Attachment 4: Study Protocol
OMB Number: TBD

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

Karin van den Berg, Marion Vermeulen, Elizabeth Montgomery, Tshilidzi Muthivhi, Rob Crookes
Charl Coleman, Solomuzi Ngcobo, Neo Moleli, Charlotte Ingram, Edward Murphy, Brian Custer

January 5, 2014

1. Concept Synopsis and Study Schema

The South African National Blood Service (SANBS) uses both Nucleic Acid Testing (NAT) and serology to screen blood donors for Human Immunodeficiency Virus (HIV) and Hepatitis-B Virus (HBV). Positive NAT precedes seroconversion by days to weeks in newly acquired HIV and HBV infections; a combined testing strategy using NAT and serology therefore, confers the ability to detect acute infection and discriminate between recent (incident) and more remotely acquired (prevalent) infection. Additional post-seroconversion techniques that exploit antibody maturation kinetics such as Limiting Antigen Avidity assay (LAg Avidity) can further assist to classify HIV seroconverters as recently acquired or prevalent infections. Hepatitis B core antibody (anti-HBc) testing of NAT only and NAT and serology concordant HBV infections allows further classification of HBV infections as recently acquired or prevalent infections. Studies suggest that the risk factors for HIV among incident infected blood donors may differ from those of prevalent infection. Similarly risk factors for recently acquired HBV may be different than for prevalent HBV infections. The demographic and behavioural risks associated with incident HIV and incident HBV infection have, as yet, not been formally assessed in South African blood donors using analytical study designs. A better understanding of these risk factors is imperative to transfusion safety; this information can be used to improve exclusion of high-risk donors through targeted questions on the donor history questionnaire, and may have critical importance for other sub-Saharan Africa countries with similar risk profiles and insufficient resources for NAT testing. Moreover, this study will identify any shift in the epidemiologic characteristics of infected donors that may have occurred in South Africa; it also imparts the ability to prospectively monitor genetic characteristics of recently acquired infections through genotyping and drug resistance profile testing. A contemporary understanding of the current risk profile for HIV and separately for HBV are critical not only to blood banking, they also directly inform broader public health initiatives in South Africa. HBV infection impacts HIV progression in co-infected individuals and vice versa. In this study the potential for identifying persons who are recently infected with both HIV and HBV also exists. Finally, the ability to identify recent HIV infections provides a unique opportunity to study the biology, host response and evolution of HIV disease at time points proximate to virus acquisition.

In order to determine risk factors for incident HIV and HBV infections (Objective 1) we will conduct a frequency matched (stratified sample) case-control study with two case groups: incident HIV infected blood donors; and incident HBV infected blood donors, both to be compared to infectious marker negative controls. Cases and controls will be accrued from a geographically diverse donor pool. We will further characterize HIV clade and drug resistance profiles and determine viral loads in all cases of incident HIV infection, and HBV genotype and viral load in all incident HBV infections (Objective 2), and follow incident and elite controller HIV infections prospectively for three additional visits at 2, 3, and 6 months following index donation (Objective 3).

2. Table of Contents

Protocol

3. Background and Significance

HIV Infection is presently the foremost public health concern in South Africa owing to a nationwide prevalence of 10.9% in persons over 2 years of age and up to 30% among pregnant women.[1] In this environment of high HIV prevalence, the South African National Blood Service (SANBS) must collect more than 800,000 units of whole blood each year from a pool of more than 350,000 donors in order to contend with the country's growing transfusion need. A number of strategies have been successfully implemented by SANBS in order to protect against HIV in the blood supply. Donor selection is central to this process and is achieved through the exclusive use of voluntary, non-remunerated donors, exclusion of high-risk donors identified through the donor history questionnaire and a product triage policy that excludes use of higher risk, first-time donations for component production. An accurate risk assessment of prospective blood donors is contingent upon contemporary knowledge of behavioural risk factors associated with recent HIV infection, since recent infection is more likely to escape detection by available laboratory tests. The selection process consequently hones in on high-risk behavioural exposure (e.g. multiple sexual partners, sexually transmitted diseases, injection drug use, sexual assault) etc. Failure to capture high-risk donors through this primary interface is in part addressed through universal laboratory screening for antibodies against HIV (HIV-1 and HIV-2) and HBV surface antigen (HBsAg) as well as HIV viral RNA and HBV viral DNA using individual donation- nucleic acid testing (ID-NAT). Although laboratory testing bolsters the safety net, it should not distract from the importance of donor selection, particularly in view of residual risk such as possible viral subtypes or very early infection that could escape laboratory capture.[2]

National surveillance of HIV risk in Africa has traditionally focused on prevalent rather than incident infection; this holds implications for prevention planning and evaluation.[3] Prevalent infection, although more amenable to measure, provides a skewed assessment of disease profile, particularly in view of expanding antiretroviral coverage that prolongs survival. Incidence surveillance reflects a more accurate representation of currently relevant risk factors given the proximity to recent infection. This has consequently been adopted effectively in the United States and other industrialized countries.[4] Unfortunately, there is a lack of HIV or HBV incidence data, particularly in resource-poor settings and specifically in sub-Saharan Africa where these diseases are prominent. Furthermore, comparative analyses of incident vs. prevalent risk factors are few. The assumption that incident risk factors parallel those of prevalent infection is not necessarily accurate. This was demonstrated in a study by McFarland et al[5] in Zimbabwe, whereby age and marital status reversed their direction of association with respect to HIV incidence vs. prevalence. This may reflect a shift in the epidemiology of HIV infection.

The use of ID-NAT by SANBS provides a unique opportunity to identify very recent HIV and HBV infections with a high degree of accuracy. Universal screening of donations by parallel testing for nucleic acids and serological assays can accurately gauge incidence given known thresholds of detection by NAT and serology. In the event that HIV is newly acquired, positivity by ID-NAT (5-10 days post-infection) precedes antibody positivity (detectable at about 21 days) by over 10 days; these so-called "NAT-yield" HIV infections refer to incident or recently acquired infections that have not yet stimulated an antibody response. Over more than 5 years (2005-2010), 165 HIV NAT-yield incident positive cases have been identified at SANBS.

Following infection by HIV or Hepatitis B virus there is a time known as the "eclipse" phase when the infection is not widely disseminated and therefore is not detectable in blood. This time period is different for each infection. Once the infection is established the risk of transmission to another individual increases as plasma viremia increases during the ramp-up phase. After progressing through this stage of infection, the presence of an infection can be determined based on the ability of available methods to identify it. Although ID-NAT testing is imperative to early detection of HIV, there remains a narrow "window period" that eludes current laboratory testing and an even longer window period for HBV infection. This underscores the importance of donor selection as the primary means to capture the high-risk donor prior to appearance of the first detectable viral marker.

In addition to detection of relatively rare NAT yield infections, a number of other methods have been used to gauge HIV incidence, each of which has both strengths and weaknesses, reflecting a balance between logistical ease vs. biological plausibility. These include prospective study, mathematical modeling[6,7], serologic testing algorithms for recent HIV seroconversion (STARHS or BED)[8-10] and laboratory testing platforms that specifically target pre-seroconversion acute infection (HIV RNA or p24 antigen).[3] Prospective evaluation is cost, labor and time intensive, as well as participant to bias; a consented cohort of HIV negative participants is informed of risk, thereby compromising true incidence estimation. Mathematical modeling, although logistically simpler than other methods, relies on both availability and quality of collected data, while interpolating gaps with statistical assumption. The post-seroconversion serological methods i.e. STARHS are particularly useful as they allow incidence estimation via cross-sectional design. STARHS are based on the understanding that following HIV seroconversion, there are described changes in the antibody levels, avidity, class/subclass type and epitope specificity that can reliably be used to backdate incidence.[11] An example of STARHS is the less-sensitive enzyme immunoassay (LS-IA) or "detuned assay" on which early/incident infections are no longer detected by conventional assays. Rapid format detuned testing provides a good indication of incident infection and is increasingly being used. In addition, there is a new Limiting Antigen (LAg) Avidity assay which was recently developed by the CDC and is now commercialized for definitive estimation of HIV incidence.[12]

Similarly for HBV, the window period for HBV ID-NAT from the time of potential infectivity that could lead to infection transmission to the ability of current technology to detect is approximately 15 days and HBV ID-NAT positivity precedes Hepatitis B surface antigen (HBsAg) positivity by approximately 17 days (pre HBsAg window period 32 days from potential infectivity) on average. If a donation tests HBV-NAT positive, HBsAg positive, and anti-HBc negative, the infection is also recently acquired (approximately between 32 and 95 days). SANBS identified 476 HBV ID-NAT yields in 5 years however over 50% were also anti-HBc positive, indicating prevalent versus recently acquired infection. Thus, there were approximately 216 true HBV ID-NAT yields in the five year period, resulting in 40-45 individuals per year that were confirmed "window period" donors. In addition SANBS detected 3414 concordant HBV-NAT positive and HBsAg positive donations of which approximately 5% (170) were anti-HBc negative in five years, giving an additional 30-35 incident infections per annum.

The significance of this study is related to its three objectives: One, determination of demographic and behavioural risk factors for incident HIV and HBV hold great promise for informing targeted prevention measures by blood banks and public health authorities. The potential for identifying persons who are recently infected with both HIV and HBV also exists. In countries where the two viruses are highly endemic the rate of co-infection can be as high as 25%.[13] In recent years, approximately 2.4 % of those SANBS blood donors with confirmed HIV or HBV infection have been co-infected. Prospective collection of incidence data allows for measurement of secular trends and the methodological approaches of behavioural and molecular surveillance we will use may also be of value to other countries with generalized HIV and HBV epidemics in Africa and Asia. Two, we will determine the viral subtype and drug resistance profile of all recently acquired HIV infections, and the genotype of recently acquired HBV infections, allowing study of viral evolution, viral fitness and pathogenicity. While we expect few HIV-HBV co-infections, persons with co-infection are known to have up to five-fold faster disease progression[13] and so efforts to understand the epidemiology of both HIV and HBV, among individuals who are co-infected within the SANBS donor population has the potential for direct impact on public health and health care services in South Africa. In addition for HIV we will be able to determine the frequency at which drug-resistant strains are being transmitted in the population which has clear implications for changing pharmacogenetics and appropriate antiretroviral medicines. Three, evaluation of donors with recently acquired HIV infection and "elite controllers" (HIV antibody positive, ID-NAT negative infections) at multiple time points will be valuable to virologists and immunologists studying the natural history of early HIV infection. These studies will be useful in identifying appropriate HIV drug therapy regimens for this context, as well as strategies for producing an effective HIV vaccine, which has eluded 30 years of HIV research.

4. Objectives

1a: To identify the demographic and behavioural risk factors associated with incident HIV infection (based on "NAT yield" or LAg Avidity testing) among blood donors in South Africa.

Hypotheses: 1) The risk factors for incident HIV in blood donors in South Africa will include a 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.

1b: To identify the demographic and behavioural risk factors associated with incident HBV infection among blood donors in South Africa identified as "NAT-yields" or concordant serology and NAT positive and anti-HBc negative.

Hypotheses: 1) The risk factors for incident HBV in blood donors in South Africa will include greater number of recent sexual partners, recent scarification/tattoo/body markings, and history of personal contact with an HBV-infected person.

2a: 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 findings with donor demographics and risk behaviors derived in Objective 1.

Hypotheses: 1) The proportion of donors identified with incident HIV infection containing drug resistance mutations will be positively related to the proportion of HIV-positive individuals receiving anti-retroviral therapy at a population-level in South Africa

2b: 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 1

Hypotheses: 1) Incident HBV infections will show differences in genotypes with genotype E increasing when compared to public health data on prevalent infections.

3: 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.

4.1 Study Population

We will conduct a case-control study of risk factors and molecular surveillance for incident HIV and HBV infections in blood donors in South Africa. Accrual, enrollment and follow-up is planned to occur during the period of 3 years (April 2014 – March 2017).

Our Two Case Groups will be: (1) 300 incident HIV infected SANBS blood donors, as established through either combined ID-NAT/serology ("NAT yield") or LAg Avidity assay testing, and (2) 150 incident HBV infected SANBS blood donors, as established through combined ID-NAT/serology ("NAT yield") and anti-HBc negative testing.

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 that (a) come from the same geographic areas as the cases (distributed across up to 5 of the 7 regional zones within SANBS) and will further be frequency matched for (b) population group (race), and (c) age. We will enroll three controls for each incident HIV case. These participants will serve as controls for HBV cases also.

Prospective Cohort: Approximately 50 persons with very recent HIV infection (NAT yield only) 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 PBMC and other specimen preparations as soon possible after the index donation followed by additional specimen collection at 2, 3 and 6 months.

4.1.1 Inclusion Criteria

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

4.1.2 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 participants 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 designee, would preclude informed consent, make study participation unsafe, complicate interpretation of study outcome data, or otherwise interfere with achieving the study objectives.

4.2 Study Enrollment or Specimen Procurement

4.2.1 Screening/Recruitment/Specimen Acquisition

SANBS operations are divided into seven zones, covering eight of the nine provinces of South Africa. Each zone has a Medical Liaison Officer (MLO) who has the primary responsibility for managing donors who test positive for either HIV antibodies or HBV antigen, as well as donors who are antibody/antigen-negative but NAT-positive (NAT yields). 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. Testing is performed at two testing centres 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 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. Donations found negative by serology but repeat reactive by NAT testing are classified as a potential NAT yield. 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 ; and the following for HBV: seroconversion to either HBsAg or anti-HBc on follow up sample, anti-HBc reactivity on current sample, detection of viral load or repeat reactivity on plasma from the fresh frozen plasma unit from the index donation. Figures 1 and 2 provide detailed flow diagrams of the planned study activities for HIV and HBV positive donors, respectively.

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 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.

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 centre 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 laboratory 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 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 centre for follow-up, they will be 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 testing 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 pre-test counseling. Wherever possible, follow-up appointments will be made for the donor to return for the final confirmatory results notification at this time.

The tests that will be performed as part of this study depend on SANBS standard testing procedures and results from the index donation. 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 centre. Tables 1 and 2 show the standard testing and REDS-III protocol testing that will be conducted.

Figure 1 Approaches for HIV-positive donor testing and study entry.

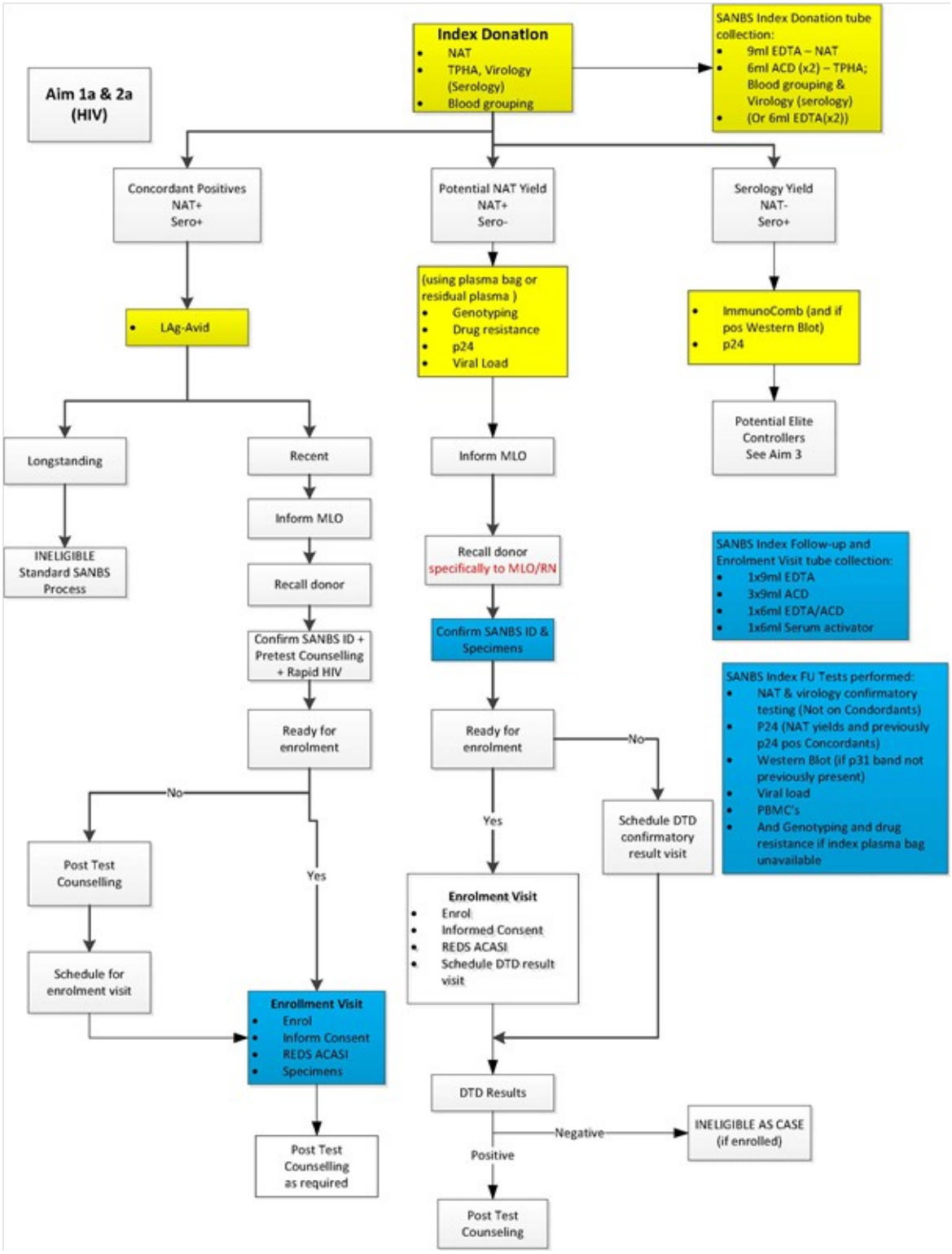


Figure 2 Approaches for HBV-positive donor testing and study entry.

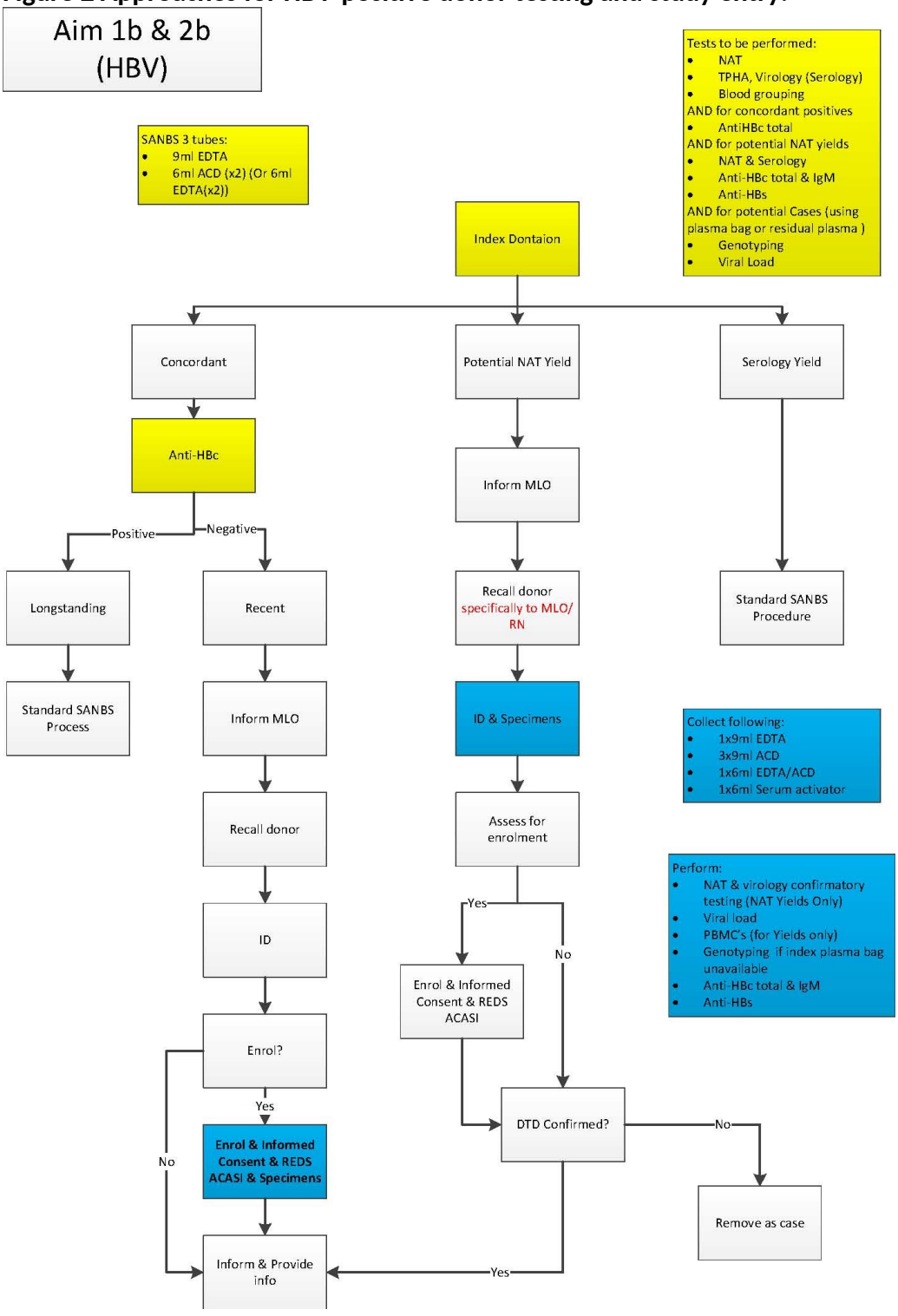


Table 1 Planned HIV testing based on index donation test results.

Index Donation Testing Result	Tests to be Conducted on Index Donation Specimens	Tests to be Conducted on Follow-up Samples	SANBS Standard Procedure Testing	REDS-III Study Protocol Testing
Serology Only Positive	Serology confirmatory (Immunocomb)	NAT	X	
	Western Blot	Serology	X	
		Western blot	X	
NAT and Serology Concordant Positive	LAg Avidity	LAg Avidity (if no index sample available)		X
	Serology confirmatory (Immunocomb)			X
	Western Blot if LAg avidity negative			X
	p24 Ag if LAg avidity negative			X
	Viral Load if LAg avidity negative			X
	Genotyping if LAg avidity negative	Genotyping (in no index sample available)		X
	Drug resistance if LAg avidity negative	Drug resistance (if no index sample available)		X
		NAT confirmatory		
		Serology confirmatory		
		Viral load		X
		Western Blot if p31 negative on previous WB		X
		p24 Ag (if p24 Ag negative on index)		X
		Rapid HIV test and counseling	X	
NAT Only Positive	NAT Confirmatory		X	
	p24 Ag		X	
	Viral Load		X	
	Genotyping	Genotyping (in no index sample available)		X
	Drug resistance	Drug resistance (if no index sample available)		X
		NAT confirmatory	X	
		Serology confirmatory	X	
		Viral load	X	
		Western Blot		X
		p24 Ag	X	

Table 2 Planned HBV testing based on index donation test results.

