Infectious disease testing of blood for transfusion in the United States in 2006 includes the routine use of conventional serologic tests for antigens and/or antibodies to human immunodeficiency virus, type 1 and type 2 (HIV-1 and HIV-2); hepatitis B virus (HBV); hepatitis C virus (HCV); human T-cell lymphotropic viruses, type I and type II; and syphilis. In addition, incident cases of HIV-1 and HCV infections are detected by nucleic acid amplification testing (NAT); NAT is also used by some blood centers for HBV DNA detection and by virtually all blood collection facilities for West Nile virus (WNV) RNA detection. Nucleic acid amplification testing for the release of blood for transfusion is performed in small pools of equal aliquots of 6 to 24 donations each, referred to as minipool (MP) NAT, or performed individually (ID) by some smaller systems (ID NAT).1 Nucleic acid amplification testing was originally introduced in some European countries in 1995 as a method to reduce viral loads for plasma-derived products, but NAT was implemented for all donations in the United States, Canada, Australia, Japan, and much of Europe in 1998 to 2000 to reduce the residual risk of HIV and HCV transmission by all components.2 Similarly, because septic transfusion reactions due to bacterial contamination of platelets have been recognized as a serious hazard of transfusion, various methods for detecting bacterial contamination of platelets were introduced in the United States in 2004.3-4

Safety and adequacy remain the central goal of blood donor screening programs. In the United States, residual risk estimates since the introduction of MP NAT plus serologic screening remain at 1 in 2 million for donations from HIV-1 and HCV infected individuals5 (Table). An almost 10-fold reduction in residual risk is observed for HCV with NAT and serology as opposed to serology alone.5 The current HIV and HCV NAT assays can detect 14 copies per milliliter of HIV-1 RNA and 12 copies per milliliter of HCV RNA at the 50% detection level or 30 to 60 copies per milliliter for both at the 95% detection level.1 Differences in identifying additional HIV-1 and HCV infected donors between MP and ID NAT, although significant by sensitivity analysis (odds ratios ranging from 45.3 to 93.4), only translates to 1 to 2 additional infections detected in 10 million donations screened (ie, 1 in 5 million for HIV and 1 in 6.7 million for HCV)5 or, based on a different model, 1 in 3.0 or 3.7 million for HIV and 1 in 2.6 or 2.7 million for HCV.7 Yield by ID NAT is supported by known breakthrough cases of HIV and HCV that were undetected by MP NAT methods that are discussed further in the text. Window period closures for MP versus ID NAT of 3.4 days for HIV and 2.5 days for HCV compare with total window period reductions relative to serologic detection of 57% achieved by MP NAT for HIV and 97% achieved for MP NAT for HCV.8 For HBV, unlike HIV and HCV, which has low viral loads not detected by sensitive hepatitis B surface antigen (HBsAg) methods during longer periods, MP NAT does not have the same benefit.9

The low frequency, which only can be estimated through mathematical models, of released blood compo-
Current Risks of Infection From Test-Negative Blood Components, United States

<table>
<thead>
<tr>
<th>Agent*</th>
<th>Risk</th>
</tr>
</thead>
<tbody>
<tr>
<td>HIV-1</td>
<td>1 in 2 135 000†</td>
</tr>
<tr>
<td>HIV-2</td>
<td>None‡</td>
</tr>
<tr>
<td>HCV</td>
<td>1 in 1 935 000‡</td>
</tr>
<tr>
<td>HBV</td>
<td>1 in 205 000–488 000‡</td>
</tr>
<tr>
<td>WNV</td>
<td>None§</td>
</tr>
<tr>
<td>Bacteria (cultured apheresis platelets), 2004–2006</td>
<td>1 in 75 000∥</td>
</tr>
<tr>
<td>Bacteria (untested whole blood platelets), 2004–2006</td>
<td>1 in 33 000¶</td>
</tr>
</tbody>
</table>

