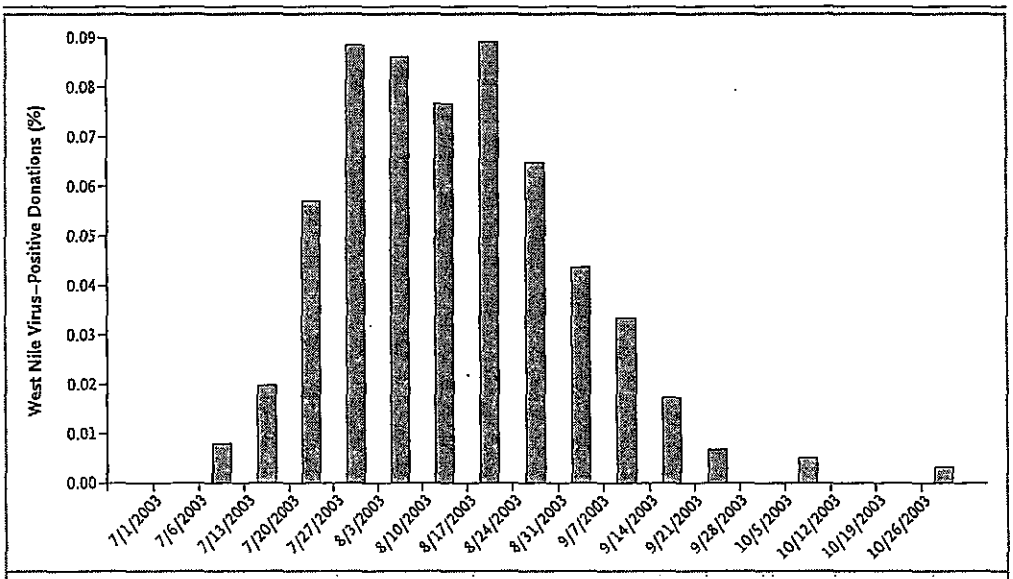

Figure 1. Results of Minipool Nucleic Acid Amplification Testing during the 2003 West Nile Virus Season.

Nile virus–specific IgM antibody appeared a median of four days after donation (interquartile range, one to six) and virus-specific IgG antibody appeared a median of two days later (interquartile range, one to five).

The median viral load for 143 of the 183 specimens confirmed to be positive on minipool testing with sufficient volume for quantitative PCR analysis was 3519 copies per milliliter (range, less than 50 to 690,159). Twelve IgM-positive donations identified by minipool testing had significantly lower viral loads than 131 IgM-negative donations identified by minipool testing (median, less than 50 and 5325 copies per milliliter, respectively; $P<0.001$ by the two-sample Wilcoxon rank test). As expected, viral loads were very low in donations detected only by nucleic acid amplification testing of individual donations: 21 of these 44 samples (48 percent) were reactive on only one of two replicate tests, indicating that the viral load was near the limit of detection of the transcription-mediated amplification assay, and 16 of 22 evaluated by TaqMan PCR (73 percent) had RNA levels that were below the limit of quantitation (i.e., fewer than 50 copies per milliliter).

We performed a subanalysis of 113 confirmed viremic donations identified from July 1 through September 30, 2003, in North Dakota and South Dakota from a donor population that had been screened with the use of both minipool and individual nucleic acid amplification testing. Table 1 shows the overall rates of detection of units confirmed positive by minipool testing and by individual testing alone, as well as the incremental rate of detection of viremia by nucleic acid amplification testing of individual donations throughout the epidemic, with adjustment to account for the proportion of units tested individually. Minipool testing detected 66 percent of viremic units detected by nucleic acid amplification testing of individual donations. Five percent of viremic donations were detectable by individual testing alone and were negative for West Nile virus antibody; 7 percent were reactive on individual testing alone and were positive for IgM but negative for IgG; and 22 percent were reactive on individual testing alone and were positive for both IgM and IgG (Table 1).

On the basis of previous studies of inoculation of West Nile virus in humans[17] and animals,[18,19] acute-phase infection is thought to be characterized by a brief period of very-low-level viremia shortly after inoculation (as reflected by the viral-load data presented above and the finding of IgM-negative specimens that were reactive on nucleic acid amplification testing of individual donations). This period is followed by a longer interval (approximately seven days) with an increasing and then decreasing viral load, which makes the viremia de-

Table 1. Yield of Minipool and Individual Nucleic Acid Amplification Testing of Donations from North Dakota and South Dakota, July 1 through September 30, 2003.

