Abstract

The increasing incidence of invasive fungal infections which are associated with significant morbidity and mortality in immunocompromised patients and critically ill patients and the recent availability of expensive antifungal agents with better safety profiles, emphasized the need to improve the currently limited diagnostic tools. We have established the Light-Cycler PCR and detection system (Roche Diagnostics, Mannheim, Germany) that specifically amplified a DNA fragment between inter-spacer 1 (ITS1) region and ITS2 region. Methods for extraction of DNA of fungi from whole blood were established which was designed to isolate DNA from yeasts and molds and to avoid contamination from common reagents. Using whole blood spiked with serial dilution of yeast suspension, the sensitivity of this assay was determined. Up to one colony-forming unit of Candida per mL of whole blood was detectable. A total of 58 positive blood cultures containing yeasts were analyzed by the real-time PCR for species identification. From these blood cultures, 59 strains of yeasts were isolated, which included C. albicans (17 strains) , C. tropicalis (10 strains), C. glabrata (8 strains), C. parapsilosis (7 strains), C. neoformans (7 strains), and C. krusei (10 strains). All strains of the above species were correctly identified, resulting in a test sensitivity of 100% for each of the above seven species. Coexisting bacteria in blood specimens did not produce any detectable PCR products and did not interfere with yeast identification. To apply this PCR assay in the clinical practice, several issues were important and discussed. In conclusion, the real-time Light-Cycler PCR assay combines rapid in vitro amplification of DNA with real-time species determination and quantification. This method is simple, rapid, sensitive, and cost-effective and seems to be very promising.

Keywords：Real-Time Light-Cycler Polymerase Chain Reaction；serological diagnosis；invasive fungal infection；Candida