A new strategy for estimating risks of transfusion-transmitted viral infections based on rates of detection of recently infected donors

Michael P. Busch, Simone A. Glynn, Susan L. Stramer, D. Michael Strong, Sally Caglioti, David J. Wright, Brandee Pappalardo, and Steven H. Kleinman for the NHLBI-REDS NAT Study Group

BACKGROUND: Estimates for human immunodeficiency virus (HIV)-1 and hepatitis C virus (HCV) transfusion-transmitted risks have relied on incidence derived from repeat donor histories and imprecise estimates for infectious, preseroconversion window periods (WPs).

STUDY DESIGN AND METHODS: By use of novel approaches, WPs were estimated by back-extrapolation of acute viral replication dynamics. Incidence was derived from the yield of viremic, antibody-negative donations detected by routine minipool nucleic acid testing (MP-NAT) of 37 million US donations (1999-2002) or from sensitive/less-sensitive HIV-1 enzyme immunoassay (S/LS-EIA) results for seropositive samples from 6.5 million donations (1999). Incidences and WPs were combined to calculate risks and project yield of individual donation (ID)-NAT.

RESULTS: The HIV-1 WP from presumed infectivity (1 copy/20 mL) to ID-NAT detection was estimated at 5.6 days, and the periods from ID to MP-NAT detection and from MP-NAT to p24 detection at 3.4 and 6.0 days, respectively; corresponding estimates for HCV were 4.9, 2.5, and 50.9 days (the latter represents period from MP-NAT to antibody detection). The HIV-1 incidence projected from MP-NAT yield or from S/LS-EIA data was 1.8 per 100,000 person-years, resulting in a corresponding HIV-1 transfusion-transmitted risk of 1 in 2.3 million. The HCV incidence from MP-NAT yield was 2.70 per 100,000 person-years with a corresponding risk of 1 in 1.8 million donations. Conversion from MP-NAT to ID-NAT was projected to detect two to three additional HIV-1 and HCV infectious units annually.

CONCLUSIONS: MP-NAT yield and S/LS-EIA rates can accurately project transfusion risks. HCV and HIV-1 risks, currently estimated at 1 per 2 million units, could be reduced to 1 in 3 to 4 million units by ID-NAT screening.

ABBREVIATIONS: ID = individual donation; I-WP = incidence window period; MP(s) = minipool(s); S/LS-EIA = sensitive/less-sensitive HIV-1 enzyme immunoassay; SOD = sample optical density; T-I = time (days) between infectivity and ID-NAT detection (Stage I); T-II = time (days) between ID-NAT and MP-NAT detectability (Stage II); T-IIIa = time (days) between MP-NAT detectability and p24Ag detection (Stage IIIa); T-IIIb = the time (days) between p24Ag and antibody detection by Western blot (Stage IIIb); T-IV = time (days) between Western blot and S/LS-EIA detectability; WP(s) = window period(s).

From the Blood Systems Research Institute, San Francisco, California; Blood Systems, Inc., Scottsdale, Arizona; the University of California at San Francisco, San Francisco, California; Westat, Inc., Rockville, Maryland; the American Red Cross, Gaithersburg, Maryland; Puget Sound Blood Center, Seattle, Washington; the Blood Systems Laboratory, Tempe, Arizona; and the University of British Columbia, Victoria, British Columbia, Canada.

Address reprint requests to: Michael P. Busch, MD, PhD, Blood Systems Research Institute, 270 Masonic Avenue, San Francisco, CA 94118; e-mail: mpbusch@itsa.ucsf.edu.

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tests. The residual risk, or probability of having a potentially infectious donation released into the blood supply, has been estimated both in the US and internationally with the incidence window period (I-WP) model.  

Previous applications of the I-WP model have relied on the assumption that viral incidence for all donors can be projected from seroconversion rates derived from repeat donors. Incidence is derived by dividing the number of incident or new infections observed in a donor population by the number of person-years during which donors are at risk; incidence has been calculated only for repeat donors because their serial donation histories can be compiled in a database that can then be used to identify seroconverters and calculate person-years of observation. To estimate incidence in all donors, it has then been necessary to adjust the observed repeat donor incidence upward to account for the fact that first-time donors have a higher incidence than repeat donors.

A second assumption of the I-WP model has been that recently infected persons are as likely to donate in early and late stages of infection. There are multiple factors, however, that could influence a recently infected person’s decision to donate in the early stages of infection. Recent risk behaviors or acute viral symptoms could lead to a reduced probability of donation. Conversely, test seeking following a recent exposure could result in increased donations during the critical early infection phase.

