LAPS-II FINAL PROTOCOL

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LAPS II: A retrospective data collection study of TRALI in recipients who are transfused with blood components from well characterized donors with or without leukocyte antibodies

A. Rationale

The recently completed Leukocyte Antibody Prevalence Study (LAPS) provides the largest well characterized population of blood donors with a detailed pregnancy and transfusion history and concomitant HLA class I and II, neutrophil, and endothelial cell antibody test results. Transfused blood components from these donors provide a unique opportunity to study the relationship between potential donor risk factors and the incidence of TRALI. The availability of a consensus definition of TRALI now facilitates establishing a more precise incidence of TRALI. A retrospective data collection (referred to as look back) study of components from these donors can provide valuable new information regarding TRALI with policy implications.

The major rationale for this study, referred to as LAPS-II, is to determine if TRALI occurs more frequently from components with HLA antibodies vs. components without HLA antibodies.

Other secondary aims of the study include generating data to examine the following questions:

1. Does TRALI occur more frequently from components with HLA and HNA (Human Neutrophil Antigen) antibodies vs. components with HLA antibody but without HNA antibody?

2. Are there antibody characteristics that are correlated with a higher incidence of TRALI such as HLA class I vs. class II, antibody strength as indicated by signal intensity on the screening assay, or antibody specificity.

B Background

B1. TRALI incidence in relation to method of detection: The precise incidence of TRALI is unknown. A wide range of estimates have been reported in the literature as described in Table-1. The detailed review of published literature regarding the incidence is included in the appendix A.

Table-1 Case finding methods and TRALI incidence

No.	Type of study	Risk of TRALI per unit
1	Passive surveillance, e.g. Serious Hazard of Transfusion (SHOT)	1:8264 for whole-blood derived platelets (Kleinman 2003) to 1:557,000 for cryoprecipitate (SHOT 2004 Annual Report)
2	Enhanced surveillance with extra steps for case finding	1:5000 (all types of blood components; Popovsky 1985)
3	Single hospital based reports	1:312 for whole blood derived platelets (Clark 1994) to 1:7896 for FFP (Wallis 2003)
4	Specialized treatment category and/or monitoring	75% of liver transplant patients with pulmonary edema (Yost 2001); 1:200 FFP (Palfi 2001)
5	Retrospective medical record review	33% of mechanically ventilated patients (Rana 2005); 1:1,722 (all types of blood components; Finlay 2005)
6	"Classic" lookback of recipients of donations previously made by implicated donors (Retrospective data collection)	From 0% to 42% of the recipients (See Table-3)

B2. TRALI Incidence based on retrospective medical record review: The incidence data derived from retrospective medical record review are particularly relevant to our proposed study because of our plan to conduct such a review. Such studies in critically ill patients who are mechanically ventilated have detected high rates of post-transfusion lung injury (Rana et al. and Gajic et al.). Another study reported by Finlay et al. is particularly interesting as it was based on a general hospital population rather than patients selected exclusively from critical care units. In addition, they employed a stepwise comprehensive review process which we propose to use in this study. Finlay et al. provide an estimate of TRALI without other risk factors for ALI as 1:1,722 transfused units, TRALI with other risk factors for ALI as 1:2,296 transfused units, and a combined rate (without and with other risk factors) as 1:984 transfused units.

B3. Stepwise record review to detect TRALI: Finlay's group performed first level screening of the University of California at San Francisco (UCSF) Medical Center hospital's computer data for arterial blood gas analysis before and after transfusion to detect new hypoxia (PaO2/FiO2 of 300 or less). For those patients who developed new hypoxia within 6 hours of transfusion, the investigators performed a second level screening of electronic medical records and evaluated radiology interpretations of chest radiographs to identify patients who had bilateral infiltrates with pulmonary edema. For patients who had new hypoxia and had bilateral lung infiltrates on chest radiographs, further electronic records (discharge summary, electrocardiogram reports) were reviewed to identify those patients who had cardiogenic pulmonary edema. If cardiogenic pulmonary edema was excluded, then the medical records underwent a full record review by the investigators.

Table 2: A stepwise approach to retrospective record review of transfused recipients to detect TRALI cases (data from Finlay et al.)

Parameter	Finlay et al. Data
Total number of patients transfused	820
Number with new pO2/FiO2 <300	88
No. with chest x-ray	66
New bilateral pulmonary infiltrates	23
Cardiogenic pulmonary edema/fluid overload	10
TRALI cases identified (3 cases without and 4 cases with other risk factors for ALI)	**7**

B4. Type of blood component transfused and TRALI risk: We should also note that although Fresh Frozen Plasmas (FFPs) comprised 34% of the 6888 components transfused during the study period in Finlay's study (47% were RBCs, 15% apheresis platelets, 4% others), 61% of the 59 components received by the 7 patients identified with TRALI were FFPs (29% were RBCs, 2% apheresis platelets, 8% others), suggesting a higher TRALI risk in recipients of FFP. Theoretically, one would expect a higher incidence of TRALI from FFP transfusion when compared to red blood cell transfusion because the amount of plasma in a unit of FFP is about six times greater than that present in a unit of red blood cells (250 vs. 40 ml).

An American Red Cross study analyzed 38 cases of probable fatal TRALI reported between 2003 and 2005(Eder et al). This study found that the rate was higher for plasma components (odds ratio = 12.5; 95% CI=5.4-28.9) and apheresis platelets (odds ratio = 7.9; 95% CI = 2.5-24.8) when compared to RBC. These data indicate that the per unit risk of TRALI is eight to 12 fold higher for products (FFP and apheresis platelets) that are plasma-rich as compared to the lower-risk (plasma-poor) products.

B5. Incidence of TRALI based on lookback: For transfusion complications, detection of previously undiagnosed cases is attempted using "Lookback", a process in which previous donations from a donor whose subsequent donation is implicated in TRALI are identified. Recipients of blood components from the previous donations are followed either by reviewing medical records or testing them to see if the transfusion complication occurred. Such lookback studies for detection of TRALI among recipients of previous donations by donors subsequently implicated in TRALI have been reported and their results are summarized in Table-3.

Table 3: Summary of lookback studies

Study	No. Patient Charts Reviewed	No. TRALI cases Detected
Kopko	36	15*
Win	30	0
Toy	103	1
Cooling	20	3
Nicolle	18	1
Total	**207**	**20**
Total w/o Kopko	**171**	**5**

*The study from Kopko et al. had the highest prevalence of pulmonary reactions on lookback (42%), but this included reactions that were not severe enough to be diagnosed as TRALI. The high rate of reactions may be due to the fact that their donor had granulocyte 5b (HNA3a) antibody while all other lookback TRALI cases were from donors with HLA antibodies.

Excluding the study by Kopko et al. that appears to be an outlier, the above studies show that the prevalence of unreported TRALI is about 2.9% (5/171) among recipients of blood components from implicated donors. It should be noted that Win et al. failed to detect any case of unreported TRALI among 30 recipients despite the fact the medical records were reviewed by hematologists. To avoid biasing the incidence rate towards the high side, our calculation of an aggregate 2.9% incidence includes the data from Win et al.

B6. Limited data regarding identification of TRALI cases in recipients who are transfused with leukocyte antibody positive blood components: We are aware of only two studies describing retrospective review of medical records designed to detect TRALI cases among recipients of blood components from HLA antibody positive donors which were not previously implicated in TRALI (referred to as un-implicated donors). In a report by Maslanka et al., 62 HLA antibody positive women donors were found out of 633 tested. These alloimmunized women had donated a total of 211 blood components. One case of TRALI was reported after a transfusion of a unit of RBC from a woman who had multi-specific HLA class I and class II antibodies. This case was previously reported to the blood bank. Retrospective record review revealed no additional unreported cases of TRALI. In the second report by Fadeyi et al., blood component transfusions from four donors with HLA class I or II antibodies and 12 matched control donors (without HLA antibodies) were reviewed. Transfusion reactions were detected in 3 of 167 (1.8%) and 3 of 295 (1%) of transfusions from HLA antibody positive and control donors respectively. None of the patients developed TRALI.

Because of the scarcity of data from un-implicated donors, this study proposes to assess the incidence of unreported TRALI among recipients of blood components from leukocyte antibody positive and antibody negative donors from the LAPS study. Such a study is important because patients continue to receive leukocyte antibody untested units, of which approximately 10% may contain leukocyte antibodies. It is important to know if TRALI incidence is different among recipients of antibody positive units when compared to recipients of antibody negative units. This comparative risk assessment will provide much needed information, which can then be used to estimate the degree of TRALI prevention from different product and donor management

strategies. As part of TRALI prevention, in some countries in Europe, especially in the UK, and more recently in the U.S., blood collection centers have stopped using certain plasma products from female donors for transfusion. Preliminary data suggest that there might be a reduction in incidence of TRALI following this step (Williamson L AABB 2006 Annual Meeting). However, it is recommended that TRALI incidence be carefully monitored to assess if the preventive strategies are working (Kleinman S 2006 Annual AABB meeting).

B7. Types of antibodies implicated in TRALI: A number of case reports or small case series have implicated HLA class I and HLA class II antibodies. Among class I antibodies, both HLA-A and HLA-B locus antibodies have been implicated. There are no published cases implicating HLA-C locus antibodies. Among HLA class II antibodies, HLA-DR antibodies are most often implicated. In addition, HLA-DQ5 antibody has been implicated in one case. (Nakagawa M et al.). Besides the antibodies with a well defined single or multiple antigen specificities, HLA-A2 CREG (cross reactive group) (Eastlund DT) and DR 3/5/6 CREG antibody (Wallis JP 2003) have been implicated in one case each. Antibodies with a wide-ranging PRA (panel reactive antibody) from 16% to 99% without demonstrable antigen specificities have been implicated (Cooling L; Davoren A et al.). One report indicates strong antibodies as being responsible for TRALI (Marques MB) while another report shows that low-titer pan-reactive IgG antibody in intravenous gamma globulin (IVIG) was responsible (Rizk et al.). A high titer (256) antibody against neutrophil antigen 5b was responsible in one case (Nordhagen R). Neutrophil antibodies with or without defined specificities have also been implicated. Anti-monocyte antibody seemed responsible in one report (Dooren MC) although the gamma globulin responsible for TRALI in this case also had HLA class I & II antibodies in addition to the monocyte antibodies.

As described above, the data suggest that many different leukocyte antibodies have been reported as possibly causing TRALI. However, it should be noted that most of these data on the specificity of leukocyte antibodies implicated in TRALI are from isolated case reports or small case series. The relative rates at which various antibodies lead to TRALI as determined by a population based study such as is proposed in this protocol is currently not known.

B8. TRALI in relation to plasma rich vs. plasma poor components: It makes intuitive sense that TRALI incidence might be higher with plasma rich components compared to plasma poor components due to the fact that donor antibodies are present in plasma. In fact, recent data from the American Red Cross show that the rate of fatal TRALI was higher for plasma components (odds ratio = 12.5; 95% CI=5.4-28.9) and apheresis platelets (odds ratio = 7.9; 95% CI = 2.5-24.8) compared to RBC (Eders et al.). The current study is designed to measure TRALI incidence with plasma rich components. Plasma poor components, e.g., red blood cells, from LAPS donors will not be systematically studied in the present protocol. However, lookback investigations will be performed for recipients who receive red cells from a LAPS study donor whose plasma rich component was associated with TRALI in another recipient. This additional investigation will permit a full evaluation of all components transfused from a donor who is associated with a TRALI case.

C. Objectives

C1. Primary objective: The primary objective of this study is to evaluate whether TRALI occurs more frequently in recipients of at least one known HLA antibody-positive blood product (cases) vs. recipients of at least one known HLA antibody-negative blood product (controls). We propose to evaluate case and control recipients for the presence of TRALI using the Canadian Consensus diagnostic criteria and also by the NHLBI criteria. TRALI cases with other risk factors for ALI will be included in our overall definition of having TRALI. Both the Consensus definition & NHLBI diagnostic criteria require the presence of bilateral pulmonary infiltrates on chest x-ray in order to make a diagnosis of TRALI.

Potential cases will be identified by blinded retrospective review of medical charts of recipients of transfusions from donations that were given by donors enrolled in the LAPS protocol. Initial screening of recipients will include a search for a chest x ray result within 24 hours of the index transfusion. A further medical record review (described elsewhere in the protocol) will be performed only on recipients with a chest x-ray result that shows new or worsening bilateral pulmonary infiltrates.

C2. Secondary objectives: Secondary objectives will include the risk of TRALI associated with HLA antibodies in the presence and absence of HNA antibodies and HLA antibody characteristics such as HLA class I vs. class II, antibody strength (as indicated by signal intensity on the screening assay), and antibody specificity.

D. Hypotheses

D1. Estimation of TRALI in relation to number of blood components: Based on experience of Finlay et al. who performed detailed retrospective record review, and on additional assumptions outlined in G3 and H3, we hypothesize that the incidence of TRALI from high risk products is 7.1 per 1,000 HLA antibody-positive components, 0.45 per 1,000 HLA antibody-negative components, and 1.8 per 1,000 components of unknown HLA antibody status. It should be noted that Finlay et al. included TRALI cases with or without associated risk factors for acute lung injury in accordance with the NHLBI diagnostic criteria for TRALI as shown in Table-2 above.

D2. Estimation of TRALI in relation to number of recipients: Based on the experience of Finlay et al. who performed detailed retrospective record review, and on additional assumptions outlined in G3 and H3, we project that the incidence of TRALI (with and without other risk factors for ALI) in recipients of at least one known HLA antibody-positive component will be about 14.6 per 1,000, while the corresponding estimate in recipients of at least one known HLA antibody-negative component will be about 7.9 per 1,000.

E. Significance

TRALI is now the leading documented cause of transfusion-related morbidity and mortality in the US. The transfusion community has begun implementing measures to reduce the risk of TRALI with a recommended deadline by the AABB of November 2007 for plasma components

and November 2008 for apheresis platelets. Most centers have adopted a strategy of using primarily or exclusively male donors for plasma for transfusion. The limited availability of group B and AB plasma makes this strategy problematic. The same issues apply to apheresis platelets to an even greater extent. As a result, centers are interested in strategies that include testing for leukocyte antibodies to identify donors at risk for causing TRALI. However, prior to the recent completion of LAPS-I, there has been a paucity of data establishing the incidence of HLA or HNA antibodies using current methodologies in donors who are well characterized by gender and alloimmunization history. There is also controversy regarding the clinical significance of these antibodies and the true incidence of TRALI using the Canadian Consensus or the NHLBI definition. Lastly, none of the measures currently contemplated to reduce the risk of TRALI address non-immune mechanisms by which TRALI may occur. The LAPS data provide needed information to establish the relationship between a donor's history of pregnancy or transfusion and leukocyte alloimmunization using the most sensitive methods available. LAPS II is the logical follow up study of components from these donors to answer the crucial question regarding the clinical relevance of these antibodies.

The results of LAPS II will have major policy implications including whether HLA or HNA antibody testing is of value in predicting a higher risk of TRALI; and whether certain antibody characteristics such as in-vitro reactivity strength or specificity pose a greater risk of TRALI. With future protocol additions, it will be possible to determine whether plasma rich components pose a higher risk compared to plasma poor components. In addition LAPS II will provide an estimate of the incidence of TRALI based on a retrospective cohort study. The advantages of the proposed study design include: the ability to perform retrospective record review on a large number of both antibody positive and negative components, the use of blinded reviewers, the use of a standardized data collection instrument, and the use of a blinded central adjudication panel that will use the currently accepted definitions for TRALI diagnosis.

The ideal method for precise determination of TRALI incidence would be a multi-institutional prospective clinical study that might include special monitoring of transfusion recipients at the time of transfusion (e.g., pulse oxymetry to detect oxygen desaturation). Such a study would permit interactions of TRALI researchers with the clinicians caring for the patient and allow for concurrent dialogue between these groups that would permit better delineation of data for differential diagnosis. Such an interactive clinical diagnostic deliberation is not possible with the retrospective cohort study proposed here. Also, a prospective study avoids the limitation of missing clinical data that is inherent to a retrospective study. .

Despite clear advantages of a prospective study, we still believe that a retrospective cohort study is of value for several reasons. Firstly, a prospective study in which recipients are randomized to receive leukocyte antibody positive or antibody negative blood components cannot be ethically conducted. Secondly, because of the extensive work done in LAPS-I, we have the unique opportunity to evaluate the recipients of transfused plasma rich components from a large number of donors with well characterized leukocyte antibodies. Such an opportunity is unlikely to present again. As diversion of plasma products from female donors away from transfusable products becomes more widespread, future studies of the type proposed here would not be possible. Also, it is unlikely that anyone will perform a study of recipients of blood components from a large cohort of donors who are as well characterized for the presence and the absence of

leukocyte antibodies. The answer to the question of TRALI incidence among recipients of blood components from leukocyte antibody-positive donors in comparison to the leukocyte antibody-negative donors will likely not be established unless the proposed study is performed. Clinical data such as chest x-ray, arterial blood gas (ABG) analysis results, BNP levels etc. that are needed for a diagnosis of TRALI are unlikely to be missing as they are routinely captured unless clinicians did not entertain a possible diagnosis of TRALI. Therefore, for patients with severe respiratory distress, such data should be available for the vast majority of patients. Although it remains a possibility that retrospective data collection may not allow detection of mild cases of TRALI, the current consensus definition does not include diagnostic criteria for mild TRALI.

The NHLBI is currently funding a prospective TRALI study that is being conducted at UCSF and Mayo Clinic (PI is Dr. Pearl Toy) under a Specialized Centers of Clinical Research (SCCOR) mechanism. Our retrospective cohort study will clearly complement this prospective study and will be less expensive than a new prospective study that enrolls a similar number of recipients. In addition, Dr. Toy has been participating in the design of this study to permit inclusion of important elements of her research design into the present study.

Overall, we believe that a well constructed retrospective multi-institutional cohort study might provide a reasonable estimate for TRALI incidence in a cost effective manner for the following reasons: (a) The unique opportunity presented to us by the donor cohort studied in LAPS, (b) It is unlikely that a similar opportunity will ever arise to conduct a retrospective study such as ours, and (c) The cost effectiveness of retrospective data collection study is favorable compared to a similarly powered prospective clinical study.

F. Study design/Methods

F1. Case and control recipients: The current protocol is designed to evaluate the incidence of TRALI in recipients of at least one known HLA-antibody positive plasma rich component by performing a retrospective evaluation of medical records; and to compare the incidence of TRALI in such recipients to that among recipients of at least one known HLA antibody-negative plasma rich component. The following blood components are considered to be plasma rich and only these components will be subject to the study: Plasma (that includes fresh frozen plasma (FFP), cryoprecipitate- reduced plasma, plasma frozen within 24 hours of phlebotomy, plasma collected by automated methods, thawed plasma, and any transfusable plasma), Platelets, Apheresis (single donor plateletpheresis units) and whole blood (which is currently used very sparingly). A retrospective study on plasma-poor components may be performed in the future to ascertain whether there is a difference in TRALI rate between the two types of components (plasma-rich vs. plasma-poor).

