

ORIGINAL ARTICLE

Detection of HIV-1 and HCV Infections among Antibody-Negative Blood Donors by Nucleic Acid–Amplification Testing

Susan L. Stramer, Ph.D., Simone A. Glynn, M.D., M.P.H.,
Steven H. Kleinman, M.D., D. Michael Strong, Ph.D.,
Sally Caglioti, M.T. (A.S.C.P.), S.B.B., David J. Wright, Ph.D.,
Roger Y. Dodd, Ph.D., and Michael P. Busch, M.D., Ph.D.,
for the National Heart, Lung, and Blood Institute Nucleic Acid Test Study Group

ABSTRACT

BACKGROUND

From the American Red Cross, Gaithersburg, Md. (S.L.S.), and Rockville, Md. (R.Y.D.); Westat, Rockville, Md. (S.A.G., S.H.K., D.J.W.); the University of British Columbia, Victoria, B.C., Canada (S.H.K.); Puget Sound Blood Center, Seattle (D.M.S.); Blood Systems Laboratory, Tempe, Ariz. (S.C., M.P.B.); Blood Systems Research Institute, Blood Centers of the Pacific, San Francisco (M.P.B.); and the University of California, San Francisco (M.P.B.). Address reprint requests to Dr. Stramer at the American Red Cross National Testing and Reference Laboratories, 9315 Gaither Rd., Gaithersburg, MD 20877, or at stramers@usa.redcross.org.

Testing of blood donors for human immunodeficiency virus type 1 (HIV-1) and hepatitis C virus (HCV) RNA by means of nucleic acid amplification was introduced in the United States as an investigational screening test in mid-1999 to identify donations made during the window period before seroconversion.

METHODS

We analyzed all antibody-nonreactive donations that were confirmed to be positive for HIV-1 and HCV RNA on nucleic acid–amplification testing of “minipools” (pools of 16 to 24 donations) by the main blood-collection programs in the United States during the first three years of nucleic acid screening.

RESULTS

Among 37,164,054 units screened, 12 were confirmed to be positive for HIV-1 RNA — or 1 in 3.1 million donations — only 2 of which were detected by HIV-1 p24 antigen testing. For HCV, of 39,721,404 units screened, 170 were confirmed to be positive for HCV RNA, or 1 in 230,000 donations (or 1 in 270,000 on the basis of 139 donations confirmed to be positive for HCV RNA with the use of a more sensitive HCV-antibody test). The respective rates of positive HCV and HIV-1 nucleic acid–amplification tests were 3.3 and 4.1 times as high among first-time donors as among donors who gave blood repeatedly. Follow-up studies of 67 HCV RNA–positive donors demonstrated that seroconversion occurred a median of 35 days after the index donation, followed by a low rate of resolution of viremia; three cases of long-term immunologically silent HCV infection were documented.

CONCLUSIONS

Minipool nucleic acid–amplification testing has helped prevent the transmission of approximately 5 HIV-1 infections and 56 HCV infections annually and has reduced the residual risk of transfusion-transmitted HIV-1 and HCV to approximately 1 in 2 million blood units.

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SCREENING OF POTENTIAL BLOOD DONORS has historically relied on the use of immunoassays to detect viral antibodies or antigens. In 1999, new screening methods involving nucleic acid amplification to detect human immunodeficiency virus type 1 (HIV-1) and hepatitis C virus (HCV) RNA were implemented in the United States under an investigational new drug protocol approved by the Food and Drug Administration (FDA).¹⁻³ This new technique was used to test multiple samples in small pools, referred to as "minipools." The decision to implement this technique was based on its ability to identify HIV-1- and HCV-infected donors early in the infectious window period, before seroconversion,⁴ and the experience of plasma-derivative manufacturers showing the practicality of this approach for pooled specimens.¹ Finally, it was recognized that the availability of nucleic acid-based tests would support future testing of emerging agents.^{5,6}

The advent of nucleic acid-amplification testing has led to the discontinuation of two less effective screening tests. HIV-1 p24 antigen screening was recommended by the FDA in 1996 for the early detection of HIV-1 infection,⁷ and the FDA allowed this approach to be discontinued on the licensure of the HIV-1 nucleic acid-amplification test. Elevated levels of alanine aminotransferase have been used as a surrogate (nonspecific) marker for HCV infection since 1986.⁸ The use of this screening approach was never an FDA requirement, so blood centers have voluntarily discontinued this test. RNA-based donor screening has afforded an opportunity to study events occurring early in HIV-1 and HCV infection.⁹⁻¹² To quantify the relative risk of transmission of HIV-1 and HCV from first-time blood donors and those who donated blood repeatedly, we analyzed the number of RNA-positive, antibody-nonreactive allogeneic blood donations from donors infected with HIV-1, HCV, or both that were identified in the first three years after the implementation of nucleic acid-amplification testing in the United States.

