Multiplex PCR system for fungal pathogen detection

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Introduction: The rapid and correct identification of pathogenic species is crucial for several reasons that differ according to the area of interest. In clinical laboratories it is important since knowing the pathogen guides appropriate treatment, dose and duration of therapy. In clinical microbiology laboratories culture and microscopic examination remain the "gold standard". Biochemical and mass spectometry identification systems, such as the Vitek systems (BioMerieux®) or MALDI-TOF are examples of commercial systems for microbial identification with the advantage of being semi automatic. However, these methods are based on the regrowth and isolation of the microorganism from samples, which in some cases, such as from blood, is time consuming and the success rate of cultivation can be as low as 20%. Recently, several techniques based on the polymerase chain reaction (PCR) have been developed for microbial pathogenic detection and identification particularly Real-Time PCR methods. In this study we developed an alternative PCR based method for the detection of fungal pathogens involved in systemic infections. This is a multiplex PCR method that attributes to each species specific amplicon lengths and a fluoresce dye, according to previously design panels, enabling a fast and reproducible co-amplifying of several loci in a single PCR reaction.
Materials and methods: First a panel for species identification, combining fluorescence with molecular weight of specific PCR fragments for *Candida* and *Asprgillus* was designed. The PCR fragments obtained are then analysed by capillary electrophoresis and GeneScan fragment analysis. This methodology was optimized using DNA extracted from strains previously identified and, in order to optimize the methodology to clinical samples, we also used serum from healthy donors spiked with different concentrations of fungal DNA. Then the method was tested in DNA extracted from different types of clinical samples, including blood, biopsies and bronchoalveolar lavages of patients with invasive fungal infections (IFI).
Results: The optimization of the method, by using DNA from known strains belonging to the target species and strains form other species, showed 100% of specificity. The calculated yield from DNA extracted from serum spiked with fungal DNA was of around 80%. This DNA was then used to determine the sensitivity of the technique and results showed that we were able to obtain amplification products within a range of 1 to 10 pg of the total DNA extracted. Results obtained with DNA extracted from samples of IFI patients, showed that we were able to detect the specific fungal species in 75% of the samples. Several optimizations are being performed.
Discussion and conclusions: This new methodology is a promising method since interpretation of results is easy, based on presence/absence of a particular peak of the panel, it is fast, accurate and reproducible and due to the design of the identification panel it is able to identify the pathogenic fungal species involved in mixed infections. Several optimizations are being performed to enhance sensitivity of the method.

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