* HIV indicates human immunodeficiency virus; HCV, hepatitis C virus; HBV, hepatitis B virus; and WNV, West Nile virus.
† Dodd et al. reported using observed incidence calculated for repeat donors (80% of total donors); rates higher when adjusted for all donors (adding 20% for first-time donors and adjusted for 3-fold higher incidence in first-time donors), or for HIV-1 of 1 in 1 525 000 and for HCV of 1 in 1 390 000. HBV total incidence adjusted using a correction factor of 2.38 multiplied by the observed hepatitis B surface antigen (HBsAg) incidence (to adjust for the transient nature of HBsAg relative to the long interdonation interval in repeat donors).
‡ No direct or modeled measurements; fewer than 10 positive donors reported since 1992.
§ None observed since successful use of minipool/individually nucleic acid amplification testing strategy.
∥ Seventeen reported cases of septic transfusion reactions associated with 1 277 508 single-donor platelet components issued (American Red Cross internal data, March 2004 through February 2006).
¶ Based on 30 reported cases of septic transfusion reactions per 1 million whole blood–derived platelet components released (American Red Cross internal data, March 2004 to February 2006).

In the American Red Cross program, the overall NAT yield per unit, that is, RNA-confirmed positive but seronegative, since the implementation of NAT screening in 1999 to the end of 2005 is 1 in 1.7 million donations. However, this yield has increased significantly during time with 16 (62%) of the 26 total HIV-1 NAT yield donors identified in 2004 and 2005 (P = .003). In contrast, during the same time, the HIV antibody frequency has remained stable at 1 in 33 000. Of the 26 NAT yield donors, 46% were first-time donors and 81% were men with a median age of 31 years. The majority (60%) was from the southeastern portion of the United States. In contrast, of the antibody positives studied, 81% were first-time donors with only 36% from the southeastern portion of the United States; similarly, the median age was 33 years and 86% were men. The increase in observed recent HIV-infected donors identified uniquely by NAT is of concern, but as of yet has not been explained.

Even with the benefit of HIV NAT added to sensitive antibody tests, lookback procedures in the United States have led to the identification of 4 breakthrough HIV cases (nonreactive by MP NAT) originating from 3 donations. In the first transmission, the implicated unit of red blood cells (RBCs) contained fewer than 150 copies per milliliter of HIV-1 RNA. The seroconverting repeat donor presented for a subsequent donation 4 months posttransfusion of the seronegative but RNA-positive component; no risk factors were identified in this 29-year-old male donor.

The second and third HIV transmissions came from a repeat seroconverting 18-year-old donor. The seropositive donation occurred 2 months following her seronegative, MP NAT-nonreactive donation. The route of infection for the donor is unknown but likely was acquired sexually. On repeat questioning, the donor did recall 5 of her recent sexual partners of which 4 were located and each tested HIV nonreactive. The fifth, who could not be located for testing, did have sexual contact with the donor within 10 days of her MP NAT-nonreactive donation. Phylogenetic studies of the red cell recipient in the first case, and the red cell and fresh frozen plasma recipients in the second case, each linked them to the identified seroconverting donor.

In the third case, a donation from a first-time seroconverting 33-year-old female donor infected a fresh frozen plasma recipient but not the co-component RBC recipient (RBC component 6 days old at the time of transfusion). The differential transmission is possibly linked to a low viral load in the donation and the fact that a far larger viral inoculum would have been received by the fresh frozen plasma recipient. At the 5-month seroconverting donation, the donor did recall that her sexual partner died (HIV status of the partner was unknown to the donor). Interestingly, at the time of her seropositive donation, the donor did indicate that her unit should not be used for transfusion and had told the blood bank staff on questioning, following the identification of her HIV status, that she had started antiretroviral therapy 2 months following her transmitting donation (which was 3 months prior to the seropositive donation).

Recently, another case of differential transmission was reported from South Africa; in this case, the platelets transmitted, but again the RBCs (that were 12 days old at the time of transfusion) did not. In this case the donor was a 53-year-old man who had donated 53 times previously; 3 months following the transmitting donation, the donor returned for a subsequent donation and tested HIV

HUMAN IMMUNODEFICIENCY VIRUS

In the American Red Cross program, the overall NAT yield per unit, that is, RNA-confirmed positive but seronegative, since the implementation of NAT screening in
antibody confirmed positive. The transmitting donation tested nonreactive by p24 antigen and antibody; the fresh frozen plasma that was available for testing was RNA positive with an estimated viral load of 12 copies per milliliter. Again, the infected donor and recipient were linked by phylogenetic analysis.

