BACKGROUND
Because of the potential severe clinical consequences of Zika virus (ZIKV) infection, the large numbers of asymptomatic travelers returning from ZIKV-active areas, the detection of ZIKV nucleic acid in blood, and reports of transmission of ZIKV through transfusion, in 2016 the Food and Drug Administration released recommendations for individual-unit nucleic acid testing to minimize the risk of transmission of ZIKV through blood transfusions.

METHODS
The American Red Cross implemented investigational screening of donated blood for ZIKV RNA by means of transcription-mediated amplification (TMA). Confirmatory testing of reactive donations involved repeat TMA, TMA testing in exploratory minipools, real-time reverse-transcriptase polymerase chain reaction, IgM serologic testing, and red-cell TMA. Viral loads in plasma and red cells were estimated by means of endpoint TMA. The costs of interdicting a donation that was confirmed to be positive were calculated for the 15-month period between June 2016 and September 2017.

RESULTS
Of the 4,325,889 donations that were screened, 393,713 (9%) were initially tested in 24,611 minipools, and no reactive donations were found. Of the 3,932,176 donations that were subsequently tested individually, 160 were initially reactive and 9 were confirmed positive (a 1:480,654 confirmed-positive rate overall; positive predictive value, 5.6%; specificity, 99.997%). Six (67%) of the confirmed-positive donations were reactive on repeat TMA, of which 4 were IgM-negative; of these 4, all 3 that could be tested were reactive on minipool TMA. Two confirmed-positive donors had infections that had been transmitted locally (in Florida), 6 had traveled to ZIKV-active areas, and 1 had received an experimental ZIKV vaccine. ZIKV RNA levels in red cells ranged from 40 to 800,000 copies per milliliter and were detected up to 154 days after donation, as compared with 80 days of detection in plasma at levels of 12 to 20,000 copies per milliliter. On the basis of industry-reported costs of testing and the yield of the tests in our study, the cost of identifying 8 mosquito-borne ZIKV infections through individual-unit nucleic acid testing was $5.3 million per ZIKV RNA–positive donation.

CONCLUSIONS
Screening of U.S. blood donations for ZIKV by individual-donation TMA was costly and had a low yield. Among the 9 confirmed ZIKV-positive donations, only 4 were IgM-negative; of these donations, all 3 that were tested were reactive on minipool TMA. (Funded by the American Red Cross and Grifols Diagnostic Solutions.)
Zika virus (ZIKV), an arthropod-borne virus (arbovirus) of the genus flavivirus, was identified in a sentinel rhesus monkey in the Zika Forest of Uganda in 1947 and was isolated from mosquitoes (Aedes africanus) in 1948.1 Outbreaks of ZIKV infection were observed in Yap Island, Federated States of Micronesia, in 20072 and in French Polynesia in 2013.2 ZIKV is transmitted through the bite of infected female A. aegypti mosquitoes4 and potentially A. albopictus mosquitoes,5 as well as through nonvector routes, including vertical (i.e., mother-to-infant),6,7 transfusion,8,9 and sexual transmission.10,11 Acute ZIKV infection is often asymptomatic (in 50 to 80% of cases), but it can manifest as a self-limited disease that usually resolves within 1 week.2,3,15 Severe complications related to ZIKV infection have been identified, including congenital defects, such as microcephaly,7,6,16 and noncongenital neurologic disorders — notably encephalitis,17-19 myelitis,20 and the Guillain–Barré syndrome.21-23

On the basis of the large proportion of asymptomatic cases, the severe clinical consequences to an infected fetus, the detection of ZIKV RNA in asymptomatic blood donors,12,24 and the reports of suspected cases of transfusion transmission of ZIKV in Brazil,10,11 ZIKV infections represent a threat to blood safety. Accordingly, the Food and Drug Administration (FDA) issued recommendations to minimize the risk of ZIKV transmission through blood components.25,26 On June 20, 2016, the American Red Cross initiated screening for ZIKV RNA using the Procleix Zika Virus Assay (Grifols Diagnostic Solutions) under an FDA-approved investigational-new-drug protocol. Data collected during investigational-new-drug testing and donor follow-up were used to assess the performance of the assay and the effect of donor screening on blood safety.

