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PERFORMANCE CHARACTERISTICS OF THE VALIDATED AND IMPROVED QUALITATIVE AND QUANTITATIVE TARGET-CAPTURE PCR WNV NAT ASSAYS

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Background:

The year 2003 registered the highest number of reported cases for West Nile Virus. There is a critical need for nucleic acid detection tests as an indicator for WNV due to low viral titers. Here we describe the results from a validated assay, and an improved assay based on Target-Capture PCR technology for Qualitative and quantitative detection of WNV RNA.

Methods:

A magnetic bead-based protocol was used to isolate WNV RNA and a related internal control RNA in a single tube using a semi-automated method from 0.5/mL of plasma. The capsid region of isolated targets on the beads was amplified by real-time PCR technology. A blinded panel of 25 WNV samples distributed by Blood Centers of the Pacific (BCP) was tested by both the validated and improved assays. The validated assay has been used at Bayer Reference and Testing Laboratories (BRTL) to confirm WNV positives. In collaborations with BCP and Bonfils Blood Center, the quantitative Target Capture-PCR assays have been used to determine viral load in WNV positive samples to track the 2003 epidemic of infected blood donations.

Results:

The results with the BCP WNV panel for analytical sensitivity by the validated assay indicate 100% detection of 30 copies/mL, with the improved assay detecting 10 copies/mL. The assay has high specificity and tolerates a variety of anti-coagulants, interfering substances, and plasma from pathological conditions. The validated assay implemented at BRTL confirmed 682 of the 3811 possible WNV positives (17.9%) between July 2003 - January 2004. The profile of positives mirrors the profile of the reported cases. Eighty samples from January 2004 tested at BRTL were also tested by the improved assay. Despite the increase in sensitivity between the validated and the

improved assay, no gain in detection of WNV positive donors was observed. The quantitative assay estimates WNV in the range of 10²-10¹⁰copies/mL. The results from quantitative assay performed on blinded samples from Bonfils closely tracks the results from BRTL.

Conclusions:

The Target Capture-PCR WNV assay is a rapid, sensitive, easy to use, accurate assay for WNV RNA detection and quantitation.