Section B

Introduction

B. Collection of Information Employing Statistical Methods

B.1. Respondent Universe and Sampling Methods

B.1.1. Donor Universe

The population consists of donors at the six REDS-II blood collection centers. The inclusion criteria are different for the two cohorts. For the First time/Reactivated cohort, donors whose last donation was over 2 years ago and are age 18 and older, presenting to give whole blood or double red cell donation are eligible to participate in the study. Frequent repeat donors who are age 18 and older, with a history of three or more annual whole blood donations in the last year for men, and two or more annual whole blood donations in the last year for women, or double red cell equivalent are qualified to take part in the study. FT/reactivated donors meeting the above criteria who will commit to 2 additional donations or more per year for two years and to give a final blood sample and complete a follow-up survey about 19-24 months after the baseline visit will be enrolled in the study. Repeat donors meeting the above criteria who will commit to maintain or exceed their current (last year) donation frequency for two more years and to give a final blood sample and complete a follow-up survey about 19-24 months after the baseline visit will be enrolled at the baseline visit. In addition, donors who are hemoglobin-deferred at the baseline (enrollment) visit will be asked to enroll for the baseline study procedures (laboratory measures/questionnaire at baseline). There will be no further follow-up of these donors.

B.1.2. Donor Sample

Making reasonable assumptions for deferral rates (20% in FT/reactivated females, 10% in FT/reactivated males, 15% in RPT females, and 5% in RPT males), non-deferred donor consent rates (30% for FT/reactivated donors, 50% for repeat donors), loss at enrollment for specimen loss and incomplete blood unit drawn (10%), and donor compliance with the protocol requirements (50% FT donor cohort, 75% RPT donor cohort), we estimate initial requirement of approximately 3970 FT/reactivated non-deferred male donors and 4470 FT/reactivated female donors to identify 3570 male and 3570 female non-deferred FT/reactivated donors, enroll about 1070 males and 1070 females and have final follow-up data on 480 male and 480 female FT/reactivated donors. Similarly, for RPT donors, we estimate that 1890 female and 1685 male donors will need to be evaluated so that 1600 female and 1600 male RPT donors who are not deferred can be approached for possible enrollment. We would then expect to enroll 800 female and 800 male RPT donors and have final follow-up data for 540 female and 540 male RPT donors. We also estimate that centers should also approach about 1850 FT/reactivated donors (300 per center) who are deferred for Hb to enroll about 550 for baseline assessment and have baseline test data on 500. An additional 1,000 Hb-deferred FT/reactivated donors (165 per center) would need to be approached if we assume that 30% will consent to enroll in the study and we aim to enroll 550 FT/reactivated donors.

Table B.1-2 Monthly Recruitment Goals by Donor Subgroup.

	Eligible Donors at Selected sites	Recruitment Goal	Recruitment Goal * (% of eligible donors)	Center-specific Recruitment Goal
FT/Reactivated donors	1657	384	23.0	64
Repeat donors	9151	288	3.1	48
Hgb deferred donors	1837	500	27.2	17

First time/Reactivated Donors Sample: Dividing enrollment equally among centers, each center will have an enrollment goal of 160 female and 160 male FT/Reactivated donors. With available staff we anticipate 4 FT/Reactivated donors can be enrolled per

day. Thus, a center can realistically meet enrollment goals in 90 days (22 days per month).

Repeat Donors Sample: Dividing enrollment of 1440 donors equally among centers, each center will have an enrollment goal of 240 repeat donors. Based on available staffing resources, it will be possible to recruit about 3 repeat donors per day per center.

Hgb Deferred Donor Sample: It is proposed to enroll 500 first time hemoglobin deferred donors in the study. Therefore each center will need to enroll about 85 such donors over a period of 5 months. The 5 month accrual period is therefore expected to be adequate.

B.1.3. Site selection by Centers

Each center will select sites to ensure racial/ethnic and first-time/repeat donor distribution that best represent their overall donor population. Centers will select sites in a manner that 20 eligible donors are available each day, to be approached for the study. Centers may choose to recruit at fixed, mobile, or apheresis sites. All eligible donors at the site will be approached for study enrollment. Once the recruitment target for a particular subgroup has been reached, the CC will provide feedback to the blood center, so that further enrollment into that subgroup will cease.