METHODS

SCREENING PROTOCOL

We informed donors who met current donor-suitability criteria about the study and included them in prospective screening unless they opted out. Investigational nucleic acid testing was initiated on June 20, 2016, at the American Red Cross National Testing Laboratories; nucleic acid testing was conducted in 16-member minipools with the Procleix Zika Virus Assay on the Panther System (Grifols Diagnostic Solutions). Testing was performed on collections of blood donations from five southeastern U.S. states that were presumed to be at high risk (Fig. 1). The assay uses magnetic-based target capture, transcription-mediated amplification (TMA), and chemiluminescent detection, with an estimated 95% limit of detection of 3.9 copies per milliliter (95% fiducial limits, 3.2 to 4.8), as determined by probit analysis.27,28 In accordance with the FDA revised guidance from August 26, 2016, investigational nucleic acid testing was extended to all U.S. blood donations, including conversion from minipool TMA to individual-donation TMA. Phased implementation of individual-donation TMA was completed on December 12, 2016 (Fig. 1). Donations that were initially found to be reactive on individual-donation TMA were retested in triplicate with the use of the same testing platform; donations with reactivity in any of the triplicate retests were considered to be repeat reactive. The screening protocol remains active, with 15 months of the results presented here.

CONFIRMATORY TESTING

Surplus sample tubes from all initially reactive donations were obtained; corresponding plasma units and red-cell components were quarantined and retrieved from collection regions. Samples from retrieved plasma units were each retested by TMA in 20 replicates. In addition, samples of plasma were sent to the Wadsworth Center (New York State Department of Health) for confirmatory testing, including alternate nucleic acid testing by in-house real-time, reverse-transcriptase polymerase chain reaction (RT-PCR), which had an estimated limit of detection of 70 copies per milliliter,29 and testing for anti-ZIKV IgM with an IgM antibody-capture enzyme-linked immunosorbent assay (MAC-ELISA) (performed under emergency-use authorization).30 IgM-positive donations (considered presumptively positive) were tested with an arbovirus plaque-reduction neutralization test to determine the presence of ZIKV, dengue viruses 1 through 4, or West Nile virus (WNV) neutralizing antibodies. When available, samples of red cells or residual whole blood and samples of plasma were sent to Grifols Diagnostic Solutions for further assessment of reactivity and estimation of viral loads by end-point TMA.31 Donors were classified as confirmed positive if their plasma, red cells, or whole blood was repeat reactive on TMA or if they were found to be positive by subsequent serologic or molecular testing.
To assess the sensitivity of minipool TMA, donations that were confirmed to be positive after individual-donation TMA were further tested in triplicate in exploratory 16-sample minipools that were created by combining the reactive donation with 15 donations that had been found nonreactive on TMA.

**DONOR FOLLOW-UP**

All donors whose samples tested reactive by TMA were contacted within 24 hours of the reactive test result to inquire about potential risk factors, including travel in the previous 28 days or sexual contact with a person who received a diagnosis of ZIKV infection or traveled to or resided in an area with active ZIKV transmission in the 3 months before sexual contact. All donors of reactive samples were notified of their reactive results, temporarily deferred from future blood donation, and invited to participate in the Zika Virus Follow-up Study, for which the participants provided additional blood samples beginning 1 week after the index reactive donation. Deferred donors were reinstated for ongoing donation when a follow-up sample tested nonreactive and 120 days had elapsed after the last reactive donation.

**STUDY OVERSIGHT**

This study was a collaboration between the American Red Cross and Grifols Diagnostic Solutions and included a signed confidentiality

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**Figure 1. Timeline of Zika Virus (ZIKV) Investigational Testing at the American Red Cross (ARC).**