Index Donation Testing Result	Tests to be Conducted on Index Donation Specimens	Tests to be Conducted on Follow-up Samples	SANBS Standard Procedure Testing	REDS-III Study Protocol Testing
Serology Only Positive	Serology (confirmatory)	NAT	X	
	Anti-HBc IgM	Serology (confirmatory)	X	
	Anti-HBs	Anti-HBs	X	
	Anti-HBc Total	Anti-HBc total and IgM	X	
NAT and Serology Concordant Positive	Anti-HBc Total			X
	Viral Load if anti-HBc negative			X
	Genotyping if anti-HBc negative	Genotyping (if no index sample available)		X
		NAT confirmatory		X
		Abbott Prism HBsAg		X
		Viral load		X
		Anti-HBc IgM & Total		X
		Anti-HBs		X
NAT Only Positive	NAT Confirmatory	NAT Confirmatory	X	
	Abbott Prism HBsAg	Abbott Prism HBsAg	X	
	Viral load	Viral load	X	
	Anti-HBc IgM & Total	Anti-HBc IgM & Total	X	
	Anti-HBs	Anti-HBs	X	
	Genotyping	Genotyping (if no index sample available)		X

Procedures for donors with ID NAT yield results

Blood specimens will be tested at either of the two donation testing centres and the results entered in to the centralized database. Donors will be recruited into the study at the time of return to the blood centre for notification. In addition to the standard tubes collected for confirmation of infection (described below) one 9mL sample will be collected. Once the samples have been collected, the donors will be approached for participation and enrolled into the study. Those interested will undergo a written informed consent process with study staff. Following provision of consent, participants who agree will complete the ACASI research questionnaire. Participants whose confirmatory laboratory 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.

Procedures for donors with incident 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 performing of an HIV rapid test when the donor returns for counseling.

The donors with an 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**.**

4.2.2. Control Recruitment and Enrollment

SANBS collection sites are administratively organized into zones, branches, and clinic sites. Clinics can be fixed or mobile collection sites. 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. On recruitment days at the randomly selected clinics, individuals that are demographically matched potential controls (age within 5 years, and by population group) will be randomly 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 invited 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 as a 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 serve the dual purpose as the control 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 more HIV-controls in the database, for each HBV case 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 the HBV study within the already selected HIV study matched controls to achieve a similar case control ratio of 1:3 HBV case to controls ratio. 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.

SANBS schedules blood drives up to 6 weeks in advance. Based on previous collections and expected turnout, SANBS is able to predict with a good degree of accuracy the number of blood collections that will be obtained from a given mobile or fixed site on a specific day. We will use

this information to guide the random selection of the clinics that research staff will target on a given day for control recruitment. A list of clinics or mobile sites based on the size of the clinic or mobile site from which cases are likely to come from will be created according to each SANBS zone and branch. From this list the clinic(s) (fixed and/or mobile) will be selected for study recruitment on a given day.

Within each branch, only a subset of clinics will be randomized. To determine the clinics from which controls will be recruited, we will use LAg Avidity assay results from the REDS-III Molecular Surveillance fast track study to identify the geographic area (clinic level), age and population group characteristics of donors with recently acquired HIV. Using 3 years of previous LAg Avidity results (2010 – 2012) to define the likely characteristics of donors who will meet our HIV case definition, we can enumerate the clinics that will be included in the randomization scheme from which we will seek to recruit control donors. 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 match the age and population group demographics of expected cases based on the analysis of the persons from the LAg Avidity study. However, 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. Particular clinics may not be appropriate for control recruitment due to reasons such as 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 existing knowledge of particularly challenging venues for research study activities.

Every three months during the first two years of participant 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, we will shift the control recruitment to an approach which will rely either solely on the characteristics of the cases who enrolled in this study as the information used to determine targeted recruitment of controls, or on a combination of historical data and 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. The planned study activities for Objective 3 participants are shown in Figure 3. Enrollment will take place at the time these donors attend the post-test HIV counseling visit, or, where the donors are not assessed to be 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 specimens collected for CD4, viral load and cytokine profiling as well as PBMC preparations. 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. All samples will be sent to the Donation Testing Laboratory in Constantia Kloof, Gauteng from where it will be sent to the appropriate laboratories for additional testing as required. 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 questionnaire will be completed just after collection of the blood samples, using a Teleform-style form. 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 the visit schedule, including the enrollment visit expected to occur 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, as described above. At scheduled follow-up visits, participant samples will be collected, labeled and sent to the Donation Testing Laboratory in Constantia Kloof as detailed above.

Every year SANBS identifies approximately 50 NAT-only HIV infections. With a planned enrollment period of 3 years and an enrollment target of 50 HIV NAT-only infections for

Objective 3, and assuming 50% enrollment, we anticipate two years of accrual to meet the enrollment goal, and allow 6 months of prospective follow up.

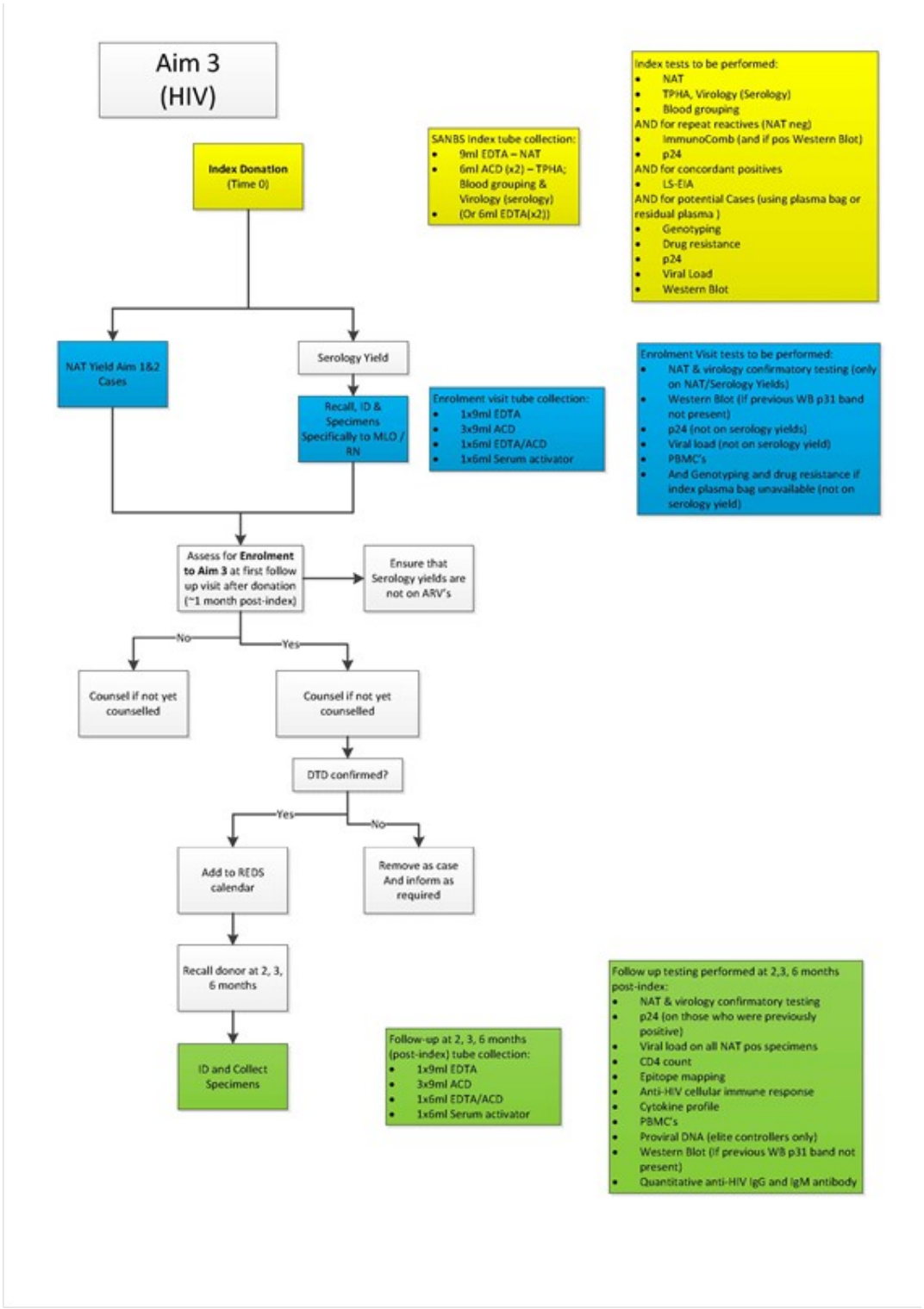
The number of elite controllers identified each year has been increasing (Table 4). Projecting that the proportion of donors who are elite controllers in 2014 -2017 will be similar to those observed in recent years and assuming a 50% enrollment rate it will be feasible to enroll 20 elite controllers during the first 2.5 years of the study, allowing for a tail-end follow-up of 6 additional months of return follow-up 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**

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.

Figure 3 Objective 3 Prospective cohort testing and study entry.



4.2.2 Participant Enrollment

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). 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 LAg Avidity testing for HIV and anti-Hepatitis B core negative 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.

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

1. SANBS will seek to inform and counsel all early HBV infections (NAT positive, HBsAg positive or negative and anti-HBc core negative donors) using similar procedures to those used for new HIV infections.
2. SANBS will seek to inform and counsel all of the NAT yields (HIV & HBV). The standard notification letter will be changed to stress that the donor has an early or recently acquired infection. Given the range of testing that is performed on the index donation specimens we expect few notifications to be false positive notifications (See Tables 1 and 2 for standard SANBS index donation testing conducted).
3. All confirmed cases will be urged to seek medical care that conforms to South Africa national treatment guidelines for each infection.

Cases Objectives 1 and 2: The prospective enrollment will build on the existing notification mechanism with additional research nurse staff; we will consent and enroll donors during the retest visit during which they will receive pre-test counseling and be informed of the likelihood of being either HIV or HBV positive (**Consent in Appendix 2**). Following the provision of written informed consent and enrollment, participants will complete the ACASI questionnaire. Although

we plan to administer the ACASI questionnaire immediately following enrollment, we will provide study participants the option of returning to enroll and complete the interview at a later date.

For recent HIV (NAT yield and LAg Avidity recent) and recent HBV (NAT yield and HBsAg positive/anti-HBc negative) infections, all eligible cases will be approached for potential enrollment into the study since we anticipate that no more that 50% of those individuals with a recent infection will return to SANBS for their results following an index donation, but that we will have high success in enrolling these individuals into the study

Controls: Controls will be identified from among donors who are negative for all infections for which SANBS screens (HIV, HBV, HCV, and syphilis), and who are stratum-matched by blood centre, age within 5 years, population group. (**Consent in Appendix 3**).

We are proposing on-site recruitment of controls, meaning recruitment of potential controls following successful whole blood donation. To enhance our ability to recruit controls that will be able to be *post-hoc* matched on geographic region, population group, and age, we will select donation sites (both fixed and mobile clinics) with donor characteristics that are similar to donors who are incident HIV-positive. Following completion of the donation, we will invite random donors at predetermined collection sites that have similar demographic characteristics to enrolled cases, to participate in the risk factor study by completing the ACASI. Following completion of testing, donors who are negative for all infections for which SANBS screens will be included in the study.

To minimize any 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.

Objective 3 Participants: Selected donors with incident HIV infections who were identified and enrolled during the first two years of Objective 1a and 2a recruitment will be prospectively enrolled into Objective 3 of the study. Participants in Objective 3 will be a subset of Objective 1a and 2a participants who meet eligibility criteria and are willing to participate in this objective. Elite controllers will include those identified in the first 2.5 recruitment years of the study. Donors will be consented and enrolled either during the post-test HIV counseling visit or shortly thereafter timed with Objective 1 enrollment and specimens collected on the day of enrollment and at 3 follow-up visits for CD4, viral load and cytokine profiling as well as PBMC preparations (**Consent in Appendix 4**). The total volume of blood collected at each follow-up visit will not exceed 48 mL.

Study Locations: The study will be located in geographic areas within the 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. We will conduct the study in 5 locations 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.

HIV

The monthly success in notifying donors who are HIV-positive has been documented. These data include both incident and prevalent infections. However, emphasis within SANBS is placed on notifying NAT-yield donors. For the first six months of 2012 cumulative total success in notification was 49% (Table 5). If notification by outside agencies, which can occur for donors who reside in remote locations, is included this proportion increases to 57%. Notification requires that the donor return to SANBS or the alternate notification testing location. This helps us to get past the first barrier to participation in our proposed study; donor return. Because approximately 50% of donors are notified and we are seeking to enroll donors at the time of notification, we believe we are likely to be able to recruit most of the donors who receive notification. However, success in notification is related to the geographic area for two reasons. First is the presence of an active MLO for the region and scale of the geographic area which the MLO has to cover. Table 6 shows that HIV prevalence in the donor population varies by region and rates of successful notification vary by month and by region. During the REDS-III study enrollment period, both a MLO and research assistant will be coordinating efforts to notify and recruit participants for this study. The proportion of successful notifications is expected to increase during the study period. We expect enrollment in our study to be close to the number of successful notifications conducted by SANBS, i.e. approximately 50% of donors with recently acquired HIV.

Table 5. Notification and counseling success rates, all SANBS regions, January-June 2012

All SANBS Zones	**Jan 2012**	**Feb 2012**	**March 2012**	**April 2012**	**May 2012**	**June 2012**	**Total**
Total HIV Positive	83	97	114	80	134	149	657
Contacted	73	90	97	68	9	143	480
Could not be reached	29	20	19	12	13	38	131
Counseled by SANBS	39	51	48	32	76	76	322
Counseled Outside SANBS	9	5	13	7	10	11	55
Percentage counseled by SANBS	47%	52%	42%	40%	57%	51%	49%

Table 6. Notification and counseling success rates, by SANBS regions, January-June 2012

	January 2012	February 2012	March 2012	April 2012	May 2012	June 2012	Totals for 6 months
KwaZulu Natal (Durban)							
Whole Blood Donations							67429
Number HIV Positive	26	33	38	10	33	32	172
Proportion HIV Positive							0.26%
Number Counseled by SANBS	19	23	22	8	27	25	124
Counseled Outside SANBS	1	1	4	0	0	1	7
Percentage counseled by SANBS	73%	70%	58%	80%	82%	78%	72%
Egoli (Johannesburg)							
Whole Blood Donations							82973
Total HIV Positive	14	29	22	22	22	30	139
Proportion HIV Positive							0.17%
Counseled by SANBS	1	4	9	10	3	5	32
Counseled Outside SANBS	4	8	6	2	10	5	35
Percentage counseled by SANBS	29%	28%	27%	9%	45%	17%	25%
Vaal							
Whole Blood Donations							57537
Total HIV Positive				21	23	19	63
Proportion HIV Positive							0.11%
Counseled by SANBS				11	17	12	40
Counseled Outside SANBS				0	0	2	2
Percentage counseled by SANBS	No MLO	No MLO	No MLO	52%	74%	63%	63%
Eastern Cape							
Whole Blood Donations							36070
Total HIV Positive	16	14	17	4	13	16	80
Proportion HIV Positive							0.22%
Counseled by SANBS	6	5	4	2	3	5	25
Counseled Outside SANBS	3	0	5	0	0	1	9
Percentage counseled by SANBS	38%	36%	24%	50%	23%	31%	31%
Free State/Northern Cape							
Whole Blood Donations							37642
Total HIV Positive	12	14	9	10	9	7	61

Proportion HIV Positive							0.16%
Counseled by SANBS	7	11	9	9	9	5	50
Counseled Outside SANBS	3	1	0	1	0	1	6
Percentage counseled by SANBS	58%	79%	100%	90%	100%	71%	82%
Mpumalanga							
Whole Blood Donations							42493
Total HIV Positive	16	20	22	13	23	24	118
Proportion HIV Positive							0.28%
Counseled by SANBS	4	3	3	7	14	13	44
Counseled Outside SANBS	2	2	2	1	1		8
Percentage counseled by SANBS	25%	15%	14%	54%	61%	54%	37%
Northern (Pretoria)							
Whole Blood Donations							79499
Total HIV Positive	23	15	30	13	18	21	120
Proportion HIV Positive							0.15%
Counseled by SANBS	7	9	13	3	9	11	52
Counseled Outside SANBS	4	1	3	1	1	1	11
Percentage counseled by SANBS	30%	60%	43%	23%	50%	52%	43%
All Locations							
Whole Blood Donations							404842
Total HIV Positive	107	125	138	93	141	149	753
Proportion HIV Positive							0.19%
Counseled by SANBS	44	55	60	50	82	76	367
Counseled Outside SANBS	17	13	20	5	12	11	78
Percentage counseled by SANBS	41%	44%	43%	54%	58%	51%	49%

HBV

To date, notifying donors with HBV NAT-yield infections has not been as successful as HIV notifications. In addition, tracking of this information is not currently routine in SANBS. Based on information obtained from the Reference Laboratory in Constantia Kloof with data covering a three month period January – March 2012, successful return and notification for donors with HBV-yield donations is significantly higher in Durban area compared to Johannesburg (Table 7). This is also an area with an appreciably higher burden of HBV infection. As with HIV, we believe we will be able to enroll nearly all persons who return for notification for recently acquired HBV infection into the study given that they will be on site for notification.

Table 7. HBV notification and counseling success rates, January – March 2012.

	JHB (Zone 1)	DBN (Zone 2)	Total
HBV	28	15	43
HBV returns	6	6	12
Return rate HBV	**21%**	**40%**	**28%**
*Untyped	6	5	11
Untyped returns	4	4	8
Total returns	10	10	20
Combined return rate	**29%**	**50%**	**37%**
*Most untyped yields that confirm are HBV therefore are included.			

4.3 Stratification or Randomization

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 to HIV-cases by geographic region, age (5-year intervals), and population group from among donors who test negative for all infections (HIV, HBV, HCV, and syphilis) at randomly selected clinics.

4.4 Measurement

4.4.1 Schedule of Measurement

Risk Factor Questionnaire: A new questionnaire for the South Africa setting has been developed in English from a template previously used in Brazil for the REDS-II study. It assesses risk exposure over two intervals; ever and within the 6 months preceding the index donation. **Appendix 7** provides content 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 centre, but we will allow the interview to be conducted at the study participants' location of preference, such as at home. We propose to use 10 laptop computers (2 per zone) which will permit simultaneous interviews at different locations throughout SANBS. It will take approximately 30-45 minutes to complete the questionnaire. 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 Centre.

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 to use varies in different parts of the country and also 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. 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. English is also the language of business in South Africa.

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 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. Descriptions of the tests used at SANBS are provided in **Appendix 5**.

Serological assays for anti-HIV, anti-HCV and HBsAg are performed in parallel with Nucleic acid testing (NAT) using Ultrio Plus in individual testing format. The serological and NAT assays used are manufactured by Abbott Diagnostics and Hologic/Novartis Diagnostics respectively. Donations found initial reactive on serology are retested in duplicate and a final screening outcome (FSO) determined as negative if both duplicate replicates are non-reactive and positive if either one of the replicates is reactive. Initial reactive NAT test results are repeated in duplicate on the Ultrio Plus assay as well as a discriminatory assay being performed for HIV

(dHIV), HBV (dHBV) and HCV (dHCV) to determine which virus is reacting. A donation that is non-reactive for all supplementary testing is classified as a Non repeatable reactive (NRR) and the unit destroyed but the donor is not notified. Any donation that has 1 or more of the supplementary tests reactive is classified as repeat reactive. If the NAT and serology are both considered repeat reactive the donor is classified as a concordant confirmed positive. If only FSO is reactive, but NAT non-reactive, an HIV Western Blot is performed for HIV and HBsAg neutralization performed for HBV. If these tests are reactive the donor is classified as a potential serology yield. Conversely If NAT is repeat reactive and the FSO is negative the donor is classified as a potential NAT yield. All potential yields are recalled to confirm the result. Concordant confirmed positives are counseled and deferred. To classify a concordant confirmed positive donation as recently acquired or longstanding, the Donation Testing department will perform a Limiting antigen (LAg) avidity assay for HIV and an anti-HBc (total) for HBV infections. All test results will be captured onto the Meditech mainframe, which is the SANBS blood establishment computer system (BECS).

Samples for confirmatory testing. 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 follow-up results at the time of collecting the follow-up samples. Additional sample collection in addition to the standard tubes collected for confirmation will require the additional collection of one 9mL sample. 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 centres and the results entered in to the centralized database.

Storage of plasma: While the routine testing is taking place the whole blood donation is processed into a red blood cell (RBC), fresh frozen plasma (FFP) and buffy coat. If any of the screening test results are positive the FFP unit is sent to the National Biorepository housed in Boksburg. If the positive donation is classified as a recent concordant positive the FFP is thawed and three 3.5mL aliquots are sampled and the remaining unit and aliquots are refrozen at -20^{o}C. One aliquot is used to confirm that the blood in the bag is positive, one aliquot is used to determine viral load using the Abbott RT PCR and p24 antigen reactivity using Immunogenetics Innotest mAB in the Virology reference laboratory and one aliquot will be sent to the National Institute of Communicable Diseases (NICD) to have the genotype and drug resistance determined.

A FFP from a donation that is classified as a potential NAT yield will be thawed and the entire product sampled into 3.5 mL aliquots and frozen at -80^{o}C. One aliquot will be sent to the Donation Testing laboratory for replicate testing to eliminate contamination of the index sample. One aliquot will be tested to determine p24 antigen reactivity using the

Immunogenetics mAB Innotest assay and viral load using the Abbott RT PCR assay. One aliquot will be sent to NICD for genotyping and drug resistance. The FFP from a donation classified as a potential serology yield will be thawed and sampled into 3.5mL aliquots and re frozen at -80^{o}C. One aliquot will be sent to Donation Testing for replicate testing to estimate viral load using Probit analysis.

Follow up samples collected from all HIV or HBV cases that return to the blood centre: 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 tube

Tests performed on recent concordant follow up samples: An initial screen will be performed on Ultrio Plus and Abbott Prism to confirm that the correct donor has presented. A viral load determination will be performed and if the plasma bag was not available at time of index donation, the tests normally performed on the index FFP will also be performed. All remaining samples will be separated and frozen for other possible studies.

Tests performed on potential HIV NAT yield follow up samples: A 9mL EDTA specimen tube will be tested on Ultrio Plus and Discriminatory HIV. If both of these tests are negative further replicates are performed to confirm the absence of virus before possible reinstating of the donor. One 6mL EDTA tube is used to test for Abbott anti-HIV to determine if seroconversion has occurred and p24 antigen testing in conducted. One 6ml EDTA tube is used to perform an Abbott RT PCR viral load. The 9mL ACD tube will have peripheral blood mononuclear cells (PBMC) isolated and stored in liquid nitrogen (LN2) for further studies. The remaining tubes will be used if the index FFP was not available to perform genotyping and drug resistance testing. All remaining specimen tubes will be separated and frozen and used for further SANBS replicate testing.