In the United States, the Food and Drug Administration (FDA), via approval variance requests, has permitted the use of HIV NAT in place of a licensed confirmatory test for donations testing anti-HIV repeat reactive if an RNA positive result is obtained. For donations testing RNA (MP or ID NAT) nonreactive, use of an FDA licensed confirmatory test remains required. The agreement between NAT and antibody positivity is high, that is, 99.2% of RNA positive donors are anti-HIV confirmed positive; however, the disadvantage of the algorithm is that fewer than 5% of anti-HIV screen reactive donors are HIV RNA positive.12 Therefore, the vast majority of samples would still require Western blot (or alternate supplemental test) to be performed. Combining the HIV-1 NAT results with the Western blot indeterminate and negative results demonstrates that less than 0.04% of these donors are truly infected with HIV-1. Of the anti-HIV confirmed positive samples that tested NAT reactive, 91.6% had a signal-cut-off ratio on the screening enzyme immunoassay (Abbott Laboratories, Abbott Park, Ill) of 15 or greater. In contrast, of those anti-HIV repeat reactive samples that tested NAT nonreactive and Western blot negative or indeterminate, 98.5% had an Abbott enzyme immunoassay signal-cutoff ratio less than 15.

HEPATITIS C VIRUS

Since the introduction of NAT for HCV in the United States, more than 48 million donations have been tested by the American Red Cross NAT program with 196 yield donors (RNA confirmed positive, seronegative) identified, or a yield of 1 in 245,000 donations. The yield per year has remained relatively consistent at 30 to 35. Of yield donors identified, further risk questioning has revealed that only 43% of donors will acknowledge any risk factors; recent injection drug use or incarceration were each independently associated with HCV infection in this subset of HCV-infected donors.17 Three long-term immunosilent donors have been described1 with 1 having been followed for 7 years without seroconversion. Although the donor remains immunocompetent to other immunogens, no HCV antibody has been detected. The donor continues to be without complications (normal liver enzyme levels) but has several million copies of circulating HCV RNA at all times sampled. The virus is infectious in the case of this donor (genotype 2b by InnoLIA); he did transmit HCV to a platelet recipient early in the American Red Cross NAT program (prior to the release of NAT-negative components).18 Recently the recipient has been documented to be anti-HCV positive proving that the virus is a competent immunogen. Other reports of immunosilent donors have documented eventual seroconversion.19 However, it remains unknown why this single long-term immunosilent donor has never seroconverted.

During the time since NAT has been introduced worldwide, there has only been 1 documented breakthrough case of an HCV transmission reported from a MP-NAT nonreactive component,20 although other unreported cases have likely occurred. Similar to 2 of the reported cases of HIV breakthrough transmission, 1 component transmitted infection to a recipient (platelets), whereas the other (RBCs) did not. Replicate testing by sensitive NAT assays was required to detect viremia, indicating the very low viral load in the donation.

Data on the agreement between HCV NAT and confirmed antibody positivity for the first 4 years of NAT screening have been recently published.21 The data support a revision of the current HCV confirmatory serologic algorithm by combining NAT with the recombinant immunoblot assay (RIBA). In the algorithm, donation samples that test NAT reactive are not further tested by RIBA. Because the population of donors that is affected is considerably larger than that for HIV, this algorithm is a significant improvement relative to logistics and costs as compared with the prior algorithm in which all donations that are anti-HCV screen reactive are tested by RIBA. Of 49.3% of donors who were RIBA positive, 79.2% were also NAT reactive. Less than 3% of the remaining donation samples that tested either RIBA indeterminate or negative were RNA positive.

