Detection of West Nile virus RNA and antibody in frozen plasma components from a voluntary market withdrawal during the 2002 peak epidemic

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BACKGROUND: The US West Nile virus (WNV) epidemic in the summer and fall of 2002 included the first documented cases of transfusion-transmitted WNV infection. In December 2002, the FDA supported a voluntary market withdrawal by the blood banking community of frozen blood components collected in WNV high-activity areas. At the time, the prevalence of viremia and serologic markers for WNV in the blood supply was undefined.

STUDY DESIGN AND METHODS: In collaboration with America's Blood Centers, 1468 frozen plasma components (of approx. 60,000 frozen units voluntarily withdrawn from the market) were selectively retrieved from the peak epidemic regions and season (June 23, 2002-September 28, 2002). These units were unlinked, subaliquoted, and tested by WNV enzyme immunoassays (EIAs; Focus Technologies and Abbott Laboratories) and nucleic acid amplification tests (NATs; Gen-Probe Inc. and Roche Molecular Systems).

RESULTS: Of the 1468 EIA results from Abbott and Focus, 7 were anti-immunoglobulin M (IgM)- and anti-immunoglobulin G (IgG)-reactive by both assays, 8 and 1 were IgM-only-reactive, and 8 and 23 were IgG-only-reactive, respectively. NAT by Gen-Probe and Roche Molecular Systems yielded one RNA-positive, antibody-negative unit containing approximately 440 RNA copies per mL. An additional 10-fold replicate NAT testing by Gen-Probe on 14 of 15 IgM-reactive specimens yielded 2 additional IgM- and IgG-reactive units with low-level viremia (i.e., 7/10 and 2/10 replicates tested reactive).

CONCLUSION: The prevalence of acute (RNA-positive) and recent (IgM-seroreactive) WNV infections indicates that transfusion risk in high-risk areas could have been considerable and that voluntary market withdrawal of frozen components likely averted some WNV transfusion transmissions. The existence of very-low-level viremic units raises concerns, because WNV minipool NAT screening will miss such units and individual NAT may not completely correct this situation.

West Nile virus (WNV) is a mosquito-borne flavivirus transmitted primarily among birds with humans as incidental hosts. The virus is endemic in parts of Africa, Europe, the Middle East, and Asia.[1] In 1999, WNV emerged in Queens, New York,[2,3] and subsequently spread epidemically in the eastern and central United States. The first well-documented cases of transfusion-transmitted WNV infection occurred during the 2002 WNV epidemic. During the course of this epidemic, 23 patients were confirmed to have acquired WNV infection after transfusion of leukoreduced and nonleukoreduced red cells, platelets, or fresh frozen plasma. Sixteen blood donors with evidence of WNV viremia at the time of their donation were linked to

ABBREVIATIONS: ABC = America's Blood Centers; BSRI = Blood Systems Research Institute; ID = individual donor; MP = minipool; PRNT = plaque reduction neutralization; SLE = St Louis encephalitis; TMA = transcription-mediated amplification.

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these recipients by the Centers for Disease Control and Prevention (CDC) and collaborating blood organizations.[4]

Subsequent to the recognition that WNV was transfusion-transmitted in the summer of 2002 and in the absence of blood donor screening assays, members of America's Blood Centers (ABC) in geographic areas with WNV activity voluntarily withdrew from the market and quarantined noncellular blood products that had been collected during the epidemic.[5] These units were an invaluable resource in addressing a number of important issues remaining at the end of the 2002 epidemic: 1) prevalence of both viremia and WNV serologic markers in the blood supply; 2) comparative use of WNV serology versus nucleic acid amplification tests (NAT) to potentially detect infectious units;[6,7] and 3) the relative value of voluntarily market withdrawal, quarantining, and discarding noncellular blood products in geographic areas of high WNV activity. In this article, we report the results of a study that evaluated 1468 voluntarily withdrawn from the market and quarantined frozen plasma units provided by ABC blood centers that collected blood in selected geographic areas during CDC-defined periods of high WNV activity.

