Thesis Title: Detection and Identification of Dengue Viruses in Suckling Mice : Comparison Studies Between the Western Blot and Viral Gene Amplification Processes

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ABSTRACT

Dengue fever patients' sera, in the year 1990, were obtained from Ramathibodi Hospital. Detection and identification for the presence of dengue viruses that caused the hemorrhagic fever in children were done by passage of viruses to suckling mouse's brain. This classical method was employed more than 40 years ago. The moribund were killed on day 6 or 7 and various infected organs were collected. The infected brain and liver were the first two organs that demonstrated the presence of dengue viruses. In this study, investigation were extended to cover the spleen, lung, lymph nodes and plasma. The results from Viral Antigen Strip / Enzymeimmunoassay (VAS/EIA) was compared to PCR technique. For PCR technique, both primers, sense and complementary, were synthesized and purified. Using the positive specimens screening by VAS/EIA as the starting material, the first PCR

experiment was done by using suckling mice that were infected with dengue virus type 2 as a model. Since thespecimens were from different type of tissue, several methods to extract viral RNA from these tissues were employed and the results were evaluated. The results indicated that the PCR method can detect viral genes in brain, liver, spleen, lymph nodes and which confirmed previous study using VAS/EIA. Improvement can be done via better extraction method which may lead to a sensitive system to detect dengue viral RNA in plasma and serum of infected mouse with an ultimate goal to identify the virus in clinically confirmed dengue fever cases.