Attachment 4: Protocol for Prevalence, Incidence, Epidemiology and Molecular Variants of HIV in Blood Donors in Brazil

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Abstract

Establishing and monitoring viral prevalence and incidence rates, and identifying behavioral risk behaviors for HIV incidence among donors, are critical steps to assessing and reducing risk of HIV transmission through blood transfusion. Identifying donation samples from donors with recent HIV infection is particularly critical as it enables characterization of the viral subtypes currently transmitted within the screened population and hence most likely to "break-through" routine screening measures (i.e., peri-seroconversion window period donations). In addition to characterizing genotypes of recently infected donors for purposes of blood safety, molecular surveillance of incident HIV infections in blood donors enables documentation of the rates of primary transmission of anti-viral drug resistant strains in the community, and serves a public health role in identifying new HIV infections for anti-retroviral treatment. We plan to enroll eligible HIV positive subjects detected at our four blood centers: Sao Paulo, Recife, Rio de Janeiro and Belo Horizonte, and analyze molecular variants and their correlation with risk behaviors. **Aims.** Determine risk factors associated with HIV infection, HIV subtype, and drug resistance profile among HIV positive donors according to HIV infection status (nucleic acid testing (NAT) yield versus recent versus long-standing seropositives), year of donation and site of collection.

Previously we conducted a case control study of HIV seropositive donors and a comparison population of uninfected donors as part of the NHLBI-funded Retrovirus Epidemiology Donor Study (REDS-II) International Brazil. The new study will build off of the previous study to continue risk behavior and molecular surveillance at the four REDS-III blood centers. Note that the formal name of the study has changed to Recipient Epidemiology and Donor Evaluation Study (REDS-III), but our study objectives for this HIV project remain similar to the previous REDS-II study.

NAT testing for HIV (and HCV) is currently being implemented in Brazil. It will be important to continue to collect molecular surveillance and risk factor data on HIV infections, especially now that infections that might have been missed by serology testing will be identified through the use of NAT. NAT-only infections represent recently acquired infections. Therefore the results of our HIV surveillance study will be especially useful for interpreting the findings of HIV NAT testing in Brazil. For example, HIV test seeking at blood banks is already a concern. If we find that NAT-only donors are test seeking this will raise critical policy issues. Even after NAT is implemented, the residual risk of HIV infection is likely to remain substantially higher than in the USA because of the approximately 10-fold prevalence of infection in Brazil compared to the USA. Additional measures towards safe donor recruitment and deferral continue to be essential in further reducing the risk of transfusion-transmitted HIV infection. The continuation of these important HIV activities will build directly off of the capacity established at all 4 blood centers during participation in the REDS-II Brazil HIV case control study.

Specific Aims

Aim. Determine risk factors associated with HIV infection, HIV subtype, and drug resistance profile among HIV positive donors according to HIV infection status (NAT yield vs recent *vs* long-standing seropositives), year of donation and site of collection.

Hypothesis 1: Having multiple heterosexual partners, male-to-male sex, and toa lesser extent injection drug use (IDU) will continue to be the predominant risk factors for HIV in Brazil among both seroprevalent infections and incident infections detected by NAT or recent seroconversion.

Hypothesis 2: There will be clinically relevant increases in the diversity of HIV subtypes and increasing rates of primary drug resistance among recently infected donors in all 4 blood centers. Non-B subtypes and drug resistant strains will be seen in recently infected persons from all risk factor categories.

