PCR diagnosis of tinea unguium with specific detection of Trichophyton rubrum on clinical samples

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Dermatophytes are keratinolytic fungi responsible for skin infections. The affinity for keratinised tissues implies that the infection remains restricted to the cornified layers of the skin, nails, and hair. Onychomycosis is the general term used for fungal infection of the nails and dermatophytes, non-dermatophytes moulds or yeasts are causative agents. However, tinea unguium is the term more appropriate when mycological diagnosis confirms that the agent is a dermatophyte being Trichophyton rubrum the most frequently isolated species all over the world. Conventional methods routinely used in laboratory for mycological analysis included both direct microscopic examination and cultures. Therefore the confirmation of the etiologic agent relies on macroscopic and microscopic morphology. This approach is time-consuming (3-4 weeks); it requires specialized staff and frequently delays the treatment. Thus false-positives and false-negatives are reported in a high percentage of cases. Alternatively molecular-based methods have been proposed by some authors. Amplification of DNA sequences specific for certain classes of fungi by PCR allows the identification of the etiologic agent most likely in about two days. In this study we applied the multiplex PCR-based method developed by Brillowska-Dabrowska et al. (2007) directly from 96 nail specimens clinically suspected of onychomycosis and comparison with conventional mycological analysis was performed. PCR diagnosis was positive for dermatophytes for 43 samples and in 91% T. rubrum was the etiologic agent. Nineteen samples that could not grow in culture media were found positive for tinea unguium and from those 17 (90%) T. rubrum was present. The application of multiplex PCR-based diagnosis and the T. rubrum detection allow obtaining a rapid, specific and low-cost diagnosis for tinea unguium in routine clinical laboratory.