LAPS PROTOCOL

Leukocyte Antibodies Prevalence Study

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American Red Cross Blood Services - Southern Region
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The REDS-II Leukocyte Antibodies Prevalence (LAP) Study

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Leukocyte Antibodies Prevalence (LAP) Study

A. Protocol synopsis

The Leukocyte Antibodies Prevalence (LAP) Study is a cross-sectional multi-center study to measure the prevalence of HLA and neutrophil antibodies in blood donors with or without a history of blood transfusion or pregnancy. Over a six month period, 7,900 adult blood donors from all six REDS-II blood centers will be enrolled in the study. Eligible donors will be provided with detailed information regarding the study and given sufficient opportunity to ask questions. Donors who are willing to participate will be consented (Attachment 6). Donors will also be asked to complete a short questionnaire (Appendix 3) relating to their transfusion history (ever, number, and date of last transfusion) and, for female donors, their pregnancy history (ever, number and outcome of pregnancies, date of last pregnancy). Male donors will be asked if they have ever had a transfusion and if yes, will be asked for the number of prior transfusions and the date of the last transfusion episode. Female donors who have never been pregnant will only need to answer one additional question. Females who have been pregnant will be asked five additional questions about the number, outcome, and date of last pregnancy. Each donor will also be asked to provide a sample of blood to be tested for the presence of HLA class I and class II antibodies using Flowcytometry techniques. These data will be used to evaluate variations in HLA antibody prevalence based on blood transfusion and pregnancy history and time since the last immunizing event (transfusion or pregnancy). This study will have sufficient power to establish with confidence the relationship between number of pregnancies and HLA antibody prevalence in US blood donors. Neutrophil specific antibodies will be measured in blood donors who have HLA antibodies since this group represents donors who are immune responders and are therefore considered more likely to have formed anti-neutrophil antibodies. Conducting neutrophil antibody testing only on this group of donors is a cost effective testing strategy since fewer samples will need to be tested to estimate the highest prevalence of neutrophil antibodies in blood donors. Specimens from donors found to have neutrophil antibodies will undergo further testing to determine their neutrophil phenotype using routine serologic and DNA methods, since individuals homozygous for certain neutrophil antigens are more prone to develop certain neutrophil antibodies. The results from testing HLA positive donors for neutrophil antibodies in this primary study could be used to develop an optimal testing strategy for larger number of donors in the future using the stored repository samples. These data will provide the basis for calculating donor loss in the event that a TRALI prevention strategy is implemented that includes deferring donors with a history of transfusion or pregnancy or those with HLA or neutrophil antibodies. These data are critical since the U.S. is already faced with blood shortages and any policy to restrict donations by individuals transfused or with multiple pregnancies will have a serious adverse effect on the ability to deliver blood when needed.

The second major goal of this study is to develop a repository of blood samples from well characterized blood donors whose detailed transfusion and pregnancy histories are known. This would be the largest repository of its kind. Repository samples will be stored indefinitely. Although future research on repository samples is yet to be determined, they may be tested for

studies designed to help transfusion safety and transfusion biology. Donors will be informed of the plan to establish this research repository and have the option to opt out if they so choose.

The current project includes human subject research. To a large degree, risks of phlebotomy to obtain blood samples are obviated by using a portion of the blood sample that is collected at the time of each blood donation. Minimal risk is expected if separate phlebotomy is employed to collect the study specimen. Risks of notification of test results for HLA and neutrophil antibodies and neutrophil typing are considered minimal. Safety measures have been included in this protocol to minimize the risk related to loss of privacy involved with a linked repository. For instance, the protocol includes procedures for double coding of the blood samples, procedures for the manner in which the codes can be linked to allow confidential communication of the results between participating centers, coordinating center and the testing laboratory, and procedures to be met before donors can be approached in any follow-up studies.

The protocol is designed to involve the six REDS-II Participating Centers and thus provides a platform for coordinating similar studies in the future. The six REDS-II centers are: Blood Centers of the Pacific, San Francisco, California; Blood Center of Wisconsin, Milwaukee, Wisconsin; American Red Cross Southern Region, Atlanta, Georgia; Hoxworth Blood Center, Cincinnati, Ohio; Institute for Transfusion Medicine, Pittsburg, Pennsylvania; American Red Cross New England Region, Dedham, Massachusetts. Westat, in Rockville, Maryland serves as the coordinating center. Blood Systems Research Institute, San Francisco, California is the central laboratory and SeraCare BioServices in Gaithersburg, Maryland is the central repository.

B. Study schema

The study schema for the LAP study includes the following benchmark tasks: protocol review and approval by the REDS-II Steering Committee, Executive Committee and OSMB; protocol dissemination to the participating centers, central laboratory and the coordinating center; IRB approval at all participating facilities; protocol-specific training; protocol implementation to include identification and enrollment of study participants; collection and processing of blood samples; labeling of blood samples to ensure proper coding; sample aliquot preparation and freezing; shipping of the samples to the central laboratory for HLA screening and to Blood Center of Wisconsin, the designated laboratory for Neutrophil antibody screening and characterization; shipping of the samples to an approved storage facility for repository development and maintenance; central management of the donor and sample data at the coordinating center; and data analysis, interpretation and publication.

C. Objectives

This study has the following objectives:

1. Establish the prevalence of HLA antibodies in relation to donor characteristics, such as sex, age, number of pregnancies (deliveries, miscarriages/terminated pregnancies), history of blood transfusion, and time elapsed since the immunizing event/s.

2. Measure the prevalence of HLA class I and class II antibodies, including their titer in blood donors, and determine the type of HLA antibody present (mono-specific, multi-specific, or non-specific).

3. Measure the prevalence of neutrophil antibodies in a group of donors who are alloimmunized to HLA.

4. Characterize the neutrophil antibodies in blood donors by DNA testing.

5. Create a repository of linked blood samples from well-characterized blood donors (age, sex, detailed history of transfusion/pregnancy). Future studies related to transfusion safety and transfusion biology will be possible by accessing the repository samples. These latter studies will require protocol development, review and approval before the samples can be accessed.

D. Significance and rationale for studies to measure prevalence of anti-leukocyte antibodies in blood donors

Two current hypotheses for pathogenesis of transfusion-related acute lung injury (TRALI) include the development of acute pulmonary insufficiency from immune and non-immune causes.[1-10] The immune-mediated mechanism postulates that passively transferred anti-leukocyte antibodies from blood donors are responsible for causing activation and aggregation of the recipient's leukocytes that subsequently lead to pulmonary capillary leakage and alveolar edema.[1-2] Rarely, the recipient's anti-leukocyte antibodies that bind and activate passively transfused donor leukocytes initiate the immune mechanism.[1] The donor antibodies involved in TRALI include antibodies directed towards HLA class I and HLA class II antigens. [1,6,8,11]Anti-neutrophil antibodies have also been implicated.[1,8,12,13] The LAP study will not address the pathogenesis of non-immune causes of TRALI.

D1. Prevention of TRALI

Approaches to prevention of TRALI could include (a) routine questioning of blood donors for a history of pregnancy or blood transfusion, (b) permanent deferral of donors implicated in TRALI cases, (c) testing of donated units for the presence of anti-leukocyte antibodies, and (d) diversion of plasma from high-risk donors defined above.[2, 7, 14-15]

The blood collection program in the Netherlands implemented a preventive approach in 1970 in which all donors who gave a history of pregnancy or blood transfusion were excluded from plasma donation.[7] The program did allow the donation of platelet concentrates from whole blood and red blood cells that generally contained less than 50ml of plasma. However, the lack of details on the results of this preventive approach does not allow generalization of their experience.

More recently, the United Kingdom implemented plasma diversion as a preventive step. In this plan, fresh frozen plasma (FFP) units from women donors were not used for blood transfusion and were discarded.[16] However, the UK imports a significant number of FFP as a prevention measure for variant Creutzfeldt Jacob Disease (vCJD) and whether the plan would have been successful without importation of FFP is not certain.[17]

Additional regional blood center-specific reports describing diversion of plasma have appeared in the literature.[7,18-19] However, insufficient information is available to indicate that such programs can be applied nationally in the U.S.

Arguments against deferring multiparous donors from donating plasma components are based on the following: First, TRALI cases are not limited to plasma containing products only and in fact, one third of the cases are from transfusion of red blood cells, which contain only a small amount of plasma.[10] Second, although prevalence of anti-leukocyte antibodies in multiparous blood donors is high, the prevalence of TRALI is considerably lower.[1] Third, more than 15% of the donors and more than 25% of multiparous donors would be deferred for alloimmunization against HLA antigens.[15] In addition, pregnancy or blood transfusion may also result in anti-neutrophil antibodies in a small proportion of individuals resulting in even greater donor deferral.[20-21] Thus application of a strategy consisting of deferring donors with anti-leukocyte antibodies will result in significant rates of deferrals.[2, 14-15] Fourth, there is infrequent concordance between donor antibody specificity and recipient's leukocyte antigens.[10, 22-23] Even when concordance exists, TRALI does not necessarily occur.[24] Fifth, women without a history of pregnancy can possess anti-HLA antibodies.[20] Sixth, loss of antibody can occur over time after pregnancy or transfusion.[25] Finally, deferral of multiparous women would have significant adverse effects on blood component availability that could lead to shortage and adverse impact upon patients who need non-elective transfusions.

D2. HLA class I antibody prevalence in previously pregnant blood donors – old literature

In the past 30 years or so, a number of published studies have documented that women with a history of pregnancy frequently develop circulating anti-HLA antibodies.[26-36]These studies were conducted using the standard microlymphocytotoxicity (LCT) assays, and the definition for the presence of antibodies varied considerably among them. Definitions for a positive screen ranged from 1 positive cell out of 23 panel cells to 5 positive cells out of 100 panel cells. This variability may have contributed to more than a two-fold difference (12% vs. 38%) in the prevalence of HLA antibodies seen in pregnant women between different studies.[35, 30] In addition, LCT assays use live lymphocytes and are therefore subject to lymphocyte viability problems during storage in Terasaki trays. LCT assays are increasingly being replaced with HLA antigen-coated beads that utilize either enzyme-immunoassays or fluorescence measurements by a flow cytometer.[37] Newer assays are more sensitive and better able to simultaneously differentiate mixtures of HLA antibodies present in donor sera than the LCT assays.

