

## **Clinical sensitivity of WNV screening and supplemental NAT assays for detection of very low-level viremic donations**

### Background

Recent studies have established that a substantial fraction (15%-25%) of WNV RNA positive blood donations collected in high incidence regions have low level viremia, in the absence or presence of IgM +/- IgG antibody. Such donations may be missed by NAT screening and/or supplemental assays, especially when screening is performed on mini-pools (MPs). This has led to concern over the residual risk of WNV transmission from MP-NAT screened blood transfusions, with consequent consideration of targeted conversion to ID-NAT, reduced collections and frozen component recalls in high MP-NAT regions. There is also evidence that some very low-level viremic donations and follow-up specimens detected as WNV RNA positive by the screening NAT assays, may test negative on currently employed supplemental WNV NAT assays.

### Objective

Develop a panel of frozen plasma specimens, for which large volumes of plasma are available (>200 mL), which represent donations identified with low level-viremia (<50 copies/mL) and various profiles of IgM and IgG reactivity. This panel will be coded along with controls, and distributed to each manufacturer of WNV RNA screening and supplemental assays (Roche, Gen-Probe, NGI, Chiron). Screening NAT assays will be run on all panel members both neat and at dilutions consistent with relevant MP-screening practices (Roche, 1:6; Gen-Probe, 1:16 [plus 1:8?]). Supplemental NAT assays will be run neat. All testing will be performed in replicates of 10 at each input level (neat and mock-MP dilutions). The results will be compiled and distributed to all participants including FDA. Multiple frozen aliquots (1, 2 and 50 mL) from each panel member will be retained for evaluation of modified or additional WNV NAT assays, and for use in in vitro and animal infectivity studies

### Panel composition

Unit volumes of plasma (FFP or RP) with low level viremia have been identified by BSL MP-NAT (16 member MPs) or reflex ID-NAT WNV screening studies using the Gen-Probe screening TMA assay, or through the ABC-REDS plasma recall study. These have been subaliquoted by BSL and BCP. We have selected 15 low-level viremic donor specimens representing serial stages of WNV primary infection, including: 3 samples detectable by Gen-Probe ID-NAT (through the reflex ID-NAT study) that had been missed by MP-NAT and which lack detectable WNV antibodies (infection stage I/II); 3 samples lacking WNV antibodies which were detected by MP-NAT but have very low level viremia based on borderline reactive s/c values by MP-NAT and viral load results that were <LOD or < 100 copies/mL (stage IIIa); 3 samples detected by MP-NAT which had detectable WNV IgM antibodies as well as very low level viremia based on borderline reactive MP-NAT s/c values and viral load results below LOD or < 100 copies/mL (stage IIIc); 3 samples missed by routine MP-NAT but detected by reflex ID NAT which contain detectable IgM +/- weak IgG reactivity (stage IV); and 3 samples that have high level IgM and IgG reactivity and very low level viremia based on

inconsistent detection of RNA by replicate ID-NAT (stage V). These 15 units were all initially carefully thawed, mixed and subaliquoted into a repository containing small (1-10 mL) and large (25-50mL) volumes of frozen plasma (-70C). For the present study a subset of frozen aliquots will be shipped to and processed at BCP as follows: Each unit will be handled separately in a laminar flow hood. Tubes containing sufficient volume for all panels will be thawed at RT to allow cryoprecipitate to fully dissolve, followed by mixing and low speed centrifugation to clear any non-soluble particulate matter. Supernatant plasma will be distributed into coded tubes with appropriate volume for each company's planned testing (12-15 mL), and immediately refrozen to -70C. Five control units will be similarly processed. The panels will be coded so as to not allow distinction of viremic specimens from different stages of controls; a linkage will be retained to allow reference of study results back to predicate data, and to subsequent use of reserve aliquots for future test evaluation and infectivity studies. Panels, each comprised of 20 coded tubes, will be shipped on dry ice to each company along with an electronic file for reporting results according to pre-agreed testing formats (# of reps at neat +/- relevant MP dilutions for each assay). The companies evaluating mock MP dilutions will be responsible for performing those dilutions using WNV-negative plasma as diluent. For replicate tests the assays should be performed independently including viral particle concentration and nucleic acid extraction steps. Results must be reported within 10 days of shipment of the panels to preclude concerns over determination of other results, which might assist in breaking the study code.

#### Assays to be evaluated

*Gen-Probe*: Primary WNV screening and alternate (supplemental) TMA assays performed in 10 replicates on each panel member neat and at 1:16 (and possible 1:8) dilutions.

*Roche*: WNV Ampliscreen PCR performed in 10 replicates on each panel member neat and at 1:6 dilutions.

*Chiron/Bayer*: Supplemental qualitative WNV PCR assay performed in 10 replicates on each panel member neat.

*NGI*: Supplemental PCR qualitative WNV PCR assay performed in 10 replicates on each panel member neat.

#### Reporting, compilation and distribution of study results

Each company will report their results to Drs. Busch/Tobler at BCP. A summary database will be compiled that includes predicate and all study results. The hit rates (# reactive/# replicate tests performed) of each assay, neat and at mock MP-dilutions, will be calculated. Results will be summarized by stage of infection. The summary table of results will be reported back to all participants simultaneously, along with a database that includes the predicate results on each specimen that were the basis for selection.

#### Presentation, publication and other use of study results

The results of the study may be prepared in abstract and slide show format for presentation at policy or scientific meetings, and in manuscript format for submission for publication in a scientific journal. Representatives of each company will be recognized on all such presentations and publications, and each company will have the right to

preview and comment on all such materials prior to distribution beyond the study participants. The results from this study may be used for submission of performance data to FDA in support of regulatory review and approval of assays.