Abstract

Yellow fever, dengue fever, and Japanese encephalitis are three reportable infectious diseases in Taiwan belong to the yellow fever, dengue fever and Japanese encephalitis subgroups of flaviviruses, respectively. Development of rapid, molecular diagnostic system to monitor these already existing (dengue and Japanese encephalitis) and other potentially invading emerging infectious diseases (such as yellow fever, West Nile fever/encephalitis) is important. We had recently successfully developed a SYBR Green I-based quantitative one-step real-time RT-PCR assay for routine diagnosis of various flavivirus infections. This assay system had the advantages of rapidity, high sensitivity, high specificity and low contamination rate in the differential diagnosis of various flavivirus. To further improve the assay efficiency of real-time RT-PCR assay, a four colors multiplex real-time PCR system based on TaqMan amplification was developed to simultaneously detect and differentiate four different viruses in a single tube. Two sets of four colors multiplex real-time PCR systems were developed. The flavivirus-specific assay can detect and differentiate dengue virus, Japanese encephalitis virus, yellow fever virus, and West Nile virus, while dengue virus-specific assay can detect and differentiate four different dengue virus serotypes. Isolated flaviviruses and confirmed serum samples from flavivirus-infected patients reported to CDC, Taiwan were used to evaluate the specificity and sensitivity of these two multiplex real-time PCR assays. The results showed that the multiplex four colors real-time PCR assay constitutes a specific and sensitive alternative to SYBR Green I-based quantitative one-step real-time RT-PCR assay. Specific and sensitive results within 6 hours are important in a clinical setting, and therefore, this assay could improve patient management by appropriate therapy following rapid diagnosis of a viral infection. The enhanced laboratory capacity to handle large amount of serum samples due to multiplex PCR assay in a single tube could greatly improved the assay efficiency in an outbreak and contribute significantly to the prevention and control measures.

Keywords：flavivirus；multiplex；molecular diagnosis；PCR