Previous studies also suffered from small sample sizes.[26-36] The largest study was reported in 1974 and included screening of 3,662 sera from multiparous women.[26] This sample size gives a power of >85% when differences in antibody prevalence are compared between women with a different number of pregnancies. However, when this study was published, HLA

class I loci were not well established, most of the class I antigens were yet to be discovered, and HLA class II antibodies were not studied.[26] Also, data regarding the persistence of HLA antibodies after the last pregnancy are limited. The two previous studies are not in agreement regarding the prevalence of HLA antibodies found in women who were screened more than 15 years after their last pregnancies (approximately 8% and 26% prevalence rates respectively).[26,15]

D3. HLA class I antibody prevalence in previously pregnant blood donors – recent studies

Two recent studies have been published. A study from Washington University in St. Louis reported 332 apheresis female donors who were tested for HLA class I and class II antibodies using microlymphocytotoxicity.[15] Because of the small sample size, the study is underpowered to assess the prevalence of antibodies in relation to the number of previous pregnancies. The second study is from the United Kingdom (UK) and is published in abstract form.[20]This study answers some of the questions that need to be addressed in order to develop recommendations for donor management to prevent TRALI. For instance, it provides information about HLA antibody prevalence in blood donors in relation to the number of previous pregnancies and the history of blood transfusion. It also shows that in UK blood donors, the prevalence of anti-human neutrophil antibodies is quite low. However, several limitations of this study exist and our proposed project will address them (see section D8 below).

D4. HLA class II antibody prevalence in previously pregnant blood donors

The two studies referred to above provide some information regarding HLA class II antibodies. However, the total number of women included in the two studies was small (N=1,498). Other limitations of these studies are described below.

D5. Human neutrophil antigen (HNA) specific antibodies in previously pregnant blood donors

In the literature, we could identify only two large studies. In the first study, 2,313 sera from multiparous women were screened.[21]In the second study, 1,416 sera were screened.[20]There was a large difference in the prevalence of anti-HNA antibodies between the studies (0.1% vs. 1.8% respectively). These differences may relate to the differences in the underlying donor population (US vs. UK) or the methods used to detect the antibodies. More definitive studies are needed to characterize the prevalence of anti-HNA antibodies in multiparous women.

D6. History of blood transfusion in blood donors and rates of HLA alloimmunization – a review of the older literature

A minority of the blood donor population has a history of blood transfusion (119,626 of 2,860,999 blood donors or 4.2%)[38] According to the "Sixth Edition of Mollison" published in 1979, anti-HLA and anti-neutrophil antibodies are known to be produced in *patients* who receive blood transfusions.[39] This has been studied by several investigators in the 1950s to 1970s using mostly the leukoagglutinin assay to detect the presence of anti-leukocyte antibodies. Leukoagglutinins were found in 16.3% of multiple-transfused patients.[39] Many (69%) of these also reacted with neutrophils.[39] Leukocyte sensitization rates in transfused individuals are

directly correlated with the number of units transfused.[39] Anti-leukocyte antibodies can be found in approximately 5% of patients who have received fewer than 10 units of blood, in 25% to 35% of those transfused with 50 to 100 units, and in as many as 80% of patients who received more than 100 units.[39] A study published in 1974 employed the lymphocytotoxicity method and found lymphocytotoxic antibodies in 25% of patients who received ≤ 10 units of blood and in 44% of patients who received more than 30 transfusions.[39]

Important points to consider regarding the previous observations on the prevalence of anti-leukocyte antibodies after blood transfusion are that these studies were conducted in patients shortly (i.e., weeks) after their transfusion and that the blood components were not leukoreduced. More recent data suggest that the rates of anti-leukocyte antibodies in transfused blood donors are much lower (see below). Thus, the information available regarding patients is not directly applicable to blood donors. A carefully conducted study is needed to determine prevalence of HLA antibodies in blood donors who have been transfused previously.

D7. History of blood transfusion in blood donors and rates of HLA alloimmunization – a review of the more recent literature

One recent study from the UK detected anti-HLA antibodies in 4/205 (2.0%, 95% CI 0.5%-4.9%) un-transfused and 1/48 (2.1%, 95% CI 0.1% - 11.1%) transfused male donors.[20] These investigators concluded that there was a lack of effect of blood transfusion on rates of HLA prevalence in male donors.[20] However, the confidence intervals were wide because of the small sample size.

D8. LAP Study will address limitations of previously published studies

1. We will use improved (Flow vs. EIA/LCT) methods to study HLA class I and class II antibodies in a sufficient number of blood donors with a history of pregnancy to establish with confidence the relationship between the number of pregnancies and HLA antibody prevalence in US blood donors.

2. Previous studies did not address the titer of HLA antibodies detected in multiparous women donors; our study plans include measurement of the titers. Preliminary evidence suggests that antibody titers may be important based on the fact that HLA antibodies are not detected in solvent detergent (SD)-treated plasma, which is prepared from a pool of a large number of plasma donations that sufficiently dilutes antibodies until they are no longer detectable.[40] Also, there are preliminary data suggesting that TRALI incidence may be lower with SD-plasma compared to FFP.[41]

3. Previous data conflict regarding the influence of the interval from the last immunizing event on the presence or absence of antibodies. We will collect data on the time of the last immunizing event and evaluate its association with HLA antibody results. In subsequent studies, such an association can also be evaluated for neutrophil antibodies. Our study may also generate new data regarding antibody titer in relation to the number of pregnancies or blood transfusions and the interval since the last immunizing event.

4. Our study could confirm the UK data regarding HLA antibody prevalence rates in multiparous donors. However, it is possible that we might detect differences in prevalence in our donors when compared to the UK donors because their population genetics might differ. Furthermore, prevalence of antibodies might be lower in transfused blood donors in the UK compared to the US due to the fact that the UK adopted pre-storage leukocyte-reduced blood components earlier than the US. While our study will not directly address the possible salutary effect of transfusions of universally leukocyte-reduced blood components on leukocyte antibody formation, we will be able to examine the persistence of the antibodies in relationship to the time since the last transfusion.

5. We will test a selected group of blood donors for anti-neutrophil antibodies. The group will be composed of donors known to have anti-HLA antibodies. This group represents donors who are immune responders and are therefore considered more likely to have formed anti-neutrophil antibodies. This strategy will require testing of a smaller number of samples and will thus be more cost effective as an initial step to estimate the highest prevalence of neutrophil antibodies in blood donors.

6. The samples from donors who have the anti-neutrophil antibodies will be phenotyped for neutrophil antigens using the standard serological and DNA typing methods.[43-49] Neutrophil antigen phenotypes have been found to be associated with the type of antibody produced. For instance, women with NA1/NA1 phenotype, that is women who are homozygous for NA1 antigen, are likely to produce anti-NA2.[50] The consent form will include consent for WBC typing of the research study samples.

7. In addition to testing the selected number of donors for anti-neutrophil antibodies described above, we will also establish a repository of blood samples from donors with known demographics, transfusion/pregnancy history, and anti-HLA (Class I/II) antibody status. Such a repository will allow for testing of a larger number of specimens for neutrophil antibody in the future. The repository will also allow us to select the optimal method for neutrophil antibody testing when available, evaluate the association between neutrophil phenotype (as determined by DNA/serological methods) and the ability to produce neutrophil antibodies, and allow the comparison of methods for HLA antibodies (e.g. Flow PRA vs. Luminex to determine false positive rates for these methods). This latter approach addresses one particular concern regarding the non-reproducibility of results between laboratories using current methods.[42] Because anti-neutrophil antibody tests are costly, the repository will present opportunities to reduce cost by selecting the desired types of donors to test and reducing the total number of samples that will be tested.

8. Our protocol will study a larger number of transfused donors to establish the prevalence of HLA antibodies in this population with greater confidence.

D9. Other practical reasons to perform the study

The above observations provide the scientific rationale for the study. More practical benefits of the study include utilization of information obtained by supplemental blood donor

questions related to ever having received blood transfusion or ever having been pregnant. This information will be used in the current study to recruit the desired number of donors with such histories. The study will be conducted in all six REDS-II blood centers, providing sufficient donor base to conduct the study. HLA antibody testing methods are readily available at the REDS-II central Laboratory and the coordinating center already has considerable experience with the management of blood samples, including coding of the samples to preserve confidentiality and data analysis.

E1. Future scientific research using the repository samples to increase the understanding of transfusion biology

Future scientific research will also be possible with the use of blood samples from the repository.[51-52] For instance, the repository can be used to assess the frequency of microchimerism after blood transfusion or pregnancy.[53-56] In order to facilitate such future research, appropriate donor consent will be obtained at the time of blood sample donation for the repository.

E2. Privacy risk to the participants of the repository

The potential studies to be conducted with the repository samples would have a low level privacy risk. The participants will be informed that future research may include testing for genetic (inherited) factors relating to WBC's and the body's immune response. The National Institutes of Health will give access to these repository samples only to its employees or approved researchers. Any future study will be reviewed by an Institutional Review Board that will protect the rights of the research participant.

E3. Repository of linked samples

Our protocol calls for a linked repository so that the results of the testing can be linked to the donor. The linked repository offers certain advantages including the ability to notify the donor of test results that may have clinical significance to the donor; re-testing to confirm the results if this is needed; recall the donor for additional samples and recruit other members of the donor's family if family studies might be of value.

E4. Procedures to protect privacy and confidentiality

Although the repository will be linked, procedures will be established to protect the confidentiality of the donor information, the repository data and future test results. In order to ensure the highest level of confidentiality, repository samples will be double coded. Blood centers will have the key (or link) between the blood center donation number and the donor's identifying information (name, address, etc.). The coordinating center will never possess this link. The key linking the blood center donation number and the study specific Subject Identification Number will be located at the coordinating center and at the participating blood centers so that laboratory test results can be communicated between the coordinating center and the participating centers to allow donor notification. The laboratory that will perform the testing will only be given the Subject Identification Numbers so that the laboratory cannot link the

results to the donors. The laboratory will send the results to the coordinating center and the coordinating center will store all test results. The coordinating center will communicate test results to the participating centers to permit donor notification as described above. During REDS-I, a donor/recipient repository was successfully established and experience gained with REDS-I will form the basis for creating the presently proposed repository.[51-52] It should be recognized that one disadvantage of a double-coded repository is that the results are difficult to share with the participants because of the delays caused by the double coding. The delay is unlikely to be of significance because these are repository samples that have been stored for sometime to begin with. Nonetheless, for those test results that might be time sensitive, processes will be established to communicate time-sensitive results in a timely manner by a coordinated effort between the laboratory, the coordinating center and the participating centers.

The steps described above will allow distancing the test results two codes away from the donor identifying information and thus permit maximum confidentiality without compromising the ability to communicate the test results to the donors and the ability to have the donors participate in follow-up research. These control processes will contribute to obtaining maximum individual and societal benefit while safeguarding the privacy of the participating donors. In our project, an anonymized sample repository design was not selected because it would limit the research potential of the repository and prevent the participants from learning potentially important results obtained using their repository samples.