Other limitations in previous applications of the I-WP model relate to estimates used for the preseroconversion WPs. Previous estimates have been based on information on the period from a probable infectious exposure to when viral or serologic markers are detectable. These data are primarily derived from cases of transfusion or needle-stick transmissions or from incident cases identified in prospective cohort studies of high-risk populations. Given the limited number of such cases and the limited frequency of available serial samples from these cases, however, past WP estimates have been imprecise. Moreover, there appears to be a period following exposure termed the eclipse phase during which viruses are restricted to the inoculation site or parenchyma target organs; during this phase viral particles may not yet be in the blood stream at sufficient levels to be transmissible by transfusion. Previous applications of the I-WP model have assumed that the entire period between exposure and marker positivity is infectious.

We present a new approach to accurately estimate the duration of the infectious WPs, based on back-extrapolation of viral replication rates in plasma during the so-called ramp-up phase of primary viremia. We also introduce a novel strategy to derive incidence for the overall donor population using the rate of viremic seronegative donations detected by nucleic acid amplification testing (NAT) and the rate of donations in the recent HIV-1 seroconversion WP detected by the sensitive/less-sensitive (S/LS)-EIA strategy (which identifies persons with low-titer and low-avidity antibodies characteristic of recent seroconversion). This incidence derivation is based on the rates of detection of donations in early phases of infection and does not necessitate knowledge of first-time or repeat donor status or serial donation histories. These newly derived incidence and WP estimates are combined using the I-WP model to estimate transfusion-transmission risks. This report thus provides an alternative method to viral risk estimation that can be easily implemented in donor settings where NAT yield data are compiled.

MATERIALS AND METHODS

Periods estimations

For ease of presentation, we can divide early HCV and HIV-1 infection into three or four major stages (I to IV), respectively. For HIV-1, we refer in the text to the period or time in days between infectivity and individual donation (ID)-NAT detection (Stage I) as T-I; the time (days) between ID-NAT and minipool (MP)-NAT detectability (Stage II) as T-II; the time (days) between MP-NAT detectability and p24Ag detection (Stage IIIa) as T-IIIa; the time (days) between p24Ag and antibody detection by Western blot (Stage IIIb) as T-IIIb; and the time (days) between Western blot and S/LS-EIA detectability as T-IV (Fig. 1). The corresponding time frames of interest for HCV are T-I (the time between infectivity and ID-NAT detection), T-II (the time between ID-NAT and MP-NAT detectability), and T-III (the time between MP-NAT detectability and third-generation EIA detection).

Fiebig and coworkers estimated that T-IIIb for HIV-1 lasted 5.3 days (standard error [SE], 1.0 days) based on analysis of serial data from 95 plasma donors who became infected with HIV-1, and Pappalardo and colleagues (Pappalardo et al., submitted for publication) estimated the HCV T-III as 50.9 days (SE, 2.5 days) based on analysis of longitudinal data collected on 58 plasma donors detected by HCV NAT and followed to third-generation EIA seroconversion (Fig. 1). These estimates are consistent with other published data. Further, the HIV-1 T-IV associated with the Vironostika S/LS-EIA has been shown to be 170 days (SE, 10 days).

All other periods presented in Fig. 1 were estimated with the following assumptions. First, infectivity was assumed to begin at 1 viral copy per 20 mL of plasma, which is consistent with animal model inoculation studies and findings from transfusion lookback studies (see Discussion). Hence, we assumed that a unit of red blood cells (RBCs), processed with the additive solution procedures...
common in the US, would contain approximately 20 mL of plasma and would be infectious if it contained as little as 1 viral copy, a worst-case assumption. Second, we assumed that, on average, on the day of ID-NAT detectability, the viral load was at the 50 percent sensitivity level reported for the NAT assays. The 50 percent sensitivity levels, or viral loads at which ID-NAT has a 50 percent chance of yielding a positive test result, reported for the Gen-Probe (San Diego, CA) assay system, are 4.8 copies per mL (95% confidence interval [CI], 4.4-5.2) for HIV-1 ID-NAT and 12.1 copies per mL (95% CI, 11.1-13.2) for HCV ID-NAT. Because MPs screened with the Gen-Probe transcription-mediated amplification system consist of 16 samples, we estimated that, on the day of MP-NAT detectability, the viral load was 16 times the ID-NAT detection viral load, that is, 76.8 copies per mL for HIV-1 MP-NAT and 193.6 copies per mL for HCV MP-NAT. Third, the viral load was presumed to increase at a constant rate (i.e., in a linear fashion) on a log scale around the time of detectability. HIV-1 and HCV viral load doubling times in this ramp-up period have been estimated to be 20.5 hours (95% CI, 18.2-23.4; Fiebig et al.\textsuperscript{11}) and 14.9 hours (95% CI, 12.9-17.7; Pappalardo et al., submitted for publication; Nubling et al.\textsuperscript{24}), respectively, corresponding to rates of increase, or slopes, of 0.35 for HIV-1 and 0.48 for HCV (log increase per day). Hence, the mean period from the day of infectivity to the day of detectability by NAT was approximated as (the log of the ID- or MP- NAT detection viral load) minus (the log of the viral load corresponding to infectivity, that is, log(1 copy/20 mL)) divided by the slope. The SE associated with each period was estimated with a Taylor series approximation.\textsuperscript{27} We also calculated the periods with the 50 percent sensitivity levels associated with the HIV-1 and HCV assays (COBAS AmpliScreen, Roche Molecular Systems Inc., Pleasanton, CA\textsuperscript{26,28} with screening with the Roche platform performed on MPs of 24 samples.\textsuperscript{30} This allowed us to compare residual risks and yields estimated with either the Roche or the Gen-Probe assays’ 50 percent sensitivity values.

