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### **PROCLEIX® WNV ASSAY: A TRANSCRIPTION-MEDIATED AMPLIFICATION BASED ASSAY FOR SCREENING BLOOD DONATIONS FOR WEST NILE VIRUS RNA.**

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#### **Background:**

We are developing the Procleix® WNV Assay for screening blood donations for West Nile virus (WNV) RNA. The Procleix WNV Assay is a Transcription-Mediated Amplification (TMA) Assay that uses the same semi-automated instrumentation and assay procedures as the FDA licensed Procleix® HIV-1/HCV Assay.

#### **Methods:**

To assess analytical sensitivity, we tested serial dilutions of WNV lineage 1 and 2 virus and WNV RNA transcript. To examine specificity, we tested 1180 normal negative donor samples, including both frozen and fresh samples. We also conducted a reproducibility study including 3 operators, 3 instrument sets, and 2 reagent lots. For this study, heat-inactivated virus at 100 copies/mL and WNV transcript at 100 and 30 copies/mL were tested. For a preliminary assessment of clinical sensitivity, we tested 383 plasma samples from CDC transfusion transmission case studies provided by the American Red Cross. The positive samples were confirmed by repeat testing and then testing in three replicates at 1:8 and 1:16 dilutions.

#### **Results:**

Analytical sensitivity data indicated that the Procleix WNV Assay 95% detection level is about 10 copies/mL of transcript. For the serially diluted viruses, the 95% detection levels were 15 and 9 copies/mL for the lineage 1 and 2 viruses, respectively. Initial specificity was 99.8% and resolved specificity was 100%. In the reproducibility study, all replicates tested at 100 and 30 copies/mL were positive and no invalid results were observed. Results indicated that the largest source of variation in relative light unit (RLU) signals occurred within run. The Procleix Assay detected WNV in 12 samples from the blinded set of 383 samples. Eleven of the 12 reactive samples were reproducibly detected when diluted 1:8 and 1:16. One sample that was detected undiluted had equivocal IgM results and was detected only intermittently at the 1:8 and 1:16 dilutions, indicating a very low viral titer.

#### **Conclusions:**

Analytical sensitivity results indicate that the assay is capable of very sensitive detection of WNV RNA, with 95% detection levels ranging from 9 to 15 copies/mL. The specificity and reproducibility results are comparable to those observed with the Procleix HIV-1/HCV Assay. Preliminary clinical sensitivity results show feasibility of pooled testing for WNV, but additional studies are necessary before efficacy can be fully established.