Supporting Statement B for
Incident HIV/ Hepatitis B virus infections in South African blood donors: Behavioral risk factors, genotypes and biological characterization of early infection

OMB Number: 0925-XXXX

September 2013

Sponsored by:

The National Heart, Lung, and Blood Institute
Transfusion Medicine and Cellular Therapeutics Branch
National Institutes of Health

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Objectives 1 and 2

We will characterize HIV clade and drug resistance profiles and determine viral loads in all cases of blood donors who are found to be recently infected with HIV (incident HIV infection), as well as characterize HBV genotype and viral load in all incident HBV infections. We will also conduct a case-control study of risk factors for incident HIV and HBV infections in blood donors in South Africa. Accrual, enrollment and follow-up is planned to occur during a period of 3 years (April 2014 – March 2017). The study will be located in geographic areas within the South African National Blood Service (SANBS) catchment where the proportion of donors with HIV and HBV infectious markers is high and where there is documented evidence of good success in notifying donors of infectious disease testing results. SANBS divides its collection regions into 7 zones. We will conduct the study in 5 zones in 4 Provinces; Gauteng (Johannesburg and Pretoria), KwaZulu Natal (Durban), Mpumalanga Province and the Eastern Cape Province. For operational purposes, SANBS divides the Gauteng Province into 3 operational zones, namely "Egoli", " Vaal" and "Northern", with Johannesburg the main metropolitan area in Egoli and Pretoria the main metropolitan area in the Northern zone.

The study sample will include HIV and HBV cases who are recruited from among those with identified incident infection who return to SANBS for their results (anticipated to be ~50% of donors with incident infections). The study sample controls will be selected to achieve stratum-matching by select characteristics (see below) at randomly selected clinics.

Our Two Case Groups will be: (1) 300 incident HIV infected SANBS blood donors (see Study enrollment or Specimen Procurement Objective 1a for definition), and (2) 150 incident HBV infected SANBS blood donors (see Study enrollment or Specimen Procurement Objective 1b for definition).

Controls will comprise a sample of up to 900 SANBS blood donors who are negative for all infectious markers for which SANBS currently tests and who (a) come from the same geographic areas as the cases (distributed across up to 5 of the 7 regional zones within SANBS) and will be frequency matched to cases for (b) population group (term used to define race in South Africa), and (c) age within 5 years. We will enroll three controls for each incident HIV case. A portion of these control participants will be identified to serve as controls for HBV cases also. In the absence of knowledge about important matching variables, our working premise is that a subset of controls for the HIV study can fulfill the dual purpose of serving as controls for the HBV study, so we will begin with the expectation that the distribution of HBV cases will be similar to the HIV distribution for geography, population group, and age. Because there will be six times as many controls as HBV cases, if we discover that the distributions for HBV and HIV cases do differ, there will still be an adequate number of controls to match to HBV cases for geography, population group, and age.

Objective 3

Prospective Cohort: Approximately 50 persons with very recent HIV infection (HIV test results of NAT positive, antibody negative – these are termed NAT yield or NAT only cases) and up to 20 elite controllers identified during this study (mid-2014 – mid-2017) will also be eligible for enrollment in a six month prospective longitudinal study with samples collected for peripheral blood mononuclear

cells (PBMC) and other blood samples as soon as possible after the index donation followed by additional specimen collection at 2, 3 and 6 months following blood donation.

Inclusion Criteria

For Objective 1a cases: Allogeneic whole blood donors who test positive for incident HIV infection (per NAT or LAg Avidity Assay) at a participating SANBS blood center during the period of enrollment.
For Objective 1b cases: Allogeneic whole blood donors who test positive for incident HBV infection at a participating SANBS blood center during the period of enrollment.

For Objective 1a and 1b controls: donors who successfully donated and are negative for all infections for which SANBS screens (HIV, HBV, HCV, and syphilis).

All participants must be able and willing to provide informed consent and complete the ACASI in the study language, English. South Africa has eleven official languages. However, producing the ACASI in multiple African languages is not feasible for this study. English is the language that is most dominant in the media and government in South Africa, and is commonly understood in the urban areas where SANBS operates.

Exclusion Criteria

The following will be excluded from participation: (1) autologous and directed donors; these persons inherently have an altered risk profile that is different from allogeneic donors and consequently not generalizable; (2) deferred donors; (3) donors less than age 18, prisoners, or subjects otherwise unable to provide informed consent; (4) donors from whom insufficient volume of blood was collected to complete their virology testing, i.e. failed phlebotomies; and (5) donors with any other condition that, in the opinion of the Investigator of Record or his/ her designees, would preclude informed consent, make study participation unsafe, or otherwise interfere with achieving the study objectives.