METHODS

Since 1999, allogeneic blood donations in the United States have been screened for HIV-1 and HCV RNA in a minipool format with the use of one of two nucleic acid-amplification tests.^{1,2,13} The Gen-Probe Transcription-Mediated Amplification sys-

tem uses a multiplex HIV-1 and HCV assay and minipools of 16 donor samples.¹⁴ All donation samples within a reactive minipool are tested individually to identify both the sample that was reactive and the viral cause of the reaction. The Roche Molecular Systems Cobas AmpliScreen HIV-1 and HCV tests separately detect HIV-1 and HCV RNA in minipools of 24 donor samples.¹¹ Both assays are highly specific and sensitive, with 50 percent detection limits (i.e., the level at which 50 percent of test results would be expected to be reactive) of 14 or fewer copies of HIV-1 per milliliter and 12 or fewer copies of HCV per milliliter on the basis of probit analyses.^{13,14} The 95 percent detection limits as defined in the package inserts for both tests range from 30 to 60 copies per milliliter for HIV-1 and HCV. Both systems have received FDA approval for routine screening of blood donors.

All major laboratories in the United States participating in nucleic acid-amplification screening (accounting for over 98 percent of tested blood donations) participated in this study and reported data collected on cases identified between March 1999 and January 2002, and in some instances from March 1999 through April 2002. A case was defined as an allogeneic donation that was nonreactive to antibody against HIV-1, HCV, or both but that was reactive on minipool nucleic acid-amplification screening and confirmed to be positive for HIV-1 or HCV RNA. Five testing programs used the Gen-Probe assay and reported cases of HIV-1 and HCV viremia identified on screening of 27,956,758 donations. The Roche Cobas AmpliScreen was used in 13 laboratories, which tested a total of 9,207,296 donations for HIV-1 RNA and 11,764,646 donations for HCV RNA. All participating sites received approval of this study from their institutional review board. Data were contributed by the blood-collection organizations and the manufacturers of the nucleic acid assays (Roche Molecular Systems, Gen-Probe, and Chiron).

The date of donation, the donor's status as a first-time or repeat donor, and whether the unit would have qualified for transfusion if not for the result of the nucleic acid-amplification test (i.e., whether the unit was transfusable) were collected for each case. Furthermore, the results of HIV-1 p24 antigen testing were compiled for cases of HIV-1 viremia, whereas data on alanine aminotransferase levels and the presence or absence of antibody against hepatitis B core antigen (anti-HBc) were col-

lected for cases of HCV viremia. When applicable, we also compiled the results of repeated serologic analyses, repeated nucleic acid–amplification testing of the index sample with the use of a different type of RNA method (e.g., different techniques, primers, or probes), nucleic acid–amplification testing of an independent sample from the index donation, and serologic and nucleic acid–amplification testing of samples collected from donors participating in the follow-up analysis. For HIV-1, antibody was detected with the use of enzyme immunoassays and confirmed by Western blotting; for HCV, antibodies were detected by either second- or third-generation enzyme immunoassays and confirmed by recombinant immunoblot assay (RIBA, Chiron). Laboratories that routinely used second-generation HCV-antibody tests to screen donations were also asked to report the results of third-generation HCV-antibody tests performed on the HCV RNA–positive donations. This allowed categorization of cases of HCV viremia into those in which antibodies were detectable only by the more sensitive third-generation test and those with no detectable HCV antibody on both second- and third-generation HCV-antibody tests.¹¹ A case was considered confirmed if the index donation was reactive to HIV-1 or HCV RNA with the use of a second type of nucleic acid–amplification test, if another sample from the index donation was reactive on the nucleic acid assay, or if at least one follow-up sample was reactive on nucleic acid–amplification testing or antibody testing.

An expanded data set was developed by the largest participating program (the American Red Cross) to study the dynamics of HCV infection. This data set included follow-up of HCV RNA–positive donors identified from March 1999 through mid-June 2003, thus providing an additional 15 months of follow-up on a well-characterized group of donors with acute HCV infection. For this program, a standardized prospective protocol was used to enroll donors, with specimens collected at approximately four-week intervals through the time of seroconversion, as confirmed by third-generation HCV-antibody tests, and beyond.

To determine whether trends observed for HIV-1–positive and HCV RNA–positive donors were constant beyond this three-year study, an additional two years of data from the American Red Cross were analyzed. Data on HIV-1–positive and HCV RNA–positive donors from March 1999 through March 2002 were compared with those for the

subsequent two-year period from April 2002 to April 2004.

To evaluate rates of positive nucleic acid–amplification tests for HIV-1 and HCV RNA in specific subgroups (first-time and repeat donors and donors with otherwise transfusable donations), data were included only from laboratories that routinely reported this information. These subgroups represented about 37.0 million of the 39.7 million total donations. On the basis of data from the American Red Cross for 1999 through 2002, it was estimated that 23 percent of allogeneic donations were collected from first-time donors and 1 percent of all donations were discarded owing to reactivity to another routine serologic screening test in addition to nucleic acid–amplification testing.¹⁵

Rates of positive nucleic acid–amplification tests per million donations were calculated by dividing the number of cases by the number of known donations screened (or for samples from first-time or repeat donors and samples that were otherwise transfusable, by the estimated number of donations) and multiplying by 10⁶. When the number of donations was known, the associated 95 percent confidence interval for the rate was computed.¹⁶ When the number in a subgroup of donations was estimated, an approximate 95 percent confidence interval was computed incorporating the uncertainty around the estimated number of donations.^{16,17} Fisher's exact tests and Wilcoxon's tests were used to compare categorical and continuous variables, respectively. All reported P values are two-sided.

