itive, IgM-negative) sample detectable only by nucleic acid amplification testing of individual donations for every four such samples detected by *minipool testing.*[11] *For 2003 and 2004, we therefore* chose to initiate testing of individual donations in any blood-collection region after the identification of four RNA-positive donations and consequent calculation of a detection frequency of 1 in 1000 (the epidemic frequency documented in 2002[11,19]), on the basis of the date of collection of the first reactive donation. Once nucleic acid amplification testing of individual donations had been initiated, a seven-day period with no RNA-reactive donations was required before a collection region could revert to the use of minipool testing.

### NUCLEIC ACID AMPLIFICATION TESTING AND IgM AND IgG TESTING OF INDIVIDUAL DONATIONS

In 2003, 30,501 Red Cross donations from Kansas residents (August 19 to September 27) and Nebraska residents (August 25 to October 4) underwent individual nucleic acid amplification testing. Prospective testing identified 181 confirmed positive donations. Of these, 96 (53 percent) were nonreactive at a 1:16 dilution, of which 88 (92 percent) were IgM-positive and 8 (8 percent) were IgM-negative. In addition, as requested by the FDA, individual nucleic acid amplification testing was performed *retrospectively on frozen samples from 18,049 do*nations collected from July 10 (the date of the first confirmed positive donation identified by minipool testing) to August 22 from donors who lived in Nebraska to determine whether donations that were nonreactive on minipool testing and had therefore been released for transfusion would be identified as reactive on testing of individual donations. This retrospective evaluation identified 21 additional confirmed positive donations: 19 were IgM-positive samples and 2 were IgM-negative samples. During the same period, minipool testing had previously identified 80 confirmed positive donations (or 79 *percent of the total detected during this period):* 7 were IgM-positive samples and 73 were IgM-negative samples.

Overall, 117 of the 436 confirmed positive donations identified in 2003 (27 percent) were detected only by individual nucleic acid amplification testing (although this may be an underestimate, since not all donations were tested by this method), and of these 117, 10 (9 percent) were IgM-negative (Table 1). Of the remaining 319 donations that were reactive on minipool testing, 283 (89 percent) were IgM-negative (Table 1). For the 143 IgM-positive donations, the median viral load was be*low 100 copies per milliliter (range, less than 5 to* 14,000), as compared with 5800 copies per milliliter (range, less than 5 to 580,000) for the 293 IgM-negative donations ($P<0.001$ by the Wilcoxon rank-sum test). Donations that were positive for West Nile virus RNA and IgM were identified more frequently as the season progressed (Fig. 2A).

In 2004, the trigger for nucleic acid amplification testing of individual donations was reached for collections tested by the Red Cross in three areas: southern California (July 25 to October 8), Kansas (September 12 to 27), and Arkansas (August 28 to September 6), for a total of 92,460 donations tested individually. *No reactive donations were identi*fied by individual testing of Arkansas donors. However, 48 of 54 confirmed positive donations from southern California residents (89 percent) and 3 of 7 confirmed positive donations from Kansas residents (43 percent) were identified during the period of individual testing, for a combined positive rate of 0.056 percent, or 1 in 1791 donations. Of the 51 confirmed positive donations identified by nucleic acid amplification testing of individual donations, 31 (61 percent) were nonreactive on minipool testing. These 31 donations represented 30 percent of the total 104 positive donations identified in *2004; 26 (84 percent) were positive for IgM or IgG,* and 5 (16 percent) were negative for IgM and IgG (Table 1). Figure 2B shows that the identification of confirmed positive donations with IgM or IgG antibody reactivity increased as the 2004 season progressed; this increase was less than that observed for 2003. For the 35 IgM- or IgG-positive donations, the median viral load was below 100 copies per milliliter (range, less than 5 to 47,000), as compared with 3200 copies per milliliter (range, less than 5 to 160,000) for the 69 IgM- and IgG-negative donations ($P<0.001$ by the Wilcoxon rank-sum test).

*In both 2003 and 2004, two thirds of all viremic* donors were negative for West Nile virus antibody, according to two different IgM-antibody testing strategies: the Abbott Laboratories IgM assay (Fig. 2A) and the Focus Technologies IgM and IgG assays (Fig. 2B).

