TITLE: PCR/ARRAY LAB-ON-A-CHIP DEVICE FOR RAPID DIAGNOSIS OF SEPSIS-CAUSING PATHOGENS

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ABSTRACT

Sepsis is the multiple organ dysfunction syndrome caused by an irregular body inflammatory response to infection. It is the major cause of death in intensive care units worldwide, which is associated to the lack of appropriate and timely diagnosis and treatment. Blood culturing is the gold standard technique for blood stream infection (BSI) diagnosis. However, it generally takes 5 to 7 days to a result. Furthermore, antimicrobial administration prior to blood collection and fastidious pathogens render this method unsuitable to guide proper therapy. Advances in molecular biology offer more sensitive and faster alternatives. According to the literature, PCR-based tests directly from whole blood, without prior incubation or culture, are considered the most promising among them. Here we developed a PCR/Array multitest that allies PCR amplification and hybridization of nucleic acids on a chip, coupled to an automated microbial DNA enrichment and purification solution direct from patient's whole blood. Microorganisms covered in the panel were listed by specialists according to the national epidemiology data. Genome sequences from the microorganisms on the test's panel from the databanks plus sequenced samples were retrieved and aligned for primers and probes design. Primers were generated to target and amplify conserved regions spanning internal variability where probes were designed to detect and identify each species. Primers and probes as well as PCR amplification and hybridization protocols were optimized using DNA purified from cultured pathogens previously characterized. Analytical specificity and sensitivity were investigated using DNA purified from whole blood artificially spiked with known cell density of pathogens. The prototype showed the ability to detect and identify 22 species of bacteria and 5 species of *Candida*. Additionally, probes were added to differentiate GramN and GramP bacteria and *Candida* spp. The detection limit for the tested microorganisms is between 10 and 100 CFU/mL, which has clinical relevance considering reported bacteria density in peripheral blood of septic patients. The test may provide faster and more accurate detection of the sepsis-causing agents, and allow a more efficient treatment before progression to septic shock and potentially contribute to reduce mortality rates. It also has the benefit to promote antimicrobial stewardship that lowers toxicity, reduces treatment costs and limits the selection of resistant strains.

Keywords: Sepsis, Blood Stream Infection, Diagnosis, PCR-Array

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