The proposed study design is that of a retrospective cohort study where cases will be defined as recipients of at least one known antibody-positive plasma rich component and controls as recipients of at least one known antibody-negative plasma rich component. We estimate that we will be able to conduct reviews on 1,175 plasma rich components donated by HLA antibody-positive donors identified in the LAPS-I protocol and their corresponding 1,175 recipients (cases). We will conduct the same review on 1,175 plasma rich components known to be HLA antibody-negative and their corresponding 1,175 recipients (control). To this number, a 10%

margin is added to account for deaths of patients within six hours of transfusion, missing records, outdate or loss of the distributed products before transfusion or other unforeseen factors. Therefore, the total number of components traced to participating hospitals will need to be 2,590 (1,175 + 118 = 1,293 rounded to 1,295 case and similarly 1,295 control). Control donors will be gender and parity frequency-matched with the antibody positive donors. Additionally, the antibody status of all the available donors who are involved in any TRALI cases found in our study will be determined following a standardized protocol for donor investigation at the participating REDS-II blood centers. A similar procedure will be conducted for control cases.

F2. Management of TRALI cases that are previously reported to the hospital blood bank: It is possible that the hospital blood bank may have received a report of suspected TRALI in recipients of the study units. These reported cases will also be identified during record review and will be subject to the same rigorous process described in this protocol to establish or reject a diagnosis of TRALI. Only those cases that meet the diagnostic criteria of this protocol for TRALI will be included in determining the prevalence of TRALI.

F3. Radiology report/digital image capture and review: Retrospective data collection procedures will include an initial review of radiology data to ascertain if a chest x ray was done within 24 hours of the transfusion. If yes, the post-transfusion chest x-ray will be reviewed and a written verbatim radiologist's interpretation will be recorded in the data collection form. If available, a pre-transfusion chest x-ray will also be reviewed and a verbatim report will be recorded in the data collection form. If more than one radiology report for chest x-ray exists within 24 hours of the study unit transfusion, all the reports will be captured in the data collection forms. Those patients with new or worsening bilateral lung infiltrates, pulmonary edema, or ARDS, will have their data forms forwarded to the local REDS-II investigator for review.

Written radiology reports will be transcribed verbatim into the data collection forms for each study participant. When available, digital images will be collected for those cases that are submitted for review by the triage physician. These images will be provided to the expert panel for review. Recipient name, medical record number and the hospital name will be removed from the digital image by the REDS-II study staff or by the radiology staff. Each digital image will be labeled with the study ID number and the date and time the x-ray was taken. The digital images will subsequently be submitted to the Central Coordinating Center as part of the Power Point summaries that will be prepared by the nurse coordinator. It is recognized that in some cases, the digital image may not be available because the hospital does not have capability to obtain digital images. In some facilities, the hospital may have instruments to capture x-ray film into a digital image. Digital images captured from the x-ray films will be acceptable for the study. It is also recognized that a given hospital's policies may not permit sending such images outside of their institution and such cases digital images will not be available for the study.

The local REDS-II investigator will be blinded as to the type of recipient (case vs. control). The investigator will determine if the description in the x-ray report shows the presence of (or is consistent with) new or worsening bilateral lung infiltrates/pulmonary edema/ARDS (acute respiratory distress syndrome). If this is the case, the investigator will indicate that further chart

review is indicated; the next step in the review will be for the record review coordinator to determine the presence or absence of hypoxemia (indicative of ARDS) as described below.

If the report does not indicate the presence of bilateral lung infiltrates or other findings consistent with a diagnosis of TRALI, then the investigator will indicate that no further record review is needed. The local REDS-II investigator may consult the radiologist if any clarification is needed for the radiology reports.

As noted above, the total number of blood components subject to review is 2,590 (1,295 case and 1,295 control components). In order to streamline the record review process, pilot data were generated to determine the proportion of patients who would have a chest x-ray after transfusion. Since the diagnosis of TRALI requires bilateral lung infiltrates on chest x-ray, it seemed appropriate not to perform a detailed record review if there was no post-transfusion chest x-ray. Pilot data were generated at the REDS-II Center in Pittsburgh for 106 transfused blood components which showed that approximately 40% of transfusion recipients have a chest x-ray done within 24 hours of the time of issue from the blood bank of a blood component. These data when applied to the record review process suggests that 40% of the 2,590 or 1,036 recipients would have a post-transfusion chest x-ray.

The expected number of patients with a pre-transfusion chest x-ray is estimated at 20% which is approximately half the percentage of post-transfusion x-rays. Therefore, a total of 1,554 (1,036 post-transfusion and 518 pre-transfusion) x-rays will have to be reviewed and data recorded in the data collection form.

According to the article by Finlay et al., 23 of the 66 (35%) chest x-rays had bilateral pulmonary infiltrates consistent with new acute lung injury. In order to be somewhat more conservative for the planning purposes, we will assume that 50% of the chest x-rays would have new or worsening bilateral pulmonary infiltrates/pulmonary edema. This stepwise approach will then result in having to perform further medical record review on 1,036 x 0.5 = 518 recipients.

F4. Review to detect presence or absence of hypoxemia indicative of ARDS: In order to further eliminate the unnecessary detailed record review, a focused review of the transfusion episode will be performed to detect hypoxemia occurring within six hours of completion of transfusion. Medical records will be reviewed for the respiratory status within six hours after the completion of the study unit transfusion to detect the following: Oxygen saturation (SpO2) less than 90% by pulse oxymetry without or with oxygen therapy; ratio of SpO2/FiO2 <315; and ratio of PaO2/FiO2 <300 on arterial blood gas analysis. FiO2 will be calculated for patients receiving oxygen by cannula or mask as follows: 1 liter = 0.21; 2 liters = 0.23; 3 liters = 0.25; 4 liters = 0.3; 5 liters = 0.35; 6 and 7 liters = 0.45, and 8, 9, and 10 liters = 0.49 (Rice TW 2007).

If hypoxemia is documented, a detailed medical record review will be performed by the record review coordinator. The full extended data form will be completed. A Power Point summary of the major events and timeline will be prepared. The completed extended data forms and summaries will be sent to the Triage MD for further review.

Precise incidence rates for hypoxemia as defined in this manner in hospitalized patients are not available. Most of the data on incidence of acute respiratory failure (ARF) are reported in patients who are treated in the intensive care unit (ICU). For example, Flaatten et al have reported an incidence of ARF of 63% among 832 patients treated in the ICU. In 13,346 ICU admissions, 1,231 (9.2%) patients were found to have acute respiratory failure by Luhr et al. Pettila et al have described an incidence of ARF of 32.5% in 520 ICU patients while Vincent et al found that 32% of the 458 ICU patients had ARF at admission to the ICU and 35% of the 991 ICU patients developed ARF during their stay in ICU. Roupie et al report that 43% of the ICU patients required mechanical ventilation. A combined rate of 16% for ARDS and ALI in 1977 ICU admissions has been observed by Bersten et al. These data show a wide range for ARF in ICU patients. In order to be more conservative in our estimates for the number of charts requiring detailed review, we have estimated that 60% of the study patients would have hypoxemia/acute respiratory failure and would therefore require detailed chart review along with a review by a triage critical care physician (see below).

F5. Triage critical care physician review: Based on these considerations, the number of charts that would require a review by a triage critical care specialist will be 311 (60% of 518). The triage physician would receive the case summaries in the form of several PowerPoint slides per case along with the detailed medical chart data consisting of completed extended data form for each case and digital images of chest x-rays. The triage MD will be blinded as to the type of study component transfused. The review will be performed by a single MD and all REDS-II Centers will send records for review to the triage MD. The major aspect of this review is to determine if there is sufficient information to conclude that the patient has moderate to severe TACO. If such is the case, the triage MD will complete the form indicating the presence of TACO and no further reviews will be required. The number of cases that will be excluded from further review by the triage MD is uncertain, but may amount to 50%. If the percent is lower, then the workload for the Medical Review Board may need to be reassessed during the conduct of the study.

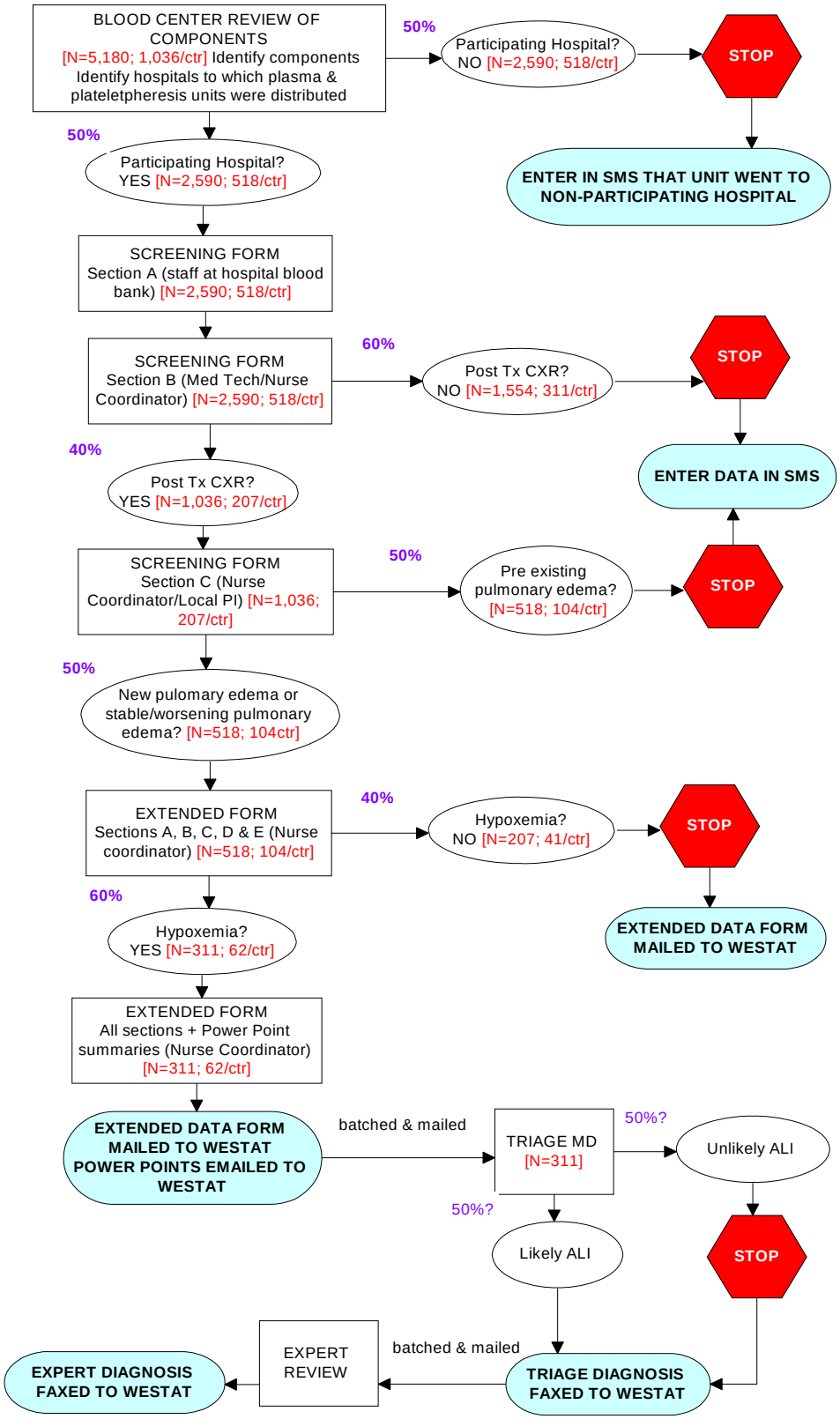
The above described processes are shown in the flow chart on next page and in Table-4 below.

Table-4: The stepwise approach to record review with corresponding estimated activity numbers for the five REDS-II Centers

Parameter	Number of patients	Comment
Number of patients needed for each study arm	1175	Based on 90% power calculations, for each arm of the study
Including ten percent margin	1,275	Margin added to account for missing records, deaths within six hours etc.
Total number of patients	2,590	Two equal groups of antibody positive and antibody negative component recipients
40% of patients with a post-transfusion chest x-ray	1,036	No. of post-transfusion chest x-rays needing a review
20% of patients with a pre-transfusion chest x-ray	518	No. of patients with a pre-transfusion chest x-ray
Total number of chest x-rays	1,554	No. of chest-rays needing a review

50% of post-transfusion chest x-rays showing new or worsening bilateral infiltrates/pulmonary edema	518	No. of patients requiring hypoxemia determination review
60% of the study patients would have hypoxemia	311	No. of patients with evidence of hypoxemia
No. medical charts requiring detailed chart review including preparation of PowerPoint synopsis and then further Triage MD review	311	An unknown number of cases will be diagnosed as TACO and will have no evidence for ALI; these will not need to be forwarded to the Medical Review Board for adjudication. Total number of forwarded charts will be less than 311

LAPS-II DATA COLLECTION FLOW CHART



F6. Pilot study to validate assumptions for proportions of patients with chest x-ray, pulmonary edema, and hypoxemia: A medical record review was conducted at Pittsburgh Center by research nursing staff on 66 randomly selected units of FFP transfused to 66 patients in Pittsburgh area hospitals. Twenty two patients were at a community hospital and 44 patients at a tertiary care hospital. Chest x-ray data was extracted from a radiology electronic database with radiologist's interpretation. A chest x-ray was considered present if done within 24 hours of the end of the index transfusion. Those reports indicating pulmonary edema, bilateral pulmonary congestion or bilateral infiltrates were then evaluated for hypoxemia. Hypoxemia was sought in the laboratory section of the patient's electronic medical record and was defined as: SO2/FiO2 of <315 or pO2/FiO2 of <300 within 6 hours of the end of the FFP transfusion.

At the community hospitals, 5 of the 22 (23%) had a chest x-ray within 24 hours of the transfusion; 3 of the 5 (60%) had pulmonary edema, and 2 of the 3 (66%) had hypoxemia. At the community hospitals, 2 of the 22 (9.1%) of the recipients would require an extended record review.

At the tertiary care hospitals, 24 of the 44 (55%) recipients had a chest x-ray within 24 hours of the transfusion. Twelve of the 24 (50%) had pulmonary edema and 6 of the 12 (50%) had hypoxemia. These data indicate that 6 of 44 FFP transfusion recipients at tertiary care hospitals would require extended record review.

It should be noted that of the six recipients at the tertiary care hospitals who had pulmonary edema on chest x-ray after the transfusion, four had pre-existing pulmonary edema on pre-transfusion chest x-ray.

The above pilot study supports our assumptions regarding the stepwise record review process.

F7. REDS-II Blood Centers to identify blood components from LAPS donors:

The total number of components that would require review is 1,295 antibody positive plasma rich components and 1,295 antibody negative plasma rich components. These components will include all types of transfusable plasma (FFP, Plasma frozen within 24 hours of phlebotomy, cryoprecipitate-reduced plasma, concurrent plasma, thawed plasma and other transfusable plasma), apheresis platelets (plateletpheresis), and whole blood. Further, we estimate that 50% of components identified by the blood centers will be distributed to those hospitals where the record review will be performed. This is a conservative estimate and the percentage may in fact be higher. Based on this 50% rate, the number of plasma rich components that the project needs to identify totals 2,590 (1,295 x 2.0) from antibody positive donors and 2,590 (1,295 x 2.0) from antibody negative donors.

F8. Estimate of number of donations and components from LAPS donors: A preliminary analysis at the five participating REDS-II Centers has been performed to determine the number of HLA antibody positive plasma rich components that will be available for recipient record review. This analysis included the components from the index LAPS-I donations, all subsequent donations after the index LAPS donation, and all components distributed within 2 years prior to the index LAPS donation. Data collected from 75 LAPS-I donors have shown that during this

defined time interval, there were 694 plateletpheresis and 705 plasma components distributed from donations made by these donors. Therefore, we estimate that a total of 1,399 blood components (Plasma + Plateletpheresis) were available from 75 donors in this preliminary study; this averages to 18.5 components per donor. In order to obtain 2,590, antibody positive plasma rich components, we estimate that we will need to trace records from 140 antibody positive donors (28 per participating center). Similarly, we estimate the need for 140 antibody negative donors.

LAPS-I has identified 879 donors with HLA antibody at the five participating REDS sites, with a range of 108 to 219 HLA antibody positive donors per site. Thus the number of antibody positive LAPS donors at each one of the REDS-II site is sufficient to conduct the study.

F9. Long term persistence of HLA antibodies in blood donors: The above calculations require that donations made up to two years prior to the LAPS index donation will be subject to recipient record review. These calculations also assume that HLA antibody in blood donors will persist for a prolonged period. Pilot data to support this assumption were obtained on multiparous female donors at two REDS-II Centers (Norris PJ et al.). A total of 44 donors who were previously shown to have HLA antibody by lymphocytotoxicity at least 10 years previously were re-tested for HLA antibodies. 43 of these 44 (98%) donors had antibodies detected by screening Luminex assay when a new sample was collected 10 or more years after their HLA antibodies were first detected. This study involved observations that go forward ten years since the antibodies were detected. The present protocol intends to go backward in time from the index LAPS donation to perform the record review on previous donations. However, the interval for going back will be much shorter and is expected to be two years or less based on the prevalence of HLA antibodies in LAPS donors and the pilot data obtained on the number of donations made by the LAPS donors within two years prior to the index donation. To the extent possible, prior components from LAPS donors who have had a pregnancy in the two years preceding their LAPS index donation will not be selected for the record review in order to remove the possibility that the last immunizing event may have altered the type/s of antibodies in the donor. Such exclusion is permissible because the number of donors that will have an immunizing event in the past two years is expected to be low as pregnant women are deferred from blood donation during the pregnancy and for several weeks after the delivery.

F10. REDS-II center-specific numbers of blood components for record review: Each Center will be asked to identify a total of 1,295/5 = 259 antibody positive plasma rich components and 259 antibody negative plasma rich components sent to participating hospitals. Since only 50% of the distributed products will be sent to the participating hospitals, each center must identify 259 x 2 = 518 plasma rich components from antibody positive donors. Similarly, each center must identify 518 plasma rich components from antibody negative donors.

The number of components requiring a record review at participating hospitals at each Center will be 518. Of these, 40% will have a post-transfusion chest x-ray resulting in 207 post-transfusion chest x-rays to be reviewed. Additionally, 20% would have a pre-transfusion chest x-ray resulting in 104 pre-transfusion chest x-rays to be reviewed. The total number of chest x-rays (pre- and post-) to be reviewed will be 311 at each Center.

Of the total 207 post-transfusion chest x-rays, only 50% or 104 per Center will be expected to show new or worsening bilateral lung infiltrates, pulmonary edema, and/or ARDS. All these charts will require a review to determine the presence or absence of hypoxemia (hypoxemia review). As described above, 60% of the study recipients are expected to show hypoxemia. Therefore, we estimate that 104 x 0.6 = 62 recipients at each Center will have hypoxemia and will require a detailed chart review including a review by a critical care MD. The activities at the blood centers with their corresponding estimated numbers are shown in Table-5 below.