Background

The HIV epidemic continues to be a major public health problem in Brazil. The HIV/AIDS epidemic began in Brazil in the early 1980s, and Brazil now has the largest HIV-1 infected population in South America, with 544,846 reported cases of AIDS and 630,000 individuals known to be living with HIV in 2009[1]. The epidemic in Brazil is considered a "concentrated epidemic", with an overall prevalence below 1% in the general population[2], but levels as high as 50% among vulnerable population such as males who have sex with males (MSM), injection drug users (IDUs) or sex workers [3]. Incidence of AIDS cases vary across Brazilian regions, with the highest incidence rates concentrated in the South and the Southeast (29.3 and 19.2/100,000), followed by the Midwest, North and Northwest (16.4; 15.4; and 11/100,000[1]. Although incidence of AIDS has stabilized in the South, Southeast and Midwest, HIV transmission and clinical AIDS case report rates are increasing in the North and Northeast. According to the criteria of the World Health Organization (WHO), Brazil has an HIV epidemic with prevalence of HIV infection of 0.6% in 15 to 49 year old age strata, and concentrated foci in specific sub-populations with prevalence greater than 5%. In 2005, 35,965 new cases of AIDS were reported, representing an incidence rate of 19.5 AIDS cases per 100,000 inhabitants. Sao Paulo, Rio de Janeiro and Minas Gerais States (all in the Southeast) are responsible for 64% of AIDS cases in Brazil, while Pernambuco (in the Northeast) has a higher rate of new infections than the other regions[1]. Studies evaluating the prevalence of drug-resistant virus among recently infected individuals are thus extremely important in Brazil. A survey developed by the Brazilian Ministry of Health which included 535 patients from the entire country, found that the frequency of primary resistance was low, 4.4% for nucleoside reverse transcriptase inhibitors (NRTI) and non-nucleoside reverse transcriptase inhibitors (NNRTI) and 2.2% for protease inhibitors[4]. (Dr Sabino was a co-investigator on this project.) More recently, Sucupira and colleagues analyzed 75 drug naïve HIV positive individuals in the city of Santos and found 21 (28%) harboring resistant strains, raising grave concern that in certain areas of Brazil drug resistant strains may be rapidly increasing and spreading[5].

HIV/AIDS health care in Brazil is provided by the state to all HIV infected individuals. As early as 1991, the Brazilian Ministry of Health provided antiretroviral (ARV) drugs through its extensive public health system. In 1996 a law was enacted guaranteeing free access of

antiretroviral therapy to all Brazilians who required treatment, according to Brazilian guidelines. The widespread use of ARV has led to a decline in AIDS related mortality in Brazil[6,7]. However, it is expected that the proportion of patients experiencing virologic failure and consequently harboring resistant strains will increase in time. Depending on the behavioral characteristic of these individuals, transmission of drug resistant strains may occur with increased frequency[8]. The transmission of resistant variants to uninfected individuals raises serious clinical and public health consequences and may dramatically impair the capacity of treating HIV in the near future[9-14]. Moreover, the genetics of the virus can be used to track the movement of HIV-1 into new groups or new at-risk populations. It is also conceivable that the diversity of the virus may impair the immune response to candidate vaccines, cause false negative results in blood screening, diagnostic and monitoring laboratory tests (e.g. especially viral load assays targeting nucleic acids)[15,16],and lead to differences in the observed disease progression[11],[10] and therapeutic response[14,17]. The predominant HIV-1 clade in Brazil is B (~70%), but unlike the U.S., Brazil also has appreciable numbers of HIV-1 infections caused by clade F (~15%), C (~3%), and circulating recombinant forms (CRFs, ~12%).[18-20] Furthermore, approximately 50% of the clade B strains circulating in Brazil comprise a cluster of genetically and antigenically distinct strains relative to US clade B strains[21]. Monitoring HIV viral subtypes and drug resistance patterns, and identifying risk behaviors for incident HIV infections among donors (NAT yield and recent seroconvertors) are critical steps to assessing and reducing risk of HIV transmission through transfusion[22]. In addition to characterizing genotypes of recently infected donors for purposes of blood safety, molecular surveillance of HIV infections in blood donors enables documentation of the rates of primary transmission of anti-viral drug resistant strains in the community, and serves a public health role in identifying new HIV infections for which subjects can then obtain anti-retroviral treatment.

In Brazil[23] the prevalence and incidence of HIV infection varies widely by geographic area and by demographic and behavioral subgroup. In this sense, it is essential at the State and local levels that HIV prevention activities be targeted to those geographic areas and population groups currently affected by HIV and to those into which the virus may be spreading. The impact of prevention activities is reflected in the prevalence and trends of infection over time. At regional and national levels, knowledge of the current patterns of HIV infection and estimates of the total number of infected persons are important for anticipating future health needs and setting public health policies in Brazil.

