DONOR INFECTIOUS DISEASE TESTING

Assessment of HIV transfusion transmission risk in South Africa: a 10-year analysis following implementation of individual donation nucleic acid amplification technology testing and donor demographics eligibility changes

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BACKGROUND: In 1998 we estimated that 34/million infectious window period donations were entering the blood supply at the South African National Blood Service. Selective use of donations based on donor race-ethnicity reduced this risk to 26/million donations but was deemed unethical. Consequently, in 2005 South African National Blood Service eliminated race-ethnicity–based collection policies and implemented individual-donation nucleic acid testing (ID-NAT). We describe the change in donor base demographics, human immunodeficiency virus (HIV) detection rates, and transfusion-transmissible HIV risk.

STUDY DESIGN AND METHODS: In ten years 7.7 million donations were tested for anti-HIV and HIV RNA. Number of donations, HIV prevalence, ID-NAT yield rate, serology yield rate and residual transfusion-transmissible HIV risk were analyzed by donor type, race-ethnicity, age, and sex. Multiple regression analysis was performed to investigate the determinants of HIV-positive and nucleic acid testing yield donations.

RESULTS: The combined strategy of increasing donations from black donors and implementing ID-NAT increased the proportion of donations from black donors from 6% in 2005 to 30% in 2015 (p < 0.00001), and reduced the transfusion-transmissible risk from 24 to 13 per million transfusions. ID-NAT interdicted 481 (1:16,100) seronegative window period donations, while one transfusion-transmissible case (0.13 per million) was documented. Race-ethnicity and donor type were highly significant predictors of HIV positivity, with adjusted odds ratio for first-time donors of 12.5 (95% confidence interval, 11.9-13.1) and for black race-ethnicity of 31.1 (95% confidence interval, 28.9-33.4). The proportion of serology yields among HIV-infected donors increased from 0.27% to 2.4%.

CONCLUSION: ID-NAT enabled the South African National Blood Service to increase the number of donations from black donors fivefold while enhancing the safety of the blood supply.

According to a “between-census” community survey done in 2016 that sampled 1.3 million households of the 56 million people living in South Africa, approximately 7.06 million people are human immunodeficiency virus (HIV) positive, providing an estimated adult HIV prevalence and incidence of 18% and 0.91 per 100 person-years, respectively. In this environment, the South African National Blood Service (SANBS) collects approximately 800,000 blood donations annually from voluntary nonremunerated blood donors, which are processed into blood components that are provided to approximately 400,000 patients each year.

ABBREVIATIONS: ART = antiretroviral therapy; FT = first-time; ID-NAT = individual donation nucleic acid testing; MID50 = 50% minimum infectious dose; p24Ag = p24 antigen; SANBS = South African National Blood Service; TT = transfusion-transmitted; WP = window period.

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In 1998, despite screening donations with a sensitive HIV p24 antigen (p24Ag) test, it was estimated that a significant rate (34 per million) of infectious window period (WP) donations could be entering the blood supply, a projection supported by confirmed reports of transfusion-transmitted (TT) HIV infections each year. A structured risk management program, which included stringent education, product triage, and donor race-ethnicity as risk markers, was implemented in 1999, and these measures were successful at reducing the estimated residual risk of TT HIV infection from 34 per million to 26 per million red blood cell (RBC) transfusions. However, the selective use of donations based on donor race-ethnicity, where donations from black African donors were discarded, was unethical and unsustainable. Consequently, it was decided, after estimating that individual donation nucleic acid testing (ID-NAT) would reduce the TT HIV risk to 17 per million RBC transfusions, to implement ID-NAT and change the risk management program by eliminating race-ethnicity-based collection and utilization policies. It was anticipated that the implementation of ID-NAT would provide a safety buffer as donor eligibility policies were progressively modified to increase donations by the black African majority population.

South Africa was the first country globally to implement ID-NAT nationwide. On October 3, 2005, 1) all blood donations were screened for HIV RNA, hepatitis B virus deoxyribonucleic acid (DNA), and hepatitis C virus ribonucleic acid (RNA); 2) p24Ag testing was discontinued; 3) donor race-ethnicity was removed as a marker of risk categorization; and 4) a donor education and motivation campaign was launched to increase the black African donor base. With these measures, SANBS aimed to maintain a sufficient and safe blood supply for the patients of South Africa. In this paper, we describe how the changes in donor base demographics over 10 years and the implementation of ID-NAT have affected HIV detection rates and residual TT HIV risk estimates.