HEPATITIS B VIRUS

Hepatitis B virus NAT for routine use in screening of blood donors has not been adopted; indeed, the use of sensitive HBsAg assays coupled with anti–hepatitis B core (anti-HBc) testing in many HBV nonendemic countries has resulted in relatively low estimates of HBV residual risk.5 In the United States, estimates of 1 in 205,000 to 1 in 488,000 have been calculated for donors donating between 1995 and 2001.5 The 2 serologic screening measures (sensitive HBsAg and anti-HBc tests) together interdict virtually all HBV-infected individuals and reports of HBV transfusion transmission in countries using both tests are virtually nonexistent. Through the National Notifiable Diseases Surveillance System (Centers for Disease Control and Prevention, unpublished data, 2003) in the United States, an enhanced surveillance for acute HBV occurred. Of 7381 cases of acute hepatitis B reported in 2003, 49 were initially reported as transfusion associated; of those, 10 were confirmed by the Centers for Disease Control and Prevention as acute cases of hepatitis B that involved transfused blood components within the past 6 months. Of the 10, only 1 case could be associated with a single infected donor.22 Therefore, although the calculated residual risk for HBV is higher than that for HIV or HCV, clinical cases of transfusion-transmitted hepatitis B infection are only infrequently reported. There are many reasons for this, possibly relating to overestimates of HBV incidence (for which an anti-HBc correction factor is applied) or overestimates of the length of the HBsAg-negative window period. Other reasons may include incomplete investigations and underreporting of hepatitis B in recipients, infection with inapparent disease (as is true of greater than 95% of hepatitis B infection in adults), or widespread use of the HBV vaccine.22

Two recent studies demonstrate the performance of the 2 commercially available HBV DNA assays relative to HBsAg. The first was the US clinical trial of the Roche AmplicScreen HBV Test (Roche Diagnostics, Pleasanton, Calif)23 reporting a yield during the formal study and extended investigation period of 1 HBV DNA positive, HBsAg and anti-HBc nonreactive donor in 340,000 donations screened (5 yield donors in 1.7 million). The test sensitivity was 5 IU/mL (20 copies per milliliter), or 480 copies per milliliter in the operating pool size of 24. Although
this test was FDA licensed in the United States, its use is optional in both the MP and ID NAT formats. The FDA considers the use of the test voluntary because the estimated individual and public health benefits of adding this test to the available screening tests already performed (ie, HBsAg and anti-HBc) are thought to be very limited. The second study is the European evaluation of the Gen-Probe Ultrio triplex assay (Gen-Probe, Inc, San Diego, Calif) in which HBV DNA analytic sensitivity was 11 IU/mL. A window-period reduction of 14 days (range, 1–18 days) was observed when seroconversion panels were tested by ID NAT; the window period reduction decreased to 6 days (range, 3–8 days) in pools of 8 and 3 days (range, 0–5) in pools of 16. No window period reduction was observed in a pool of 24 in comparison to the sensitive HBsAg assay used (Abbott PRISM), with PRISM HBsAg being more sensitive in about half the panels studied. These 2 studies demonstrate that MP NAT for the detection of HBV-infected donors has little if any value compared with the most sensitive HBsAg screening methods (soon to be FDA licensed and introduced in the United States).

The major advantage in coupling HBV DNA with anti-HBc testing is that donors may be counseled that, although HBsAg nonreactive, they nevertheless have evidence of active HBV infection.

WEST NILE VIRUS

West Nile virus entered North America in 1999 and in 2002 was shown to be transfusion transmitted. With competent mosquito and bird vectors throughout North America, WNV clinical disease continues at epidemic proportions. Routine blood donation screening for WNV began in July 2003 in the United States and Canada in response to documented cases of transfusion transmission in 2002. A combination of MP NAT during the ‘‘non-season’’ coupled with the conversion to the more sensitive ID NAT in epidemic locations during epidemic times has been successful in detecting approximately 1500 infected blood donors in the United States from 2003 to 2005. Assuming that each donation was infectious, and manufactured into 1.45 blood components, testing has therefore prevented close to 2200 recipient infections and potential clinical disease.