In accordance with Food and Drug Administration (FDA) guidance, the ARC modified the donor-history questionnaire on March 14, 2016, and implemented donor deferral that was based on whether the donor had traveled to an area with active transmission of ZIKV or had had sexual contact with a man who had ZIKV infection or who resided in or had traveled to an area with active transmission of ZIKV. On June 20, 2016, the ARC voluntarily implemented ZIKV testing under an FDA-approved investigational-new-drug (IND) protocol, using the Procleix Zika Virus Assay on the Panther System (Grifols Diagnostic Solutions) with minipool transcription-mediated amplification (MP-TMA) in five states that were presumed to be at risk for ZIKV transmission — Alabama, Florida, Georgia, Mississippi, and South Carolina. On July 21, 2016, after the identification of the first autochthonous cases of ZIKV infection in Florida, the ARC started testing all Florida collections by individual-donation transcription-mediated amplification (ID-TMA). On August 26, 2016, the FDA issued a revised guidance recommending the individual screening by nucleic acid testing (ID-NAT) of all blood donations collected in the United States and its territories. On August 29, 2016, ZIKV testing by MP-TMA was initiated for selected states and regions: all donations from Arizona, Oklahoma, and Texas, as well as from additional areas of Mississippi. On September 6, 2016, MP-TMA was implemented for California, Oregon, and Washington collections. On September 26, 2016, MP-TMA started for New York collections and additional regions in California. On October 3, 2016, conversion from MP-TMA to ID-TMA took place for collections from Alabama, Arizona, California, Georgia, Mississippi, New York, Oklahoma, South Carolina, and Texas. On November 14, 2016, the ARC implemented MP-TMA in all remaining states and converted from MP-TMA to ID-TMA for donations collected in Idaho, Montana, Nevada, Oregon, Utah, and Washington. On December 12, 2016, the ARC finished converting all remaining states to ID-TMA. The ZIKV-related travel question was removed from the donor-history questionnaire on January 23, 2017.
agreement. The study was supported by internal funds of the American Red Cross with FDA-approved cost recovery (the FDA allows investigators to recover the actual costs of the investigational testing program). The costs of both minipool and individual-donation TMA were calculated on the basis of the mean of a range of cost-recovery figures reported by the industry. The protocol was approved by the institutional review board of the American Red Cross and by the FDA (Investigational New Drug application 17003). The authors vouch for the accuracy and completeness of the data and analyses presented.

RESULTS

ZIKV RNA DETECTION AND ASSAY PERFORMANCE

Between June 20, 2016, and September 9, 2017, a total of 4,325,889 donations were tested; 393,713 (9%) of the donations were tested in 24,611 minipools without a reactive result (Fig. 2). Of the other 3,932,176 donations (91%) that underwent subsequent testing by individual-donation TMA, 160 were initially reactive, of which 9 (5.6%) were confirmed positive by repeat TMA or additional testing (Fig. 2), for a rate of confirmed ZIKV-positive donations of 1 per 480,654. The remaining 151 initially reactive donations were found to be nonreactive on alternate nucleic acid testing, serologic testing, or additional TMA testing (or a combination of the three tests) of plasma and red cells or whole blood and were classified as false positive. The positive predictive value was 9 confirmed-positive donations among 160 reactive donations, or 5.6%. The specificity was the percentage of donations with nonreactive test results that were truly negative: 4,325,729 of 4,325,880, or 99.997% (Table 1).

Of the 9 confirmed-positive donations, 6 (67%; from Donors 1, 2, 3, 5, 8, and 9) were repeat reactive on individual-donation TMA; 3 were initially reactive on individual-donation TMA, but ZIKV infection was confirmed by the detection of ZIKV IgM in plasma and ZIKV RNA in red cells or whole blood (Donors 4, 6, and 7) (Fig. 2). Among the 6 donors of repeat-reactive blood, the index donations of 4 (67%; Donors 1, 3, 5, and 9) were IgM-negative — that is, they were window-period donations (collected before seroconversion had occurred). The results of alternate nucleic acid testing were positive in 2 of 4 donors (Donors 1 and 5) and equivocal in the other 2 window-period donors (Donors 3 and 9). All repeat-reactive donations were confirmed positive, for a positive predictive value of 100% (Table 1). Detection of ZIKV RNA at index by alternate nucleic acid testing in the 5 IgM-positive donors (Donors 2, 4, 6, 7, and 8) was unsuccessful. Plasma from confirmed-positive donations had an estimated 12 or fewer to 20,000 copies per milliliter at donation, as compared with red cells, which had 40 or fewer to approximately 800,000 copies per milliliter, with the exception of Donor 9, from whom index samples were not available (Table S1 in the Supplementary Appendix).