Characterization of donor demographics
Demographics, including sex, race-ethnicity, date of birth, and donation site, are routinely collected from donors from the self-reported predonation questionnaire. The type of blood donation, that is, from a first-time (FT), repeat (<1 year from previous donation) or lapsed (>1 year from previous donation) donor, is recorded in the blood establishment computer system.

HIV infection detection rates
We calculated HIV prevalence for FT donors (by year and demographic subcategories) by dividing the number of HIV-confirmed-positive donations (concordant positive, confirmed NAT yield, and serology yield donations) by the total number of blood donations from FT donors, with results expressed as percentages. The rates of HIV-infected donations from repeat and lapsed donors were calculated similarly and expressed per 100,000 donations, as were the NAT yield and serology yield rates in the different donation categories.

TT risk analysis
To calculate the TT HIV risk for the 10-year period we used the WP NAT yield ratio model. The proportional increase in the black African donor base and the residual
TT HIV risk was estimated annually for donations from all donors, FT donors, and repeat donors. The annual TT HIV residual risk was estimated for ID-NAT screened blood transfusions, as well as on the assumption that blood had been screened by p24Ag testing instead of ID-NAT. For the ratio modeling we used detection periods of 10.1 days for the NAT-positive/p24Ag-negative/anti-HIV negative detection period\(^7\) and 5.3 days for the p24Ag-positive/anti-HIV-negative detection period.\(^7\) To determine the infectious pre-ID-NAT WP in risk day equivalents, formulas published by Weusten and colleagues\(^9\) were used, with 50% and 95% lower limits of detection by ID-NAT of 2.7 and 18.4 copies/mL, a transfusion plasma volume of 20 mL for RBC components, a 50% minimum infectious dose (MID\(_{50}\)) of 3.1 (range, 1-10) HIV virions\(^\text{10}\) and a doubling time of the virus during the ramp-up phase of infection of 0.85 days; this yielded a residual infectious WP for ID-NAT screened blood of 2.9 days. To determine the pre-p24Ag infectious WP in risk day equivalents we used 50% and 95% limits of detection for p24Ag enzyme immunoassay of 10,000 and 64,000 copies/mL,\(^8\) which yielded an infectious WP of 13.0 days for p24Ag and anti-HIV screened RBC donations. We also investigated the impact of increasing the MID\(_{50}\) to less conservative values for stored RBCs of between 10 and 1000 virions\(^\text{10}\) which reduced the infectious WP for ID-NAT/anti-HIV screened blood from 2.9 to between 1.7 and 0.04 days, respectively, and for p24Ag/anti-HIV screened blood from 13.0 days to between 11.6 and 5.9 days, respectively. The rates of detection of donations confirmed as NAT yields were used to estimate the ID-NAT residual TT HIV risks, and the rates of NAT yield donations that tested p24Ag positive were used to estimate the p24Ag residual TT HIV risks.

**Lookback and traceback**

Lookback was performed on the recipient of the previous donation when a subsequent donation from the donor was confirmed HIV positive (donor triggered), whereas traceback was performed when a recipient alleged potential acquisition of HIV from a transfusion (recipient triggered). If the recipient and donor are both HIV positive, phylogenetic sequencing was performed by the National Institute of Communicable Diseases to confirm transmission. Due to inconsistent lookback documentation until 2010, we limited reporting from 2010 to 2015.