Table-5: Estimated activities at each participating REDS-II Center

Parameter	**Estimated Number**	**Comment**
Antibody positive components to be identified	518	Total for 5 centers for record review of 1,295 components or 259 per center. Each Center needs to identify 259 x 2 = 518 components because only about 50% of the components will be able to be traced and reviewed at participating hospitals
Antibody negative components to be identified	518	Total for 5 centers for record review of 1,295 components or 259 per center. Each Center needs to identify 259 x 2 = 518 components because only 50% of the components will be able to be traced at participating hospitals.
No. distributed to the participating hospitals (50%)	518	Includes antibody positive and antibody negative components in equal proportions
Post-transfusion chest x-ray in 40%	207	Post-transfusion chest x-ray requiring a review
Pre-transfusion chest x-ray (20%)	104	Pre-transfusion chest x-ray requiring a review
Total number of chest x-rays needing a review	311	Includes pre- and post-transfusion chest x-rays
New or worsening bilateral lung infiltrates/pulmonary edema in post-transfusion chest x-ray (50%)	104	Number of charts requiring hypoxemia review
Number of charts showing hypoxemia and requiring detailed review (full extended data form, Power Point summaries with X-ray images)	62	Sixty percent of the study recipients with bilateral lung infiltrates are expected to show hypoxemia

F11. Multi-center study: This study will take place at the five of the six REDS-II blood centers. Logistical constraints at the New England Region of the American Red Cross prevent their participation in the study. Specifically, their blood component inventory is distributed to hospitals in three different states (Massachusetts, Connecticut, and New Hampshire). The ability to review the transfusions of the study units would have required participation of a large number

of hospitals with each hospital not contributing more than a handful of cases. Therefore, these logistics constraints make it impractical for the study to be conducted at this REDS-II Center.

We assumed in the calculations presented below that each center will be able to identify a sufficient number of interested hospitals that on average receive about 50% of the blood components distributed by the blood center. Identified and interested hospitals will perform retrospective record review on essentially 100% of the components for which they will be requested to perform a record review. If REDS-II blood centers cannot identify sufficient number of interested hospitals, then the interval for previous donations for the LAPS donors will need to be extended back in time to allow for identifying additional previous donations for which a record review can be performed. However, our data for HLA antibody prevalence in LAPS donors and the pilot data on number of donations per donor suggest that this will not be necessary.

F12. REDS-II Blood center processes

F12A. Identification of blood components from LAPS donors: The blood center will receive notification from the coordinating center indicating which of the LAPS donors (donor-ID and BUI) and their donations require recipient record review. For purpose of this study, individuals at the blood center who are retrieving records on the LAPS donors will not be informed of the antibody status of the donors. It is possible that the blood center's other staff may have previously been informed of the antibody test results and that some leukocyte antibody donors have been identified and notified of their antibody status per the blood center's procedures. The blood center will identify all donations made by each donor: index donation + all donations after the index donation + all donations made within two years prior to the index donation. The blood center will identify all the plasma rich components made from these donations and where these components were distributed.

LAPS donor enrollment began in September 2006 and was completed in May 2007. Donations made after the index donation will be available for the study in the REDS-II database. However, plasma products from some of these subsequent donations may not have been transfused due to implementation of TRALI risk reduction programs (which preferentially manufacture transfusable plasma components from donations by male donors) in late 2007 or early 2008.

F12B. Data management of blood components from LAPS donors: The blood center will enter all the donation information and the component distribution data for each donation into a web based system developed and maintained by the Coordinating Center. The information needed will be donation date, blood unit identifier (BUI), type of component, if/where (hospital) each component was distributed to, and the date the component was distributed. Once all the data have been entered into the computer, the blood center staff will identify all blood components (i.e. plasma components, apheresis platelets, and whole blood) that are eligible for study.

F12C. Notification of hospital transfusion service regarding blood components requiring record review: The blood center staff will notify by a letter the medical director of the hospital transfusion service that the recipient record review is needed for the list of blood components. The letter will also ask the transfusion service/blood bank to determine the disposition of each

component and notify the record review coordinator of which components were issued for transfusion to whom (See Appendix B for a draft letter).

F12D. Record review coordinator's approach to radiology reports and hypoxemia data collection: The record review coordinator(s), who would either be a staff at the hospital, hired by each blood center or one approved at the hospital, will perform a search of radiology records. This search can involve either electronic records or paper records. The coordinator will collect the reports as described above for the local PI's determination as to the need for further record review. The coordinator will perform the medical record review of those records that are pertinent (24 hours before and 24 hours after the transfusion of the study unit) to detect hypoxemia.

F12E. Training of record review coordinator: Depending upon the requirements of the hospitals where the record review is to be conducted, the record review coordinator(s) may undergo training similar to the one that honest brokers have to undergo to become certified. The certification protocol to become an honest broker is available from the University of Pittsburgh Medical Center and is attached as Appendix C. Also, depending upon the hospital's specific requirements, a possible alternative to hiring the staff/s at the hospital or at the blood center as record review coordinator/s may include a contracted service to conduct the record review. Additional training specific to the manner by which medical record reviews are to be done will be provided to each coordinator (see below).

F13. Survey to identify interested hospitals, Institution Review Boards (IRB), and the level of difficulties expected in accessing patient records at each REDS-II Center: In July 2007, all five REDS-II Centers were surveyed to identify the number of interested hospitals to assess the feasibility of conducting the study. The Centers were advised to classify the hospitals as definitely interested or tentatively interested. Definitely interested hospitals are either those that expressed after discussion with the local REDS-II investigator or those at which the local investigator/s has the staff privileges that would facilitate the conduct of the study. If the local investigator/s did not have the time to discuss the study with the hospitals, but the local investigators felt that the hospital would be interested, such hospitals were classified as tentatively interested. The Centers were asked to provide the number and percentage of total distribution of FFP and plateletpheresis units to the interested hospitals. The data for July 2004 to June 2007 were requested and are described below in the Table-6.

As seen in the table, the hospital participation rate by the interest category of definite and tentative varied between the Centers. For FFP, for the last three years, interested hospitals represented 60 to 66% of the total distribution of FFP and 61 to 65% of plateletpheresis. . These survey data show that sufficient number of interested hospitals can be identified such that >50% of distributed high plasma volume components can be studied at each REDS-II Center.

Table-6: Survey results of hospitals interested in LAPS-II participation: FFP and Plateletpheresis distributions (Definite interest = D; Tentative Interest = T)

	REDS-II Center	No. of FFP distributed July 06 to June 07 (%)	No. of FFP distributed July 05 to June 06 (%)	No. of FFP distributed July 04 to June 05 (%)	No. of Platelets distributed July 06 to June07 (%)	No. of Platelets distributed July 05 to June06 (%)	No. of Platelets distributed July 04 to June05 (%)
1	SARC-D	0	0	0	0	0	0
2	SARC-T	89,842 (61)	90,920 (62)	100,244 (62)	34,996 (59)	30,622 (58)	30,026 (61)
3	SARC – D+T	89,842 (61)	90,920 (62)	100,244 (62)	34,996 (59)	30,622 (58)	30,026 (61)
4	BCW - D	20344 (43)	20085 (41)	19288 (38)	11592 (69)	11407 (73)	10597 (65)
5	BCW - T	5003 (10)	6628 (14)	8940 (17)	1810 (11)	1430 (9)	1762 (11)
6	BCW – D+T	25347 (53)	26713 (55)	28228 (55)	13402 (80)	12837 (82)	12359 (76)
7	BCP - D	14483 (30)	13519 (29)	16281 (34)	8787 (37)	8984 (38)	8869 (38)
8	BCP - T	7875 (17)	9122 (20)	9982 (21)	2378 (10)	2629 (11)	2744 (12)
9	BCP – D+T	22358 (47)	22641 (49)	26263 (55)	11165 (47)	11613 (49)	11613 (50)
10	ITxM - D	50092 (75)	49502 (73)	52344 (72)	3759 (37)	4112 (36)	5953 (49)
11	ITxM - T	0	0	0	0	0	0
12	ITxM – D+T	50092 (75)	49502 (73)	52344 (72)	3759 (37)	4112 (36)	5953 (49)
13	HUC - D	0	0	0	0	0	0
14	HUC - T	30944 (81)	33068 (81)	43461 (85)	10210 (82)	9200 (83)	8159 (89)
15	HUC – D + T	30944 (81)	33068 (81)	43461 (85)	10210 (82)	9200 (83)	8159 (89)
16	**Average % All Centers D+T**	**63**	**60**	**66**	**61**	**62**	**65**

A follow-up survey conducted in January 2008 sought to more definitively identify the participating hospitals by name, the type of IRB (University or hospital-based), and the level of difficulty expected for access to the hospital's records by the record review coordinators. The

level of difficulty was assigned a score of 1 (easy) to 5 (extremely difficult). The detailed data for each REDS-II Center are presented in Appendix E. Overall, 29 hospitals have been identified by the five REDS-II Centers as the candidate hospitals for participation in the study. They all are either university-based IRB (N=17) and/or hospital-based IRB (N=13). Sixteen hospitals had electronic records and fifteen had paper records (one hospital had both the electronic and paper records). The REDS-II Centers anticipate the following levels of difficulty in accessing the records at the hospitals [level 1 (very easy) to level 5 (very difficult)]: level 1 = 1; level 2 = 15, level 3 = 10, and level 4 = 3 and level 5 = 0.

F14. Hospital process for retrospective data collection: There are several distinct activities for each participating hospital. These include obtaining approval of the study protocol, approval of the designated honest broker or the individual serving as the record review coordinator at the hospital and identifying the recipients who received the study blood components. The record review coordinator will then review radiology records, complete appropriate data forms, identify any adverse reactions that were reported to the blood bank from transfusion of the study blood components, perform the detailed medical record review when indicated and return the completed data forms to the blood center or to the coordinating center. For those cases that are submitted for review by the triage physician and the Expert panel, digital images of chest x-rays will be collected for the study when available.

F15. Project and record review coordinator approval at the hospital: Each participating hospital will have the study and the designated honest broker/record review coordinator approved at the hospital before participation by the hospital can begin. The study approval will be by existing mechanism/s at the hospital. The approval may be from the institutional review board (IRB) at the hospital. In some instances, the hospital may elect to use a centralized IRB approval or approval from another local IRB with which the hospital has previous agreement. In some cases in which the hospital does not have an IRB, existing mechanisms at the hospital will be used to have the study approved. For instance, medical executive committee or a privacy board at the hospitals may carry out such responsibilities. Once an approval is obtained, approval documentation will be forwarded to the medical staff of the hospital's blood bank and to the REDS-II site investigator. The blood bank medical staff will notify the blood center regarding the approval and provide a copy of the written approval to the center. Copies of the approvals will also be sent to the Coordinating Center.

F16. Identification of study recipients, record retrieval, medical records review, and data collection: Hospital blood bank staff will identify the disposition of study blood components. For those components that were issued for transfusion, the blood bank staff will complete a form that indicates that the component was issued for transfusion, name of the patient, the patient's medical record number, blood unit identification number, the date and time the product was issued, type of component and whether there was any adverse event reported within 24 hours after the issue of the product.

The record review coordinator will record information from those patients who had a chest x-ray within 24 hours after the blood component was issued for transfusion. Each center will need to work with their hospitals to find the best way to review radiology records. The radiologist's interpretation of each chest x-ray will be transcribed verbatim on to a data collection form

provided by the Coordinating Center and forwarded to the site investigator. The site investigator will review the radiology reports and determine which patients' charts will require further detailed review. The site investigator review and decision will be documented on to a data collection form. The record review coordinator will request the medical charts from the medical records department. At some hospitals, electronic records may be available and the coordinator may access the electronic records after having been approved to do so. Digital images of chest x-rays will be obtained for cases that need the triage physician and Expert panel reviews when available.

F17. Record review coordinator qualification and training: Qualifications of personnel engaged in record review can include medical technologists; nurses with critical care unit or operating room experience; respiratory care professionals; and other suitable individuals. The designated individuals at each Center will be provided with reading material and will undergo training at Dr. Pearl Toy's program which will involve review of actual cases of TRALI, Acute lung injury (ALI), TACO, and transfusion recipients without these complications. The nurses working with Dr. Toy's prospective study on TRALI who are very familiar with record reviews will provide training. The training will also include writing up case summaries for the Medical Review Board.

F18. Data collection by record review coordinator: The coordinator will perform medical records review and enter patient data in the data collection form (Appendix F & G). The data collection form will contain a unique patient identification number, which will be different from the hospital's medical record number in order to protect the confidentiality of the data. If the Medical Board identifies any TRALI cases, the coordinator will identify the recipient and the hospital where the recipient was treated and will notify the medical director of the blood bank. A crosscheck will be done to find out if the case had previously been reported to the blood bank. If not, the case will then need to be reported by the hospital to the blood center as the blood center will not be capturing this data from the research database. The blood center will perform a serological work up for TRALI using, to the extent possible, a standardized approach described below for donor investigations. For suspected TRALI cases in control patients who were transfused with a component from an HLA antibody negative LAPS donor, the involved donor's repository sample will be tested for HNA antibody to ascertain the presence or absence of HNA antibody; this will also occur for HLA antibody positive donors if they were not already tested for HNA antibody. As per standard procedures for investigation of the transfusion complications, the hospital may share the results of investigation of TRALI cases identified in this study with the REDS-II Center. Subsequently, these results will be available for data analysis. At the end of the study, the record review coordinator will destroy the link between the study ID and the patient's demographic information.

F19. Data entry and centralized database creation: REDS-II Center staff will enter the data from the Screening Form about distribution of the study components into a web based data collection system developed and hosted by the Coordinating Center. Each REDS-II Center will be provided with a user name and password that will allow them access to the system. The Center based data will only include the Blood Unit Identifier and will not include name or other identifying information about the donors. A similar system will be developed and hosted by the Coordinating Center that will permit data entry by the coordinator at each Center. Again, for

patient data, patient's name, hospital name, and medical record number will not be entered into the system. For clinical diagnoses of the study recipients, descriptive diagnoses, including ICD-9 codes will be entered into the Data Forms by the coordinator at each Center. The REDS-II Center's staff will manually record data into the Data Forms and mail them to the Coordinating Center for coding and data entry. The data from the web based system and the Data Forms will eventually be compiled into a single dataset for analysis.

F20. Standardized protocol for donors/recipients testing for cases of TRALI identified in the study: The basic requirement for the protocol would be to test all involved donors for HLA and HNA antibodies as described above for LAPS donors. Depending upon hospital's cooperation for case investigation, recipient testing for HLA and HNA antibody, HLA and HNA phenotyping will be pursued, but not required. . Because it is difficult to coordinate sample acquisitions on donors and recipients simultaneously, crossmatch between donor serum and recipient leukocytes will not be performed.

F21. Medical Board for adjudication (aka Expert Panel): A Medical Board will be created by the Coordinating Center and will be composed of three physicians that will be qualified by training, certification, and/or experience as critical care specialists, pulmonologists, and/or intensivists. The Board will review submitted data without the knowledge of HLA antibody status of the transfused blood components. Two members of the Board will review each case and if there is an agreement regarding diagnosis, the case will be assigned the agreed upon diagnosis. If there is a disagreement between the members, then such cases will be discussed during periodic conference calls of the Board to arrive at an adjudicated diagnosis. These conference calls may be attended by the REDS-II investigators. The Board will be asked to make a determination of the case as: 1. TRALI: classified by whether or not other risk factors for acute lung injury are present or not; 2. Acute lung injury (ALI); 3.Transfusion-related cardiac overload (TACO); 4.TRALI/TACO (overlap cases); and 5.Other (e.g. transient hypoxemia due to transient airway obstruction at bronchoscopy or extubation or other causes of transient hypoxemia). These categories will allow cases to be grouped either by the Canadian Consensus Conference definition or the NHLBI Working Party definition, which differ only slightly. For cases that are assigned a diagnosis of other, the Board may be able to assign a more specific diagnosis such as Febrile Non-Hemolytic Transfusion Reaction (FNHTR), bacterial sepsis, etc. Each member of the Board will record their diagnosis on the Expert Review Log Form (Appendix I) and fax it to the coordinating center.

F22. Deaths identified during record review: If during hypoxemia review or detailed chart review, deaths occurring within 24 hours after completion of the study unit transfusion are identified, they will be recorded in the data collection form along with the reason/cause of death. This will occur whether or not the case goes on to further record review. Such deaths along with deaths occurring within 24 hours of transfusion of the study unit in which TRALI, TACO or other serious adverse transfusion reaction is a possibility will also be recorded in the data collection form.

F23. Expected number of TRALI cases: Using the Finlay data and reasonable assumptions concerning HLA prevalence among donors, then the estimate of TRALI incidence is 2.32% in the case recipient group and 0.79% in the control recipient group. With a sample of 1,295 in each

of the two groups, we expect to observe 30 cases of TRALI in case recipients and 10 cases of TRALI in control recipients.

TRALI cases in the study recipients already previously reported to the blood bank will also be included in calculating the incidence of TRALI if the cases meet all the review requirements of the study.

F24.Coordinating Center process: The coordinating center (Westat) will be responsible for development of the Manual of Procedures, all forms, and for providing a data tracking and a data management system for the study. Westat will also establish the medical review board.

F24A. Tracking of HLA antibody positive and HLA antibody negative study donors: The coordinating center will first access the database created for LAPS-I to identify and list by each REDS-II center, the HLA (class I only, class II only and class I & II) antibody positive donors and their index donation that tested antibody positive. The **Table-7-9** below shows REDS-II Center-specific numbers of HLA antibody positive donors.

Table-7: HLA Class I antibody positive LAPS donors

REDS-II Center	HLA Ab Negative	HLA Ab Positive	Total
BCW	1236	102	1338
BCP	1221	70	1291
SARC	1206	126	1332
Hoxworth	1152	108	1260
ITxM	1344	116	1460
Total	**6159**	**522**	**6681**

Table-8: HLA Class II antibody positive LAPS donors

REDS-II Center	HLA Ab Negative	HLA Ab Positive	Total
BCW	1226	112	1338
BCP	1225	66	1291
SARC	1208	124	1332
Hoxworth	1126	134	1260
ITxM	1310	150	1460
Total	**6095**	**586**	**6681**

Table-9: Any HLA antibody positive LAPS donors

REDS-II Center	Class I & II Ab positive	Class I Ab only positive	Class II Ab only positive	Any HLA Ab positive	Ab negative	Total
BCW	46	56	66	168	1170	1338
BCP	28	42	38	108	1138	1291
SARC	53	73	71	197	1135	1332
Hoxworth	55	53	79	187	1073	1260
ITxM	47	69	103	219	1241	1460
Total	**229**	**293**	**357**	**879**	**5802**	**6681**

HLA antibody positives are those donors whose samples show a normalized background ratio (NBG) of 10.8 or higher in HLA Class I antibody testing and an NBG ratio of 6.9 or higher in HLA class II antibody testing by the Luminex method. These cut-offs represent a mean + 3 standard deviations of a log transformed distribution of values in non-transfused males enrolled in LAPS-I. For purposes of this protocol, LAPS HLA antibody negative donors will be defined as having NBG ratios for both class I and class II antibodies that are less than the mean + 2 standard deviations of the non-transfused LAPS males; this equates to an NBG value of <4.6 for Class I and <3.4 for Class II. A random sample consisting of an equal number of gender and parity frequency-matched antibody negative donors will also be selected for each participating Center.