RESULTS

VIREMIC, SERONEGATIVE DONATIONS DETECTED BY NUCLEIC ACID–AMPLIFICATION TESTING

In the three years after the implementation of mini-pool nucleic acid–amplification testing, 12 donations that were not reactive to HIV-1 antibody and 170 donations that were not reactive to HCV antibody were confirmed to be positive for HIV-1 RNA and HCV RNA, respectively, among approximately 37 million to 40 million donations screened (Table 1). Hence, 1 per 3.1 million donations screened was confirmed to be positive for HIV-1 RNA and antibody–nonreactive, whereas 1 donation per 230,000 was confirmed to be positive for HCV RNA and antibody–nonreactive. Rates did not differ significantly between users of the Gen-Probe nucleic acid–amplification test and users of the Roche test (P=0.74, data not shown).

EFFECT OF SEROLOGIC SCREENING ASSAYS ON THE DETECTION OF HCV RNA

In the United States, laboratories use one of two licensed assays, which differ significantly in window-period sensitivity, to screen donations for HCV antibody.^{11,18} Of 156 HCV RNA–positive donations, 17 that were antibody–nonreactive on the second-generation assay would have been identified as reactive by the third-generation assay, adjusting the rate of HCV–positive donations to 1 in 270,000 donations (Table 1).

Some of the donations that were positive on minipool nucleic acid–amplification testing would not have been released for transfusion even if such testing had not been performed. Among the 12 donations that were positive for HIV-1 RNA, 2 were confirmed to be positive for HIV-1 p24 antigen (Table 1), and 33 percent of donations that were identified as positive for HCV RNA (51 of 156) by laboratories that reported subgroup information would have been deemed nontransfusable (Table 1), including 45 of 51 units (88 percent) with an elevated alanine aminotransferase level. The remaining 6 were nontransfusable owing to reactivity to other routine screening tests; none of 155 HCV RNA–positive donations evaluated for anti-HBc reactivity were reactive. Thus, HCV nucleic acid screening prevented the release of 1 viremic donation for every 350,000 donations screened.

ALANINE AMINOTRANSFERASE PATTERNS IN DIFFERENT STAGES OF HCV INFECTION

To compare the distribution of alanine aminotransferase levels associated with various stages of HCV infection, we evaluated donor alanine aminotransferase levels compiled by the American Red Cross from 1999 through 2002. As shown in Figure 1, HCV–seronegative donors had significantly different alanine aminotransferase distributions depending on their HCV RNA status; donors confirmed to be positive for HCV RNA had higher median enzyme levels than HCV RNA–negative donors (54 vs. 21 IU per liter, $P < 0.001$). Donors who were confirmed to be positive for HCV RNA had elevated enzyme levels independent of their HCV antibody status (median, 56 IU per liter for seropositive donors vs. 54 IU per liter for seronegative donors; $P = 0.99$). However, enzyme elevations of 120 IU per liter or more were noted more frequently among HCV RNA–positive, seronegative donors than among HCV RNA–positive, seropositive donors (30 percent vs. 15 percent, $P < 0.001$). Lastly,

Table 1. Rates of Positivity for HIV-1 and HCV RNA, March 1999 to April 2002.

	No. of Donations	RNA-Positive Donations	
		Total No.	Rate per 10 ⁶ Donations (95% CI)*
HIV-1			
Total	37,164,054	12	0.32 (0.17–0.56)
Otherwise transfusable	36,792,000†	10	0.27 (0.13–0.50)
HCV			
Total	39,721,404	170	4.3 (3.7–5.0)
Subtotal‡	36,974,722	156	4.2 (3.6–4.9)
Third-generation antibody–nonreactive‡	36,974,722	139	3.8 (3.2–4.4)
Otherwise transfusable‡	36,605,000†	105§	2.9 (2.3–3.5)

* CI denotes confidence interval.

† Ninety-nine percent of donations were assumed to be transfusable (nonreactive on all screening tests and suitable for transfusion).

‡ Data from three laboratories were excluded because they did not report data on transfusability according to donors' first-time or repeat status, or the results of third-generation assays for the RNA-positive index donations.

§ One HCV RNA–positive donation with missing data on transfusability was weighted according to the distribution of transfusable and nontransfusable units among the remaining HCV RNA–positive donations, so that 104.67 (rounded to 105) such donations were assumed to be otherwise transfusable.

among seropositive donors, enzyme levels were again higher among HCV RNA–positive donors than among HCV RNA–negative donors (56 vs. 22 IU per liter, $P < 0.001$).