Variable	Total No. of Donations	Minipool Testing	Individual Nucleic Acid Amplification Testing Alone			
			Total No.	IgM−	IgM+, IgG−	IgM+, IgG+
No. of donations tested	27,009	27,009	22,641	22,641	22,641	22,641
No. of confirmed positive units	113	79	34*	5	6	21
Rate of detection (no. of confirmed positive units/100 donations)	0.443	0.292	0.150	0.024†	0.028†	0.099†
Adjusted no. of confirmed positive units‡	119	79	40	6	8	26
Proportional yield (%)	100	66	34	5	7	22

* Two units had insufficient volume for serologic testing.
† The value was adjusted to account for serologic classification of 32 of 34 units that were identified by individual testing alone.
‡ The value was adjusted to account for individual testing of 22,641 of the 27,009 units that underwent minipool testing.

tectable by minipool testing. Finally, as IgM and IgG seroconversion evolves, the viral load decreases to a level detectable only by testing of individual donations. Given this natural history, Figure 2 presents the biweekly yield data for North Dakota and South Dakota according to the results of individual and minipool tests and antibody tests. The figure shows that although testing of individual donations identified additional donations with a low level of viremia throughout the epidemic, the characteristics of these units shifted from primarily antibody-negative early in the epidemic to predominantly IgM-positive and IgG-positive late in the season ($P<0.001$ by Fisher's exact test).

Case investigations were initiated for 17 recipients of blood components from 14 donations that were negative on minipool testing but identified as having a low level of viremia on retrospective nucleic acid amplification testing of individual donations. On the basis of clinical symptoms and serologic analysis for West Nile virus, two recipients of seronegative donations with low-level viremia were infected with West Nile virus, probably as a result of transfusion, whereas two recipients of components from one donation that was reactive on individual testing alone and was positive for IgM and IgG were not infected.[12] The evaluation of the remaining 13 recipients was deemed inconclusive owing to a lack of follow-up laboratory data to support or rule out West Nile virus infection (Montgomery S and Brown J, CDC: personal communication).

During the 2004 epidemic (from May 1 to October 23, 2004), analysis of 1,065,212 donations by minipool testing yielded 71 confirmed viremic donations (Table 2). An additional 58,679 donations (5 percent of all donations tested) were prospectively tested individually with the use of previously described triggers,[16] and 54 donations were confirmed to be viremic. Sufficient volume was available to test 48 of these 54 donations at a 1:16 dilution: 27 were negative (and thus classified as reactive on individual testing alone), and 21 were positive (and thus classified as detectable by minipool testing). Of the 27 donations identifiable by individual testing alone, 23 were IgM-positive and 4 were IgM-negative. Thus, as seen in Table 2, targeted testing of individual donations in the regions of the 2004 epidemic yielded percentages of units detectable by minipool testing (76 percent) and by individual testing alone (24 percent) and a serologic profile for units detectable by individual testing alone that were similar to the percentages and profile observed in North Dakota and South Dakota during the 2003 epidemic (66 percent and 34 percent, respectively).

DISCUSSION

The implementation of nucleic acid amplification testing for West Nile virus RNA in 2003 resulted in the identification of 183 confirmed viremic units, with 47 additional infected units detected by targeted testing of individual donations. Nationally, the combination of minipool testing and targeted testing of individual donations resulted in the identification of approximately 1000 viremic donations.[11,15] Since, on average, each unit is made into 1.45 transfusable components,[20] the transfusion

of almost 1500 viremic components (most of which lacked antibody and would be expected to be infectious) was averted in 2003.

Our study confirms previous data from clinical cases and experimental inoculation studies indicating that infected persons would probably have low titers of West Nile virus.[2,3,12,15,17-19] Unlike seronegative donors with HIV and HCV infection and positive results on minipool testing, who usually have viral titers of 10^5 to 10^7 copies per milliliter,[6] in our study, the median number of copies of West Nile virus RNA was only about 3500. This observation of a low viral load combined with data indicating that proven transfusion-transmitted cases of West Nile virus infection occurred from donors with low viral titers[3,15] prompted us to study retrospectively the incremental value of individual nucleic acid amplification testing as compared with minipool testing and to implement individual testing prospectively in selected high-prevalence regions in late 2003 and 2004. We found that individual testing identified up to 50 percent more viremic donors than were detected by minipool testing. Five percent of all viremic donations were identifiable by individual testing alone and were antibody-negative and thus were donations that have been shown to be infectious. (Both recipients of units that were antibody-negative and reactive on individual nucleic acid amplification testing alone in our study were infected.) These donations were detected at a fairly constant rate throughout the epidemic. In contrast, the additional yield of antibody-positive donations identified by means of individual testing alone was minimal when tests were performed during the early weeks of the epidemic, but toward the end of the epidemic it increased to levels greater than those observed with minipool testing.