**Incidence estimations**

**Incidence based on MP-NAT yield cases.** We referred to the report by Stramer and coworkers\textsuperscript{23} to ascertain the number of HIV-1 and HCV MP-NAT-positive, antibody-negative donations (referred to as MP-NAT yield cases in the text) identified at all major US NAT screening laboratories in the first 3 years of MP-NAT screening (April 1999-April 2002). In this surveillance effort conducted by the National Heart, Lung, and Blood Institute NAT Study group, approximately 28 million allogeneic donations were screened for HIV-1 and HCV RNA by the Gen-Probe transcription-mediated amplification system,\textsuperscript{26} whereas the Roche COBAS AmpliScreen HIV-1 and Roche COBAS AmpliScreen HCV assays were used to screen approximately 9 and 12 million donations, respectively.\textsuperscript{30,31} To apply the model accurately, the present analysis was restricted to NAT yield cases that tested negative by all serologic assays, that is, HIV-1 MP-NAT yield cases that were also p24 antigen-negative (10 of 12 total HIV-1 NAT yield cases) and HCV MP-NAT yield cases that did not react by third-generation EIA (139 of 156 cases detected by either second- or third-generation EIA screening).\textsuperscript{23} We also abstracted from this report the first-time to repeat donor MP-NAT yield rate ratios that were reported to be 2.7 (95% CI, 0.5-12.7) and 3.1 (95% CI, 2.0-5.0) for HIV-1 and HCV, respectively.\textsuperscript{23}

The period during which a donation can be considered at risk for having MP-NAT-positive and antibody- (and p24Ag-) negative test results is the number of days during which RNA is detectable by MP-NAT but during which antibodies (or p24Ag) have not formed.\textsuperscript{11,32} Hence, on average, each donation is at risk of being a NAT yield case for the duration of the T-III or T-IIIa period, that is, the MP-NAT to antibody (or p24Ag) period. The MP-NAT yield cases represent newly detected infections or incident cases, where for the purposes of estimating incidence the number of yield cases have a SE equal to the square root of the number of yield cases (assuming a Poisson distribution). Person-years can be estimated as the sum of all periods during which donations were at risk of being yield cases, that is, by multiplying the total number of screened donors for each period by the average person-days at risk per donation.

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**Fig. 1.** Periods in days (SE) for HIV and HCV. Calculations based on the HIV and HCV Gen-Probe transcription-mediated amplification 50 percent sensitivities. WB = Western blot.
donations by T-IIIa for HIV-1 or by T-III for HCV expressed in years. The person-years SE is then a function of the SE around T-IIIa or T-III.

Incidence based on S/LS-EIA assay yield cases. To identify HIV-seropositive donors with recent infection and project donor incidence we used the standardized testing algorithm for recent HIV seroconversion (STARHS), developed by the Centers for Disease Control and Prevention (CDC, Atlanta, GA) to detect persons with low-titer HIV antibody responses characteristic of recent infection. This testing strategy uses the Vironostika HIV-1 microelisa (bioMérieux Industry, Raleigh, NC) assay for anti-HIV, which has been modified for “detuned” EIA application by increasing the sample dilution from 1:76 to 1:20,000 and reducing the sample incubation time from 100 to 30 minutes while retaining the kit-specified conjugate incubation time of 30 minutes. Western blot confirmed-positive donor samples are run in parallel with CDC-supplied low-positive control, high-positive control, and a calibration panel composed of five specimens with predetermined antibody levels. The standardized sample optical density (SOD) value is calculated for each clinical sample as follows:

\[
\text{SOD} = \frac{(\text{sample OD} - \text{average negative control OD})}{(\text{average calibrator OD} - \text{average negative control OD})}.
\]