F. Study population

All blood donors age 18 and older at the selected sites will be eligible for the study. Both male and female donors will be enrolled. The participants must be eligible to donate whole blood or apheresis products in order to participate in the study. Donors who test reactive or positive in any of the infectious diseases tests will not be eligible to participate in the study and their specimens will not be stored in the repository. The recruitment goals for the minority donor enrollment will be consistent with the overall proportion of minority donors at each participating center.

G. Study enrollment

The goal is to compile laboratory information on approximately 7,100 donors. In order to achieve this number, a 10% margin is added to account for loss of samples, discarding of samples due to positive infectious diseases tests, and for inadequate samples. Including the 10% margin, the enrollment goal will be 7,900. This goal will be divided among the six REDS-II blood centers. Therefore, each center will recruit about 1,320 donors. **Appendix 1** shows the total number of donors for the study and the number of donors to be enrolled at each center. The total number of donors to be enrolled shown in Appendix 1 is higher than 1,320 needed at each site because the numbers were rounded. Each center is expected to achieve a minimum enrollment level of 50% of the center-specific goal and the maximum enrollment for each center should not exceed 125% of the goal. Appendix 1 also shows the center-specific minimum and maximum numbers. Pre-determined goals for different ethnic groups of study donors to be enrolled will be established for each center based on the race/ethnic center-specific distribution.

Periodically during the conduct of the study, the number of donors recruited for each desired category of donors at each site will be monitored. If needed, the LAP Study working group will review the difficulties and assist in developing strategies to enhance study enrollment at sites experiencing difficulties. In this manner, timely enrollment of the study participants is expected.

G1. Number of Female Donors in the Study

A total of about 5,700 non-transfused women will be enrolled in this study. Assuming a 10% loss (inadequate volume, broken vial, etc.), the estimated target for this study is to compile laboratory information on a total of 5,100 non-transfused women.

Appendix 2 details how the sample size for non-transfused women was selected and delineates the assumptions used in the statistical power calculations. The prevalence in women of parity 0 is expected to be 1.6%, the prevalence in women of parity 1 is expected to be 10.5%, the prevalence in women of parity 2 is expected to be 15.8%, and the prevalence in women of parity ≥3 is expected to be 22.4%.[20] Three plausible parity distributions were evaluated: 1) the parity distribution provided by McLennan et al.[20] representing the parity distribution in British woman donors; 2) the parity distribution provided by Densmore et al. representing the parity distribution of apheresis women giving at a hospital-based blood program in the US;[15] and 3) the estimated parity distribution of REDS-II donors based on the parity distribution of US women as reported by the 1995 National Survey of Family Growth (NSFG) and on the preliminary age, and race/ethnicity distributions for REDS-II collections (available on the REDS-II website). The chosen sample size is driven by the ability to have ≥90% power to detect prevalence differences between successive parity groups for any of the three plausible distributions. While we believe that the estimated NSFG distribution probably represents our best guess at the parity distribution of donors in the US, we cannot rule out having a parity distribution such as seen by Densmore et al.[15] Hence, we have selected the most conservative sample size estimate for this study, namely a sample size of 5,100 non-transfused women, because this scenario gives for any of the three plausible parity distributions a power of ≥90% to conclude that the prevalence in non-transfused women with parity 0 is less than the prevalence in women of parity 1, which is less than the prevalence in women of parity 2, which is less than the prevalence in women of parity ≥3, given HLA antibody prevalences of 1.6%, 10.5%, 15.8% and 22.4%.[20]

As shown in Table G1.1 below, we have used the NSFG/REDS-II distribution to estimate the expected number of non-transfused women in each parity category if 5,100 are enrolled, because this distribution represents our best guess. We have then used the expected number of women in each parity group to estimate the number of non-transfused women donors with HLA I/II antibodies based on HLA antibody prevalence estimates from McLennan et al.[20]

Table G1.1: Suggested sample size for non-transfused women

Pregnancy History	Number of Women	Transfusion History	Estimated Percent of Female Donors in each category (%)*	Estimated HLA I/II Ab prevalence (%)	Estimated Number of donors with HLA I/II abs
Never pregnant	1173	None	23.0	1.6	19
One pregnancy (includes miscarriage alone)	765	None	15.0	10.5	80
Two pregnancies (includes miscarriage)	1224	None	24.0	15.8	193
Three or more pregnancies (includes miscarriage)	1938	None	38.0	22.4	434
Total	**5100**		**100.0**		**726**

*The estimated percent of female donors in each category reflects the 1995 NSFG distribution applied to REDS-II donors.

G2. Number of Male Donors in the Study

The sample size for male donors was based on the ability to estimate the HLA antibody prevalence with more precision than previous studies. Since the prevalence of HLA antibody in males transfused or non-transfused is expected to be about 2%, we selected a sample size of 1,000 in each group (male non-transfused, male transfused) so the width of a 95% confidence interval (given a 2% prevalence estimate) would be ± 0.9. A targeted approach to the recruitment of transfused male donors will be necessary since this group represents only about 4% of the donor population. We also expect that in about 10% of cases, test results will not be available (inadequate volume, broken vial, etc.) and therefore propose to enroll 2,200 male donors (1100 transfused; 1,100 non-transfused) to end up with 2,000 with HLA antibody test results.

Table G2.1: Characteristics of male study donors

Transfusion History	Number	Percent of Male Donors	Estimated HLA I/II Ab prevalence (%)	Estimated # of donors with HLA I/II Abs
Previously transfused	1000	4.0	2.0	20
Non-transfused	1000	96.0	2.0	20
Total	**2000**	**100.0**		**40**

It should be noted that the proposed sample sizes for male donors do not provide the power to detect a difference in HLA prevalence between the two groups since the estimated HLA Ab prevalence in each of these 2 groups is expected to be similar (see section H2). This

study is not designed to compare the prevalence between transfused and non-transfused males but rather to estimate with more precision than in the past what the prevalence of HLA-Ab is in a representative group of transfused males (n=1000) and in a representative group of non-transfused male donors (n=1000). The demographic characteristics (i.e. age, race/ethnicity, education, country of birth) are known to differ among transfused and non-transfused males.[38] Nonetheless, matching by any or all of these demographic characteristics is not warranted. First, the transfusion effect on HLA-Ab prevalence can be estimated in a model adjusting for these demographic characteristics if need be. Second, none of the demographic characteristics are thought to be major risk factors for HLA-Ab prevalence. Hence, matching is unlikely to result in an appreciable benefit and an adjusted model is unlikely to differ from an unadjusted model.

G3. Expected numbers of donors with different types of HLA antibodies

The estimates below are derived from two studies: Densmore et al.[15]and McLennan et al.[20].

Table G3.1: Expected number of study donors with HLA antibodies

Antibody	Number of donors	Estimated contribution of each type of HLA antibodies (%)
HLA class I	337	44
HLA class II	199	26
HLA class I and class II	230	30
Total	**766**	100

H. Additional Sample size and Power Considerations

H1. Parity and interval since last pregnancy

An expected number of 3,927 women with a previous pregnancy or miscarriage will be recruited for the study. Based on the distribution of the number of pregnancies and the intervals since the most recent pregnancy (see Table H1.1 below derived using data from Densmore et al.[15]), a 0.05 level one-sided test will have >99% power to detect a higher HLA antibody prevalence among women who were more recently pregnant (i.e. a comparison of a ≤5 vs. >5-year interval since the most recent pregnancy).

Table H1.1: Estimated HLA Ab prevalence estimates in relation to parity

Number of pregnancies	HLA antibody prevalence and its relation to interval since the most recent pregnancy	
	0-5 years	**>5 years**
1-2	5/14 (33%)	10/90 (11%)
>2	5/8 (62%)	17/75 (23%)
Total	10/22 (45%)	27/165 (16%)

H2. Limitations of sample sizes selected for the study

A limitation of the sample size selected is that it is insufficient to detect a difference in antibody prevalence between non-transfused and transfused male donors. If HLA antibody prevalence estimates for these two groups of male donors are 1.6% and 2.0% respectively, we estimate that 14,000 transfused and 14,000 non-transfused male donors would be required to achieve a one tailed α=0.05 with 80% power. A study of such a large sample size would be quite expensive. This limitation would also apply to transfused vs. un-transfused females who have never been pregnant if transfused females would have been enrolled. For male non-transfused and male transfused donors, we have selected sample sizes that would allow determination of prevalence estimates with narrower confidence intervals than in previous studies. In the UK study, the confidence interval for HLA antibody prevalence in such groups ranged from 0.1% to 11.1%. This study's expected confidence interval for HLA antibody prevalence will range from 1.1% to 2.9% around a point estimate of about 2.0%.

An analysis sample size of 3,927 female donors with a pregnancy will not have sufficient power to detect an interaction effect between the number of pregnancies and the interval since the most recent pregnancy; i.e., a differential effect of the interval since the last pregnancy as a function of the number of pregnancies. However, the apparent interaction (based on published data, Densmore et al.,[15] see table H1.1 above) appears to be negligible due to the fact that the prevalence after 2 pregnancies is almost double the prevalence seen with 1-2 pregnancies (62% vs. 33%) when the interval from the most recent pregnancy ranged from 0-5 years, or when the interval is >5 years (23% vs. 11%). Similarly, there was about a threefold difference in prevalence estimates seen when groups were compared in the other direction (33% vs. 11% and 62% vs. 23%).

I. Statistical Analysis

The aims of this analysis include:

1. Estimation of HLA- class I and HLA-class II antibody prevalence within specific subgroups of blood donors; specifically, subgroups based on gender, history of blood transfusion, number of pregnancies/miscarriages (females only), or time elapsed since the immunizing event. These prevalence estimates will also be stratified by additional demographic groups of interest such as age, race/ethnicity and education.

2. Estimation of neutrophil antibody prevalence in HLA-class I or HLA-class II antibody positive blood donors (expected to be primarily women) and in a small number of HLA-negative non-transfused male blood donors. Neutrophil antibody positive donors will be characterized in terms of their demographics (gender, age, race/ethnicity, education), number of pregnancies/miscarriages, history of blood transfusion, and time elapsed since the immunizing event.

3. Estimation of HLA antibody type (mono, multi, non-specific, etc.) prevalence within specific subgroups of blood donors; specifically, subgroups based on their gender, history of blood transfusion, number of pregnancies/miscarriages (females only), or

time elapsed since the immunizing event. Prevalence estimates stratified by age, race/ethnicity and education will also be calculated.

