

The Fast Diagnosis of Acanthamoeba Keratitis Using Real-Time PCR Assay

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Abstract : Acanthamoeba genus belongs to kingdom protozoa, and it is known as free-living amoebae. Acanthamoeba genus has been isolated from human bodies, swimming pools, bottled mineral water, contact lens solutions, dust, and soil. The members of the genus Acanthamoeba causes Acanthamoeba Keratitis which is a painful sight-threatening disease of the eyes. In recent years, the prevalence of Acanthamoeba keratitis has been high rate reported. The eight different Acanthamoeba species are known to be effective in Acanthamoeba keratitis. These species are Acanthamoeba castellanii, Acanthamoeba polyphaga, Acanthamoeba griffini, Acanthamoeba hatchetti, Acanthamoeba culbertsoni and Acanthamoeba rhyssodes. The conventional diagnosis of Acanthamoeba Keratitis has relied on cytological preparations and growth of Acanthamoeba in culture. However molecular methods such as real-time PCR has been found to be more sensitive. The real-time PCR has now emerged as an effective method for more rapid testing for the diagnosis of infectious disease in decade. Therefore, a real-time PCR assay for the detection of Acanthamoeba keratitis and Acanthamoeba species have been developed in this study. The 18S rRNA sequences from Acanthamoeba species were obtained from National Center for Biotechnology Information and sequences were aligned with MEGA 6 programme. Primers and probe were designed using Custom Primers-OligoPerfectTMDesigner (ThermoFisherScientific, Waltham, MA, USA). They were also assayed for hairpin formation and degree of primer-dimer formation with Multiple Primer Analyzer (ThermoFisherScientific, Watham, MA, USA). The eight different ATCC Acanthamoeba species were obtained, and DNA was extracted using the Qiagen Mini DNA extraction kit (Qiagen, Hilden, Germany). The DNA of Acanthamoeba species were analyzed using newly designed primer and probe set in real-time PCR assay. The early definitive laboratory diagnosis of Acanthamoeba Keratitis and the rapid initiation of suitable therapy is necessary for clinical prognosis. The results of the study have been showed that new primer and probes could be used for detection and distinguish for Acanthamoeba species. These new developing methods are helpful for diagnosis of Acanthamoeba Keratitis.

Keywords : Acanthamoeba Keratitis, Acanthamoeba species, fast diagnosis, Real-Time PCR

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