### DEMOGRAPHIC CHARACTERISTICS OF DONORS

Among the confirmed positive donors, as compared with the group of donors with false positive

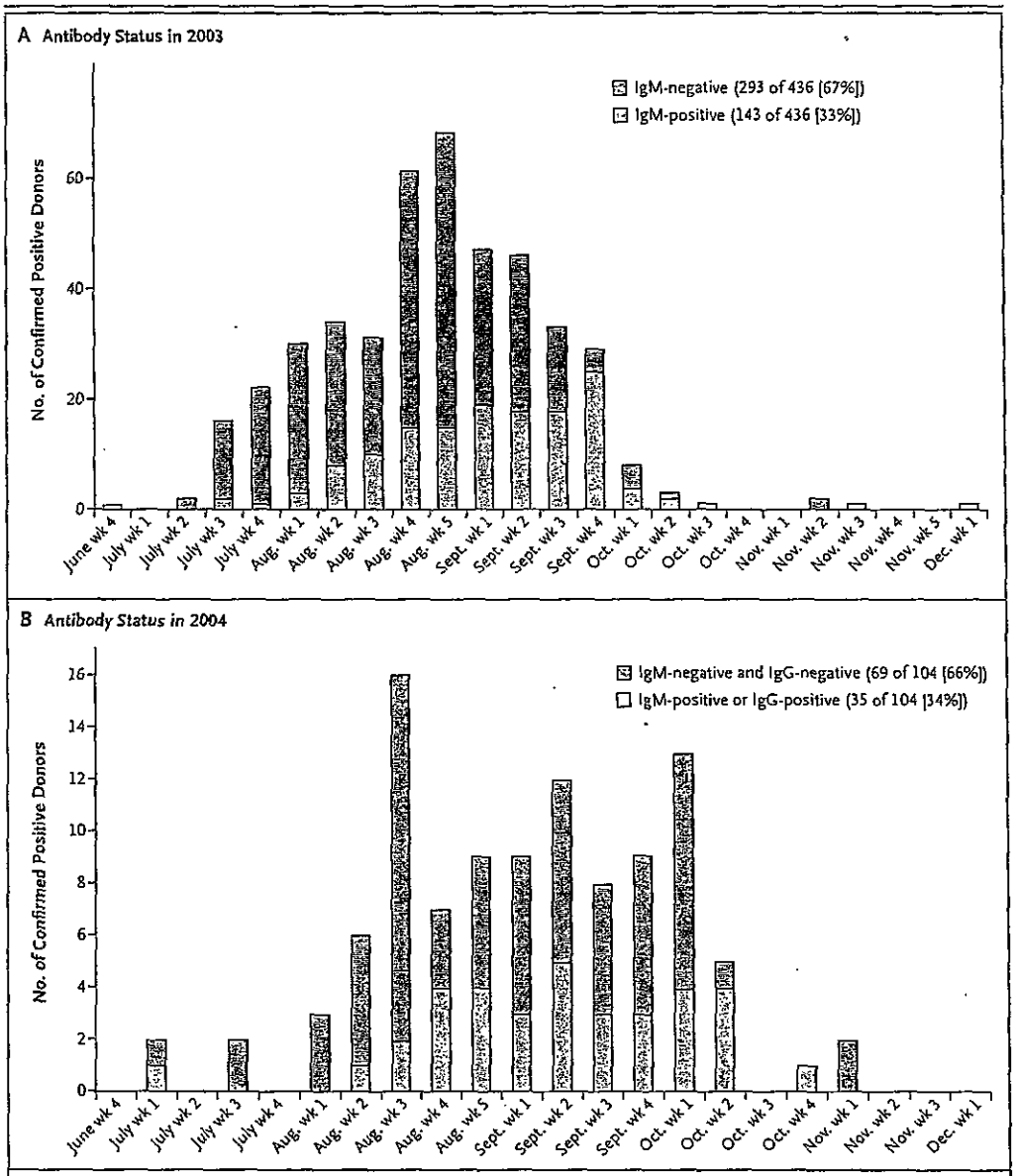


**Figure 2. IgM and IgG Antibody Status of Blood Donors Who Were Confirmed to Be Positive for West Nile Virus RNA on *Prospective Screening in 2003 and 2004*, According to the Week of Collection.**