4. Estimation of the percentage of HLA-positive samples with low (<1:8) or high (≥ 1:8) titers, overall, and as a function of gender, history of blood transfusion, number of pregnancies/miscarriages, or time elapsed since the immunizing event (and by age, race/ethnicity, and education).

The parity distribution among enrolled female donors may vary from expectations for primarily one of two reasons: 1) the parity distribution among female donors at the REDS-II centers is very different from expectations based on previously published data; and/or 2) the consent rate may depend on parity. Thus, an interim analysis will be performed (after approximately one month) to assess the parity distribution and consent rate. If the statistical power for a sample with parity distribution as found in the interim analysis is less than 90%, then modifications will be made in the recruitment process. For example, targeted sampling within each parity group could be implemented or the enrollment period could be extended. If the consent rate is markedly less than 50%, then modifications will be considered. For example, reasons for refusal could be assessed to evaluate if attempts at converting "soft" refusals would be useful.

Descriptive statistics will first be used to evaluate the distributions of all variables. We will probably use exact tests to evaluate if a categorical characteristic (presence of HLA-I antibody, yes/no) is statistically significantly different among groups (e.g. transfused vs. non-transfused; or, in females, by parity groups, 0, 1, 2, ≥3). For comparison of a continuous characteristic (such as time elapsed since the immunizing event) among groups, means will be compared among 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).

Prevalence estimates (e.g., the proportion of donors with a particular characteristic such as HLA-class I antibody) and their associated 95 percent confidence interval (CI), will then be calculated for each group of interest. Further, we will conduct binary logistic regressions to compare prevalence between groups. Logistic models provide odds ratios (and 95% CIs) that compare the odds of having a certain outcome (such as the odds of being HLA-class I antibody positive) between two groups. For example, a logistic model with HLA- class I antibody prevalence (yes/no) as outcome variable and independent variables of interest (gender, transfusion history, parity classification, time elapsed since the immunizing event, age race/ethnicity, education) will be built. Similarly, logistic regression models with other outcome variables (e.g., low vs high titer HLA antibody prevalence) will be built if warranted.

Logistic models will first be conducted with one independent variable at a time (e.g., transfused yes/no). We then propose to build parsimonious models that will include independent variables that individually, and when adjusted for one another, predict the outcome of interest such as having HLA-I antibodies. We will use an appropriate modeling process to build the most parsimonious models such as backward modeling (whereby all potential independent variables that can be associated with the outcome are first included in the model and non-significant

variables are then removed one at a time). Models with more than one independent variable at a time ("adjusted" models) permit evaluation of whether the association observed in the unadjusted model between an independent variable (e.g., number of pregnancies) and the outcome of interest (e.g., prevalence of HLA antibody) is in fact explained by other independent variables (e.g., transfusion history, age, or race/ethnicity).

The rate at which donors consent to participate in the study is expected to be unrelated to the outcome variables (prevalence of HLA antibody or neutrophil antibody) primarily because donors are unaware of their antibody status. However, the consent rate may be related to potential predictor variables (e.g. older donors may be more likely to consent or minority donors may be less likely to consent). The logistic regression model that will help identify predictors of HLA-antibody prevalence (or other outcomes) is relatively unaffected by differential consent rates among demographic subgroups. For example, if older donors are more likely to consent, then a logistic regression model adjusted for age will yield an unbiased estimate of the parity effect. Further, a logistic regression model unadjusted for age (or any other predictor variable associated with consent rate) will yield, at most, an expected small bias in the parity effect, since the dominant effect on HLA-antibody prevalence (and other outcome variables) is expected to be parity.

Population (REDS-II donors) based estimates of HLA-antibody prevalence (and other outcome variables) by certain demographic subgroups will need to include a 'weighting' adjustment for those variables that are significant predictors of HLA-antibody prevalence. For example, if a logistic model conducted on female donors showed that parity, race/ethnicity, and center were significantly associated with having HLA-antibodies and we wanted to estimate the prevalence of HLA antibodies in REDS-II black female donors, we would 'weight' the model-based estimates of HLA-antibody prevalence in each black female donor parity group by the number of REDS-II female donors in each black donor parity group at each center.

J. Interventions

No interventions are planned.

K. Methods

K1. Donor recruitment

Blood donors will be recruited at the time of blood donation, after they have been determined to be eligible to donate blood. Such an approach will prevent recruiting donors who are otherwise disqualified from donating blood. Qualified donors already have blood samples drawn as part of the donation process for routine infectious disease testing and extra samples for the study can be collected as part of this routine procedure. The participating blood centers will develop plans to recruit donors so that the enrolled subjects are representative of their overall population. **Appendix 4** contains general guidelines for recruiting donors. Recruitment of Hispanic and African-American subjects will be monitored to ensure that distribution is similar to the center's overall donor population prevalence. Donors will only be eligible to enroll one time. Prospective donors will be asked if they have already been enrolled in the study previously

to prevent duplicate enrollment. In addition, the web-based Subject Management System (SMS) will prevent duplicate entry of the same participant.

As part of the recruitment plan, each center will select a few sites that are likely to allow for enrollment of donors with desired characteristics. For instance, fixed donation sites located in general communities, including urban and rural sites will be selected, whereas certain sites will be avoided as they are unlikely to allow for recruitment of donors with the desired characteristics. For example, high school blood drives are likely to have fewer donors with a history of pregnancy and will not be selected.

All donors at selected sites will be approached for the study. Non-consenting donors will be identified at each site where enrollment occurs. This is possible because we will capture the site code and date of donation information on those enrolled and will thus be able to identify and characterize all donors who presented to donate at those sites on those dates using the REDS-II donation database, an aggregate database formed on an ongoing basis as part of the general REDS-II program.

It should be noted that the number of donors to be enrolled into the study at each blood center is a small fraction of the total number of donors seen. Therefore, the approach described above is expected to allow for enrollment of the desired number of participants for all the categories with relative ease except for transfused males.

The category of blood donors that may prove more difficult to recruit in sufficient numbers is transfused males. As previously mentioned, the proportion of such donors is approximately 4% of the total donor population. In order to recruit 1,100 such donors, we will need to possibly approach 42,500 male donors to identify 1,700 with a history of blood transfusion and enroll 1,100 (if about 2/3 consent to participate). There is a sufficient donor base to achieve these numbers among the six participating REDS-II blood centers, although enrollment may take longer than for other categories of donors. For this reason, centers will be given two options to supplement their on-site recruitment of transfused male donors.

Option 1: On a daily basis, blood centers will review REDS-II short forms and flag the transfused male donors from that day's donations. Specimens from these flagged donors will be processed, aliquoted and saved. Specimens will be accessioned into the Specimen Tracking System (STS) with a "dummy" subject ID. Study staff will mail a recruitment letter to these donors along with the consent form, questionnaire and a postage paid envelope for mailing it back**.** The donors will also be contacted by phone to provide them with an opportunity to ask the study staff questions about the LAP study or the consent form. Once consent is obtained a "real" subject ID will be assigned to this donor and his information will be entered into the SMS. The STS will be updated with the new subject ID and consent information. Before shipping, specimens will be reconciled against consent to avoid shipping non-consented specimens. At the end of the study recruitment period all non-consented specimens will be discarded. Processing and holding of samples until consent is obtained is necessary since samples need to be processed within 72 hours of collection.

Option 2: Westat will query the donation database and provide centers with the Blood Unit Identifier (BUI) numbers of transfused male donors from the previous three months. These BUIs will be pre-loaded into the SMS for convenient tracking. Blood centers will have to merge these BUI numbers with identifying information at the center and subsequently mail recruitment letters to the transfused male donors in 3 waves**.** The blood center's own telephone recruitment staff will contact the donors by phone, explain the LAP study and schedule initial appointments for a donation (if the donor does not wish to make a blood donation an appointment can be made for the donor to provide study specimen only). Study staff will be responsible for re-contacting no-shows, and following up to reschedule missed appointments. When these donors come to donate, study staff will obtain consent, and the questionnaire and specimen following the regular on-site recruitment protocol.

K2. Donor consent

Donors will be provided with information regarding the study and asked to participate. **Appendix 5** contains an example of the LAP study information sheet that each center can modify and use locally. Donors will be given sufficient opportunity to ask questions and after the donor is satisfied and agrees to participate in the study, the donor will be asked to sign the consent form. Donors can consent to participate in either the study or the repository, or both. The consent form template is included in **Appendix 6.** It can be minimally customized at each center. All consent forms will be reviewed by the coordinating center to ensure consistency across all six centers.

As consents are received by the study staff, they will be entered into the Subject Management System (SMS). Each consent form will have a bar coded subject ID label attached, which will be scanned into the system on a daily basis. The Donor ID and BUI number will also be scanned into the SMS to allow for linkage to the Specimen Tracking System (STS) and the REDS-II donation data.

K3. Donor history questions

Eligible donors will be asked questions regarding blood transfusion (ever, number of transfusions, and date of last transfusion). In addition, female donors will be asked questions about their pregnancy history (ever, number, live births, still births, miscarriage/terminated pregnancy, tubal pregnancy, date of last pregnancy). Special LAP study questionnaires will be utilized to record the donor information. **An example of the questionnaire with precise questions to be used is included in Appendix 3.** Each participating center will use the same questions that are found in the form contained in this appendix. Every completed questionnaire will be identified by the subject identification number, allowing linkage to other study information collected. Subject data will be captured in a computer database by either manual entry or by scanning.

K4. Blood sample requirement and collection

7ml of whole blood will be collected from each donor participating in the study. It is anticipated that centers can collect this volume without an additional phlebotomy from the

following sources: retention tube, additional EDTA tube at collection, or sample first pouch. If sufficient quantity of whole blood specimen cannot be obtained as part of regular operations, then an additional phlebotomy may be necessary. When possible, an additional red top tube will be collected to obtain serum. Specimen tubes will be labeled with BUI, subject ID and tube ID. All specimens will be accessioned into the Specimen Tracking System (STS), processed and aliquoted at the processing area.

K5. Blood sample transportation to the participating blood center

All specimens from donation collections will be transported by the operational staff at each blood center per their routine collection process. The designated REDS-II Study staff or their designated operational staff at each blood center will be responsible for the acquisition of the blood samples of consenting donors and for transport of the samples from the collection area to the location determined for REDS-II processing and storage. The samples will be kept at a refrigerated temperature of 4 – 7 °C until processing can occur within 48-72 hours of collection.