**Regression analysis**

We fit logistic regression models for total HIV-positive donations (concordant, NAT yield, and serology yield) and for NAT yield donations. Crude and adjusted odds ratios were computed (using single and multiple predictors, respectively) and 95% confidence intervals (CIs) estimated using the profile likelihood method. Owing to potential bias resulting from the small number of events, we applied the Firth correction to NAT yield models.\(^\text{11}\) The predictors investigated were donor type, donor race-ethnicity, donor age, sex, and geographic region of collection. Estimation was performed using the *logistf* R package.\(^\text{12}\)

**RESULTS**

**Donor demographics**

In the 10 years following implementation of revised donor eligibility and recruitment policies and implementation of ID-NAT, SANBS collected 7,736,125 whole blood donations of which 947,594 (12.2%) were collected from FT donors, 877,385 (11.3%) from lapsed donors (>1 year interdonation interval) and 5,910,737 (76.4%) from frequent repeat donors (<1 year interdonation interval). The majority of blood donations came from white (67%), male (59%), and > 30-year-old donors (58%) (Table S1, available as supporting information in the online version of this paper). The only significant change in demographics in the donor base over the decade was the number and proportion of collections by different racial-ethnic groups (Fig. 1). An additional 203,417 donations were collected from black African donors in 2015 compared to 2005, with the proportion of donations from black African donors increasing from 6% in 2005 to 30% in 2015 (p < 0.00001). The proportion of donations collected from black African FT donors increased from 19% in 2005 to 54% in 2015 by an additional 44,171 donations. Moreover, black African repeat donors contributed an additional 132,989 donations in 2015 compared with 2005, increasing the proportion of repeat donations from black African donors from 5% to 26% (p < 0.00001).

**HIV infection rates by donation and donor demographic categories**

There were a total of 15,702 (0.2%) HIV-positive donations over the 10-year period of which 10,765 (69%) were from FT donors, 2430 (15%) from lapsed donors and 2504 (16%) from frequent repeat donors (Table 1). In the first 5 years, there was a significant increase in HIV prevalence in FT donors, from 0.70% to 1.27% (p < 0.00001), but that rate later decreased to 1.14% (Table S2, available as supporting information in the online version of this paper; and Fig. 2). During the 10 years, the prevalence of HIV in black African FT donors decreased from 3.18% to 1.97% (p < 0.00001) (Table S2 and Fig. 2).

Bivariate (Models 1-5) and multivariate (Models 6-7) logistic regressions on HIV-positive donations are shown in Table 2. In bivariate models, very large crude odds ratios (ORs) were observed for FT donation status (OR, 27.10; 95% CI, 25.95-28.31) and black race-ethnicity (OR, 57.26; 95% CI, 53.43-61.45), while female sex was also highly significant (OR, 2.36; 95% CI, 2.29-2.44). A multivariate model including these variables and also
controlling for age and geographic region (Model 7) resulted in lower adjusted ORs, but FT donation status (adjusted OR, 12.49; 11.93-13.07), black African race-ethnicity (adjusted OR, 30.20; 95% CI, 28.13-32.47) and female sex (adjusted OR, 1.54; 95% CI, 1.49-1.59) remained highly significant.

### HIV NAT yield rates by donation and donor demographic categories

ID-NAT interdicted 481 (1:16,100) HIV-confirmed-positive donations that were anti-HIV negative, of which 137 (1:7000) were in FT donors, 44 (1:20,000) in lapsed donors, and 300 (1:19,700) in frequent repeat donors. Of the...

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### TABLE 1. Total HIV-positive donations and NAT yield and serology yield rates by donation status, donor demographics, and SANBS region

<table>
<thead>
<tr>
<th></th>
<th>Collections</th>
<th>Total HIV-positive cases</th>
<th>NAT yield cases</th>
<th>Serology yield cases</th>
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<tr>
<td></td>
<td>N*</td>
<td>N</td>
<td>%</td>
<td>N x:10^5</td>
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</table>

* A total of 409 donors had unknown donor type and were not included in the table.*
462 confirmed HIV NAT yield donations that were tested for p24Ag, 285 (61.7%) tested p24Ag negative and 177 (38.3%) tested p24Ag positive. Of the p24Ag negative NAT yield donations, 183 were collected from frequent repeat donors (1:32,300), 80 from FT donors (1:11,800), and 22 from lapsed donors (1:39,900).