Study Enrollment or Specimen Procurement

SANBS operations are divided into 7 zones, covering 8 of the 9 provinces of South Africa. (Western Cape Province is not included in this study.) Each zone has a Medical Liaison Officer (MLO) who has the primary responsibility for notifying, counseling and managing donors who test positive for either HIV or HBV antibodies with or without being NAT positive for the same infection, as well as donors who are serology-negative but NAT-positive (NAT yields) for each infection. Each unit of blood collected by SANBS is tested for HIV, HBV, Hepatitis C Virus (HCV) and syphilis. Donation testing results influence whether standard operational procedures for donor recall and retesting or study protocol procedures will be used. Laboratory testing is performed at two testing centers which are located in Constantia Kloof, Gauteng (Johannesburg area) and Pine Town in KwaZulu-Natal (Durban area). Serological assays for anti-HIV, anti-HCV and HBsAg using Abbott Prism are performed simultaneously with nucleic acid testing (NAT) using Ultrio Plus in individual donation (ID) testing format. Donations found repeat reactive by serology but NAT non-reactive or NAT non-repeatable reactive are confirmed using either an HIV Western Blot for HIV or an HBsAg neutralization assay for HBV. If these confirmatory tests are reactive then the donor is classified as a potential serology yield (possible elite controller in the case of HIV infection).

Objective 1a:

Donations found negative by serology but reactive for HIV by repeat NAT testing are classified as a potential "NAT yield" or incident case and are eligible to be approached for enrollment. Confirmation of NAT yield status is achieved by assessing the following for HIV: seroconversion in a follow-up sample, detectable viral load, p24 antigen positive result or repeat reactivity on plasma from the fresh frozen plasma unit from the index donation for HIV RNA. In addition donations which are both serology positive and NAT repeat reactive will be tested using the LAg Avidity assay which will also allow us to determine if the donor recently seroconverted or not. If the LAg Avidity assay results show that seroconversion occurred within the last 6 months, the donor will be eligible to be included as an HIV incident case.

Objective 1b:

Donations found negative by serology but reactive for HBV by repeat NAT testing are classified as potential "NAT yield" or incident cases and are eligible to be approached for enrollment. Confirmation of NAT yield status is achieved by finding HBV DNA by NAT in a follow up sample in a donor whose index sample is negative for anti-HBc. Viral load or repeat reactivity on plasma from the fresh frozen plasma unit from the index donation for HBV DNA will also be used to confirm initial laboratory findings. Donors who are HBV NAT and HBsAg concordantly positive are also eligible if they test anti-HBc negative.

Each positive donation is entered into a centralized database maintained at SANBS headquarters in Johannesburg (Constantia Kloof). Information pertaining to viral positive donations is forwarded to the regional Medical Liaison Officer (MLO) on a weekly basis through an automated report generated from the database. Basic demographic information for all recently acquired HIV and HBV infections identified at the point of index donation will be available to the study team so that we may evaluate the potential for non-response bias among those who are enrolled vs. not enrolled among those eligible.

We will leverage existing standard operation procedures at SANBS. Per procedures, the MLO, in cooperation with the research nurses who will be employed specifically for this study, will coordinate recall of the donors to the blood center for follow-up counseling and testing. Initial contact with donors who have positive HIV or HBV test results consistent with recently acquired infection will be made as soon as possible after receiving the lab results reports. All NAT yield donors will, wherever possible, be contacted by a staff member who speaks the donor's home language as the initial contact will be via telephone. During this telephone call, an appointment will be made for the donor to return for follow-up testing at a specific date, time and venue. If the donor cannot, after repeated attempts, be contacted via telephone, a first letter and second text message will be sent to the donor. If the donor were to respond to the letter or text message, an appointment will be confirmed for that donor. Letters confirming the date, time and venue of the appointment will be sent to each donor and will be followed by a text message the day before the appointment. Where donors refuse to return to the blood center for follow-up, they are strongly urged to go to their preferred doctor or health care clinic to which the initial results will be made available. Study staff will systematically document all contact attempt methods, dates, and outcomes for all individuals with positive test results in a Contact Log.

When potential study participants return to the SANBS clinic for follow-up testing, collection of the follow-up samples for repeat/confirmatory testing will occur and enrollment onto the study will be

managed by a combination of the MLO and the research nurses. (The extent of the research questionnaire and the availability of laptops will preclude the use of SANBS collections staff to perform this function.) Each donor will receive counseling.

The tests that will be performed as part of this study depend on SANBS standard testing procedures and results from the index donation testing. Based on whether a donor tests serology-only positive, concordant-serology and NAT, or NAT-only positive, different tests will be conducted on both the index donation residual specimen volumes that remain available and additional specimens that are collected at the time of returning to the blood center.

Enrollment Procedures for Donors with ID NAT Yield Results

Specimens from donors with ID NAT yield results (potential incident cases) will be tested at either of the two donation testing centers and the results entered into the centralized SANBS database. Donors will be recruited into the study at the time of return to the blood center for notification. In addition to the standard tubes collected for confirmation of infection (described below) one additional 9 mL sample will be collected. Blood samples totaling no more than 48 mL will be collected. Once the samples have been collected, the donors will be approached for study participation. Those interested will undergo a written informed consent process with study staff. Participants who provide consent will be classified as "enrolled" into the study, and will complete the ACASI research questionnaire. Participants whose confirmatory lab results are positive for either incident HIV or incident HBV will be allocated as cases. Participants whose infections are subsequently not confirmed will be excluded from analysis.
If it is not possible to make follow-up notification appointments with the donors at the time of collecting the samples for infection confirmation, follow-up appointments will be made as soon as the results become available. A similar process will be followed as with the initial letter and telephone contact of the donor as shown in Figures 1 and 2 of the Protocol (Attachment 4).