The confirmed-positive donors resided in Texas, California, Florida (3 donors), Massachusetts (2 donors), New York, and West Virginia (Table 2, and Fig. S1 in the Supplementary Appendix). Four (67%) of the 6 donors of repeat-reactive blood reported travel to a ZIKV-active area (Donors 1, 2, 5, and 8), with a mean of 14 days (range, 2 to 31) elapsing from travel return to blood donation (Table 2). Two of 3 donors of non-repeat-reactive confirmed-positive blood — Donors 4 and 7 — returned to the United States from a ZIKV-active area 73 and 59 days before donation, respectively, and had “tail-end” infections (i.e., residual, low-level infection), as reported previously. In addition, Donor 7 reported sexual contact with a person who traveled to an area with active ZIKV transmission (Table 2). Two of 9 confirmed-positive donors (Donors 4 and 7) became symptomatic after travel (Table 2), for a rate of symptomatic infection among donors of 22% — a finding similar to that previously reported for this virus, although the rate of symptomatic infection is probably higher. Each confirmed-positive donor is described in detail in the Supplementary Appendix.

EXPLORATORY MINIPool TESTING

Confirmed-positive index donations were tested by TMA in simulated minipools of 16 donations. The results of minipool TMA were 100% reactive for all tested window-period donations (from Donors 1, 5, and 9) (Table S1 in the Supplementary Appendix). Conversely, ZIKV IgM–positive donations were found to be nonreactive in TMA minipool testing (Donors 4, 7, and 8); the exception was that of Donor 2, whose blood was found to be reactive in one of three tests. Donors 3 (IgM-negative at index) and 6 (IgM-positive at index) were not included in minipool testing...
4,325,889 Donations were tested between June 20, 2016, and September 9, 2017. 4,325,729 Were tested by MP-TMA. 3,932,176 Were tested by ID-TMA. 160 Were initially reactive. 4,325,729 Were nonreactive. 2 Had positive result for alternate NAT testing (Donors 1 and 5). 3 Had positive IgM test result (Donors 4, 6, and 7). 148 Had negative IgM test result. 2 Had negative result for alternate NAT (Donors 2 and 8). 151 Had negative result for alternate NAT. 2 Had positive IgM test result (Donors 2 and 8). 0 Had negative IgM test result. 2 Had negative IgM test result (Donors 1 and 5). 6 Were repeat reactive (Donors 1, 2, 3, 5, 8, and 9). 154 Were non-repeat reactive. 9 Were confirmed ZIKV-positive donations. 2 Were reactive in whole-blood test (Donors 1 and 5). 0 Were not reactive in whole-blood test. 2 Were reactive in whole-blood test (Donors 2 and 8). 0 Were not reactive in whole-blood test. 3 Were reactive in whole-blood test (Donors 4, 6, and 7). 0 Were not reactive in whole-blood test. 1 Was reactive in whole-blood test (Donor 3). 1 Did not undergo whole-blood testing (Donor 9). 2 Had equivocal result for alternate NAT (Donors 3 and 9). 1 Did not undergo alternate NAT (Donors 1 and 5). 2 Had negative result for alternate NAT (Donors 2 and 9). 0 Had positive IgM test result. 0 Had negative IgM test result (Donors 3 and 9). 2 Had positive IgM test result (Donors 1 and 5). 0 Had positive IgM test result. 0 Had negative IgM test result. 2 Had negative result for alternate NAT (Donors 1 and 5). 2 Had positive IgM test result (Donors 1 and 5).
because of inadequate sample volume. TMA signal levels were higher in IgM-negative donations.