The NAT yield rate in all race-ethnicities combined was significantly higher in FT (14.5 per 100,000) compared with repeat donors (5.1 per 100,000) (OR, 2.85; 95% CI, 2.33-3.48). However, as shown in Table 2, when controlling for race, sex, age, and geographic region in multivariate models, the coefficients for donor type were no longer significant (adjusted OR, 1.04; 95% CI, 0.84-1.28). In the full model (Model 14), black African race-ethnicity remained a substantial and significant predictor (adjusted OR, 32.63; 95% CI, 23.36-46.99), while colored race-ethnicity (adjusted OR, 6.44; 95% CI, 3.66-11.05) and female sex (adjusted OR, 2.19; 95% CI, 1.82,2.65) also remained significant.

**HIV serology yield rates by donation and donor demographic categories**

Serologic testing detected 206 confirmed HIV antibody-positive, NAT-negative donations (2.6 per 100,000), of which the majority (186) were from FT donors (Table 1 and Table S2). There was a significant increase in the rate of serology yield donations from FT donors during the 10-year period, from 3 per 100,000 to 39 per 100,000 (p = 0.0001; Table S2). When expressed as a percentage of all HIV-positive donations, there was a similar increase over time from 0.27% in 2006 to 2.43% in 2015 (Fig. 3).
Impact of screening strategy and changing donor demographics on WP transmission risk

Using a MID$_{50}$ of 3.16 virions in the transfused RBC component plasma, the WP NAT yield ratio model estimated an overall residual risk of 11.9 per million RBC transfusions for the 10-year period. Figure 4A compares the TT risk by year when ID-NAT testing is performed compared to p24Ag screening (both in combination with a third-generation anti-HIV assay). Figure 4A also shows the TT risk estimated at 26 per million RBC transfusions by Heyns and colleagues$^3$ in the years immediately prior to the implementation of ID-NAT. The TT risk in 2005 would have been 24 per million RBC transfusions had ID-NAT not been implemented in place of p24Ag testing, and would have increased to 71.2 per million RBC transfusions in 2015 given the increasing proportion of donations by black African donors. However, the WP TT risk was estimated to be much lower, at 13.4 per million RBC transfusions, in Year 10 following implementation of ID-NAT. Figure 4B shows the TT risk by donor type and year when NAT testing is performed. FT donors have a threefold higher TT risk than repeat and lapsed donors.

Figure S1 (available as supporting information in the online version of this paper) shows the impact of alternative estimates of infectivity on the TT risk estimates. When the MID$_{50}$ estimate is increased to between 31.6 and 316 virions in an RBC unit, the TT risk using ID-NAT and antibody screening is between 3.47 and 0.52 per million RBC transfusions compared to between 47.31 and 44.16 per million if the previous screening strategy of p24Ag plus antibody testing had been maintained.

HIV genotypes and drug resistance profiles in NAT yield donors

We tested each NAT yield donation with sufficient volume and viral load for drug resistance and genotyping to establish these virological parameters for cases with recently acquired infections. Of the 481 NAT yields, 168 were unable...
to be tested due to viral loads of less than 1000 copies/mL (minimal viral load for genetic testing at the National Institute of Communicable Diseases), and 86 could not be amplified, probably also due to low viral load or RNA stability. Of the 227 donations genotyped, 204 (90%) were subtype C without any drug resistance mutations, and an additional 18 (8%) were subtype C, with one or more drug resistance mutations. Five (2%) were non-subtype C (one A1, three B, and one CRF02_AG), of which two (0.9%) of the subtype B donations had drug resistance mutations. The fact that these 20 donors are HIV RNA only positive and have drug resistance mutations suggests recent acquisition of HIV with drug resistance mutations.

**Lookback and traceback investigations**

Between 2010 and 2015, 2887 lookback investigations (of 5.8 million HIV-negative transfusions) were initiated based on lapsed or repeat donors who seroconverted to HIV antibody positivity. Of these, 1166 cases (40%) remained unresolved (i.e., no recipient outcome data were provided by hospitals), 396 (14%) patients died after transfusion, 262 (9%) patients tested HIV positive prior to the transfusion, 236 (8%) patients tested HIV negative following investigation, 23 (0.85%) declined further testing, and 15 (0.5%) patients tested HIV positive following transfusion without documentation of their infection status prior to the implicated transfusion. Of these 15 HIV-positive patients identified as possible TT cases, eight showed no genetic linkage between the donor and recipient viruses, two were unlikely transfusion related due to a very short time interval to HIV positive results following transfusion (<6 days), three were unresolved but were unlikely to be TT cases (one was already on ART 3 months after the transfusion and could not be amplified, and two never returned for testing), and one case was a confirmed HIV transmission following transfusion of a WP RBC unit. This case was confirmed by 100% sequence
DISCUSSION