Enrollment Procedures for Donors with Incident HIV Infection Identified Through Lag Avidity Assay (serology and NAT concordant positive, with LAg-Avidity results)

LAg avidity testing will be performed in real time along with routine SANBS supplementary testing and the test results will be available at the time of notification of positive test results. All donors with concordant results, both LAg avidity negative and positive, will initially be counseled in accordance with the SANBS standard procedures which includes the performance of an HIV rapid test when the donor returns for counseling.

The donors with incident HIV infection identified through the LAg Avidity assay will be assessed for suitability to be enrolled on to the study. If the donor satisfies the inclusion criteria for this study, consent will be sought and the donor enrolled. Blood samples will be collected and the ACASI questionnaire completed.

All other HIV positive donors (serology only and/or non-recent concordant infections) will complete the standard SANBS HIV risk inventory survey as part of the standard SANBS operational procedure.

Control Recruitment and Enrollment

SANBS collection sites are administratively organized in the following hierarchical levels: zones, branches, and then clinic sites (mobile or fixed). To meet the study criterion of matching by geographic area, controls will be selected from clinics within the branches from where persons with recently acquired HIV infection have been identified. To minimize selection bias, clinics within each branch will be randomly selected by the study statisticians, from among a subset of clinics likely to have appropriate controls based on historical data (additional detail below). On recruitment days at the randomly selected clinics, individuals that are demographically matched (age within 5 years, and by population group) will be selected using systematic sampling and approached for enrollment into the study as a "potential control". Our study documents and consents indicate the "potential control" designation, and explain the meaning of this term. Following successful donation the potential controls will be asked to complete the ACASI study questionnaire. After each donation has been tested and if the donor tests negative for all infections, the person will be reclassified in the database from "potential control" to "(actual) control".

The characteristics of individuals with recent HIV and HBV infection (per NAT-only over a 5 year period) are substantially similar for the three matching variables for the study cases and controls (see Table 3). As such, we intend to use a subset of the controls matched for the HIV portion of the study to serve as controls for the HBV objective.

Table 3. Demographic characteristics of HIV NAT only and HBV NAT only positive donors at SANBS for the years 2006 – 2010.

Demographic Characteristic		HIV (n=300)		HBV (n=248)	
		Number	Percent	Number	Percent
Gender	Female	174	58.0	108	43.5
	Male	126	42.0	139	56.0
	Missing	0	0.0	1	0.4
Population Group	Asian	6	2.0	12	4.8
	Black	251	83.7	161	64.9
	Coloured	12	4.0	10	4.0
	White	29	9.7	63	25.4
	Missing	2	0.7	0	0.0
Age Group	16-19 years	49	16.3	43	17.3
	20-25	90	30.0	69	27.8
	26-30	59	19.7	43	17.3
	31-40	60	20.0	52	21.0
	41-50	32	10.7	24	9.7
	51-60	10	3.3	10	4.0
	61-70	0	0.0	7	2.8
SANBS Region	Eastern Cape	24	8.0	18	7.3
	Egoli (Johannesburg)	43	14.3	34	13.7
	Free State/Northern Cape	29	9.7	28	11.3
	KwaZulu Natal (Durban)	83	27.7	60	24.2
	Mpumalanga	35	11.7	30	12.1
	Northern (Pretoria)	52	17.3	31	12.5
	Vaal	34	11.3	47	19.0

There are two reasons why we believe that the controls for the HIV cases can fulfill the dual purpose of serving as controls for the HBV cases. First, we expect the distribution of HBV cases to be similar to the distribution of HIV cases for geography, population group, and age. Second, because there will be an excess of controls in the database relative to HBV cases (a ratio of 6:1), even if the characteristics of specific HBV and HIV cases do differ, there should still be an adequate number of controls to match on geography, population group, and age for each HBV case and thereby achieve a HBV case-control ratio of 1:3 which is the same ratio as for the HIV study. The study team will be critically reviewing enrollment on a regular basis, will monitor the accurate distribution of controls for both HIV and HBV, and make modifications as needed.

To determine the subset of clinics from which controls will be recruited, we will use LAg Avidity assay results already available in the SANBS database for the period 2010-2012 to identify the clinics where donors with recently acquired HIV infection have been identified, and include those clinics in the randomization scheme from which control donors will be recruited. The selected clinics will be determined in advance based on SANBS blood drive scheduling and included in the study management system so that research staff can plan for recruitment of potential controls on given days at specific venues. Using systematic sampling, we will randomly select donors presenting at a clinic who frequency match the age (within 5 years) and population group demographics of expected cases. If we are unable to recruit 3 controls from a given clinic we will seek to recruit the controls from other randomly selected clinics within the same SANBS branch. In addition to the number or proportion of HIV incident cases at a clinic, particular clinics may not be appropriate for control recruitment due to operational reasons (e.g., a very short period when a mobile clinic may be in operation or where there is no place at the collection site with a set up such that the donor can complete the ACASI in private). Research staff may have to make this determination based on visiting the actual collection venue or using their existing knowledge of particularly challenging venues for research study activities.

Every three months during the first two years of subject enrollment we will evaluate how well our controls match our enrolled cases with respect to the three key characteristics of geographic area, age, and population group. If the use of historical recently HIV-infected donors is not leading to correct frequency matching for controls, we will shift the targeted control recruitment to an approach which will rely either solely on the characteristics of the HIV cases enrolled in this study or on a combination of historical data and data on enrolled cases.