Enrollment in the REDS-II HIV study is closed and we are currently analyzing the data. A total of 343 HIV positive blood donors and 901 HIV seronegative controls enrolled in the study. Data reporting molecular variants and demographic characteristics of HIV positive donors was presented at the 2011 AABB meeting held in San Diego. Of clear concern, thirty-two of 343 cases were taking anti-retroviral therapy. These donors were excluded from the analysis of primary transmitted drug resistance. A total of 274 samples could be typed and the distribution by subtype is shown the table below. Non-B subtypes represent a quarter of HIV infections in the blood donor at the four REDS-II blood centers. Thirty-six of the 274 subtypes (13%) had evidence of primary drug resistance. Specific demographic and behavior risk factors were not associated with primary drug resistance. Further analyses are underway at this time.

Total number of specimens available for subtyping	Subtypes n (%)					
	A	B	C	F	C/F	B/F

274	1 (0.4)	212 (77)	13 (5)	37 (14)	2 (1)	9 (4)

With respect to the larger HIV risk factor analysis, preliminary results suggest that the use of audio computer assisted self-interview (ACASI) methodology has been very important for disclosure of risks because even seronegative donors disclosed deferrable risk behaviors. This by itself has important implications for blood safety in Brazil.

Significance

Defining prevalence and incidence in blood donors and residual risk of HIV transmission by transfusions in Brazil may lead to new blood safety approaches in Brazil. The data we will collect can be used to project the yield, safety impact and cost-effectiveness of implementing enhanced testing strategies that include nucleic acid testing.. Determination of HIV risk factors in donors according to additional characteristics (first time versus repeat donor status; volunteer versus replacement status; demographics and risk behaviors) will support policy discussions over strategies to recruit the safest possible donors in Brazil, and will also yield significant information for HIV surveillance in Brazil when combined with prevalence and incidence data derived from general populations and high risk surveillance studies. The documentation of incident HIV infections allows for clinical identification of recently transmitted strains of virus in donor settings in the different cities of Brazil. This surveillance will monitor the trafficking of non-B subtypes and rates of transmission of drug resistant viral strains in low risk blood donors that can be compared with data from similar studies in higher risk populations. Monitoring drug resistance strains is extremely important in a country that provides free ARV therapy for HIV infected individuals, many of whom have low level education and modest resources, thus making compliance with drug regimens and hence the risk of drug resistant HIV a serious problem. The findings from this project will also complement similar monitoring of HIV prevalence, incidence, transfusion risk and molecular variants in the USA and other funded international REDS-III sites in South Africa and China, thus allowing direct comparisons of these parameters on a global level. By combining HIV data collected in Brazil during REDS-II and REDS-III we will also be able to examine time trends in HIV molecular variants and risk factors associated with HIV infection in donors from 2007 through to 2017.

Approach

All blood donations are routinely screened by two HIV-1 antibody assays in parallel, as mandated in Brazil. These assays have been selected to have comparably high sensitivity to early seroconversion and diverse clades, as well as excellent specificity with non-overlapping populations of false reactivity. Samples reactive by both EIAs will be considered positive for prevalence calculations. In Brazil, only subjects who return for counseling will have a follow-up sample obtained that will be tested by western blot (WB) (~80% of HIV reactive subjects return and WB is performed, of which 95% confirm as WB positive). In addition, a NAT screening assay for HIV and HCV in pools of 6 donations has been developed for use in blood centers in Brazil. The NAT assay will be used at all of the REDS-III blood centers in Brazil during the planned HIV case surveillance activities. In addition, to detect recently infected donations, samples from all HIV dual-EIA reactive donations and/or NAT positive donations will be tested by the Recent Infection Testing Algorithm (RITA) which is based on use of a sensitive/less-sensitive enzyme immunoassay ("detuned" EIA)[24,25]. RITA testing will be performed by BSRI (Blood Systems Research Institute, the REDS-III Central Laboratory) because these test kits are not registered for use in Brazil and would be difficult for the local laboratory to import. For enrolled subjects, this testing will be performed on linked index and enrollment specimens from WB-confirmed donors, whereas for subjects who do not return for counseling we will anonymize

the donation samples. False positive samples will be identified by performing sensitive EIA and WB on samples lacking reactivity on the LS-EIA (i.e., standardized optical density <0.1). Incidence data derived using the RITA methods will be used to project risk and yield of enhanced donor screening strategies. Subtype and drug resistance profiles will be determined in Dr. Sabino's laboratory on all donors who returned for counseling and consented and enrolled into the interview study (see below). Risk factors and donor demographics will be correlated with recent infection status, as well as with clade and acquisition of drug resistant virus.

