

Supporting Statement B for

Prevalence, Incidence, Epidemiology and Molecular Variants of HIV in Blood Donors in Brazil

Extension without Change

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TABLE OF CONTENTS

	Page
B. Collection of Information Employing Statistical Methods.....	1
B.1 Respondent Universe and Sampling Methods.....	1
B.2 Procedure for the Collection of Information.....	2
B.2.1 Questionnaire.....	2
B.2.2 Phlebotomy for Clinical Testing.....	2
B.2.3 Detection of Recent Infections by LS-EIA Testing.....	3
B.2.4 HIV-1 Clade typing and Drug Resistance Testing.....	3
B.2.5 Counseling and Medical Referrals.....	3
B.2.6 Data Analysis.....	3
B.2.6.1 Analysis of Incidence, Residual Risk and NAT Yield.....	3
B.2.6.2 Analysis of Risk Behaviors.....	4
B.2.6.3 HIV Subtype and Drug Resistance Analysis.....	4
B.3 Methods to Maximize Response Rates and Deal with Non-response.....	4
B.4 Test of Procedures.....	4
B.5 Individuals Consulted on Statistical Aspects and Individuals Collecting and/or Analyzing Data.....	4

B. Collection of Information Employing Statistical Methods

B.1. Respondent Universe and Sampling Methods

Surveillance will be performed to identify HIV NAT yield donors and HIV seropositive donors. All eligible cases will have had dual HIV EIA testing and Western blot performed to confirm their HIV-positivity. In order to distinguish between recent seroconversion and long-standing infection, samples from all HIV antibody dual 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" Enzyme Immunoassay). A questionnaire on risk factors, developed for the current, first phase of the study, approved by OMB, and utilized at all 4 centers in Brazil for the previous phase of data collection, will continue to be administered through ACASI 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. 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. The study plans 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:

- Blood donors confirmed HIV-positive by EIA and Western blot or identified as HIV NAT yield will be included.
- Autologous blood donors will be excluded.
- Donors under the age of 18 will be excluded.

Subject Enrollment:

For this next phase of the Study, subjects will be enrolled for a five-year time 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, 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 who do not return for confirmatory testing, the samples will be anonymized and sent to Blood Systems Research Institute (BSRI), San Francisco, CA to perform the Recent Infection Testing Algorithm (RITA).

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 of the 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.

Sample Size Calculations:

This will be an ongoing surveillance activity for a 5-year period of the REDS-III study. Across all 4 centers we expect that as many as 2 NAT-only yield cases and 200 HIV seropositive donors per year will be identified through standard testing. All 200 seropositive donors will be tested for recent acquisition of infection (RITA). 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.

B.2. Procedure for the Collection of Information

B.2.1. Questionnaire

A detailed HIV risk factor questionnaire will be administered to all subjects. A self-administered audio computer-assisted self-interview (ACASI) on a computer will continue to be used in order to maximize reporting of stigmatized behaviors. A research assistant or nurse will provide the ACASI (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.2.2 Phlebotomy for Clinical Testing

In addition to blood saved from their index blood donation, 30 mL of blood will be drawn from consented cases at the time of the enrollment and interview. Specimens will be sent for genotype testing in Sao Paulo, Brazil and RITA testing in San Francisco, CA, 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.

B.2.3 Detection of Recent Infections by LS-EIA Testing

All eligible cases will have had dual HIV EIA testing and HIV Western blot to confirm their HIV seropositivity, per core procedures at the Brazilian central laboratory. Per routine procedures the blood centers will also have minipool (pools of six donations) Nucleic Acid Testing (NAT) results throughout the proposed study enrollment period. Recently infected individuals will be defined through the Standardized Testing Algorithm for the RITA protocol.

B.2.4 HIV-1 Clade Typing and Drug Resistance Testing

Sequencing of the entire HIV-1 protease gene (99 amino acids [aa]) and of the reverse transcriptase (RT) gene through amino acid 240 will identify all mutations known to confer resistance to protease, nucleoside and non-nucleoside RT inhibitors.⁸

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 and complementary DNA will be obtained using Superscript reverse transcriptase. A nested PCR will be used to obtain one fragment containing the protease gene and approximately 700 base pairs of the RT gene.

B.2.5 Counseling and Medical Referrals

Prior to enrollment, all subjects will have received counseling regarding their HIV infection by trained personnel at the blood centers, per standard procedures and according to operational protocols. The genotype and drug-resistance results will be sent to the donor by mail and they will be advised to share these with their physician. If desired by the subject, a new visit will be provided to discuss the results of this testing.

B.2.6 Data Analysis

B.2.6.1 Analysis of Incidence, Residual Risk and NAT Yield

Data from this study will be merged with the core REDS-III Brazilian donation database to allow calculation of incidence and univariate and multivariate analyses 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 are 5.6, 9.0, 15.0 and 20.3 days, respectively, as described by Busch et al.⁹

Confidence intervals for prevalence rates will assume that 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 that incident cases are Poisson distributed. Poisson regression will be used to assess differences in incidence 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.

B.2.6.2 Analysis of Risk Behaviors

We will report the frequency of risk behaviors according to demographic characteristics, recent versus long-standing infection, and HIV genotype categories. We will compare recent and longstanding infections to identify statistical differences in the factors associated with recent infection. 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.

B.2.6.3 HIV Subtype and Drug Resistance Analysis

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:¹⁰ 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.

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

Every donor who consents to participate in the study will be asked to complete the questionnaire. Since the questionnaire will be conducted in a more user friendly ACASI format, and also a research staff or a nurse will be available to provide assistance and answer questions, it is assumed that all donors participating in the study will respond to the questionnaire.

B.4. Test of Procedures

The pretesting of the ACASI questionnaire was conducted at Sao Paulo Blood Center before the initiation of the HIV case-control study conducted under the previous (current) OMB authorization by asking 9 donors to complete the questionnaire using a touch screen computer. Two of the donors had minimal (very low) education and they took approximately 40 minutes to complete the questions but had no problems using the ACASI tool. The average time spent to complete the questionnaire was 24 minutes, and we have also used this for the calculation of burden hour. Also, the pretest provided valuable comments, leading to further refinement of questionnaire format and content.

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

We have consulted biostatisticians on statistical aspects of the study design, the blood centers researchers responsible for enrollment, administering questionnaires and collection of samples as well

as the Coordinating Center staff for protocol development, study monitoring, and data management. Data analysis will be performed by the analytic staff at the Coordinating Center that includes epidemiologists and biostatisticians, with assistance and oversight provided by the REDS-III International Advisory Committee (see Attachment 3.3 for a complete list of IAC members). The REDS-III OSMB (attachment 3.1) will monitor the study.