MATERIALS AND METHODS

Center and time period eligibility criteria

ABC blood centers meeting the following two inclusion criteria were invited to participate in this study: 1) estimated incidence of WNV meningoencephalitis or illness greater than 2 per 100,000 in their collection area based on CDC data or 100 or more human cases of WNV illness reported to the CDC Web site as of November 6, 2002, from their collection area; and 2) available quarantined collections drawn between June 23, 2002, and September 28, 2002, because 90 percent of WNV cases occurred during this period. Twelve blood centers from seven states chose to participate: Heartland Blood Centers (IL), LifeSource Blood Services (IL), Central Illinois Community Blood Bank (IL), Michigan Community Blood Centers (MI), Hoxworth Blood Center (OH), Community Blood Center (OH), LifeShare Blood Centers (LA), United Blood Services Lafayette (LA), United Blood Services Meridian (MS), Mississippi Blood Services (MS), Community Blood Bank of the Lancaster County Medical Society (NE), and Gulf Coast Regional Blood Center (TX). All Gulf Coast Regional units included in the study had been exported to Houchin Blood Services in California and were supplied by Houchin Blood Services.

Sample shipping

Quarantined frozen plasma products were shipped via Federal Express "priority overnight" to the Viral Reference Laboratory and Repository Core at Blood Systems Research Institute (BSRI, San Francisco, CA). Collection center blood unit identification number and either draw date or expiration date identified samples. All incoming units were either included or excluded from the study based on comparing their draw date to the study date criteria.

Sample aliquoting and anonymization

Upon receipt, units were anonymized and subaliquoted by BSRI personnel. All blood unit identification numbers were replaced with identification numbers based on the state of origin, the month of collection, and a sequential four digit number, for example, IL-8-0554. After relabeling, the frozen units were thawed and subaliquoted for NAT and serology testing. Five identical sets of specimens were prepared, with identical labels for each aliquot from a given unit. Residual plasma volume was retained in the plasma bag, frozen, and stored at –80°C.

Controls

Both serologic and nucleic acid controls (Boston BioMedica Inc., West Bridgewater, MA) were part of the final set of specimens. These controls were composed of seven and six members, respectively. The serologic controls consisted of serial specimens from a plasma donor collected while in the process of WNV immunoglobulin M (IgM) and immunoglobulin (IgG) seroconversion. Owing to the study's volume requirements for enzyme immunoassay (EIA) testing, the seroconversion controls were diluted 1:5 whereas the nucleic acid-conversion panel members remained undiluted. The controls for this NAT were composed of a proficiency panel with 0, 30, 100, 300, 1000, and 10,000 copies per mL of WNV RNA. All controls were subaliquoted into tubes designated for either EIA or NAT, and randomly distributed throughout the sequence of study identification numbers.

Anti-WNV IgM and IgG testing

Both Abbott Laboratories (Abbott Park, IL) and Focus Technologies (Cypress, CA) received 1475 specimens (i.e., 1468 specimens from frozen plasma units plus 7 controls) for anti-WNV IgM and IgG testing. A brief description of the manufacturers' assays follows. Both Abbott and Focus developed assays to detect IgM to WNV; both assays use the capture assay format wherein the solid phase is coated with antibody directed against human IgM.[8] After plasma or serum incubation and washing, the captured IgM is incubated with WNV-specific antigen. The presence of WNV-specific antibody is detected with horseradish peroxidase–labeled flavivirus group-specific monoclonal antibody.[9] Additionally, the Focus IgM assay has a background subtraction step to detect possible false-positive IgM results.[10] Briefly, serum is added to duplicate wells of