**Follow-up of Confirmed ZIKV-Positive Donors**

Each confirmed-positive donor consented to participate in the Zika Virus Follow-up Study and provided their first follow-up sample between 4 and 38 days (mean, 19.5 days) after their index donation (Table S1 in the Supplementary Appendix). Confirmed-positive donors provided between 1 and 25 follow-up samples (mean, 8). In 3 of 4 window-period donors (75%), seroconversion occurred at between 7 and 17 days of follow-up (mean, 11 days). The fourth window-period donor was the recipient of an experimental ZIKV vaccine (ZIKV purified inactivated vaccine [ZPIV]); the donor provided three additional samples at 14, 23, and 38 days, and none of the samples were reactive on ZIKV IgM testing (Table S1 in the Supplementary Appendix). ZIKV IgM detection continued in all seropositive donors for the duration of the follow-up study (range, 4 to 176 days; mean, 82) (Fig. 3). When the estimated time of ZIKV exposure of imported cases was included, IgM detection continued for a mean of 113 days after exposure (range, 18 to 235). All follow-up samples were nonreactive on alternate nucleic acid testing but were TMA-reactive in plasma or red cells. The longest duration of detection of ZIKV RNA in red cells was 154 days (Fig. 3B), as compared with 80 days in plasma (Fig. 3G).

**Cost of Testing**

On the basis of a per-donation cost of $6 for minipool nucleic acid testing (range, $3 to $9), which is similar to that for other transfusion-transmitted viruses for which minipool nucleic acid testing is currently used; $10 for individual-

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### Table 1. Performance Characteristics of the Procleix Zika Virus (ZIKV) Assay Based on the Number of Reactive Donations, June 20, 2016, to September 9, 2017.

<table>
<thead>
<tr>
<th>Test</th>
<th>Tested number of donations</th>
<th>TMA Reactive</th>
<th>Confirmed Positive</th>
<th>False Positive</th>
<th>Specificity percent</th>
<th>Positive Predictive Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Minipool</td>
<td>393,713</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>100.000</td>
<td>—</td>
</tr>
<tr>
<td>Individual donation</td>
<td>3,932,176</td>
<td>160</td>
<td>9</td>
<td>151</td>
<td>99.996</td>
<td>5.63</td>
</tr>
<tr>
<td>All</td>
<td>4,325,889</td>
<td>160</td>
<td>9</td>
<td>151</td>
<td>99.997</td>
<td>5.63</td>
</tr>
<tr>
<td>Repeat reactive</td>
<td>4,325,889</td>
<td>6</td>
<td>6</td>
<td>0</td>
<td>100.000</td>
<td>100.00</td>
</tr>
</tbody>
</table>

* Of the 160 donations tested by individual-donation testing that were ZIKV RNA–reactive, 9 (5.63%) were confirmed positive by repeat transcription-mediated amplification (TMA) or subsequent testing, for an overall confirmed-positive rate of 1 confirmed-positive donor per 480,654 tested donations. The assay specificity for minipool TMA was greater than that for individual-donation TMA (100.000% vs. 99.996%). The overall TMA specificity was 99.997%, and the positive predictive value was 5.63%. No reactive donations were identified by minipool TMA (100% specificity), which prevented the comparison of the positive predictive value of minipool versus individual-donation TMA. All repeat-reactive donations were confirmed positive by subsequent testing, yielding a positive predictive value and specificity of repeat reactivity of 100%.
Table 2. Confirmed ZIKV-Positive Donor Demographic Characteristics and Risk Factors. *