The Coordinating Center will provide the BUI for each index donation and BUI for each subsequent donation for the study and control donors. Each blood center will then identify all the components made from these donations and their distribution to the hospitals. In addition, the REDS-II Center will identify donations made by the study donor within two years prior to the index donations, components (by component type) made from such donations and the hospitals that received these components. The component distribution data will be entered into the web based screening data forms developed and maintained by the Coordinating Center.

F24B. Other activities at the Coordinating Center: Some blood components made after the index donation may not be subject to recipient record review due to the fact some of the REDS-II participating centers may already be diverting female plasma away from transfusion to the fractionation. The interval prior to the index donation may be extended to get sufficient numbers of components for recipient record review. However, the need to go back further in time for donations is likely to be negligible based on the data on HLA antibody prevalence in LAPS donors and their prior donations.

The coordinating center will review these reports to evaluate the number of plasma rich components that were distributed to the participating hospitals. If the number of components from the antibody positive LAPS donors exceeds the number needed for the study, selection criteria will be developed to choose which components will be subject to record review.

A list of the components that were supplied to each participating hospital will then be printed by the blood center and provided to each corresponding transfusion service medical director or designated individual to identify corresponding recipients and to have the radiology and the medical records be abstracted by the record review coordinator(s). All information will be tracked and compiled into the tracking and data management systems either directly by research staff at the blood center/blood bank or by Westat, if forms are mailed to the coordinating center for data entry.

The coordinating center will be responsible for formation of the medical review board. The medical review board will assess all completed data forms in a blinded manner to determine the presence or absence of TRALI as described above. The coordinating center will compile the results of these reviews into the data management system to allow for formation of a database that will be used for analyses. The proposed diagnostic criteria for TRALI are detailed in the next section.

F25. Proposed Diagnostic criteria for TRALI: Using the Canadian Consensus Conference criteria, the medical review board will be responsible for reviewing the completed recipient forms and determining the presence or absence of TRALI (Kleinman et al).

1. New hypoxemia must be present and hypoxemia is defined as pO2/FiO2 ratio of <300 within 6 hours of completion of transfusion or venous oxygen saturation (SpO2) of less than 90% on room air; or other clinical evidence of hypoxemia (e.g., cyanosis). Based on the recent report from Rice TW et al., additional criteria to determine hypoxemia representative of ARDS will be included as follows: ratio of SpO2/FiO2 <315; where FiO2 will be calculated for patients receiving oxygen by cannula or mask as follows: 1 liter = 0.21; 2 liters = 0.23; 3 liters = 0.25; 4 liters = 0.3; 5 liters = 0.35; 6 and 7 liters = 0.45, and 8,9, and 10 liters = 0.49 (Rice TW 2007).

2. Bilateral infiltrates on frontal chest radiograph (obtained within 24 hours of Completion of transfusion) must be present. There must be absence of left atrial hypertension (i.e., circulatory overload). However, if ALI is diagnosed by the expert panel in the presence of left atrial hypertension, the case may be classified as TRALI/TACO (overlap case).

3. For definition of left atrial hypertension, one or more of the following criteria must be present: EKG evidence for recent MI, echocardiogram indicative of heart failure or an ejection fraction <50%, a plasma troponin value greater than the upper reference range of the laboratory, a plasma brain natriuretic peptide of >100 pg/mL, pulmonary edema fluid/plasma fluid protein ratio less than 0.60, and pulmonary artery diastolic pressure or pulmonary artery wedge pressure of 18 mm Hg or more.

   In addition to the above, clinical criteria for congestive heart failure, e.g., elevated jugular venous pressure, presence of S3 and S4 sound on auscultation, hepatojugular reflux, and excess input of fluid compared to output, and response to diuretic will be considered in order to differentiate TACO from TRALI.

4. No pre-existing acute lung injury before transfusion

5. No temporal relationship to an alternate risk factor for acute lung injury

   Note: Recipients who meet the above criteria but also have a clear relationship to an alternate risk factor for acute lung injury will be considered to have possible TRALI. Also, in accordance with the NHLBI definition, such cases will be classified as TRALI with other risk factors for acute lung injury, provided that the expert panel determines that transfusion is the most likely cause of the ALI. ALI risk factors could be those that cause direct lung injury or those that cause indirect lung injury. Factors that cause direct lung injury are: aspiration, pneumonia, toxic inhalation, lung contusion, and near drowning. Factors that cause indirect lung injury are severe sepsis, shock, multiple trauma, acute pancreatitis, cardiopulmonary bypass, and drug overdose.

6. If the chest x-ray report review indicates absence of bilateral pulmonary infiltrates, the cases will be designated as “Not TRALI”.

F.26. Once a case is classified as TRALI (whether from an antibody positive or antibody negative donor), the recipients of all other components (including red cells) made from donations by the LAPS antibody positive and antibody negative study donors in the approximate three year time frame covered by the lookback study (two years backward and one year forward) will be investigated as described for high plasma volume components. The study projects a total of 40 TRALI cases (30 from HLA antibody positive high plasma volume components and 10 from HLA antibody negative high plasma volume components), of which 70% will come from transfusable plasma and 30% from apheresis platelets. Thus 28 cases will arise from components made from whole blood; assuming each of these donors gave an average of 1.5 donations per year over the 3 year time frame under investigation and assuming that 1.6 components are made from each whole blood donation, we estimate 202 blood compoents (red cells, whole blood-derived platelets, and cryoprecipitate) will require tracing and 101 recipients (assuming 50% of these units went to participating hospitals) would require chart review. In addition, there will be similar investigations for an equal number of control donors (defined as LAPS study donors whose traced components did not cause TRALI, matched to the case donors by gender and parity). Thus, a total of 202 recipients of blood components from associated donors and control donors will be followed for all five participating REDS-II Centers. This number is in addition to the 2,590 study components record review described in Table- 4.

G. Study Design Limitations and Justification

G.1. Limitations of retrospective medical record review: One of the limitations of a retrospective medical record review study is that some clinical findings may not be recorded in a patient’s chart. However, it is highly unlikely that major clinical findings such as severe respiratory distress would not be recorded. Conversely, milder signs and symptoms may not be recorded and it is therefore possible that milder cases of TRALI (e.g., those in which hypoxemia is not severe) as well as milder cases of pulmonary dysfunction that fall short of the diagnosis of TRALI may be missed. However, TRALI experts are unsure if mild forms of TRALI exist. Since certain clinical data are almost always recorded including arterial blood gas analysis results, chest x-ray interpretations, electrocardiogram results, brain natriuretic peptide (BNP) measurements, echocardiogram interpretation, white blood cell counts, and vital signs including temperature, the retrospective study design proposed in this research should allow for comprehensive detection of clinically-significant forms of TRALI.

A large number of blood recipients must be studied to confidently identify a difference in TRALI risk from an antibody positive vs. antibody negative component. To accomplish this practically, a stepwise method for recipient record review is proposed. First, a patient must have had a chest x ray within 24 hours of the time the blood was issued. Second, the radiology report must indicate the presence of new or worsening bilateral lung infiltrates/pulmonary edema for the patient’s record to be reviewed further. Third, hypoxemia must be documented. Fourth, a critical care specialist will review the cases at this point primarily to exclude or triage cases of TACO

prior to forwarding the case to the medical board for review. Since a retrospective data collection study is by design inefficient compared to a prospective study, this stepwise approach described reduces the labor needed for medical record review and yet allows follow up of a large number of recipients.

We have performed a pilot study at one REDS-II Center in Pittsburgh which showed that 30% of patients (weighted by size of institution and transfusion volume) have a chest x-ray within 12 hours. The rate is 42% for a 24-hour interval and 52% for a 48-hour interval. Because the consensus definition of TRALI requires a chest x-ray result, and symptoms of TRALI must occur within 6 hours of the transfusion, we believe that an interval of 24 hours, "the planned interval for this protocol" will balance sensitivity and specificity in identifying TRALI cases. The 24-hour interval has also been employed in recent studies in which findings on chest x-ray taken the next day (Finlay et al.) or within 24 hours of transfusion (Higgins S et al.) were included to arrive at a diagnosis of TRALI. This approach will limit our ability to detect mild cases which do not have a chest x-ray and thus do not meet a definition of TRALI. Those cases which have a chest x-ray done more than 24 hours after the transfusion will also be missed. Despite these limitations, our study design clearly includes collection of data that permit us to make a diagnosis of TRALI according to the generally accepted consensus criteria and therefore, we will not be missing a TRALI diagnosis in any significant number of cases.

Further, to help accurately identify TRALI in our study, we are proposing to create a medical review board. Gajic et al. conducted a retrospective medical chart review and found that 60 patients (of a total of 181) had developed acute lung injury after transfusion (Gajic O et al.). In this study, the inter-observer agreement for the diagnosis of acute lung injury was moderate (kappa 0.6) and the final diagnosis had to be assigned using a consensus mechanism when differences existed. Therefore, for our study, we propose to create a medical review board that will be responsible for reviewing data extracted from medical charts and determining if a given case meets the criteria for a diagnosis of TRALI or not. The board will be blinded as to the recipient group (i.e., the board members will not know whether the recipient received at least one known HLA antibody-positive component, or one known HLA antibody-negative component). The board will be composed of physicians from the subspecialties of critical care, pulmonary medicine and intensive care.

G2. Two Consensus Definitions of TRALI: The Finlay study estimated TRALI risk by including TRALI cases with and without other risk factors for acute lung injury (ALI) based upon diagnostic criteria that were developed by the NHLBI Working Group. Another consensus definition has been developed by the Canadian Consensus Conference Group which classified "TRALI with other risk factors for ALI" as "possible TRALI". For research purposes, we believe that in order to better characterize the incidence of TRALI (and not underestimate it), we should include "TRALI cases with other risk factors for ALI" when estimating our overall TRALI incidence. The identification of all possible TRALI cases would give the health care professional a more accurate picture of the potential for TRALI and will allow better decisions concerning the costs and benefits associated with various preventive steps that may be envisioned. Our data collection form will permit classification by both the Canadian Consensus Conference and the NHLBI working group criteria and will permit a comparison of cases diagnosed by these two slightly different definitions.

G3. Generalizability of Finlay's data to the proposed study setting: Since certain blood components (e.g. Plasma, Apheresis Platelets) are more likely to cause TRALI, it is important to review whether the blood component mix transfused at UCSF Medical Center (47% were RBCs, 34% FFPs, 15% apheresis platelets, 4% others) is similar or not to what could be expected at hospitals associated with the REDS-II centers. We have therefore reviewed published data on blood component utilization for various types of hospitals. The data shown below in Table-10 include the results from the US Department of Health and Human Services (DHHS) which had funded a nationwide blood collection and utilization survey in 2005.

As shown in **Table-10**, the data from 2001 described by Novis et al. show that among small hospitals, platelet concentrates derived from whole blood continue to be used. However, these data are now seven years old and in the intervening period, the use of apheresis platelets has grown and that of platelet concentrates from whole blood has decreased nationally. The DHHS data (**Table-10**) provide evidence for this change in transfusion practice.

Another important aspect of Finlay's data includes the fact that FFP represented 34% of all components transfused. Since plasma volume in FFP is greater than that in red blood cells and in whole blood-derived platelets, the greater use of FFP in Finlay's patients might have contributed to a higher risk for TRALI. We therefore compared the blood component usage in Finlay's patients to other published data.

FFP utilization as a proportion of total blood component transfusion is less in small hospitals (<200 beds) compared to Finlay's data which represents use in a large, university based, tertiary care hospital (Novis et al.). Assessed in another way, the ratio of FFP to RBC unit transfusion at UCSF Medical Center (Finlay et al.) was 1:1.4. For small hospitals, the ratio was 1:7.8 (Novis et al.). An average ratio of 1:3.6 has been reported (Wallis and Dzik).

The above data indicate that the ratio of FFP to RBC transfusion at Finlay's institution is higher than that for the US. Since FFP (including other transfusable plasmas) is more likely to cause TRALI than other types of blood products (e.g., red blood cells), and if more FFP units were transfused in Finlay's study than what would be expected at hospitals enrolling in our study, we may overestimate the number of recipients who may develop TRALI in our study if we base our estimates solely on Finlay's data. Therefore, we have used the recent data from the DHHS survey to calculate the estimated TRALI cases among control and case recipients.

In our estimates for number of patients needed for the study (control vs. case recipients), we assigned different weights of TRALI risk to the plasma rich products compared to the plasma poor products. We derived the numbers of needed study subjects based on incidence per number of units transfused with and without assigning any weights. In assigning a higher risk to the plasma-containing products, we have used the assumption that the risk with FFP (and other transfusable plasmas) and apheresis platelets is five to ten fold greater than the risk with red blood cells. This assumption is supported by recent data from the American Red Cross which show an odds ratio (relative to red cells) in fatal TRALI cases of 12.5 with the plasma-containing products and an odds ratio of 7.9 for apheresis platelets. The assumption is also biologically plausible in that the amount of plasma in the higher risk components is approximately six fold

greater than the amount in the lower risk components; i.e. each unit of FFP contains approximately 220-250 ml of plasma, each apheresis platelet transfusion contains 250-300 mL of plasma and a unit of red blood cells contains approximately 20-40 mL of plasma. Based on these considerations, we have calculated our sample size in different ways as described in the section on statistical considerations.

Table-10: Blood component utilization by type of hospitals

Study/Type of hospitals	RBC transfused	FFP transfused	Apheresis Platelets transfused	Whole blood-derived platelets transfused	Other	Total units transfused
US Dept HHS (DHHS)	13,912,000 (63%)	4,089,000 (19%)	1,389,667* (6%)	1,537,000 (7%)	1,079,000 (5%)	22,006,667
Finlay (major academic)	3258 (47%)	2319(34%)	1003(15%)	0	308(4%)	6888
Novis (small community <200 beds)	365263((73%)	47031(9%)	12829(3%)	75031(15%)	0	500,154

*The survey reported whole-blood derived platelet (WBDP) equivalents. Each apheresis platelet unit was assumed to be six WBDP units.

H. Statistical considerations

H1. Number of donors with and without HLA antibody and their donations/components: HLA antibody positives are those donors whose samples show a normalized background ratio (NBG) of 10.8 or higher in HLA Class I antibody testing and an NBG ratio of 6.9 or higher in HLA class II antibody testing by the Luminex method. These cut-offs represent a mean + 3 standard deviations of a log transformed distribution of values in non-transfused males enrolled in LAPS-I. There are 879 such donors at the five REDS centers which are more than sufficient for this study. If the number of confirmed positive antibody donors exceeds the capacity of the study to perform the record review, a selection procedure will need to be introduced. The number of LAPS donors without antibody will be even higher and will therefore not present a problem for the number needed for the study.

H2. Hospital participation rate: Assuming that the participating hospitals receive about 50% of blood components distributed by the REDS-II centers, a total of 2,590 blood components will be evaluated from antibody-positive donors and 2,590 blood components from antibody-negative donations. The number of components donated by the LAPS donors that would allow the total number of components (2,590 in each group) needed for the study is described in section F8 above.

H3. Assumptions underlying power considerations: We have assumed that about half of the donations are given by male donors and half by female donors with about 17% of the latter having HLA antibodies. We also assumed that 1% of male donors are HLA antibody positive. Thus, we assumed that about 9% of components received by a recipient on average are HLA antibody-positive. These numbers are supported by the recently analyzed data from LAPS-I in which 17.2% of female donors were HLA antibody positive as compared to 1% of male donors. They are further supported by three published studies. First, the study of 324 apheresis female donors showed a prevalence of HLA class I & II antibodies of 16.7% (Densmore 1999). Second, a study of 1416 UK blood donors (males and females) showed a prevalence of HLA class I & II antibodies of 15.0%. Third, a study of 308 blood components from unselected donors showed a prevalence of 22% for HLA class I & II antibodies (Bray et al. 2004). The results from the LAPS study indicate the prevalence will be about 9% in all (males plus females) donors.

We also further assumed that there will be only one study component transfused per patient and that there will not be new onset hypoxemia in any given patient more than once during the transfusion episode involving the study component.

Another assumption for the power calculation is that 80% of the TRALI cases result from leukocyte antibodies. This assumption is supported by the original studies of Dr. Popovsky reported in 1985. More recent data also support this assumption. For instance, antigen-antibody correspondence was demonstrated in 87% (13 of 15) of cases in one recent series (Kopko et al. 2002). An analysis of fatal TRALI cases reported to FDA also has demonstrated the presence of donor leukocyte antibodies in 83% (40 of 48) of the cases. (Holness L et al.).

Based on these assumptions and data from the study by Finlay et al., we estimate

- the probability of a HLA antibody-positive high risk component causing TRALI to be 1.59%
- the probability of a HLA antibody-negative component causing TRALI to be 0.04%
- the probability of a component of unknown HLA antibody status causing TRALI to be 0.18%

The Finlay data suggest a transfusion episode consists of 8 blood components per recipient. The number of subjects needed for the study decreases significantly if the number of blood components transfused per patient is less than 8.0 (i.e. there would be fewer components in the transfusion episode with unknown HLA status; these ‘unknown’ components ‘muddy’ the analysis). In order to determine the number of components per transfusion, data from the Pittsburgh hospitals were collected and analyzed. These data were collected for October 2006 for five hospitals that represent small and large community hospitals and a University Medical Center with trauma and transplant services. Data are shown below in **Table-11**.

Table-11. Number of blood components transfused during a hospital admission among different categories of hospitals located in Pittsburgh area

Hospital Category	# RBC tx'd/yr	# Patients Analyzed (1 month data)	Tot RBC / # per pt	Tot plasma/ #per pt	Tot Plts/ #per patient	Tot components/ # per pt
A. Small community	3500	107	246/2.3	50/0.5	32/0.3	328/3.1
B. Medium Community	7500	240	482/2.0	91/0.4	44/0.2	617/2.6
C. Large community w cancer ctr	11,000	301	868/2.9	633/2.1	1740/5.8	3241/10.8
D. Large community	15,000	404	1175/2.9	621/1.5	580/1.4	2376/5.9
E. (University Trauma/trans-plant)	30,000	676	3066/4.5	1786/2.6	2196/3.2	7048/10.4
Combined data		1728	5837/3.4	3181/1.8	4592/2.7	13610/7.9

Cumulative data from all Pittsburgh hospitals indicate approximately 8 components per patient admission, very similar to Finlay et al. However, this could include multiple transfusion episodes (defined as a gap of six hours between transfusions of any two blood components) during a given patient admission. Therefore, 24 patient records in hospital A and 30 patient records for the University Medical Center were further reviewed. At Hospital A, there were 38 transfusion episodes representing 1.6 episodes per admission. For the University Medical Center, there were 69 episodes representing 2.3 episodes per admission. Thus, using a roughly weighted estimate of 2 episodes per patient appears appropriate. These hospitals use pooled platelets almost exclusively at a standard dose of 5 units per pool. If one were to recalculate the number of transfused platelet components based on the use of single donor apheresis platelets (SDP), the 4592 whole blood platelets would translate to 918 SDPs and the number of total components per patient's admission would be 5.8. Assuming that the REDS sites issue a mix of SDP and pooled platelets and that there are 2 transfusion episodes per patient admission, it is more likely that the actual number of components per transfusion episode will be 3-4 components. In order to be somewhat conservative, we have assumed that six blood components are transfused per episode of plasma transfusion. For plateletpheresis transfusions, we have assumed a one unit transfusion per episode based on reports that the majority of plateletpheresis units are prophylactic transfusions to hematology/oncology patients. (McCullough J et al. and Meehan KR et al.).