the IgM-capture plate and processed according to the package insert. After addition of unknown serum or plasma and washing of the wells, specimen diluent is added to one well, and WNV antigen is added to the second well. Following an additional incubation and washing procedure, peroxidase-labeled mouse monoclonal anti-flavivirus antibody is added to both wells. IgM index values are determined with the net absorbance value, calculated by subtracting the absorbance value of the well receiving diluent only from the absorbance value of the well receiving the WNV antigen. The Focus WNV IgG enzyme-linked immunosorbent assay uses the preM/E recombinant protein in a standard indirect EIA, whereas the Abbott WNV IgG assay uses the capture format wherein the solid phase is coated with antibody directed against human IgG (anti-IgG capture).[11] Finally, Focus results are interpreted as follows: values of less than 0.9 are considered negative, 0.9 to 1.1 equivocal, and greater than 1.1 positive for WNV IgM antibodies. For the IgG assay, values of less than 1.30 are considered negative, 1.30 to 1.50 equivocal, and greater than 1.50 positive. For Abbott's anti-WNV IgM and IgG assays, any samples with a signal-to-negative ratio of at least 5.0 are considered positive for either WNV IgM or IgG antibodies.

WNV polymerase chain reaction and transcription-mediated amplification testing

Gen-Probe Inc. (San Diego, CA) and Roche Molecular Systems (Branchburg, NJ) received 1474 specimens for undiluted WNV NAT screening (i.e., 1468 specimens from frozen plasma units plus 6 controls). Each assay manufacturer performed testing in compliance with their respective testing protocols. A brief description of the manufacturer's assays follows.

The transcription-mediated amplification (TMA) assay (Gen-Probe) has three main steps: 1) sample preparation, 2) RNA target amplification by TMA, and 3) detection of the amplification products with chemiluminescent probes with the hybridization protection assay.[12] During sample preparation, viral RNA is isolated from plasma and separated from potentially inhibitory substances by target capture onto paramagnetic microparticles. Nucleic acid amplification of the viral target occurs via TMA, a transcription-based technology that uses two enzymes, MMLV reverse transcriptase and T7 RNA polymerase.[13] Reverse transcriptase creates a DNA copy of the viral target, in which a promoter sequence for T7 polymerase is incorporated. T7 polymerase then catalyzes the production of multiple RNA transcripts from the DNA copy template. Finally, detection of amplicon is achieved with single-stranded nucleic acid probes that contain chemiluminescent acridinium ester labels.[14,15]

The TaqScreen West Nile virus system (Roche Molecular Systems) combines automated specimen preparation on the COBAS AmpliPrep instrument, together with automated amplification and detection with the COBAS TaqMan (CTM) analyzer and a real-time polymerase chain reaction (PCR). The purified nucleic acids are amplified and detected on the COBAS TaqMan. Controls are handled in the same manner as specimens on the two instruments and are taken through the entire process of specimen preparation, amplification, and detection.

Target-capture PCR WNV RNA quantitative assay

The quantitative target-capture PCR WNV viral load assay consists of two steps: 1) target capture and 2) amplification-detection. A total of 400 µL of capture reagent and 500 µL of each specimen were used per test. This suspension was incubated in a 60°C water bath for 20 minutes to lyse the virus, solubilize the proteins, and release viral RNA. The samples were then cooled and the beads with the hybridized target were washed three times in a HEPES buffer (0.3 mol/L NaCl, 0.5% Nonidet P-40, pH 7.5). After removal of the wash buffer, 100 µL of one-step reverse transcription-PCR master mix (ABI, Foster City, CA) containing both amplification and detection primers corresponding to the capsid region of WNV was added to the beads. The WNV target and internal control specific signals were monitored with the ABI Prism 7900 system. Results were quantified with a nine-member standard curve (40-160,000 copies/mL) generated from serial dilutions of WNV cultured in Vero cells (African green monkey kidney) and computer software (SDS, ABI, Foster City, CA).