Tests performed on potential HIV serology yield follow up samples: One 9mL EDTA tube will be used to perform Ultrio Plus and dHIV. One 6mL EDTA tube will be used to perform Abbott Prism anti-HIV and western blot. A 9mL ACD sample will be used to collect PBMCs. All remaining tubes will be separated and the plasma frozen at -80^{o}C.

Tests performed on HBV potential yields: One 9mL EDTA sample is used to perform Ultrio plus and dHBV and one 6mL EDTA tube is used to perform Abbott Prism HBsAg assay and anti-HBc IgM, anti-HBc total and anti-HBs titer. One 6mL EDTA sample is used to perform the Abbott RT PCR to determine viral load. A 9mL ACD tube will have peripheral blood mononuclear cells (PBMC) isolated and stored in liquid nitrogen (LN2) for further studies. The remaining tubes will

be used if the index FFP was not available to perform genotyping and drug resistance testing. All remaining specimen tubes will be separated and frozen and used for further SANBS replicate testing.

Tests performed on HBsAg potential serology yields: One 9mL ACD sample will be used to collect PBMC. All remaining tubes will be separated and the plasma frozen at -80^{o}C.

4.4.2 Definitions

dHIV – HIV discriminatory assay
dHBV – HBV discriminatory assay
RBC – Red blood cells
FFP – Fresh frozen plasma
EDTA - Ethylenediaminetetraacetic acid
ACD – Acid citrate dextrose
PBMC – Peripheral blood mononuclear cells

4.5 Assessment and Measurement Procedures

Detection of Recent HIV Infections by LAg Testing: LAg avidity testing of concordant HIV RNA and anti-HIV positive donations collected during routine donation will be performed in the SANBS virology reference laboratory to determine whether the donor has recently acquired the infection or whether it is longstanding. LAg avidity incidence assays are focused on measurement of antibody avidity, which increases over time following seroconversion. Antibody avidity is believed to be more robust than antibody titer incidence assays because it is a functional property of maturing antibodies. Duong recently described two novel assays, including two-well avidity index EIA (AI-EIA) and single-well limiting antigen avidity EIA (LAg-Avidity EIA), to measure avidity of HIV antibodies. Key features of the one-well assay are: 1) use of rIDR-M, a multi-subtype recombinant protein that covers the immunodominant region (IDR) of gp41 of HIV-1, group M, 2) the limiting amount of antigen available to allow binding of only high-avidity antibodies, and 3) use of pH 3.0 buffer to further assist dissociation of low avidity antibodies.[12]

Detection of recent HBV infections by NAT and anti-HBc testing: Recently infected individuals will be defined by either DNA+/HBsAg-/anti-HBc- or DNA+/HBsAg+/anti-HBc-. Each index donation is screened using NAT and HBsAg and if either or both are repeatable reactive then anti-HBc testing will be performed to eliminate HBV NAT yield occult infections or HBsAg long standing infections. The potential NAT yields will be tested using anti-HBc IgM, total and anti-HBs. The concordant DNA+/HBsAg+ will be tested using anti-HBc total alone.

HIV-1 Clade Typing and Drug Resistance Testing: Subtype and resistance analysis will be performed by the South Africa HIV reference laboratory as previously described. Following RNA isolation, complementary DNA will be obtained using Superscript reverse transcriptase and random primers. A nested PCR approach will be used to obtain one fragment containing the protease gene and approximately 700 base pairs of the RT gene. In the first round the primers K1/K2 will be used followed by DP10 and F2 primers. Three other sets of primers, RT4/DP16, F1/F2 and DP10/DP11, will be used for samples that are not amplified using the initial primers. All amplification products will be analyzed on a 1% ethidium bromide-stained agarose gel and purified using QIAquick PCR purification kit. To obtain sequence results for the entire amplified segment in both strands we will use at least 6 primers for sequencing each sample, including F1, F2, DP10 and DP11 primers and a new pair of primers - GABO 1 and GABO-2. Sequence data will be obtained using the ABI Prism Big Dye Terminator Cycle Sequencing Ready Reaction kit, according to manufacturer's protocol in an automated sequencer. Following phylogenetic analysis of the pro-RT region we will identify the subtype of each recently transmitted HIV-1 strain. Sequencing of the entire HIV-1 protease gene and of the RT gene through amino acid 240 will identify all mutations known to confer resistance to protease, nucleoside and non-nucleoside RT inhibitors. The only class of approved anti-HIV-1 drug resistance not detected by this analysis will be envelope gene mutations known to confer resistance to the more recently introduced HIV-1 fusion inhibitor Fuzeon™, which are not currently available in South Africa. To detect possible PCR contamination, sequences will be compared to each other using a web interface that uses the Blast program and which highlights any pair of sequences that have a percentage of similarity higher that a specific threshold (we will use 99% for pol gene).[14]

HBV Genotype Testing: The INNO-LIPA HBV Genotyping assay (BioWeb, Innogenetics, Germany) is a line probe assay designed to identify HBV genotypes A to H. Extracted positive DNA is amplified using biotinylated PCR primers supplied in the assay. The outer primers will amplify part of domain B and C of the HBV polymerase gene, yielding an amplified target sequence of 409 bp. The nested PCR amplification yields an amplified sequence of 342 bp. After gel electrophoresis, detection of genotypes is performed using the nitrocellulose strips supplied. Amplified DNA is hybridized to specific oligonucleotide probes that are immobilized as parallel lines on strips. After hybridization, unhybridized DNA is washed from the strip; conjugate-streptavidin labelled with alkaline phosphatase is added and bound to any biotinylated hybrid. Incubation with substrate-BCIP/NBT chromogen results in a purple/brown precipitate. The reading card and interpretation chart supplied in the assay are used to interpret the lines on each strip. Internal negative and positive quality controls are included in each run. The negative control should have only the Conjugate Control line visible. All positive controls and positive samples should have the Conjugate Control line and Amplification Control lines visible. The INNO-LIPA HBV Genotyping assay is a SANAS accredited test.

Objective 3 Biological characterization and evolution of incident HIV infection: This study of incident HIV infections in South African blood donors provides a unique opportunity to

investigate the early virologic and immunologic course of HIV infection. Capturing these donors early in the course of HIV infection will allow us to prospectively monitor the time course of early HIV viremia, production of antibody, host immune response, and potential suppression of HIV infection in a small subset (elite controllers). Specifically, we propose to accomplish focused studies of dynamics of viremia and adaptive and innate immune responses based on follow-up laboratory studies of donors detected with acute HIV as well as studies of HIV Elite controllers. Because of the expected high proportion of HIV clade C infections these studies will contribute substantially to the understanding of whether clade C has a prolonged early viremic phase, and whether suppression of virus in elite controllers follows the same dynamics as for clade B. These studies are significant because differences in clade C early dynamics may account for higher infectivity in South Africa, and a better understanding of these dynamics are also relevant for clade C vaccine development.

We will obtain serum, plasma and PBMC specimens at time of enrollment and at 3 follow-up visits at 2, 3, and 6 months post-donation for CD4, viral load and cytokine profiling from the following two subsets of HIV-infected donors detected by NAT screening: i) early incident HIV infections, defined as HIV NAT+/ HIV antibody negative (N~50); and ii) HIV elite controllers defined as HIV NAT-/HIV antibody+ (N~20).

The testing of samples will be conducted in South Africa and in the US by BSRI (cell associated HIV RNA, pro-viral HIV DNA, cytokines). We will also have sufficient specimen volumes that will permit future studies and research collaborations. Consent for specimens to be stored and used for this purpose is included in the consent form (Appendix 3).

Serum, plasma and PBMC specimens obtained at enrollment and at 2, 3, and 6 month follow up will be tested as follows:

- *SANBS and collaborating South African laboratories:* HIV genotyping and drug resistance, CD4 lymphocyte counts and anti-HIV IgG and IgM antibody.
- *BSRI virology core*: quantitative cell-associated HIV RNA, pro-viral DNA, and cytokine panels. These data, together with clinical data will be used to perform Fiebig staging on all participants at each time point.

Donors enrolled in the prospective follow-up study will have additional specimens collected at 3 time points.

At each visit, 48mL of whole blood will be collected:

- 1 x 9mL EDTA specimen tubes
- 3 X 9mL ACD specimen tube for PBMC preparation
- 1 x 6mL EDTA/ACD specimen tube
- 1 x 6mL serum tube

4.5.1 Specimen collection procedures

All specimens will be collected using standardized aseptic technique, accessing the median cubital vein through the antecubital fossa. Venous blood will be collected after applying a superior tourniquet. Sufficient blood will be collected for the requirements detailed above. Specimens will be labeled, stored and transported as per the standard SANBS operating procedures. Additional study-specific specimen labels will be prepared and supplied by the Data Coordinating Centre (DCC). All disposables will be discarded in biohazardous containers as per local regulations and standard operating procedures.

4.6 Survey Considerations and OMB Requirements

An extensive risk factor interview will be completed by both cases and controls who participate in this study (**Appendix 7**). Objective 3 participants will also complete a brief clinical questionnaire at each visit (**Appendices 8 and 9**). All interview instruments will have to be approved for use by OMB. We are planning to allow for up to nine months to obtain clearance to use the instruments.

4.7 Data Management

The specifics of at least four data management systems will need to be developed by the REDS-III DCC, Research Triangle Institute:

1. Tracking system. SANBS is currently developing an operational tracking system for notification and counseling of donors with positive viral test results which will serve to identify potential participants. The specifications for that tracking system are included as **Appendix 6**. This document can serve as guide to the components that are necessary for a tracking system. In addition, a web-based Study Management System (SMS) will be designed to monitor enrollment of cases and controls, completion of questionnaires, blood sampling tasks and other required visit procedures for each enrolled participant in Objectives 1 and 2 and at follow-up visits for those enrolled in Objective 3. More specifically, this will require the development of a regular reporting system with which to closely monitor the demographic characteristics of cases to ensure the enrollment of properly matched controls at the specified ratio (1:3).

2. Laboratory database. Test result data for the additional laboratory testing mandated by this protocol and not part of usual SANBS procedure, will be entered into the SANBS main frame and uploaded via the supplementary file to the REDS-III South Africa HIV/HBV Biological Specimen Inventory (BSI) database.

3. Questionnaire database. ACASI interviews will be consolidated from laptops to a central database on a daily basis at SANBS using QDS Software, and then transferred weekly to RTI via a secure FTP server for QC and analysis.

4. Clinical questionnaires. Teleform forms completed at each visit during Objective 3 of the study will be scanned into a database at SANBS headquarters (Constantia Kloof), and transferred to RTI using standard procedures already in use for the REDS-III South Africa project.

4.8 Statistical Considerations

To estimate the required sample size for the incident HIV and incident HBV groups and the control negative donors we focused on two different behaviors that have been separately associated with HIV and HBV infection in South Africa. For HIV infection we considered the risk factor of multiple sexual partners. For HBV we assumed scarification or other forms of body modification would be a potential route for HBV acquisition. Power analyses below indicate that with expected enrollment we will have sufficient power to detect odds ratios between 1.6 and 2.0 for putative incident HIV and HBV risk factors.

4.8.1 Sample size and power

Sample size and power calculations for this study cannot be based on the assumption of independent donor sample selection. We will be selecting blood donation clinics that are similar to those clinics where the study cases were donated, and, then, from the selected clinics selecting 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 the assumptions of the exposure prevalence in the donor populations and the expected number 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. Calculations are based on alpha equal to 0.05, power equal to 0.8, and a two-sided test.

HIV Power Calculations

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 that the prevalence of multiple sexual partners in the control and case populations.[15] 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 8). In the Monte Carlo, simulation estimates for odds ratios were similar regardless of the ratio of cases to controls, 1:1, 1:2, or 1:3. That is, a 1:1 ratio would allow for similar odds ratios to be detected. This analysis assumes that the selection of controls from the same geographic regions as cases will be consistent with the assumptions of inter cluster correlations that were defined for the Monte Carlo simulation power analysis. However, there is substantial uncertainty around how similar the behavioural exposures of cases and controls between different clusters (clinics) will be. For this reason we believe including a larger number of controls per case may be required in order for the team to ensure the study meets its primary objectives. Recall that we will be accepting virtually all cases so the only way to enlarge the sample is to include more controls. Also, including more controls will allow for secondary analyses of behavioural prevalence in donors without infection. For example, 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. We want to be able to detect and report findings across a range of behavioural exposures. 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 in exposure prevalence between cases and controls within each clinic. For these reason 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 around 50%, it will take 3 years to accrue the target number of 300 recently acquired HIV cases in this study. Our enrollment projections suggest that over a 3-year period we will be able to enroll up to 300 recently acquired HIV infections. 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 because controls will be easier to obtain within the available time frame and infrastructure available to conduct the study.

Table 8. Sample size and achieved odds ratios assuming 80% power for incident HIV infections.

Behavioural 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 Power Calculations

For HBV infections we considered scarification prevalence in rural South Africa[16] and similar body modification such as tattoo and body piercing and more recent study from Nigeria[17]. These two papers reflect divergent proportions of scarification in HBV negative individuals 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 behavioural exposures associated with incident HBV (Table 9). 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 prevalence scarification and similar body modification in the control population.

Previously SANBS has reported approximately 40-45 true HBV window period ID-NAT yields per year. In addition, in preliminary anti-HBc testing conducted in early 2012, during 6 months 29 donors were HBsAg-positive, anti-HBc-negative, giving an estimate annual number of expected HBsAg-positive, anti-HBc-negative participants of ~60. These numbers together 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 in any risk factors assessment estimated to be around 50%, it should take 3 years to accrue the target number of 150 recently acquired HBV cases in this study. The controls identified for the HIV case comparison will be used for the HBV comparison. Separate controls to compare to incident HBV will not be selected.

Table 9. Sample size and achieved odds ratios assuming 80% power for incident HIV infections.

Behavioural 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

4.9 Analytic Approach

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 behavioural 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. That 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., behavioural 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 behavioural 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 blood donor has HBV infection. That 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 correlate findings with donor demographics and risk behaviors derived in objective 1a. The outcome variable is HIV subtype. That 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 drug resistant to HIV treatment. That 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.

4.10. Human Participants

Procedures & risks: The participant will complete a self-administered computer based questionnaire on HIV and/or HBV risk behaviors. There is risk of lost 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 drug resistance results for participants in Objective 1a. Similarly, for the Objective 3 prospective study there may be minor risk of psychological stress from providing longitudinal CD4 and HIV viral load testing results.

Recruitment, consent and protection against risks: Recruitment will occur during the initial notification visit, and or at the final notification visit. Participants will be given the opportunity to complete the study procedures including the risk factor interview at another time. The study will be conducted in accordance with approval of IRB's at both UCSF and SANBS. All measures will be implemented to protect privacy and confidentiality including using participant ID

numbers instead of names on all study questionnaires and blood sample labels, maintaining a secure database, limiting the number of research staff with access to patient identifiers needed for contact.

Potential benefits to participants: If the results of these tests for drug resistance in Objective 1a indicate a participant has an HIV infection resistant to medications, these results will be provided to participant, and clinically important for the participant to be able to obtain appropriate medical care. Recently infected participants in the Objective 3 prospective study may benefit from the provision of free CD4 and HIV viral load testing.

Importance of knowledge to be gained: Significant benefit to public health in South Africa relating to better understanding of the epidemiology of incident HIV and incident HBV infections, and to SANBS in terms of knowledge applicable at the recruitment of safer blood donors.

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 behavioural factors differ significantly between the White and Black populations in South Africa. In order to obtain an accurate assessment of behavioural risks, we have decided that stratified matching on race is essential. Nevertheless, we expect overall enrollment into the study for both cases and controls to contain a much higher percentage of Black and population groups other than White when compared to the SANBS donor population, because of projected HIV and HBV infection rates. No restrictions based on gender will be used, we also 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.

4.10.1 Data Security

Data in this study will be kept confidential, and US investigators and members of the DCC will not have access to personally identifiable information (PII). At the SANBS study sites, key study personnel will have access to records with PII. For example, written signed consent forms. Study documents with PII will be stored in secure locations separate from study data (eg. laboratory results and questionnaires). Identifying information from donors that is temporarily linkable to the laboratory results, routine donation records and our study specific-questionnaires for the

purpose of proper identification and notification of each donor will only be available to SANBS operational staff. We will take several steps to minimize the possibility of inadvertent disclosure of the identity and information of participants. First, data are tracked and linked with a donation identification number (DIN) created at the time a person donates at SANBS. In addition a donor number is created to track individuals longitudinally. For this study, none of the datasets transferred to the US will contain PII, rather the DIN and an assigned study participant identification number will be used to link the donation history, health and risk screening data, laboratory results and study specific questionnaires entered into the study database.

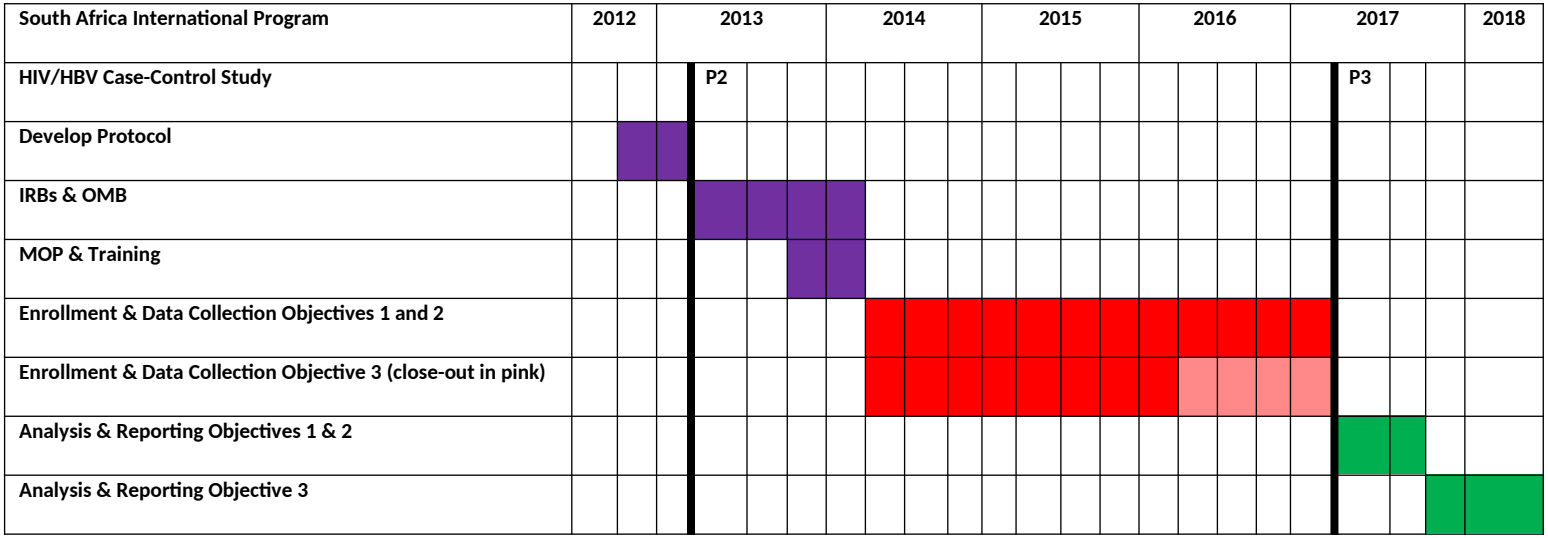
Limitations on who can access donor information already exist within SANBS. Only those individuals with job responsibilities that require access to PII have access to donor names and contact information. Such access is controlled through computer systems that only allow working on password protected workstations that require individual usernames and passwords. Similar restrictions on access will be in place for the study research staff.

Due to the sensitive nature of the study, the following steps are to be taken to protect the personal information of individuals recruited and/or enrolled in the study. The goal of this security plan is to put in place procedures that will reduce the risk of release of personal or sensitive information related to the study participants. The below items are the key points to achieve data security and participant privacy during the study period.

- Require the use of a two-factor authentication process to access the study management system (SMS).
- Store study materials in access-controlled rooms (use of key or access card to gain entry)
- Use computers with access-level control for SMS, including:
    - Individual level username and password requirements for all staff with access to study computers
    - Separately store files with PII. These data files reside on local systems that will be backed up on a regular weekly schedule.
    - Impose a mandatory screen saver requiring re-login to the SMS after 5-minutes of inactivity
    - Study-specific usernames assigned to staff
- Instruct staff to lock all study computers when they leave rooms
- Open the study files only when needed.
- If printing is required, print reports and other materials with identifying information on local printers in access-controlled spaces/rooms
- SANBS staff working on the REDS-III study will sign a non-disclosure agreement as part of their training and certification on this HIV/HBV protocol.

4.11. Timeline

Below is the timeline for development activities and administrative approval for the study. We plan to use 2013 to finalize the protocol and specific sampling plans. Development and pilot testing of the ACASI, and training of will occur in late 2013 to early 2014. Validation of the Lag assay, rapid LS-IA and PBMC preparation procedures will be done during 2012 and 2013. Enrollment is intended to occur starting in 2014 and to continue through 2016. Analysis and reporting of the finding is planned for 2017.



5. References

1. National Department of Health. The national HIV and syphilis prevalence survey, South Africa. Report 2007.
2. Van der Bij AK, Coutinho RA, Van der Poel CL. Surveillance of risk profiles among new and repeat blood donors with transfusion-transmissible infections from 1995 through 2003 in the Netherlands. Transfusion 2006;46:1729-36.
3. Braunstein SL, van de Wijgert JH, Nash D. HIV incidence in sub-Saharan Africa: a review of available data with implications for surveillance and prevention planning. AIDS Rev 2009;11:140-56.
4. Hall HI, Song R, Rhodes P, et al. Estimation of HIV incidence in the United States. JAMA 2008;300:520-9.
5. McFarland W, Mvere D, Katzenstein D. Risk factors for prevalent and incident HIV infection in a cohort of volunteer blood donors in Harare, Zimbabwe: implications for blood safety. AIDS 1997;11 Suppl 1:S97-102.
6. Ghys PD, Kufa E, George MV. Measuring trends in prevalence and incidence of HIV infection in countries with generalised epidemics. Sex Transm Infect 2006;82 Suppl 1:i52-6.
7. Williams B, Gouws E, Wilkinson D, Karim SA. Estimating HIV incidence rates from age prevalence data in epidemic situations. Stat Med 2001;20:2003-16.
8. Janssen RS, Satten GA, Stramer SL, et al. New testing strategy to detect early HIV-1 infection for use in incidence estimates and for clinical and prevention purposes. JAMA 1998;280:42-8.
9. Le Vu S, Pillonel J, Semaille C, et al. Principles and uses of HIV incidence estimation from recent infection testing--a review. Euro Surveill 2008;13.
10. Parekh BS, Kennedy MS, Dobbs T, et al. Quantitative detection of increasing HIV type 1 antibodies after seroconversion: a simple assay for detecting recent HIV infection and estimating incidence. AIDS Res Hum Retroviruses 2002;18:295-307.
11. Parekh BS, McDougal JS. Application of laboratory methods for estimation of HIV-1 incidence. Indian J Med Res 2005;121:510-8.
12. Duong YT, Qiu M, De AK, et al. Detection of recent HIV-1 infection using a new limiting-antigen avidity assay: potential for HIV-1 incidence estimates and avidity maturation studies. PLoS One 2012;7:e33328.
13. Kourtis AP, Bulterys M, Hu DJ, Jamieson DJ. HIV-HBV coinfection--a global challenge. N Engl J Med 2012;366:1749-52.
14. Altschul SF, Gish W, Miller W, Myers EW, Lipman DJ. Basic local alignment search tool. J Mol Biol 1990;215:403-10.
15. Minister of Health - Government of the Republic of South Africa. Getting to Success: Improving HIV Prevention Efforts in South Africa. South Africa HIV Epidemic, Response and Policy Synthesis 31 May 2011.
16. Karim SSA, Thejpal R, Singh B. High prevalence of Hepatitis B virus infection in rural black adults in Mseleni, South Africa. AJPH 1989;79:2.
17. Eke AC, Eke UA, Okafor CI, Ezebialu IU, Ogbuagu C. Prevalence, correlates and pattern of hepatitis B surface antigen in a low resource setting. Virol J 2011;8:12.
18. Hunt GM, Ledwaba J, Basson AE, et al. Surveillance of transmitted HIV-1 drug resistance in Gauteng and KwaZulu-Natal Provinces, South Africa, 2005-2009. Clin Infect Dis 2012;54 Suppl 4:S334-8.