Samples are initially tested individually with both standard and detuned assay conditions. Samples that test positive in the standard sensitive assay and have a screening SOD below 1.5 in the detuned assay are retested in triplicate with the detuned assay. For samples with an average SOD below the prescribed cutoff value of 1.0, the corresponding donor is classified as having a putative recent infection. This S/LS-EIA testing strategy was used to test for recent infections in 208 of 221 HIV-1 Western blot confirmed antibody-positive donations (13 specimens were exhausted) that had been identified by the American Red Cross upon screening of 6,511,094 allogeneic donations in 1999. Fifty of the 208 cases were identified as recent infections (i.e., S/LS-EIA yield cases) based on previously defined test criteria. HIV-1 incidence was then estimated by dividing the number of new infections detected by the S/LS-EIA assay by the sum of all periods during which donations were at risk of being an HIV-1 S/LS-EIA yield case. As shown in Fig. 1, the T-IV period during which a confirmed seropositive donation is at risk for being reactive on the sensitive Vironostika viral lysate EIA test but nonreactive on the less sensitive modified assay has been estimated to be 170 days (SE, 10.0 days).

Incidence based on repeat donors donation history: the classical method. For comparison purposes, we calculated incidence in repeat donors with the most current and representative data available for repeat donors (2000-2001 data) in the US as reported by Dodd and colleagues. We then adjusted the reported repeat donor incidence by the first-time to repeat incidence ratio. This ratio was estimated with the MP-NAT yield rates (number of MP-NAT yield cases/number of screened donations) obtained in first-time and repeat donors by the NHLBI NAT Study. First-time donor incidence can be calculated by dividing the number of first-time MP-NAT yield cases by person-years for first-time donors (i.e., number of first-time donations × T-III for HCV or T-IIIa for HIV-1). Similarly, the repeat donor incidence can be estimated by dividing the number of repeat MP-NAT yield cases by person-years for repeat donors (i.e., number of repeat donations × T-III for HCV or T-IIIa for HIV-1). When dividing the first-time donor incidence by the repeat donor incidence to obtain the first-time to repeat donor incidence ratio, T-III or T-IIIa cancels out. Hence, the first-time to repeat donor incidence ratio is equal to the first-time to repeat MP-NAT yield rate ratio; and incidence in first-time donors can be estimated by multiplying the first-time to repeat donor incidence ratio by the repeat donor incidence.

Finally, if the proportions of first-time and repeat donations are FT percent and RPT percent, respectively, the incidence in the overall donor population can be estimated as

\[
(\text{FT\% × first-time donor incidence}) + (\text{RPT\% × repeat donor incidence}).
\]

Estimating the 95 percent CIs. Wald-type 95 percent CIs are used for time ratios, incidence estimates, yield estimates, and residual risk estimates. The SEs were estimated with Taylor series approximations.

Residual risks and ID-NAT yield rate estimations

Residual risks can be estimated with one of two methods. We can either multiply the incidence derived by any of the three methods previously described by the WP between infectivity and detection by MP-NAT, which is the sum of T-I and T-II, or multiply the yield rate (number of MP-NAT or LS-EIA yield cases/number donations screened) by the ratio between the two periods of interest. For example, the residual risk associated with MP-NAT screening can be directly estimated by multiplying the MP-NAT yield rate by (T-I + T-II)/T-III for HIV-1 or by (T-I + T-II)/T-III for HCV. We chose to present the intermediate “by-product” of the residual risk calculation, that is, the incidence rates, to allow the reader to compare incidence estimates obtained with different sources of data and between countries for which incidence estimates and 95 percent CI are available. The results from the simpler time ratio approach are identical and allow for direct derivation of risk and yield projections for any donor population for which MP-NAT or LS-EIA yield are known.
We can also estimate the number of ID-NAT-positive, MP-NAT-negative, antibody (p24Ag)-negative donations (ID-NAT yield cases) that would be newly detected if ID-NAT were implemented by multiplying the yield rate by T-II/T-IIIa for HIV or by T-II/T-III for HCV, or alternatively, by multiplying the incidence by T-II. Wald-type 95 percent CIs around residual risk estimates and ID-NAT yield rate estimates were estimated with Taylor series approximation of the residual risk estimate standard errors and yield rate estimate standard errors. 27

Human subjects approval

All components of this study received approval by the appropriate institutional review boards associated with the participating blood center programs. All data were devoid of personal identifiers and sent to the NAT surveillance program’s coordinating center (Westat, Rockville, MD) for compilation in a database. The NAT surveillance study 23 received approval by the coordinating center’s institutional review board.