Objective 3 Prospective Cohort

From the group of donors identified as having incident and “elite controller” HIV infections, a smaller convenience group of willing participants will be enrolled into Objective 3 of the study. Enrollment will take place at the time these donors attend the post-test HIV counseling visit, or, if the donors are assessed as not ready for enrollment at that time, shortly thereafter.

Donors who agree to participate in the study will be fully consented as to the requirements of Objective 3 of the study. Donors who consent will then be enrolled on to the study and additional study-specific specimens will be collected to obtain CD4, viral load and cytokine profiling as well as PBMC preparations. Following sample collection, the participant will complete the ACASI questionnaire for Aim 1 of this study. In addition, at each follow-up visit a short paper questionnaire will be completed just after collection of the blood samples, using scannable forms to allow for electronic data capture. The content of this questionnaire will assess healthcare-seeking behavior and any medicines or natural remedies the study participant has taken or initiated since the last study visit,

possible sides effects of these medicines and other exposures that may influence the viral dynamics of early HIV infection. Women will also be asked if they are currently pregnant.

Clear information will be provided to participants on required visit schedules for the cohort study, including the enrollment visit expected to occur as late as approximately 1 month after the index donation visit and repeat sampling at 3 follow-up visits at 2, 3, and 6 months following the index blood donation. The importance of adherence to the visit schedule will be emphasized, and full contact details recorded for each participant. Follow-up contact of these donors will be centrally coordinated and each MLO notified when a donor in the prospective cohort must be contacted to return for follow-up samples. Donors will be contacted via telephone. During this telephone call, an appointment will be made for the donor to return for follow-up testing at a specific date, time and venue. If the donor cannot, after repeated attempts, be contacted via telephone, a letter and text message will be sent to the donor. If the donor were to respond to the letter or text message, an appointment will be confirmed for that donor. Letters confirming the date, time and venue of the appointment will be sent to each donor and will be followed by a text message the day before the appointment. All successful and unsuccessful contact attempts with the participant will be documented in a Contact Log. At scheduled follow-up visits, participant samples will be collected, labeled and sent to the Donation Testing Laboratory in Constantia Kloof.

Every year SANBS identifies approximately 50 NAT-only HIV infections. The number of elite controllers identified each year has been increasing (Table 4). The planned enrollment period is 3 years for Objectives 1 and 2. We project that the proportion of donors who are elite controllers in 2014 -2017 will be similar to that observed in recent years. We further assume a 50% enrollment rate for both groups. For Objective 3, we have an enrollment target of 50 HIV NAT-only infections and 20 elite controllers during the first 2.5 years of the study, allowing for a tail-end follow-up of 6 additional months of return visits during the same time as the Objective 1 and 2 enrollment takes place.

Table 4. Number of elite controller HIV infections identified each year by SANBS while ID-NAT for HIV has been in use.

Year	**Elite Controllers**
2007	2
2008	11
2009	8
2010	9
2011	13
2012	21
2013 (January – June only)	11
Total	**75**

Modification to Current SANBS Notification Procedures:

All donors with NAT-only infections (HIV, HCV, or HBV) are contacted by letter and telephone to request return to SANBS for additional confirmation of infection. Donors are not informed of the testing results in the letter or during the phone call. Examples of standard notification letters are included (Appendix 1 in Attachment 4). Currently, all NAT yields are called back to SANBS for follow-up sample collection. SANBS staff does not discuss which virus is suspected based on the NAT yield result. Counseling is done following the testing of the additional samples. A similar letter and

approach will be developed and used for persons who are determined to be recently infected based on serology results (i.e, LAg Avidity testing for HIV and anti-Hepatitis B core testing for HBV).

Currently, donors who test concordant NAT and serology positive for HBV are contacted by telephone if possible and notified of their results with a follow-up letter of the results sent by mail. During the telephone conversation as well as in the letter, donors are advised to visit their doctors or local clinic.

Every effort is made to get HIV positive donors back to SANBS for MLO's to do full HIV notification and counseling. Part of this process includes referral to their doctors and/or clinics for further investigation and management. Donors are provided a letter detailing their results which they are urged to take to their doctors.

All NAT yields (HIV, HCV, or HBV) are called back to SANBS for follow-up sample collection. SANBS staff does not discuss which virus is suspected based on the NAT yield result. Counseling is done following the testing of the additional samples. The primary reason for this is the relatively high numbers of false positives among the supposed NAT yields. Infection status must be confirmed before notification.

In order to support the objectives of this study, SANBS will make changes to the following donor notification procedures:

1. SANBS will seek to inform and counsel all early HBV infections (NAT positive, and anti-HBc core negative donors) using similar procedures to those used for new HIV infections.
2. SANBS will inform and counsel all of the NAT yields (HIV & HBV). The standard notification letter will be changed to stress that the donor may have an early or recently acquired infection. Given the range of testing that is performed on the index donation specimens, we do not expect any notifications to be false positive notifications (See Tables 1 and 2 for standard SANBS index testing conducted).
3. All confirmed cases will be urged to seek medical care that conforms to South Africa national treatment guidelines for each infection.

ACASI

To minimize any potential community rumors or individual anxieties linking the research to HIV status, recruitment scripts and study informational materials will emphasize that both HIV-positive, HBV-positive, and HIV and HBV-negative participants are being enrolled in order to identify and compare prevailing risk factors among the positive and negative groups. All donors will complete the same ACASI questionnaire.