RELATIVE YIELD OF NUCLEIC ACID–AMPLIFICATION TESTS AMONG FIRST-TIME DONORS AND REPEAT DONORS

Viremic donations were more likely to be detected from first-time rather than repeat donors. Although only marginally significant ($P = 0.05$), the rate of positivity for HIV-1 RNA was 4.1 times as high among the former group as the latter group; this ratio was 2.7 for the donations that were nonreactive to HIV-1 p24 antigen but positive for HIV-1 RNA (Table 2). The rate of positivity for HCV RNA was 3.3 times as high among first-time donors as among repeat donors ($P < 0.001$) (Table 2); the rate among first-time donors was similarly elevated when the calculations were restricted to HCV RNA–positive donations that were nonreactive on third-generation assays (7.9 per 10⁶ units from first-time donors vs. 2.5 per 10⁶ units from repeat donors; rate ratio, 3.2; 95 percent confidence interval, 2.0 to 5.0).

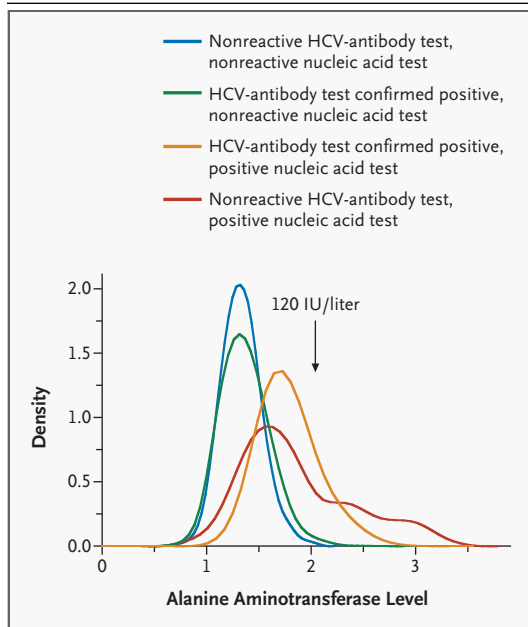


Figure 1. Distribution of Alanine Aminotransferase Levels According to HCV Status.

The alanine aminotransferase values, which are given on a base 10 logarithmic scale, in each of the four groups were used to estimate probability densities.

FOLLOW-UP INVESTIGATIONS OF RNA-POSITIVE DONORS

Follow-up studies of seronegative donors who were confirmed to be positive for viral RNA demonstrated that these donations were virtually all made in the early stage of infection when viremia is present but an antibody reaction cannot be detected. Eight of 12 donors with positive HIV-1 nucleic acid–amplification tests enrolled in follow-up. All eight seroconverted within six weeks after the positive test. The median interval between the RNA-positive index donation and the first antibody-reactive sample was 11.5 days (range, 6 to 42), and the median interval between the donation and the first confirmed seropositive sample was 20.5 days (range, 15 to 42). These intervals probably represent an overestimate of the actual time to seroconversion, since the interval between follow-up samples varied and the sample size was small. Data were available for six additional HIV-1 RNA–positive donors (identified from April 2002 through April 2004) and demonstrated a similar time to seroconversion (8.6 days to antibody reactivity and 20.5 days to confirmed positivity).

For HCV, 90 of the 139 HCV RNA–positive donors who had nonreactive third-generation assays

Table 2. Rates of Positivity for HIV-1 and HCV RNA among First-Time and Repeat Donors, March 1999 to April 2002.*

Donor Status	Total No. of Donations	Total No.	RNA-Positive Donations	
			Rate per 10 ⁶ Donations (95% CI)	Ratio of First-Time Donors to Repeat Donors (95% CI)
HIV-1†				
First-time	8,178,000‡	6	0.73 (0.24–1.81)	4.1 (1.0–17.0)§
Repeat	27,692,000‡	5	0.18 (0.06–0.43)	
HCV¶				
First-time	8,430,000‡	77	9.1 (6.4–12.9)	3.3 (2.1–5.1)
Repeat	28,544,000‡	79	2.8 (2.1–3.6)	

* CI denotes confidence interval.

† Data from one laboratory were excluded because the laboratory did not report donors' status (first-time or repeat).

‡ A total of 22.8 percent of donations were assumed to be from first-time donors and 77.2 percent from repeat donors.¹⁵

§ If calculations were restricted to RNA-positive donations that were nonreactive to HIV-1 p24 antigen, the rates were 0.49 per million first-time donors (95 percent confidence interval, 0.12 to 1.41) and 0.18 per million repeat donors (95 percent confidence interval, 0.06 to 0.43), resulting in a ratio of 2.7 (95 percent confidence interval, 0.5 to 12.7).

¶ Data from three laboratories were excluded because they did not report donors' status, data on transfusability, or the results of HCV third-generation assays for the HCV RNA–positive index donations. Five HCV RNA–positive donations with missing data on donor status were weighted according to the distribution of first-time and repeat donors among the remaining HCV RNA–positive donations.

enrolled in the follow-up study; 75 of the 90 seroconverted. In the majority of those who did not seroconvert, the duration of follow-up was too short (range, 12 to 58 days) to allow determinations of their eventual seroconversion status.