In **Table-12** below, the numbers of subjects needed for case and control recipients in view of the above assumptions are presented for different blood donor HLA antibody prevalence rates, different rates of antibody -mediated TRALI and differences in average number of components transfused per episode. We have found in our pilot study of 75 LAPS donors that among high risk components (i.e. transfusable plasmas and apheresis platelets), 30% are apheresis platelets and 70% are plasma components.

Table-12: Required sample sizes for comparing TRALI rate among 'case' recipients and TRALI rate among 'control' recipients for various powers in a one-sided hypothesis under various assumptions.

HLA antibody prevalence in blood donors*	Percent of TRALI that are antibody mediated**	Average # components per transfusion episode***	Number required in each of 'case' and 'control' recipients arms for different power estimates		
			80%	85%	90%
8.5%	80%	6	806	917	1067
9.0%	80%	6	886	1007	1175
10.0%	80%	6	1034	1178	1379

* Results for LAPS show the HLA prevalence to be about 16% for women and about 1% for men, hence overall prevalence of HLA antibodies in units transfused in hospitals participating in LAPS-II is likely to be in the range of 8.5% to 10%.

** Approximately 80% to 85% of TRALI cases are thought to be antibody mediated. The required sample size decreases as the immune mediated percentage increases, thus conservatively 80% is used in the table.

*** Recipients receiving an apheresis component are assumed to receive no other components during the transfusion episode. Recipients receiving an FFP (or other plasmas) component are assumed to receive on average a total of 6 components during the transfusion episode, and as a worst case scenario the additional components are assumed to be high risk TRALI components.

H4. Sample size and power selected for the study: From the above table and the details provided in Appendix D, the sample size selected was 1,175 for the antibody positive components and 1,175 for the antibody negative components. This number will give 90% power to detect a TRALI rate difference between the two groups of recipients. This conservatively assumes that a transfusion episode consists of 6 components although apheresis platelet component transfusion is most often a single unit transfusion episode. The percentage of TRALI that is antibody mediated is assumed to be 80% which may also be a conservative estimate. To the above number, a 10% margin is added to account for missing records etc. Therefore, the total number of antibody positive and antibody negative components is 1,295 for each group.
Based on OSMB recommendations we plan to conduct an interim analysis to see if the estimated sample size is adequate. The interim analysis will assess the average number of components per transfusion episode (assumed to be 6) and the TRALI incidence per component of unknown HLA status (assumed to be 0.18%). If the average is much greater than 6 or the incidence much lower than 0.18%, then increasing the sample size will be considered. A detailed description of the interim analysis is provided in Appendix H.

H5: Pilot study for record review: In collaboration with Dr. Edward Murphy, Dr. Pearl Toy and her co-investigators, and REDS-II Working group for LAPS-II, a pilot study has been conceived and is being conducted as of this writing. The study will assess the feasibility of: (a) whether a non-physician can perform the medical record reviews, (b) the amount of time needed to retrieve the information, (c) the amount of time needed per chart review, and (d) the expert

(medical) panel's ability to diagnose TRALI from the extracted data collected during the chart review.

For the pilot, LAPS-II data collection forms were utilized by a research nurse (pilot nurse) working with Dr. Edward Murphy's group. Dr. Murphy is the Principal Investigator for the REDS-II site of Blood Centers of the Pacific/BSRI. This blood center is one of the five REDS-II Centers that will participate in LAPS-II. The pilot nurse was trained by the nursing staff working with Dr. Toy for the prospective TRALI study at the University of California at San Francisco (UCSF). The pilot nurse was given blood unit identifier (BUI) numbers from 20 plasma or plateletpheresis units transfused to 20 patients with the following diagnosis: 3 patients who were previously diagnosed as TRALI cases; 3 patients who were previously diagnosed as having had Acute Lung Injury (ALI) that did not meet the criteria for a diagnosis of TRALI; 3 patients who were diagnosed as Transfusion-associated cardiac overload (TACO); and 11 cases who did not have ALI, TACO, or TRALI. These cases were selected from the case files of Dr. Toy's current prospective study. The pilot nurse was not informed about the type of cases she was reviewing. As far as was possible, FFP and plateletpheresis products were selected for the pilot.

As a part of the pilot, a time study was performed to assess the time needed to retrieve the information (retrieval time) for completing the data collection form for the blood bank information (e.g., patient demographic, date/time of product issue etc.). Time needed to perform exclusions (including completing the data collection forms) was measured for those cases in which TRALI wasn't a differential diagnosis. Finally, time required for the extended chart review, data collection, and the preparation of a narrative summary for those cases in which TRALI was a differential diagnosis was measured. In Dr. Toy's on-going prospective study, the prospective study nurse prepares several PowerPoint slides to summarize such cases and the summary is then reviewed by the Expert Panel to classify cases as TRALI, not TRALI, ALI, TACO etc. For the pilot, the pilot nurse similarly prepared a summary for the cases in which TRALI was a differential diagnosis. These data are available to share with the record review coordinators for training. The results of the pilot demonstrated that the process to exclude the cases in which TRALI was not in the differential diagnosis was effective.

H6. Study population, enrollment, and human subject research: There are two types of study subjects in this protocol, namely, blood donors and transfusion recipients. Study donors are from the REDS-II supported Leukocyte Antibody Prevalence Study-I (LAPS-I). All these donors have been tested for HLA class I and HLA class II antibodies and some have been tested for neutrophil antibodies per the LAPS-I protocol. All HLA antibody positive donors at the five REDS Centers participating in LAPS-II will be eligible for the current study (and a proportion of HLA antibody negative donors will also be selected as controls). It should be noted that most will not been tested for neutrophil antibodies. Any TRALI cases in recipients will prompt testing of the donor's repository specimen for HNA antibodies (if not already tested). All donations made after the HLA antibody tested donation and donations made by these donors in the previous years from the time of antibody testing may be eligible for the study. Current estimates are that we would perform retrospective review of donations going back for a period of two years.

There is no risk to the study donors that will arise from this proposed research except that very few donors might be deferred from donating blood in the future if found to be implicated in TRALI; the possibility of such deferral is already included in the original LAPS-I informed consent. Also, donors with HLA antibodies but who are not implicated in TRALI may need to be diverted from apheresis platelet donation based on recent AABB recommendations; however, this outcome would result from donor participation in the original LAPS protocol and is not a consequence of this proposed protocol. Donor consent for proceeding with investigation of the recipients of their donations will be waived because such investigation falls within the expected transfusion medicine practice.

The study recipients of blood transfusion will be selected from participating hospitals and are those patients who have already received the study blood components from antibody positive and negative LAPS study donors. Transfusion recipient consent will be waived because: (a) this study involves record review of previously recorded clinical information and therefore poses minimal risk related to loss of confidential health information (b) all data will be managed in a confidential manner, in particular, the centralized database will not contain patient's name or the hospital's medical record number, (c) the access to recipient data will be restricted to authorized individuals, and (d) the study cannot be performed if the recipient consent is required because of the significant number of recipients who may have already expired after the transfusion due to the known high mortality rates after transfusion from their primary disease.

The recipient data will be linked and the linking would allow follow up of those transfusion recipients who are believed to have had TRALI discovered by the current research. For follow up, other blood components transfused within 6 hours of onset of TRALI will be identified and the donors of involved units will be investigated for leukocyte antibodies using a standardized investigation protocol. In case of any deaths identified by current research that are believed to be due to TRALI, the blood bank medical staff of the hospital will be notified for donor investigation and reporting the case to the FDA as per local hospital protocol. The only risk involved for the recipient is the possibility of loss of confidentiality of private health information. Safeguards will be taken to prevent the loss of confidentiality. Additionally, a certificate of confidentiality will be obtained (see below).

H6A. Certificate of confidentiality: A certificate of confidentiality from the National Institute of Health will be obtained. With this certificate, the researchers cannot be forced to disclose information that may identify the donor or the recipients even by a court subpoena, in any federal, state, or local civil, criminal, administrative, legislative, or other proceedings. The researchers will resist any demands for information from personnel of the United States Government that is used for auditing or evaluation of federally funded projects or for information that must be disclosed in order to meet the requirements of the federal Food and Drug Administration (FDA). Researchers do not plan to voluntarily disclose information obtained with this research.

I. Inclusion criteria

For blood donors, the inclusion criteria include those with HLA antibodies and some of the donors who do not have antibodies. Not all donors with HLA antibodies may be needed. Among

donors without HLA antibodies, the selection will be based on gender and parity frequency-matching at each REDS-II Center with the antibody positive donors.

For transfusion recipients, inclusion criteria will be all those recipients who received the study blood components at participating hospitals. Further record review will be conducted on those recipients who had a chest x-ray within 24 hours after the study transfusion.

J. Exclusion criteria

For blood donors from the LAPS, some donors without leukocyte antibodies will be excluded due to the fact that the number of antibody negative LAPS donors exceeds the numbers needed for this study.

K. Informed consent

Consent will be waived for blood donors since recipient investigation on blood donations from routine blood donors is a known part of general transfusion medicine practice. Also, there is no risk to the donors except for potential for loss of confidentiality of private health information. Safeguards will be in place to protect the confidentiality of the information. There will be a small risk that a donor who is not part of LAPS may be deferred from donating blood in the future if the donor is clearly implicated in a case of TRALI or is found to have antibodies upon recall testing. However, this risk is no different than if such a donor was found to be implicated or involved in a TRALI case that occurred outside of this study.

Informed consent from transfusion recipients is also waived because all recipient data will be obtained and maintained in a confidential manner and therefore the risk to the recipients is minimal from the loss of confidential private health information. The Centralized data will be maintained at Westat, the coordinating center for REDS-II research program. Westat has considerable experience in managing centralized database to ensure confidentiality of the donor and recipient data. All resulting publications will not contain recipient names or other personal identification information. The data will be linked to permit investigation of additional donors in those TRALI cases which are identified through the research and which were not previously reported to the blood bank. Potential for loss of confidentiality of private health information exists, but several safeguards will be in place to protect the information.

L. Data analysis

The primary analysis will compare the TRALI incidence among case recipients to the TRALI incidence among control recipients. Fisher's exact test for 2x2 tables will be used. The presumed statistical significance will imply HLA antibody in a transfused blood component is a risk factor for TRALI. Then secondary analysis will estimate;

- the probability of a HLA antibody-positive high risk component causing TRALI
- the probability of a HLA antibody-negative component causing TRALI
- the probability of a component of unknown HLA antibody status causing TRALI
- the proportion of TRALI due to HLA antibodies (i.e. immune-mediated)

Additional analyses will involve estimating TRALI incidences and other incidences. Analysis will be conducted to assess the incidence of TRALI and possible TRALI per unit transfused and per transfusion recipient. For this analysis the number of units transfused per episode per patient will be derived from the data available in the data collection forms. Incidence will also be calculated, when possible, for (a) TRALI without other risk factors and TRALI with other risk factors, (b) among those with other risk factors, incidence will be calculated for ALI which cannot be distinguished from TRALI and for ALI that is not TRALI but that occurred within 6 hours or >6 hours of transfusion.

Analysis of other incidences is described below. In addition to the transfusion complications described above, incidence for transfusion-associated cardiac overload will be based on cases that are classified by the Medical panel as TACO.

Using appropriate statistical methods, associations will be explored between different transfusion complications described above (TRALI, no-TRALI, possible TRALI, ALI of undetermined origin, TACO) and the underlying clinical information, such as, demographics, diagnosis (CPT codes), co-morbid conditions (e.g. renal failure), degree and severity of hypoxemia, chest x-ray and cardiac abnormalities. Data, when available, will be correlated with the presence of HLA and HNA antibodies (signal strength and/or specificity of antibodies) in the recipients as well as the involved donors and their HLA and HNA phenotypes to assess the antibody specificity and the corresponding cognate antigens in donor/recipient pairs among different groups of patients with complications described above. Frequencies of clinical symptoms of transfusion complications will be ascertained. Recipient outcome data, when available, will be tabulated for different types of transfusion complications. Clinical characteristics of the cases that were reported to the blood bank before the study and after the study will be compared.

M. Timeline

The protocol will be submitted to local IRBs (university/hospitals) for review and approval and also to each participating REDS-II Blood Center's IRB for review and approval. For some hospitals, the hospital's Medical Executive Committee or Privacy Board may review/approve the protocol as appropriate. The study protocol submitted to the hospital's IRBs (or Privacy Board) may include deployment of a certified honest broker. Also, record review coordinators for data collection will be subject to local hospital's requirements. Once IRB (Privacy Board) approvals have been received, blood centers can identify blood components needing record review at the hospitals and notify the hospitals. We expect that identification of components by the blood centers and hospital-based record review will be completed by April 2009. Anticipated start date for accruing patient data is October 2008. Data compilation, analysis, and reports are expected to be completed in six months.

Table-13: Time table

Item	Target Date
Finalize protocol & Forms	June 2008
Centers IRB submission and approval	June -July 2008
*Rolling IRB approvals of participating hospitals	June – Sep 2008
Obtain Certificate of Confidentiality	Aug – Sep 2008
Develop SOP	July – Sep 2008
Systems Development	June - Sep 2008
Nurse coordinator training at UCSF	Sep/Oct 2008
Blood center staff training (systems training)	Sep/Oct 2008
Data Collection	Dec 2008 – June 2009
*Perform Interim Analysis	March 2009
Data cleaning and coding	July 2009
Data analysis/reports	Aug - Oct 2009

* We envision obtaining "rolling IRB approvals". Submissions for IRB approval would be made in June 2008. Data collection would start with as many hospitals as we are able to obtain IRB approvals from. Interim analysis would be performed after 1/3 of the data is collected.

N. Budget

LAPS-II Blood Center Budget Guidelines

An itemized budget estimate along with justifications is presented below.

(a) Record review coordinator: $91,000.00

Initial training 60 hours (50% effort for 3 weeks)
Coordinating by phone with each hospital, 30 minutes/day for 5 days per week for 24 weeks = 60 hours
On-site coordination of access to medical records includes waiting to have records pulled, 15 minutes/chart, 518 charts = 130 hours
Screening form completion: 30 minutes/per form X 518 forms = 259 hours
Data entry of the screening forms: 15 minutes/form x 518 forms = 130 hours
Detailed chart review: 3 hours/chart, 62 charts = 186 hours
Data transmission of the detailed forms to the Coordinating Center: 15 minutes/form for 62 forms = 16 hours
Preparation of PowerPoint slides, 1 hours/chart, 62 charts = 62 hours
+ Travel time for six months, one hour each day of the week for 5 days per week for 26 weeks = 130 hours

Subtotal hours = 1,033 hours

Add 40% additional time for unforeseen delays in getting access to medical records and increased numbers needed due to interim analysis= 1,033 X 0.4 = 413 hours

Total hours for record reviewer = 1,033 + 413 = 1,446.

0.7 FTE (based on 2080 hours/FTE).

*Salary = $100,000/FTE X 0.7 FTE = $70,000.00 + 30% fringe = $91,000.00

+ Depending on geographic location of hospitals this could be more or less than one hour/day.
*Salary estimate based on ICU/Critical Care Nurse. It is likely that a Physician's Assistant or Respiratory Therapist with similar experience would have comparable salary requirements.

(b) REDS-II Blood Center Study Coordinator

It is expected that before the record review coordinator starts the review, the Study Coordinator will spend 25% of his/her time getting IRB approvals, training, organizing etc. for 6 months and then 10% for 6 months. However, this cost is already budgeted in the overall Center budget.

(c) Donor services department associate: $4,333.00 (To prepare a list of components distributed to the participating hospitals)

One month effort at $40,000.00/year salary = $3,333.00 + 30% fringe = $4,333.00. This individual will prepare a list of blood components donated by the LAPSI study donors for the index donation, all subsequent donations, and all donations in previous two years. This individual will prepare a list of the components from these that are distributed to the participating hospitals. The goal is to identify 518 components from the antibody positive donors and 518 components from the antibody negative donors. The total number of components to be studied is 518.

Total for donor services department associate = one month effort at $40,000.00/year = $3,333.00 + 30% fringe = $4,333.00

(d) Blood bank labor: $6.00/product = $3,108.00

Blood bank to retrieve the information regarding which recipient received the product and the time of issue, etc. Ten minutes per product. Total products per center = 518 = 518 x 10.0 = 5,180.00 minutes = 86 hours = 86/2080 = 0.04 FTE.

Total blood bank labor = 0.04 FTE at an annual salary of $40,000.00 + 30% fringe rate = $2.080.00 + 50% overhead = $3,120.00.

For 518 products, this comes to $6.00/product.

(e) Medical record/radiology department labor: Total cost =$5,046.00.

Medical record retrieval at $6.00/record, 518 records to retrieve = $6.00 X 518
= $3,108.00.

Radiology record retrieval at $6.00/record, 311 records to retrieve = $6.00 x 311 = $1,866.00

(f) Local travel: $1,212.00

One record review coordinator travel to local hospitals five days a week for 24 weeks. Five travel events per week for 24 weeks = 120. Each travel involves 10 miles one way or 20 miles round trip. Total miles traveled = 120 X 20 = 2,400 miles. Mileage reimbursement is 50.5 cents/mile, total travel reimbursement =2,400 X 0.505 = $1,212.00.

(h) Printing of forms: 550 forms at $1.00/form = $550.00

(j) Project coordinator and record review coordinator training at UCSF

Airfare ($500.00/person)	**$1,000.00**
Hotel two nights ($250.00/night/person)	**$1000.00**
Limousine to/from airport ($50/one way/person)	**$ 200.00**
Incidental ($70/day/person)	**$ 280.00**
Local transport ($10/trip/person)	**$ 80.00**
Total	**$2560.00**

(k) Total cost REDS-II Centers

Record review coordinator:	$ 91,000.00
Donor services associate:	$ 4,333.00
Hospital blood bank labor:	$ 3,108.00
Hospital medical record/radiology dept labor	$ 5,046.00
Local travel	$ 1,212.00
Printing of forms	$ 550.00
Travel for training	$ 2,560.00
Total center cost (each Center)	**$ 107,809.00**
Total cost for five centers	**$ 539,045.00**

(l) Central Laboratory budget

HLA Antibody testing

Salary (10% for 6 months) $2524.00 + $550.00 fringe = $3074.00

LSM12 screening kits (5 kits) at $1000.00/kit = $5000.00

Note: The above budget does not include the Central Coordinating Center's budget and that of the Central Laboratory.