K6. Processing and storage of blood samples at the participating blood center

As the batched samples are brought to the study processing area the bar coded BUI, subject ID and tube ID will be scanned into the Specimen Tracking System (STS) and be processed and aliquoted. 5 plasma aliquots (4 cryovials of 0.5ml and 1 cryovial of 1ml) and 2 red blood cell aliquots of 0.5ml will be made. If red top tubes are available 2 serum aliquots will be made. All cryovials will be labeled with the appropriate aliquot ID. Cryovials will be stored in freezer boxes in a -70 degree freezer until the shipping date. Before shipping, all specimens will be checked for consent. Blood centers will ship one 0.5 ml aliquot to the central laboratory, BSRI, for testing. All remaining cryovials will be shipped to the central repository, Sera Care. At the end of the recruitment period a reconciliation process will occur to notify Sera Care of specific aliquots to destroy associated with donors who did not consent to repository storage.

K7. Transporting of blood samples to the central laboratory and the repository

On a set schedule, each blood center will ship batched specimen freezer boxes on dry ice via overnight FedEx (or other designated carrier) to the central laboratory, Blood Systems Research Laboratories in San Francisco, CA and/or the NHLBI central storage facility, Sera Care in Gaithersburg, MD. These shipments will be scheduled and monitored by the REDS-II Coordinating Center staff. Based on test results, the coordinating center will requisition necessary samples (positives) from the repository to ship to Blood Center of Wisconsin for further testing.

K8. Coding (de-identifying) donor data

Blood centers routinely assign each donor a unique identifying number at the time of blood donation. This donor number is placed on donor records that contain donor's identifying information (name, address, etc.). In addition, each blood sample tube that is collected for routine donor testing at the time of blood donation is labeled with a unique Blood Unit Indentifier (BUI). This represents the first level of coding and such a procedure is an established

method of tracking donors, donations, and tubes in regional blood centers. In order to achieve double coding, a unique subject identification number will be assigned to each donor's consent form, study-related data collection questionnaire, and the study-related blood sample. The study-related unique identification numbers will be generated by the coordinating center which will provide these numbers to the participating regional blood centers. Coded samples labeled with ssubject identification numbers will be tested at the central laboratory and the results of the testing will be forwarded to the coordinating center. The coordinating center will forward the results to the blood center for those donors who are to be notified of their test results. These steps ensure that the coordinating center and the testing laboratory have only one link, namely, the subject identification number and the test results. The blood center has both links, namely, donor identifying information linked to blood donation numbers, and blood donation numbers to subject identification number.

K9. Methods to detect, characterize and titer HLA Class I & Class II antibodies

Samples will be screened first for HLA class I and HLA class II antibodies using the One Lambda Luminex-based LABScan 100 flow analyzer.[57-58] The Luminex-based method of antibody detection affords high-throughput capability combined with excellent sensitivity and specificity. Samples will be screened using antigen panels containing 55-Class I and 32-Class II antigens (LABScreen PRA Class I and Class II). The initial screening will provide information on whether class I, class II, or both classes of HLA antibodies are present. Initial screening will require 20 µl per sample and will be performed in batch mode on 96-well plates. Screen-reactive samples will be further tested by the Luminex method to confirm the screening results as well as to determine antibody specificities. Class I positive samples will be tested using LabScreen PRA Class I, which contains positive and negative control beads and 55 beads coated with combinations of antigens representing 78 individual class I HLA A, B, and C alleles. Class II positive samples will be similarly screened using LabScreen PRA Class II, containing 25 DR and DQ antigens on 35 beads plus positive and negative control beads. Both the class I and class II PRA will provide identification of individual alleles targeted as each allele is represented on multiple beads. Proprietary software provides analysis of the pattern of positive beads to identify antibodies reactive to individual HLA alleles. Confirmation and antibody specificity definition will require an additional 20 µl of plasma for each class of antibody tested. All samples that screen positive will be tested at 1:8 dilution. Samples that show reactions at 1:8 dilution will be titered at higher dilutions. It is expected that the vast majority of samples will be low titer (i.e., <1:8 titer).

K10. Neutrophil Antibody Detection and identification strategy and methodology

A selected subset of donors will be tested for granulocyte antibodies, specifically those donors that show the presence of HLA class I or HLA class II antibody. It is anticipated that approximately 766 such donors will be identified and require granulocyte antibody testing. Selection of donors who are alloimmunized to HLA is based on the hypothesis that these donors are responders and are therefore likely to also be alloimmunized to granulocyte antigens. Selection of this group does present some special considerations because HLA antibodies must be differentiated from granulocyte antibodies. This can be achieved by testing with the standard assay to measure granulocyte antibodies, including the immunofluoresence and MAIGA assays.

Platelet absorption of test sera will be needed to remove HLA class I antibodies before testing for granulocyte antibodies.

Rather than testing all 7900 samples with a prevalence of 0.1 to 1.8%, we propose testing the approximately 766 samples that show the presence of HLA antibodies as these HLA alloimmunized donors are considered immune responders. We will also test approximately 300 samples from non transfused men to establish the background rate of positivity for the test including autoantibodies. The results of the selective testing can then be used to develop the sample size analyses to determine how many additional samples, if any, would need to be tested.

K11. Neutrophil antibody testing by the Blood Center of Wisconsin

The Platelet & Neutrophil Immunology Laboratory (PNIL), Blood Center of Wisconsin (BCW), Milwaukee, Wisconsin will provide serologic and DNA-based testing for the detection and identification of granulocyte antibodies and antigens in blood donor samples **(See Appendix 7)**. Class I HLA antibody results obtained from the Central Laboratory will be used to aid in determining tests required to distinguish Class I HLA antibody reactivity from granulocyte-specific reactivity in plasma samples. These tests will include the MAIGA and GIFT-FC testing using plasma absorbed with normal platelets to remove Class I HLA antibodies that may mask detection of granulocyte-specific antibodies present. Samples with negative Class I HLA antibody test results will be screened (Level I Testing) for the presence of granulocyte antibodies using a granulocyte immunofluorescence flow cytometry assay (GIFT-FC)[43-46] against sufficient normal donor granulocytes to cover all of the common granulocyte alloantigens (HNA-1a, HNA-1b, HNA-1c, HNA-2a, HNA-3a, Table 1).[46]

Table K11-1. Human Neutrophil Alloantigens

Alloantigen	Antigen Frequency (Caucasian)	Glycoprotein Location	Alleles
HNA-1a (NA1)	54%	FcγRIIIb, CD16	FCGR3B*01
HNA-1b (NA2)	88%	FcγRIIIb, CD16	FCGR3B*02
HNA-1c (SH)	5%	FcγRIIIb, CD16	FCGR3B*03
HNA-2a (NB1)	97%	CD177	CD177*01
HNA-3a (5b)	97%	unknown	Not Known
HNA-3b (5a)	33%	unknown	Not Known
HNA-4a(Mart)	92%	MAC-1, CD11b	CD11B*1
HNA-5a(OND)	99%	LFA-1, CD11a	CD11A*1

Plasma samples with positive Class I HLA test results will be subjected to the same testing using both unabsorbed and platelet absorbed serum/plasma (Level II Testing). Samples testing positive with granulocytes will undergo additional testing (Level III testing) to determine the antigen-specificity of the antibodies. This testing will include flow cytometry tests against larger panels of typed granuloctyes and the monoclonal antibody immobilization of granuloctye antigens (MAIGA) assay.[47] The MAIGA is an ELISA, in which alloantigen specificity of patient

serum/plasma antibodies can be determined using granulocyte glycoproteins that have been captured on the wells of a microtiter plate by specific monoclonal antibodies. In cases where a granulocyte-specific antibody has been identified, genotyping of donor and/or patient DNA or serologic typing of donor and patient granulocytes for the corresponding granulocyte antigen may be required. However, it is estimated that only 1-2 cases will require this more extensive testing. Genotyping will be performed with established methods involving amplification of 50 µl of 20 ng/µl of genomic DNA by PCR with sequence-specific primers (PCR-SSP), followed by electrophoresis of PCR products on ethidium bromide stained agarose gels, and inspection for specific allelic bands. [48-49]

Of the 1000 samples screened, approximately 570 samples are estimated to require Level II testing to distinguish Class I HLA from granulocyte-specific antibodies. As mentioned previously, only a small percentage of samples are estimated to require Level III testing.

K12. Strategy for notifying donors of their test results and their future eligibility to donate blood

There are only a few case reports in the literature that have suggested an adverse impact on newborns of mothers with HLA or neutrophil antibodies.[59-61]despite the fact that as many as 30% of women are thought to possess HLA antibodies and approximately 0.5% of women might show the presence of neutrophil antibodies.[15, 20] Moreover, a thorough search for neutropenia in the newborns among women with neutrophil antibodies did not detect a single case of neutropenia among 1,038 women.[50] despite the fact that 203 women delivered a neutrophil antigen-incompatible child.[50] In another study, cord blood platelet and granulocyte counts were found to be normal in women with HLA and granulocyte antibodies.[62] Also, the effect of maternal HLA antibodies on pregnancy evolution has been measured previously and, nocorrelation was found between the presence of such antibodies and obstetric complications, fetal wastage, placental weight, or infant birth weight.[63] These findings support the conclusion that HLA and neutrophil antibodies in pregnant women are generally without adverse effect to their newborns. In fact, some investigators have postulated a protective role of Ia-like antibodies for the newborn.[64] HLA antibodies have also been shown to confer protective effect on the severity of the hemolytic disease of the newborn.[65] Besides these observations, it is also recognized that it is not routine clinical practice to screen pregnant women for leukocyte antibodies nor is there a recommended change in prenatal management for women who are incidentally found to have leukocyte antibodies.

In addition to maternal-fetal transfer of leukocyte antibodies discussed above, there are other clinical circumstances in which such antibodies might be of clinical significance. This includes the development of febrile non-hemolytic transfusion reactions after blood transfusion, poor response after platelet transfusion, and rare instances of TRALI.

Leukocyte antibodies are clearly implicated in febrile non-hemolytic transfusion reactions. Nonetheless, detection of such antibodies prior to transfusion is not a routine clinical practice. It is clear that some patients with leukocyte antibodies might respond poorly to platelet transfusions. In such circumstances, HLA_matched or cross-matched platelet transfusions are

indicated. In very rare cases, TRALI might be due to the presence of leukocyte antibodies in the recipient.

A survey of REDS-II Centers was conducted to determine how individual center medical directors evaluated the need for donor notification, donor deferral and recall of previously donated blood components from donors with leukocyte antibodies. Based on the scientific literature, results of the survey of the participating centers, and detailed discussion within the LAP study group and the REDS-II Steering Committee, a recommended approach has been developed and is presented in Table-K12 below. There was general agreement that donor notification, donor deferral and blood component recalls are not recommended for those donors who possess HLA class I and class II antibodies and those who show non-specific or weak reactions in neutrophil antibody testing. It is recommended that donors with neutrophil antibodies that are specific for one or more known neutrophil-specific antigen be notified and deferred from future donations. However, these donors may be allowed to continue donating non-plasma containing blood components if the blood center has sufficient controls in place. Recall of in-date blood components still in inventory from donors with neutrophil antibodies with a defined specificity is also recommended.