The expanded data set from the American Red Cross allowed more extensive evaluation of the dynamics of HCV seroconversion. Figure 2 provides the follow-up results for 67 HCV RNA-positive donors (48 identified from March 1999 through April 2002, plus 19 identified from May 2002 through June 2003). Of these, 7 (10 percent) discontinued follow-up before either seroconversion or three

months of follow-up, the interval during which seroconversion generally occurred (Fig. 2A), and 55 (82 percent) seroconverted. The median time to seroconversion (from the RNA-positive, antibody-nonreactive index donation to a reactive third-generation antibody test) was 35 days. This is likely to be an underestimate of the viremic, antibody-nonreactive window period, since the period of viremia before the index donation is unknown. Of the 55 donors who seroconverted, 47 remained viremic during continued follow-up; 3 donors had fluctuating viremia in the presence of HCV antibody, and in 5, the HCV infection resolved after seroconversion.

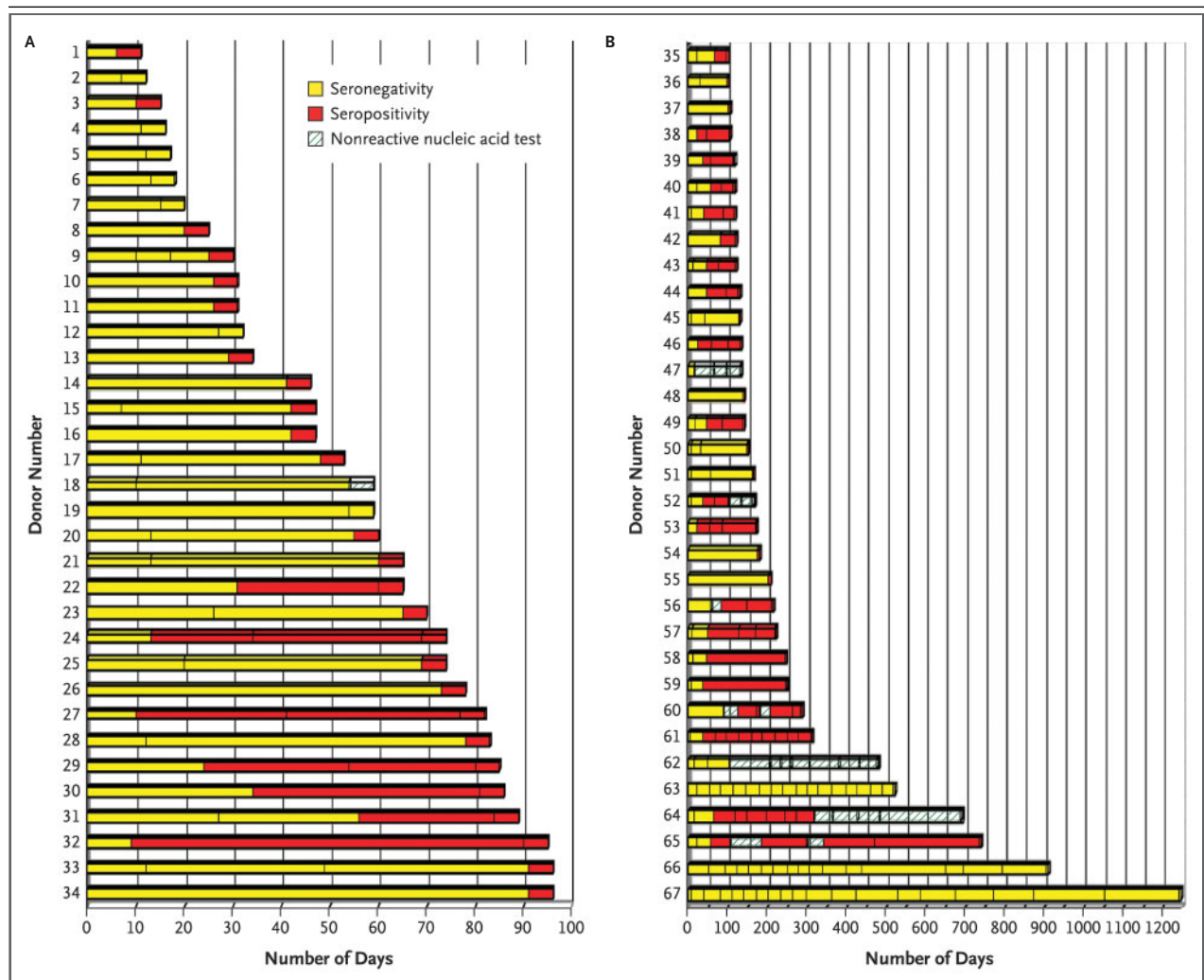


Figure 2. Follow-up Results for 67 HCV RNA-Positive, HCV Antibody-Nonreactive Donors.