<table>
<thead>
<tr>
<th>Donor</th>
<th>State</th>
<th>Collection Date</th>
<th>Sex</th>
<th>Age in Yr</th>
<th>Travel†</th>
<th>Days from Return to Donation</th>
<th>Symptoms‡</th>
<th>Sexual Contact§</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Texas</td>
<td>Nov. 2, 2016</td>
<td>Male</td>
<td>62</td>
<td>Yes</td>
<td>8</td>
<td>No</td>
<td>No</td>
</tr>
<tr>
<td>2</td>
<td>California</td>
<td>Nov. 18, 2016</td>
<td>Male</td>
<td>61</td>
<td>Yes</td>
<td>14</td>
<td>No</td>
<td>No</td>
</tr>
<tr>
<td>3</td>
<td>Florida</td>
<td>Dec. 5, 2016</td>
<td>Male</td>
<td>68</td>
<td>No</td>
<td>—</td>
<td>No</td>
<td>Yes</td>
</tr>
<tr>
<td>4</td>
<td>Massachusetts</td>
<td>Dec. 27, 2016</td>
<td>Female</td>
<td>58</td>
<td>Yes</td>
<td>73</td>
<td>Yes</td>
<td>No</td>
</tr>
<tr>
<td>5</td>
<td>Florida</td>
<td>Jan. 10, 2017</td>
<td>Female</td>
<td>20</td>
<td>Yes</td>
<td>2</td>
<td>No</td>
<td>No</td>
</tr>
<tr>
<td>6</td>
<td>Florida</td>
<td>Jan. 12, 2017</td>
<td>Male</td>
<td>22</td>
<td>No</td>
<td>—</td>
<td>No</td>
<td>No</td>
</tr>
<tr>
<td>7</td>
<td>New York</td>
<td>Jan. 31, 2017</td>
<td>Female</td>
<td>26</td>
<td>Yes</td>
<td>59</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td>8</td>
<td>West Virginia</td>
<td>Mar. 13, 2017</td>
<td>Male</td>
<td>67</td>
<td>Yes</td>
<td>31</td>
<td>No</td>
<td>No</td>
</tr>
<tr>
<td>9</td>
<td>Massachusetts**</td>
<td>May 16, 2017</td>
<td>Female</td>
<td>19</td>
<td>No</td>
<td>—</td>
<td>No</td>
<td>No</td>
</tr>
</tbody>
</table>

* Of 160 donors identified by individual-donation transcription-mediated amplification (ID-TMA), 9 were confirmed as ZIKV-positive by supplemental testing. Five confirmed-positive donors were male, and 4 were female. The age distribution was 19 to 68 years (mean, 45 years). The donors resided in California, Florida (3 donors), Massachusetts (2 donors), New York, Texas, and West Virginia.

† Six confirmed-positive donors reported traveling to a ZIKV-active area; donors traveled to the following destinations: Donor 1, Puerto Rico; Donor 2, St. Maarten; Donor 4, St. Thomas; Donor 5, Curaçao; Donor 7, St. Kitts and Nevis; and Donor 8, Aruba.

‡ Donor 4 reported symptoms consistent with ZIKV infection, including low-grade fever, aches, and rash and vasculitis in the hands. Donor 7 reported clinical symptoms between December 5 and December 8, 2016, after her return to the United States, including a rash and a head cold.

§ Donors 3 and 7 reported sexual contact with someone who traveled or resided in an area of active ZIKV transmission. The sex partner of Donor 3, also a resident of Miami-Dade County with no travel risk, was invited to participate in the follow-up study. The partner provided five samples that were TMA-reactive in whole blood and seropositive by IgM antibody-capture enzyme-linked immunosorbent assay and plaque-reduction neutralization testing analyses.

¶ Donors 3 and 6 were residents of Miami-Dade County, an area of active ZIKV transmission at the time of donation; these donors did not report a travel risk and therefore had probable cases of autochthonous ZIKV transmission.

‖ Donors 4 and 7 had tail-end ZIKV infections; these donors provided TMA-reactive samples 73 and 59 days after their return from a ZIKV-active area, respectively.

** Donor 9 did not report traveling to a ZIKV-active area and is a resident of Massachusetts; the donor is the recipient of an experimental ZIKV vaccine.

Figure 3 (facing page). Long-Term Follow-up of Selected Confirmed-Positive Donors.

All confirmed-positive donors consented to participate in the Zika Virus Follow-up Study. Confirmed-positive donors provided between 1 and 25 follow-up samples, which were tested by TMA with the Procleix Zika Virus Assay and by RT-PCR to determine the presence of ZIKV RNA in plasma and red cells or whole blood, as well as by MAC-ELISA and plaque-reduction neutralization testing (PRNT) to determine the presence of ZIKV antibodies in serum. PRNT results are expressed as antibody dilution titers. Samples with neutralizing ability at a dilution greater than 1:10 are considered positive and represented in the figure. ZIKV RNA loads in plasma and red cells or whole blood were determined by limiting-dilution TMA from samples that were 100% TMA-reactive; these are expressed as RNA copies per milliliter. RT-PCR results are expressed as cycle threshold (Ct) values. Ct values of less than 40 are considered positive and are shown in the figure. MAC-ELISA results (IgM) are represented as the ratio of the mean optical density (OD) of the test specimen reacted with Zika viral antigen and the mean OD of the normal human serum reacted with Zika viral antigen. Results greater than 2 are considered presumptively positive and are shown in the figure. All results are expressed in logarithmic scale. Detailed descriptions of each donor’s test results can be found in the Supplementary Appendix.
Testing for Zika Virus among U.S. Blood Donors

