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Detection of JEV and WNV by quantitative real-time reverse-transcription (qRT)-PCR

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ABSTRACT

you can use this protocol to detect the RNA of your sample

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MATERIALS TEXT

RNA of mosquito grinding supernatant , serum sample and CSF samples,AgPath-IDTM One-step RT-PCR Kit (AM1005, Thermo Fisher Scientific, USA) ,Stratagene real-time PCR instrument (model MX300; Thermo Fisher Scientific, Waltham, MA, USA) .

BEFORE STARTING

RNA was extracted from all collected samples (serum, CSF, and mosquito grinding supernatant) using a Tianlong Nucleic Acid Automatic Extractor with the Tianlong Nucleic Acid Extraction Kit(EX-RNA/DNA virus, Suzhou Tianlong Biotechnology Co., Ltd, Suzhou, China).

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1 The instrument used was a Stratagene ™, real time PCR machine (manufacturer: Thermo Scientific, model: MX300) for quantitative fluorescence qRT-PCR detection of JE virus and West Nile virus. AgPath-IDTMOne-step RT-PCR Kit (REF: AM1005, manufacturer: Thermo Fisher Scientific.) was the detection kit used, the total volume of the detection system was 20 µl, containing 10 µl of 2Xbuffer4 µl of RNAse Free Water4 µl, 1 µl of primers, probes, and enzyme mixture (Mix), and 2 µl of template RNA.

2 The reaction program was: 45 ° C for 10 min, 95 ° C for 10 min for 1 cycle, 95 ° C for 15 s and 60 ° C for 1 min for 40 cycles. Using primers and probes specific for the JE virus gene and the West Nile virus gene.

3 The final result is checked on the real time PCR machine,the sample with CT value over 35 thought to be negetive, sample with CT value lower than 35 thought to be positive.

4 The positive sample will be sent to do PCR specific to JEV genes to be ferther tested.

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