19. Nwobegahay J, Selabe G, Ndjeka NO, Manhaeve C, Bessong PO. Low prevalence of transmitted genetic drug resistance in a cohort of HIV infected naive patients entering antiretroviral treatment programs at two sites in northern South Africa. J Med Virol 2012;84:1839-43.

6. Appendices

Appendix 1 Standard SANBS NAT-only Notification Template and Serology Positive Notification Letters

CONFIDENTIAL PERSONAL INFORMATION

SANBS
South African National Blood Service

CONFIDENTIALITY NOTE: This letter is intended only for the confidential use of the intended recipient. The information may be protected by privilege. Please note that any review, dissemination, distribution or copying of this letter is strictly prohibited if you are not the intended recipient. If you have received this letter in error, please desist from reading the contents, notify the sender immediately and destroy this letter

Registration No 2000/026390/08

<Donor's Address>

<Date>

<Donor Number>

<Dear Donor>

Thank you for your recent blood donation. The South African National Blood Service (SANBS) screens all blood donations for certain infections which may be transmitted by blood transfusion, specifically hepatitis B, hepatitis C, HIV and Syphilis. The screening tests used are sometimes over-sensitive and, although an abnormal result may be detected, this does not necessarily mean that you have, or have ever had, an infection.

On your last blood donation the results of a nucleic acid screening test (PCR) was positive. The Nucleic Acid Test is a sensitive test designed to detect early viral infections. Additional tests performed, for the above infections, were however, negative i.e. no corresponding antibodies to any of the above infections were detected.

Because of the discrepancy in these test results, you are urged to revisit a Blood Donor Centre to have another blood sample taken so that the tests can be repeated.

If this result is confirmed, you will unfortunately not be able to continue to donate blood. Because these tests are designed to detect infections which may be transmitted by blood transfusion and, since they may be sexually transmitted, neither you nor your partner may donate blood until this matter has been resolved.

Since it is possible that these results indicate a recent transmissible infection please do not donate blood until this matter is resolved. .

If you have any questions relating to this letter, please contact (MLO and contact number).

Yours sincerely,

Dr……
Medical Officer (SANBS)

Information from the Letterhead

Registration No.
2000/026390/08

<Title> <First Name Initial> <Surname>
<Street Address>
<Suburb>
<postal code>

<Print Date>

<Venue Mnemonic> -

<donor Number>

CONFIDENTIAL PERSONAL INFORMATION

CONFIDENTIALITY NOTE: This letter is intended only for the confidential use of the intended recipient. The information may be protected by privilege. Please note that any review, dissemination, distribution or copying of this letter is strictly prohibited if you are not the intended recipient. If you have received this letter in error, please desist from reading the contents, notify the sender immediately and destroy this letter.

Dear <title> <surname>

Thank you for your recent blood donation. The South African National Blood Service (SANBS) screens all blood donations for infections which may be transmitted by blood transfusion, especially Hepatitis B, Hepatitis C, HIV and Syphilis. The screening tests used are sometimes over-sensitive and even if an abnormal result may be detected, this does not necessarily mean that you have, or have ever had an infection.

Following your recent blood donation, we obtained a result that needs further investigation. You are advised to contact <MLO> at <number> or consult with your own doctor or clinic as soon as possible. If you prefer to visit your own doctor, please ask your doctor to contact the above mentioned medical staff. Your doctor (or Clinic Health Care Professional) will discuss the significance of this finding with you and perform further tests to verify the result.

Because these tests are designed to detect infections which may be transmitted by blood transfusion and, since they may be sexually transmitted, neither you nor your partner may donate blood until this matter has been resolved.

If you have any queries relating to this letter, please contact:
<MLO> at <number>.

Yours sincerely,

Dr <…… >
<Zone MO SANBS designation>

Appendix 2 Informed Consent – Case Participant

INFORMED CONSENT – CASE PARTICIPANTS

South African National Blood Service

Consent for Research

Research Study Title: Incident HIV/ Hepatitis B virus infections in South African blood donors: Behavioural risk factors, genotypes and biological characterization of early infection

Dear Blood Donor,

Thank you for taking the time to review the information below before considering whether you are willing to participate in this research project. You are being invited to take part in a research study titled, "Incident HIV / Hepatitis B virus infections in South African blood donors: Behavioural risk factors, genotypes and biological characterization of early infection"

The person in charge of this study in South Africa is Dr. Charlotte Ingram from the South African National Blood Service. Before you decide if you want to join this study, we want you to learn about the study. The study staff will talk with you about the study and answer your questions. Before you agree to join this study, please read this consent form carefully. Take your time in deciding if you wish to join this study. This consent form might contain some words that are not familiar to you. Please ask questions about anything you do not understand.

Who is conducting this research study?

The study is part of an international project known as the "Recipient Epidemiology and Donor Evaluation Study-III (REDS-III)". The **South Africa National Blood Service (SANBS)** is leading this study in collaboration with researchers from the University of California San Francisco and Blood Systems Research Institute in the United States. The data collected for this study will be analyzed in South Africa and in the United States (by the data coordinating centre for the REDS-III research program, Research Triangle Institute, Inc. located in Rockville, Maryland, US) and results reported in medical journals. The results of the study may be used to improve blood safety in South Africa and other countries in Africa. The study is supported financially by the National Heart, Lung, and Blood Institute of the U.S. National Institutes of Health.

What is the purpose of this research study?

1. The first purpose is to find out how many donors were recently infected by HIV and/or Hepatitis B virus, and what subtype of virus these donors have.
2. The second purpose of this research is to study donors who, following the donation of a unit of blood to SANBS, were found to have been recently infected with HIV and/or Hepatitis B, and to identify behaviors that may have caused them to become infected.

We are inviting you to participate because you have recently had a test result indicating possible HIV and/or HBV at the blood centre.

What will happen if you participate in this study?

This study consists of two parts, namely:

1. Taking a new sample of your blood.
2. Completing a computer interview.

Procedures:

If you agree to participate, the following will happen:

1. We will collect an additional 48 ml (about 3 tablespoons) of blood from you, which will be used to perform the following tests: measure the stage of infection and amount of virus in your body and determine the genetic make-up of the HIV and/or Hepatitis B virus you have. This will allow us to determine the type of HIV or HBV you have and, if it is HIV, whether it is resistant to some of the antiretroviral medicines used to treat HIV/AIDS.
    a. The results of tests we plan to conduct on your blood samples will not be available at the same time. If you are confirmed positive for infection we will inform you of the results of the tests to measure the amount of virus in your body when you come back to SANBS for test results notification. Also we will inform your doctor if you grant us permission to do so. If you are HIV positive, we will also ask you to return to SANBS in a few months to tell you about the results that indicate whether or not you have an HIV infection resistant to some antiretroviral medicines. The results of tests that will not influence your care for HIV or Hepatitis B virus will not be provided to you.

b. Your blood samples will be kept in case some tests need to be repeated. Some of the samples will be sent to other research laboratories in South Africa, and may be sent to the project's central laboratory, Blood Systems Research Institute, located in San Francisco, United States, for additional testing. These laboratory tests are not part of the routine testing at SANBS. These samples may also be used in other future studies about HIV and/or HBV infection. Your blood samples may be stored indefinitely at SANBS, but any future research not related to HIV and/or HBV research as described in this consent will require additional or new approval by appropriate ethics committees.

2. You will complete a confidential questionnaire using a computer to answer questions about your sexual history, other factors that may be associated with HIV or Hepatitis B virus infection, your knowledge about HIV/AIDS and about the motivations that took you to donate blood. You may skip any questions that you are not comfortable answering.

Please note that we will provide information on local services in your community that can provide counseling and other medical services to you, should you require this. In addition, you should feel free to speak to the research staff from SANBS or physicians from the local SANBS blood centre after the computer interview if you have any questions or need anything to be explained to you again.

Are there risks to you for participating in the study?

Risks:

1. There is a small risk, such as bruising or a little pain, when collecting a blood specimen. A trained nurse or other health care professional will collect the blood

samples. SANBS will provide you the same assistance given to all blood donors in case this happens to you.

2. There is a small chance that your personal information may become public because of an unintentional or accidental data security breach. However, to avoid this, the questionnaire as well as the samples will be identified by code numbers and not your name.
3. The results of the testing we will conduct could influence the types of medical care you may require, such as which medications are prescribed for you. Some of the testing may reveal that, if you are HIV-infected, your HIV infection is resistant to some of the antiretroviral medicines and this could be upsetting to you.

Are there benefits to you for participating in the study?

Benefits:

1. For you personally, participating in this study may result in you learning about the stage of your infection. If you have HIV you will also learn whether it is resistant or not to antiretroviral medicines used in HIV/AIDS treatment. This information will help you and your doctor find the most effective treatment for your HIV infection.
2. Beyond this there is no other specific benefit for you in participating in this study. You will, however, be helping to better the understanding of the HIV and Hepatitis B epidemics in our country.

Will I be paid and are there any costs to the research?

You will be paid 80 Rand to compensate you for your transportation to the study centre following completion of the interview. All of the research tests will be done free of charge.

What if I don't want to participate after I have completed the study?

You will not be forced to participate in this study and you may retract your consent for participating at any time by contacting the investigator listed on this consent form. If you decide to remove yourself from the study, your blood samples will be destroyed and your questionnaire responses will be deleted from the study databases. However, if the data have already been analyzed and reported in medical journals we will not be able to remove you from the study. Your decision to remove yourself from the study will not affect your relationship with SANBS in any way.

Questions you may have:

You can have any questions you may have answered by the responsible investigator, before and during the research. If you have questions right now please ask them before signing this consent form.

Informed Consent Signature Page – CASE PARTICIPANTS

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

If you have any questions about this research study, your blood donation test results, or if you are injured as a result of the research you may contact the following at any time:

South African National Blood Service Contact Person

Name: TBN

Telephone Number: TBD

You may also contact the Secretariat of the Ethics Committee of SANBS, at telephone number (TBD) if you have questions about your rights as a research participant.

Your participation in this research is voluntary, and you will not be penalized or lose benefits in anyway if you refuse to participate or decide to stop participating.

If you agree to participate, you will be given a signed copy of this entire informed consent document, which provides you with a written summary of the research.

Do you consent to allow the researchers to send the results of tests that may influence the care your doctor provides to you for HIV or Hepatitis B infection directly to your doctor or medical care provider's address?

___ Yes **____ No**

Contact information for your doctor or medical care provider:

Name:

Address:

Telephone number (if known):

I DECLARE THAT I HAVE READ AND UNDERSTOOD ALL THE INFORMATION CONTAINED IN THE CONSENT DOCUMENT AND I AGREE TO PARTICIPATE IN THIS RESEARCH STUDY. I AM FREE TO RETRACT MY CONSENT IN ANY PART OF THE RESEARCH IF I DECIDE THAT I DO NOT WANT TO CONTINUE PARTICIPATING.

Name: _____

Signature: _____

Date: _____/_____/_____

Signature of study staff taking consent:
I declare that the above participant has been fully informed about the nature, conduct and risks of the above study.

Name: _____

Signature: _____

Date: _____/_____/_____

Appendix 3 Informed Consent – Control Participant

Informed Consent – Control Participants

South African National Blood Service Consent for Research

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

---

Dear Blood Donor,

Thank you for taking the time to review the information below before considering whether you are willing to participate in this research project. You are being invited to take part in a research study titled, "Incident HIV / Hepatitis B virus infections in South African blood donors: Behavioural risk factors, genotypes and biological characterization of early infection" **You are being approached to be a control in this study which means that if you have donated before you tested NEGATIVE for HIV and Hepatitis B infection. Blood from your donation today will be tested and we will include you in the study as a comparison donor if your blood tests negative for all of the tests that South Africa National Blood Service uses to test blood donations.**

The person in charge of this study in South Africa is Dr. Charlotte Ingram from South African National Blood Service. Before you decide if you want to join this study, we want you to learn about the study. The study staff will talk with you about the study and answer your questions. Before you agree to join this study please read this consent form carefully. Take your time in

deciding if you wish to join this study. This consent form might contain some words that are not familiar to you. Please ask questions about anything you do not understand.

Who is conducting this research study?

The study is part of an international project known as the "Recipient Epidemiology and Donor Evaluation Study (REDS-III)". The **South Africa National Blood Service (SANBS)** is leading this study in collaboration with researchers from the University of California San Francisco and Blood Systems Research Institute in the United States. The data collected for this study will be analyzed in South Africa and the United States (by the coordinating centre for the REDS-III research program, Research Triangle Institute, Inc. located in Rockville, Maryland, US) and results reported in medical journals. The results of the study may be used to improve blood safety in South Africa and other countries in Africa. The study is supported financially by the National Heart, Lung, and Blood Institute of the U.S. National Institutes of Health.

What is the purpose of this research study?

1. **We are inviting you to participate because you just donated blood. Studies like this need donors who are NEGATIVE for HIV and Hepatitis B infections to serve as a comparison group. Your blood will be tested and we will include you in the study as a comparison donor if your blood tests negative for all of the tests that South Africa National Blood Service uses to test blood donations.** If by chance your donation tests positive for HIV or Hepatitis B virus, Hepatitis C virus or syphilis when it is tested you will be removed from the study as a comparison person. In that case you will be contacted and counseled using standard procedures of the SANBS.

What will happen if you participate in this study?

The study consists of one main activity, namely:

1. Completing a computer interview.

Procedures

If you agree to participate, the following will happen:

1. You will complete a confidential questionnaire using a computer to answer questions about your sexual history, other factors that may be associated with HIV or Hepatitis B infection, your knowledge about HIV/AIDS and about the motivations that took you to donate blood. You may skip any questions that you are not comfortable answering.

Please note that we will provide information on local services in your community that can provide counseling and other medical services to you, should you require this. In addition, you should feel free to speak to the research staff from SANBS or physicians from the local SANBS blood centre after the computer interview if you have any questions or need anything to be explained to you again.

Are there risks to you for participating in the study?

1. There is a small chance that your personal information may become public because of an unintentional or accidental data security breach. However, to avoid this from happening, the study questionnaire will be identified by code numbers and not your name.

Are there benefits to you for participating in the study?

Benefits:

1. There is no personal benefit to you from participating in this study, but you will be helping to improve our understanding of the HIV epidemic, Hepatitis B virus, and ways to keep blood safe in our country.

Will I be paid and are there any costs to the research?

You will be paid 80 Rand to compensate you for your transportation to the study centre following completion of the interview.

What if I don't want to participate anymore after I have completed the study?

You will not be forced to participate in this study and you may retract your consent for participating at any time by contacting the investigator listed on this consent form. If you decide to remove yourself from the study your questionnaire responses will be deleted from the study databases. However, if the data have already been analyzed and reported in medical journals we will not be able to remove you from the study. Your decision to remove yourself from the study will not affect your relationship with SANBS in any way.

Questions you may have:

You can have any questions you may have answered by the responsible investigator, before and during the research. If you have questions right now please ask them before signing this consent.

Informed Consent Signature Page – CONTROL PARTICIPANTS

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

If you have any questions about this research study, your blood donation test results, or if you are injured as a result of the research you may contact the following at any time:

South African National Blood Service Contact Person
Name: TBN
Telephone Number: TBD

You may also contact the Secretariat of the Ethics Committee of SANBS, at telephone number (TBD) if you have questions about your rights as a research participant.

Your participation in this research is voluntary, and you will not be penalized or lose benefits in anyway if you refuse to participate or decide to stop participating.

If you agree to participate, you will be given a signed copy of this entire informed consent document, which provides you with a written summary of the research.

I DECLARE THAT I HAVE READ AND UNDERSTOOD ALL THE INFORMATION CONTAINED IN THE CONSENT DOCUMENT AND I AGREE TO PARTICIPATE IN THIS RESEARCH STUDY. I AM FREE TO RETRACT MY CONSENT IN ANY PART OF THE RESEARCH IF I DECIDE THAT I DO NOT WANT TO CONTINUE PARTICIPATING.

Name: _____

Signature: _____

Date: _____/_____/_____

Signature of study staff taking consent:
I declare that the above participant has been fully informed about the nature, conduct and risks of the above study.

Name: _____

Signature: _____

Date: _____/_____/_____

Appendix 4 Informed Consent – Cohort Participant

Informed Consent – Longitudinal Follow-up Participants

South African National Blood Service
Consent for Research

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

---

Dear Blood Donor,

Thank you for taking the time to review the information below before considering whether you are willing to participate in this research project. You are being invited to take part in a research study titled, "Incident HIV / Hepatitis B virus infections in South African blood donors: Behavioural risk factors, genotypes and biological characterization of early infection"

The person in charge of this study in South Africa is Dr. Charlotte Ingram from South African National Blood Service. Before you decide if you want to join this study, we want you to learn about the study. The study staff will talk with you about the study and answer your questions. Before you agree to join this study please read this consent form carefully. Take your time in deciding if you wish to join this study. This consent form might contain some words that are not familiar to you. Please ask questions about anything you do not understand.

Who is conducting this research study?

The study is part of an international project known as the "Recipient Epidemiology and Donor Evaluation Study (REDS-III)". The **South Africa National Blood Service (SANBS)** is leading this study in collaboration with researchers from the University of California San Francisco and Blood Systems Research Institute in the United States. The data collected for this study will be analyzed in South Africa and the United States (by the data coordinating centre for REDS-III, Research Triangle Institute, Inc. located in Rockville, Maryland, US) and results reported in medical journals. The results of the study may be used to improve blood safety in South Africa and other countries in Africa. The study is supported financially by the National Heart, Lung, and Blood Institute of the U.S. National Institutes of Health.

What is the purpose of this research study?

1. The first purpose is to study donors with very recent HIV infection in order to find out how the HIV virus begins to reproduce and how the body's natural defenses (immune system) reacts to the HIV virus over the first few months of infection.
2. The second purpose is to study donors who have HIV infection but whose immune systems seem to have controlled the virus without treatment so that it is difficult to detect any HIV virus in the body. Finding out how these people control the virus could help develop new treatments or vaccines against HIV.

You are being invited to participate in this research study because you have already participated as a case participant in the larger research study, and you have either (1)

recently become infected with HIV or (2) have an HIV infection but with a very low or undetectable amount of HIV virus in your body.

What will happen if you participate in this study?

There are two main study activities that will happen to you on four different visits to SANBS, namely:

1. A new sample of your blood will be taken at four different dates over the next ~6 months.
2. You will complete a brief questionnaire each time a blood sample is collected.

Procedures

If you agree to participate, the following will happen:

We will ask you to agree to participate in a 6-month long follow-up study in which we will ask you to attend research study visits at the blood centre a total of 4 times (today, in 1 month, in 2 months, and in about 5 months from now.)

1. At each visit we will collect 6 tubes of blood totaling 48 ml (3 tablespoons) from your vein.
2. We will use these samples to conduct special tests that measure the type of HIV infection you have and you body's immune response to that infection. The results of this project will not be sent to you because they are research tests and will not influence the healthcare you receive. Please note that results from the larger research study will be given to you when you come back to SANBS to participate in this part of the study. The results that will be given to you have been described in the INFORMED CONSENT – CASE PARTICIPANTS document you already signed. For

this part of the study some of the samples will be sent to the Project's Central Laboratory, Blood Systems Research Institute, located in San Francisco, United States for additional testing. These tests are not part of the routine testing at the blood service.

3. The sample you give to us may be used in future studies to understand how people's bodies respond differently to HIV infection. Your specimens may be stored indefinitely at SANBS or Blood Systems Research Institute in San Francisco, US, but additional approval by Ethical Committees will be necessary for future research use outside of HIV research consent you are providing for this study.
4. At each follow-up visit, you will be asked to complete a short paper questionnaire on your current medical status, including questions about visits to health care providers, any medications you may have started taking, and symptoms you may have experienced since your last study visit.
5. Our research nurses will stay in contact with you to schedule your follow-up visits. We will stay in contact with you using telephone calls, text messages and emails. We would like to call you every month just to check-in with you.

Are there risks to you for participating in the study?

Risks:

1. There is a small risk, such as bruising or a little pain, when collecting a blood specimen. A trained nurse or other health care professional will collect the blood samples. The blood service will provide you the same assistance given to all blood donors in case this happens to you.
2. There is a small chance that your personal information may become public because of an unintentional or accidental data security breach. However, to avoid this, the questionnaire as well as the samples will be identified by code numbers and not your name.

Will I be paid and are there any costs to the research?

You will be paid 80 Rand to compensate you for your transportation to the study centre after each follow-up visit. All of the research tests will be done free of charge.

What if I don't want to participate after I have completed the study?

You do not have to participate in this study and you may retract your consent for participating at any time by contacting the investigator listed on this consent form. If you decide to remove yourself from the study, your blood samples will be destroyed and your questionnaire responses will be deleted from the study databases. However, if the data have already been analyzed and reported in medical journals we will not be able to remove you from the study. Your decision to remove yourself from the study will not affect your relationship with SANBS in any way.

Questions you may have:

You can have any questions you may have answered by the responsible investigator, before and during the research. If you have questions right now please ask them before signing this consent.

Informed Consent Signature Page – LONGITUDINAL FOLLOW-UP PARTICIPANTS

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

If you have any questions about this research study, your blood donation test results, or if you are injured as a result of the research you may contact the following at any time:

South African National Blood Service Contact Person

Name: TBN

Telephone Number: TBD

You may also contact the Secretariat of the Ethics Committee of SANBS, at telephone number (TBD) if you have questions about your rights as a research participant.

Your participation in this research is voluntary, and you will not be penalized or lose benefits in anyway if you refuse to participate or decide to stop participating.

