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DETECTION AND QUANTITATION OF WEST NILE VIRUS RNA BY THE ALTERNATIVE NAT WNV ASSAY.

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Introduction:

West Nile Virus (WNV) is a positive strand arbovirus of approximately 11 kb. Although humans are incidental hosts, there have been 4156 WNV positive cases and 284 fatalities reported in US as of April 15 of 2003. The use of antibody detection tests as indicators of WNV infectivity are inconclusive due to persistent circulation of IgM following infection. The detection of WNV antigens is complicated due to cross reactivity with other members of the flavivirus family and the presence of the antigen & antibody immunocomplex during the seroconversion phase. Therefore, there is a critical need for nucleic acid detection tests (NAT) as an indicator for the presence of WNV. Here we describe a method based on Bead capture-Taqman technology for Qualitative and Quantitative detection of WNV RNA.

Materials and Methods:

A magnetic bead based protocol was used for the isolation of WNV RNA, which significantly simplifies the isolation of nucleic acid compared to standard precipitation/centrifugation methods. The isolated target on the beads was amplified by Taqman technology, with amplification primers corresponding to Capsid, NS1/NS2, or 3' NC genomic regions. An Internal Control RNA of 900 nt for Capsid region with altered probe sequence served as a control for both target capture and amplification for each sample. The analytical sensitivity of the assay was tested with a commercially available WNV RNA qualification panels of Lineage 1 and 2.

Results:

The WNV Taqman assay that amplifies and detects the Capsid region proved to be the most robust. Using the commercial WNV panel as few as 30 copies/ml of the WNV RNA could be detected. Assay specificity with fifty normal donors indicated no cross-reactivity and the presence of HCV and HIV RNA did not affect or interfere with the assay. A discriminatory test has been developed for Lineage 1-the US and Lineage 2-the Ugandan strain. The Quantitative assay was used to quantitate WNV propagated in Vero cells and hamsters. The assay has a range of 100-1011 copies/mL.

Conclusion:

Using a combination of magnetic bead based target capture and Taqman technology, a rapid, sensitive, user-friendly WNV qualitative and quantitative assay has been developed.