Data were provided by the American Red Cross. Panel A shows the results for those with no more than 92 days of follow-up, and Panel B the results for those with more than 92 days of follow-up. A red tip at the end of a yellow line indicates that the donor was seropositive on the last day of follow-up.

sion, with persistent RNA negativity for up to one year (Fig. 2B). Two additional donors (3 percent) had an abortive HCV infection, in which HCV RNA could initially be repeatedly demonstrated shortly after enrollment but disappeared in the absence of HCV seroconversion. Lastly, three donors (4 percent) remained viremic without elevated alanine aminotransferase levels, but they did not seroconvert after a follow-up period ranging from 1.5 to more than 3 years (so-called immunologically silent infections) (Fig. 2B). The donor with the longest immunologically silent period was infectious during this period, since his RNA-positive donation transmitted HCV to a platelet recipient early in the nucleic acid–amplification testing program before the American Red Cross began withholding all blood components until the results of such tests were available.¹²

HIV-1-POSITIVE AND HCV-POSITIVE DONORS IDENTIFIED FROM APRIL 2002 TO APRIL 2004

An additional two years of data from the American Red Cross demonstrated no changes in the rates of positivity for HCV RNA — from 1 in 251,000 for the period from March 1999 through March 2002 (79 per 19,811,809 donations screened) to 1 in 222,200 for the period from April 2002 through April 2004 (60 per 13,332,257 donations screened, $P=0.49$). For HIV-1, the rates were 1 in 4 million and 1 in 2.2 million, respectively. A similar number of HIV-1 RNA–positive donations were identified during the two periods (five and six, respectively). Even though the frequency of HIV-1 RNA–positive donations increased for the period from April 2002 through April 2004, this increase was not significant ($P=0.37$).

DISCUSSION

Assuming that each of the 13.6 million allogeneic units of blood donated annually in the United States is converted on average to 1.45 transfusable components,^{19,20} our data indicate that the implementation of minipool nucleic acid screening likely prevented about 5 cases of transfusion-transmitted HIV-1 infection and 56 cases of HCV infection annually. The documented findings are consistent with those predicted from mathematical models.^{4,15,21} Despite the fact that these rates are relatively low and have remained stable for five years, implementation of these tests was consistent with the goal of maximizing blood safety.^{1,3} It has been

estimated that nucleic acid screening has reduced the residual risk of transfusion-associated infection for both HIV-1 and HCV to about 1 in 2 million blood units from repeated donors.¹⁵ This is a reduction from rates of 1 in 276,000 for HCV and 1 in 1.5 million for HIV-1 with the use of serologic testing alone.¹⁵ The residual risk after the implementation of nucleic acid–amplification testing results from the presence of virus below the limit of detection of minipool testing²²; individual nucleic acid screening of each sample, rather than screening of small pools of multiple samples, would further decrease the residual risk but at a substantially greater cost.

With the licensure of nucleic acid–amplification tests, the FDA has permitted the discontinuation of HIV-1 p24 antigen testing on the basis of data showing that HIV-1 RNA screening is better able to detect infection in the window period shortly after infection and that all p24 antigen–positive donations are also RNA-positive.^{7,23} This policy is supported by our data, in which HIV-1 nucleic acid screening identified 12 infected donors, only 2 of whom were identified by p24 antigen testing; in contrast, there were no RNA-negative donations from HIV-1–infected donors that were identified as positive by p24 antigen testing. The detection of p24 antigen in the absence of antibody corresponds to the peak viremic period when blood donors are likely to defer donations owing to influenza-like symptoms.^{7,9,24}

Approximately one third of the units detected by HCV nucleic acid–amplification testing would have been discarded anyway owing to elevated alanine aminotransferase levels. Because of the relative non-specificity of this surrogate marker, the absence of evidence of additional transfusion-transmissible hepatitis agents,^{8,25} and the implementation of a sensitive screening method for the detection of HCV RNA, the continued use of alanine aminotransferase screening for preventing transfusion-associated hepatitis is no longer justified; consequently, many blood centers have stopped using this test. In addition, the presence of circulating HCV RNA is a direct marker of viral replication and indicates a diagnosis of HCV infection with greater sensitivity and specificity than does the presence of elevated liver enzymes.

Our data show that new HCV and HIV-1 infections occur three to four times as often among first-time donors as among repeat donors, substantiating previous observations.^{15,26} This finding sup-

ports the general principle that retention of repeat donors enhances both the adequacy and safety of the blood supply. Possible reasons for higher rates among first-time donors include inappropriate use of blood donation to obtain the results of viral tests; failure to understand the questions for donors and, hence, the donor-selection criteria; and self-deferral of the donor after the first donation owing to the realization that his or her donation was unsuitable.

The routine use of nucleic acid–amplification tests and serologic assays for donor screening has made possible the identification of persons in the very early stages of HIV-1 and HCV infection; this information can provide insights into risk factors associated with viral infection and potentially contribute to studies of the natural history, pathogenesis, and treatment of these infections.^{9,10} For example, an analysis of recent risk-related behavior among HCV-infected donors identified by nucleic acid–amplification testing may identify behavioral and demographic characteristics that could be used to improve donor-qualification criteria, provided effective questions could be designed.²⁷ The addition of HCV RNA testing to routine HCV-antibody screening has also allowed seropositive donors to be subdivided into those with active infection (plasma RNA–positive) and those with either resolved HCV infection or intermittent viremia (plasma RNA–negative at the time of donation). Enrollment of these donors into natural-history and early-treatment trials could enhance our understanding of the pathogenesis of HCV infection, including the factors underlying the spontaneous resolution of HCV viremia.^{10,28}

