Abstract

The aim was to develop a LightCycler PCR method for the rapid detection of fungal DNA in clinical samples such as whole blood and cerebral spinal fluids. LightCycler PCR assays were established for 6 species-specific primer sets and 2 pan-fungal primer sets. For each primer set LightCycler melting points were defined by amplification of DNA from 21 reference strains and clinical isolates. The sensitivity was determined by amplification of serial dilutions of fungal DNA or fungal cells spiked in whole blood. Analysis of clinical samples showed positive results in all of 58 blood cultures bottles positive of different *Candida* or Cryptococcus neoformans, 18 of 19 whole blood (all culture negative) collected from 4 patients with disseminated cryptococcosis, and 33 of 35 cerebrospinal fluids (11 culture positive) from 5 patients with cryptococcal meningitis and elevated intracranial pressure. The copy numbers of PCR-positive clinical samples ranged from 9 to 553,130 per mL. Thus the LightCycler PCR protocol established here represents a rapid diagnostic tool that may aid in the diagnosis of invasive fungal infections and quantitation of fungal load in clinical samples.

Keyword : Real-time Light-Cycler PCR, Candida, Cryptococcus