The incremental safety to be achieved by the use of individual testing over minipool testing is difficult to quantify because of the unknown risk of transmission by donations with low-level viremia that contain West Nile virus antibody. The absence of transmission of West Nile virus from two antibody-positive components identified by individual testing alone in this study is consistent with the observation that no documented case of post-transfusion infection has been attributed to a seroreactive donation, despite the relatively high frequency of such donations during the later stages of West Nile virus epidemics.[5,10,15] This is also consistent with in vitro and animal infectivity experiments suggesting that IgM and IgG antibodies neutralize infectivity.[19] Similar experience with hepatitis A virus, an acute infection that also leads to the production of neutralizing IgM antibody, has indicated that the concurrence of viremia and IgM production does not result in infectivity.[7,21] Thus, one working hypothesis is that viremic units that contain IgM (and particularly those that also contain IgG) are not infectious when transfused.

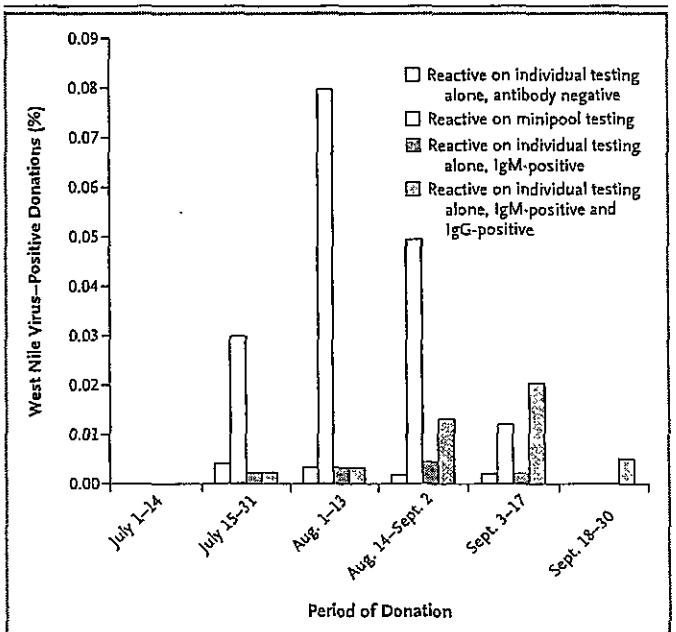

Figure 2. Rate of West Nile Virus–Positive Donations Detected in North Dakota and South Dakota by Minipool and Individual Nucleic Acid Amplification Testing.

The pattern of observed viremia (i.e., the percentage of West Nile virus RNA–positive donations detected by minipool testing as compared with the percentage detected by individual testing) and seroreactivity (presence of IgM and IgG) of viremic units changed dramatically through the 10 weeks of the epidemic, strongly suggesting that the greatest benefit of individual testing can be obtained by implementing such screening early in the epidemic when new infections are on the rise. This observation led Blood Systems Laboratories to adopt a new nucleic acid amplification testing strategy during the 2004 epidemic. A designated level of reactive minipool tests in a defined geographic region was used to determine when sufficient risk existed to implement individual testing.[16] This strategy

Table 2. Actual and Adjusted Systemwide Yield of Minipool and Individual Nucleic Acid Amplification Testing of Donations Screened from May 1 through October 23, 2004.

Type of Nucleic Acid Amplification Test	Total Tested	Confirmed Positive Results		Adjusted Confirmed Positive Results*	
	no. (%)	*no. (%)*	*no./100 donations*	*no. (%)*	*no./100 donations*
Minipool	1,065,212 (95)	71 (57)	0.007	95 (76)	0.009
Individual	58,679 (5)	54 (43)*	0.092	30 (24)	0.051
Total	1,123,891 (100)	125	0.012	125	0.012

* Of the 54 samples confirmed to be positive by nucleic acid amplification testing of individual donations, 48 were available for retesting at a 1:16 dilution; 27 of the latter (56 percent) were found to be negative and therefore classified as reactive on individual testing alone. This percentage was applied to the 54 positive units detected by individual testing to project that 30 of these donations would be positive with the use of this method alone and that 24 would have been detected by minipool testing. The reclassification is reflected in the column that provides adjusted confirmed positive results.

was designed to balance the residual risk of transfusion-transmitted West Nile virus infection accompanying the use of minipool testing against the limited capacity for individual testing, given the current limitations of automation.[5] This strategy proved highly effective, with the identification and removal of at least 27 units that would have been missed by minipool testing in regions with West Nile virus epidemic activity. They included at least four units that were IgM-negative and hence likely to transmit West Nile virus to recipients. Moreover, the similarity of the systemwide distribution of the yields of the two tests in 2004 (an increase in the rate of detection by approximately 32 percent with the use of individual over minipool testing) with that observed in a region of epidemic infection in 2003 indicates that our targeting of individual testing was appropriately directed to regions with an increased yield.