In 2003 (Panel A), IgM antibody testing was performed by Abbott Laboratories; in 2004 (Panel B), IgM and IgG antibody testing was performed by Focus Technologies with the use of a reduced cutoff value (the standard cutoff value was multiplied by a correction factor of 0.67).

results, there were more male than female donors in 2002,[11] 2003, and 2004 (Table 2). In 2003, 50 percent of the overall donor population of the American Red Cross was male. Combining the confirmed positive donors from 2002 through 2004, 13 percent were first-time donors, with a mean age of 46 years (range, 16 to 83). These observations did not differ significantly from those observed for the donors with false positive test results.

### FOLLOW-UP OF DONORS AND RECIPIENTS

In 2003, 350 of 415 confirmed positive donors identified prospectively participated in the follow-up study, of whom 335 (96 percent) were IgM-positive

Table 2. Demographic Characteristics of Blood Donors with Reactive Nucleic Acid Amplification Tests.

| Period | No. of Donors | Male Donors | 1st-Time Donors | Age | |
|---|---|---|---|---|---|
| | | | | Mean | Range |
| | | no. (%) | | yr | |
| Sept. 3–28, 2002* | | | | | |
| Confirmed positive | 46 | 24 (52) | 9 (20) | 43.8 | 17–77 |
| False positive | 52 | 23 (44) | 10 (19) | 42.7 | 17–73 |
| June 29–Dec. 1, 2003 | | | | | |
| Confirmed positive | 436 | 258 (59)† | 43 (10)† | 46.5 | 17–83 |
| False positive | 382 | 187 (49) | 58 (15) | 47.5 | 13–89‡ |
| June 16–Oct. 16, 2004 | | | | | |
| Confirmed positive | 104 | 62 (60) | 24 (23) | 44.9 | 16–83 |
| False positive | 73 | 37 (51) | 12 (16) | 41.8 | 17–75 |

* Retrospective nucleic acid amplification testing was performed on frozen samples of individual donations from six high-incidence regions.[11]
† $P<0.05$ for the comparison with the false positive group during the same period.
‡ Frozen plasma was obtained from a 13-year-old repeat autologous donor.

or seroconverted during follow-up. Of 186 donors who participated in long-term follow-up, 166 (89 percent) retained specific IgM reactivity for 100 days or longer. However, of the 17 of 46 confirmed positive donors identified in 2002 for whom follow-up data were available,[11] 10 (59 percent) had IgM reactivity for more than 398 days, consistent with the observations of Roehrig and coworkers.[21] Of 104 confirmed positive donors identified in 2004, 82 (79 percent) participated in follow-up studies.

Only two recipients of donations confirmed to be positive on retrospective testing of individual donations in 2003 could be identified and consented to follow-up studies. Both were seronegative for West Nile virus and had had no reported symptoms associated with West Nile virus infection during the year after transfusion. In the case of both recipients, the transfused component was IgM-positive and had an RNA level that was too low to quantitate. In contrast, a 2002 recipient of an IgM-negative unit with a viral load of 6300 copies per milliliter had West Nile virus–related symptoms and antibody seroconversion.[11] Although these numbers are small, the data are consistent with reports of seroconversion and disease related to West Nile virus infection only among recipients of viremic, IgM-negative blood components.[7,22-25]

## DISCUSSION

In 2002, transfusion-transmitted West Nile virus infection was confirmed in 23 recipients of blood components, with the true number of transmissions believed to be much higher. By early summer of 2003, blood-collection agencies had implemented blood-donor testing for West Nile virus RNA, identifying and reporting a total of 1041 RNA-positive donations to the CDC through Arbonet for the 2003 and 2004 seasons.[19,26] The Red Cross program identified 540 of these viremic donations, of which 362 (67 percent) were negative for West Nile virus antibodies and likely infectious. The major epidemic focus in 2003 was the upper Plains states, moving to the Southwest in 2004. Most important, on the basis of prospective screening for West Nile virus RNA performed in 2003 and 2004 in our program, 1023 components manufactured from 519 prospectively screened viremic donations were not released for use and therefore not transfused.

