Abstract

As a national laboratory responsible for routine diagnosis of reported cases of dengue fever (DF), Japanese encephalitis (JE), Yellow fever (YF), and hantavirus diseases, we have set up a diagnostic laboratory with the capability to perform routine tests including cell culture and virus isolation, molecular diagnosis, serological diagnosis, and molecular epidemiology. For rapid detection and differentiation of dengue and JE viruses, a diagnostic system was developed and applied to daily routine. For acute-phase sera, virus isolation by cell culture and real-time one-step reverse transcription-polymerase chain reaction (RT-PCR) were performed. For all of the sera reported, serological diagnosis of specific antibodies based on envelope and membrane (E/M)-specific capture IgM and IgG enzyme-linked immunosorbent assay (ELISA) were tested. In this report, a quantitative one-step SYBR Green I reverse transcription-polymerase chain reaction (RT-PCR) system was developed for the detection and differentiation of four different dengue virus serotypes in acute-phase serum samples. A set of group- and serotype-specific primer pairs was designed against conserved sequences in the core region and evaluated for clinical diagnosis. A linear relationship was obtained between the amount of input RNA and cycle threshold (Ct) value over a range of 10 to 107 plaque forming units (PFU) per milliliter of cell culture-derived dengue viruses. The detection limit of group-specific primer pair was between 4.1-43.5 PFU/ml for four dengue serotypes. The detection limit of each of the serotype-specific primer pairs was calculated to be 10 PFU/ml for DEN-1, 4.6 PFU /ml for DEN-2, 4.1 PFU/ml for DEN-3, and 5 PFU/ml for DEN-4. Comparison between one-step SYBR Green RT-PCR and conventional cell culture method in the clinical diagnosis of dengue virus infection from acute phase serum samples of confirmed dengue patients were performed. The results showed that 83% vs. 67% out of 193 acute phase serum samples tested were positive for one-step SYBR Green RT-PCR and cell culture method, respectively. Further analysis showed that one-step SYBR Green RT-PCR could detect twice much more acute phase serum samples with positive dengue-specific IgM and/or IgG antibodies than cell culture method. Our results demonstrate the potential clinical application of one-step SYBR Green I RT-PCR for the detection and differentiation of dengue virus RNA. Based on the combined analyses of Real-time one-step RT-PCR and E/M-specific Capture IgM and IgG ELISA, 95% of acute phase sera from confirmed cases can be identified as positive or probable cases within 24-48 hours of receiving serum samples. Therefore, we recommend that the insecticide spray will be hold for suspected cases unless laboratory diagnosis comes out positive or probable in areas with no indigenous dengue cases.

Keywords：vector-borne infectious diseases；real-time quantitative PCR；Flavivirus