B.2. Procedure for the Collection of Information

Recruitment and consent: Recruitment will occur during the initial SANBS results notification visit, and/or at the final notification visit. Participants will also be given the opportunity to complete the study procedures including the risk factor interview (ACASI) at another time. The study will be conducted in accordance with approval of IRBs at the University of California, San Francisco, SANBS, and RTI (the data coordinating center). All measures will be implemented to protect privacy and confidentiality including using subject ID numbers instead of names on all study questionnaires and blood sample labels, maintaining a secure database, and limiting the number of research staff with

access to patient identifiers needed for contact.

Risk Factor Questionnaire: A new self-administered computer-based questionnaire for the South Africa setting has been developed in English from a template previously used in Brazil for the REDS-II study and currently being used in Brazil for HIV case surveillance in donors as part of REDS-III. It assesses risk exposure over two time intervals: ever and within the 6 months preceding the index donation. Supporting Statement A (SSA) provides content and justification for the risk factor questionnaire. The interview will be an audio computer-assisted structured interview (ACASI) as done successfully in a previous REDS-II and current REDS-III study in Brazil. Participants will be able to read and hear the questions using ear phones for privacy. Interviews will be conducted using portable laptops so as to provide ease of access to the study participants. The ACASI interview will be conducted in a private location, ideally at a SANBS blood center, but we will allow the interview to be conducted at the study subjects' location of preference, such as at home. We will use 10 laptop computers (two for each of the 5 selected study zones) which will permit simultaneous interviews at different locations throughout SANBS. It will take approximately 30-45 minutes to complete the questionnaire.

The ACASI instrument will be developed in English only. South Africa has eleven official languages. However, producing the ACASI in multiple African languages is not possible. This study is unable to use multiple additional languages because the language that would be appropriate as a second study language varies in different parts of the country and also is based on heritage. At minimum if multiple languages were to be used, we would require at least 3 more; Afrikaans, Zulu, and Xhosa, but still this might not meet the needs of all participants. In addition the cost and complexity of creating an ACASI application for more than one language is impossible to overcome. Please note that one of the questions on the interview captures information on the respondent’s home language, so we will have a basic understanding of the first language of all cases and controls. All persons who are schooled in South Africa are required to learn English. It represents a common denominator language, making it an appropriate language for this study. Furthermore, English is the language that is most dominant in the media and government in South Africa, and commonly understood in the urban areas where SANBS operates.

Objective 3 – Brief Clinical Questionnaire

A new instrument has been developed specifically for this study that will allow us to collect information relevant to the prospective cohort objectives. The questionnaire will be administered using paper at each study visit. It is an electronically scannable form to permit data capture without requiring keypunch entry. At each visit the questionnaire will take less than 10 minutes to complete. The questionnaire will have only study ID numbers on it – no personal identifying information will be collected.

All of the donors who participate in the study will be nominally compensated for travel expenses and inconvenience of study participation for each study visit in Objectives 1, 2 and 3. The reimbursement amount will be approved in accordance with the local South African IRB, and will represent an amount in local currency equivalent to approximately USD$10.

Procedures: The subject will complete the audio self-administered computer based questionnaire on HIV and/or HBV risk behaviors. There is risk of loss of confidentiality regarding HIV and HBV status and risk behaviors, minor risk of discomfort and bruising related to phlebotomy, and a risk of

psychological stress due to disclosure of some laboratory results (e.g. drug resistance results, results indicating acute infection) for participants in Objective 1a. Similarly, for the Objective 3 prospective study there may be minor risk of psychological stress from additional laboratory testing. Results that might have an impact on clinical care and treatment will be made available to study participants. Interview data will be backed-up from the laptops onto a server or dedicated desktop computer at SANBS central offices on a daily basis, and uploaded weekly via a secure FTP site to the Data Coordinating Center (Research Triangle Institute, US).

Women and minorities: Within the South African context the majority population is Black and one of the minority populations is White. Enrollment of controls will be stratified to cases on the basis of race (population group). This is necessary for the scientific design of the study because HIV prevalence and incidence is significantly higher in the Black population, and socioeconomic and behavioral factors differ significantly between the White and Black populations in South Africa. In order to obtain an accurate assessment of behavioral risks, we have decided that stratified matching on race is essential. We expect overall enrollment into the study for both cases and controls to contain a much higher percentage of Black and non-White population groups when compared to the SANBS donor population, because of projected HIV and HBV infection rates. No restrictions based on gender will be used; we expect women to be represented in the recent infection cases at or above their proportion in the donor base due to higher incidence of HIV in women compared to men in South Africa. For HBV we are unsure of whether women will be over-represented as cases.

Children: Children under the age of 18 will not be enrolled.

Collection of index donation and routine donation testing specimens: During the routine collection of a blood donation at SANBS, 3 specimens are taken from a sample pouch attached to the blood bag. One 9mL EDTA and two 6mL EDTA specimen tubes are collected and dispatched to the 2 Donation Testing sites. The 9mL specimen tube is used to perform the Novartis Ultrio Plus ID-NAT, one 6mL specimen tube is used to perform the Abbott Prism anti-HIV, HCV and HBsAg assays and one 6mL specimen tube is used to perform the ABO and Rh blood grouping, Treponema palidum haemagluttination assay (TPHA) and atypical antibody screening tests.