The New England Journal of Medicine

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donation nucleic acid testing (range, $7 to $13), including all expenses related to testing; and the number of collections tested by the American Red Cross between June 20, 2016, and September 9, 2017, the cost of ZIKV testing was approximately $41.7 million over the course of the 15-month study.

**Discussion**

The ZIKV outbreak in the Americas in 2015 and 2016 revealed unprecedented characteristics for an arbovirus, including causal association with a severe congenital syndrome and sexual transmission. These findings, together with the large proportion of asymptomatic cases and reports of probable transfusion-transmitted ZIKV infections, triggered a rapid response to minimize the risk of ZIKV transmission to pregnant women and blood-transfusion recipients, including the rapid development of ZIKV nucleic acid testing assays for donor screening. The American Red Cross implemented the Procleix Zika Virus Assay in minipool TMA testing on June 20, 2016. After the identification of the first cases of local mosquito transmission in Florida, the American Red Cross converted from minipool to individual-donation TMA for donations collected in Florida, subsequently extending this to all U.S. collections.

Of 4,325,889 donations, 160 were initially reactive, including 9 (5.6%) confirmed-positive samples, for a rate of 1 per 480,654 donations screened. The specificity of minipool TMA was higher than that of individual-donation TMA (100% vs. 99.996%), for an overall TMA specificity of 99.997% and a positive predictive value of 5.6%. All repeat-reactive donations were confirmed positive by subsequent testing, yielding a positive predictive value of 100%. The calculated specificity of the Procleix Zika Virus Assay in individual-donation TMA was similar to that previously published for ZIKV nucleic acid testing. The overall performance characteristics of ZIKV TMA are similar to those of another assay, the Procleix WNV Assay (Table 1, and Table S2 in the Supplementary Appendix). However, the Procleix Zika Virus Assay had a substantially lower positive predictive value because individual-donation TMA for ZIKV is performed throughout the year in areas in which the virus is not endemic, as opposed to WNV, for which individual-donation TMA occurs only during the active WNV season of June through November, with minipool TMA used during other times. Minipool TMA has the advantage of an additional cycle of reactive minipool resolution testing before the identification of an individual TMA-reactive donation (i.e., each donation in a reactive minipool is tested individually in a second round of testing).

Four window-period donations were identified by ZIKV screening; seroconversion had occurred in all window-period donors with the exception of the vaccine recipient, Donor 9, at the time of the first follow-up. The absence of seroconversion in Donor 9 may have been due to receipt of only a single vaccine dose of two planned by the protocol. Alternatively, 38 days of follow-up may have been insufficient to detect seroconversion. It is possible that receipt of only a single vaccine dose plus subsequent plateletpheresis in Donor 9, which occurred 6 hours 20 minutes after intramuscular administration of the vaccine, together contributed to the absence of seroconversion. Nonetheless, this study shows that ZIKV RNA can be detected in plasma by a sensitive TMA method after ZPIV administration. In all other confirmed-positive donors, IgM detection persisted for at least 113 days after exposure.

