Thesis Title	Detection for Dengue Virus Infection Using Suckling Mice as a Biological Amplification System and Western blot/ Enzymeimmunoassay
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Abstract

Detection for dengue viral proteins in patients' sera with dengue virus infection was investigated by using suckling mouse as a biological amplification system evaluated in conjunction with the Viral antigens strip/ enzymeimmunoassay (VAS/EIA). Dengue viral proteins can not be detected directly from patients'sera by VAS/EIA method due to the high concentration of other serum proteins in human plasma. Therefore, an additional step must be inserted in order to facilitate the use of VAS/EIA. Classical biological amplification system using dengue type 2 virus inoculated intracerebrally to suckling mouse brain was used as the first step to amplify the number of virion circulated in patients' sera. Dengue type 2, new guinea C strain, was employed for titration. Two groups of patients' sera with clinical diagnosis of dengue infection collected

from Ramathibodi hospital were tested for this study. Three dilutions, 1:1, 1:3 and 1:10 of patients' sera were compared to select the proper serum dilution for an inoculation. The signs and symptoms of infected mice were closely observed. After inoculation, they were sacrified on day 5, 6, and 7. Infected mouse organs including the brain, spleen, liver, and also plasma were examined for the presence of dengue viral proteins by VAS/EIA.

The best dilution for serum specimen to be used for an inoculation was found to be at 1:1 ratio, and the positive brain collected on day 7^{th} clearly showed dengue viral proteins, eventhough the mice had no sign of infection which was used previously to indicate the establishment of dengue virus in mouse brain. Rabbit anti-dengue sera, dengue human hyperimmune sera, specific polyclonal anti-D2 (NS3), and MAb-D2 (E) were used as the specific primary antibody to identify dengue viral proteins. Human hyperimmune sera was found to be the most useful antibody for this purpose. Dengue viral proteins from each positive case revealed different patterns which were useful for further typing of dengue virus strains in another study which was done at this laboratory. Besides the brain, dengue viral proteins were also detected in the liver, but not in the spleen and plasma. However, the intensities of protein bands appeared after immunoenzymatic assay from infected liver specimens showed lower reaction than those from infected brain of the same animal.