

Evaluation of different trigger criteria for West Nile Virus ID NAT Testing

Background

Individual donation (ID) WNV NAT can identify viremic donations that are not detectable by minipool (MP). Seasonal ID is not feasible for large testing labs. Optimal ID triggering criteria have not been determined. In 2007, AABB recommended minimal triggering criteria of 2 MP presumed viremic donations (PVDs) and a >1:1000 rate in a rolling 7-day interval ("primary" trigger). However, because of PVDs detected in overlapping and/or adjacent geographic areas, some blood centers served by our lab triggered on 1-PVD ("neighbor" trigger) or no PVDs ("self" trigger).

Methods

Donations were tested in the Procleix Assay as part of 16-sample pools or as ID. All NAT initially reactive donations (either from MP resolution testing or ID screening) were retested in the ID format by the WNV TMA. These units were tested also for WNV IgM, IgG antibodies using FDA-licensed enzyme-linked immunosorbent assay (ELISA) kits (Focus Diagnostics, Cypress, CA).

True positives (TP) were defined based on repeat reactivity or presence of anti-IgM. ID TPs were diluted 1:16 to determine their final designation as ID or MP detectable. ID yield (not detectable by MP) was determined for each of the 3 different triggering criteria.

Results

WNV RNA test results were obtained for 1,217,929 donations collected from June 4 through November 30, 2007. Although 93.9% of donations were screened by MP testing and 6.1% by ID testing, the number of tests performed by each method were approximately equal (~72,000 and ~74,000 respectively), due to the fact that MP testing requires approximately 16 fold fewer tests than ID testing. The weekly number of WNV RNA positive donations identified is shown in **Figure 1**.

Table 1 shows 162 WNV RNA TPs identified, 87 were detected by MP testing (TP rate of 0.008%) and 75 by ID testing (TP rate of 0.10%); a more than 10-fold increase; $p < 0.0001$. NAT initially reactive donations that confirmed as TP varied by testing format: 97.8% (87 of 89) for MP testing versus 78.1% (75 of 96) for ID testing.

Of the 75 ID-NAT, 41 (55%) were positive at a 1:16 dilution and 34 (45%) were detectable only by ID NAT. Four were IgM and IgG antibody negative.

Overall, WNV RNA positive ID NAT yield donations occurred at a frequency of 1 in 35,000 donations. WNV RNA positive, IgM negative ID NAT yield donations occurred at a frequency of 1 in 300,000 over the course of the 2007 WNV season.

The FP rate was 0.0002% for MP testing vs. more than one hundred-fold higher rate of 0.028% for ID testing ($p < 0.0001$). FP rate did not differ between automated testing using the Tigris platform and eSAS, a semi-automated method; $p = 0.10$. However, when the analysis was confined only to those samples tested by ID NAT, there was a statistically significant difference in false positive rates between the two laboratories; the rate was 0.02% in Tempe (Tigris) and 0.1% in Bedford (eSAS) ($p < 0.0001$).

Table 1: WNV Procleix Assay Test Results: June-November 2007

Test Format	Donations Tested	Initial Reactive	True Positives	False Positives
MP	1,143,679	89 (0.008%)	87 (0.008%)	2 (0.0002%)
ID	74,250	96 (0.13%)	75 ¹ (0.10%)	21 (0.028%)
Total	1,217,929	185 (0.015%)	162 (0.013%)	23 (0.002%)

¹ 34 of these (45%) were ID NAT yield donations; the other 41 (55%) were reactive at a 1:16 dilution thus 34 of 162 (21%) required ID NAT

Conclusion

These data demonstrate that the recommended minimal AABB trigger criteria of 2-PVDs and a rate > 1:1000 missed viremic donors; therefore it is reasonable to adopt more stringent triggers, including elimination of the rate criterion and triggering on 1 PVD for regions adjacent to centers which have already triggered. However, blood centers which self trigger prior to the detection of any PVDs had very limited yield and required a significant proportion of testing capacity.

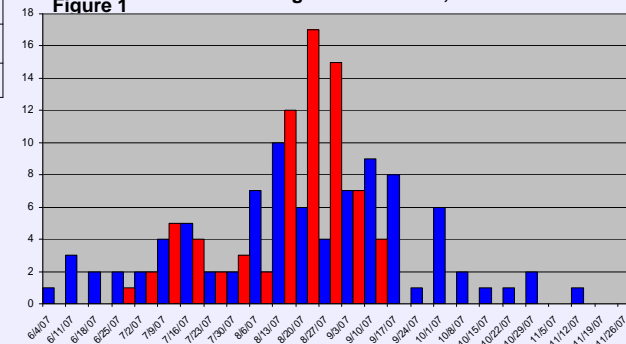
As shown in **Table 2**, ID NAT testing volume was approximately equal for sites using each of the three trigger criteria. However, ID NAT yield rates differed: these were 0.077% for primary trigger sites, 0.052% for neighbor trigger sites, and 0.004% for self trigger sites ($p = 0.0003$). ID NAT at self trigger sites produced only one yield case and one additional case resulted from testing an HCTP donor. Overall, 62% of the ID NAT yield cases were detected by ID NAT initiated as a result of the primary trigger criteria. The percentage of blood collectors with ID NAT yield cases was approximately equal for those that triggered ID NAT based on primary or neighbor trigger criteria but was much lower for blood collectors that self triggered.

Table 2: ID NAT results by triggering criterion

Trigger	Donations tested	Yield donations detected (%)	Blood collectors with yield/# triggering ^{1,2}
Primary	27,211	21 (0.077%)	6 of 10
Neighbor	21,301	11 (0.052%)	8 of 15
Self	25,210	1 (0.004%)	1 of 21
HCTP	264	1 (3.8%)	NA
Total	74,250	34 (0.046%)	15 of 46

¹ Some blood collectors had multiple triggers (primary, neighbor and/or self) at different time intervals during the monitoring period. ² There were 48 blood centers/hospitals that did not trigger ID NAT during the 2007 season; 13 (27%) had at least one NAT TP identified by MP NAT.

Figure 1
West Nile Virus RNA True Positive Results
June 4 through November 26, 2007 N=162



Weekly distribution of all 162 WNV RNA true positive donations. Results are plotted by testing format (blue bars for MP, red bars for ID) used for WNV RNA detection.

Session I
TTID1: Arboviruses
Abstract: SP155

Joan Dunn Williams¹
Gene Robertson¹
Sally Caglioti¹
Steve Kleinman²
Robert C. Williams³
Randy Spizman¹
Larry Morgan¹
Michael Busch⁴

¹Blood Systems Laboratories, Tempe, AZ

²University of British Columbia Vancouver BC;

³Arizona State University, Tempe, AZ;

⁴Blood Systems Research Institute, San Francisco, CA.