Serum dilution-plaque reduction neutralization procedure

Among arbovirus testing methods, the serum dilution-plaque reduction neutralization (PRNT) procedure is the most specific method for determining the presence of virus-specific antibodies. All samples that tested anti-WNV IgM-reactive by either manufacturer's assay were sent to CDC for PRNT. Plasma demonstrating WNV antibody reactivity by EIA was initially heat-inactivated at 56°C for 30 minutes and then diluted 1:5 followed by successive twofold dilutions. Reference WNV and St. Louis encephalitis (SLE) virus were diluted to a final concentration of 200 plaque-forming units per 0.1 mL. Equal volumes of virus and plasma dilution were mixed and incubated overnight at 4°C. The virus-plasma mixtures were then added to Vero cell monolayers, and the virus was allowed to absorb to the Vero cells before addition of a nutrient-agar overlay mixture. A second overlay containing neutral red as a vital stain was then added. The plates were incubated upside down in a 37°C CO_2 incubator. Plates were inspected daily, and the well yielding a plaque count of 30 to 100 plaques was used to calculate the plaque reduction titer. Neutralization was defined as a

90 percent reduction in the plaque count when compared with control plates. The reciprocal of the dilution of plasma that reduced and/or neutralized the challenge inoculum was used as the titer.[16]

Statistical analysis

All test result data were compiled with computer software by the study's coordinating center (SAS data set, Westat, Rockville, MD). All analyses were performed with computer software (SAS, Release 8.2, SAS Institute Inc., Cary, NC).

Human subject approval

The study protocol was reviewed by Westat's institutional review board and the University of California at San Francisco's Committee on Human Research before implementation and was found exempt from the regulation requirements because the study involved existing biologic specimens and study subjects were not identifiable.

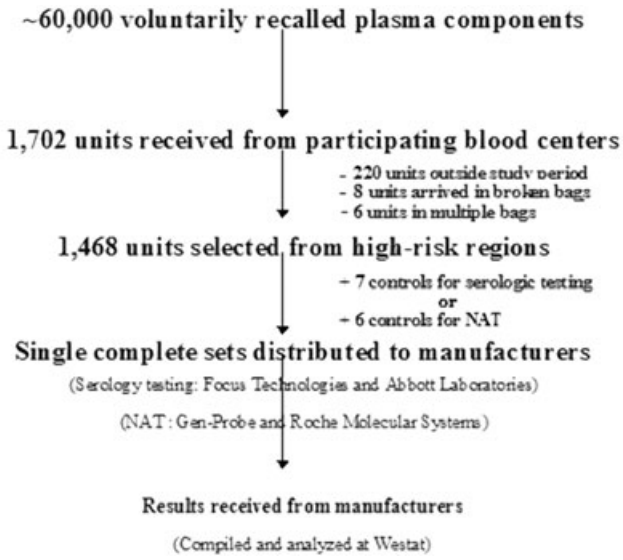
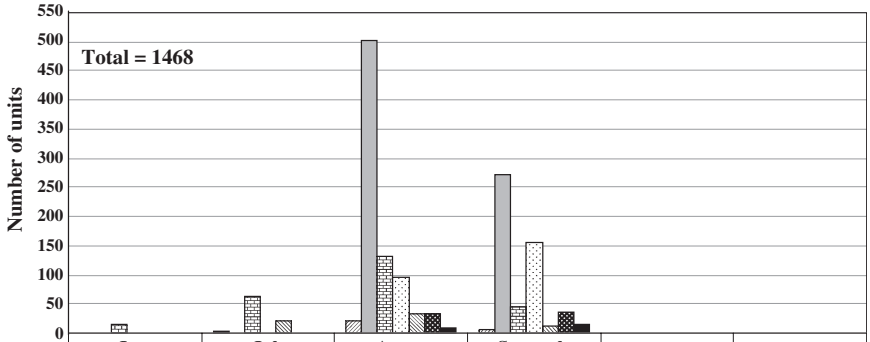


Fig. 1. Study flow diagram.

RESULTS

In 2002, the voluntary market withdrawal by blood centers in WNV epidemic areas resulted in withdrawal and quarantine of approximately 60,000 frozen noncellular blood products (ABC survey data, C. Bianco, personal communication). BSRI received 1702 frozen plasma units from participating blood centers. As seen in Fig. 1, 220 (13%) of the submitted plasma units were collected outside the study time period, whereas 14 (0.82%) either arrived broken (8) or were in multiple plasma bags (6). Figure 2 shows the distribution of the 1468 plasma units included in the study, by state and month. Fifty-three percent of all frozen plasma units included in the study were from the state of Illinois. The remaining 47 percent were from the states of Louisiana, Michigan, Mississippi, Nebraska, Texas, and Ohio in descending order of contribution.



