

Detection of Dengue Virus RNA in Blood Donors From Honduras and Brazil with a Prototype Transcription-Mediated Amplification Assay (S103-040H)

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Background: The WHO estimates that in 2004 there were 50-100 million cases of dengue fever and 500,000 cases of dengue hemorrhagic shock syndrome, resulting in 20,000 deaths. Dengue is considered to be the most significant arboviral disease of humans. We developed a prototype nucleic acid test (NAT) for detection of dengue virus RNA in blood donations that is suitable for high-throughput screening. To demonstrate the feasibility of detecting dengue virus in blood donors and to determine prevalence in highly endemic regions, we screened donor samples from regions in Brazil and Honduras. Screening of additional samples from selected regions in Australia is in progress.

Methods: The prototype assay uses the same technology as the PROCLEIX[®] HIV-1/HCV Assay, consisting of lysis and target capture of viral RNA followed by Transcription-Mediated Amplification (TMA) and chemiluminescent detection by Hybridization Protection Assay (HPA). Samples tested included 2994 donations from Honduras and 4858 from Brazil. The Honduran plasma samples were collected in the Tegucigalpa region from August 31, 2004-January 24, 2005 and in the San Pedro Sula region September 6, 2004-January 13, 2005. The Brazilian samples were archived donor samples from a dengue outbreak in the Sao Paulo area February 2003-April 2003. All samples were screened on the fully automated PROCLEIX[®] TIGRIS[®] System. Quantitation of positive samples was done using a kinetic polymerase chain reaction (PCR) assay.

Results: Screening of the Honduran samples yielded 12 (0.40%) initially reactive and 9 (0.30%) repeatedly reactive results. Eleven of the 12 initially reactive results were confirmed to be positive by an alternate NAT assay. The inability to show repeat reactivity with 2 samples suggests that the viral titers were below the reliable detection level (previously determined to be about 50 copies/mL). Genotyping revealed all 4 dengue genotypes among the confirmed positive samples from Honduras. Viral loads ranged from < 0.01 to 518 plaque forming units (PFU)/mL. For the Brazilian samples, 8 (0.16%) donations were initially reactive and 3 (0.06%) were repeatedly reactive. Confirmation testing and genotyping of the initially reactive Brazilian samples is in progress.

Conclusions: Using a prototype dengue TMA assay we demonstrated the feasibility of detecting dengue virus in blood donors. Prevalence rates observed in these studies, ranging from 0.37% (confirmed positive results Honduras) to 0.06% (repeatedly reactive results Brazil) raise questions about the rate of transfusion transmission and consequences to recipient health. Future studies are planned to address these questions.