the panel were tested. Pathogen identification was confirmed by high-resolution melt profiling. The FilmArray software call is “mecA detected”, as mecA is sometimes located in some coagulase-negative staphylococci.

The FilmArray pouch contains the film array, which consists of 150 microfluidic channels, microporous membranes, and disposable, thin-film plastic disposables, all assembled in a self-contained, fluid manipulation system per a-pouch system called “Fil-A”. During sample preparation, patient blood is placed on solid media, followed by biochemical testing. Overall, it can take up to 1-4 hours to perform all steps of the assay, from nucleic acid extraction to nested multiplex PCR (nmPCR) and data analysis.

CONCLUSIONS

These data show proof-of-principle that nmPCR targeting of conserved housekeeping genes and virulence genes can accurately identify bacteria involved in sepsis. Broad-range outer primers provide the potential to easily modify the inner targets for different patient populations. When fully developed the FilmArray Sepsis System will provide a tool for the rapid and accurate evaluation of bacteria in blood cultures.

Rapid Automated Multiplex PCR Diagnostics for Blood Pathogens

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ABSTRACT

Sepsis, the syndrome of infections leading to a systemic inflammatory response, is a leading cause of death in the U.S. Delay in diagnosis and treatment of appropriate therapy is the most common contributor to increased mortality and morbidity. Current microbiological methods used to identify responsible pathogens are slow and tedious, requiring days to result. As a large number of pathogens can cause sepsis, broad spectrum antibiosis therapy must be initiated, and continued until an isolate is ruled out or a pathogen is identified. Recently, molecular tests, such as those using polymerase chain reaction (PCR) have emerged and shown promise for rapid diagnosis of infectious diseases. Primarily due to complexity and cost, these have not been widely adopted.

Idaho Technology, Inc. (ITI) has developed the FilmArray™ System (Figure 1), an innovative molecular diagnostic device that streamlines pathogen identification in human samples. The FilmArray System can simultaneously identify up to 32 organisms in ~1 hour, and requires only a minimally trained operator to perform the test. The FilmArray System comprises a uniquely designed ‘lab-in-a-pouch’ and a custom-built instrument which together automatically perform all steps of the assay, from nucleic acid extraction to nested multiplex PCR (nmPCR) and data analysis.

ITI’s objective is to develop the FilmArray Sepsis System to detect and identify 22 sepsis-causing pathogens from blood cultures. Toward this end, we have designed nmPCR assays for both bacterial housekeeping genes and species-specific virulence targets. First, we performed the detection and identification of 13 organisms and the staphylococcal resistance gene, mecA. These targets have been tested in the FilmArray, and show specific and sensitive detection of pathogen nucleic acid templates. In addition, blood culture samples positive for a variety of pathogens included in the panel were tested. Pathogen identification was confirmed by high-resolution melt profiling. The FilmArray software call is “mecA detected”, as mecA is sometimes located in some coagulase-negative staphylococci.

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