If you agree to participate, you will be given a signed copy of this entire informed consent document, which provides you with a written summary of the research.

I DECLARE THAT I HAVE READ AND UNDERSTOOD ALL THE INFORMATION CONTAINED IN THECONSENT DOCUMENT AND I AGREE TO PARTICIPATE IN THIS RESEARCH STUDY. I AM FREE TO RETRACT MY CONSENT IN ANY PART OF THE RESEARCH IF I DECIDE THAT I DO NOT WANT TO CONTINUE PARTICIPATING.

Name: _____

Signature: _____

Date: _____/_____/_____

Signature of study staff taking consent:
I declare that the above participant has been fully informed about the nature, conduct and risks of the above study.

Name: _____

Signature: _____

Date: _____/_____/_____

Appendix 5 Descriptions of Standard Laboratory Assays at SANBS

Novartis Procleix Ultrio Plus assay: The PROCLEIX® ULTRIO® Plus Assay* is a qualitative in vitro nucleic acid amplification test for the detection of human immunodeficiency virus type 1 (HIV-1) RNA, hepatitis C virus (HCV) RNA, and/or hepatitis B virus (HBV) DNA in plasma and serum specimens from human donors, tested individually or in pools. The PROCLEIX ULTRIO Plus Assay utilizes target amplification nucleic acid probe technology for the detection of HIV-1 RNA, HCV RNA, and HBV DNA. The assay contains reagents which may be used for simultaneous detection of all three viruses or the individual viruses: HIV-1, HCV, and HBV. The PROCLEIX® Assays incorporate an Internal Control for monitoring assay performance in each individual specimen. The PROCLEIX® ULTRIO® Plus Assay involves three main steps, which take place in a single tube: Sample Preparation; HIV-1 RNA, HCV RNA, and HBV DNA target amplification by Transcription-Mediated Amplification (TMA); and detection of the amplification products (amplicons) by the Hybridization Protection Assay (HPA).

Abbott Prism HIV 1/2: The ABBOTT PRISM HIV O Plus assay is an in vitro chemiluminescent immunoassay (ChLIA) for the qualitative detection of antibodies to HIV-1 (anti-HIV-1) Groups M and O and/or antibodies to HIV-2 (anti-HIV-2) in human serum and plasma specimens. The ABBOTT PRISM HIV O Plus assay is intended to screen individual human donors, including volunteer donors of Whole Blood and blood components and other living donors, for the presence of anti-HIV-1 Groups M and O and/or anti-HIV-2. The ABBOTT PRISM HIV O Plus assay uses recombinant DNA-derived antigens corresponding to three viral proteins (HIV-1 Group M envelope, HIV-1 Group O envelope, and HIV-2 envelope) and one synthetic peptide corresponding to HIV-2 envelope. The ABBOTT PRISM HIV O Plus assay is a three-step sandwich ChLIA. The reactions occur within the ABBOTT PRISM System in the following sequence:

- Microparticles coated with HIV antigens (recombinant proteins) are incubated with sample (plasma, serum, calibrator, or control) in the incubation well of the reaction tray. During incubation, HIV-1 and/or HIV-2 antibodies present in the sample bind to the antigen(s) on the microparticles.
- After this first incubation is complete, the reaction mixture is transferred to the glass fiber matrix (matrix) of the reaction tray using the transfer wash. The microparticles are captured by the matrix, while the remaining mixture flows through to an absorbent blotter.
- A probe mixture (probe) consisting of biotinylated HIV-1 recombinant proteins and biotinylated HIV-2 peptide is added to the microparticles on the matrix and incubated. The probe binds to the microparticle antibody complex created during the first incubation process. After the second incubation, the unbound probe is washed into the blotter with the probe wash.
- The acridinium-labeled anti-biotin conjugate is added to the microparticles on the matrix and incubated to bind any probe that is present. After the third incubation, the unbound conjugate is washed into the blotter with the conjugate wash.
- The chemiluminescent signal is generated by addition of an alkaline hydrogen peroxide solution. The resultant photons are counted. The amount of light emitted is proportional to the amount of anti-HIV-1 and/or anti-HIV-2 in the sample. The presence or absence of anti-HIV-1/HIV-2 in the sample is determined by comparing the number of photons collected from the sample to a cutoff value determined from a calibration performed in the same batch. If the number of photons collected from a test sample is less than the cutoff value, the sample is considered nonreactive for anti-HIV-1 and anti-HIV-2 by the criteria of the ABBOTT PRISM HIV O Plus assay. These specimens need not be further tested. If the number of photons collected from a test sample is greater than or equal to the cutoff

value, the sample is considered reactive for anti-HIV-1 and/or anti-HIV-2 by the criteria of the ABBOTT PRISM HIV O Plus assay.

Abbott Prism HBsAg: The ABBOTT PRISM HBsAg assay is an in vitro chemiluminescent immunoassay (ChLIA) for the qualitative detection of hepatitis B surface antigen (HBsAg) in human serum and plasma specimens. The ABBOTT PRISM HBsAg (ChLIA) is intended to screen individual human donors, including volunteer donors of whole blood and blood components, and other living donors for the presence of HBsAg. Hepatitis B virus (HBV) is a small, partially double stranded, DNA virus and a member of the Hepadna virus family. The HBV genome contains four overlapping reading frames representing the core, polymerase, surface, and X genes. The ABBOTT PRISM HBsAg assay is a two-step sandwich ChLIA. The reactions occur within the ABBOTT PRISM System in the following sequence:

• Microparticles coated with mouse monoclonal anti-HBs are incubated with sample (either plasma, serum, calibrator, or control) in the incubation well of the reaction tray. During incubation, HBsAg present in the sample binds to the antibody on the Microparticles.

• After this first incubation is complete, the reaction mixture is transferred to the glass fiber matrix (matrix) of the reaction tray using the Transfer Wash. The Microparticles are captured by the matrix while the remaining mixture flows through to the absorbent blotter.

• The Acridinium-Labeled Goat Polyclonal Anti-HBs Conjugate is added to the Microparticles on the matrix and incubated. After this second incubation, the unbound Conjugate is washed into the blotter with the Conjugate Wash.

• The chemiluminescent signal is generated by addition of an alkaline hydrogen peroxide solution. The resultant photons are counted. The amount of light emitted is proportional to the amount of HBsAg in the sample. The presence or absence of HBsAg in the sample is determined by comparing the number of photons collected from the sample to a cutoff value determined from a calibration performed in the same batch. If the number of photons collected from a test sample is less than the cutoff value, the sample is considered nonreactive for HBsAg by the criteria of the ABBOTT PRISM HBsAg assay. These specimens need not be further tested. If the number of photons collected from a test sample is greater than or equal to the cutoff value, the sample is considered reactive for HBsAg by the criteria of the ABBOTT PRISM HBsAg assay.

Roche Elecsys anti-HBc assays: Immunoassay for the in vitro qualitative determination of IgG and IgM antibodies to the hepatitis B core antigen in human serum and plasma. The electrochemiluminescence immunoassay "ECLIA" is intended for use on Elecsys and cobas e immunoassay analyzers. The hepatitis B virus consists of an external envelope (HBsAg) and an inner core (HBcAg). The hepatitis core antigen comprises 183-185 amino acids. During an infection with the hepatitis B virus, antibodies to HBcAg are generally formed, which often persist for life. Anti-HBc appears shortly after the onset of infection with hepatitis B virus and can usually be detected in serum soon after the appearance of HBsAg. Anti-HBc antibodies persist both, in persons who have recovered from a hepatitis B infection and in those who develop HBsAg-carrier status. Accordingly, they are an indicator of existing or past hepatitis B infection.

Innogenetics mAB Innotest p24 antigen: INNOTEST® HIV Antigen mAb is an enzyme immunoassay for the qualitative detection of HIV p24 antigen in human serum, plasma, and cell culture supernatant.

Abbott Real-Time HIV-1 PCR: The Abbott RealTime HIV-1 assay is an in vitro reverse transcription-polymerase chain reaction (RT-PCR) assay for the quantitation of Human Immunodeficiency Virus type 1

(HIV-1) on the automated m2000 System in human plasma from HIV-1 infected individuals over the range of 40 to 10,000,000 copies/mL. The Abbott RealTime HIV-1 assay is intended for use in conjunction with clinical presentation and other laboratory markers for disease prognosis and for use as an aid in assessing viral response to antiretroviral treatment as measured by changes in plasma HIV-1 RNA levels. Quantitative measurement of HIV levels in peripheral blood has greatly contributed to the understanding of the pathogenesis of HIV infection and has been shown to be an essential parameter in prognosis and management of HIV infected individuals. Decisions regarding initiation or changes in antiretroviral medicine are guided by monitoring plasma HIV RNA levels (viral load), CD4+ T cell count, and the patient's clinical condition. HIV RNA levels in plasma can be quantitated by nucleic acid amplification or signal amplification technologies. The Abbott RealTime HIV-1 assay uses Polymerase Chain Reaction (PCR) technology with homogenous real-time fluorescent detection. Partially double-stranded fluorescent probe design allows detection of diverse group M subtypes and group O isolates. The assay is standardized against a viral standard from the Virology Quality Assurance (VQA) Laboratory of the AIDS Clinical Trial Group, 23 and against World Health Organization (WHO) 1st International Standard for HIV-1 RNA (97/656). The assay results can be reported in copies/mL or International Units/ mL (IU/mL).

Abbott Real-time HBV PCR: Abbott RealTime HBV assay is an in vitro polymerase chain reaction (PCR) assay for use with the Abbott m2000 SystemDNA reagents and with the Abbott m2000sp and m2000rt instruments for the quantitation of Hepatitis B Virus (HBV) DNA in human serum or plasma (EDTA) from chronically HBV-infected individuals. The assay is intended for use as an aid in the management of patients with chronic HBV infection undergoing anti-viral therapy. The assay can be used to measure HBV DNA levels at baseline and during treatment to aid in assessing response to treatment. The results from the Abbott RealTime HBV assay must be interpreted within the context of all relevant clinical and laboratory findings. Quantitation of HBV DNA is important in the evaluation and management of patients with chronic HBV infection. Current guidelines recommend HBV viral load to determine which chronic HBV patients should be treated and to monitor their response to therapy. A low baseline viral load has been shown to be predictive of response to therapy. Conversely, a high baseline viral load is predictive of resistance to therapy as well as relapse following therapy, and has also been found to be an independent risk factor for hepatocellular carcinoma. HBV DNA in serum or plasma can be quantitated using nucleic acid amplification or signal amplification technologies. The Abbott RealTime HBV assay uses PCR technology combined with homogeneous real time fluorescent detection for the quantitation of HBV DNA. The selection of a highly conserved region in the Surface gene provides for the detection of HBV genotypes A, B, C, D, E, F, G, and H. The location of the target region in the N terminal third of the Surface gene ensures that the assay is not impacted by YMDD mutants, HBsAg escape mutants, or drug-resistant mutants, as this region is essential for the assembly and secretion of subviral particles, and tolerates only minor structural changes. The assay is standardized against the World Health Organization (WHO) International Standard for Hepatitis B Virus DNA (NIBSC).11 Results are reported in International Units per milliliter (IU/mL) or copies/mL.

Appendix 6 Marker Positive Donor Database Functional Requirements – Example of Relevant Aspects of Study Management System

A.Project / Request Name and Helpdesk number:

57653 – Marker Positive Donor Database

B.Detailed Analysis:

Overview:

A mechanism is required by the South African National Blood Service to manage the counseling of donors who are confirmed positive for one of the viral markers used in the organization. The database will be used to register new confirmed donors and manage the counseling process of the donors on the database.

The database will be created on a SQL 2008 R2 database. This database will be hosted on one of the SQL servers connected to the VNX SAN. The database will be backed up according to the current backup methodologies.

The database will consist of 15 tables that are explained in the section below.

Database Design:

1.Donor

- The main table for the database is the Donor table. This table will keep the donor records for the viral positive donors.

Donor	
PK	**DonorID**
	DonorNumber
	DonationNumber
	DateBled
FK1	DonorStatusID
FK3	ViralogyID
	dHxV
FK4	ProvinceID
FK2	ZoneID
	DateMLOReceivedResults
	DateFileCreated
	DateFirstTelephoneCall
	DateDonorContacted
	DateFirstAppointment
	DateCounselled
FK5	CounseledByID
	DateCaseClosed
	TimeStamp
	Mnemonic
	Place
	StaffNumber
FK7	UserID

Field	Description	Field Type
DonorID	Unique number generated internally by system.	INTEGER
Donor Number	The donor's MEDITECH number.	VARCHAR(10)
DonationNumber	The unit number of the unit donated by the donor as found in MEDITECH.	VARCHAR(10)
DateBled	The date on which the donor donated the unit.	DATE
DonorStatusID	The status of the donor as per the MEDITECH system. (Foreign key with DonorStatus table.)	INTEGER
ViralogyID	The virology marker for which the donor are positive. (Foreign key with Virology table.)	INTEGER
ProvinceID	The province in which the donor resides. (Foreign key with Province table.)	INTEGER
ZoneID	The zone in which the donor resides. (Foreign key with Zone table.)	INTEGER
DateMLOReceivedResults	The date that the Medical Liaison officer received the information.	DATE
DateMLOReceivedResults**TS**	The date that the above value has been added to	DATE

	the system. Added by system.	
DateFileCreated**TS**	The date that the file is created. Added by system.	DATE
DateDonorContacted	The date that the donor was successfully contacted.	DATE
DateDonorContacted**TS**	The date that the above value has been added to the system. Added by system.	DATE
DateFirstAppointment	The date of the first appointment with the donor.	DATE
DateFirstAppointment**TS**	The date that the above value has been added to the system. Added by system.	DATE
DateCounselled	The date that the donor was counselled.	DATE
DateCounselled**TS**	The date that the above value has been added to the system. Added by system.	DATE
CounseledByID	The staff member that counselled the donor. (Foreign key with User table.)	INTEGER
DateCaseClosed	The date that the case was closed.	DATE
DateCaseClosed**TS**	The date that the above value has been added to the system. Added by system.	DATE
Mnemonic	The mnemonic of the donor clinic at which the unit was collected.	VARCHAR(10)
Place	Where the donor was counselled, at SANBS or other venue.	VARCHAR(10)
UserID	The user ID of the user who has created the initial record. (Foreign key with User table.)	INTEGER

2.DonorStatus

- The table will keep a list of donor statuses.
    - Lapsed
    - New
    - Repeat

DonorStatus	
PK	**DonorStatusID**
	DonorStatus

Field	Description	Field Type
DonorStatusID	Unique number generated internally by system.	INTEGER
DonorStatus	The different statuses for a donor.	VARCHAR(10)

3.Virology

- The table will keep a list of virology markers for which donors can be counseled.
    - HIV
    - HBS
    - HCV

Virology	
PK	**VirologyID**
	Virology

Field	Description	Field Type
VirologyID	Unique number generated internally by system.	INTEGER
Virology	The different type of virology markers.	VARCHAR(10)

4.Province

- The table will keep a list of the provinces. This will enable reporting per province.
    - Gauteng
    - Free State
    - Northern Cape

Province	
PK	**ProvinceID**
	Province

Field	Description	Field Type
ProvinceID	Unique number generated internally by system.	INTEGER
Province	A list with the different provinces.	VARCHAR(10)

5.Zone

- The table will keep a list of the zones. This will enable reporting per zone.
    - Egoli
    - Northern

oEastern Cape

Zone	
PK	**ZoneID**
FK1	Zone ProvinceID

Field	Description	Field Type
ZoneID	Unique number generated internally by system.	INTEGER
Zone	Names of different zones.	VARCHAR(10)
ProvinceID	The province in which the zone operates. This will ensure that if you pick province that you can only pick a zone in that province.	INTEGER

6.User

•The table will keep the details of the different staff interfacing with the system.

User	
PK	**UserID**
	Username Password Name Surname Tel Cell Email JobTitle

Field	Description	Field Type
UserID	Unique number generated internally by system.	INTEGER
Username	The user name of the user as defined in MEDITECH.	VARCHAR(10)
Password	The password that the user uses to log into the system.	VARCHAR(15)
Name	The user's name	VARCHAR(30)
Surname	The user's surname	VARCHAR(30)
Tel	The landline telephone number for the user.	VARCHAR(15)
Cell	The cellular number of the user.	VARCHAR(15)
Email	The email address of the user. Can be used in future to notify MLO's of new cases.	VARCHAR(60)

JobTitleID	The job title of the staff member. (Foreign key with JobTitle table)	VARCHAR(30)
Link staff member to Zone so they can see own donors.		

7.JobTitle

- The table will keep a list of job titles of staff in SANBS.
    - Medical Liaison Officer
    - Secretary
    - Medical Doctor

JobTitle	
PK	**JobTitleID**
	JobTitle

Field	Description	Field Type
JobTitleID	Unique number generated internally by system.	INTEGER
JobTitle	A list of job titles in SANBS will be kept in this field.	VARCHAR(30)

8.Counselor

- The table will keep a list of all the external counsellors.

Counselor	
PK	**CounselorID**
	Counselor

Field	Description	Field Type
CounsellorID	Unique number generated internally by system.	INTEGER
Counsellor	What data must be captured in this field? Name, title, institution??	VARCHAR(30)

9.Letter

- The table will keep a list of letters that ***can*** be sent to donors.

Letter	
PK	**LetterID**
	Letter

Field	Description	Field Type
LetterID	Unique number generated internally by system.	INTEGER
Letter	A description of the letters that can be send to the donors.	VARCHAR(10)

10.DonorLetter

- The table will keep a history of the letters that ***have been*** sent to the donor.

DonorLetter	
PK	**DonorLetterID**
FK1 FK2	LetterID DonorID DateSent

Field	Description	Field Type
DonorLetterID	Unique number generated internally by system.	INTEGER
LetterID	The letter that was sent to the donor. (Foreign key with Letter table)	VARCHAR(10)
DonorID	The donor to whom the letter was sent. (Foreign key with Donor table)	INTEGER
DateSent	The date that the letter was sent to the donor.	DATE
UserID	Will this be the MLO or can it also be Donation testing. Do we want to know which user? The user who has sent the letter. (Foreign key with User table)	INTEGER

11.TelephoneEvent

- The table will keep a list of telephone events that can be applied to a donor.
    - First Call
    - Follow-up Call
    - Reminder

oReturned Call

TelephoneEvent	
PK	**TelephoneEventID**
	Event

Field	Description	Field Type
TelephoneEventID	Unique number generated internally by system.	INTEGER
Event	The telephonic event that can be applied to a donor	VARCHAR(20)

12.TelephoneHistory

- The table will keep a list of telephone correspondence between SANBS and the donor. If the donor phones SANBS that event can also be recorded on the donor's record.

TelephoneHistory	
PK	**TelephoneHistoryID**
FK1 FK2	TelephoneEventID DonorID TelephoneNumber Description UserID TelephoneDate TelephoneDateTS

Field	Description	Field Type
TelephonHistoryID	Unique number generated internally by system.	INTEGER
TelephoneEventID	The telephonic event that can be applied to a donor	VARCHAR(20)
DonorID	The donor record to which the event is linked (Foreign key with Donor table)	INTEGER
TelephoneNumber	The telephone number that was dialled.	VARCHAR(20)
Description	A description of what the call was about.	VARCHAR(200)
UserID	The user who added the telephone event to the donor record.	INTEGER
TelephoneDate	The date on which the call was made or received	DATE
TelephoneDateTS	The date that the above value has been added to the system. Added by system.	DATE

13.DonorHistory

- The table will keep a history of changes made to the donor. The table will have a field for the old value as well as a field for the new changed value.

DonorHistory	
PK	**DonorHistoryID**
FK1	DonorID Field OldValue NewValue DateTS
FK2	UserID

Field	Description	Field Type
DonorHistoryID	Unique number generated internally by system.	INTEGER
DonorID	The donor record to which the event is linked (Foreign key with Donor table)	INTEGER
Field	The field that has changed e.g. donor name , number etc.	VARCHAR(30)
OldValue	The original value of the field that has changed.	VARCHAR(30)
NewValue	The new value of the field that has changed.	VARCHAR(30)
DateTS	The date that the change was made to the system. Added by system.	DATE
UserID	The user who has made the change. (Foreign key with User table)	INTEGER

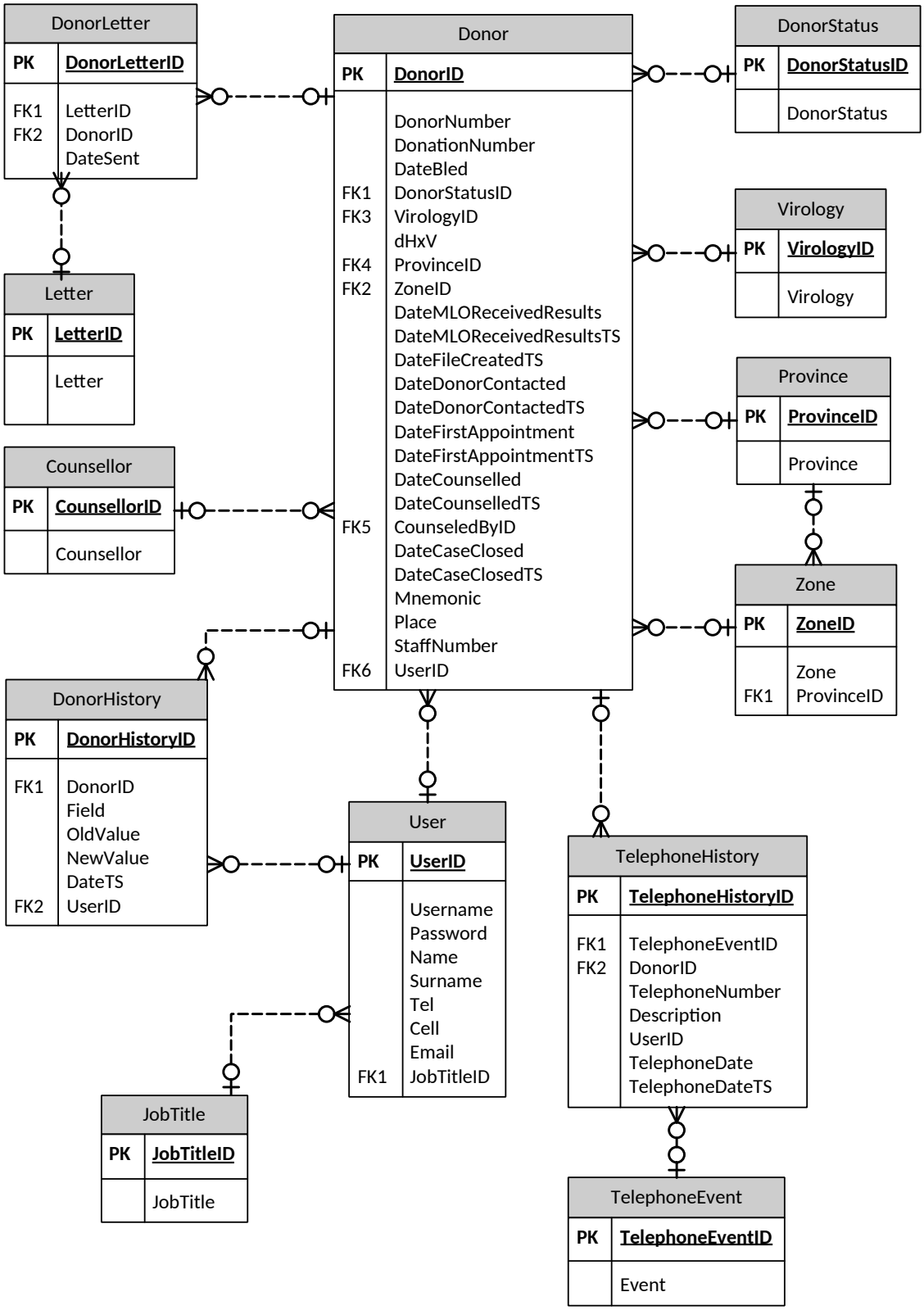
14.Appointment Outcome

- We need a table that can keep track of the outcome of each appointment whether successful or not for example,
    - donor may commit to come, but does not pitch up or
    - donor refuses to come
    - counseled in person
    - counseled and tested

15.Case Outcome

- We need a table that can keep track of the outcome of each case, i.e. when it is “closed”; what was the final outcome of the case, for example:
    - Never located

- Firm refusal
- Passive refusal
- Successful counseling


DonorLetter
PK DonorLetterID
FK1 LetterID
FK2 DonorID
DateSent
Letter
PK LetterID
Letter
Counsellor
PK CounsellorID
Counsellor
Donor
PK DonorID
DonorNumber
DonationNumber
DateBled
FK1 DonorStatusID
FK3 VirologyID
dHxV
FK4 ProvinceID
FK2 ZoneID
DateMLOReceivedResults
DateMLOReceivedResultsTS
DateFileCreatedTS
DateDonorContacted
DateDonorContactedTS
DateFirstAppointment
DateFirstAppointmentTS
DateCounselled
DateCounselledTS
FK5 CounseledByID
DateCaseClosed
DateCaseClosedTS
Mnemonic
Place
StaffNumber
FK6 UserID
DonorStatus
PK DonorStatusID
DonorStatus
Virology
PK VirologyID
Virology
Province
PK ProvinceID
Province
Zone
PK ZoneID
Zone
FK1 ProvinceID
DonorHistory
PK DonorHistoryID
FK1 DonorID
Field
OldValue
NewValue
DateTS
FK2 UserID
User
PK UserID
Username
Password
Name
Surname
Tel
Cell
Email
FK1 JobTitleID
JobTitle
PK JobTitleID
JobTitle
TelephoneHistory
PK TelephoneHistoryID
FK1 TelephoneEventID
FK2 DonorID
TelephoneNumber
Description
UserID
TelephoneDate
TelephoneDateTS
TelephoneEvent
PK TelephoneEventID
Event

C.Timeline:

	Planned Date	Actual Date
Initial Investigation:		
Execution:		
Testing:		
Training:		
Planned Go Live:		

D.Costs:

Resources	Man Days	Estimated Cost	Actual Man Days	Actual Cost
Business Analyst:				
Developer:				
Consultant:				
Total IT Cost				
User:				
Training:				
Other:				
Total Cost				

E.Deliverables:

A database that will contain records for the counselling of marker positive donors. The database will enable SANBS to develop reporting functionality that can be used for statistical purposes.