Table-K12: Recommended management of donors and products for those donors who test positive for leukocyte antibodies

Antibody	**Notification**	**Deferral**	**Recall**
HLA class I or II	Not recommended	Not recommended	Not recommended
Neutrophil: Weak and/or non-specific	Not recommended	Not recommended	Not recommended
Neutrophil: Defined specificity	Recommended	Not recommended*	Recommended

* Donors with neutrophil antibodies with defined specificity may continue to donate non-plasma containing blood components if the blood center has control procedures in place so that it can ensure production of plasma-free blood components.

An individual center may elect to vary from these recommendations based on local procedures and IRB considerations. If an individual center decides to defer a donor because of the presence of leukocyte antibodies, then the donor should be notified of such a deferral and the consent form should list the possibility of deferral as a risk. An example of a donor notification letter is included in **Appendix 8**. This letter can be modified for local use as deemed necessary.

K13. Limitations of the current protocol

This protocol includes a sample size that will not be able to detect a difference in HLA or neutrophil alloimmunization rates between transfused and non-transfused donors. The sample size needed with a sufficient power to detect such a difference would be prohibitively large due to the expected low antibody prevalence in this population. We also expect that not undertaking this task would have minimal consequence in our ability to come up with a preventive strategy for TRALI due to the fact that the alloimmunization rates in such groups are expected to be quite low. The sample size also does not have sufficient power to detect an interaction effect between

the number of pregnancies and the interval since the most recent pregnancy. Lastly, the study does not address risk factors for non-immune causes of TRALI. Available evidence suggests that such factors are patient related and/or present in the blood component at the time of transfusion and cannot be answered in the proposed study. A follow up study is planned in recipients of plasma components to examine these issues.

L. Budget

Below are the cost estimates for the LAP Study.

Table L1: LAP Study Budget

Category	Amount
Blood Centers	$543,920.39
Central Laboratory	$263,990.00
Coordinating Center	$380,000.00
Contracted Laboratory	$ 45,210.00
Total	$1,233,120.30

M. Timeline (Project length: 24 months)

The table below shows the timeline for the project. Major steps for the project are tabulated along with their completion date.

Table-M.1: Timeline

Step	**Date of completion**	**Comment**
OSMB submission and approval	12/05	
60-day notice	1/06	
OMB submission	3/06	Expected approval in July 2006
IRB submission packet	4/06	Includes preparation of consent forms
IRB approval	5/06-7/06	REDSII centers and Westat IRB review and approval
Begin donor enrollment	8/06	May be earlier if we receive early OMB approval
Finish donor enrollment	2/07	Six months to enroll all donors
Begin laboratory testing	2/07	Testing begins six months after the first donor is enrolled to allow batch testing
Complete laboratory testing	8/07	Six months to complete all testing
Data compilation and analysis	8/07	Four months for data compilation and analysis

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Appendix 1: Center-specific study participant enrollment goal (N=1,320 for each center)*

Males	Entire Study Goal	Center-Specific Goal	Center-specific lower limit (50% of total)	Center-specific upper limit (125% of total)
Transfused	1,100	185	95	230
Untransfused	1,100	185	95	230
Total Males	**2,200**	**370**	**190**	**460**
Females (non-transfused; non-targeted)				
Expected never pregnant	1,311	220	110	275
Expected one pregnancy	855	145	75	180
Expected two pregnancies	1,368	230	115	285
Expected ≥ 3 pregnancies	2,166	360	180	450
Total females	**5,700**	**955**	**480**	**1,190**
Total Males + Females	**7,900**	**1,325**	**670**	**1,650**

Numbers in the above table are rounded up and therefore, the total number of the study donors to be enrolled at each site is somewhat higher than the goal of 1,320.

Appendix 2: Sample Size Statistical Considerations for Women

Table A1 below shows four proposed sample sizes (of 12 possibilities) necessary to achieve sufficient power depending on three plausible parity distributions: 1) the parity distribution provided by McLennan et al. representing the parity distribution in British woman donors; 2) the parity distribution provided by Densmore et al. representing the parity distribution of apheresis women giving at a hospital-based blood program in the US; and 3) the estimated parity distribution of REDS-II donors based on the parity distribution of US women as reported by the 1995 National Survey of Family Growth (NSFG) and on the preliminary age, and race/ethnicity distributions for REDS-II collections (available on the REDS-II website). While we believe that the estimated NSFG distribution probably represents our best guess at the parity distribution of REDS-II donors, we cannot rule out having a parity distribution such as seen by Densmore et al, although the latter may have included more highly educated younger donors who may be less likely to have had any pregnancy than REDS-II female donors.

Table A1: Possible sample sizes for women based on three different parity distributions

	Parity	**Number of female donors**				**Estimated Prevalence HLA I/II Ab +ve**	**Expected Parity Distribution**		
		A*	**B***	**C***	**D***		**British**	**Densmore**	**NSFG†**
Female donors not transfused									
	0	1050	1632	1000	1000	1.6%	25%	32%	23%
	1	462	510	652	872	10.5%	11%	10%	15%
	2	1596	1122	1044	872	15.8%	38%	22%	24%
	3+	1092	1836	1653	677	22.4%	26%	36%	38%
	Total	4200	5100	4350	3421				
Statistical Power‡	British	≥90%	≥94%	≥91%	≥90%				
	Densmore	≥83%	≥90%	≥85%	≥90%				
	NSFG	≥92%	≥96%	≥93%	≥90%				

* See section A2.3. † See section A2.2. ‡ See section A2.1.

A2.1 Statistical Power

Parity has been established to be a risk factor for HLA Ab seroprevalence. In this study we want to quantify the additional risk of each additional pregnancy. Statistical power in this study is the power to conclude that the prevalence in women of parity 0 is less than the prevalence in women of parity 1 which is less than the prevalence in women of parity 2 which is less than the prevalence in women of parity ≥3, given the estimated HLA Ab prevalence estimates given by McLennan et al. (1.6%, 10.5%, 15.8% and 22.4% for 0, 1, 2, and 3 or more pregnancies, respectively).

The null hypothesis is $H_0: p_0=p_1=p_2=p_{3+}$. In this study, by rejecting the null hypothesis, we do not want the statistical conclusion to be that the parity prevalence estimates are not all equal. Rather, we would like for the statistical conclusion to be that $p_0<p_1<p_2<p_{3+}$. Towards this end, consider

the following three sub-null hypotheses; $H_0^{'}: p_0 = p_1, H_0^{''}: p_1 = p_2, H_0^{'''}: p_2 = p_{3+}$. Performing one-sided 0.05 level tests on each of these three sub-null hypotheses, means that upon rejecting all three sub-null hypotheses, we indeed can statistically conclude $p_0 < p_1 < p_2 < p_{3+}$. A 'multiple comparison' adjustment using 0.017 level tests was considered but we did not feel this was necessary and used 0.05 level tests. As defined 'test rejection' is rejecting all three sub-null hypotheses, thereby inferring 'test acceptance' as accepting any of the three sub-null hypotheses. In this sense the test level, α, is at most 0.05, since $1-\alpha = \Pr(\text{accept } H_0^{'} \text{ or } H_0^{''} \text{ or } H_0^{'''}) \geq \Pr(\text{accept } H_0^{'}) = 0.95 \Rightarrow \alpha \leq 0.05$. Given a sample of 5,100 female donors under the Densmore et al. distribution (scenario B in Table A1), the expected powers for each of the three sub-null hypotheses are >99.99% for $H_0^{'}$, 90.24% for $H_0^{''}$, and 99.76% for $H_0^{'''}$.

Using the probability inequality, Pr(A or B)≤Pr(A)+Pr(B), a lower bound for the overall power can be determined.

$$\Pr(\text{accept } H_0^{'} \text{ or } H_0^{''} \text{ or } H_0^{'''}) \leq \Pr(\text{accept } H_0^{'}) + \Pr(\text{accept } H_0^{''}) + \Pr(\text{accept } H_0^{'''})$$

then

$$\Pr(\text{reject } H_0^{'} \text{ and } H_0^{''} \text{ and } H_0^{'''}) \geq 1 - \left(\Pr(\text{accept } H_0^{'}) + \Pr(\text{accept } H_0^{''}) + \Pr(\text{accept } H_0^{'''})\right)$$

which can be re - expressed as

$$\text{Power}(H_0) \geq 1 - (1 - \text{Power}(H_0^{'})) - (1 - \text{Power}(H_0^{''})) - (1 - \text{Power}(H_0^{'''}))$$

Thus, the overall power in this study is at least 1-(1-100%)-(1-90.24%)-(1-99.76%)=90%.

Note: Although the power to detect a slope is >99% in all scenarios, assuming a slope is unreasonable since we do not expect that each additional pregnancy adds an incremental risk to the HLA prevalence.

A2.2 Estimating the REDS-II parity distribution using the 1995 National Survey of Family Growth (NSFG) parity distribution

From the REDS-II baseline donation file available on the REDS-II website, we estimate (averaged over the 6 centers) that 2.9% of donors are Hispanic, 85.6% donors are White, 6.7% donors are Black, and 4.9% donors are from the category Other. Additionally, 50.7% donors are 45+ years old. Thus, there are an estimated 1.4% Hispanic 18-44 donors, 42.2% White 18-44 donors, 3.3% Black 18-44 donors, 2.4% Other 18-44 donors, and 50.7% 45+ donor.

The CDC data from the 1995 National Survey of Family Growth (NSFG) gives estimates of parity by race/ethnicity for women 15-44 (Fertility, Family Planning and Women's Health: New Data From the 1995 National Survey of Family Growth. Vital and Health Statistics series 23, No19, May 1997). It also gives estimates of parity for women 40-44 (and it is assumed the parity for donors 45+ will be akin to the 40-44 group). Combining the CDC estimates with the REDS-II estimates, an estimate of the parity distribution of REDS-II donors is obtained. This NSFG based

parity distribution estimate among female donors is 23% for parity 0, 15% for parity 1, 24% for parity 2, 18% for parity 3, and 20% for parity 4+.

A2.3 Sample sizes for Various Scenarios and Selection of Final Sample Size for Women:
Table A1 above shows four different scenarios and corresponding sample sizes to conclude that parity 0 prevalence is less than parity 1 prevalence which is less than parity 2 prevalence which is less than parity ≥3 prevalence, given the estimated HLA Ab prevalence estimates given by McLennan et al. (1.6%, 10.5%, 15.8% and 22.4% for 0, 1, 2, and 3 or more pregnancies, respectively) and three possible parity distributions.