Several reports have suggested that serologic testing may miss a substantial proportion of infected persons.²⁹⁻³¹ We found that only three seronegative donors with persistent hepatitis C viremia did not seroconvert during the expected time frame. During this same time at the American Red Cross, more than 800 HIV-seropositive donors and more than 16,000 HCV-seropositive donors were identified. Thus, persistent immunologically silent infections are extremely rare, reinforcing the continued reliance on serologic analyses for HIV-1 and HCV as the primary tools for diagnostic testing.³²

Because blood centers had already implemented nucleic acid–amplification testing for HIV-1 and HCV, it was feasible in 2003, in collaboration with the Centers for Disease Control and Prevention and

the FDA and with the rapid development of nucleic acid–amplification tests by manufacturers, to implement screening for West Nile virus in less than nine months.³³⁻³⁵ Results indicate that close to 1000 donors with West Nile virus infection were identified by nucleic acid–amplification testing in 2003 and their donations discarded, probably preventing more than 1000 transfusion-related infections.³⁵

The relatively low yield and poor cost effectiveness of HIV-1 and HCV minipool nucleic acid–amplification testing have led some to question the value of such screening. Using somewhat different analyses and assumptions, two independent groups studying the cost-effectiveness of HIV-1 and HCV minipool nucleic acid–amplification testing, both in the context of eliminating p24 antigen screening, estimated costs of \$1.5 million to \$4.3 million per quality-adjusted year of life.^{19,36} Costs increase further if each donated blood unit is to be tested rather than combined in minipools, with yet further increases in cost for the automation required to perform large numbers of individual screening tests. Therefore, the cost of HIV-1 and HCV nucleic acid–amplification testing would need to decrease substantially to bring it in line with that of most other accepted medical practices. However, the aggregate cost-effectiveness of nucleic acid–amplification testing may have substantially improved with the implementation of such screening for West Nile virus. The rapid development and introduction of nucleic acid screening for West Nile virus and the ability to expand nucleic acid–amplification testing to include other emerging infections in the future further serve to support the adoption of this important tool for the screening of blood donations.

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APPENDIX

The National Heart, Lung, and Blood Institute Nucleic Acid Test Study involves the following sites and investigators: National Heart, Lung, and Blood Institute, National Institutes of Health — G. Nemo; Blood Centers of the Pacific and Blood Systems — M. Busch (principal investigator); Westat — G. Schreiber, M. King, S. Kleinman, S. Glynn; American Red Cross (Gen-Probe site) — S. Stramer, R. Dodd, J. Brodsky, J. Davis; America's Blood Centers (Gen-Probe sites: Blood Center of Southeastern Wisconsin, Blood Systems Laboratories, and Florida Blood Services, and Roche sites: Blood Center of Southeast Louisiana, BloodSource, Bonfils Blood Center, Central Florida Blood Bank, Community Blood Center of Greater Kansas City, Gulf Coast Regional Blood Center, LifeSource Blood Services, LifeSouth Community Blood Centers, Memorial Blood Centers of Minneapolis, New York Blood Center, Oklahoma Blood Institute, and Puget Sound Blood Center) — S. Caglioti, D.M. Strong; Association of Independent Blood Centers (Gen-Probe site) — R. Gammon; Center for Biologics Evaluation and Research, FDA — I. Hewlett; Roche — J. Gallarda, Y. Yang; Gen-Probe — L. Mimms, C. Giachetti, S. McDonough; Chiron — B. Phelps; Stanford Medical School blood bank.