RESULTS

We first derived new estimates for relevant acute infection WPs based on: 1) the assumption that infectivity by blood transfusion begins when donor viral load reaches 1 copy per 20 mL of plasma, 2) the known doubling times for ramp-up phase HIV and HCV viremia, 3) the documented viral load detection limits for HIV and HCV MP- and ID-NAT assays, and 4) the estimated times from MP-NAT positivity to detection of relevant serologic markers (p24Ag for HIV and third-generation antibody EIA for HCV). As shown in Fig. 1, we estimated the duration of the HIV-1 WP between infectivity (1 copy/20 mL) and ID-NAT detection (T-I) as 5.6 days, T-II (ID- to MP-NAT detection) as 3.4 days, and T-IIIa (MP-NAT to p24Ag detection) as 6.0 days for the Gen-Probe and 4.2 days for the Roche assays, respectively. Corresponding estimates for HCV were 4.9 days for T-I, 2.5 days for T-II, and 50.9 days for T-III. Figure 1 also presents the SEs associated with each of these period estimates.

The first 3 years of MP-NAT screening in the US resulted in detection of 1 HCV-viremic-seronegative donation per 266,000 donations screened (3.76 per million; 95% CI, 3.16-4.44). As expected, the MP-NAT yield rate was lower for HIV-1 with identification and interdicting of 1 viremic seronegative (p24Ag- and antibody-negative) donation per 3.7 million donations screened (0.27 per million; 95% CI, 0.13-0.49). Further, 50 of 6.1 million donations were reactive on the S-EIA assay and confirmed by Western blot and NAT but did not react on the LS-EIA, and thus were considered recent HIV-1 infections, for an S/LS-EIA yield rate of 1 in 123,000 donations (8.16 per million; 95% CI, 6.05-10.77). Table 1 summarizes these yield data and presents the period ratios used for residual risk and yield projections.

### TABLE 1. HIV-1 and HCV MP-NAT and S/LS-EIA detection rates for US blood donors and time ratios used for projecting risk and ID-NAT yield

<table>
<thead>
<tr>
<th>Virus and detection method</th>
<th>Yield of ID-NAT</th>
<th>Residual risk for ID-NAT</th>
<th>Residual risk for MP-NAT</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>HIV-1</strong></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>MP-NAT</td>
<td>0.27 (0.13-0.49)</td>
<td>1.51 (1.00-2.02)</td>
<td>0.049 (0.039-0.059)</td>
</tr>
<tr>
<td>S/LS-EIA</td>
<td>8.16 (6.05-10.77)</td>
<td>0.053 (0.044-0.062)</td>
<td>0.146 (0.117-0.175)</td>
</tr>
<tr>
<td><strong>HCV</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>MP-NAT‡</td>
<td>3.76 (3.16-4.44)</td>
<td>0.053 (0.044-0.062)</td>
<td>0.146 (0.117-0.175)</td>
</tr>
</tbody>
</table>

* The yield of ID-NAT or residual risks associated with MP-NAT and ID-NAT, respectively, can be directly calculated by multiplying the yield rate by the appropriate time ratio (see Materials and methods and Fig. 1 for time periods used to derive these ratios); hence, incidence derivation per se is not needed to calculate yield or residual risks. The period between detection by MP and detection by p24Ag (T-IIIa) varies for HIV-1 as a function of the assay used. This period was calculated to be 6.0 (SE, 1.1 day) if the Gen-Probe transcription-mediated amplification system with a MP of 24 is used (as shown in the table) and 4.2 days (SE, 1.2 days) if the Roche COBAS AmpliScreen HIV-1 assay with MP of 24 is used. For HCV, T-III (period between detection by MP-NAT and EIA-3.0 seroconversion) was estimated to be 50.9 days (see text). Other time ratios, not used in our calculations of risk variables in the context of NAT screening in the US, were developed for use in calculating HIV residual risk in settings of serologic screening: HIV residual risk for p24 screening: (T-I + T-II + T-III)/T-III = 2.51 (95% CI, 2.00-3.02); (T-I + T-II + T-III)/T-III = 3.39 (95% CI, 2.71-4.08); HIV residual risk for antibody screening: (T-I + T-II + T-III)/T-IV = 0.09 (95% CI, 0.07-0.11); (T-I + T-II + T-III)/T-IV = 0.12 (95% CI, 0.10-0.14).

† Total number of donations (6,511,094) reduced by a factor 208/221 (208 S/LS-EIA tested of 221 HIV-1-confirmed-seropositive donations).

‡ Yield represents cases detected as RNA-positive and third-generation EIA-nonreactive; donations from three testing laboratories were not included because they did not report third-generation EIA results on the index donation.
Incidence rates per 100,000 person-years and 95 percent CIs are presented in Table 2. For HIV-1, incidence was similar when derived from MP-NAT yield (1.77; 95% CI, 0.55-2.99) or S/LS-EIA data (1.75; 95% CI, 1.23-2.28). The incidence derived from the repeat donation history data set appeared slightly higher (2.16; 95% CI, 0.55-2.99) or S/LS-EIA data (1.75; 95% CI, 1.23-2.28). Incidence derived from MP-NAT yield data or from S/LS-EIA data (for HIV-1 only) data were not significantly different from one another (p = 0.76). HCV incidence estimates derived from MP-NAT yield data or from repeat donation histories were similar (2.70 vs. 2.80; p = 0.82).