Samples for confirmatory and research testing. For the purpose of this study, at each study visit for cases whether for objectives 1 and 2 or objective 3, a total of no more than 48 mL of whole blood will be collected for use as part of this project. Depending on the type of infection, the case participant will have some of the blood samples already collected because of standard procedures whereas for other participants the entire blood sample will need to be collected based on participation in the protocol. Blood samples for follow-up testing will be collected in accordance with SANBS standard operating procedures, which place emphasis on identification of the donor and correct labeling of samples to avoid any sample mix-up. Wherever possible, follow-up appointments will be made for the donor to return for the subsequent visit results at the time of collecting the index enrollment samples. Samples will be labeled, packed and transported according to SANBS standard operating procedures which comply with all local requirements for the transportation of bio-hazardous material. These specimens will be tested at the two donation testing centers and the results entered in to the centralized database.

Follow up samples collected from all HIV or HBV cases that return to the blood center: At the time that a blood donor is counseled by the MLOs a further set of specimen tubes will be collected and sent to the Donation Testing department:
1 x 9mL EDTA specimen tubes

3 x 9mL ACD/EDTA specimen tube
1 x 6mL EDTA/ACD specimen tube
1 x 6mL serum activator tube

The same sample collection will be obtained at each study visit for Objective 3.

B.2.1 Data Analysis

Sample Size and Power

The study is interested in understanding the range of relatively common to less common risk factors for newly acquired infections in blood donors in South Africa. Sample size and power calculations for this study cannot be based on the assumption of independent donor sample selection. We will be randomly selecting blood donation clinics where we anticipate HIV incident donors will donate based on historical data, and will then select the controls. Consequently, the sampling and analytic approaches account for clustering at the clinic level. Computer simulations (Monte Carlo simulation) of the study population were run to determine the odds ratios we can detect based on assumptions of the exposure prevalence in the donor populations and the expected number of HIV and HBV cases that we will be able to enroll in the study during a 3-year period. There were several factors examined in the Monte Carlo simulation, i.e., the expected number of cases, the expected number of controls per case, the correlation between proportion exposed for the cases and controls, the proportion exposed in the control group, and the difference in proportions between the controls and cases. Thus cross-classification of all of these factors created multiple experimental groups and 1,000 Monte Carlo simulations were run for each group.

HIV

For HIV we focused on multiple sexual partners (more than one partner at the same time, or at least two partners in the previous year) as a primary risk factor in South Africa, and estimated the prevalence of multiple sexual partners in the control and case populations. We assumed a 10% difference in the prevalence of multiple sexual partners between cases and controls. Depending on the prevalence of multiple sexual partners in the control population the odds ratio that we will be able to detect with 80% or higher power is between 1.65 and 1.94 for a study with 300 cases and 900 controls (Table below). In the Monte Carlo simulation, estimates for odds ratios were similar regardless of the ratio of cases to controls. That is, a 1:1 ratio would allow for similar odds ratios to be detected, assuming that the selection of controls from the same geographic regions as cases will be consistent with the assumptions of the underlying correlations that were defined for the Monte Carlo simulation power analysis. However, there is substantial uncertainty around how similar the behavioral exposures of cases and controls will be between different clusters (clinics) and it is not expected the distribution of all behaviors will conform to the assumptions made for the Monte Carlo power analysis. Risks other than multiple sexual partners will have different prevalence in the donor population and may have smaller differences between cases and controls within each clinic. For these reasons, the inclusion of a larger number of controls per case is necessary in order to ensure the study meets its primary objectives, such that we can detect and report findings across a range of behavioral exposures. We are planning to enroll cases and controls in a ratio of 1:3.

Each year approximately 50 HIV NAT-only (anti-HIV negative) infections are identified in SANBS donors. Using LAg Avidity testing strategies an additional ~160 HIV-positive donations per year can

be classified as recently acquired infections. With a likely participation proportion in the study estimated to be approximately 50%, it will take 3 years to enroll the target number of 300 recently acquired HIV cases in this study.. We will not be able to enroll more cases and so to slightly enhance power we will increase the number of controls in a ratio of 1:3 as described above.

Sample size and achieved odds ratios assuming 80% power for incident HIV infections.

Behavioral Exposure Proportion		**Detectable Odds Ratio**	**Case:Control = 1:3**		
Control	**Case**		**Case**	**Control**	**Total**
0.15	0.25	1.94	300	900	1,200
0.20	0.30	1.74	300	900	1,200
0.25	0.35	1.65	300	900	1,200

HBV

For HBV infections we considered scarification prevalence in rural South Africa and similar body modification such as tattoo and body piercing from a more recent study in Nigeria. These two papers reflect divergent proportions of scarification in HBV negative individuals from as high as 90% in the older paper from South Africa to as low as 3% in the more recent paper from Nigeria. Because we anticipate that the prevalence of body scarification in the population we wish to include in this study will be somewhere between these values, we chose an approximate midpoint for the purpose of sample size estimation as the prevalence of scarification and other body modification in the HBV negative population.

