Abstract

The laboratory of Vector-borne viral and Rickettsial diseases in the Center of Research and Diagnostics is responsible for the laboratory diagnostics of various reportable Rickettsial diseases in CDC Taiwan. Due to increased international traffic exchanges and global warmer climate, the spread of vector-borne diseases have expanded rapidly in whole world with increased intensity and severity. Similarly, the tendency of these infectious diseases has increased in Taiwan in recent years. Although molecular diagnosis based on polymerase chain reaction (PCR) method had been developed for years, laboratory diagnosis of Rickettsial infections was largely relied on serological assay detecting antibody serum conversion between acute and convalescent phase serum samples. The main obstacle in adopting PCR for routine laboratory diagnosis is that traditional PCR method is less reliable due to sample contamination in the running process. In addition, although assay sensitivity could be improved by using nested PCR method, much longer time (usually more than 12 hours) was needed. In this study, we reported the development of a real time SYBR Green I-based quantitative PCR system that can be used to rapidly detect Rickettsial infections in acute-phase blood samples. For scrub fever, two sets of O. tsutsugamushi-specific primer pairs against conserved sequences in the 56kDa, type-specific antigen gene and groEL gene were successfully designed and evaluated for clinical diagnosis in a total of 600 acute phase whole blood samples. The amplification product from real time SYBR Green I-based quantitative PCR can be further sequenced to differentiate various serotypes of O. tsutsugamushi. For typhus fever, primer pairs against conserved sequences in the groEL gene and 17 kDa gene, were successfully designed to detect both epidemic and endemic typhus infections. The amplification product from real time SYBR Green I-based quantitative PCR can then be sequenced to differentiate epidemic or endemic typhus infection. For Q fever, primer pairs against conserved sequences in the com-1 gene and conserved region of plasmid (HF 1 & HF 2), were successfully designed to detect Q fever infection. In the future, real time quantitative PCR will replace traditional Nested PCR method gradually and become the new standard of molecular diagnostic method. This improvement will have great impact on the clinical treatment of patients with Rickettsial infections.

Key words: Rickettsia, , Real-Time SYBR Green I-based PCR, scrub typhus, endemic typhus, Q fever