By use of the periods and their ratios (Fig. 1 and Table 1), we estimated how many more HIV-1 or HCV viremic donations would be captured if ID-NAT was implemented; the residual risk or probability of having an HIV-1 or HCV infectious donation released in the US at the present time, that is, with MP-NAT screening; and the HIV-1 and HCV residual risks if ID-NAT screening was implemented. As discussed earlier, yield and residual risks derived from NAT yield data or from S/LS-EIA data (for HIV-1) could be calculated by either multiplying the yield rate by the corresponding time ratio (Table 1) or by multiplying the incidence (Table 2) by the appropriate time (numerator of the time ratios presented in Table 1).

As shown in Table 3, we estimated that implementation of ID-NAT would result in the identification of 1 HIV-1 viremic donation for every 5 to 6 million donations screened. Hence, if approximately 13.8 million allogeneic donations are collected annually in the US, we would expect to detect 2 to 3 additional potentially HIV-1-infectious donations per year across the US with ID-NAT implementation. Similarly, HCV ID-NAT implementation was predicted to detect an additional 2 to 3 HCV viremic donations per year in the US because yield was estimated to be 1 in 5.2 to 5.4 million donations.

Residual risks under current screening conditions were estimated to be 1 in 1.8 million donations for HCV and to vary from 1 in 1.9 to 1 in 2.3 million donations for HIV-1 (Table 3). ID-NAT implementation was estimated to reduce these risks to 1 in 2.6 to 1 in 2.7 million donations for HCV and to 1 in 3.0 to 3.7 million donations for HIV-1.

### DISCUSSION

This study presents new approaches for estimating durations of infectious WPs relevant to blood transfusion safety and for assessing viral incidence and residual risks in the context of contemporary NAT screening of US blood donors. The risk of recipient infection by HIV-1 with MP-NAT screened blood was estimated at 1 in approximately 2 million based on MP-NAT yield or S/LS-EIA data. The risk of HCV infection with MP-NAT screening was also estimated at approximately 1 in 2 million. These findings offer reassurance that the US blood supply is now extremely safe with respect to these major viral infections.

Several advantages to estimating incidence and residual risks based on rates of detection of donations in early stages of recent infection by NAT or LS-EIA screening methods are noteworthy. Residual risk and yield estimates based on seroconversion incidence rates derived with repeat donation histories assume that donors are as likely to donate in early and late stages of acute infection. Infected donors could either delay their return or stop donating following high-risk behaviors or symptomatic acute infections. Because flu-like symptoms are often associated with the p24Ag-positive phase of primary HIV-1 viremia, infected donors may be less likely to donate in this early stage of infection. This hypothesis is supported by the finding that only 2 of 12 HIV-1 MP-NAT yield donations tested p24Ag-positive, despite comparable lengths.
of the p24Ag-positive and -negative phases. To avoid this bias, we restricted the HIV-1 MP-positive yield cases to the p24Ag-negative phase. To evaluate the hypothesis that infected donors may delay their return around seroconversion, Schreiber and associates used repeat donation histories collected between 1991 and 1997 at five US blood centers and confirmed that on average, HIV-1 seroconverters returned to donate 42 percent later than predicted. The lack of a significant difference between residual risks derived from the MP-NAT, S/LS-EIA, or repeat donation history data, however, suggests that such a delay in return does not appreciably impact risk estimation.

Conversely, test seeking, which has been documented to occur by follow-up interviews of HIV-1- and HCV-seropositive donors and random surveys of seronegative donors, could result in increased rates of donations during the critical early postexposure period. Our finding of similar residual risk estimates for HIV-1 and HCV, whether derived from MP-NAT or from repeat donation history data, suggests that such a test-seeking effect, if present, is small and does not appear to impact residual risk estimates.

