REDS-II DONOR IRON STATUS EVALUATION (RISE) STUDY

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SUPPORTING STATEMENT B

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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(FT)/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.

B.1.2 Donor Sample

A sample of 225 female FT/reactivated donors will yield a sample of about 200 female FT/reactivated donors who are not iron depleted at enrollment. A sample of 200 female FT/reactivated donors who are not iron depleted at enrollment will have 85% power in a one-tailed 0.05 level test to assess high donation intensity effect on development of iron depletion in female FT/reactivated donors and 99% power to assess effect on hemoglobin deferral in female FT/reactivated donors. An equal sample of FT/reactivated male donors is planned.

A sample of 540 RPT female donors will have 85% power in a one-tailed 0.05 level test to assess high donation intensity effect on development of iron depletion in RPT female donors and 94% power to assess effect on hemoglobin deferral in RPT female donors. An equal sample of RPT male donors is planned.

Assumptions concerning 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 (4%), and donor compliance with the protocol requirements (57% FT donor cohort, 75% RPT donor cohort), are discussed in section E.3 of the protocol. These assumptions lead to the conclusion that approximately 1400 FT/reactivated non-deferred male donors and 1400 FT/reactivated non-deferred female donors need to be identified. Among these eligible donors the recruitment goals will be 420 male and 420 female non-deferred FT/reactivated donors, leading to final follow-up data on 225 male and 225 female FT/reactivated donors. Similarly, for RPT donors, we estimate that 1500 female and 1500 male RPT donors who are not deferred need to be identified. We would then expect to recruit 750 female and 750 male RPT donors and have final follow-up data for 540 female and 540 male RPT 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	168	10.1	28
Repeat donors	9151	300	3.3	50

First time/Reactivated Donors Sample: Dividing enrollment equally among centers, each center will have an enrollment goal of approximately 70 female and 70 male FT/Reactivated donors. With available staff we anticipate 4 FT/Reactivated donors can be enrolled per day. Thus, a center should easily meet enrollment goals within the allotted 5 months (i.e. Recruiting 140 donors is anticipated to take 35 days. Assuming 22 ‘working’ days per month, the required time is less than 2 months).

Repeat Donors Sample: Dividing enrollment of 1500 donors equally among centers, each center will have an enrollment goal of 250 repeat donors. Based on available staffing resources, it will be possible to recruit about 4 repeat donors per day per center. Again, a center should easily meet enrollment goals. (i.e. Recruiting 250 donors is anticipated to take 63 days. Assuming 22 ‘working’ days per month, the required time is about 3 months).

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 Procedure for the Collection of Information

Data collection occurs at time of donation. At the baseline visit and final visit there is a self administered questionnaire. Much of the laboratory data is collected routinely as part of the donation procedure. This data will be available to this study. Some supplemental laboratory data will be collected for enrolled donors at each donation time. The procedures are outlined in section F.2 of the protocol.

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 Procedures

We will use descriptive statistics to evaluate the distributions of all variables. We will use log-likelihood χ^2 statistics (or exact tests if cell sizes are too small) to evaluate if the distribution of a categorical characteristic (e.g., high vs. low baseline hemoglobin in first-time/reactivated donors; high vs. low HYPOm) is significantly different among groups (e.g. gender, race/ethnicity, iron supplementation vs. not). For comparison of continuous characteristics among groups, we will compare means among several groups by conducting t-test

(two groups) or analysis of variance (> 2 groups); or if a non-parametric method is more appropriate by conducting a Wilcoxon rank-sum test (two groups) or a Kruskal-Wallis test (> 2 groups). Correlations and coefficients of determination may also be used to evaluate the association between two continuous variables. To evaluate whether two continuous variables are equivalent (such as evaluating if the hemoglobin level obtained by fingerstick HemoCue® is similar to that obtained from a pre-donation venous draw), a paired t-test would be suggested.

B.2.2.1 Hemoglobin Measures

Hemoglobin is assessed in a variety of ways. Several analyses are planned (as described in section H.2.1 of the protocol) that will compare the various measures. A comparison of hemoglobin and hematocrit will be done. The difference between pre-donation and post-donation measures will be evaluated. Also, the difference between fingerstick and venous measures will be evaluated.

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.

Primarily the 'elaboration' is to develop repeated measures models for iron status (iron depletion, IDE, and/or iron deficient donor deferral) using additional data derived from interim visits (i.e. donations made between baseline visit and final visit). Such analyses are outlined in section H.4 of the protocol.

B.2.2.3 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.2.2.4 Prevalence of RLS and Pica in blood donors

Associations of RLS and Pica with iron status (iron replete, iron deficiency, and iron deficiency anemia) among blood donors will be based on log-likelihood χ^2 statistics of corresponding 2x2 tables. Since both disorders (RLS and Pica) are known to be strongly associated with iron deficiency, our sample of at least 1200 blood donors with final questionnaire data concerning RLS and Pica is expected (with statistical power exceeding 95% in both instances) to confirm these known associations. Our longitudinal data concerning blood donation history will allow unique exploratory analyses relating iron status with RLS and Pica.

B.2.3 Quality Control

Upon completion of the baseline visit and final visit questionnaire, study coordinators will visually check for accurate completion. 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.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 two cohorts (first time/reactivated donors group, the repeat 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:

- Reminder Postcards (Attachment 7):
  - Blanket Mailings: A blanket mailing of reminder postcard will be sent 11 months into the study to all study participants.
  - Targeted mailings: Towards the end of the Interim Phase (16 months into the study), targeted mailings will be conducted for monthly cohorts in the order donors were enrolled during the Baseline Phase.
- Posters will be placed in the reception and canteen areas of the research sites.

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 Individuals Consulted on Statistical Aspects and Individuals Collecting and/or Analyzing Data

Attachment 5 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 4 for a complete list of Steering Committee members)