During the time following the implementation of WNV NAT, transfusion transmission has occurred from 7 MP NAT-nonreactive/ID NAT-reactive units (6 in 2003 and 1 in 2004) or a total of 30 transfusion transmitted cases since WNV has been identified in North America; all 30 donations have been antibody negative. Since the full implementation of the MP to ID NAT trigger strategy in epidemic areas, there have been no documented WNV transfusion transmissions.

Because WNV is an acute infection that only occurs seasonally, other strategies have been proposed such as seasonal testing, which has been implemented successfully in Canada (Quebec) coupled with a screening question used in the ‘‘non-season’’ of whether the donor has been in the United States during the past 56 days, in which case WNV NAT is performed. West Nile virus is an example of an emergent agent in which a rapid series of interventions has been successful in controlling transmission through blood transfusion. Two recent publications document the success of WNV NAT screening programs in the United States during the 2003 and 2004 WNV seasons.

Due to the high cost and relative poor cost effectiveness of WNV screening using the MP/ID NAT approach, other prevention strategies have been proposed that minimize or eliminate testing. In addition to seasonal testing, selective testing for only immunocompromised patients or the use of questionnaires to eliminate at-risk donors was proposed. These additional strategies, however, did not fully consider regulation, cost, or logistics. Although WNV screening of blood donations is not yet required in the United States by the FDA, testing is controlled via the Investigational New Drug or licensure process. The FDA perspective is one of risk versus benefit as opposed to cost versus benefit. It is clear that the United States cannot use the same approach as Canada because the Canadian approach to testing in the ‘‘off season’’ is dependent on absence of travel to the United States. In a seasonal or geographic model in which fewer reagents are used, it is not clear that a proportional reduction in cost would occur. The proposed strategies of WNV symptom-related predonation questioning and/or transfusion of WNV-screened components to at-risk recipients ignored the other factors involved in system cost such as labor and computer systems that can manage tested versus nontested components. Also, the use of a predonation questionnaire would not have detected the 74% of donors who later developed symptoms. Stratifying recipients based on need is also problematic because not all recipients who developed WNV clinical disease were strictly classified as immune compromised. Lastly, the maintenance of a dual-tiered blood inventory is a source of potential errors. Selective testing protocols create operational difficulties and frequently fail to meet their objectives in that patients who need a specific component may not necessarily receive it. The MP/ID NAT targeted testing strategy serves as a bridge strategy between no testing and very expensive year-round ID NAT throughout the United States and may serve as a model of testing for new agents if the disease epidemiology is clearly related to specific geographic regions. This approach may not be the least costly, but it does achieve blood safety and public health goals.

The real proof of the effectiveness of the MP/NAT current strategy has been the elimination of documented cases of WNV transfusion transmission.

BACTERIAL DETECTION IN PLATELETS

In March 2004, the AABB (formerly the American Association of Blood Banks) standards required that blood banks and transfusion services implement methods to limit and detect bacterial contamination in all platelets for transfusion. This standard reflected the concern from the reports in the literature of septic transfusion reactions of 1 in 5000 to 1 in 15 000, and culture-positive results of 1 in 1500. In the reported study from the Hopkins group, risk decreased as the use of single-donor apheresis platelets increased from 52% in 1987 to greater than 99% in 1999; this translated to a 5.4-fold reduction in septic transfusion reactions. However, in the Centers for Disease Control and Prevention study looking at bacterial contamination (BaCon), there were no significant differences in reported cases of septic transfusion reactions observed between the use of whole blood–derived platelet concentrates and single-donor apheresis platelets (both at approximately 1 in 100 000 transfusions) even with the use of up to 6 different donor platelet units per transfused platelet dose. Differences in findings, including the lower
rate of bacterially derived transfusion reactions in the BaCon study, may relate to the stricter criteria used for a confirmed case (unequivocally linking donor platelet unit and recipient) in the BaCon study.