Before the initiation of blood-donor screening, information from experimental studies of the inoculation of West Nile virus conducted in the early 1950s in patients with advanced cancer indicated that the duration of viremia (as assayed by intracerebral injection of virus into mice) was approximately six or seven days.[17] Contemporary data from primate and murine models of West Nile virus infection are consistent with this estimate.[7,18,19] On the basis of the proportional rates of detection of viremia in the early phases of infection in asymptomatic viremic blood donors, we estimate that the duration of antibody-negative viremia detectable only by nucleic acid amplification testing of individual donations is shorter (one or two days). Our data cannot be used to estimate the length of the phase in which specimens are reactive on individual testing and antibody-positive, since a cross-sectional analysis would be biased: the frequency of donation in this convalescent phase is reduced as a result of the signs and symptoms of West Nile virus infection.[2] As compared with other transfusion-transmissible infections (e.g., HIV and HCV), West Nile virus has a similar interval in which it is detectable only by nucleic acid amplification testing of individual donations.[22] In contrast, the duration of viremia detectable by minipool testing is much shorter for West Nile virus infections than it is for HIV and HCV infections, in which high-titer viremia is detected for weeks or months before seroconversion and usually persists for many years after seroconversion.

In conclusion, although the use of minipool screening in 2003 prevented hundreds of West Nile virus infections, it failed to detect donations with a low level of viremia, some of which were antibody-negative and infectious. Our 2003 data supported the use of targeted nucleic acid amplification testing of individual donations in high-prevalence regions, a strategy that was successfully implemented in 2004. On the basis of the price of reagents for previously licensed nucleic acid amplification tests, the costs of performing minipool and individual screening at Blood Systems Laboratories, and the observed yields of minipool testing, the cost of minipool screening was $120,000 per unit intercepted in 2003 and $232,000 per unit intercepted in 2004. The reduced cost utility in 2004 reflects the decreased rate of viremic donations detected, illustrating the close inverse relationship between yield and cost-effectiveness. The targeted individual screening of approximately 60,000 donations in the summer of 2004 resulted in a 33 percent increment in yield and cost only $32,000 per incremen-

tal case detected. In contrast, had individual testing been performed for West Nile virus during all of 2004, the cost per viremic donation detected would have been $281,000. This approach of performing targeted testing of individual donations on the basis of real-time monitoring of the yield of minipool testing may prove to be a rational and cost-effective donor-screening paradigm for other agents similar to West Nile virus that cause seasonal and regional epidemics.

Supported in part by a grant (R01-CI-000214) from the Centers for Disease Control and Prevention (CDC) and the Blood Systems Foundation.

Dr. Busch is an employee of Blood Systems, a not-for-profit company that collects and tests donated blood for West Nile virus and other infections as described in this article; is a member of Scientific Advisory Boards of Chiron and Gen-Probe, the manufacturers of the West Nile virus assays that were evaluated in this study; and reports having received an unrestricted research grant from Chiron and speaking honoraria from Chiron and Gen-Probe. Ms. Caglioti, Dr. Robertson, Ms. McAuley, Dr. Tobler, Dr. Kamel, and Dr. Tomasulo are employees of Blood Systems. Dr. Linnen is an employee of Gen-Probe and reports owning equity stock and stock options in Gen-Probe. He is named on patents filed by Gen-Probe for the West Nile virus nucleic acid amplification assay described in this article. Dr. Shyamala is an employee of Chiron and is named on patents filed by Chiron for the West Nile virus nucleic acid amplification assay described in this article.

Reagents for performing the retrospective and prospective West Nile virus transcription-mediated amplification testing on collections in 2003 were supplied to Blood Systems at no charge by Gen-Probe. The costs for reagents to perform minipool screening in 2003 and 2004 and individual screening in 2005 were paid to Chiron and Gen-Probe on a cost-reimbursement basis, as detailed in an FDA Investigational New Drug (IND) application. All other sample-collection, shipping, and labor costs related to West Nile virus screening and acquisition of follow-up specimens were funded by Blood Systems, with reimbursement from hospitals or other blood centers. The cost of follow-up serologic and PCR testing was covered by Gen-Probe and Chiron as part of the IND application.

We are indebted to the staff at Blood Systems Laboratories and the United Blood Services and other donor centers for their effort in support of this study and to Susan Montgomery and Jennifer Brown at the CDC for coordination of look-back investigations of recipients exposed to West Nile virus.

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