Screening of blood donations for West Nile virus RNA was initiated in minipools of 16 samples, leading to concern that donations with low-level viremia might escape detection.[11,17,23] Our data from the 2002 West Nile virus season suggested that, at the height of the epidemic, there might be one viremic donation undetectable by minipool testing for every four that were detected. Accordingly, in areas with a high prevalence of RNA-positive donations (i.e., more than 1 in 1000 samples), we initiated nucleic acid amplification testing of individual donations after identifying a total of four RNA-positive donations on minipool testing in any given blood-collection region. This policy was supported by the observation of West Nile virus infec-

tions associated with the transfusion of blood units with RNA levels that could not be detected by minipool testing.[22-25] In addition, the effectiveness of this evidence-based trigger is demonstrated by the absence of any confirmed cases of transfusion-transmitted West Nile virus infection associated with blood components from our system in 2003 and 2004. An assessment of the effectiveness of various trigger strategies has been published elsewhere.[27] The continued use of a trigger strategy for the screening of blood donations for West Nile virus appears justified in order to focus available resources at times and locations of peak incidence. In contrast, at times and locations in which there are few or no identified viremic donations, minipool screening provides adequate safety.

Through careful follow-up studies of all RNA-reactive donors, we were able to establish the natural history of West Nile virus infection, finding that IgM antibodies against the virus were detectable about 11 days after the detection of viral RNA on minipool testing (13 days after the detection of viral RNA on testing of individual samples), followed rapidly by the appearance of IgG antibodies.[12,13] The transmission of West Nile virus through transfusion has not been linked to an RNA-positive component that is also positive for IgM or IgG antibodies against the virus. Although we identified 148 viremic donations that were detectable only by nucleic acid amplification testing of individual donations, only 15 of them (10 percent), or 1 in 9400 samples, were IgM-negative and thus represent the earliest stages of donor infection. Therefore, in programs dependent on trigger strategies, careful, real-time monitoring is critical; in the absence of timely system readiness and monitoring, breakthrough infection has been documented.[24]

The vast majority of the yield of nucleic acid amplification testing of individual donations was IgM-positive, demonstrating the long duration of positivity for IgM antibody in the presence of low-level viremia. It is likely that such donations would be noninfectious, especially in the presence of high titers of IgM and IgG, but studies to confirm this possibility have not been performed. Studies tracing recipients of blood components generally have a low yield, and our study is no exception; however, two recipients of IgM-positive, viremic blood components had no evidence of West Nile virus infection, in contrast to recipients who received IgM-negative, viremic units.[7,11,22-25]

In future years, will the need to screen blood donors for West Nile virus continue? We have now seen three West Nile virus epidemic seasons in the United States, with an expanding geographic range of viremic blood donors and clinical infections. The number of reported cases of West Nile virus neuroinvasive disease peaked in 2002 and 2003 (2946 and 2866 cases, respectively[20]) and decreased (to 1108 cases) in 2004.[26] This same trend was observed by our identification of 436 viremic blood donors in 2003 and of 104 in 2004, as well as by the total number of viremic blood donors reported to the CDC during these years (818 and 223, respectively).[20,26] West Nile virus infection may eventually follow the same pattern as St. Louis encephalitis, with only infrequent, localized recurrences.

To our knowledge, the implementation of nationwide testing for West Nile virus RNA has been the most rapidly instituted test-based intervention in the history of transfusion safety, taking only about nine months from the decision to develop a test to its implementation. The program is a model of cooperation among public health agencies, regulators, manufacturers, and the blood-supply system.[28] It is to be hoped that this process will be effectively replicated should there be a similar outbreak in the future.

Dr. Stramer reports holding stock in Abbott Laboratories and having received consulting fees from Gen-Probe, Chiron, and Abbott Laboratories. Dr. Fang reports holding stock in and having received consulting fees from Chiron. Ms. Brodsky reports holding stock in Abbott Laboratories. Dr. Dodd reports having received consulting fees from Gen-Probe, Chiron, and Abbott Laboratories.

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