A similar computer simulation approach as that used for HIV was used to estimate the detectable odds ratios for behavioral exposures associated with incident HBV (Table below). Our enrollment projections suggest that over a 3-year period we will be able to enroll approximately 150 recently acquired HBV infections. With the ratio of cases to controls of 1:3 we will have sufficient power to be able to estimate the odds of HBV in people with scarification and similar body modification between 1.87 and 1.96 depending on the prevalence of scarification and similar body modification in the control population.

Previously SANBS has reported approximately 40-45 true HBV ID-NAT yields per year. In addition, in preliminary anti-HBc testing conducted over a 6 month period in early 2012, 29 donors were HBsAg-positive, anti-HBc-negative, giving an estimated annual number of expected HBsAg-positive, anti-HBc-negative subjects of ~60. Taken together, these numbers suggest that at best we might have ~100 recently acquired HBV infections per year that we could include in the study. With a likely participation proportion estimated to be approximately 50%, it should take 3 years to accrue the target number of 150 recently acquired HBV cases in this study. As described and scientifically justified above, the controls identified for the HIV case comparison will be used for the HBV comparison.

Sample size and achieved odds ratios assuming 80% power for incident HBV infections.

Behavioral Exposure Proportion		Detectable Odds Ratio	Case:Control = 1:3		
Control	**Case**		**Case**	**Control**	**Total**
0.45	0.60	1.87	150	450	600
0.50	0.65	1.90	150	450	600
0.55	0.70	1.96	150	450	600

Statistical Analysis

Data analysis will be conducted in four phases: exploratory, adjustment for missing data, descriptive, and modeling. The first two phases, exploratory data analysis and adjustment for missing data, will be conducted independently of the analyses for the specific objectives. The second two phases, descriptive data analysis and modeling, will be specifically tailored for each objective.

The exploratory data analysis phase will investigate the information provided in the data sets to determine: type of variable, valid values, level of missingness, and, if applicable, coding schemes. In addition, if there is any information available about relationships among variables, these relationships will be checked for logical consistency. Any data anomalies or logical inconsistencies identified during this phase of the analysis will be resolved. This will provide a relatively clean data set for the following phases of the data analysis.

The adjustment for missing data phase will minimize the potential bias from missing data. Virtually all data collection efforts experience the challenge of missing data. Typically, the missing data are not missing completely at random. Consequently, failure to account for the missing data, e.g., available or complete case analysis, can potentially lead to bias in the estimates produced. To investigate the potential for bias from the nonresponse, we will conduct a nonresponse bias analysis. Given the results of this analysis, which usually indicates that there is the potential for bias, we will use weight adjustments and/or multiple imputation to account for the missing values, and, therefore, minimize the potential bias.

The purpose of objective 1a is to identify the demographic and behavioral risk factors associated with incident HIV infection among blood donors in South Africa previously identified as “NAT-yield” and other recently acquired infection based on LAg Avidity testing as compared to controls. The outcome variable is whether or not a blood donor has HIV infection, which is a binomial variable. The predictor variables believed to be associated with HIV in blood donors will include recent change in sexual partner, older male/younger female sexual partnership, a greater number of recent sexual partners, unprotected receptive anal intercourse, and lower socioeconomic status. Other possible predictor variables, e.g., behavioral variables, will be examined to understand their relationship to whether or not the blood donor has HIV infection. The association between the outcome variable and a predictor variable will be examined using categorical data analysis techniques. Bivariate analysis will be conducted using Chi-square tests. Analyses that include more than one predictor variable will be

conducted using multiple logistic regression, which may include interaction terms. In addition, nonparametric modeling will be used when there are multiple predictor variables.

The purpose of objective 1b is to identify the demographic and behavioral risk factors associated with incident HBV infection among blood donors in South Africa previously identified as "NAT-yields" and which are anti-HBc negative. The outcome variable is whether or not a blood donor has HBV infection, which is a binomial variable. The predictor variables believed to be associated with HIV in blood donors will include greater number of recent sexual partners, recent scarification/tattoo/body markings, and history of personal contact with an HBV-infected person. Other possible predictor variables, e.g., demographic variables, will be examined to understand their relationship to whether or not the blood donor has HBV infection. The association between the outcome variable and predictor variable will be examined using categorical data analysis techniques. Bivariate analysis will be conducted using Chi-square tests. Analyses that include more than one predictor variable will be conducted using multiple logistic regression, which may include interaction terms. In addition, nonparametric modeling will be used when there are multiple predictor variables.

The purpose of objective 2a is to determine HIV subtype and drug resistance profiles among HIV positive donors with recently acquired infection based on year of donation and site of collection, and examine the findings in relation to donor demographics and risk behaviors derived in objective 1a. The outcome variable is HIV subtype which is a nominal categorical variable. The predictor variables believed to be associated with HIV subtype are year of donation and site of collection. Other variables, e.g., demographic variables and predictor variables identified in objective 1a, will be examined to understand their relationship to HIV subtype. The association between the outcome variable and predictor variable will be examined using categorical data analysis techniques. Bivariate analysis will be conducted using Chi-square tests. Analyses that include more than one predictor variable will be conducted using generalized logit models, which may include interaction terms. In addition, nonparametric modeling will be used when there are multiple predictor variables.