F.Risk Analysis:

To be defined.

G.Test Plan:

The test plan will be defined in the next draft after the graphical user interface has been scoped.

H.Implementation Issues:

Too early to define as database structure is only being developed.

I.Implementation Plan (Including Training and DRP):

To be defined.

J.Configuration Changes:

A database with the following tables:

- Donor
- DonorStatus
- Virology
- Province
- Zone
- User
- JobTitle
- Counsellor
- Letter
- DonorLetter
- TelephoneEvent
- TelephoneHistory
- DonorHistory

Appendix 7 HIV and HBV Risk Factor Questionnaire

Incident HIV/Hepatitis B Virus infections in South African blood donors:
Behavioural risk factors, genotypes and biological characterization of early infection

Blood Donor Risk Factor Study Questionnaire (English)

SECTION A – GENERAL STUDY DATA

<u>This section is to be completed by the research assistant or other research staff.</u>

A1. Participant ID ***(Internal study number to be assigned by Study Management System)***

__ __ __ __ __ __ __

A2. Participant donor number ***(Number that will link with donor's Meditech info)***

_____

97	Don't Know
98	Refuse to Answer
99	Not Applicable

A3. Blood collection site. ***(Blood collection site neumonic (clinic site code). The neumonic can be mapped back to Branch, Zone or Province – this will have to be coded during analysis phase)***

_____

UID = Concatenated(A3, A1)

A4. Month of interview (Choose one)

01	January
02	February
03	March
04	April
05	May
06	June
07	July
08	August
09	September
10	October
11	November
12	December

A5. Year of interview (please enter four numbers)

__ __ __ __ yyyy

YEAR2 = A5 - 1

A6. Research Staff Initials: __ __ __ __ __

A7. Type of Participant (Choose one)

1	Study group 1 (won't appear on screen: HIV case)
2	Study group 2 (won't appear on screen: HBV case)
3	Study group 3 (won't appear on screen: Control)

INSTRUCTION to RESEARCH STAFF: If study participant is not already sitting at the computer, at this time please make sure the study participant is sitting at the computer and has put the headphones on.

SECTION B - DEMOGRAPHIC DATA

APPEARS ON SCREEN and HEARD if ACASI: This study has been approved by Ethical Committees in South Africa and the USA. This study also has been approved by the Office of Management and Budget; OMB XXXX, OMB approval expires XX, XXXX, 201X.

APPEARS ON SCREEN and HEARD if ACASI: We are asking you to respond as truthfully as you can. Please keep in mind that the questions are part of a scientific study, and the researchers will make every effort to keep your responses confidential. However, there is a small chance that your responses may not be kept confidential, but your name is not collected on the questionnaire and your responses cannot be easily traced back to you. Please answer these questions to the best of your knowledge and as truthfully as you can. You may skip any questions that you are not comfortable answering.

In this section of the questionnaire the research assistant will show you how to use the computer to answer the interview questions. After completing this section, with help from the research assistant, you will be left to complete the interview in private. If you have any questions at any time or are unsure of what to do, please ask for help.

B1. What is your gender? (Choose one)

1	Male
2	Female
3	Transgender
97	Don't Know
98	Refuse to Answer

B2. What is your birth year?

— — — —

9997	Don't Know
9998	Refuse to Answer

B3. What is your birth month? (Choose one)

__ __ January

__ __ February

__ __ March

__ __ April

__ __ May

__ __ June

__ __ July

__ __ August

__ __ September

__ __ October

__ __ November

__ __ December

__ __ Don't Know

__ __ Refuse to Answer

B4. What is your birth day?

__ __

97 Don't Know

98 Refuse to Answer

B5a. What is your country of birth? (Choose one)

1	South Africa
2	Zimbabwe
3	Malawi
4	Mozambique
5	Swaziland
6	Botswana
7	Lesotho
8	Namibia
9	Nigeria
10	Other
97	Don't Know
98	Refuse to Answer

If B5a is not equal to 10, then skip to instruction before B6.

B5b. Please tell us your country of birth.

_____

READ and HEARD: From now on, you will be left alone. It means that you will have total privacy to answer all these questions. Please, if you have any questions call the research assistant for help.

B6. What is your race / ethnic origin? (Choose one)

1	Black
2	White
3	Coloured
4	Asian
6	Other
97	Don't Know
98	Refuse to Answer

B7. What is the primary language that you speak at home? (Choose one)

1	isiZulu
2	isi Xhosa
3	Afrikaans
4	Sepedi
5	Setswana
6	English
7	Sesotho
8	Xitsonga
9	siSwati
10	Tshivenda
11	isiNdebele
12	Other
98	Refuse to Answer

B8. What is your current marital status? (Choose one)

1 Single, never married.

2 Living with partner, but not married.

3 Married to one partner (including traditional marriage).

4 Married to more than one partner (including traditional marriages).

5 Separated/divorced.

6 Widowed.

97 Don't Know

98 Refuse to Answer

B9a. Is your primary sexual partner?

1 Male

2 Female

3 Transgender

4 Do not have a primary sexual partner ***Skip to B10.***

97 Don't Know

98 Refuse to Answer

B9b. Are you currently living with your primary sexual partner? ***(ONLY ask if B8 = 2, 3, or 4)***

0 No

1 Yes

98 Refuse to Answer

B10. What is the highest level of education you have completed? (Choose one)

0 Never been to school

1 Up to Grade 7 / Standard 5

2 Up to Grade 10 / Standard 8

3 Up to Grade 12 / Standard 10

4 Incomplete further degree or qualification (some college or technical school)

5 College or technical qualification

6 University or professional degree

97 Don't Know

98 Refuse to Answer

B11. What is your religion or affiliation? (Choose one)

1. Christianity
2. Islam
3. Hinduism
4. Judaism
5. African traditional beliefs
6. Other faiths
7. No religion

97 Don’t Know

98 Refuse to Answer

B12a. Do you have medical aid?

0 No

1 Yes

98 Refuse to Answer

B12b. Do you self-fund access (pay out of pocket) to private hospital?

0 No

1 Yes

98 Refuse to Answer

B13c. Are you currently working?

1 Yes, self-employed

2 Yes, employed full or part-time

3 No, unemployed ***SKIP to Section C***

97 Don't know

98 Refuse to Answer

B13b. What type of work are you doing?

1 Mining

2 Transport/cargo delivery

3 Military/police

4 Medical/healthcare

5 Business/sales/retail

6 Farming

7 Teacher/ education/ student

8 General labour (domestic worker, gardener, janitorial)

9 Civil service (examples including working in government office, post office)

10 Other

97 Don't Know

98 Refuse to Answer

B13c. ***If B13b=10 Else SKIP*** What is your occupation?

_____

B14a. In the last 6 months have you spent a total of four weeks or more away from your primary residence (home)?

0 No

1 Yes

97 Don't Know

98 Refuse to Answer

If B14a equals 0, then skip to B15a.

B14b. What was the primary purpose for being away during this period?

1 Work

2 Looking for work

3 Holiday

4 Other, specify: _____

B15a. How many nights did you sleep at your primary residence (home) in the past six months?

1 Every night

2 Most nights

3 About half of the nights

4 Fewer than half of the nights

B15b. How long has your pattern of sleeping at your primary residence (home) been the same as it has been in the past six months?

1 It has been like this only in the past 6 months

2 It has been like this for only the past year

3 For one to five years

4 For five years or more

SECTION C - PREVIOUS DONATION AND HIV TESTING

C1. Before your recent donation, have you ever donated blood? (Choose one)

0	No	***Skip to C4***
1	Yes	
97	Don't Know	
98	Refuse to Answer	

C2. Before your recent donation, how many times have you donated blood? (Choose one)

1	1 time
2	2 or more times
97	Don't Know
98	Refuse to Answer

C3. At the time of your donation, were you given information about who should not donate blood? (Check all that apply)

0	No
1	Yes, written information (pamphlets)
2	Yes, through discussion with the donor staff at the donation centre
97	Don't Know
98	Refuse to Answer

C4. Do you know of places in your community where you can be tested free of charge for HIV?

0 No

1 Yes

97 Don't Know

98 Refuse to Answer

C5. Not including HIV testing conducted as part of blood donation, have you ever been tested for HIV? (Choose one)

0 No ***Skip to instruction before D1***

1 Yes

97 Don't Know

98 Refuse to Answer

C6. Not including HIV testing conducted as part of blood donation, how many times have you been tested for HIV? (Choose one)

0 Never

1 1 time

2 2 or more times

97 Don't Know

98 Refuse to Answer

C7a. Not including HIV testing done as part of blood donation, what was the primary reason for the most recent HIV test? (Choose one)

1 Pregnancy care

2 Health insurance

3 Doctor’s order, routine medical care, hospitalisation or surgery

4 I wanted to know my HIV status

5 Other

97 Don't Know

98 Refuse to Answer

If C7a is not equal to 5, then skip to C8a.

C7b. Please tell us the reason for the HIV test.

—————————————————————————————————

C8a. Not including HIV testing done as part of blood donation, where else have you been tested for HIV? (Choose one)

1 General Practitioner

2 Local clinic / health facility

3 Hospital

4 HIV Testing Centre (known as a Voluntary Counseling and Testing or VCT Centre)

5 Other test site

97 Don't Know

98 Refuse to Answer

If C8a is not equal to 5, then skip to Section D.

C8b. Please tell us the other test site.

——————————————————————————————

SECTION D - INCENTIVES AND MOTIVATIONS FOR DONATING

READ and HEARD: The following questions will ask you about things that you may do in your daily life.

D1. Apart from your involvement in blood donation, do you do any volunteer work for any clubs, groups, societies or religious groups in your community?

0 No

1 Yes

97 Don't Know

98 Refuse to Answer

READ and HEARD: The following questions will ask you about factors that may have influenced your decision to donate blood. Use the following scale to indicate how much the factors influenced your decision to donate blood. 1- Not at all, 2 - Very little, 3 - Somewhat, 4 - Very much

D2a. My decision to donate blood was influenced by my desire to anonymously help someone else who needs blood. (Choose one)

1 Not at all

2 Very little

3 Somewhat

4 Very much

97 Don't Know

98 Refuse to Answer

D2b. My decision to donate blood was influenced by my desire to help a friend or relative who is sick or needs blood. (Choose one)

1	Not at all
2	Very little
3	Somewhat
4	Very much
97	Don't Know
98	Refuse to Answer

D2c. My decision to donate blood was influenced by a campaign on TV or radio. (Choose one)

1	Not at all
2	Very little
3	Somewhat
4	Very much
97	Don't Know
98	Refuse to Answer

D2d. My decision to donate blood was influenced by a telephone call or sms (text message) from the blood bank asking me to donate. (Choose one)

1	Not at all
2	Very little
3	Somewhat
4	Very much
97	Don't Know
98	Refuse to Answer

D2e. My decision to donate blood was influenced by my belief that it is important to give blood. (Choose one)

1	Not at all
2	Very little
3	Somewhat
4	Very much
97	Don't Know
98	Refuse to Answer

D2f. My decision to donate blood was influenced by my desire to get my blood test results. (Choose one)

1	Not at all
2	Very little
3	Somewhat
4	Very much
97	Don't Know
98	Refuse to Answer

D2g. My decision to donate blood was influenced by my belief that my blood type is in high demand. (Choose one)

1	Not at all
2	Very little
3	Somewhat
4	Very much
97	Don't Know
98	Refuse to Answer

D2h. My decision to donate blood was influenced by my belief that I am doing something important for society. (Choose one)

1	Not at all
2	Very little
3	Somewhat
4	Very much
97	Don't Know
98	Refuse to Answer

D2i. My decision to donate blood was influenced by my belief that I may need blood myself someday. (Choose one)

1	Not at all
2	Very little
3	Somewhat
4	Very much
97	Don't Know
98	Refuse to Answer

D2j. My decision to donate blood was influenced by my belief that blood donation is good for my health. (Choose one)

1	Not at all
2	Very little
3	Somewhat
4	Very much
97	Don't Know
98	Refuse to Answer

D2k. My decision to donate blood was influenced by my desire to know about my health and blood donation is a good way to find this out. (Choose one)

1	Not at all
2	Very little
3	Somewhat
4	Very much
97	Don't Know
98	Refuse to Answer

D2l. My decision to donate blood was influenced by someone offering me an incentive to donate. (Choose one)

1	Not at all
2	Very little
3	Somewhat
4	Very much
97	Don't Know
98	Refuse to Answer

D2m. My decision to donate blood was influenced by my belief that blood banks always need blood and so donating is the right thing to do. (Choose one)

1	Not at all	
2	Very little	
3	Somewhat	
4	Very much	
97	Don't Know	
98	Refuse to Answer	

D2n. My decision to donate blood was influenced by pressure to donate that I received from other people (such as friends, family, colleagues, fellow students, church or temple members).

1	Not at all	
2	Very little	
3	Somewhat	
4	Very much	
97	Don't Know	
98	Refuse to Answer	

D2o. Is there another reason that best explains why you came to donate? (Choose one)

0	No	***Skip to D3***
1	Yes	
97	Don't Know	***Skip to D3***
98	Refuse to Answer	***Skip to D3***

D2p. What is the reason that best explains why you came to donate?

____

D3. Some people feel they must donate blood because family, friends, co-workers or other people in an organization they know donate or encourage others to donate blood. Did this happen to you when you last donated blood? (Choose one)

0	No
1	Yes
97	Don't Know
98	Refuse to Answer

D4. Do you believe that the blood service uses better HIV tests than are available at other places? (Choose one)

0	No
1	Yes
97	Don't Know
98	Refuse to Answer

D5. Did you donate blood mainly because you wanted to be tested for HIV? (Choose one)

0	No	***Skip to D8***
1	Yes	
97	Don't Know	
98	Refuse to Answer	

D6. What were all the factors that contributed to your decision to come to the blood centre to be tested for HIV? Please check all the boxes that apply to your answer. When you have selected all of your answers, please touch the "Next Question" box. (Check all the answers that apply)

a __ Only place I know of that offers HIV testing

b __ HIV testing is free

c __ HIV testing is confidential

d __ HIV testing is more accurate than at other sites

e __ HIV testing is more convenient than at other test sites

f __ Other reason

e __ Don't Know

g __ Refuse to Answer

If D6_f is equal to 0, then skip to D8.

D7. Please specify other reason

__ __

D8. Did you donate blood mainly because you wanted to be tested for hepatitis? (Choose one)

0 No ***Skip to D11***

1 Yes

97 Don't Know

98 Refuse to Answer

D9. What were all the factors that contributed to your decision to come to the blood centre to be tested for hepatitis? Please check all the boxes that apply to your answer. When you have selected all of your answers, please touch the "Next Question" box. (Check all that apply)

__ Only place I know of that offers Hepatitis testing

__ Hepatitis testing is free

__ Hepatitis testing is confidential

__ Hepatitis testing is more accurate than at other sites

__ Hepatitis testing is more convenient than at other sites

__ Other reason

__ Don't Know

__ Refuse to Answer

If D9_f is equal to 0, then skip to D13.

D10. Please specify other reason

____

D11. Did a health worker such as a doctor, nurse, or someone from a clinic suggest that you go to the blood centre for a blood test for HIV, hepatitis, or for some other reason? (Choose one)

0	No	***Skip to instruction before D13***
1	Yes	
97	Don't Know	
98	Refuse to Answer	

D12. Please tell us who suggested you come to the blood service for a blood test? (Choose one)

1 Doctor

2 Nurse

3 Someone else from the clinic

4 Other

97 Don't Know

98 Refuse to Answer

READ and HEARD: Now we will ask a few questions about what you think about HIV and HBV risks and donating: In the following section, please indicate whether you think each statement is true or false.

D13. You can donate blood if you have engaged in risk behaviors for HIV/AIDS because the blood service tests all blood and throws away any infected blood. (Choose one)

1 True

2 False

97 Don't Know

98 Refuse to Answer

D14. You can donate blood even if you engage in risk behaviors for HIV/AIDS as long as you have a negative HIV test. (Choose one)

1 True

2 False

7 Don't Know

8 Refuse to Answer

D15. You can donate blood even if you engage in risk behaviors for HIV/AIDS as long as you use condoms. (Choose one)

1 True

2 False

97 Don't Know

98 Refuse to Answer

D16. The blood test for HIV identifies everyone who is infected with the virus. (Choose one)

1 True

2 False

97 Don't Know

98 Refuse to Answer

D17. The blood test for hepatitis identifies everyone who is infected with a hepatitis virus. (Choose one)

1 True

2 False

97 Don't Know

98 Refuse to Answer

SECTION E - SEXUAL HISTORY

READ and HEARD: Now, we want to ask about the people you have had sex with and your sexual partners. We understand that these questions are about intimate and private matters, which could make you uncomfortable. Please keep in mind that the questions are part of a scientific study, and the researchers will keep your responses confidential. Your individual responses will not be known by SANBS. Please answer these questions to the best of your knowledge and as truthfully as you can.

E1. Do you consider yourself to be? (Choose one)

1	Straight/heterosexual
2	Bisexual
3	Gay/homosexual
97	Don't Know
98	Refuse to Answer

READ and HEARD: The following questions will ask you about your sexual experiences. In these questions, include only those people you have had oral, vaginal, or anal sex with. Do not include people that you have only kissed. Please try to be as accurate and honest as possible. If you cannot remember the precise answers below, please provide your best estimates.

Please note: For the next few questions the terms "sexual contact" and "sex" refer to any of the following activities, whether or not a condom or other protection was used:

1. ***Vaginal sex (contact between penis and vagina)***
2. ***Oral sex (mouth or tongue on someone's vagina, penis, or anus)***
3. ***Anal sex (contact between penis and anus)***

E2a. (Ask of Men Only) How many different women have you had sex with since you first began having sex?

— — — —

9997	Don't Know
9998	Refuse to Answer
9999	Not Applicable

E2a.1 ***If Answer is Don't Know, Else Skip.*** If you Don't Know, can you give an approximate answer? __ _

E2b. (Ask of Men Only) How old were you when you had sex with a woman for the first time?

__ __

9997 Don't Know

9998 Refuse to Answer

9999 Not Applicable

E3a. (Ask of Men Only) How many different men have you had sex with since you first began having sex?

__ __ __ __

9997 Don't Know

9998 Refuse to Answer

9999 Not Applicable

E3a.1 ***If Answer is Don't Know, Else Skip.*** If you Don't Know, can you give an approximate answer? __ _

E3b. (Ask of Men Only) How old were you when you had sex with a man for the first time?

__ __

9997 Don't Know

9998 Refuse to Answer

9999 Not Applicable

E2a. (Ask of Women Only) How many different men have you had sex with since you first began having sex?

__ __ __ __

9997 Don't Know

9998 Refuse to Answer

9999 Not Applicable

E2a.1 ***If Answer is Don't Know, Else Skip.*** If you Don't Know, can you give an approximate answer? __ _

E2b. (Ask of Woman Only) How old were you when you had sex with a man for the first time?

__ __ __ __

9997 Don't Know

9998 Refuse to Answer

9999 Not Applicable

E3a. (Ask of Women Only) How many different women have you had sex with since you first began having sex?

__ __ __ __

9997 Don't Know

9998 Refuse to Answer

9999 Not Applicable

E3a.1 ***If Answer is Don't Know, Else Skip.*** If you Don't Know, can you give an approximate answer? __ _

E3b. (Ask of Women Only) How old were you when you had sex with a woman for the first time?

__ __ __ __

9997 Don't Know

9998 Refuse to Answer

9999 Not Applicable

E4a. Have you ever been physically abused, physically assaulted, or beaten by a sexual partner?

0 No ***Skip to E5***

1 Yes

7 Don't Know

8 Refuse to Answer

E4b. In the six months before your donation were you physically abused, physically assaulted or beaten by a sexual partner?