This study will be conducted at all four participating REDS III centers in Brazil; Sao Paulo, Recife, Rio de Janeiro and Belo Horizonte.

Methods

Study Design. Surveillance of all HIV positives during a period of 5 years, with serological confirmation and RITA, will allow calculation of HIV prevalence and incidence. A detailed risk factor questionnaire testing for HIV genotype, and drug resistance will be done on all consented HIV cases. Data analysis will compare the frequency of reported risk behaviors between first-time *vs* repeat donors, community *vs* replacement, recent vs long standing infections, year of donation and site of collection.
Secondary analyses will provide evidence for changes (declining or increasing) HIV prevalence and incidence in selected sites and explore the reasons for the changing patterns in particular the role of behavioral change; and also evaluate if the frequency of specific risk factors in the blood donor population such as MSM, multiple heterosexual partners, and IVDU are changing over time. [To assess this, we will compare REDS-II HIV case risk factors to those we determine using the identical ACASI in REDS-III]Analyses on the cumulative data will be repeated at annual intervals to assess secular trends in HIV risk factors.

Study Population. Surveillance will be performed to identify HIV NAT yield cases and HIV seropositive donors. All eligible cases will previously have had dual HIV EIA testing, NAT and Western blot performed to confirm their HIV seropositivity, per core REDS-III procedures at Fundação Pró Sangue/Hemocentro de São Paulo. Recently infected individuals will be defined through the Standardized Testing Algorithm for Recent HIV Seroconversion (RITA) protocol. An ACASI on risk factors, developed for REDS-II and in place at all 4 centers, will be administered to subjects who return for counseling and consent to participate in the study. Testing for HIV genotypes and drug resistance will be performed on all consented HIV cases at Fundação Pró Sangue/Hemocentro de São Paulo as previously described. The genotype result will be sent to the donor by mail and they will be counseled to take it to their physician. If desired by the subject a new visit will be provided to discuss the results of genotype testing. We plan to enroll 25 cases per year at each center or approximately 1 case every other week. For these subjects, in addition to Western blot testing, we will perform linked genotype and drug resistance testing on the sample obtained at the time of counseling. For those individuals who do not return for confirmatory testing, the samples will be anonymized and tested using RITA.

Inclusion & Exclusion Criteria. Portuguese-speaking blood donors aged 18–65 years at the four participating blood centers who were confirmed HIV-positive by EIA or NAT and Western blot. Autologous blood donors will be excluded.

Subject Enrollment: Subjects will be enrolled for a 5-year period from March (or when OMB approval is received) 2012 – 2017. According to the Brazilian guidelines blood donors are requested to return to the blood bank for confirmatory testing (Western blot) and HIV counseling. At the time the donors return for the final HIV results and counseling and they will be invited to

participate in the study, and if consent is obtained the ACASI questionnaire about risk factors and motivations to donate will be administered. For these subjects, we will perform linked genotype and drug resistance testing on the sample obtained at the time of counseling. For those individuals that do not return for confirmatory testing, the samples will be anonymized and sent to BSRI to perform the RITA.

Procedures:

A -Questionnaire

A detailed HIV risk factor questionnaire will be administered to all subjects. This is the same instrument that was previously used in REDS-II except for a change in the name of the instrument and removal of one of the data capture fields that was used by study research assistants to define case or control status for the REDS-II study. As the REDS-III case surveillance study will only include HIV positive donors this data capture field is no longer necessary. A self-administered audio computer-assisted self-interview (ACASI) on a laptop computer will continued to be used in order to maximize reporting of stigmatized or socially sensitive behaviors. A research assistant or nurse will provide the ACASI laptop (including earphones to be able to listen to the questions confidentially) to each subject at the blood center. The study subject will be shown how to use the computer to complete the interview by entering basic demographic data with the help of the research assistant or nurse, but will be given privacy to complete the rest of the questionnaire. The research assistant or nurse will remain available to answer questions and provide help as necessary.

