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## **CLONING OF WEST NILE VIRUS FRAGMENTS FOR PRODUCTION OF RNA STANDARDS TO SUPPORT NUCLEIC ACID TESTING**

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### **Background:**

West Nile virus (WNV) is an enveloped, positive single-stranded RNA virus of approximately 11 kb that is naturally transmitted between birds and other vertebrates through mosquitoes feeding on infected animals. Although humans are incidental hosts, the fast spread of human WNV infections in the US, along with the confirmation that the virus can be transmitted through blood transfusion, led to the implementation of nucleic acid amplification tests. Blood centers started routine screening in July 2003 of all donations so as to quarantine and retrieve potentially infectious blood components.

### **Objective:**

Clone WNV fragments into vectors suitable for in vitro transcription of viral RNA to allow preparation of transcripts from different viral genomic regions. The resulting transcripts could be further characterized and used as RNA standards to compare the assay performance between different WNV Nucleic Acid Tests.

### **Methods:**

RNA extracted from cultures of Vero cells infected with WNV strain NY385-99 was used to synthesize, by RT-PCR, a number of DNA fragments spanning the full-length WNV genome. The fragments were cloned into a shuttle vector and their nucleotide sequences verified by DNA sequencing. WNV nucleotide fragments purified from the different shuttle plasmids were cloned into pGEM-4z for further in vitro transcription of different segments of the WNV genome. Construction of a recombinant plasmid encoding for a full-length non-infectious WNV (nucleotide deletion that causes frame-shift and creates a stop codon at residue 576) was also pursued.

### **Results:**

A total of thirty-one RNA transcripts spanning the entire WNV genome were prepared in vitro using the WNV recombinant plasmids

***Conclusions:***

The availability of a set of WNV transcripts synthesized from specific genomic regions will facilitate the preparation of well-characterized RNA standards to assist in the performance evaluation of different WNV nucleic acid tests.