A targeted approach to enrollment would allow for recruitment of the least number of women in the study (n=3,421) while achieving ≥90% power for any of three plausible parity distributions. However, this approach is operationally complex and a non-targeted enrollment approach for women of different parities is preferred. For a non-targeted approach, three scenarios were considered:

1. Scenario A: Sample 4,200 female donors who have never been transfused. Numbers are those expected given the British parity distribution (McLennan et al).
2. Scenario B: Sample 5,100 female donors who have never been transfused. Numbers are those expected given the Densmore parity distribution.
3. Scenario C: Sample 4,350 female donors who have never been transfused. Numbers are those expected given the NSFG parity distribution. (For the NSFG distribution, 90% power could be achieved with a sample of 4000, but in order to expect a sample of 1000 female donors with parity 0, the sample is increased to 4350).

For a targeted approach,

4. Scenario D: Targeted samples within each parity group (thus power does not depend on parity distribution, but the difficulty in attaining targets depends on the parity distribution).

For this study, we have selected the most conservative sample size estimate, namely a sample size of 5,100 non-transfused women (Scenario B in table A1 above) because this scenario gives for any of the three expected parity distributions, a power of ≥90% to conclude that the prevalence in non-transfused women with parity 0 is less than the prevalence in women of parity 1 which is less than the prevalence in women of parity 2 which is less than the prevalence in women of parity ≥3, given HLA antibody prevalence estimates of 1.6%, 10.5%, 15.8% and 22.4% for women of parity 0, 1, 2, and ≥3, respectively (McLennan et al).

Appendix 3:

OMB No. 0925-XXXX
Expiration Date: XX-XX-XXXX

REDS-II LEUKOCYTE ANTIBODIES PREVALENCE (LAP) STUDY QUESTIONNAIRE

TODAY'S DATE: |__|__| |__|__| |__|__|__|__|
M M D D Y Y Y Y

Question 1: Have you ever received someone else's blood?

☐ Yes → **How many times in your life have you received someone else's blood?**
☐ No

☐ Don't Know

☐ Once
☐ Twice
☐ Three or more times

When was your last transfusion? |__|__| |__|__|__|__| **;** ☐ Don't Know
(best estimate) M M Y Y Y Y

For Female Donors Only (Male Donors skip to end statement):

Question 2: Have you ever been pregnant? Please include live births, miscarriages, terminated pregnancies, still births, and tubal pregnancies.

☐Yes
☐No **SKIP TO END STATEMENT**
☐Don't Know

Question 3: How many times have you been pregnant in your life? Again, be sure to include all pregnancies including live births, miscarriages, terminated pregnancies, still births, and tubal pregnancies.

☐☐
Enter Number of Pregnancies

☐ Don't Know

Question 4: How many of your pregnancies resulted in a live birth? Please count the total number of pregnancies which resulted in children. For example, if you had twins or other multiple births, count as a single pregnancy.

☐☐
Enter Number of Pregnancies Resulting in Live Birth

☐ None
☐ Don't Know

Question 5: How many of your pregnancies resulted in still birth? Again, please count the total pregnancies.

☐☐
Enter Number of Pregnancies Resulting in Still Birth

☐ None
☐ Don't Know

Question 6: How many of your pregnancies resulted in miscarriages or terminated pregnancies?

☐☐
Enter Number of Pregnancies Resulting in Miscarriage/Terminated pregnancy

☐ None
☐ Don't Know

Question 7: The last time you were pregnant, in what month and year did the pregnancy end?

|__|__| |__|__|__|__|
M M Y Y Y Y

☐ Don't Know

END STATEMENT

Thank you for your participation in the Leukocyte Antibodies Prevalence (LAP) Study. We appreciate you taking the time to complete this questionnaire.

Appendix 4 : Recruitment plans for the LAP study participants

I. Enrollment goal: 7,900 donors

II. Recruitment goal for each center: proposed at 1,320 donors

III. Donors to be approached:

A. Females: 5,700 donors (age 18 and older)
   i. Never pregnant
   ii. One pregnancy
   iii. Two pregnancies
   iv. Three or more pregnancies

B. Males: 2,200 donors (age 18 and older)
   i. Previously transfused
   ii. Never transfused

IV. Plan for representative recruitment:

A. Each blood center should develop and submit to WESTAT a recruitment plan to include the following:
   i. outline the current demographics of each center's donor population
   ii. describe how each center will ensure that the donors they recruit into the LAP study are representative of their donor population

B. Criteria to be considered will include:
   i. Race
   ii. Ethnicity
   iii. Age
   iv. Geography

C. Recruitment at fixed, mobile, or apheresis sites is appropriate, and may be decided by the individual center, as long as the sites selected are representative of the demographics of the overall donor population of that center.

V. Methods of recruitment:

A. On-site recruitment

   i. Approaching all donors who are ≥ 18 years old upon presentation at donation site
   ii. The on-site approach will be used to recruitment of all donor groups.

iii. It is estimated that this type of approach will result in the capture of data for all types of female donors and all non-transfused male donors (see "Part B", below, for details of recruiting approaches for transfused male donors).

B. "Targeted" recruitment

i. It is believed that while non-targeted recruitment will satisfy the necessary numbers and types of female and non-transfused male donors needed to power the study appropriately, targeted recruitment (primarily of transfused male donors) will likely be necessary.

ii. Two options for targeted recruitment:

Option 1:

1. Review short forms at the end of each day and flag transfused male donors
2. Process specimen and save with "dummy ID".
3. Mail invitation to participate in study, consent form and questionnaire.
4. Follow-up with phone call to answer questions.
5. When consent is received, assign real subject ID in SMS.
6. Update STS with real subject ID and consent information.

Option 2:

1. Westat will query previous three month's REDS-II donation database and upload all transfused males into SMS.
2. BC will contact donors by phone and mail inviting them to participate in the study.
3. When donor comes in for his next blood donation study coordinator will consent the donor and collect questionnaire and specimen using regular on-site recruitment protocols.

When study numbers for certain groups have been filled, the coordinating center will provide feedback to all blood centers, so that more targeted recruitment may proceed in any unfilled groups.

VI. Potential tools for recruitment:

A. Posters, table tents etc for announcing the study
B. Information sheets
C. Letters for direct mailings

D. Stickers or buttons for collections staff to wear, advertising the study ("Ask me about the LAP study")

E. Sharing of ideas regarding potential recruitment tools among all participating blood centers should save time, creative effort, and cost.

VII. Education of research and lab staff members:

A. Training plans will need to be developed for each donor center, depending on that center's educational and regulatory process, to instruct the appropriate staff members as to the recruitment process

B. PI's, Co-PI's and/or research staff at each donor center should meet with their Education and Training Department Managers, to develop and implement a training plan for donor recruitment into the LAP study.

C. Important topics for training will include:

i. Understanding of basic tenets of TRALI and LAP study
ii. Understanding and ability to explain concepts of study
iii. Ability to explain to donor what study will involve on donor's part
iv. Understanding of consent form
v. Ability to take informed consent from donor
vi. Ability to guide donor through the process
vii. Ability to give donor contact information, when requested

Appendix 5: Information sheet for the LAP study participants

The _____Blood Center is participating in a research study, the Leukocytes Antibodies Prevalence (LAP) Study, sponsored by the National Heart, Lung, and Blood Institute.

Leukocyte antibody testing research:

One part of this research study involves the study of a disease process called "Transfusion-Related Acute Lung Injury (TRALI)". TRALI is a rare condition. However, it is the second leading cause of death resulting from blood transfusion in the United States. Blood recipients who develop TRALI feel sudden difficulty breathing, and they have serious injury to their lungs, after transfusion of certain blood products. Most of these people survive, but as many as 5-10% may die following the reaction.

It is possible that TRALI is caused by transfusion of unusual special antibodies produced in the bloodstream of blood donors, especially those donors who have been previously transfused or been pregnant. These antibodies are thought to be common and do not usually harm the person who has them, just by being there. In some cases, it is possible that these antibodies may cause harm (without knowing it) to certain people who receive that blood. This is why the investigators in this study are trying to find out how many blood donors have these special antibodies, and what kind of antibodies they have.

If you would like to join the study, you will be asked to answer a few questions about your medical history including whether or not you have been transfused with blood in the past, or for female donors, whether you have been pregnant in the past. You will also be asked to provide a blood sample that will be tested for White Blood Cell (WBC) antibodies. In the event that you have these antibodies then your blood sample will be used for typing your WBCs. A portion of your sample will also be frozen and kept in a repository. Whenever possible, this blood sample will be obtained from your routine blood donation. However, if an insufficient sample is available from your routine blood donation, you may be asked to donate one extra tube of blood when you donate your unit of blood today. Generally, this extra tube of blood can be taken from the arm used for the donation. The investigators have also made sure that not too much blood will be taken from your body if you choose to donate the tube of blood for this study. It will take 10-15 minutes of your time to participate in this study.

If you are interested in participating in the research described above, please read the "Informed Consent" form. This form will tell you more about the goals of this study. It will answer your questions about when you may be notified of certain test results and what this might mean for you as a blood donor. It will also explain to you about the potential risks and benefits associated with participation in this study.

Thank you for thinking about joining this study. For all those people who may receive blood transfusions in the future, it is very important to find out more about the presence of these antibodies. By participating in this study, you will be contributing to important medical knowledge for the future.

Appendix 6:

REDS-II Leukocyte Antibodies Prevalence (LAP) Study Consent Form

INVITATION

Thank you for coming to donate blood at <*BLOOD CENTER*>. Today we are asking blood donors to participate in a research study called the Leukocyte Antibody Prevalence (LAP) Study.

WHO IS DOING THIS RESEARCH?

This research study is being conducted as part of a large blood safety and availability research program called REDS-II, and is funded by the National Heart, Lung, and Blood Institute of the National Institutes of Health. As one of six participating REDS-II blood centers, <*BLOOD CENTER*> is enrolling eligible donors in this important research study.

WHY IS THIS RESEARCH PROJECT BEING DONE?

This research is designed to help improve the safety of the blood supply. White Blood Cells (WBC) help provide immunity and fight infections. Sometimes, people make antibodies to WBCs either when they receive a transfusion or in women, when they are exposed to their child's blood during pregnancy. These antibodies generally do not cause harm when transfused to patients, but in rare cases, they may contribute to a reaction called transfusion-related acute lung injury or TRALI. In this reaction, the patient can have severe difficulty breathing and become very sick. Our research aim is to find out how many blood donors have WBC antibodies (leukocyte antibodies) and to further characterize these antibodies in donors who have them. We need approximately 8,000 donors from 6 different blood centers across the country to take part in the LAP study.

