

DETECTION OF DENGUE VIRAL RNA IN MOSQUITOES (*Aedes SP.*) BY NUCLEIC ACID SEQUENCE-BASED AMPLIFICATION (NASBA) AND REVERSE TRANSCRIPTASE-POLYMERASE CHAIN REACTION (RT-PCR)

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Virologic surveillance for dengue viruses has been used as an early warning system to predict outbreaks. In this study, the NASBA technique was used to detect serotypes specific of dengue viruses in artificially-infected and in field-caught female adult *Aedes* mosquitoes, which were compared with the RT-PCR technique. In laboratory experiments, NASBA could detect dengue virus serotypes 2 and 4 below 1 PFU/ml, which was more sensitive than RT-PCR, but this technique was as sensitive as RT-PCR when detecting dengue virus serotypes 1 and 3. Most dengue viruses were found at the thorax of mosquitoes in 0, 7, and 14 days after inoculation with dengue virus serotype 2. In the field, female adult *Aedes* mosquitoes were collected in the rainy season (June-August, 2002) and the dry season (April, 2003) in Nong Khai Province. These mosquitoes were caught from selected dengue epidemic areas and assayed by NASBA, compared with RT-PCR. From 630 mosquito samples, viral infection rates were 1.61% and 1.13% for NASBA and RT-PCR detection, respectively. The results showed the mosquitoes were infected only with dengue virus serotypes 1 and 2. The sensitivity and specificity of NASBA when compared with PCR were 100% and 99.52%, respectively. The sensitivities of NASBA for two dengue virus serotypes were 100%, whereas the specificities were 99.68% and 99.84% for serotypes 1 and 2, respectively. For serotyping, NASBA showed similar sensitivity and specificity RT-PCR (= 0.70). These results indicate that the NASBA assay is another tool for the surveillance of infected mosquitoes that is useful for decreasing the dengue problem.

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EVALUATION OF A SINGLE-DILUTION PLAQUE REDUCTION NEUTRALIZATION TEST (PRNT) AS A DIAGNOSTIC TOOL FOR DETECTING INTERCURRENT DENGUE VIRUS (DENV) INFECTIONS

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Studies of DENV transmission require diagnostic tools capable of detecting symptomatic and asymptomatic infections. The hemagglutination-inhibition (HAI) test is valuable for the diagnosis of DENV infections, but possesses low specificity. Since HAI antibody may wane, the HAI test as a DENV surveillance tool may require frequent cohort serum sampling. The PRNT is considered the gold standard bioassay for measuring DENV specific neutralizing antibody. The PRNT is labor-intensive and requires affinity maturation to provide specificity. Previously reported studies have relied on a single-dilution neutralization test (SDNT) to detect intercurrent

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