Molecular serotyping of dengue viruses in field-caught Aedes mosquitoes by in-house RNA extraction/RT-PCR reagent kits


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Abstract

We developed in-house RNA extraction and RT-PCR reagent kits for the molecular serotyping of dengue viruses in field-caught Aedes mosquitoes. Mosquitos that showed positive results by ELISA or IFA were selected for the identification of dengue viruses in order to predict the distribution of the four dengue serotypes. Total RNA was extracted from one whole mosquito as well as from one dissected mosquito by our in-house RNA extraction reagents using the modified method of guanidinium thiocyanate denaturation and isopropanol precipitation. The extracted RNA was amplified by our in-house RT-PCR reagents specific for each dengue serotype under optimized conditions. Dengue viral RNA extracted from a single mosquito as well as from the head and thorax of one dissected mosquito could be detected successfully; it could not be found in the abdomen, however. These results indicated that most of the dengue viruses were located in the head and thorax rather than in the abdomen. The results of dengue serotyping showed a pure specific PCR product for each dengue serotype at 490, 230, 320 and 398 bp for DEN-1, DEN-2, DEN-3, and DEN-4 respectively. In addition, the detection sensitivity was very high: an amount of RNA template equivalent to approximately 1/80 of a single mosquito could be detected by agarose gel electrophoresis and ethidium bromide staining. The coupling of RT-PCR-based surveillance of dengue viral infection with disease mapping data (Geographical Information System, GIS) could serve as an alternative epidemiological means of providing an early warning of dengue fever/dengue hemorrhagic fever epidemics.

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