

DENV infections. AFRIMS conducts prospective DENV infection surveillance in Thailand. In an attempt to decrease the frequency of cohort phlebotomy, the authors conducted a prospective comparison of the SDNT and HAI test as methods for detecting intercurrent DENV infections. Serum samples collected in 1998 and 1999 from previous surveillance activities were accessed. 100 samples collected in JUN 1998 and JAN 1999 were chosen at random for testing by HAI, PRNT (expressed as the antibody titer producing 50% plaque reduction), and the SDNT (expressed as the % plaque reduction). Test results were compared between time points and assessed as indicating or not indicating an intercurrent DENV infection using standard diagnostic criteria. Qualitative assessments were completed independently and the results of HAI and SDNT testing compared. PRNT results were considered the 'gold standard'. All test results were concordant in 67/100 of cases. Compared to the PRNT, the HAI possessed 7 false + and 16 false - results while the SDNT possessed 12 false + and 16 false - results. Compared to the PRNT, the SDNT was more discordant than the HAI test but without an increased number of false -'s. The SDNT was concordant with the HAI in 85/100 of cases. Of the 15 SDNT/HAI discordant results the PRNT agreed with the HAI test results in 10 and with the SDNT in 5 cases. Historic and current data indicate a SDNT may be used as effectively as the HAI test to identify intercurrent DENV infections. Utilizing a SDNT for surveillance cohort studies may lead to more false + results and require subsequent further characterization by PRNT testing. The authors will discuss the clinical characterization and additional statistical analysis of the 100 random samples and an additional, non-random, sampling of 250 specimens.

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### **HIGHLY CONSERVED NUCLEOTIDE SEQUENCE OF THE 3'-TERMINAL 111 BP - NONCODING REGION (3'-NCR) OF THAI DENGUE -3 VIRUSES, BANGKOK ISOLATES, DURING 1973-2000**

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Flavivirus genomic RNAs contain 3' noncoding regions (NCRs) with approximately 400 to 600 nucleotides (nts). The 3'-terminal 90 to 100 nts of the 3' NCR is predicted to form thermodynamically stable, adjacent stem-loop (SL) structures. Accumulate evidences suggest that the secondary structure formed by the well conserved 3'-NCR of flaviviral RNA may be very important for viral replication. Overall, a high degree of nucleotide sequence identity was also observed among the four dengue (DEN) serotypes, especially in the 3'-terminal region where there are highly conserved sequences that form secondary structures. We presented herein the highly conserved 111 bp- nucleotide terminal sequences of the 3'-NCR among 37 Thai DEN-3 viruses, Bangkok isolates, during the years 1977-1999 with different degrees of disease severity ranging from DF, DHF/DSS provided by USAMC-AFRIMS, Bangkok, Thailand. The genetic diversity as well as the evolutionary relationships of these Thai DEN-3 viruses was analyzed by using the following computer softwares including the "CLUSTAL X-1.8 1" for aligning the multiple sequences and the "MFOLD" for creating the stem and loop secondary structures. The

highly conserved 111 bps at the 3'-terminal sequence observed among these Thai DEN-3 viruses suggests that it may represent a functional role of the dengue viral 3'-NCR where all or most of the important elements in viral translation, replication and assembly are concentrated. However, this highly conserved 111 bps at the 3'- terminal may not involve the viral pathogenicity as well as severity since the same sequence was existed in the genomes of all these Thai DEN-3 viruses with different severity.

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## **IDENTIFICATION OF CONCURRENT INFECTIONS BY MULTIPLE DENGUE VIRUS SEROTYPES IN THAI CHILDREN**

**Zhang C, Chinnawirotpisan P, Klungthong C, Thirawuth V, Narupiti S and Mammen MP Jr**

Concurrent infection with more than one dengue virus (DENV) serotype has been reported from Puerto Rico, New Caledonia, Taiwan and Brazil. We provide, to our knowledge, the first case report in the medical literature of a concurrent infection with three dengue virus serotypes (DENV-1/DENV-2/DENV-3). In addition, the first report of dual dengue virus infections occurring in Thailand (DENV-1/DENV-3 or DENV-2/DENV-4). Serotype identification was determined from serum by reverse transcriptase-polymerase chain reaction (RT-PCR) and further confirmed by sequence analysis of each amplified PCR product. Phylogenetic analyses of the amplified DNA fragments further support concurrent infections by two or three dengue virus serotypes. Our report suggests molecular diagnostic tests (RT-PCR) supporting dengue virus surveillance must be designed to adequate sensitivity to detect the presence of multiple dengue virus serotypes in a single physiologic sample.

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## **IDENTIFICATION OF CONCURRENT INFECTIONS BY TWO OR THREE DENGUE VIRUS SEROTYPES IN THAI CHILDREN**

**Zhang C, Chinnawirotpisan P, Klungthong C, Thirawuth V, Narupiti S and Mammen MP Jr**

Concurrent infections with two dengue virus (DENV) serotypes in the same patient have been reported previously in Puerto Rico, New Caledonia, Taiwan and Brazil. We provide, to our knowledge, the first report of a case of dengue fever associated with viremia with concurrent infection in the same individual by three dengue virus serotypes (DENV-1/DENV-2/DENV-3). Additionally, we provide, to our knowledge, the first report of concurrent infection by two dengue virus serotypes (DENV-1/DENV-3 or DENV-2/DENV-4) in Thailand. Serotype identification was determined directly from serum by reverse transcriptase-polymerase chain reaction (RT-