B - Phlebotomy for Clinical Testing

In addition to blood saved from their index blood donation, 30 ml of blood will be drawn from cases at the time of the enrollment and interview. Specimens will be sent to Sao Paulo for genotype testing and to the USA for RITA testing, and the remaining specimens will be processed into aliquots and saved in the study repository in Sao Paulo for future testing, including repeated genotyping and drug resistance, if necessary. Specimens will be stored in Brazil in accord with Brazilian law. Specimens sent to the USA for RITA will be kept until the study is concluded (March 20, 2018). On this date remaining specimens and residual aliquot volumes in the USA will be destroyed.

C - Detection of Recent Infections by Less Sensitive-Enzyme Immunoassay (LS-EIA) Testing

All eligible cases will previously have had dual HIV EIA testing and HIV Western blot to confirm their HIV seropositivity, per core procedures at our Brazilian central lab. Recently infected individuals will be defined through RITA testing at BSRI in San Francisco, CA, USA.

D - HIV-1 Clade Typing and Drug Resistance Testing

Subtype and resistance analysis will be performed at Fundação Pró Sangue Hemocentro de São Paulo as previously described[25,26]. Sequencing of the entire HIV-1 protease gene (99 amino acids [aa]) 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 [26]. 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™, a drug that is not yet in use in Brazil. Following phylogenetic analysis, sequencing of the pro-RT region will also identify the subtype of the recently transmitted HIV-1 strain. RNA will be isolated using QIAamp Viral RNA Mini kit (Qiagen Inc., Valencia, CA) according to the manufacturer's

instructions. Complementary DNA will be obtained using Superscript reverse transcriptase (Invitrogen, Carlsbad, CA) and random primers (Pharmacia, Uppsala, Sweden). A nested PCR 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.[31] 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. Conditions for both rounds of PCR will be 94°C for 1 minute followed by 35 cycles at 94°C for 45 seconds, 55°C for 45 seconds and 72°C for 2 minutes, with a final elongation step at 72°C for 10 minutes All amplification products will be analyzed on a 1% ethidium bromide-stained agarose gel. PCR products will be purified using QIAquick PCR purification kit (Qiagen Inc.). 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 (sense -5' -CTC ARG ACT TYT GGG AAG TTC- 3') and GABO-2 (antisense - 5' -GCA TCH CCC ACA TCY AGT ACT G-3'). Sequence data will be obtained using the ABI Prism Big Dye Terminator Cycle Sequencing Ready Reaction kit (Applied BioSystems Inc., Foster City, CA, USA), according to manufacturer's protocol in an automated sequencer (ABI 377 Sequencer-Applied Biosystems). 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)[15,16].

Counseling and Medical Referral.

Prior to enrollment, all subjects will have received counseling regarding their HIV infection by trained personnel at the blood centers, per operational protocols. The genotype result will be sent to the donor by mail and they will be counseled to take it to their physician. If desired by the subject a new visit will be provided to discuss the results of genotype testing.

Data Analysis

Analysis of Incidence, Residual Risk and NAT Yield.

