D-38: Detection of the St. Louis encephalitis virus (SLEV) in mosquitoes using a non-radiolabelled probe

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St. Louis encephalitis (SLEV) is a major arboviral disease in the USA. SLEV (Flavivirus:Flaviviridae) is transmitted by mosquitoes of Culex spp *viz. Cx. tarsalis, Cx. quinquefasciatus and Cx. nigrapalpus*. Man, birds, rodents and bats are major hosts of SLEV. Mosquitoes acquire a life long infection through the ingestion of blood from an infected vertebrate. A quick and accurate method of identifying SLEV in mosquitoes is a valuable aid to studying the epidemiology of the disease. A method is presented that allows polymerase chain reaction (PCR) amplification of the most conserved region of viral RNA in the total RNA isolated from mosquitoes with specific primers and the subsequent detection of SLEV RNA by a non-radio-labelled DNA probe.

A relatively conserved 260 base sequence in the SLEV RNA was identified. SLEV RNA was extracted from cultures of SLEV using the guanidium thiocyanate-phenol-chloroform extraction method. Virus RNA was converted to double stranded DNA (dsDNA) by reverse transcriptase and then amplified by PCR. The amplified region was detected by a 18bp oligonucleotide probe labelled at the 3' end with digoxisgenin-11-ddUTP. After PCR, the amplified dsDNA was denatured at 95°C for 1 min and the samples were spotted on positively charged nylon membrane strips. The strips were dried at 120°C for 15 min to bind DNA to the nylon membrane. Nylon strips with the DNA spots were pre-hybridized

and then hybridized with 3' end-labelled dig-probe for 1 h at room temperature. The strips were then washed and incubated with alkaline phosphatase conjugated with anti-digoxigenin antibody. Colour was developed with 5-bromo-4-chloro-3 inolyl phosphate and nitroblue tetrozolium salt.

The cDNA fragment from PCR amplification was purified by HPLC, and treated with restriction endonuclease enzymes in separate reactions. The fragments obtained by DNA digests were run by electrophoresis on an agarose gel (3%) confirmed the expected PCR amplification of a 260 bp of DNA. Amplified DNA fragment was also sequenced by using the primers and the ampli-taq cycle sequencing kit (Perin-Elmer Cetus).

Results of dot-blot assays showed that the Dig-labelled probe consistently detected 1-2 x 10^{-15} g of SLEV RNA. However increasing the amount of mosquito RNA to 700ng interfered with the PCR amplification. The specificity of the probe was also tested against western equine encephalitis virus (WEEV), eastern equine encephalitis virus (EEEV), Venezuelan equine encephalitis virus (VEEV), La-Crosse virus and Flender virus. False positive result was shown with Flender virus in dot-blot assays. Although the DNA probe detects the Flender virus non-specifically the DNA fragment from the PCR amplification did not show the 260 pb of SLEV fragment in agarose gel electrophoresis.

Hybridization based tests using nucleic acid probes are more specific and reliable than immunoassays, because of the cross reactive antigens present in related viruses. Alternatively amplified region of RNA can be detected by agarose gel electrophoresis and viewing under UV light. However this method was not sensitive enough to detect the DNA fragment obtained from PCR amplification when 1.3 x 10^{-12} g of SLEV RNA was incorporated into the PCR reaction. The sensitivity of the DNA probe was shown to be about 1-2 x 10^{-15} g SLEV RNA. However this DNA probe did not show an absolute specificity to detect the SLEV.