Our 10-year analysis shows that screening blood donations by ID-NAT allowed for a substantial increase in the number and proportion of donations from the majority black African population without reducing the safety of the blood supply in South Africa. Over 10 years, the proportion of donations from black African donors increased fivefold. The increase was seen in both FT and repeat donors, with the number (percentage) of donations from black African FT donors increasing from 12,333 (19%) donations in 2005 to 56,504 (54%) donations in 2015 and collections from black African repeat donors increasing from 26,657 donations in 2005 (5%) to 159,646 donations (26%) in 2015. The TT HIV risk was estimated at 26 per million RBC transfusions prior to the implementation of ID-NAT screening and is expected to reduce to 17 per million RBC transfusions if screening by ID NAT is implemented. We estimated in this study that over 10 years following implementation of revised donor eligibility criteria, recruitment policies, and ID-NAT, the TT HIV risk was reduced from 24 per million in 2005 (with p24Ag and antibody but without ID-NAT testing) to 13.4 per million RBC transfusions in 2015 (with ID-NAT testing).

Black African race-ethnicity, female sex, and donor age of less than 30 years were associated with higher prevalence of HIV infection, consistent with HIV infection demographic associations in the larger population in South Africa. The HIV prevalence in black African FT donors was reduced by one-third during the 10 years, which we attribute to our improved predonation education programs.

When HIV NAT yield cases (representing recent infections within 1–4 weeks prior to donation) are analyzed with all race-ethnicities together, the rate is approximately threefold higher in FT donors compared to repeat donors (OR, 2.85; 95% CI, 2.33–3.48), while the rate in black African donors was 38-fold higher than in white donors. This higher rate of NAT yields in FT donors is therefore confounded by race. When controlled for race ethnicity, NAT yield donations were detected at the same rate in FT and repeat donors (Table 2), suggesting that noncompliance to the risk behavior questions in the donor questionnaire is comparable, irrespective of prior donation education and experience. The fact that there was a significantly higher NAT yield rate in FT donors than in repeat donors overall, but no difference observed within ethnic groups, is thus explained by the higher proportion of black African donors in FT than in repeat donors. Similarly, in a large international study of HIV infection rates in donors, there was no difference in HIV-NAT yield rates between FT and repeat donors in Asia and in Europe. The higher rate of HIV NAT yield cases in black African female donors may well be due to sex inequality, which is often compounded by cultural, legal, and political factors that impede a woman’s ability to protect herself from HIV. In this case, a prospective female donor may still be compliant to the risk behavior question that asks about multiple sexual partners. In this study, women were almost threefold more likely to be HIV positive and had double the NAT yield rate than men. Currently, a study to determine risk factors that are not covered by the predonation questionnaire is under way to inform future enhancements of education and eligibility policies.

ID-NAT interdicted 481 HIV-confirmed-positive donations that tested negative for HIV antibodies, the majority of which would have been made into two or three blood components. Most transfused components from these interdicted donations would likely have caused TT HIV in a recipient, considering the viral loads ranged from low levels to more than 1 million copies/mL. We identified only one confirmed case of TT HIV during the 10-year period linked to an RBC component derived from an ID-NAT–negative donation. This contrasts with one to two annual confirmed TT HIV cases documented by SANBS’s lookback programs during the 5 years prior to the implementation of ID-NAT. To our knowledge, this is the first and only reported case of TT HIV by ID-NAT tested blood in the world.