Like WNV, ZIKV associates with red cells in the blood. The presence of ZIKV RNA in red cells has been reported for as long as 101 days when tested by RT-PCR with a limit of detection exceeding 100 copies per milliliter, as compared with 54 days in serum as determined by Trioplex RT-PCR with an estimated limit of detection of 40 copies per milliliter. The longest period of ZIKV RNA detection in red cells in our study was 154 days, as compared with 66 days in plasma from the same donor, whose sample was repeat-reactive on TMA, a finding consistent with the use of a more sensitive assay with an estimated limit of detection of 3.9 copies per milliliter. Viral RNA loads in reactive samples were generally low, ranging from approximately 12 to 200 copies per milliliter in plasma and approximately 40 to 800 copies per milliliter in red cells. The sole exception was Donor 5, a window-period donor whose index plasma and red-cell samples contained 20,000 and 800,000 copies per milliliter, respectively; 7 days later, the ZIKV RNA levels in this donor underwent a several-log decrease, concomitant with seroconversion. Red
cells may be the source of ZIKV RNA detected in plasma by TMA (see the Supplementary Appendix).

The correlation between ZIKV genome copies and viral infectivity is currently unknown, but it has been estimated to be within the range of 200 to 500 genome copies per infectious viral particle in culture.\(^2\) On the basis of this range, the number of infectious units in all but one of the confirmed-positive donations in this study would be approximately 1 infectious unit per milliliter in plasma and approximately 1.6 infectious units per milliliter in red cells (the exception again being Donor 5, whose sample was repeat-reactive on minipool TMA and who had returned from travel just 2 days before blood donation).

Donors 3 (repeat reactive) and 6 (initially reactive) did not report a travel risk and are residents of Miami-Dade County, an area previously designated as ZIKV active. ZIKV infection in Donor 3's partner was confirmed by RNA reactivity in plasma and whole blood and by seropositivity at the same time point as the donor. The partner also resided in Miami-Dade County and had not traveled. In the absence of additional epidemiologic data, Donor 3 and his partner represent two potential cases of autochthonous ZIKV infection or one case of autochthonous ZIKV infection with associated sexual transmission. Similarly, Donor 6 is another patient with a probable case of local ZIKV infection.

Given that most cases of ZIKV infection are asymptomatic and that there are unusual non-vector routes of ZIKV transmission, donor-deferral policies that are based on travel and symptom reporting may have limited sensitivity for blood safety. Consequently, the FDA has recommended individual-donation nucleic acid testing of all U.S. blood donations. Such an implementation came at a substantial expense: $42 million over a period of 15 months. If ZIKV screening after the investigational-new-drug assessment follows current FDA guidance, and given that the American Red Cross collects 42% of the U.S. blood supply, the projected annual cost for national screening is estimated to be $137 million (range, $109 million to $167 million),\(^11\) which will pose an additional strain on the blood industry.\(^10\) Our data for 8 donations that were confirmed positive for mosquito-borne ZIKV translates to a cost of $5.3 million for each RNA-positive donor identified per year by individual-donation TMA, whether infectious or not, or $21 million for each of 2 donors without travel risk. Note that these costs do not reflect increases attributable to commercial pricing. In addition, further testing beyond the period covered here (6.32 million donations through February 3, 2018) has yielded only one additional confirmed-positive donation: a window-period donation from a traveler who donated blood 4 days after return from Puerto Vallarta, Mexico. The estimated viral loads in this donor were 1,000,000 and 20,000 copies per milliliter in plasma and red cells, respectively. There is currently no conclusive evidence that low-level, antibody-positive donations, as in Donors 2, 4, 6, 7, and 8, are infectious, whereas previous experience with WNV indicates that seronegative donations can result in transfusion transmission,\(^14,40\) which suggests that the donations from Donors 1, 3, and 5 were potentially infectious.

We also explored the detection of ZIKV RNA in confirmed-positive donations in simulated pools of 16 samples. Minipool TMA results were reactive for all window-period donors who could be tested (including the most recently identified donor), whereas seropositive donations that were tested in pools were found to be nonreactive. The exception was the repeat-reactive donation from Donor 2, for whom one of three pool results was found to be reactive.

In conclusion, screening of U.S. blood donations for ZIKV by means of individual-donation TMA had a low yield and a high cost. Among the nine donors whose blood was confirmed as ZIKV-positive, only four had acute infection (i.e., were IgM-negative); among these four donors, all three with adequate sample volumes for testing also tested positive on minipool TMA. Red cells had higher viral loads over longer periods than plasma in the confirmed-positive donors who underwent repeat testing.

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