0 No

1 Yes

7 Don't Know

8 Refuse to Answer

If E2a is equal to 0 and E3a is equal to 0 and B1 is equal to 1, then skip to Section H.

If E2a is equal to 0 and E3a is equal to 0 and B1 is equal to 2, then skip to Section H.

E5a. Have you ever been sexually abused, sexually assaulted or forced to have any kind of sex when you did not want to?

0 No ***Skip to Next Section.***

1 Yes

7 Don't Know

8 Refuse to Answer

E5b. In the six months before your donation were you sexually abused, sexually assaulted or forced to have any kind of sex when you did not want to?

0 No

1 Yes

7 Don't Know

8 Refuse to Answer

E5c. In the six months before your donation were you sexually abused, sexually assaulted or forced to have any kind of sex when you did not want to with someone you consider to be an intimate partner, such as a spouse, husband, wife, boyfriend or girlfriend?

0 No

1 Yes

7 Don't Know

8 Refuse to Answer

SECTION F – SOCIAL/SEXUAL MATRIX

READ and HEARD: This next set of questions is about sexual experiences you may have had. While some people have had a lot of sexual experience, others have not, so questions may or may not apply to you. Please answer these questions as accurately as possible. Remember that answers that you provide will be combined with those from all other people who complete the questionnaire and we will never disclose individual responses to any question. Specifically, we will ask about sexual activities that include vaginal, anal and/or oral intercourse. Please answer these questions to the best of your knowledge and as truthfully as you can.

F1. How many people did you have sex with in the 12 months before your blood donation?

__ __

97 Don't Know

98 Refuse to Answer

99 Not Applicable

If F1 is equal to 0, then skip to instruction Section H.

F2. How many people did you have sex with in the 6 months before your donation? ***(THIS INFORMATION DOES NOT APPEAR TO THE RESPONDEN: This is the seed number for the social matrix (0 up to 5 partners in last six months - persons with more than 5 partners will only be asked about 5 partners).***

__ __

97 Don't Know

98 Refuse to Answer

99 Not Applicable

READ and HEARD: Now, we want to ask about the people you have had sex with and your sexual partners. If you had more than five partners in the 6 months before your blood donation, we are only going to ask you about the five most recent people you have had sex with and your sexual partners. Please start with the most recent person you had sex with before your blood donation and then move back in time.

F3. What is Partner 1's gender?

1 Male

2 Female

3 Transgender

97 Don't Know

98 Refuse to Answer

F4. How old is partner 1?

__ __

97 Don't Know

98 Refuse to Answer

F5. When did your relationship with partner 1 start? We recognize that you may not remember the exact date and so we are asking that you try to recall the month and the year.

Month _____ Year _____

97 Don't Know

98 Refuse to Answer

99 Not Applicable

F6a. Are you currently have sex with partner 1?

0 No

1 Yes

97 Don't Know

98 Refuse to Answer

99 Not Applicable

If F6a = yes, skip to F7

F6b When did your relationship with partner 1 end? We recognize that you may not remember the exact date and so we are asking that you try to recall the month and the year.

Month _____ Year _____

97 Don't Know

98 Refuse to Answer

99 Not Applicable

F7. What type of partner is partner 1? (Choose one)

1 Primary partner - Your husband, wife, boyfriend or girlfriend

2 Regular partner – Had sex with regularly, but not your primary partner (husband, wife, boyfriend or girlfriend)

3 Casual partner – Had sex more than once but not regularly

4 One time partner – Had sex only once

5 Anonymous partner – Did not know, met for sex, never plan to see again

6 Sex worker – Money or other goods were exchanged for sex

97 Don't Know

98 Refuse to Answer

F8. How would you describe partner 1's race or ethnicity? (Choose one)

1	Black
2	White
3	Coloured
4	Asian
6	Other
97	Don't Know
98	Refuse to Answer

F9. What country is partner 1 from?

1	South Africa
2	Zimbabwe
3	Malawi
4	Mozambique
5	Swaziland
6	Botswana
7	Lesotho
8	Namibia
9	Nigeria
10	Other
97	Don’t Know

98 Refuse to Answer

If F9 is equal to South Africa skip to F9b which province, Else skip to F10.

If F9 is equal to Other skip to F9c, Else skip to F10.

F9b. Which province in South Africa is partner 1 from?

1 Eastern Cape

2 Free State

3 Gauteng

4 KwaZulu Natal

5 Limpopo

6 Mpumalanga

7 North West

8 Northern Cape

9 Western Cape

97 Don't Know

98 Refuse to answer

F9c. Please specify which country partner 1 is from:

_ _

F10a. Do you live with partner 1?

1 Yes

2 No

99 Refuse to answer

F10b. Is partner 1 currently working (Choose one)?

1 Yes, self-employed

2 Yes, employed full or part-time

3 No, unemployed ***SKIP to F11***

97 Don't know

98 Refuse to Answer

F10c. What type of work is partner 1 doing?

0	Mining
1	Transport/cargo delivery
2	Military/police
3	Medical/healthcare
4	Business/sales/retail
5	Farming
6	Teacher/ education/ student
7	General labour (domestic worker, gardener, janitorial)
8	Civil service (examples including working in government office, post office)
9	Other
97	Don't Know
98	Refuse to Answer

F10d. ***If F10c=10 Else SKIP*** If Other, What is partner 1's occupation?

__ __

If F1a is equal to 1 and F1b is equal to 0, then skip to Section G.

F12. What is partner 1's HIV status? (Choose one)

1 Positive

2 Negative

3 Unknown

98 Refuse to Answer

If F12 is not equal to 1, skip to F14

F13a. Does partner 1 take HIV medications? (Choose one)

0 No

1 Yes

97 Don't Know

98 Refuse to Answer

F14. Does partner 1 have hepatitis?

1 Yes

2 No

97 Don’t Know

98 Refused to answer

F15. Did you become sexually involved with partner 1 because you thought that he/she would provide you with some material benefit that you wanted or needed, such as food, shelter, transport, school fees, or other goods?

0 No

1 Yes

97 Don't Know

98 Refuse to Answer

99 Not Applicable

F15a. If yes, can you tell us what you wanted or needed? It could be food, clothes, transport, school fees, residence, fees, somewhere to stay or sleep, alcohol, drugs, cash or something else.

_____

F16. Where did you first meet partner 1? (Choose one)

1 Bar, nightclub, restaurant, tavern, shebeen, coffee shop

2 Strip club, “Adults Only” club, sex club

3 Street, park, library, public transportation, minibus taxi, train

4 Party, braai, political function, stokvel or church

5 Internet

6 Dating service, newspaper ads

7 Gym, sports event

8 School, technicon, university, college

9 Work

10 Met some other way

97 Don't Know

98 Refuse to Answer

If F16 is not equal to 10, then skip to instruction before F17.

F16a. Please tell us where you met partner 1:

_ — _

If B1 is equal to 1 and F3 is equal to 1, then skip to F19

F17. Number of times you had vaginal sex with partner 1 in the 6 months before donation. (Choose one)

__ none

__ 1 to 3 times

__ 4 to 10 times

__ more than 10 times

__ Don't Know

__ Refuse to Answer

If F17 is equal to none, then skip to F19.

F18. When you had vaginal sex, how frequently did you use condoms? (Choose one)

__ never

__ some of the times

__ every time

__ Don't Know

__ Refuse to Answer

F19. Number of times you had anal sex with partner 1 in 6 months before your donation? (Choose one)

__ none

__ 1 to 3 times

__ 4 to 10 times

__ more than 10 times

__ Don't Know

__ Refuse to Answer

If F19 is equal to none, then skip to F25.

F20. When you had anal sex, how frequently did you use condoms? (Choose one)

__ never

__ some of the times

__ every time

__ Don't Know

__ Refuse to Answer

If F3 is equal to 2, then skip to F23.

F21. Number of times you had insertive anal sex (inserted your penis in partner 1's anus) in the 6 months before your donation? (Choose one)

__ none

__ 1 to 3 times

__ 4 to 10 times

__ more than 10 times

__ Don't Know

__ Refuse to Answer

If F21 is equal to none, then skip to F24.

F22. When you had insertive anal sex, how frequently did you use condoms? (Choose one)

__ never

__ some times

__ every time

__ Don't Know

__ Refuse to Answer

F23. Number of times you had receptive anal sex (partner 1 inserted his penis into your anus) in the past 6 months. (Choose one)

__ none

__ 1 to 3 times

__ 4 to 10 times

__ more than 10 times

__ Don't Know

__ Refuse to Answer

If F23 is equal to 0, then skip to F25.

F24. When you had receptive anal sex, how frequently did you use condoms? (Choose one)

__ never

__ some times

__ every time

__ Don't Know

__ Refuse to Answer

F25. Number of times you had oral sex with partner 1 in past 6 months

__ none

__ 1 to 3 times

__ 4 to 10 times

__ more than 10 times

__ Don't Know

__ Refuse to Answer

Sexual Matrix is completed with same questions for up to 4 additional sexual partners.

READ and HEARD: The next set of questions is about your SECOND sexual partner, BEFORE your blood donation.

READ and HEARD: The next set of questions is about your THIRD sexual partner, BEFORE your blood donation.

READ and HEARD: The next set of questions is about your FOURTH sexual partner, BEFORE your blood donation.

READ and HEARD: The next set of questions is about your FIFTH sexual partner, BEFORE your blood donation.

SECTION G - SEXUAL PARTNER RISKS

If E2a is equal to 0 and E3a is equal to 0 and B1 is equal to 1, then skip to Section H.

If E2a is equal to 0 and E3a is equal to 0 and B1 is equal to 2, then skip to Section H.

READ and HEARD: Now, we want to ask about some of the behaviors of the people you have had sex with and your sexual partners. As before when answering, please consider only the following activities, whether or not a condom or other protection was used:

1. ***Vaginal sex (contact between penis and vagina)***
2. ***Oral sex (mouth or tongue on someone's vagina, penis, or anus)***
3. ***Anal sex (contact between penis and anus)***

You may not know the answers to these questions. Please answer these questions to the best of your knowledge. Several of the questions refer to the period of time in the "six months before your blood donation". This means before your most recent blood donation.

G1a. To the best of your knowledge, have you ever had sex with anyone who was an injection drug user? (Choose one)

0	No	***Skip to G2a***
1	Yes	
97	Don't Know	
98	Refuse to Answer	

G1b. In the 6 months before your donation, have you had sex with anyone who was an injection drug user? (Choose one)

0	No
1	Yes
97	Don't Know
98	Refuse to Answer

G2a. To the best of your knowledge, have you ever had sex with anyone who tested positive for HIV? (Choose one)

0	No	***Skip to instruction before G4a***
1	Yes	
97	Don't Know	
98	Refuse to Answer	

G2b. In the 6 months before your donation, have you had sex with anyone who tested positive for HIV? (Choose one)

0	No
1	Yes
97	Don't Know
98	Refuse to Answer

G3a. To the best of your knowledge, have you ever had sex with a man who has had sex with another man? (Choose one)

0	No	***Skip to G4a***
1	Yes	
97	Don't Know	
98	Refuse to Answer	

G3b. In the 6 months before your donation, have you had sex with a man who has had sex with another man? (Choose one)

0	No
1	Yes

97 Don't Know

98 Refuse to Answer

G4a. To the best of your knowledge, have you ever had sex with anyone who received a blood transfusion? (Choose one)

0 No ***Skip to G5a***

1 Yes

97 Don't Know

98 Refuse to Answer

G4b. In the 6 months before your donation, have you had sex with anyone who received a blood transfusion? (Choose one)

0 No

1 Yes

97 Don't Know

98 Refuse to Answer

G5a. To the best of your knowledge, have you ever had sex with a person with haemophillia? (Choose one)

0 No ***Skip to G6a***

1 Yes

97 Don't Know

98 Refuse to Answer

G5b. In the 6 months before your donation, have you had sex with a person with haemophillia? (Choose one)

0 No

1 Yes

97 Don't Know

98 Refuse to Answer

G6a. To the best of your knowledge, have you ever had sex with anyone who has spent three or more nights in jail, prison, or a detention centre? (Choose one)

0 No ***Skip to G7a***

1 Yes

97 Don't Know

98 Refuse to Answer

G6b. In the 6 months before your donation, have you had sex with anyone who has spent three or more nights in jail, prison, or a detention centre? (Choose one)

0 No

1 Yes

97 Don't Know

98 Refuse to Answer

G7a. To the best of your knowledge, have you ever had sex with anyone who had a job that involved exposure to human blood or other body fluids? (Choose one)

0	No	***Skip to Section H***
1	Yes	
97	Don't Know	
98	Refuse to Answer	

G7b. In the 6 months before your donation, have you had sex with anyone who has a job that involves exposure to human blood or other body fluids? (Choose one)

0	No
1	Yes
97	Don't Know
98	Refuse to Answer

SECTION H – ALCOHOL AND DRUG USE

READ and HEARD: Now we are going to ask you some general questions regarding alcohol and drug use. Some types of alcohol or drug use can be related to the risk of becoming infected with HIV or HBV. Once again, the following questions are intended to help us determine how to improve the safety of donated blood. We are asking you to respond as truthfully as you can. The answers are confidential. Your individual responses will not be known by SANBS. Your answers will be reported together with all other people who complete the questionnaire.

H1. How often do you drink beer, wine, liquor, or mixed drinks? (Choose one)

0	Never	***Skip to H4***
1	1-3 times per month or less	
2	1-2 times per week	
3	3-6 times per week	
4	Everyday	
97	Don't Know	
98	Refuse to Answer	

H2. On average how many drinks do you have each time you drink? (When answering, one drink is a glass of wine or a beer or a mixed drink.)

Number of drinks __ __ __

997 Don't Know

H3a. Have you ever used dagga, marijuana, or hashish? (Choose one)

0	No	***Skip to H4a***
1	Yes	
97	Don't Know	
98	Refuse to Answer	

H3b. When was the first year you used dagga, marijuana or hashish?

__ __ __ __ yyyy

2097 Don't Know (Year)

2098 Refuse to Answer (Year)

If H3b is less than B2 then READ and HEARD: "The year that you entered for first year of dagga, marijuana, or hashish drug use is smaller than the year you were born. Please correct the year of your first dagga, marijuana, or hashish drug use." (Returns to H3b)

H3c. When was the last year you used dagga, marijuana or hashish?

__ __ __ __ yyyy

2097 Don't Know (Year)

2098 Refuse to Answer (Year)

If H3c is less than H3b then READ and HEARD: "The year that you entered for last year of dagga, marijuana, or hashish drug use is smaller than the year of your first reported dagga, marijuana, or hashish drug use. Please correct the last year of your dagga, marijuana, or hashish drug use." (Returns to H3c)

H4a. Have you ever used whoonga which is also known as nyaope? (Choose one)

0 No ***Skip to H5a***

1 Yes

97 Don't Know

98 Refuse to Answer

H4b. When was the first year you used whoonga or nyaope?

__ __ __ __ yyyy

2097 Don't Know (Year)

2098 Refuse to Answer (Year)

If H4b is less than B2 then READ and HEARD: "The year that you entered for first year of whoonga or nyaope drug use is smaller than the year you were born. Please correct the year of your first whoonga or nyaope drug use." (Returns to H4b)

H4c. When was the last year you used whoonga or nyaope?

__ __ __ __ yyyy

2097 Don't Know (Year)

2098 Refuse to Answer (Year)

If H4c is less than H4b then READ and HEARD: "The year that you entered for last year whoonga or nyaope drug use is smaller than the year of your first reported whoonga or nyaope drug use. Please correct the last year of your whoonga or nyaope drug use." (Returns to H4c)

H5a. Have you ever used any non-injected drugs (drugs that are smoked, snorted or taken orally), examples include tik, mandrax, “glue”, cocaine (crack), methamphetamines (crystal), ecstasy ("E") and LSD? (Choose one)

0 No ***Skip to H7***

1 Yes

97 Don't Know

98 Refuse to Answer

H5b. When was the first year you used non-injected drugs?

__ __ __ __ yyyy

2097 Don't Know (Year)

2098 Refuse to Answer (Year)

If H5b is less than B2 then READ and HEARD: "The year that you entered for first year of non-injected drug use is smaller than the year you were born. Please correct the year of your first non-injected drug use." (Returns to H5b)

H5c.When was the last year you used non-injected drugs?

__ __ __ __ yyyy

2097 Don't Know (Year)

2098 Refuse to Answer (Year)

If H5c is less than H5b then READ and HEARD: "The year that you entered for last year non-injected drug use is smaller than the year of your first reported non-injected drug use. Please correct the last year of your non-injected drug use." (Returns to H5c)

H6. If you have smoked or snorted drugs, did you share pipes or straws with another person? (Choose one)

1 Always

2 Sometimes

3 Never

97 Don't Know

98 Refuse to Answer

H7a. Have you ever used injection drugs (examples include heroin, cocaine, and amphetamines)? (Choose one)

0 No

1 Yes

97 Don't Know

98 Refuse to Answer

H7b. When was the first year you used injected drugs?

__ __ __ __ yyyy

2097 Don't Know (Year)

2098 Refuse to Answer (Year)

If H7b is less than B2 then READ and HEARD: "The year that you entered for first year of injected drug use is smaller than the year you were born. Please correct the year of your first injected drug use." (Returns to H7b)

H7c. When was the last year you used injected drugs?

__ __ __ __ yyyy

2097 Don't Know (Year)

2098 Refuse to Answer (Year)

If H7c is less than H7b then READ and HEARD: "The year that you entered for last year of injected drug use is smaller than the year of your first reported injected drug use. Please correct the last year of your injected drug use." (Returns to H7c)

H8a. Have you ever injected yourself with any substances including vitamins, steroids, or hormones which were not prescribed by a doctor or nurse? (Choose one)

0 No ***Skip to Section I***

1 Yes

97 Don't Know

98 Refuse to Answer

H8b. Have you ever shared needles or syringes with another person to inject yourself with any substance including drugs, vitamins, steroids or hormones which were not prescribed by a doctor? (Choose one)

0	No
1	Yes
97	Don't Know
98	Refuse to Answer

SECTION I - MEDICAL HISTORY

READ and HEARD: In the next set of questions we will ask about some medical treatments you may have had.

I1. Have you ever had a blood transfusion? (Choose one)

0	No	***Skip to I5***
1	Yes	
97	Don't Know	
98	Refuse to Answer	

I2. How many transfusion episodes have you had?

__ __

97 Don't Know

98 Refuse to Answer

I3. When was the <u>first year</u> you received a transfusion?

__ __ __ __

9997 Don't Know

9998 Refuse to Answer

If I3 is less than B2 then READ and HEARD: "The year that you entered for first year transfusion is smaller than the year you were born. Please correct the year of your first transfusion." (Returns to I3)

I4. When was the last year you received a transfusion?

__ __ __ __

9997 Don't Know

9998 Refuse to Answer

If I4 is less than I3 then READ and HEARD: "The year that you entered for last year of transfusion is smaller than the year of your first reported transfusion. Please correct the last year of your transfusion." (Returns to I4)

I5. Have you ever had minor or major surgery in a hospital, doctor's room, or dentist's office? (Choose one)

0	No	***Skip to I9***
1	Yes	
97	Don't Know	
98	Refuse to Answer	

I6. In the 6 months before your donation, have you had minor or major medical surgery in hospital? (Choose one)

0	No
1	Yes
97	Don't Know
98	Refuse to Answer

I7. In the 6 months before your donation, have you had any surgical procedures in your doctor's room or office? (Choose one)

0 No

1 Yes

97 Don't Know

98 Refuse to Answer

I8. In the 6 months before your donation, have you had any tooth extractions or another dental procedure at a dentist's office? (Choose one)

0 No

1 Yes

97 Don't Know

98 Refuse to Answer

I9. Have you ever had an endoscopy (a medical test where a flexible tube is used to look inside of your throat and digestive system) or colonoscopy (a medical test where tube is used to look inside your colon/large intestine)? (Choose one)

0 No ***Skip to I11***

1 Yes

97 Don't Know

98 Refuse to Answer

I10. In the 6 months before your donation, have you had an endoscopy or colonoscopy? (Choose one)

0 No

1 Yes

7 Don't Know

8 Refuse to Answer

I11. Have you ever been immunized / vaccinated against Hepatitis B?

0 No

1 Yes

97 Don't Know

98 Refuse to Answer

I11b. If yes, can you tell us your age when you were immunized/vaccinated against Hepatitis B?

_____ years old

I12. In the 6 months before your donation, did you receive an injection from a traditional healer for any reason? (Choose one)

0 No

1 Yes

97 Don't Know

98 Refuse to Answer

SECTION J- OTHER POTENTIAL RISK FACTORS

READ and HEARD: The next set of questions will ask you about situations or activities that could be important for our research to improve blood safety at SANBS. Some of the questions cover topics that are very personal. Please respond as accurately as you can. You may skip any question you are not comfortable answering.

J1. In the 6 months before your donation, have you spent three or more nights in jail, prison, or a detention centre? (Choose one)

0	No
1	Yes
97	Don't Know
98	Refuse to Answer

J2. In the 6 months before your donation, have you had acupuncture treatments? (Choose one)

0	No	***Skip to J5***
1	Yes	
97	Don't Know	
98	Refuse to Answer	

J3. Who performed the acupuncture treatments? (Check all that apply)

0	A registered health care professional (such as physiotherapist, chiropractor)
1	Chinese/ Asian healer
2	Traditional healer
3	Other
97	Don’t Know

98 Refuse to Answer

J4. In the 6 months before your donation, how many times have you had acupuncture treatments? (Choose one)

1 1 time

2 2 to 5 times

3 5 or more times

97 Don't Know

98 Refuse to Answer

J5. How many tattoos do you have on your body? (Choose one)

0 0 (No tattoos) ***Skip to J8***

1 1

2 2

3 3 or more

97 Don't Know

98 Refuse to Answer

J6. In the 6 months before your donation, have you gotten a new tattoo or had one re-applied? (Choose one)

0 No

1 Yes

97 Don't Know

98 Refuse to Answer

J7. Where did you get your most recent tattoo? (Choose one)

1	Tattoo parlor
2	Informal tattoo parlor (e.g. fleamarket or street fair)
3	At home, a friends place, or at parties/raves
4	Jail, prison or detention centre
5	Other
97	Don't Know
98	Refuse to Answer

J8. How many ear or body piercings do you have? (Choose one)

0	0 (No piercings)	***Skip to J11***
1	1	
2	2	
3	3 or more	
97	Don't Know	
98	Refuse to Answer	

J9. In the 6 months before your donation, have you had new ear or body piercings? (Choose one)

0	No
1	Yes
97	Don't Know
98	Refuse to Answer

J10. Where did you get your most recent piercing? (Choose one)

1 Pharmacy or medical clinic

2 Tattoo/piercing parlor

3 At home, a friends place, or at parties/raves

4 Informal ear-piercing (for example at a flea market or street fair)

5 Jail

6 Other

97 Don't Know

98 Refuse to Answer

J11. Have you ever had a manicure or pedicure at a beauty salon or had a shave at a barbershop? (Choose one)

0 No ***Skip to instruction before J14***

1 Yes

97 Don't Know

98 Refuse to Answer

J12. How many times have you had manicures or pedicures or shaves at a beauty salon or barbershop? (Choose one)

1 1 time

2 2 to 5 times

3 5 or more times

97 Don't Know

98 Refuse to Answer

J13. In the 6 months before your donation, have you had a manicure or pedicure at a beauty salon or had a shave at a barber shop? (Choose one)

0 No

1 Yes

97 Don't Know

98 Refuse to Answer

J14a. Have you been circumcised? (Ask of men only – should we make this not gender specific?)