Data analysis will be performed by the REDS-III data coordinating center, Research Triangle Institute-RTI International in Rockville, Maryland, US. Data from this study will be merged with the large Brazilian donation database to allow calculation of incidence and univariate and multivariate analysis of correlates of HIV incidence and calculation of residual risk and yield of NAT. Since Clade B is responsible for more than 80% of the infections in Brazil, we will assume the window period corresponding to the time from seroconversion by sensitive EIA and Western blot to seroconversion by the LS-EIA would be similar to that reported for U.S. clade B infected persons, i.e., 170 days (95% confidence interval [CI] 145-200 days). We will further assume that the detection window periods (period from infectivity by blood transfusion to initial detection by the respective markers) for viral RNA by ID-NAT, MP-NAT, p24 antigen and antibody EIAs were 5.6, 9.0, 15.0 and 20.3 days, respectively, as described by Busch et al[24]. Based on our observed incidence rates and the published WP estimates, the predicted yield and associated residual risk (per 10,000 per year) for each test will be calculated using the formula: (incidence rate) ⁄ (365) × (detection window period). The statistical calculations will use the method of Busch, et al as follows. Confidence intervals for prevalence rates will assume prevalent cases are binomially distributed. Logistic regression will be used to assess differences in prevalence rates by year, type of donation, gender, and age. Confidence intervals for incidence rates will assume incident cases are Poisson distributed.

The findings from this project will add to those obtained in the REDS-II study, allowing for extended trend analyses over a 10-year period and will complement similar monitoring of HIV prevalence, incidence, transfusion risk and molecular variants in Brazil. Poisson regression will be used to assess differences in prevalence rates by year, type of donation, gender, and age. Wald type 95% confidence intervals around residual risk estimates and yield estimates will be computed using a Taylor series approximation to the residual risk standard error estimates and yield standard error estimates. These standard errors are a function of the standard errors of the Poisson distributed incidence rates and the standard error of the window periods.

Analysis of Risk Behaviors.

Independent variables will be HIV risk factors ascertained by questionnaire, including male-to-male sex, number of lifetime male and female sexual partners, number of male and female sexual partners within the past year, use of condoms, sex with a prostitute or sex work by the donor him/herself, IDU, sex with an IDU, and sex with an individual known to be HIV-positive. Secondary predictors will include demographics, socioeconomic status, drug and alcohol use. Subgroup analyses will be done in males and females and in volunteer and replacement donors, first-time and repeat donors. Data from the questionnaire will also be used in analyses of HIV subtypes and drug resistance, below. We will report the frequency of risk behaviors according to demographic characteristics, recent versus long-standing infection, and HIV genotype categories. Univariate associations of specific risk factors and recent infection compared to longstanding infection will be assessed using contingency tables with significance tests using Chi squared or Fisher's exact tests. Variables with significant or borderline univariate associations ($p<0.10$) with HIV seropositivity will be entered into a logistic regression model to assess independent associations and potential confounding.

HIV Subtype and Drug Resistance Analyses.

To define the subtype of each strain we will use interface software that automatically submits the sequences to two programs: RIP[27] and Blast[28].In the RIP program, the sequence is cut in fragments of 200 bp in 1 bp steps. Each fragment is compared to a set of reference strains and for each fragment the program determines which reference sequence has the highest similarity rate to that fragment. In the Blast approach, the sequence is cut into non-overlapping fragments of 200 bp. Each fragment is submitted to a bank of 10,000 sequences previously subtyped in the Los Alamos databank. The program detects which databank sequence is most similar to each fragment. If the results of both analysis agreed, the sample will be considered subtyped. Otherwise the sequence will be manually reviewed using SIMPLOT[29].

Interpretation of results identifying possible Protease and RT mutations that have been associated with reduced antiretroviral-drug susceptibility will be based on the International AIDS Society classification[30] Protease: D30N; M46I; M46L; G48V; I50V; V82A; V82S; V82F; V82T; I84V and I90M. Reverse Transcriptase: M41L; A62V; K65R; D67N; T69D; 69 insert; K70R; L74V; V75I; V75T; V75M; V75S; V75A; F77L; A98G, L100I; K103N; V106A; V108I; Y115F; F116Y; Q151M; Y181C; Y181I; M184V; M184I; Y188C; Y188L; Y188H; Y188C; G190A; G190S; L210W; T215Y; T215F; K219Q; K219E; P255H; P230L and P236L. Reverse transcriptase mutations that are different from wild-type T215 and T69A/N/S will be included as well.

Sample Size and REDS-III Enrollment:

Our experience in REDS-II was the following:

Overall Enrollment in the REDS-II International Component Brazil HIV Case Control Study.