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Appendix A. Summary of incidence rates based on case finding methods

Study Number	Study	Type of study	Risk per units	Risk per patients
Passive Surveillance				
1	Quebec Hemovigilance	Passive surveillance	1:9,306 for whole-blood platelets; 1:25,073 for Cryo; 1:58,279 for red cells; and 1:61,006 for FFP	1:5,952 for pooled platelets, 1:47,619 for red cells recipients
2	SHOT (Kleinman Trans Med Rev 2003)	Passive surveillance	1:74,000 for FFP to 1:557,000 for Cryo	
3	Health Canada(Kleinman Trans Med Rev 2003)	Passive surveillance	1:71,428 for red cells, and 1:8,264 for pooled platelets	
4	Health Canada and CBS/HQ(Kleinman Trans Med Rev 2003)	Passive surveillance	1:200,000 for red cells, and 1:66,667 for pooled platelets	
5	Goldman Quebec (Engelfriet Vox Sang 2001)	Passive surveillance	1:111,111	
Enhanced Surveillance				
6	Popovsky 1985	Enhanced surveillance (blood bank employees performed transfusions)	1:5000	1:625
Single Hospital-based Case Series				
7	Silliman (Canadian Patients; Blood 2003)	Single hospital-based case reports	1:1,120	
8	Wallis 2003	Single hospital-based case reports	1:7,896 for FFP	
9	Clarke 1994*	Single hospital-based case reports	1:312 platelets	
10	Weber 1995	Single hospital-based case reports		1:1,358
11	Silliman (US patients, 1997)	Single hospital-based case reports	1:2,000	
Specialized treatment category				
12	Palfi 2001	Specialized treatment category and/or monitoring (pulse oxymetry)	1:200 for FFP	

13	Yost 2001	Specialized treatment category and/or monitoring (Liver transplant)	6:8 (75%)	
14	Rana (Chest 2005);	Intensive care unit, retrospective medical record review	1:1,271 (definite TRALI); 1:524 (possible TRALI)	1:193 (definite TRALI), 1:79 (Possible TRALI)
15	Gajic 2004	Mechanically ventilated patients, retrospective record review		60:181 (33%)
Lookback				
16	Finlay 2005	Comprehensive retrospective record review	4:6,888 (TRALI without other risk factors), 3:6,888 (TRALI with other risk factors)	4:820 (0.49%, TRALI without risk factors), 3:820 (0.37% TRALI with risk factors for ALI)
17	Kopko 2002	Lookback		15:36 (42%)
18	Nicolle 2004	Lookback		1:18 (5.6%)
19	Toy 2004**	Lookback		1:103
20	Win 2002	Lookback		None of 30 patients
21	Cooling 2002	Lookback		3:20 (15%)

* Some or all of the patients in this series may also be included in the study #7.
** One patient met the criteria for TRALI, but also had diffuse alveolar hemorrhage.

Incidence of TRALI based on passive surveillance: The first five studies in Table-1 report incidence rates determined by passive surveillance done nationally or among a large number of institutions. As can be seen, incidence estimates generated by passive surveillance are usually low (1:8,000 to 1:200,000 units) depending on the study and the component transfused.

Incidence of TRALI based on enhanced surveillance: The pioneering study by Popovsky et al. from the Mayo clinic can be characterized as using enhanced surveillance because transfusions were given by nurses who were employed by the blood bank. The transfusionists were therefore quite familiar with adverse events associated with a transfusion and were probably more likely to recognize such an event, including TRALI. This study provided the incidence data of 1:5,000 transfused units that is widely quoted in the literature. Enhanced surveillance such as the one reported from the Mayo Clinic has not been replicated at other institutions.

Incidence of TRALI based on hospital case reports: Studies 7-11 in Table 1 provide single institution-based incidence rates. In each of these studies, the blood bank received TRALI case reports from the hospital's clinical staff and the number of TRALI cases was correlated with the number of units transfused within the institution. To some degree, these data can also be classified as passive surveillance, but may have some bias that will lead to a higher incidence rate due to an individual investigator's interest in case ascertainment. These reports provide a range of incidence from 1:312 transfused platelet units to 1:7,896 transfused FFP units. Study

#10 in this category observed an incidence of 1:1,358 transfused patients. Compared to the passive surveillance and enhanced surveillance categories, the incidence estimates seem to be somewhat higher when based on single hospital-based case reports. It should be further noted that the studies #7 and #11 reported by Dr. Silliman's group should be considered as incidence of non-immune mediated TRALI. Dr. Silliman's data are not directly applicable to our proposed research because their research measured the incidence of non-immune TRALI and our research is aimed to measure incidence of antibody-mediated TRALI.

Incidence of TRALI based on special patient group and special monitoring: The studies by Palfi et al. and Yost et al. are included for completeness to indicate that the incidence may be higher in certain patient population especially if any extra monitoring is undertaken. One of the two reports pertains to FFP transfusions that were carried out with extra patient monitoring by pulse oxymetry to detect milder forms of hypoxia (Palfi). The second report evaluated liver transplant patients who developed severe pulmonary edema and whose alveolar secretions were tested for protein concentration to show that these secretions were exudates rather than transudates (Yost). However, the incidence data in these two reports are not generalizable because they are very specific for the patient populations that were included in these two reports.

Appendix B: Draft letter to blood bank medical directors

Date: _____
Name of the Medical Director
Name of the hospital
Address
Re: REDS-II TRALI Research lookback study

Dear Dr. _____

Thank you for agreeing to perform the NIH supported LAPS II (REDS-II TRALI retrospective record review) study. Please note that this study was approved at your facility by the IRB/Medical Executive Board/President of the Medical Staff/Privacy Board. I have attached a list of blood numbers for blood components that were supplied to your blood bank from our program. These blood components were obtained from blood donors who were tested for leukocyte antibodies. As part of this study, the attached list of components does not include the antibody status of the donors from whom these components were derived. This step is designed to reduce bias in case ascertainment. I would like to have staff identify all those components that were issued for transfusion, the date of issue, name of the patient, patient's medical record number, and the date and time of issue. Please also identify if your blood bank had received any transfusion reaction reports on any of these patients. Once you have obtained this information for each of these components, please provide the information (including any reports of adverse reactions reported to the blood bank) to the record review coordinator that has been previously been identified and approved at your hospital. The coordinator will perform the record review and record patient information in the attached supplied data collection form. Completed data collection forms (without patient identifying information) will be sent to the REDS-II coordinating center or the blood center that will be responsible for keeping all recipient data. All recipient data submitted to the coordinating center or the blood center will be de-identified to protect the confidentiality of the patient's information. The patient data will be reviewed by the medical board of LAPS II at the REDS-II coordinating center to determine if any of the recipients had TRALI that was not reported to the blood bank. If any cases of TRALI are detected based on this record review process, the coordinating center will notify the record review coordinator of such an event. In turn, the coordinator will subsequently notify you of any such events. For TRALI cases that are identified that were not previously reported to the blood bank, you should follow your procedure for reporting such reactions to your blood supplier so that donor investigation can be performed. For fatal cases of TRALI, please also follow your procedure for reporting the cases to the FDA.

I have enclosed a copy of the protocol for your information. I am much indebted for your cooperation in the above study. Please call me at _____ if you have any questions.

Sincerely yours,

Medical Director, Blood Center

Appendix C

UPMC/UNIVERSITY OF PITTSBURGH MEDICAL CENTER

Policy and Procedure Manual

Policy: HS-RS0002

Index Title: Research

Subject: **Honest Broker Certification Process Related to the De-identification of Health Information for Research and Other Duties/Requirements of an Honest Broker**

Date: **April 14, 2003**

I. POLICY

It is the policy of UPMC/University of Pittsburgh Medical Center (UPMC) to comply with the Health Insurance Portability and Accountability Act (HIPAA) privacy rule pertaining to the use and disclosure of protected health information (PHI) and the de-identification of PHI for Research and any applicable related state laws that are not preempted by HIPAA. The HIPAA Privacy Regulations can be located at 45 CFR Parts 160 & 164 or at http://aspe.hhs.gov/admnsimp/final/PvcTxt01.htm. Terms used herein, but not otherwise defined, shall have the same meaning as those terms in 45 CFR 160.103 § 164.501.

II. BACKGROUND

The Privacy Rule of the Health Insurance Portability and Accountability Act of 1996 (HIPAA) permits protected health information (PHI) to be used without patient authorization in a number of limited cases. One such case is where the PHI is de-identified.

PHI can either be de-identified by an honest broker which is part of the covered entity (as defined by HIPAA) or by an honest broker which is a business associate of the covered entity. An honest broker is an individual, organization or system acting for, or on behalf of, the covered entity to collect and provide health information to research investigators in such a manner whereby it would not be reasonably possible for the investigators or others to identify the corresponding patients-subjects directly or indirectly. The honest broker cannot be one of the investigators. The information provided to the investigators by the honest broker may incorporate linkage codes to permit information collation and/or subsequent inquiries (i.e., a "re-identification code"), however the information linking this re-identification code to the patient's identity must be retained by the honest broker and subsequent inquiries are conducted through the honest broker.

Since neither the Federal Policy nor HIPAA regulations require prior written informed consent/authorization of patients for the research use of their de-identified health information, this approach would address satisfactorily the regulatory requirements associated with the conduct of retrospective research involving existing health information. This approach can also be used to identify eligible patients for subsequent recruitment into clinical trials. For example, based on defined search criteria, the honest broker would provide a de-identified listing of the health information of potential eligible subjects, to include re-identification code numbers, to the clinical trial investigators. The investigators would determine which of these patients appear to meet eligibility criteria and convey the respective re-identification code numbers back to the honest broker. The honest broker would subsequently provide the names of the identified

patients to the patients' personal physicians who would contact the patients to 1) introduce the research study; 2) ascertain their interest in study participation; and 3) instruct the patients to contact directly the investigators or obtain their written authorization to share their interest in study participation with the investigators and to be contacted by the investigators. **Note that direct contact of the patients by the honest broker would constitute "cold-calling", which is prohibited by the IRB.**

HIPAA defines multiple data elements that must be removed from health information in order for the information to be recognized as de-identified. A fully/completely de-identified data set is protected health information which meets the following criteria:

(1) A person with appropriate knowledge of and experience with generally accepted statistical and scientific principles and methods for rendering information not individually identifiable:

(i) Applying such principles and methods, determines that the risk is very small that the information could be used, alone or in combination with other reasonably available information, by an anticipated recipient to identify an individual who is a subject of the information; and

(ii) Documents the methods and results of the analysis that justify such determination; or

(2)…

(i) The following identifiers of the individual or of relatives, employers, or household members of the individual, are removed:

(A) Names;

(B) All geographic subdivisions smaller than a State, including street address, city, county, precinct, zip code, and their equivalent geocodes, except for the initial three digits of a zip code if, according to the current publicly available data from the Bureau of the Census:

(1) The geographic unit formed by combining all zip codes with the same three initial digits contains more than 20,000 people; and

(2) The initial three digits of a zip code for all such geographic units containing 20,000 or fewer people is changed to 000.

(C) All elements of dates (except year) for dates directly related to an individual, including birth date, admission date, discharge date, date of death; and all ages over 89 and all elements of dates (including year) indicative of such age, except that such ages and elements may be aggregated into a single category of age 90 or older;

(D) Telephone numbers;

(E) Fax numbers;

(F) Electronic mail addresses;

(G) Social security numbers;

(H) Medical record numbers;

(I) Health plan beneficiary numbers;

(J) Account numbers;

(K) Certificate/license numbers;

(L) Vehicle identifiers and serial numbers, including license plate numbers;

(M) Device identifiers and serial numbers;

(N) Web Universal Resource Locators (URLs);

(O) Internet Protocol (IP) address numbers;

(P) Biometric identifiers, including finger and voice prints;

(Q) Full face photographic images and any comparable images; and

(R) Any other unique identifying number, characteristic, or code, except as permitted by paragraph (c) of this section; and

(ii) The covered entity does not have actual knowledge that the information could be used alone or in combination with other information to identify an individual who is a subject of the information.

Alternately, HIPAA will permit, without prior patient authorization, the use and disclosure of health information (for research) in the form of a "limited data set". A limited data set may include certain indirect identifiers that are excluded in a fully/ completely de-identified data set. A limited data set is protected health information which excludes the following direct identifiers of the individual, or of relatives, employers, or household members of the individual:

(1) Names;

(2) Postal address information, other than town or city, State, and zip code;

(3) Telephone numbers;

(4) Fax numbers;

(5) Electronic mail addresses;

(6) Social security numbers;

(7) Medical record numbers;

(8) Health plan beneficiary numbers;

(9) Account numbers;

(10) Certificate/license numbers;

(11) Vehicle identifiers and serial numbers, including license plate numbers;

(12) Device identifiers and serial numbers;

(13) Web Universal Resource Locators (URLs);

(14) Internet Protocol (IP) address numbers;

(15) Biometric identifiers, including finger and voice prints; and

(16) Full face photographic images and any comparable images.

If the health information provided to the investigators is based on a limited data set, the investigators must also complete and obtain (IRB and UPMC) approval of a UPMC Data Use Agreement for Limited Data Sets. This Agreement addresses various HIPAA conditions related to subsequent uses and disclosures of limited data sets (see attached).

III. HONEST BROKER CERTIFICATION CRITERIA

For an individual, organization or system to be an Honest Broker for the UPMC, the proposed honest broker must be certified pursuant to the following process:

1. The honest broker must be initially sponsored by investigator(s) who are in good standing with a UPMC-recognized IRB of record AND who intend to use the honest broker's services.

2. The honest broker must submit an application to become a UPMC- and IRB-certified honest broker. The honest broker certification application is available at the University of Pittsburgh IRB web site (www.irb.pitt.edu). The application is to be submitted by the investigator/researcher to the IRB (of record) staff member that is designated to receive these applications. Once the IRB (of record) has approved the honest broker application, the application will then be forwarded to the UPMC Privacy Officer for approval.
3. The UPMC Privacy Officer will evaluate the honest broker application and related documentation to determine that the honest broker has presented satisfactory evidence to meet or exceed the following UPMC certification criteria:
    a. honest brokers must have written documentation of the processes and/or systems that they use to develop both fully de-identified health information data sets and limited data sets, for both electronic and paper-based records;
    b. honest brokers must have written documentation of policies, procedures and controls necessary for:
        i. compliance with the HIPAA Privacy Rule, the Federal Policy regulations for human subject protections (45 CFR 46) and UPMC's Business Associate Agreement;
        ii. security and management of all PHI in the honest broker's possession during the performance of honest broker functions;
        iii. audits and/or quality checks related to determining the efficacy of de-identification mechanisms;
        iv. security and management of re-identification keys; and
        v. documentation/maintenance/retention of all work performed (for whom, what was provided, IRB approval info, etc.).
4. All honest brokers must provide UPMC with a written statement assuring that they will abide by all relevant UPMC and IRB guidelines, policies and procedures, including continuing adherence to the UPMC honest broker certification criteria section of this policy, the duties and other requirements section (see section that follows) and the terms and conditions of the UPMC Business Associate Agreement for honest brokers.

IV. DUTIES AND OTHER REQUIREMENTS OF THE HONEST BROKER

In order for a certified honest broker to work on behalf of investigators to de-identify PHI that is owned/held by UPMC, the honest broker must perform the following UPMC-defined duties and adhere to the following UPMC-defined requirements:

1. All certified honest brokers, both UPMC and non-UPMC, must execute a Business Associate Agreement with UPMC, the terms of which will specify the continuing confidentiality requirements, duties and other expectations UPMC has of an honest broker service. The generic UPMC Business Associate Agreement can be viewed at http://purchasing.upmc.com. The generic Business Associate Agreement will be customized by UPMC to reflect the specific duties and other requirements UPMC specifies for honest broker services.

2. A certified honest broker must ensure that approval of the IRB of record has been obtained for a research study whereby the honest broker receives a request for de-identified PHI (from an investigator that is served by the IRB of record). This process may be as simple as being

copied on an IRB approval letter from the IRB to the investigator. Relative to IRB approval of the proposed research, the honest broker specified in the research application must have been prior certified by the IRB of record in order for the IRB to approve the research application.

3. A certified honest broker must adhere to all of the terms and conditions specified by the IRB of record for any research study for which the honest broker will perform de-identification services.

4. If an investigator requests a limited data set, rather than a fully/completely de-identified PHI data set, in order to be granted access to the UPMC-held PHI, an honest broker must obtain (and retain) evidence of an appropriately executed Data Use Agreement for a Limited Data Set. [Note: the IRB of record may also require evidence of a completed Data Use Agreement for a Limited Data Set as part of its application process for approval of the proposed research involving the use of a limited data set.] This Data Use Agreement will provide evidence of all of the UPMC-required detailed disclosures (honest broker data set specifications) relative to:

    a. where (what UPMC entity) the PHI is located;

    b. what HIPAA-defined limited data set elements are needed for the research;

    c. the purpose of the limited data set request (detailed uses pertinent to the limited data set); and,

    d. who (names, titles, addresses) will access, use and disclose the limited data set information other that the principal investigator.

IV. NON-COMPLIANCE

An employee honest broker's failure to abide by this policy may result in disciplinary action pursuant to UPMC policy HS-HRO704 entitled "Corrective Action and Discharge". Other non-employee work force members may be sanctioned in accordance with applicable UPMC procedures

An honest broker's (business associate) failure to abide by this policy may result in immediate termination of their UPMC certification to serve as an approved honest broker and immediate termination of their business associate agreement with UPMC.

Questions regarding this policy should be directed to the UPMC HIPAA Program Office.

SIGNED: Thomas J. Nigra
Chief Compliance Officer

ORIGINAL: Date:_____

REVIEW MONTH: Date: _____

SPONSOR: Policy Review Committee

SAMPLE AGREEMENT

Attachment A - Honest Broker Certification Process Related to the De-identification of Health Information for Research and Other Duties/Requirements of an Honest Broker

DATA USE AGREEMENT FOR LIMITED DATA SETS

*[INVESTIGATOR - THIS TEMPLATE AGREEMENT IS TO BE FILLED-IN BY THE INVESTIGATOR AND PRESENTED TO THE IRB OF RECORD AS PART OF THE STUDY PROTOCOL **SUBMISSION TO THE IRB**. THE IRB OF RECORD WILL FORWARD A COPY OF THIS AGREEMENT TO A UPMC RESPRESENTATIVE FOR UPMC APPROVAL .THE HONEST BROKER MUST BE PRESENTED WITH A COPY OF THE FULLY-EXECUTED AGREEMENT (TO INCLUDE FINAL APPROVAL FROM UPMC) IN ORDER FOR THE HONEST BROKER TO ACCESS UPMC DATA FOR THE PURPOSE OF THE CORRESPONDING RESEARCH STUDY].*

This Data Use Agreement for Limited Data Sets (the "Agreement") is made this _____ day of _____, 200_ by and between UPMC/University of Pittsburgh Medical Center ("UPMC") and _____("Recipient").

WHEREAS, 45 CFR 164, Subpart E (titled "Standards for Privacy of Individually Identifiable Health Information" and herein referred to as the "HIPAA Privacy Rule") allows UPMC to make available for the purposes of research, public health or health care operations a limited data set to Recipient, provided that Recipient agrees to be bound by the terms of this Agreement; and

WHEREAS, Recipient desires for UPMC to make available the limited data set as described below and agrees to be bound by the terms and conditions of this Agreement; and

WHEREAS, UPMC agrees to make available such limited data set, provided that Recipient agrees to abide by the terms and conditions of this Agreement as well as applicable UPMC policies and IRB requirements.

