

Japanese Encephalitis Virus Transmitted Via Blood Transfusion, Hong Kong, China

Technical Appendix

One-Step Real-Time Reverse Transcription PCR

To detect Japanese encephalitis virus (JEV), we used RealTime ready RNA Virus Master kit (Roche, Indianapolis, Indiana, USA) and real-time PCR machine LC2.0 (Roche). We used the following primers and probe: JE-F(e) (5'-GGAGCTGGATGGAATGTGAA-3', 10 μ M); JE-R(e) (5'-TCCCTCCGATGGAAGTATAGAA-3', 10 μ M); and JE-P(e) (6-FAM-CCAAAGCGTATGCACAGATGTGGC-BBQ-650, 5 μ M). Equine herpes virus primers, probes, and DNA were also included in reactions as a DNA internal control. The thermocycler conditions for the real-time reverse transcription PCR (RT-PCR) were as follows: reverse transcription at 50°C for 15 min; heat inactivation at 95°C for 30 s; 45 cycles of denaturation at 95°C for 1 s, annealing at 55°C for 20 s, and extension at 72°C for 5 s; and cooling at 40°C for 30 s.

The limit of detection of our assay (using a -7 dilution of cell culture extract, cycle threshold (C_t) 36.10–37.50; using a -8 dilution of cell culture extract, C_t 42.60 to undetectable) is comparable with that of the 1-step conventional nested RT-PCR for detection of flaviviruses when tested by using a -8 serial dilution of JEV RNA extracted from a mosquito isolate grown in C6/36 cell culture. Cross reactivity to dengue virus serotypes 1, 2, 3, and 4; chikungunya virus; and West Nile virus was not detected. Archived samples previously positive for JEV (3

mosquito isolates and 2 clinical specimens [brain tissue and cerebrospinal fluid]) were all positive by our in-house assay, and 51 JEV-negative archived RNA samples taken from cerebrospinal fluid (3 herpes simplex virus 2-positive, 3 varicella zoster virus-positive, and 45 negative) were retested and still negative by our in-house test. The 1-step real time RT-PCR with internal control has comparable limit of detection as the 1-step conventional nested RT-PCR for detection of flaviviruses and was adopted for routine use in 2014.