Subject Type	Identified	No Response to recruitment Letter	Refused	Withdrew or Dropped out	Final Enrollment
Case	564	181	27	13	343
Control	N/A*	352	18	24	901

*The identified control population is total of number of accepted blood donors who successfully donated and screened negative for all infectious markers.

Of all potential HIV positive subjects during the REDS-II study period at the four blood centers we were able to enroll 60%. Of those who responded to our recruitment efforts, nearly 90% are included I our final enrollment numbers.

Based on REDS-II experience, we expect that as many as 10 NAT-only yield cases and 200 HIV Ab-positive donors per year will be identified through standard testing at the 4 blood centers in REDS-III. All 200 seropositive donors will be tested for recent acquisition of infection (rapid detuned testing). We are assuming that 50% of these donors (approximately 100) will participate in this study, completing the ACASI risk factor interview and additional sample collection for viral subtype and resistance testing. Over the proposed enrollment period of 5 years this will provide risk factor and surveillance data for 500 HIV-positive donors.

Expected Results. Based upon our preliminary data, we expect to find that male-to-male sex and multiple heterosexual partners remain risk factors for HIV. With the larger sample size, we shall have better power to analyze risk factor profiles separately in volunteer vs. replacement blood donors. Given their age and socioeconomic status, we hypothesize that under-reported IVDU in the heterosexual population explain the unexpectedly high HIV prevalence in volunteer donors.

Potential Nonresponse Bias

During data collection, we will monitor participation and response rates to identify any potential problems that are indicated by differential response rates across sites. We assume that the responses rates HIV positive subjects for the completed REDS-II and planned REDS-III phases of the study will be similar. We will conduct nonresponse bias analysis to assess the potential for nonresponse bias. If we find that nonresponse bias may exist, we will conduct post-survey weighting based on the information produced during the nonresponse bias analysis to minimize the potential nonresponse bias when the risk behavior frequencies are reported. The factors that could be associated with non-participation and nonresponse are demographic characteristics of the donors (age, gender, race/ethnicity, previous donation history) and also potential participation rate differences by blood center. It is important to note that simple or unadjusted HIV case rates per site will not be sufficient to indicate whether there is evidence of bias because the demographic characteristics of blood donors at each of the REDS-III blood centers are not the same.

We expect low levels of item nonresponse for this study. Our use of ACASI was very successful in the previous REDS-II study with little evidence of important levels of nonresponse to any of the questions asked during the interview. For example, we asked a question about whether the donor had ever been tested for HIV outside of blood donation. Only 1 person out of 1244

respondents (<0.1%) refused to answer this question. Similarly, for a clearly social sensitive question in which we asked respondents to classify their sexual orientation 18 out 1244 respondents (1.4%) refused to answer. These data suggest that the use of ACASI was successful in eliciting responses to stigmatizing or socially sensitive questions, and we expect the same to be true for our use of the same interview in the REDS-III HIV case surveillance study.

Human Subjects Considerations.

This project will be approved by the institutional review boards (ethics committees) in Brazil and the U.S. before implementation. The main risks of this study are: 1) possible lost confidentiality regarding HIV status or risk behaviors; 2) possible discomfort due to the personal nature of the questionnaire; and 3) possible negative information risk of drug genotype test. Benefits of the study include: 1) HIV positives will receive additional HIV counseling as part of the study; 2) their clinical treatment may be improved by providing drug genotype test; and 3) benefits to Brazilian society as a whole by virtue of potential improvement blood safety and control of the HIV epidemic and 4) Provide at regional and national levels, knowledge of the current patterns of HIV infection and estimates of the total number of infected persons are important for anticipating future health needs and setting public health policies.

We shall attempt to minimize risks by stringent privacy protection of the subjects' data (see elsewhere in this application). All data will be kept on secure, password protected computers and no personal identifying information will be kept on computers used for this study. Secure data transfers procedures will be used at all stages when data is transferred from one computer or participating organization to another. For example, secure, password protected FTP procedures will be used when we transfer risk factor and HIV subtype and drug resistance information to the US DCC. We will also seek to mitigate discomfort by providing counseling and medical referral for HIV infection. Informed written consent will be obtained from all subjects prior to enrollment.

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