NOW, THEREFORE, in consideration of the mutual covenants and promises hereinafter set forth, the parties hereto agree as follows:

A. DEFINITIONS

For the purposes of this Agreement, terms used herein shall have the same definition as set forth in the HIPAA Privacy Rule.

B. DATA TO BE PROVIDED BY UPMC

The limited data set provided pursuant to this Agreement contains data acquired from *[INVESTIGATOR - SPECIFY THE UPMC LOCATION AND SOURCE INFORMATION SYSTEM/REPOSITORY*]

_____

and related to [*INVESTIGATOR - IDENTIFY THE SPECIFIC NATURE OF THE DATA AND THE SPECIFIC DATA ELEMENTS BEING REQUESTED.*]

_____

Such data shall be limited to data that is the Minimum Necessary to reasonably accomplish the Authorized Purposes identified in Section (C)(1) of this Agreement.

For the purpose of this Agreement and consistent with the HIPAA Privacy Rule, "Minimum Necessary" is defined as that protected health information that is *"reasonably necessary to achieve the purpose of the disclosure"* and is disclosed to only *"Those persons or classes of persons, as appropriate, in its workforce who need access to protected health information to carry out their duties."*

Consistent with the HIPAA Privacy Rule, in no case will the limited data set include any of the following identifiers:

1. Names
2. Postal address information (other than town or city, state and zip code)
3. Telephone numbers
4. Fax numbers
5. E-mail addresses
6. Social security numbers
7. Medical record numbers
8. Health plan beneficiary numbers
9. Account numbers
10. Certificate/license numbers
11. Vehicle identifiers & serial numbers, including license plate numbers
12. Device identifiers & serial numbers
13. Web Universal Resource Locators (URL's)
14. Internet Protocol (IP) address numbers
15. Biometric identifiers, including finger and voice prints
16. Full face photographic images and any comparable images

C. PERMITTED USES AND DISCLOSURES

1. Recipient agrees to limit the use and disclosure of the limited data set to the following purposes ("Authorized Purposes"): [*INVESTIGATOR* - SPECIFY *THE GENERAL PURPOSE(S) OF THE PROPOSED RESEARCH*.]

   _____

2. The Recipient shall allow only the following individuals access to the limited data set for the Authorized Purposes and consistent with the assurances and obligations set forth in this Agreement: [*INVESTIGATOR - ADD LIST OF AUTHORIZED INDIVIDUALS WHO WILL HAVE ACCESS TO THE LIMITED DATA SET*].

   _____

3. Recipient acknowledges that such individuals have a need to access the limited data set to carry out their duties.

D. ASSURANCES

1. Recipient shall not use or further disclose the limited data set other than as permitted by this Agreement or as otherwise required by law.

2. Recipient shall use appropriate safeguards to prevent use or disclosure of the limited data set other than as permitted by this Agreement.

3. Recipient shall report to the UPMC Privacy Officer any use or disclosure of the limited data set not provided for by this Agreement of which Recipient becomes aware.

4. Recipient shall ensure that any specified agents (see C.2., above), including a subcontractor, to whom it provides the limited data set agrees to the same restrictions and conditions that apply to the limited data set Recipient with respect to such information.

5. Recipient shall not re-identify the information or contact the individuals for whose records are contained within the limited data set.

E. BREACH AND TERMINATION

1. In the event that this Agreement is breached by Recipient, UPMC, at its sole discretion, may a) terminate this Agreement upon written notice to Recipient or b) request that Recipient, to the satisfaction of UPMC, take appropriate steps to cure such breach. If Recipient fails to cure such breach to the satisfaction of UPMC or in the time prescribed by UPMC, UPMC may terminate this Agreement upon written notice to Recipient.

2. Should this Agreement be terminated for any reason, including, but not limited to Recipient's decision to cease use of the limited data set data, Recipient agrees to

destroy or return all limited data set data provided pursuant to this Agreement (including copies or derivative versions thereof).

F. MISCELLANEOUS

1. Notices

Any notice permitted or required as provided for herein shall be in writing and to the contact and address as noted below or as may be provided by either party to the other in writing from time to time.

Notice to UPMC shall be to:

UPMC Corporate Compliance Office
Attn: Data Use Agreement Management
3600 Forbes Ave-Rear Entrance
Forbes Tower, Suite 7015
Pittsburgh, PA 15213

Notice to Recipient shall be to:

Name: _____

Address: _____

2. Governing Law

This Agreement shall be governed by, and construed in accordance with, the laws of the Commonwealth of Pennsylvania.

UPMC/University of Pittsburgh Medical Center **Recipient**

Name (print): _____ Name (print): _____

Title: _____ Title: _____

Signature: _____ Signature: _____

IRB Approval:

Name (print): _____ Title: _____

Signature: ________________________________ IRB#:_________________________________

Appendix D

Assumptions and calculations made to estimate the TRALI rate in cases and controls

Using the Finlay data and some acceptable assumptions concerning HLA prevalence among donors, then the estimate of TRALI incidence is 2.32% in the case recipient group and 0.79% in the control recipient group. A sample of 1,175 in each of the two groups will have 90% power to detect a difference in TRALI rates.

The Finlay paper gives us an estimate that 7/820 recipients will develop TRALI. However, for calculation purposes we want an estimate of the TRALI rate per component, instead of per recipient. These 820 recipients received 6,888 components, for an average of 8.4 components per recipient. Thus, another estimate is that 7/6888 components will result in TRALI.

Preliminary results from LAPS-I show about 16% of female donors are HLA positive, 1% of male donors are HLA positive, and that donors are roughly 50% female. Then we can estimate that 9% of donations are HLA positive. Components are derived from donations with no predilection towards HLA status, thus we can also then estimate that 9% of components are HLA positive.

Next, we assume most TRALI reactions are a result of an HLA positive component (literature suggests 80% of TRALI reactions implicate an HLA positive donation).

The Finlay component data consists of 51% low risk components and 49% high risk components. High risk components are assumed to be six times greater risk of causing TRALI. The overall TRALI risk of 7/6888 components can be translated into an estimate that there is a 0.18% probability of a TRALI incident per high risk component. An interim analysis is proposed to estimate this probability. If the probability is smaller than 0.18%, then increasing the sample size will be considered (see Appendix H).

Further, using the estimates that 9% of components are HLA positive and that 80% of TRALI incidents are immune mediated, then TRALI risk estimates conditional on HLA status of the component can be derived. If the HLA status of the component is positive, then the probability a high risk component causes a TRALI reaction is 1.59%. If the HLA status of the component is negative, then the probability a high risk component causes a TRALI reaction is 0.04%.

For each HLA positive donor in the study, we consider all of this donor's components in the study period, and enroll recipients of these components as case subjects. In our case recipient group, we will know that one component is HLA positive and that any other components (the number of other components can vary for each recipient) the recipient receives have unknown HLA status. For each HLA negative donor in the study, we consider all of this donor's components in the study period, and enroll recipients of these components as control subjects. In our control group, we will know that one component is HLA negative and that any other components the recipient receives have unknown HLA status. Assume the number of components a recipient receives can be anything from 1 to 11, and averages 6. (Of minor note, the case and control groups will have a higher mean number of components than the overall recipient mean. The reason is that recipients with greater number of components have a higher chance of selection into the study, i.e. since we select components and find the recipient that

received that component, then a recipient with 11 components has 11 chances to be selected, while the recipient with a single component has just 1 chance to be selected).

Using the estimates of TRALI incidence per component (conditional on whether a component is HLA positive, HLA negative, or unknown HLA status), we can estimate the TRALI incidence per case recipient and per control recipient by first calculating the probability of not developing TRALI when receiving on average of 6 components (range 1-11), and then taking the complement of that probability to estimate the probability of the recipient developing TRALI after receiving these components. As an example, a case recipient who receives one HLA antibody-positive high risk component and two high risk components whose HLA status is unknown would have a probability of not developing TRALI equal to (0.0159) x (0.0018) x (0.0018) and a probability of developing TRALI equal to 1-{(0.01.59) x (0.0018) x (0.0018)}. Using this method, we estimated that the TRALI rates would be about 2.32% for case recipients and 0.79% for control recipients.

The estimate of 0.79% for control recipients is close to the Finlay estimate (0.85%=7/820) as expected. The Finlay estimate is the TRALI incidence for recipients transfused with components of unknown HLA status. Knowing that one of the transfused components is HLA negative lowers the risk slightly, while the greater average number of components in the control group raises the risk slightly, for a net negligible change in the risk. The estimate of 2.32% in case recipients is less than the composite estimate for other lookback studies (2.7%=5/184). This too is expected, as the other lookback studies only considered components from donors who had been 'implicated' in a TRALI incident.

If the average number of transfused components per recipient were 4 rather than 6, then the TRALI rate estimates in the case recipient group and control recipients groups are lowered to 2.0% per case recipient and 0.5% per control recipient. The statistical power of the study increases as average number of components decreases. Conversely, the statistical power of the study decreases as the average number of components increases. If the interim analysis finds the average to be larger than 6, then increasing the sample size will be considered (see Appendix H). A one-sided 0.05 level test with samples of 1,175 in each of the two recipient groups will have 90% power to statistically detect a greater TRALI incidence among case recipients if 6 components are transfused (statistical power computed using StatXact for an exact conditional test comparing two binomial proportions).

Appendix E: Expected participating hospitals for LAPS-II: IRB, type of medical records, and difficulty in accessing records

No.	Participating Hospital Name	Type of IRB (check one)			Patient records (Check one)		On a scale of 1-5 how easy is it to obtain access to medical records?				
							Very easy				Very difficult
		Hosp	Univ	Other	Electro-nic	Paper	1	2	3	4	5
Hoxworth Blood Center											
1	University Hospital		x		x		x				
2	Christ Hospital	x			x			x			
3	Jewish Hospital	x			x			x			
4	Good Samaritan Hospital	x			x			x			
The Institute for Transfusion Medicine											
5	UPMC Presbyterian		x		x			x			
6	UPMC Shadyside		x		x			x			
7	Children's Hospital of Pittsburgh of UPMC		x			x			x		
8	UPMC Passavant		x			x			x		
9	UPMC St. Margaret		x		x				x		
10	Magee-Women's Hospital of UPMC		x		x				x		
11	UPMC McKeesport		x			x			x		
12	Allegheny General Hospital	x				x		x			
13	The Western Pennsylvania Hospital	x				x		x			
14	The Western Pennsylvania Hospital Forbes Regional Campus	x				x			x		
Blood Centers of the Pacific											
15	SF General	x				x				x	
16	UCSF	x			x				x		
Blood Center of Wisconsin											
17	St. Mary's Milwaukee	x				x				x	
18	Children's Hospital of Wisconsin	x			x				X (limit 25/day)		
19	Aurora-Sinai	x*			x	x**		x			
20	Aurora-West Allis	x*				x**		x			
21	Aurora-South Shore	x*				x**		x			
22	Aurora-St. Lukes	x*				x**		x			
23	Aurora-Two Rivers	x*				x**		x			

24	St. Joseph's-Marshfield				x	x			x		
25	Froedert/MCW		x		x			x			
Emory/SARC											
26	Emory University Hospital		X		x			x			
27	Emory Crawford Long Hospital		X		x			x			
28	Children's Healthcare of Atlant	x	X		x				x		
29	Grady Memorial Hospital	x	X			x				x	

*One IRB for all the sites

**Older records are likely to be microfilmed but available

Appendix F: Screening Form

LEUKOCYTE ANTIBODIES PREVALENCE STUDY – PHASE II
SCREENING FORM

STUDY ID LABEL

SECTION A: HOSPITAL Tx SERVICE FORM [completed by hospital Tx service staff]

DATE:		**COMPLETED BY:**					
BUI #:		**TYPE OF COMP:**		**Tx UNIT #:**			
DATE OF ISSUE:		**TIME OF ISSUE:** (Military Time)					
HOSPITAL:							
SERVICE ISSUED TO (Record if information is available. See Note 1)**:** (Med, Surg, Ob/Gyn, Heme/Onc, OutPt, OR, ICU, Recovery, etc.)							
PATIENT NAME: (Last, First, MI)			**MED REC #:**				
BIRTHDATE:		**GENDER:**	**MALE** ☐	**FEMALE** ☐			
PATIENT AB0/RH: (Check one)	**O+** ☐	**O-** ☐	**A+** ☐	**A-** ☐	**B+** ☐	**B-** ☐	**AB+** ☐	**AB-** ☐
Tx REACTION:	**YES** ☐ **NO** ☐						
TYPE OF REACTION (Describe)**:**							

SECTION B: HOSPITAL FORM [completed by Med Tech/ Coordinator]

DATE:		**ABSTRACTED BY:**	
SOURCE OF INFORMATION:			
ELECTRONIC RADIOLOGY REPORTS DATABASE	☐		
ELECTRONIC MEDICAL RECORDS INCLUDING RADIOLOGY REPORTS	☐		
PAPER MEDICAL RECORDS	☐		
OTHER (SPECIFY)			
		DATE	**TIME** (Military Time. See Note 2.)
PRE Tx CHEST X-RAY (If yes, record date & time of up to 3 X-rays)	**YES** ☐ **NO** ☐		
POST Tx CHEST X-RAY (If yes, record date & time of up to 3 X-rays)	**YES** ☐ **NO** ☐		

DISCHARGE DIAGNOSIS (If electronically available)**:** List up to 10 diagnosis Assign ICD-codes	**DESCRIPTIVE DIAGNOSIS**	**ICD CODE**
1.		
2.		
3.		
4.		
5.		
6.		
7.		
8.		
9.		
10.		

If NO post transfusion chest X-ray within 24 hrs of issue of blood product then end now

PRE TRANSFUSION CHEST X-RAY REPORT: (Please write complete report. If more than one, include all reports.)

POST TRANSFUSION CHEST X-RAY REPORT: (Please write complete report. If more than one, include all reports)

SECTION C: PI REVIEW FORM [completed by PI or designee nurse/MD]

DATE OF REVIEW:		**REVIEWED BY:**	
EXTENDED CHART REVIEW:		**YES** ☐ **NO** ☐	
BILATERAL LUNG INFILTRATES **YES** ☐ **NO** ☐	**PULMONARY EDEMA** **YES** ☐ **NO** ☐	**ARDS** **YES** ☐ **NO** ☐	
WORSENING PULMONARY EDEMA **YES** ☐ **NO** ☐		**NEW PULMONARY EDEMA** **YES** ☐ **NO** ☐	
PI RECCOMMENDATION: Proceed with chart review?		**YES** ☐ **NO** ☐	
COMMENTS:			

If NO extended chart review required then end now

ENTER FORM DATA IN SMS. DO NOT ENTER DATA SHADED IN YELLOW.

NOTES:

1. Service to which the product was issued from the blood bank may not always be available. For those products issued to the operating room (OR) or the recovery room, note that the issue time may differ significantly from the transfusion time since these locations may have temporary storage facility.
2. Record the date and time which is nearest to the time of study unit transfusion if there is more than one chest X-ray taken within 24 hrs prior and 24 hrs after the study unit transfusion

STUDY ID LABEL

Appendix G: Extended Data Collection Form

LEUKOCYTE ANTIBODIES PREVALENCE STUDY – PHASE II
EXTENDED DATA COLLECTION FORM

Please refer to clinical information from daily chart notes, laboratory test results, radiology reports, echocardiogram reports, discharge summary, nursing notes, blood bank issue and transfusion forms and other chart records to complete this form.

SECTION A: GENERAL INFORMATION

DATE:		**ABSTRACTED BY:**	
SOURCE OF INFORMATION: (Check all that apply)			
ELECTRONIC RADIOLOGY REPORTS DATABASE	☐		
ELECTRONIC MEDICAL RECORDS INCLUDING RADIOLOGY REPORTS	☐		
PAPER MEDICAL RECORDS	☐		
OTHER (SPECIFY)			
BUI #			
IS THERE DOCUMENTED EVIDENCE THAT INDEX BLOOD PRODUCT WAS NOT TRANSFUSED?	**YES** ☐ Stop data collection	**NO** ☐ Continue	

SECTION B: ADMISSION & DISCHARGE

		DATE	**TIME** (Military)
HOSPITAL	**ADMISSION**		
	DISCHARGE		(when available)
ICU	**ADMISSION**		
	DISCHARGE		
DISCHARGE STATUS	**ALIVE** ☐ **DECEASED** ☐		

DISCHARGE DIAGNOSIS List up to 10 diagnosis and assign ICD codes. Collect only if NOT previously entered in Screening Form	**DESCRIPTIVE DIAGNOSIS**	**ICD-9 CODE**
1.		
2.		
3.		
4.		
5.		
6.		
7.		
8.		
9.		
10.		

SECTION C: PATIENT DEMOGRAPHICS

AGE				**GENDER**	**MALE** ☐	**FEMALE** ☐	
WEIGHT		**UNIT**		**HEIGHT**		**UNIT**	
RACE/ETH	**W** ☐	**B** ☐	**H** ☐	**A** ☐	**Other** ☐		

SECTION D: TRANSFUSION ADMINISTRATION

DATE OF Tx:		**TIME OF Tx:**		**AM** ☐	**PM** ☐

List BUI numbers of all involved products and their ABO/Rh type
(within 6 hours prior to the onset of reaction)**:**

BUI #	**ABO/Rh**	**BUI #**	**ABO/Rh**	**BUI #**	**ABO/Rh**

VITAL SIGNS (See Note 1)	**BP** (mm Hg)	**PULSE/MIN**	**RESP/MIN**	**TEMP** (degree F)
BEFORE Tx:				
½ HR DURING:				
1 HR DURING:				
2 HR DURING:				
AT COMPLETION OF Tx:				

TX REACTION: (Within 6 hrs of completion of unit)	**YES** ☐ **NO** ☐	**REPORTED TO BLOOD BANK:**	**YES** ☐ **NO** ☐
TYPE OF REACTION: (Describe) See Note 2			

SECTION E: HYPOXEMIA EXCLUSION CRITERIA

Concentrate the chart review process around the time window of 24 hours prior and 24 hours after transfusion of study unit. Please complete respiratory status within **six hours** of completion of the transfusion.

Estimate FiO2 as follows, if patient is receiving oxygen by nasal cannula or mask.

Room air: **0.21**	1 Liter of O2: **0.23**	2 Liters of O2: **0.25**	3 Liters of O2: **0.27**
4 Liters of O2: **0.30**	5 Liters of O2: **0.35**	6, 7 Liters of O2: **0.40**	8, 9, 10 Liters of O2: **0.49**

If receiving oxygen therapy with mechanical ventilation, use percent oxygen recorded on the patient's respiratory care data. Review ventilator data to find FiO2 value (eg. 405 oxygen administration equals a FiO2 of 0.4).

RESPIRATORY STATUS	YES	NO	NR
Oxygen saturation (SpO2) less than 90% by pulse oxymetry without oxygen therapy?	☐	☐	☐
Oxygen saturation (SpO2) less than 90% by pulse oxymetry while receiving oxygen therapy	☐	☐	☐
Ratio of SpO2/FiO2 <315 (See Note 1)	☐	☐	☐
Ratio of PaO2/FiO2 <300 (PaO2 = oxygen saturation on arterial blood gas analysis) (See Note 2)	☐	☐	☐

If answer to **ANY** of the above respiratory status items is **YES** then further chart review is required. If answer to **ALL** of the above respiratory status items is **NO** then stop now and send copy of form to Westat.