WHAT IS INVOLVED IN PARTICIPATING IN THIS RESEARCH PROJECT?

- Participation requires about 10-15 minutes of your time.

- With your consent, a small sample of blood, an extra ½ tablespoon, will be collected from you in a separate tube. Your blood sample will be tested for white blood cell (WBC) antibodies, and be stored for later research.

- You will have to complete a short questionnaire about blood transfusion and/or pregnancy. You will be asked about whether you have ever received a blood transfusion in the past and for women, you will also be asked a few questions about previous pregnancies.

- If you screen reactive on any of the infectious disease screening tests routinely performed on your donation by the blood center then you will be de-enrolled from this study and your blood sample will be destroyed.

WHAT TYPES OF TESTS WILL BE DONE ON THE BLOOD SAMPLE?

Testing for White Blood Cell (WBC) Antibodies

The blood sample you provide for this research study will be tested for WBC antibodies. Since the presence of WBC antibodies is generally considered not to have any health consequence you will not receive the results of this testing**.** <If you plan to notify and counsel donors of neutrophil abs of defined specificity then include that language here>

Testing Your Blood to Characterize your White Blood Cells (WBCs)

If you have WBC antibodies, we may perform DNA testing of a portion of your blood sample to characterize your WBCs. WBC typing generally does not have any health consequence and therefore, you will not receive results of this test.

Storage and Future Testing of your Blood Sample

By consenting to participate in the second part of this study, you are agreeing to have a portion of your blood sample indefinitely stored in a repository maintained by the National Heart, Lung and Blood Institute. When you agree to have your blood sample stored, you are granting consent now for future uses of this sample. You may also be contacted in the future to provide additional information or an additional sample if necessary. All research on your stored or additional samples will be for the purpose of ensuring transfusion safety and understanding transfusion biology. This may include testing your blood for genetic (inherited) factors relating to WBC's and the body's immune response. The National Institutes of Health will give access to these samples only to its employees or approved researchers. Any future study must be reviewed and approved by an Institutional Review Board, the committee that protects your rights and welfare as a research participant.

ARE THERE BENEFITS TO TAKING PART IN THIS PROJECT?

If you agree to participate in this research study, there is no direct benefit to you other than the satisfaction of participating in this research for the benefit of making transfusions safer for future generations. Research performed on your blood sample will also contribute to the knowledge and understanding of transfusion and its consequences.

WHAT ARE THE RISKS?

The risks of taking part in this study are very small.

- In the unlikely event that we cannot obtain a sufficient sample from your routine blood donation, a separate needle insertion in your arm may be necessary. When blood is drawn you may feel a little discomfort as the needle goes through your skin. There may be local bruising or bleeding at the puncture site. Very rarely, the arm may become infected or you may feel faint. The risk is the same as that of having blood drawn at your doctor's office.

- There may be situations where testing will be done on the stored sample and the link between your name and the test results will be maintained. In these cases, the results will be shared with you if they are of medical significance. Notification of results from future

testing may be unexpected or upsetting to you. At the time of notification, you will be provided with more specific information about your test results and what they mean. It is your decision whether to share your test results with others.

- In the unlikely event that your blood is found to contain a rare, strong antibody to WBC that might be harmful to a blood recipient, the blood center may prevent you from giving blood in the future. Rarely, strong WBC antibodies may be dangerous to your unborn child. So you will be notified and counseled about their meaning if you are a woman who may become pregnant <Optional depending on BC deferral/notification plans>.

WILL THE INFORMATION BE KEPT PRIVATE?

Information concerning your participation in the study will be kept confidential and used only for scientific purposes, in accordance with applicable state and federal laws. Every effort will be made to maintain the confidentiality of your study records. The specimens and questionnaire data will be labeled with a study number assigned to you instead of your name. Only the research staff at your blood center will have the ability to link the study number on your samples or questionnaire to your name and other identifying information.

While we will make every effort to keep the study confidential, confidentiality cannot be guaranteed. To provide additional protection of your privacy, the blood center has obtained a Certificate of Confidentiality in accordance with Section 301(d) of the Public Health Service Act. This certificate prevents study staff from being forced to disclose information that may identify you by court order or other legal action. This protection lasts forever (even after death) for all study participants. Any results of the study, such as scientific publications, will be reported as summaries that will not reveal your identity.

WHAT ABOUT COMPENSATION?

There is no cost to you for participating in the study and you will not be paid to participate. All research tests will be free. You will not receive financial compensation for any new scientific or medical testing procedures developed and marketed using the results of the research done on your sample.

WHAT ABOUT MY RIGHTS TO DECLINE PARTICIPATION OR WITHDRAW FROM THIS PROJECT?

Your participation in this research is entirely voluntary. If you decide not to participate in this study, your decision will not adversely affect your ability to donate blood. If you decide to participate, you are free to withdraw your consent and to discontinue participation at any time. If you later decide that you do not want your sample and information to be used for future research, contact <*Principal Investigator*> at <*Phone*> and submit a written request to <*Principal Investigator*> and we will destroy any remaining identifiable samples and information.

WHOM DO I CALL IF I HAVE QUESTIONS OR PROBLEMS?

If you have any questions, please feel free to ask now. If you have any questions about your rights as a research participant, now or in the future, you may call <*Principal Investigator*> at <*Phone*>.

STATEMENT OF CONSENT

I have read this form and understand the purpose of this study, procedures to be followed, and the potential risks and benefits. I have been allowed to ask questions, and my questions have been answered to my satisfaction. I understand that I may withdraw at any time after signing this form. A signed copy of this consent form has been given to me.

I agree to participate in the research in the following ways (please check all that apply).

[ ] I consent to participate in white blood cell antibody testing and characterization research.

[ ] I consent to participate in storage of my blood sample and consent for future studies that may be performed with my blood sample that are designed to improve our understanding of transfusion biology and transfusion safety.

_____ _____

Signature of the participant Date

_____

Name of the Participant (PLEASE PRINT)

_____ _____

Witness Signature Date

Appendix 7: Neutrophil antibody testing cost proposal from the Blood Center of Wisconsin

Testing & Costs:

Level I Neutrophil Antibody Screen: Flow cytometry screen of serum against normal neutrophils covering the HNA-1a, -1b, -1c, -2a, and -3a antigens.

Cost per sample - $30

Level II Neutrophil Antibody Screen: Flow cytometry screen of serum/plasma pre- and post-absorption with normal platelets (removes interfering Class I HLA antibodies) against normal neutrophils covering the HNA-1a, -1b, -1c, -2a, and -3a antigens.

Cost per sample - $47

Level III Neutrophil Antibody Identification: Flow cytometry testing of serum/plasma against a larger panel of typed neutrophils and by MAIGA (if required) to identify the alloantigen specificity of the neutrophil-specific antibody detected in Level 1 or 2 testing.

Cost per sample (serology) - $51 (in addition to Level 1 or 2 testing performed)
Cost per sample (typing) - $120

All samples will be screened by the Core Lab in San Francisco for HLA antibodies. This information will be supplied to the Neutrophil Antibody Testing Laboratory for use in determining the "Level" of neutrophil antibody testing to be performed.

- All Class I HLA antibody positive sera will be tested in the Level II test before and after absorption with normal platelets (removes Class I HLA antibodies).

- All Class I HLA antibody negative sera will be tested in the Level I test.

The neutrophil antigen specificity will be determined by Level III testing for all sera with neutrophil antibody positive test results (not due to Class I HLA). *The need/request for this testing will be determined by the REDS II investigators.

Sample Volumes:

Level I Testing: Minimum of 150 µl of plasma or serum

Level II Testing: Minimum of 250 µl of plasma or serum.

Level III Testing: Minimum of 0.5 ml to 1 ml of plasma or serum and 100 ng DNA.

Appendix 8: Donor notification letter for HLA or neutrophil antibodies

Date:

I am writing this letter to inform you of the laboratory test results on a blood sample that you gave for research when you participated in the Leukocyte Antibodies Prevalence (LAP) Study. I apologize for the delay in reporting the results. The delay was caused by the fact that the research protocol required to have a blood sample be collected first from all donors like you before testing could occur.

As part of the research to better understand TRALI complication from blood transfusion, your blood sample was tested for white blood cell (WBC) antibodies or also referred to as leukocyte antibodies. TRALI stands for Transfusion-related Acute Lung Injury. White blood cells or leukocytes are cells in your blood that help fight infections. They also provide immunity against bacteria and viruses. WBC antibodies are produced after a blood transfusion. In women, these antibodies are also often produced due to a pregnancy. These antibodies, in rare circumstances may cause TRALI reactions.

[For HLA antibodies] Your blood sample showed the presence of WBC antibodies that are called HLA antibodies. HLA stands for Human Leukocyte Antigen. HLA antibodies are present in many blood donors. Generally, the donors who have these antibodies do not experience any adverse health effects. In rare cases, individuals with HLA antibodies experience fever after a blood transfusion or may not achieve the full expected benefit from a transfusion. In extremely rare cases, women with HLA antibodies may deliver a baby with a low WBC or a low platelet count. Platelets are cells in your blood that help to stop bleeding.

[For neutrophil antibodies] Your blood showed the presence of WBC antibodies that are called neutrophil antibodies. Neutrophils are a type of white blood cells that help fight bacterial infections. Generally, donors who have these antibodies do not experience any adverse health effects. In rare cases, individuals with neutrophil antibodies experience fever after a blood transfusion. In extremely rare cases, women with neutrophil antibodies may deliver a baby with a low neutrophil count.

As mentioned above, WBC antibodies are of no concern in the vast majority of cases. Therefore, there is no need for you to be concerned and generally no follow-up testing is required. In very specific rare circumstances, if they apply to you, I suggest that you inform your physician of the test results. These circumstances include those who are expected to receive blood transfusion in the near future and those women who are pregnant and have a history of having had previously delivered a baby with either a low WBC or a low platelet count.

[Donor not deferred] The presence of WBC antibodies in your blood does not mean that you cannot donate blood. In fact, we encourage you to continue to donate blood to help patients who need a blood transfusion.

[Donor deferred] Because of the presence of the antibodies described above, I ask that you do not donate blood again for others. We will keep the information about your deferral in your records. Please note that you can donate blood for your own use in the future when needed.

Please accept my sincere thanks for your blood donations in the past and your participation in the LAP Study.

If you have any questions, please contact [**insert name**] _____ at [**insert telephone number**] _____.

Sincerely yours,

Medical Director