Our new approach is dependent on accurate estimates for the durations of the periods between infectivity and assay detectability or between new and old assay detectability. Over the past 5 to 10 years, data based on extensive characterization of serial specimens from a variety of incident infection settings have yielded remarkably consistent and increasingly precise estimates of WP reduction for new serologic and molecular assays. In contrast to the increasing precision in estimates for time intervals between sequential appearance of laboratory markers, data are limited on estimated time periods from exposure to initial viral marker positivity and even more limited with regard to infectivity from a blood transfusion. Our approach of estimating durations for residual infectious WPs, based on viral loads at MP-NAT assay cutoffs and early viral replication kinetics, assumes that even a single viral particle in a 20-mL infused volume of plasma in a typical RBC unit may be infectious. A series of recent, well-documented cases of HIV-1 and HCV transmission by transfusions that contained very low or even undetectable levels of viral nucleic acids by highly sensitive ID-NAT systems supports this conservative modeling approach. Nonetheless, variation in the kinetics of early viremia may occur in some infected persons as a result of innate immunity or other variables. We and others have observed cases with intermittent viremia (“blips”) preceding the ramp-up phase for HIV and HCV, as well as cases with fluctuations in viral load during the preseroconversion plateau phase of HCV infection. These findings could impact the durations of the infectious WPs and MP-NAT-reactive periods used in our model projections. Consequently, further work to better characterize the length of infectious WPs and determinants of infectivity (including viral, donor unit, and recipient susceptibility factors) is warranted.

From a policy development perspective, our results allow accurate projection of the incremental safety value (i.e., reduction in residual risk) of introducing additional blood safety measures. For example, our data project the yield of ID-NAT at 2 to 3 HIV-1 and HCV infections, currently missed by MP-NAT and serologic screening, among 13.8 million units donated annually in the US. A recently published cost-effectiveness analysis of various blood screening scenarios, with similar ID-NAT yield projections, found that all scenarios involving ID-NAT resulted in cost-effectiveness estimates exceeding $7.3 million per quality-adjusted life-year, higher than those for MP-NAT ($3-$5 million per quality-adjusted life-year) and substantially higher than most other health-care interventions within or outside the transfusion arena. In

TABLE 3. HIV-1 and HCV residual risks and ID-NAT yield projections per 10^6 donations derived with repeat donation history data versus MP-NAT and S/LS-EIA yield data

<table>
<thead>
<tr>
<th>Data source</th>
<th>Yield of ID-NAT, as 1 in number of screened donations (95% CI)</th>
<th>Residual risk of transfusion of an infectious unit, as 1 in number of screened donations (95% CI)</th>
<th>1 copy/20 mL to ID-NAT</th>
<th>1 copy/20 mL to MP-NAT</th>
</tr>
</thead>
<tbody>
<tr>
<td>HIV-1</td>
<td></td>
<td></td>
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<td></td>
</tr>
</tbody>
</table>
| Repeat donation history | 1 in 5.0 x 10⁶  
(1 in 9.3 x 10⁻¹ in 3.4 x 10⁶) | 1 in 1.9 x 10⁶  
(1 in 3.5 x 10⁵-1 in 1.3 x 10⁴) | 1 in 3.0 x 10⁶  
(1 in 5.7 x 10⁴-1 in 2.0 x 10³) |                        |                        |
| MP-NAT yield    | 1 in 6.0 x 10⁶  
(1 in 20.3 x 10⁻⁵ in 3.6 x 10⁵) | 1 in 2.3 x 10⁶  
(1 in 7.7 x 10⁵-1 in 1.3 x 10⁴) | 1 in 3.7 x 10⁶  
(1 in 12.4 x 10⁴-1 in 2.2 x 10³) |                        |                        |
| S/LS-EIA yield  | 1 in 6.1 x 10⁶  
(1 in 9.1 x 10⁻⁵ in 4.6 x 10⁵) | 1 in 2.3 x 10⁶  
(1 in 3.4 x 10⁵-1 in 1.7 x 10⁴) | 1 in 3.7 x 10⁶  
(1 in 5.5 x 10⁴-1 in 2.8 x 10³) |                        |                        |
| HCV             |                                                                |                                                                                              |                        |                        |
| Repeat donation history | 1 in 5.2 x 10⁶  
(1 in 7.4 x 10⁻⁵ in 4.1 x 10⁶) | 1 in 1.8 x 10⁶  
(1 in 2.5 x 10⁵-1 in 1.4 x 10⁴) | 1 in 2.6 x 10⁶  
(1 in 3.8 x 10⁴-1 in 2.0 x 10³) |                        |                        |
| MP-NAT yield    | 1 in 5.4 x 10⁶  
(1 in 7.3 x 10⁻⁵ in 4.3 x 10⁵) | 1 in 1.8 x 10⁶  
(1 in 2.5 x 10⁵-1 in 1.4 x 10⁴) | 1 in 2.7 x 10⁶  
(1 in 3.7 x 10⁵-1 in 2.2 x 10³) |                        |                        |
fact, taking MP-NAT as the baseline, the marginal cost of introducing ID-NAT exceeded $12 million per quality-adjusted life-year.\textsuperscript{47}