Recent studies of bacterial contamination of platelets following culture continue to demonstrate rates as high as 1 in 1500\textsuperscript{39}; however, the rates decrease to 1 in 5000 following confirmatory testing. Most blood collection organizations implemented a bacterial culture method only for apheresis platelets due to limited volume and high cost associated with culture of each whole-blood-derived platelet unit. Collection organizations aseptically sample 4 mL from the apheresis bag between 12 and 36 hours after collection and culture the samples aerobically for a minimum of 12 to 24 hours prior to distributing the apheresis platelets for transfusion. Therefore, transfusion will likely occur prior to the complete incubation time of the platelet culture, which is recommended to be through the shelf life of the platelet. The American Red Cross reported that 53\% of confirmed positive cases were detected within 12 to 24 hours post-inoculation following a 24-hour platelet holding time (29\% at less than 12 hours), but 18\% required greater than 24 hours for detection with 11\% transfused by the time the positive result had been obtained. Of positive cultures identified, 47\% were Staphylococcus species and 26.5\% were Streptococcus species.

Breakthrough cases of transfusion-related sepsis are most likely linked to slow-growing skin flora that result in a sterile culture due to few or no organisms in the inoculum, but adequate growth occurred within the platelet unit during its room temperature storage prior to transfusion.\textsuperscript{39} Therefore, it has been suggested that longer platelet holding times (in excess of 24 hours) occur prior to culture bottle inoculation. Another area of controversy regarding recipient safety due to bacterially contaminated platelets is the use of aerobic bottles only instead of the package insert–required use of both an aerobic and anaerobic bottle. The argument made is that the clinically relevant aerobic or facultative anaerobic bacteria grow well in the aerobic bottle, recovered anaerobes from anaerobic culture are not clinically significant, and the aerobic culture incubation conditions parallel those of the platelets. However, it may also be argued that the anaerobic medium is richer and may encourage more rapid growth of slow-growing fastidious facultative anaerobes in addition to the fact that with the use of 2 bottles, the volume of inoculum is doubled. To address the frequency of false negativity of culture, and hence the residual risk, the FDA requested that cultures are required to be retained for the shelf life of the platelets in studies in which platelet dating extension from 5 to 7 days is being investigated.

The American Red Cross reports a residual risk from a septic transfusion reaction from a culture-negative apheresis unit (predominantly due to skin flora) at 1 in 75100 (17 reported cases of septic transfusion reactions associated with 1277508 single-donor platelet components issued) with 1 in 638800 resulting in a fatality.\textsuperscript{40} During the same period (2004–2006), septic transfusion reactions from whole blood–derived platelets that were released without culture was 1 in 33000 (based on 30 reported cases of septic transfusion reactions in 1 million whole-blood–derived platelet components released; American Red Cross unpublished data, 2006). However, whole blood–derived platelets are tested by hospital transfusion services prior to release, as required by the AABB standard and College of American Pathologists checklist, by a variety of less satisfactory surrogate methods, including urine dipsticks, pH paper or meters, glucose detection, and so forth. Further reductions in bacterial contamination rates, primarily from skin flora, can also be achieved by diverting the first 30 to 50 mL of blood containing the skin plug and associated bacteria for routine donation qualification testing rather than collecting samples at the end of the collection.\textsuperscript{39} Donor notification is recommended when an organism suspected of being endogenous to the donor is recovered by culture methods. Most studies cited\textsuperscript{37–39} report that approximately 20\% of organisms identified are gram-negative and hence would have significance to the donor.

**SUMMARY**

Although modern test methods have achieved a high degree of safety for blood components, they have also generated significant problems relating to diagnostic interpretations and counseling of test-positive blood donors. However, careful use of data from NAT and creative use of confirmatory testing algorithms can greatly improve the accuracy of the overall viral testing process. The introduction of screening for additional agents requires validated diagnostic algorithms as well as attention to all aspects of the counseling message given to donors. The implementation of bacterial testing methods for platelets has improved transfusion safety but still requires enhancements such that the risks are further reduced and all issued platelets receive adequate testing.

**References**


22. Stramer SL. Pooled hepatitis B virus DNA testing by nucleic acid amplification testing: implementation or not. Transfusion. 2005;45:1242–1246.