	June	July	August	September		
TX		3	20	6		
IL			503	272		
LA	14	63	131	46		
MI			97	155		
MS		21	33	13		
NE			32	35		
OH			8	16		

Fig. 2. Distribution of study frozen plasma units by month and state. Percent plasma unit distribution by state was: 53 percent for Illinois (IL); 17 percent for Louisiana (LA);, 17 percent for Michigan (MI);, and 13 percent for Mississippi (MS); Ohio (OH); Kentucky (KY); Texas (TX); and Nebraska (NE).

As seen in Table 1, 1445 (98%) and 1429 (97%) of the 1468 specimens sent to each manufacturer did not react for both anti-WNV IgM and anti-WNV IgG, respectively. Seven (0.48%) specimens were concordant reactive for both anti-WNV IgM and anti-WNV IgG, whereas one specimen was IgM-only-reactive by both manufacturers' assays. One manufacturer identified a total of eight anti-WNV IgM-only-reactive specimens (0.54%), whereas the second manufacturer identified only the one concordant IgM-only-reactive specimen (0.07%). Additionally, eight (0.54%) specimens were anti-WNV IgG-only-reactive by Abbott, whereas Focus identified 23 (1.57%) anti-WNV IgG-only-reactive specimens, including all 8 specimens that tested reactive by Abbott's IgG test. Only one manufacturer categorized anti-WNV IgG test results as equivocal, and 8 (0.54%) specimens fell into this category. Results

TABLE 1. Summary of WNV IgM and IgG results from two manufacturers of serology assays on 1468 plasma units withdrawn from high WNV activity regions and periods

WNV antibody type		Abbott	Focus
IgM	IgG	Laboratories*	Technologies*
Nonreactive	Nonreactive	1445 (98)	1429 (97)
Reactive	Reactive	7 (0.48)	7 (0.48)
Reactive	Nonreactive	8 (0.54)	1 (0.07)
Nonreactive	Reactive	8 (0.54)	23 (1.57)
Nonreactive	Equivocal†	0 (0)	8 (0.54)
Total		1468	1468

* Data are reported as number (%).
† One manufacturer uses an equivocal classification.

TABLE 2. Specific antibody titers for 15 withdrawn plasma unit samples that tested IgM-reactive by one or both EIAs, as measured with the PRNT assay

	Abbott (S/N*)		Focus (S/CO†)		CDC	
Case	IgM	IgG	IgM	IgG	WNV PRNT (titer‡)	SLE PRNT (titer)
1	111.1	20.1	5.29	2.59	640	40
2	78.3	45.6	4.06	3.9	640	20
3	111.1	8.4	7.2	1.64	320	20
4	109.2	38.9	4.61	3.64	320	20
5	95.9	57.8	3.83	3.69	160	20
6	108.8	20.1	4.77	1.72	160	10
7	111.1	99.8	5.04	3.46	1280	20
8	10.4		<0.90	<1.30	<10	<10
9	32.1		<0.90	<1.30	<10	<10
10	8.7		<0.90	<1.30	<10	<10
11	5.0		<0.90	<1.30	<10	<10
12	9.6		<0.90	<1.30	<10	<10
13	7.2		1.14	<1.30	<10	<10
14	5.4		<0.90	<1.30	<10	<10
15	12.4		<0.90	<1.30	<10	<10

* S/N = signal-to-negative ratio.
† S/CO = signal-to-cutoff ratio.
‡ The reciprocal of the dilution of plasma that neutralized the WNV challenge represents the titer.

of WNV serology on blinded controls were appropriate for both manufacturers.