Transfusion medicine scientists and policy makers in other countries employing MP-NAT screening may simply use the time ratios presented in Table 1 to adjust observed rates of detection of MP-NAT yield cases to project residual risks for HIV-1 and HCV, without having to separately calculate incidence. This approach can therefore be applied in donor settings where regional NAT yield (or S/LS-EIA) data are compiled from multiple blood screening programs\textsuperscript{48} or where there is limited capacity to maintain accurate databases of serial donations or to accurately determine first-time or repeat status of donors (misrepresentation of donor status is a serious problem in developing countries where paid or replacement donors are common).\textsuperscript{10,34} Moreover, the same approach can be applied to nondonor populations to estimate incidence or predict incremental yield of improved diagnostic assays (Pappalardo et al., submitted for publication).\textsuperscript{11,13,14,50}

Several limitations to the risk estimation approach presented here should be considered. Because the NAT-yield WPs (particularly for HIV-1) are short and viral incidence rates among blood donors are low, NAT screening data from large numbers of donations are required to yield accurate projections. Hence, the method may not be applicable to project risk for small donor populations and will require very large data sets to examine trends in risk over time or by donor subpopulations. Second, our projected residual risk if ID-NAT screening was implemented is based on incidence derived with 1999 to 2002 data. If incidence at time of ID-NAT implementation was higher or lower than in 1999 to 2002, the estimated number of ID-NAT yield cases would be correspondingly higher or lower than our current estimate. Third, the time ratios used to project residual risk from NAT yield data are assay specific, that is, the durations of WPs preceding and following the NAT detection time point are dependent on the analytical sensitivity of the NAT assay and the pool size used for donor screening. For generating precise risk estimates (with CIs), it is necessary to determine the 50 percent detection limit (and SE around that detection limit) of the assay under consideration. When compiling or comparing NAT yield data from multiple screening programs that use different NAT assays or pool sizes, it is necessary to first develop program-specific WP estimates and time ratios based on their NAT assay sensitivities and pool sizes and then apply these time ratios to the respective programs’ NAT yield data before proceeding to a composite analysis (as was done here to combine data from screening programs with Roche and Gen-Probe NAT assays).\textsuperscript{23}

There are also limitations regarding the use of the S/LS-EIA approach for HIV incidence and risk projections. The WP of recent seroconversion detected by the S/LS-EIA is relatively long (170 days for Clade B viruses and longer for non-Clade B viruses), when compared to the inter-donation intervals of repeat blood donors.\textsuperscript{23,33,51,52} The method presented here assumes that each donation (whether from first-time or repeat donors) has a 170-day risk period associated to it. Repeat donors, however, may have made a previous donation within this approximately 170-day risk period, such that their actual risk period is less than 170 days, and is approximately equivalent to their last inter-donation interval. Consequently, in populations with a significant proportion of frequent repeat donors, risk projections derived by applying the time ratio to the rate of recent infections detected by the S/LS-EIA among total donations will slightly underestimate risk. To account for this, an adjustment factor can be developed based on the inter-donation interval distribution of repeat donors.\textsuperscript{53} In a setting such as that in the US where 70 to 80 percent of donations are from repeat donors who give an average of 1.5 to 2.0 donations per year, the adjustment factor results in a 15 to 20 percent change in incidence and risk estimates. In most developing country settings where the S/LS-EIA approach for risk and NAT yield projections would be particularly useful, the proportion and frequency of repeat donations are low, and hence the need for development and application of an adjustment factor would be small. In contrast, in many developing countries a substantial proportion of HIV infections are due to HIV subtypes other than HIV-1B. Because various genotypes of HIV have different length seroconversion WPs with the Vironostika S/LS-EIA (which is based on HIV-1 Clade B antigen), preliminary data are required on the distribution of subtypes and their respective S/LS-EIA WPs to accurately detect incident infections and project risk in these donor populations (S/LS-EIA WPs have been estimated for several but not all of the major HIV-1 subtypes (Clades B, C, and E)).\textsuperscript{53,54} A recently developed S/LS assay, which is based on a combination of antigens from multiple HIV subtypes, may overcome this limitation.\textsuperscript{55}

In summary, we have developed and validated several new approaches for accurately estimating the duration of the infectious WPs relevant to transfusion safety analyses and deriving incidence rate and residual risk estimates for the overall donor population based on the rates of viremic seronegative donations detected by NAT or the rate of donations with laboratory evidence of recent seroconversion based on the S/LS-EIA testing strategy. Consistent with other recent report,\textsuperscript{6} we estimated that HCV and HIV-1 risks with current MP-NAT screening in the US are approximately 1 per 2 million units and that these risks could be reduced to 1 in 3 to 4 million units by ID-NAT screening. We believe that this alternative method to viral risk estimation can be effectively implemented in donor settings where NAT yield data are routinely generated, as well as in developing country settings where S/LS-EIA testing can be performed on seroreactive donations.
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