B.2. Information Collection Procedures/Limitations of the Study

B.2. Procedure for the Collection of Information

Making reasonable assumptions for deferral rates (20% in FT/reactivated, 15% in RPT females, and 5% in RPT males), non-deferred donor consent rates (30% for FT/reactivated donors, 50% for repeat donors), loss at enrollment for specimen loss and incomplete blood unit drawn (10%), and donor compliance with the protocol requirements (50% FT donor cohort, 75% RPT donor

cohort), we estimate a need to initially approach approximately 3970 FT/reactivated non-deferred male donors and 4470 FT/reactivated female donors to identify 3570 male and 3570 female non-deferred FT/reactivated donors, enroll about 1070 males and 1070 females and have final follow-up data on 480 male and 480 female FT/reactivated donors. Similarly, for RPT donors, we estimate that 1890 female and 1690 male donors will need to be evaluated so that 1600 female and 1600 male RPT donors who are not deferred can be approached for possible enrollment. We would then expect to enroll 800 female and 800 male RPT donors and have final follow-up data for 540 female and 540 male RPT donors. An additional 960 Hb-deferred FT/reactivated donors (160 per center) would need to be approached if we assume that 30% will consent to enroll in the study and we aim to enroll 550 Hb deferred FT/reactivated donors.

B.2.1. Statistical Methodology for Stratification and Sample Selection

B.2.1. Convenience Sample

A probability sample is not proposed, nor necessary, in this study. There is no reason to believe that iron depletion in first time or reactivated donors is associated with donation site. For example, whether a first time or repeat donor donates at a fixed or mobile site is expected to be independent of iron status. Therefore, allowing centers to select sites is not an issue.

Additionally, iron depletion is anticipated to be independent of consent rates. Analysis is based on the development of iron depletion in each of the study groups. Since the iron status of the donor is unknown to themselves there is no reason to believe it would bias donor consent rates. Although, a donor that develops iron depletion may be less likely to return to make a subsequent donation. Analysis techniques will be applied

to account for such donors self censoring (i.e. ‘dropping’ out of the longitudinal study due to developing iron depletion or other illness as a result of accrued blood donations).

B.2.2. Estimation Procedure

B.2.2 Estimation Procedures

B.2.2.1 Hemoglobin Measures

Several analyses are tentatively planned that will use a quantitative measure of hemoglobin/hematocrit.

1. **Conversion of fingerstick hematocrit and fingerstick hemoglobin measurements into pre-donation venous hemoglobin estimates**: The pre-donation venous hemoglobin result is anticipated to be an unbiased measure of the donor’s hemoglobin pre-donation. The fingerstick HemoCue® result is also anticipated to be an essentially unbiased measure of the donor’s pre-donation hemoglobin, albeit a more variable measure. However, to ensure that these 2 values (pre-donation venous hemoglobin and fingerstick HemoCue®) are similar we will compare the fingerstick HemoCue® results and pre-donation venous hemoglobin results for donations on which both measurements are made.
2. **Longitudinal analyses involving fingerstick hematocrit/hemoglobin measurements**: Examples of longitudinal analyses to evaluate changes in hemoglobin over time as a function of donation intensity are:
    1) Analysis where fingerstick hemoglobin (HemoCue®) is the outcome variable of interest; this analysis will be restricted to the centers that will use HemoCue® as their test-of-record.

2) Analysis where fingerstick hematocrit is the outcome variable of interest; this analysis will be restricted to the centers that use a fingerstick hematocrit as their test-of-record.

3) Analysis where pre-donation venous hemoglobin is the outcome variable of interest. In this analysis, we will not use actual observed pre-donation venous hemoglobin values even if available but rather transform the fingerstick hemoglobin or hematocrit value obtained at each visit into a pre-donation venous hemoglobin equivalent using the appropriate conversion factors.

1. **Analyses involving only baseline and final follow-up visit hemoglobin levels**: The pre-donation venous hemoglobin values will be used in the following analyses:
    1. Analyses where baseline hemoglobin is the variable of interest. For example, sex-specific median pre-donation venous hemoglobin values will be determined from the observed and projected pre-donation venous hemoglobin values among FT/reactivated donors. Baseline hemoglobin will be defined as "high" if the baseline pre-donation venous hemoglobin is greater than the sex-specific median and will be defined as "low" otherwise.
    2. Analyses comparing hemoglobin distributions at baseline visit among first-time/reactivated donors and NHANES.
    3. Analyses that evaluate changes between baseline and final follow-up visit hemoglobin as a function of donation intensity and other variables of interest.

B.2.2.2 Iron Status and Donation Intensity Association

Initial analyses of the associations between iron status and donation intensity will be based on log-likelihood χ^2 statistics of corresponding 2x2 tables. Males and females will initially be analyzed separately. Examples of 2x2 tables of interest include a 2x2 table of 'iron depletion status' by 'donation intensity' among female first-time/reactivated donors and a 2x2 table of 'iron depletion status' by 'donation intensity' among male first-time/reactivated donors. Further, two

other χ^2 statistics of interest will be conducted in repeat donors (iron depletion by donation intensity for females and males separately). Next, two χ^2 statistics of interest will be conducted in FT/reactivated donors (ever Hb deferred by donation intensity for females and males separately). Finally, two χ^2 statistics of interest will be conducted in repeat (ever Hb deferred by donation intensity for females and males separately).