Once serologic results had been obtained from both participating manufacturers, aliquots from the 15 frozen plasma units with observed anti-WNV IgM reactivity on either assay were sent to CDC for evaluation and serologic confirmation by the PRNT assay. Because both WNV and SLE viral infections occur concurrently in the United States, and there is serologic cross-reactivity between the two viruses, both a WNV PRNT and a SLE PRNT were performed at the CDC. The results of the SLE PRNT assay ruled out serologic cross-reactivity to SLE in all 15 plasma units. In addition, in the WNV PRNT assay, only those units with anti-WNV IgM and IgG reactivity on both manufacturers' assays had significant titers of WNV plaque-reducing antibodies (Table 2). Therefore, the EIA serology results could be confirmed by PRNT reduction in only 7 (47%) of the 15 plasma units with anti-WNV IgM. All 7 of these units were concordant IgM- and IgG-reactive by both manufacturers' assays.

Coded aliquots from 1474 specimens (1468 voluntarily withdrawn plasma units and 6 proficiency controls) were sent to Roche Molecular Systems and Gen-Probe Inc. for WNV RNA testing with individual donor (ID) NAT assays developed for donor screening. Both Gen-Probe and Roche detected WNV RNA in all five RNA-positive members of the proficiency panel (30, 100, 300, 1000, and 10,000 copies/mL). These samples were also tested further under code by the Gen-Probe screening and alternative-primer TMA assays at dilutions of 1:8 and 1:16 to determine the efficacy of the minipool (MP) WNV NAT screening strategy (Table 3). At a 1:8 dilution, the 10,000 through 100 copies per mL controls remained reactive in all three replicates. Conversely, only two of the three replicates were reactive at a 1:16 dilution for the 100 copies per mL control. The control containing 30 copies per mL was negative at both 1:8 and 1:16 dilutions.

A single WNV RNA-positive unit was identified by both manufacturers from the 1468 withdrawn plasma units. This unit had tested IgM- and IgG-nonreactive by both Abbott and Focus assays. The unit was donated in Illinois in August 2002. Additional data on this unit indicated that it was reactive with the Gen-Probe alternative primer pair assay as well as reactive on the Gen-Probe screening assay when retested in triplicate at 1:8 and 1:16 dilutions. The viral load in this unit was 440 copies per mL by target-capture real-time PCR quantitation.

Fourteen (93%) of the 15 plasma units with anti-WNV IgM- or IgM- and IgG-reactive results that initially tested WNV ID NAT-nonreactive with the Gen-Probe and Roche WNV assays were retested in replicates of 10 by Gen-Probe. The last plasma unit was a cytoprecipitate with limited volume; only five replicates with the WNV Procleix assay were possible. Two additional WNV RNA-positive samples were identified; one was reactive in 7 of 10 replicates (70%) and the second was reactive in 2 of 10 (20%) replicates. Both cases were anti-WNV IgM- and IgG-reactive. Based on the analytical sensitivity of the TMA assay and the percentage of positive replicates, these two withdrawn units were estimated to contain 1 to 5 WNV RNA

TABLE 3. WNV RNA results from Gen-Probe TMA assays for proficiency panel controls and one RNA-positive withdrawn noncellular component from 2002

ID	Description (copies/mL)	Procleix WNV assay (screening assay) S/CO*				Alternative TMA assay S/CO*		
		Neat	Repeat neat	1:8 dilution	1:16 dilution	Neat	1:8 dilution	1:16 dilution
Control	10,000	34.90	38.38	34.67	34.80	34.44	33.33	31.70
			35.30	33.48		32.44	31.69	
			33.11	34.44		31.99	32.07	
Control	1,000	33.78	35.84	34.63	14.43	32.80	31.54	31.75
			33.32	33.39		31.55	32.29	
			31.53	34.13		32.10	30.83	
Control	300	33.01	34.76	20.28	30.98	33.44	31.71	30.89
			26.09	2.72		31.06	31.33	
			28.92	3.71		31.99	29.85	
Control	100	30.27	32.89	27.78	0.91	30.63	32.60	31.68
			2.03	3.46		30.61	0.12	
			3.13	1.65		30.43	29.17	
Control	30	28.89	29.89	0.67	0.00	30.71	0.01	29.60
			0.55	0.49		29.49	0.10	
			0.44	0.03		29.96	29.18	
Control	0	0.00						
	~440†	33.36	35.47	34.10	34.32	31.93	31.84	31.60
			33.12	29.48		32.20	31.45	
			33.48	32.75		31.27	32.32	

* A signal-to-cutoff (S/CO) ratio of at least 1.0 is considered positive.
† Viral load was determined by target-capture PCR WNV RNA quantitative assay.

gEq per mL. All three viremic units were collected in Illinois, two during August and one in September 2002.