REFERENCES

- Nucleic acid amplification testing of blood donors for transfusion-transmitted infectious diseases: report of the Interorganizational Task Force on Nucleic Acid Amplification Testing of Blood Donors. *Transfusion* 2000;40:143-59.
- Stramer SL, Caglioti S, Strong DM. NAT of the United States and Canadian blood supply. *Transfusion* 2000;40:1165-8. [Erratum, *Transfusion* 2001;41:1079.]
- Busch MP, Dodd RY. NAT and blood safety: what is the paradigm? *Transfusion* 2000;40:1157-60.
- Busch MP. Closing the windows on viral transmission by blood transfusion. In: Stramer SL, ed. *Blood safety in the new millennium*. Bethesda, Md.: AABB Press, 2001: 33-54.
- Busch MP, Kleinman SH, Nemo GJ. Current and emerging infectious risks of blood transfusions. *JAMA* 2003;289:959-62.
- Chamberland ME. Emerging infectious agents: do they pose a risk to the safety of transfused blood and blood products? *Clin Infect Dis* 2002;34:797-805.
- Feibig EW, Wright DJ, Rawal BD, et al. Dynamics of HIV viremia and antibody seroconversion in plasma donors: implications for diagnosis and staging of primary HIV infection. *AIDS* 2003;17:1871-9.
- Busch MP. Insights into the epidemiology, natural history and pathogenesis of hepatitis C virus infection from studies of infected donors and blood product recipients. *Transfus Clin Biol* 2001;8:200-6.
- Galel SA, Strong DM, Tegtmeier GE, et al. Comparative yield of HCV RNA testing in blood donors screened by 2.0 versus 3.0 antibody assays. *Transfusion* 2002;42: 1507-13.
- Peoples BG, Preston SB, Tzeng JL, Stramer SL, Gifford L, Wissel ME. Prolonged antibody-negative HCV viremia in a US blood donor with apparent HCV transmission to a recipient. *Transfusion* 2000;40:1280-1.
- Busch MP, Stramer SL. The efficiency of HIV p24 antigen screening of US blood donors: projections versus reality. *Infusion-ther Transfusionmed* 1998;25:194-7.
- Tobler LH, Busch MP. History of post-transfusion hepatitis. *Clin Chem* 1997;43: 1487-93.
- Grant PR, Busch MP. Nucleic acid amplification technology methods used in blood donor screening. *Transfus Med* 2002;12: 229-42.
- Giachetti C, Linnen JM, Kolk DP, et al. Highly sensitive multiplex assay for detection of human immunodeficiency virus type 1 and hepatitis C virus RNA. *J Clin Microbiol* 2002;40:2408-19.
- Dodd RY, Notari EP IV, Stramer SL. Current prevalence and incidence of infectious disease markers and estimated window-period risk in the American Red Cross blood donor population. *Transfusion* 2002; 42:975-9.
- Confidence regions: definition and construction in the binomial case. In: Pratt JW, Gibbons JD. *Concepts of nonparametric theory*. New York: Springer-Verlag, 1981: 41-4.
- Lehmann EL. *Testing statistical hypotheses*. New York: John Wiley, 1959:140-3.
- Tobler LH, Stramer SL, Lee SR, et al. Impact of HCV 3.0 EIA relative to HCV 2.0 EIA on blood-donor screening. *Transfusion* 2003;43:1452-9.
- Marshall DA, Kleinman SH, Wong JB, et al. Cost-effectiveness of nucleic acid test screening of volunteer blood donations for hepatitis B, hepatitis C and human immunodeficiency virus in the United States. *Vox Sang* 2004;86:28-40.
- National Blood Data Resource Center. 2002 Nationwide Blood Collection and Utilization Survey. Bethesda Md.: American Association of Blood Banks, 2003.
- Schreiber GB, Busch MP, Kleinman SH, Korelitz JJ. The risk of transfusion-transmitted viral infections. *N Engl J Med* 1996;334: 1685-90.
- Delwart EL, Kalmin ND, Jones TS, et al. First report of human immunodeficiency virus transmission via an RNA-screened blood donation. *Vox Sang* 2004;86:171-7.
- Stramer SL, Porter RA, Brodsky JP, et al. Replacement of HIV-1 p24 antigen screening with HIV-1 RNA nucleic acid testing (NAT) for whole blood donations. *Transfusion* 1999;39:Suppl 10S:2s. abstract.
- Schreiber GB, Glynn SA, Satten GA, et al. HIV seroconverting donors delay their return: screening test implications. *Transfusion* 2002;42:414-21.
- Notari EP IV, Orton SL, Cable RG, et al. Seroprevalence of known and putative hepatitis markers in United States blood donors with ALT levels at least 120 IU per L. *Transfusion* 2001;41:751-5.
- Janssen RS, Satten GA, Stramer SL, et al. New testing strategy to detect early HIV-1 infection for use in incidence estimates and for clinical and prevention purposes. *JAMA* 1998;280:42-8. [Erratum, *JAMA* 1999;281: 1893.]
- Orton SL, Stramer SL, Dodd RY, Alter MJ. Risk factors for HCV infection among blood donors confirmed to be positive for the presence of HCV RNA and not reactive for the presence of anti-HCV. *Transfusion* 2004;44:275-81.
- Orland JR, Wright TL, Cooper S. Acute hepatitis C. *Hepatology* 2001;33:321-7.
- Beld M, Penning M, van Putten M, et al. Low levels of hepatitis C virus RNA in serum, plasma, and peripheral blood mononuclear cells of injecting drug users during long antibody-undetectable periods before seroconversion. *Blood* 1999;94:1183-91.
- Maple PA, McKee T, Desselberger U, Wreghitt TG. Hepatitis C virus infections in transplant patients: serological and virological investigations. *J Med Virol* 1994;44:43-8.
- Schmidt WN, Wu P, Cederna J, Mitros FA, LaBrecque DR, Stapleton JT. Seroepidemiology of hepatitis C virus (HCV) infection detected in the majority of patients with cryptogenic chronic hepatitis and negative HCV antibody tests. *J Infect Dis* 1997;176:27-33.
- Alter MJ, Kuhnert WL, Finelli L. Guidelines for laboratory testing and result reporting of antibody to hepatitis C virus. *MMWR Recomm Rep* 2003;52(RR-3):1-13.
- Pealer LN, Marfin AA, Petersen LR, et al. Transmission of West Nile virus through blood transfusion in the United States in 2002. *N Engl J Med* 2003;349:1236-45.
- Dodd RY. Emerging infections, transfusion safety, and epidemiology. *N Engl J Med* 2003;349:1205-6.
- Update: West Nile virus screening of blood donations and transfusion-associated transmission — United States, 2003. *MMWR Morb Mortal Wkly Rep* 2004;53: 281-4.
- Jackson BR, Busch MP, Stramer SL, AuBuchon JP. The cost-effectiveness of NAT for HIV, HCV, and HBV in whole-blood donations. *Transfusion* 2003;43:721-9.

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