These initial log-likelihood χ^2 statistics can be considered to be the results from corresponding unadjusted binary logistic regressions (e.g.; 'iron depletion' as the binary outcome variable and donation intensity as the single predictor or independent variable). Although adjusted logistic regression models could be considered (e.g. adjust for smoking, mineral supplementation, etc.), more elaborate models are planned.

B.2.2.3 Models Predicting Iron Status

The goal of the overall analysis is to develop models that will accurately predict the development of iron depletion, IDE, and/or iron deficient donor deferral in active whole blood and double red cell donors. Information derived from these models will help suggest potential guidelines to reduce the development of these outcomes among donors.

Logistic models (for binary outcomes) and linear regression models (for continuous variables) will be developed that can be either unadjusted (one independent variable) or adjusted (several independent variables). Covariates such as age, smoking, dietary supplement, menstrual history variables, genetic markers and other laboratory indices will be considered when building the adjusted models. Some continuous outcomes may require transformation (e.g. while ferritin levels tend to be on the order of 30 or 50 ng/mL, some donors have levels of 100, 200 ng/mL, or more; thus ferritin will probably need to be analyzed on the log scale).

B.2.2.4 Comparison to NHANES-III Hemoglobin distribution

The baseline venous hemoglobin measures from FT/reactivated, deferred and accepted, donors will be weighted (to reflect differential sampling probabilities of deferred and non-deferred donors) to determine estimates of the mean hemoglobin levels for the blood donor population by age, gender and race. These means will be compared to the NHANES-III data results as shown in Table B.2-1. If mean hemoglobin levels among the blood donor population differ from the U.S. populations (as determined by NHANES-III), then further analysis will be undertaken to demonstrate how the hemoglobin distribution among the blood donor population differs from the distribution among the U.S. population.

Table B.2-1. NHANES-III Mean Hemoglobin (g/dL) by age, gender, and race/ethnicity.

	Male			Female		
	White	Black	Hispanic	White	Black	Hispanic
Age (years)						
20-29	15.5	14.8	15.5	13.3	12.4	13.0
30-39	15.3	14.6	15.5	13.4	12.4	13.0
40-49	15.2	14.5	15.4	13.4	12.4	13.0
50-59	15.1	14.3	15.3	13.6	12.9	13.5
60-69	14.8	13.9	15.1	13.5	12.9	13.4
70+	14.4	13.4	14.9	13.4	12.5	13.4

B.2.3. Degree of Accuracy Needed for the Purpose Described in the Justification

Upon completion of the survey, study coordinators will visually check for accurate completion of the survey. Responses to FAQ's will be provided to coordinators so that all respondent questions will be answered in a standard manner. The questionnaire is a self administered paper survey. Following completion, study coordinators will enter data into a web-based data entry system with data capture rules programmed. The system will provide logic checks such as response dependent skip patterns and date range checks.

B.2.4. Unusual Problems Requiring Specialized Sampling Procedures

B.2.5. Use of Periodic (Less Frequent Than Annual) Data Collection Cycles

B.3. Methods for Maximizing the Response Rate and Addressing Issues of Nonresponse

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

Collection centers will designate a site for donor recruitment. The number of enrolled donors among all eligible donors at the site will define the recruitment rate which will be calculated separately for the three cohorts (first time/reactivated donors group, the repeat donors group, and the deferred donors group) and for the six centers. Posters, flyers and table top signs announcing the study will be displayed at recruitment sites. The following procedures are designed to maximize continued participation of among enrollees:

1. Periodic newsletters will be used as a means of reminders to the enrolled donors to come back and donate.
2. Posters will be placed in the reception and canteen areas of the research sites.

B.4. Tests of Procedures or Methods

B.4. Test of Procedures

Based on previous experience with similar laboratory studies of the donor populations during REDS-I we will not be conducting any pretests for the purposes of refining the data collection activities proposed.

B.5. Names and Telephone Numbers of Individuals Consulted

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

Attachment 6.1 lists those consulted: biostatisticians on statistical aspects of the study design; the blood centers researchers responsible for enrollment, administering questionnaires, and collection of samples; and the CC staff for protocol development, study monitoring, and data management. Data analysis will be performed by the analytic staff at the CC that includes epidemiologists and biostatisticians, with assistance and oversight provided by the REDS Steering Committee (see Attachment 6.1 for a complete list of Steering Committee members)