0 No ***Skip to J17***

1 Yes

97 Don't Know

98 Refuse to Answer

J15b. At what age were you circumcised?

_ _ _

97 Don’t Know

98 Refuse to Answer

J15c. Was your circumcision performed in the 6 months before your donation?

0 No

1 Yes

97 Don't Know

98 Refuse to Answer

J15d. Was the circumcision performed in hospital?

0 No

1 Yes ***Skip to J17***

97 Don't Know

98 Refuse to Answer

J15e. Who performed the circumcision performed?

0 General practitioner in doctor's room

1 Traditional surgeon/healer using old methods

2 Traditional surgeon/healer using new methods (using sterile techniques)

97 Don't know

98 Refused to Answer

J16a. (***If female (B2=2)***) In the 6 months before your donation, how often have you used anything to dry, clean, or tighten your vagina before or after having sex?

1 Every time

2 Some times

3 Once

4 Never ***Skip to J17***

5 Not Applicable, did not have vaginal sex in the 6 months before my donation ***Skip to J17***

97 Refused to answer ***Skip to J17***

J16b. Please check all the things that you have used to dry, clean or tighten your vagina.

1 Water

2 Ice

3 Soap

4 Douche

5 Detergents

6 Disinfectants

7 Acids

8 Salt

9 Herbs/snuff

10 Medications

11 Cloth

12 Paper

13 Tissues

14 A gel used as part of a research study

15 Other (please specify);_____

J17a. In the 6 months before your donation, how often have you used anything to dry, clean, or tighten your anus before or after having sex?

1 Every time

2 Some times

3 Once

4 Never ***Skip to J25***

5 Not Applicable, did not have anal sex in the 6 months prior to my last donation ***Skip to J24***

97 Refused to answer ***Skip to J25***

J17b. Please check all the things that you have used to dry, clean or tighten your anus before having sex.

1 Water

2 Ice

3 Soap

4 Douche

5 Herbs/snuff

6 A gel used as part of a research study

7 Other (please specify);_____

J18a. In the 6 months before your donation, have you had Raatib, ritual scarring, ritual piercing, ritual circumcision, blood sharing or been stabbed?

0	No	***Skip to J20***
1	Yes	
97	Don't Know	
98	Refuse to Answer	

J18b. Can you please tell us which activity? (Check all that apply)

1 Raatib

2 Ritual scarring

3 Ritual piercing

4 Ritual circumcision

5 Blood sharing

6 Been stabbed

7 Other (please specify);_____

J19. What was the reason for the activity?

1 Cultural, ritual, religious

2 Administering a substance in my skin for treatment or prevention

3 Letting out blood

4 Sealing a “blood pact”

5 Other

97 Don't Know

98 Refuse to Answer

J20. In the 6 months before your donation, did you visit a traditional healer and receive traditional medicine other than cuts and/or scarification?

0 No

1 Yes

97 Don't Know

98 Refuse to Answer

J21. In the 6 months before your donation, did you live in an environment where you were frequently bitten by mosquitos?

0 No

1 Yes

97 Don't Know

98 Refuse to Answer

J22. In the 6 months prior to your last donation, did you live in an environment where you were frequently bitten by bed bugs or lice?

0 No

1 Yes

97 Don't Know

98 Refuse to Answer

J23. In the 6 months prior to your last donation, did you have an injury such as a knife or stab wound or were you in an accident where you lost blood from a cut or injury?

0 No

1 Yes

97 Don't Know

98 Refuse to Answer

READ and HEARD: Now, we would like to know about any personal contact you have had with persons who have HIV or hepatitis. In each question, please include only family members, personal friends or acquaintances. (If you are a health care worker, please do NOT include any individuals you have given professional care to, we will ask about those contacts in a minute).

J31. How many people do you personally know who do NOT have AIDS, but have tested positive for HIV, the virus that causes AIDS? (Choose one)

0	0 (none)
1	1
2	2 to 4
3	5 or more
97	Don't Know
98	Refuse to Answer

J32. How many people do you personally know who currently have AIDS or have died of AIDS? (Choose one)

0	0 (none)
1	1
2	2 to 4
3	5 or more
97	Don't Know
98	Refuse to Answer

J33. How many people do you personally know who have hepatitis? (Choose one)

0	0 (none)
1	1
2	2 to 4
3	5 or more
97	Don't Know
98	Refuse to Answer

J34. How many people do you personally know who have died of hepatitis? (Choose one)

0	0 (none)
1	1
2	2 to 4
3	5 or more
97	Don't Know
98	Refuse to Answer

J35. In the 6 months have you been in close contact with anybody who had Hepatitis (yellow jaundice), e.g. people you live or work with?

0	No
1	Yes
97	Don't Know
98	Refuse to Answer

SECTION K - WORK PLACE EXPOSURES

K1. Do you work where you are exposed to human bodily fluids (e.g. blood, urine, feces, saliva)? (Choose one)

0 No ***Skip to Section L if Study group 1 (A7=1), Skip to Question L9 if Study group 2 (A7=2), Else Skip to Instruction After L9.***

1 Yes

97 Don't Know

98 Refuse to Answer

K2. In the 6 months before your donation, in your professional work have you had a needle stick injury (accidentally been stuck by a needle or other sharp instrument used for providing medical care to someone else)? (Choose one)

0 No

1 Yes

97 Don't Know

98 Refuse to Answer

K3. In the 6 months before your donation, did you in your professional work have someone else's blood, body fluids, or excrement splashed into your eyes, mouth or in an open skin lesion? (Choose one)

0 No

1 Yes

97 Don't Know

98 Refuse to Answer

If Study Group 3 (A7=3), Skip to Instruction After L9.

SECTION L - EXPOSURE AND TREATMENT

Study Group 1 Only (A7=1)

READ and HEARD: This is the final section of the questionnaire. It may be difficult for you to respond to these questions. Again, the responses are anonymous and will help us improve blood safety in South Africa.

L1. Did you know your HIV status before your donation? (Choose one)

0	No
1	Yes
97	Don't Know
98	Refuse to Answer

L2. How do you think you may have become infected with HIV?

____

L3. When do you think you may have been infected with HIV? (Year)

__ __ __ __	yyyy
2097	Don't Know (Year)
2098	Refuse to Answer (Year)

L4. When do you think you may have been infected with HIV? (Month) (Choose one)

__ __	January
__ __	February
__ __	March
__ __	April
__ __	May
__ __	June
__ __	July
__ __	August
__ __	September
__ __	October
__ __	November
__ __	December
__ __	Don't Know
__ __	Refuse to Answer

L5. Are you currently taking antiretroviral medicines, also called ARVs? (Choose one)

0	No
1	Yes skip to L7?
97	Don't Know
98	Refuse to Answer

L6. Have you taken antiretrovirals (ARVs) in the past? (Choose one)

0 No

1 Yes

97 Don't Know

98 Refuse to Answer

L7. (Ask of Women Only) If Female: Did you know you were HIV-positive prior to or during your most recent pregnancy?

0 No

1 Yes

96 Not applicable, never been pregnant ***SKIP to After L9.***

97 Don't Know

98 Refuse to Answer

L8. (Ask of Women Only) Did you take antiretrovirals during your most recent pregnancy to try to prevent HIV transmission to your baby?

0 No

1 Yes

96 Not Applicable

97 Don't Know

98 Refuse to Answer

Study Group 2 Only (A7=2)

L9. How do you think you may have become infected with HBV?

— —

READ and HEARD: Thank you for taking the time to complete this questionnaire. If you have any questions or concerns, please talk to the research assistant or nurse. You can also contact the medical director at our blood bank.

READ and HEARD: You have finished the questionnaire. From now on, please do not touch the computer or screen. Please, talk to the research assistant, the person who assisted you at the beginning of this questionnaire. This assistant will close the screen and acknowledge you for your participating in this study.

ETIME = ENDTIME - STARTTIM

Appendix 8 Objective 3 Brief Clinical Questionnaire

<u>This section of the form is to be completed by the research assistant or other research staff.</u>

A1. Participant ID ***(Internal study number to be assigned by Study Management System)***

__ __ __ __ __ __ __

A2. Participant Donor Number ***(Number that will link with donor's Meditech info)***

_____

A3. Location of Study Visit. ***(Blood collection site neumonic (clinic site code). The neumonic can be mapped back to Branch, Zone or Province – this will have to be coded during analysis phase)***

_____

A4. Date of Study Visit (DD/MM/YYYY)

_____

A5. Research Staff Initials: __ __ __ __ __

CURRENT MEDICAL STATUS

B1. Since your last visit for participation in this study have you gone to your doctor or sought medical care at a clinic or hospital?

0 No ***Skip to B2***

1 Yes

97 Don't Know

98 Refuse to Answer

B1a. If yes, what was the reason for seeking medical care? __ __ __ __ __ __ __ __ __ __ __ __ __ __
__ __

B2. Since your last visit for participation in this study have you gone to a traditional healer?

0 No ***Skip to B3***

1 Yes

97 Don't Know

98 Refuse to Answer

B2a. If yes, what was the reason for seeing the traditional healer? __ __ __ __ __ __ __ __ __ __ __ __
__ __

B3. Since your last study visit have you had a cold, flu, or any other infection?

0 No ***Skip to B4***

1 Yes

97 Don't Know

98 Refuse to Answer

B3a. If yes, what symptoms did you have? Please list all the symptoms you can think of such as headache, fever, body pain, chills, vomiting, diarrhea, or any other symptom that you may have had. __
__ __

B4. Since your last study visit have you started taking antiretroviral medicines, also known as ARVs?

0	No	***Skip to B6***
1	Yes	
97	Don't Know	
98	Refuse to Answer	

B4a. What are the names of the antiretroviral (ARV) medicines you are currently taking? __ __ __ __ __
__ __

To help trigger your memory, please look at the placard with pictures of medicines and then place a check mark in the box next to the medications that look like the ones you are taking:

Place √ if taking this medication	Medication
	AZT – Zidovudine
	ddI – Didanosine
	3TC – Lamivudine
	D4T – Stavudine
	ABC – Abacavir
	TDF – Tenofovir
	FTC - Emtricitabine
	IDV - Indinavir
	NVP – Nevirapine
	EFV – Efavirenz
	ETV - Etravirine
	ATV – Atazanavir
	LPV/r – Lopinavir/Ritonavir
	RAL - Raltegravir
	SQV - Saquinavir
	OTH – Other not pictured

B5. If yes, have you had any side effects from taking your current antiretroviral (ARV) medicines?

0	No
1	Yes
97	Don't Know
98	Refuse to Answer

B5a. If yes, what side effects did you have? Please list all the side effect you can think of, such as nausea, loss of appetite, vomiting, diarrhea, or any other symptom that you may have had. __ __ __ __
__ _

B5b. Did you miss taking some or all of the doses of your current antiretroviral (ARV) medicines because of the side effects you experienced?

0	No
1	Yes
97	Don't Know
98	Refuse to Answer

B6. Are you currently taking anything else for your health such as vitamins, herbs, supplements or natural medicines?

0	No
1	Yes
97	Don't Know
98	Refuse to Answer

B6a. If yes, please list the name(s) of each vitamin, herb, supplement you are taking? __ __ __ __ __ __ —

B7. Since your last study visit have you started taking traditional medicines that were recommended or provided by a traditional healer?

0	No
1	Yes
97	Don't Know
98	Refuse to Answer

B7a. If yes, please list the names of traditional medicines you are taking? __ __ __ __ __ __ __ __ __ __
__ __

B7b. If yes, have you had any side effects from taking these traditional medicines?

0	No
1	Yes
97	Don't Know
98	Refuse to Answer

B7c. If yes, what side effects did you have? Please list all the side effect you can think of, such as nausea, loss of appetite, vomiting, diarrhea, or any other symptom that you may have had. __ __ __ __
__ __

B8. (Ask of Women Only) Are you currently pregnant?

0	No
1	Yes
96	Not applicable, never been pregnant
97	Don't Know
98	Refuse to Answer

Thank you for taking the time to complete this questionnaire. Please return this questionnaire to the research staff.

If you have any questions or concerns, please talk to the research assistant or nurse. You can also contact the medical director at our blood bank.

Appendix 9 Placard Showing Antiretroviral Medicine Pictures

Antiretroviral drug chart

Generic name	Trade name	Formulation	Standard adult dose	Pills/day	Major side-effects	Food restrictions
Nucleoside reverse transcriptase inhibitors (NtRTIs)						
3TC, lamivudine	*Epivir* 	150* and 300mg tablets	150mg twice a day **or** 300mg once a day	2 1	**Common:** Nausea, vomiting, diarrhoea, headache, abdominal pain, hair loss, fever, insomnia (difficulty sleeping), rash, tiredness, runny nose, joint pain **Rare:** Lactic acidosis, liver damage	Take with or without food
Abacavir	*Ziagen* 	300mg tablet	300mg twice a day **or** 600mg once a day	2	**Common:** Rash, nausea, vomiting, diarrhoea, fever, headache, tiredness, loss of appetite **Rare:** Hypersensitivity reaction, lactic acidosis	Take with or without food
AZT, zidovudine	*Retrovir* 	100 and 250mg* capsules	250mg twice a day	2	**Common:** Nausea, vomiting, fatigue, headache, dizziness, weakness, muscle pain, loss of appetite, fever **Rare:** Blood disorders, lipoatrophy, lactic acidosis	Take with or without food
d4T, stavudine	*Zerit* 	20, 30 and 40mg* capsules	**People over 60kg:** 40mg twice a day **People under 60kg:** 30mg twice a day	2	**Common:** Lipoatrophy, peripheral neuropathy, nausea, diarrhoea, abdominal pain, heartburn, dizziness, tiredness, rash, itching **Rare:** Pancreatitis, lactic acidosis	Take with or without food
ddI, didanosine	*VidexEC* 	125, 200, 250 and 400mg* capsules	**People over 60kg:** 400mg once a day **or** 200mg twice a day **People under 60kg:** 250mg once a day **or** 125mg twice a day	1 or 2	**Common:** Peripheral neuropathy, nausea, vomiting, diarrhoea, abdominal pain, rash, headache **Rare:** Pancreatitis, lactic acidosis	Take at least two hours after and two hours before eating or drinking anything except water
FTC, emtricitabine	*Emtriva* 	200mg capsule	200mg once a day	1	**Common:** Nausea, diarrhoea, headache, raised kinase levels, skin darkening in children **Rare:** Lactic acidosis, liver damage	Take with or without food
Nucleotide reverse transcriptase inhibitor (NtRTI)						
Tenofovir	*Viread*	300mg tablet	300mg once a day	1	**Common:** Nausea, vomiting, diarrhoea, dizziness, low blood phosphate levels, weakness, rash, headache, stomach pains, fatigue, bloating, flatulence **Rare:** Kidney problems, bone thinning	Take with food
NRTI / NtRTI fixed-dose combinations						
3TC / AZT	*Combivir* 	Tablet comprising 150mg 3TC and 300mg AZT	One tablet twice a day	2	See 3TC and AZT	Take with or without food
3TC / abacavir / AZT	*Trizivir* 	Tablet comprising 150mg 3TC, 300mg abacavir and 300mg AZT	One tablet twice a day	2	See 3TC, abacavir and AZT	Take with or without food
3TC / abacavir	*Kivexa (EU)* 	Tablet comprising 300mg 3TC and 600mg abacavir	One tablet once a day	1	See 3TC and abacavir	Take with or without food
FTC / tenofovir	*Truvada* 	Tablet comprising 200mg FTC and 300mg tenofovir	One tablet once a day	1	See FTC and tenofovir	Take with food
NRTI / NtRTI / NNRTI fixed-dose combinations						
FTC / tenofovir / efavirenz	*Atripla* 	Tablet comprising 600mg efavirenz, 200mg FTC and 300mg tenofovir	One tablet once a day	1	See FTC, tenofovir and efavirenz	Take on an empty stomach, preferably at bedtime
FTC / rilpivirine / tenofovir	*Eviplera* 	Tablet comprising 200mg FTC, 25mg rilpivirine and 300mg tenofovir	One tablet once a day	1	See FTC, rilpivirine and tenofovir	Take with a meal
Non-nucleoside reverse transcriptase inhibitors (NNRTIs)						
Efavirenz	*Sustiva Stocrin* 	600mg tablet* and 200mg capsule	600mg once a day	1 or 3	**Common:** Rash, dizziness, sleep disturbance, abnormal dreams, impaired concentration, nausea, vomiting, headache, tiredness, diarrhoea, anxiety, depression **Rare:** Psychosis, severe rash, liver problems	Take on an empty stomach, preferably at bedtime
Etravirine	*Intelence* 	100 and 200mg* tablet	200mg twice daily	2 or 4	**Common:** Rash, peripheral neuropathy **Rare:** Severe rash (Stevens Johnson syndrome)	Take with food
Nevirapine	*Viramune* 	200mg tablet	200mg once a day for two weeks **then** 200mg twice a day	2	**Common:** Liver toxicity, allergic reaction, rash, nausea, headache, fatigue, stomach pain, diarrhoea **Rare:** Severe rash (Stevens Johnson syndrome)	Take with or without food
Nevirapine	*Viramune prolonged-release* 	400mg tablet	400mg once a day after introductory period on non-extended-release nevirapine	1	**Common:** Liver toxicity, allergic reaction, rash, nausea, headache, fatigue, stomach pain, diarrhoea **Rare:** Severe rash (Stevens Johnson syndrome)	Take with or without food
Rilpivirine	*Edurant* 	25mg tablet	25mg once a day	1	**Common:** Insomnia (difficulty sleeping), headache, rash, mood changes, depression **Rare:** At doses above 25mg may cause a disturbance to the heart rhythm	Take with a meal
Protease inhibitors						
Atazanavir	*Reyataz* 	150, 200 and 300mg* capsule	300mg with 100mg ritonavir once a day **or** 400mg with 100mg ritonavir once a day with efavirenz or nevirapine	2 or 3 §	**Common:** Nausea, diarrhoea, rash, stomach ache, headache, insomnia (difficulty sleeping), vomiting, hyperbilirubinaemia, lipodystrophy, liver toxicity, diabetes, heartburn, jaundice **Rare:** Kidney stones, abnormal liver function, changes in heart rhythm	Take with food
Darunavir	*Prezista* 	400 and 600mg* tablet	600mg with 100mg ritonavir twice a day **or** 800mg with 100mg ritonavir once a day	3 or 4 §	**Common:** Diarrhoea, nausea, rash, stomach pain, vomiting, headache, lipodystrophy, liver toxicity, diabetes, fever **Rare:** Abnormal liver function, changes in heart rhythm	Take with food
Fosamprenavir	*Telzir*	700mg tablet	700mg with 100mg ritonavir twice a day	4 §	**Common:** Raised lipids, nausea, vomiting, diarrhoea, rash, abdominal pain, headache, dizziness, tiredness, tingling around the mouth, changes in liver and pancreas function, lipodystrophy, liver toxicity, diabetes **Rare:** Severe rash, changes in heart rhythm	Take with or without food
Indinavir	*Crixivan* 	100, 200 and 400mg* capsules	800mg three times a day	6	**Common:** Kidney stones, abdominal pain, lipodystrophy, nausea, vomiting, heartburn, diarrhoea, rash, headache, dizziness, dry skin and mouth, tiredness, insomnia (difficulty sleeping), liver toxicity, diabetes **Rare:** Liver abnormalities, changes in heart rhythm	Take one hour before or two hours after food or take with a light, low-fat snack
Lopinavir / ritonavir	*Kaletra* 	Tablet comprising 200mg lopinavir and 50mg ritonavir	Two tablets twice a day **or** four tablets once a day	4	**Common:** Lipodystrophy, raised liver enzymes, nausea, vomiting, diarrhoea, abdominal pain, weakness, heartburn, headache, raised lipids, liver toxicity, diabetes **Rare:** Changes in heart rhythm	Take with or without food
Ritonavir	*Norvir*	100mg capsule and 100mg tablet*	**Full dose:** 600mg twice a day **To 'boost' other PIs:** 100 – 200mg once or twice a day	12 1 to 4	**Common (at full dose):** Raised lipid and liver enzymes, nausea, vomiting, diarrhoea, abdominal pain, headache, weakness, numbness around the mouth, bad taste in mouth, lipodystrophy, liver toxicity, diabetes **Common (at low dose):** Raised lipid levels **Rare:** Changes in heart rhythm	Take with food to avoid nausea
Saquinavir	*Invirase* 	200mg capsule and 500mg tablet*	1000mg with 100mg ritonavir twice a day	6 §	**Common:** Lipodystrophy, nausea, vomiting, diarrhoea, rash, tiredness, raised liver enzymes and lipids, liver toxicity, diabetes **Rare:** Changes in heart rhythm	Take within two hours of food
Tipranavir	*Aptivus*	250mg capsule	500mg with 200mg ritonavir twice a day	8 §	**Common:** Nausea, diarrhoea, vomiting, abdominal pain, tiredness, headache, fever, liver abnormalities, rash, lipodystrophy, liver toxicity, diabetes, lipid increases, flatulence **Rare:** Bleeding in brain, changes in heart rhythm	Take with food
Fusion inhibitor						
T-20, enfuvirtide	*Fuzeon* 	Powder reconstituted in water	Injection of 90mg under the skin twice a day	–	**Common:** Injection-site reaction, respiratory tract infections, peripheral neuropathy, weight loss	No food restrictions
CCR5 inhibitor						
Maraviroc	*Celsentri* 	150*, 300mg tablets	300mg twice a day **or** 150mg twice a day with all ritonavir-boosted PIs except tipranavir **or** 600mg twice a day with efavirenz or etravirine	2 to 4	**Common:** Nausea, diarrhoea, fatigue, headache	Take with or without food
Integrase inhibitor						
Raltegravir	*Isentress* 	400mg tablet	400mg twice a day	2	**Common:** Headache, insomnia (difficulty sleeping) **Rare:** Severe rash, hypersensitivity reaction	Take with or without food

*Formulation(s) shown. § Includes ritonavir capsule(s).

This drug chart is produced by an organisation called NAM. NAM provides people working in the global fight against HIV & AIDS with up-to-date and impartial information. Please visit us at our website where you can read the latest HIV news and sign up for free email updates. We welcome your feedback on our information resources.
NAM, Lincoln House, 1 Brixton Road, London, SW9 6DE. Email info@nam.org.uk **Web** www.aidsmap.com

Drugs licensed in the European Union
December 2011