Objective 2a will also include analyzing drug resistance. The outcome variable is whether or not a blood donor is resistant to drugs used for HIV treatment which is a binomial variable. The predictor variables will be HIV subtype, demographic variables, predictor variables identified in objective 1a, and predictor variables identified in the HIV subtype analysis in objective 2a. These predictor variables will be examined to understand their relationship to drug resistance. The association between the outcome variable and predictor variable will be examined using categorical data analysis techniques. Bivariate analysis will be conducted using Chi-square tests. Analyses that include more than one predictor variable will be conducted using multiple logistic regression, which may include interaction terms. In addition, nonparametric modeling will be used when there are multiple predictor variables.

Finally, objective 2a will examine the relationship between the proportion of donors identified with incident HIV infection containing drug resistance mutations and the proportion of HIV-positive individuals receiving anti-retroviral therapy at a population-level in South Africa using available published data on the overall South Africa population.[18,19] Given that we are able to identify sufficiently detailed population data, first, we will visually examine the relationship between the two types of proportions. Based on the visual examination and a sufficient amount of data, we will regress the proportion of donors identified with incident HIV infection containing drug resistance mutations on the proportion of HIV-positive individuals receiving anti-retroviral therapy at a population-level. Model diagnostics will determine whether or not an appropriate model of the relationship has been determined. In addition, nonparametric modeling will be used to determine if a relationship exists.

The purpose of objective 2b is to determine HBV genotype profiles among HBV positive donors with recently acquired infection based on year of donation and site of collection, and correlate findings with donor demographics and risk behaviors derived in objective 2a. The outcome variable is HBV genotype. That is, a nominal categorical variable. The predictor variables believed to be associated with HBV genotype are year of donation and site of collection. Other variables, e.g., demographic variables and predictor variables identified in objective 2a, will be examined to understand their relationship to HBV genotype. The association between the outcome variable and predictor variable will be examined using categorical data analysis techniques. Bivariate analysis will be conducted using Chi-square tests. Analyses that include more than one predictor variable will be conducted using generalized logit models, which may include interaction terms. In addition, nonparametric modeling will be used when there are multiple predictor variables.

The purpose of objective 3 is to prospectively characterize the virology, immunology and natural history of incident HIV infection among a cohort of recently infected blood donors and a small group of elite controllers in South Africa. Because of the prospective data collection, we will use longitudinal data analysis techniques that account for the clustered nature of the data. That is, there will be multiple time points for each blood donor. Possible outcome variables will represent the time course of early HIV viremia, production of antibody, host immune response, and potential suppression of HIV infection. The predictor variables will be HIV subtype, demographic variables, predictor variables identified in objective 1a, and predictor variables identified in the HIV subtype analysis in objective 2a. These analyses for this objective will be primarily descriptive but may include proportional hazards models**.**

B.3. Methods to Maximize Response Rates and Deal with Non-response

Every donor who consents to participate in the study will be asked to complete the risk behavior ACASI questionnaire. The procedures, content and expected duration of the questionnaire will be described during the consent process, so the participant will understand what to expect. The questionnaire will be administered via ACASI with a research staff or a nurse available to provide assistance and answer questions if needed. Hard copy versions of the questionnaire will be available as back-up if technological problems with the ACASI instrument occur. It is assumed that all donors participating in the study will respond to the questionnaire in full, and provision is made for participants to refuse to answer any questions that they are not comfortable answering. If there are a substantial number of incomplete ACASI questionnaires, or missing data from key variables, the study statisticians will perform sensitivity analyses to measure the potential effect of missing data on results. Based on the results of this nonresponse bias analysis, imputation would be used to address any potential nonresponse bias.

B.4. Test of Procedures

The questionnaire is based upon an instrument previously utilized and validated by the US Centers for Disease Control and Prevention (CDC) in its HIV surveillance at U.S. blood banks with modifications appropriate to the South African setting. Similar though not identical content was approved by OMB for a U.S. risk factor study of HIV, HBV, HCV, and HTLV infections in blood donors that used a paper and telephone administered instrument (OMB 0925-0630). Study enrollment for that project ended in April 2013. The South Africa ACASI questionnaire is similar to the questionnaire being used in Brazil for studies of HIV risk factors in blood donors (OMB 0925-0597). While a formal validation

study has not been conducted, the use of the ACASI format and the questions for the REDS-related Brazil studies have demonstrated that respondents are willing to complete the questionnaire, and that disclosure of private behavioral information is very good. This is known because in the similar study design from the REDS-II Brazil HIV Case Control study, over 10% of controls disclosed deferrable behaviors using the ACASI that would have made them ineligible to donate had they reported the same behaviors at the time of blood donation. These findings are consistent with a growing research literature that confirm that use of ACASI significantly increases the disclosure of private and/or stigmatizing behaviors compared to any other format available for collecting such behavioral data across a range of behavioral research disciplines.

B.5. Individuals Consulted on Statistical Aspects and Individuals Collecting and/or Analyzing Data

We have consulted biostatisticians on statistical aspects of the study design, the blood center researchers responsible for enrollment, administering questionnaires and collection of samples as well as the Coordinating Center data management staff for protocol development, study monitoring, and data management. Data analysis will be performed by the analytic staff at the Coordinating Center that includes epidemiologists and biostatisticians, with assistance and oversight provided by the REDS-III International Advisory Committee (see Attachment 3.2 for a complete list of International Advisory Committee members). The REDS-III OSMB (Attachment 3.1) will monitor the study.