We acknowledge that the lookback program in South Africa is not highly effective at detecting TT HIV, since we could not determine whether TT HIV occurred in 63% of the almost 3000 cases investigated (40% unresolved, 14% deceased, and 9% HIV positive at the time of transfusion). Therefore, it is possible that TT HIV risk is higher than the 1 in 7.7 million transfusions issued over 10 years that we documented. We modeled and reported a worst-case scenario of residual TT HIV risk following ID-NAT of 12 per million transfusions, or 10 TT HIV cases per year. However, the infectivity of HIV in stored RBCs is likely 10- to 100-fold reduced relative to freshly collected blood. When the MID50 estimate was changed from the conservative 3.16 virions to a more likely 31.6 to 316 virions in the transfused component inoculum, a TT HIV risk of 3.47 to 0.52 per million RBC transfusions was estimated, which is closer to the documented ID-NAT breakthrough TT rate of 0.13 per million (1 per 7.73 million). The true TT-HIV rate is
probably somewhere between the 0.13 per million donations (underestimated risk from lookback data) and 12 per million donations (overestimated worst case modeling scenario), and likely in the range of the 0.5 to 3.5 per million donations using MID$_{50}$ estimates of between 31.6 and 316 virions.

Our breakthrough case highlights that ID-NAT screening does not remove all of the risk of TT HIV. In our study, we did note that along with the increase in donations from black African donors, the TT HIV risk increased annually over 8 years from 4 per million to 19 per million RBC transfusions, but then decreased in the last 2 years to 13 per million, which could be attributed to the national rollout of ART and resultant twofold decrease in HIV incidence from 1.86% to 0.91% during the past decade in the general population,$^1$ which is reassuring for the future. Nevertheless, in the absence of pathogen inactivation for RBCs, increasing donations from donors who are at a higher risk of an acute HIV infection must be implemented carefully, optimally with targeted donor education and extensive monitoring to balance blood safety with blood sufficiency.

Ten percent of the recently acquired HIV infections in donors had sequences consistent with transmitted drug resistance, similar to the finding of 9% ART drug-resistant HIV in the first South African national survey of pretreatment resistance.$^7$ There were too few non-subtype C cases to investigate any differences in drug resistance prevalence between subtypes. This molecular surveillance aspect of our study demonstrates the important role of aligning national blood donor programs with public health reference laboratories to track changes in HIV genotypes and transmitted drug resistance in recently infected donors, which would be similar to transmitted drug resistance in the general population.$^{16,17}$

The increase in serology yields, using the same serological screening and confirmatory assays over the 10-year period from 0.27 to 2.43% of HIV-infected donors, is concerning. We are currently investigating the reasons for this increase, which is in part due to donations by donors who were aware of their HIV infections and taking ART, which suppressed their viral loads to below the detection limit of even ID-NAT.$^{18}$ This indicates that even more predonation education of donors is required.

Overall, these analyses demonstrate that the change in donor eligibility and recruitment policies linked to introduction of ID-NAT were extremely successful, resulting in a fivefold increase in donations from black donors with a reduction in modeled TT HIV risk of between 2- and 50-fold depending on infectivity of HIV in stored blood components. Moreover, in the 10-year period, the observed TT HIV case was approximately 20-fold lower as compared with the period before the introduction of ID-NAT, when two cases per annum were observed.$^{2,3}$ However, going forward, the trend in modeled residual risk must be continuously monitored to ensure the safety of the South African blood supply.

**CONFLICT OF INTEREST**

NL may be paid by Grifols, the manufacturer of the NAT assay used, for review of the draft paper. All other authors have disclosed no conflicts of interest.

**REFERENCES**


SUPPORTING INFORMATION

Additional Supporting Information may be found in the online version of this article.

Fig. S1. Impact of HIV-1 infectivity on residual window period transmission risk with individual donation nucleic acid testing (diamonds) and p24 antigen testing (squares) estimated over the 10-year screening period. Dotted lines represent 95% confidence intervals (CI) around 50% limits of detection; that is, 2.7 (2.0-3.5) and 10,000 (5000-20,000) copies/mL for individual donation nucleic acid testing and p24 antigen detection, respectively, whereby variability in analytical sensitivity of NAT and p24 antigen reagent batches, RBC plasma transfusion volume, and uncertainty in standardization in true copy or virion numbers is ignored.

Table S1. Demographic characteristics of South African blood donors over a 10-year period.

Table S2. HIV infections classified by testing results, donor type, and ethnic group per year.