DISCUSSION

The voluntary plasma withdrawal by ABC members in geographic high-incidence areas during the 2002 WNV epidemic resulted in interdiction of at least 3 viremic units among the 1468 withdrawn noncellular blood products represented in our study. Because our study included only a subset of the withdrawn frozen plasma units, the overall impact (i.e., the total number of infectious components interdicted from transfusion) of this voluntary market withdrawal on transfusion risk was likely to have been greater.

The results of the WNV serologic testing warrant several comments. Although the specificity of both manufacturers' assays was relatively high, nonconcordant reactivity that could not be confirmed by PRNT was observed. Consequently, further studies to understand the performance of these assays are needed before considering their use in donor screening. All samples that tested concordant anti-WNV IgM- and IgG-reactive by both manufacturers were confirmed by WNV-specific PRNT, suggesting a possible dual EIA approach to serologic confirmatory testing. Of the eight samples that had concordant WNV-specific IgM reactivity, seven also had WNV-specific IgG reactivity, indicating that the duration of the IgM-only phase is likely relatively brief, a finding borne out by follow-up studies of viremic donors identified during the WNV epidemic in 2003.[17]

Among the three WNV RNA-positive units identified, one was IgM-nonreactive with a viral load of approximately 440 gEq per mL. This is similar to the viral loads demonstrated by CDC during the 2002 epidemic among units implicated in transfusion transmission cases.[4] The other two WNV RNA-positive, IgM- and IgG-reactive units had very low levels of viremia (estimated at 1-5 copies/mL). The prevalence of these very-low-level viremic, IgM- and IgG-reactive units raises concern, because our data indicate that ID NAT would only interdict some low-level viremic units (i.e., IgM- and IgG-reactive units in our study were only detected by ID-NAT in 2 of 10 and 7 of 10 replicates). At present, it is unknown whether such low-level viremic units, containing anti-WNV IgM-only or IgM and IgG antibodies, are infectious. If such units are determined to be infectious, this would support arguments for consideration of ID NAT and/or IgM screening to maximally safeguard recipients during periods of peak WNV activity. In contrast, consideration of serologic screening, even if limited to epidemic periods and regions, would need to balance safety gain against the loss of units and donor deferrals attributable to detection of true seropositive units from donors with resolved infections as well as nonspecific reactivity of these assays.

Our findings have implications for the management of frozen plasma products collected during high WNV activity periods in 2004 and beyond. Despite MP-NAT

screening yielding approximately 1000 viremic units, at least six breakthrough transmissions occurred during the 2003 WNV epidemic.[18] All confirmed breakthrough transmissions tested MP-NAT-negative but ID-NAT-positive (and seronegative). Given our observations and these breakthrough transmissions, quarantine of MP-NAT-negative frozen products collected in epidemic regions may be warranted unless either ID-NAT or IgM testing can be performed on aliquots from these units during the 2004 epidemic season. Several large blood screening programs have developed and implemented targeted ID-NAT strategies that employ real-time monitoring of MP-NAT yield and specific triggers for converting MP-NAT screening to ID-NAT prospective WNV screening.[19] These programs include retrospective ID-NAT testing of plasma aliquots from MP-NAT screened units that were given several days prior to specific MP yield exceeding trigger criteria. Should this type of testing not be available, then blood organizations may want to maintain stockpiles of plasma from safer regions and periods that can be substituted for quarantined plasma products from high-risk regions. This approach would not be an option for cellular products with limited shelf life. Finally, our results support the potential value of frozen product withdrawal in the future should other epidemics of blood borne pathogens be documented.

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