SECTION F: PATIENT HISTORY & CLINICAL FINDINGS

NO.	ITEM	YES	NO	NR	START DATE (If applicable)	RESOLVE DATE (If applicable)	NOTES
1	Acute CNS Injury (within 7 days before Tx)	☐	☐	☐			Note 3
2	Acute renal failure (within 7 days before Tx)		☐	☐			Note 4
3	Aspiration	☐	☐	☐			Note 5
4	Bone marrow stem cell transplant in past 12 months		☐	☐			
5	Burn as an admitting diagnosis (if within 48 hrs before reaction)	☐	☐	☐			
6	Cancer or recent (within 1 month) chemotherapy		☐	☐			
7	Cardiac bypass during current admission (within 48 hrs before reaction)	☐	☐	☐			
8	Cardiac ischemia during current admission (within 7 days before Tx)		☐	☐			
9	Hx of chronic alcohol abuse	☐	☐	☐			Note 6
10	Hx of chronic renal failure		☐	☐			Note 7
11	Congestive heart failure during current admission (within 7 days before Tx)	☐	☐	☐			Note 8
12	Hx of coronary artery disease		☐	☐			
13	Hx of diabetes mellitus	☐	☐	☐			Note 9
14	DIC during current admission (within 7 days before Tx)		☐	☐			Note 10
15	Drug overdose as admitting diagnosis (within 48 hrs before reaction)	☐	☐	☐			
16	Exposure to high altitude as admitting diagnosis (within 48 hrs before reaction)		☐	☐			
17	Heat stroke as admitting diagnosis (within 48 hrs before reaction)	☐	☐	☐			
18	Hemorrhagic shock during current admission (within 48 hrs before reaction)		☐	☐			
19	High INR (>3.0) during current admission (within 7 days before Tx)	☐	☐	☐			
20	Hx of immunosuppression		☐	☐			
21	Just underwent surgery (within 48 hours)	☐	☐	☐			

NO.	ITEM	YES	NO	NR	NOTES
22	Hx of leukemia or lymphoma	☐	☐	☐	
23	Lung contusion during current admission (if diagnosis made within 48 hrs before reaction)		☐	☐	Note 11
24	Lung radiation in previous six months	☐	☐	☐	
25	Multiple fractures during current admission (Within 7 days before Tx)		☐	☐	
26	Near drowning as reason for admission (within 48 hrs before reaction)	☐	☐	☐	
27	Hx of chronic obstructive lung disease		☐	☐	
28	Acute pancreatitis during current admission (within 7 days before Tx)	☐	☐	☐	Note 12
29	Rapid resolution of lung infiltrates on chest x-ray		☐	☐	If within 6 hrs with diuresis/ dialysis
30	Receiving amiodarone during current admission (within 48 hrs before reaction)	☐	☐	☐	
31	Hx of restrictive lung disease		☐	☐	
32	Hx of severe liver disease	☐	☐	☐	Note 13
33	Symptomatic anemia during current admission (within 48 hrs before reaction)		☐	☐	Note 14
34	Upper airway obstruction during current admission (within 48 hrs before reaction)	☐	☐	☐	

SECTION G: OTHER CLINICAL FINDINGS

NO.	ITEM	YES	NO	NR	VALUE	DATE	TIME (military)	NOTES
1	Dialysis within 7 days before the study unit Tx (Hemo or Peritoneal)	☐	☐	☐				
2	Dialysis within 7 days after the study unit Tx (Hemo or Peritoneal)	☐	☐	☐				
3	Extubation (within 24 hrs before reaction)	☐	☐	☐				Note 15
4	FiO2 (highest value) in 24 hrs before Tx of study unit	☐	☐	☐				
5	FiO2 (highest value) in 24 hrs after Tx of study unit	☐	☐	☐				
6	Fluid balance – Excess fluid 24 hrs before Tx of study unit (mL)	☐	☐	☐				Note 16
7	Intubation within 24 hrs before Tx of study unit	☐	☐	☐				
8	Intubation within 24 hrs after Tx of study unit	☐	☐	☐				

9	Invasive procedure: Central line placement within 24 hrs before Tx.	☐	☐	☐				

NO.	ITEM	YES	NO	NR	VALUE	DATE	TIME (military)	NOTES
10	Invasive procedure: Liver biopsy	☐	☐	☐				
11	Invasive procedure: Lumbar puncture (within 24 hrs before Tx.)	☐	☐	☐				
12	Invasive procedure: Other (within 24 hrs before Tx.) Specify	☐	☐	☐				
13	Mechanical ventilation within 24 hrs before study unit Tx	☐	☐	☐				
14	List all medications given to the patient within 24 hrs prior to transfusion:							

NO.	ITEM	PROCEDURE		DATE	TIME (military)	NOTES
15	Oxygen administration method within 24 hrs <u>before</u> Tx of study unit	Nasal Cannula	☐			
		Face Mask	☐			
		CPAP	☐			
		Intubation	☐			
		Other	☐			
16	Oxygen administration method within 24 hrs <u>after</u> Tx of study unit	Nasal Cannula	☐			
		Face Mask	☐			
		CPAP	☐			
		Intubation	☐			
		Other	☐			

SECTION H: PRE-TRANSFUSION EVENTS

NO.	ITEM	YES	NO	NR	START DATE (If applicable)	RESOLVE DATE (If applicable)	NOTES
1	Pre-transfusion fever within 24 hrs before Tx (temp above 100.4F or 38C)	☐	☐	☐			
2	Pre-transfusion chills without fever within 24 hrs before Tx		☐	☐			
3	Pre-transfusion bacterial sepsis within 24 hrs before Tx	☐	☐	☐			Note 17
4	Pre-transfusion shortness of breath/dyspnea within 24 hrs before study unit Tx		☐	☐			
5	Pre-transfusion hypotension (systolic BP <90 mmHg) within 24 hrs before study unit Tx	☐	☐	☐			
6	Pre-transfusion hypertension (systolic BP >140 mmHg) within 24 hrs before study unit Tx		☐	☐			
7	Pre-transfusion shock within 24 hrs before study unit Tx	☐	☐	☐			Note 18
8	Pre-transfusion O2 sat <90% on pulse oxymetry (SpO2) with or without O2 administration within 6 hrs before Tx		☐	☐			
9	Ratio of SpO2/FiO2 <315 within 6 hrs prior to Tx	☐	☐	☐			Note 19
10	Pre-transfusion arterial blood gases within 6 hours prior to tx showing pO2/FiO2 <300 mm Hg		☐	☐			Note 20
11	Pre-transfusion lung auscultation reveals rales(crackles) within 24 hrs before study unit Tx	☐	☐	☐			
12	Pre-transfusion lung auscultation reveals rhonchi within 24 hrs before study unit Tx		☐	☐			
13	Pre-transfusion elevated jugular venous pressure within 24 hrs before study unit Tx	☐	☐	☐			
14	Pre-transfusion hepatojugular reflux detected within 24 hrs before study unit Tx		☐	☐			
15	Pre-transfusion cyanosis	☐	☐	☐			
16	Pre-transfusion central venous catheter measured elevated central venous pressure (>8 mm Hg) within 6 hrs before Tx		☐	☐			
17	Pre-transfusion central catheter measured elevated pulmonary artery wedge pressure (>20 mm Hg) within 6 hrs before Tx	☐	☐	☐			
18	Pre-transfusion peripheral pitting edema detected within 24 hrs before study unit Tx		☐	☐			
19	Pre-transfusion diagnosis with ARDS within 24 hrs before study unit Tx	☐	☐	☐			From MR or chest X-ray report
20	Pre-transfusion, patient required ICU admission within 24 hrs before study unit Tx		☐	☐			
21	Pre-transfusion, required vasopressures to support blood pressure within 24 hrs before study unit Tx (e.g., dopamine, norepinephrine, epinephrine)	☐	☐	☐			

SECTION I: VITAL SIGNS AFTER TRANSFUSION REACTION

Record 3 vital signs that were 6 -8 hrs apart. Leave blank if no transfusion reaction is noted in the medical record or reported to the blood bank.

VITAL SIGNS AFTER TRANSFUSION REACTION: (Note 21)						
NO.	DATE	TIME (military)	BP (mmHg)	PULSE/MIN	RESP/MIN	TEMP (degree F)
1						
2						
3						

SECTION J: POST TRANSFUSION EVENTS

NO.	ITEM	YES	NO	NR	NOTES
1	Post-transfusion fever ((temp above 100.4F or 38C) within 6 hrs	☐	☐	☐	
2	Post-transfusion chills within 6 hrs without fever	☐	☐	☐	
3	Acute hemolytic transfusion reactions within 24 hrs	☐	☐	☐	
4	Delayed hemolytic transfusion reaction within 7 days	☐	☐	☐	
5	Minor allergic (skin rash) within 6 hrs	☐	☐	☐	
6	Severe allergic (anaphylactic) within 6 hrs	☐	☐	☐	
7	Post-transfusion bacterial sepsis within 24 hrs	☐	☐	☐	
8	Cardiac/fluid overload or congestive heart failure within 6 hrs	☐	☐	☐	
9	Post-transfusion shortness of breath/dyspnea within 6 hrs	☐	☐	☐	
10	Post-transfusion hypotension (systolic BP <90 mmHg) within 6 hrs	☐	☐	☐	
11	Post-transfusion hypertension (systolic BP >140 mmHg) within 6 hrs	☐	☐	☐	
12	Post-transfusion shock	☐	☐	☐	
13	Post-transfusion O2 sat <90% on pulse oxymetry (SpO2) with or without O2 administration within 6 hrs	☐	☐	☐	
14	Post transfusion ratio of SpO2/FiO2 <315 within 6 hrs	☐	☐	☐	
15	Post-transfusion arterial blood gases within 6 hours after completion of transfusion showing pO2/FiO2 <300 mm Hg	☐	☐	☐	
16	Post-transfusion lung auscultation reveals rales(crackles) within 24 hrs	☐	☐	☐	
17	Post-transfusion lung auscultation reveals rhonchi within 24 hrs	☐	☐	☐	
18	Post-transfusion elevated jugular venous pressure within 6 hrs	☐	☐	☐	
19	Post-transfusion hepatojugular reflux detected within 6 hrs	☐	☐	☐	

NO.	ITEM	YES	NO	NR	NOTES
20	Post-transfusion cyanosis within 6 hrs	☐	☐	☐	
21	Post-transfusion central venous catheter measured elevated central venous pressure (>8 mm Hg) within 6 hrs		☐	☐	
22	Post-transfusion central catheter measured elevated pulmonary artery wedge pressure (>20 mm Hg) within 6 hrs	☐	☐	☐	
23	Post-transfusion peripheral pitting edema detected within 24 hrs		☐	☐	
24	Post-transfusion, was there a loss of 4.5 kg weight in 72 hours after diuretic treatment	☐	☐	☐	Note 22
25	Clinically suspect TRALI within 6 hrs		☐	☐	
26	Post-transfusion diagnosis of ARDS within 24 hrs	☐	☐	☐	
27	Post-transfusion, patient required ICU admission within 24 hrs		☐	☐	Note 23
28	Post-transfusion, required vasopressures to support blood pressure within 24 hrs (e.g., dopamine, norepinephrine, epinephrine)	☐	☐	☐	
29	Rapid resolution of lung infiltrates on chest X-ray after diuresis or dialysis within 6 hrs		☐	☐	

SECTION K: OTHER BLOOD COMPONENTS AND FLUID ADMINISTRATION

NO.	COMPONENT/FLUID	PRE-Tx AMOUNT (24 hour before Tx of the study unit started) Record if applicable	POST-Tx AMOUNT (24 hours after the Tx of the study unit finished) Record if applicable
1	RED BLOOD CELLS (No. of Units)		
2	PLATELETS, WHOLE BLOOD (No. of units)		
3	PLATELETS, APHERESIS (No. of Units)		
4	PLASMA (No. of Units)		
5	CRYSTALLOIDS - Normal saline, Half normal saline, D5W, D5/Half Normal saline, Lactated Ringer's solution (mL)		
6	COLLOIDS - albumin, plasma protein fraction or PPF (mL)		
7	Urine output (mL)		
8	Gastric tube output (mL)		
9	Surgical drain output (mL)		
10	Fluid balance – Excess fluid (mL)		

SECTION L: ELECTROCARDIOGRAM FINDINGS

PRE-TRANSFUSION EKG REPORT (Please write complete reports in box below for all EKGs that were done within 24 hrs prior to study unit transfusion. Please write date/time for each report.)

POST-TRANSFUSION EKG REPORT (Please write complete reports in box below for all EKGs that were done within 24 hrs after study unit transfusion. Please write date/time for each report.)

SECTION M: ECHOCARDIOGRAM FINDINGS (M-MODE & 2-D)

PRE-TRANSFUSION ECG WRITTEN REPORT (Please write complete report in box below; if more than one report, include all reports. Please write date/time for each report.)

POST-TRANSFUSION ECG WRITTEN REPORT (Please write complete report in box below; if more than one report, include all reports. Please write date/time for each report.)

SECTION N: LABORATORY FORM

COMPLETE BLOOD COUNT

NO.	PARAMETER	PRE-Tx (Most recent within 24 hrs. Record if applicable)			POST-Tx (Most recent within 24 hrs. Record if applicable)		
		DATE	TIME (military)	VALUE	DATE	TIME (military)	VALUE
1	Hemoglobin gm/dL						
2	Hematocrit (%)						
3	Total WBC x 10^3/uL						
4	Monocytes (%)						
5	Neutrophil (%)						
6	Lymphocyte (%)						
7	Platelet count x 10^3/uL						

OTHER LAB RESULTS

NO.	PARAMETER	PRE-Tx (Most recent within 24 hrs. Record if applicable)			POST-Tx (Most recent within 24 hrs. Record if applicable)		
		DATE	TIME (military)	VALUE	DATE	TIME (military)	VALUE
1	BUN (mg/dL)						
2	Creatinine (mg/dL)						
3	CK (IU/L)						
4	CK-MB (IU/L)						
5	Troponin I (IU/L)						
6	K (mEq/L)						
7	Na (mEq/L)						
8	B-type natriuretic peptide (pg/mL)						
9	Fibrinogen level (mg/dL)						
10	Fibrin degradation, d-dimer, or split products (record value)						
11	Total bilirubin (mg/dL)						

SECTION O: ARTERIAL BLOOD GASES

Record all values within six hours of the study unit transfusion

	DATE	TIME	FiO_2	PaO_2	$PaCO_2$	pH
PRE-Tx (Most recent within 6 hrs)						
POST-Tx Closest to time of reaction (Not more than 11 hrs and 59 min after completion of Tx)						
POST-Tx (Earliest between 12 hrs and 23 hrs & 59 min after completion of Tx)						
POST-Tx (Earliest between 24 hrs and 35 hrs & 59 min after completion of Tx)						

SECTION P: TRIAGE MD REVIEW

DIAGNOSIS OF MODERATE TO SEVERE TACO (only if clear evidence present)	**YES** ☐ **NO** ☐
EXPERT REVIEW REQUIRED:	**YES** ☐ **NO** ☐
Digital CXR Images available	**YES** ☐ **NO** ☐

****** END ******

NOTES

Note 1: All post-transfusion vital signs may not have been recorded. Complete those that are in the medical record.

Note 2: Describe the type of reaction. Following are the possible types of reactions: Febrile non-hemolytic transfusion reaction, allergic skin reaction, acute hemolytic transfusion reaction, delayed hemolytic transfusion reaction, bacterial sepsis, shock, anaphylaxis, cardiac overload, hypotension, shock, transfusion-associated dyspnea (TAD), tachycardia, tachypena (respiratory rate >22/minute, chills, fever, shortness of breath, circulatory overload, back pain, sweating, nausea, vomiting, hyperbilirubinemia, etc.

Note 3: The types of CNS injuries include sub arachnoid hemorrhage, subdural hematoma, closed head injury and stroke. Include only if the injury occurred within seven days prior to the study unit transfusion.

Note 4: If noted in medical records, or if <500 mL urine output in 24 hours or new elevated creatinine of >1.5 mg/dL.

Note 5: Select “yes” if aspiration event was witnessed and documented in the medical record. Select “no” if aspiration was suspected but not witnessed.

Note 6: Greater than 3 drinks on 5 or more days a week for males; greater than 2 drinks on 5 or more days a week for females or if documented in medical record.

Note 7: Chronic creatinine >1.5 mg/dL.
Note 8: Select "yes" if medical record indicates history of CHF or CHF present before transfusion during current admission. Select "Yes" if echocardiogram shows ejection fraction less than 40% or shows diastolic dysfunction or if there is cardiomegaly on chest x-ray.

Note 9: Diabetes can be either type I (insulin-dependent) or type-II (non-insulin-dependent).

Note 10: Select "yes" if MD note indicates presence of DIC or if platelet count <100,000/uL + fibrin degradation products or if platelet count <100,000/uL + fibrinogen concentration <100 mg/dL.

Note 11: Select "yes" if patient has lung infiltrate/s on chest x-ray within 8 hours of admission to the emergency room and has evidence of blunt trauma to the chest noted in medical record or has rib fracture/s or was involved in motor vehicle accident.

Note 12: Select "yes" if within 7 days before transfusion.

Note 13: If medical record indicate any one or more of the following: biopsy proven cirrhosis, portal hypertension, or past episodes of upper GI bleeding attributed to portal hypertension, prior episodes of liver failure/enechalopathy/coma.

Note 14: If patient received any RBC transfusion for drop in hemoglobin or hematocrit, select "yes".

Note 15: Extubation date/time should be recorded when the extubation is performed and note that this time may occur beyond the 24-48 hours after the transfusion. If intubation was performed and extubation did not take place (e.g. patient was discharged to a chronic ventilatory care facility while intubated), record No. If no record exists to determine the date/time of intubation, record NR.

Note 16: Calculate the excess fluids administered by fluids given (oral or intravenous) minus fluid losses (urine, gastric suction, thoracic or abdominal drainaige) in 24 hours prior to the transfusion. Write down the amount of excess fluid in mL.

Note 17: Pre-transfusion bacterial sepsis: If MD or nursing note indicates possibility of sepsis or a diagnosis of sepsis; other manifestations include a focus of infection (e.g. pneumonia, cellulitis, skin lucer, wound infection); manifestations of inflammation (e.g. fever, tachycardia, tachypnea), and shock (BP less than 90 mmHg systolic; sweating, cold clammy skin). Laboratory test may also show positive blood culture.

Note 18: Pre-transfusion shock: MD or nursing note indicating presence of shock. Manifestations include low systolic BP (<90 mmHg), tachycardia, cold clammy skin, distal extremity cyanosis, low urine output, mental confusion or somnolence.

Note 19: Calculate ratio by dividing the percent saturation of oxygen on pulse oxy (SO2) by FiO2 (calculate FiO2 as follows: room air = 0.21; one liter oxygen = 0.23; 2 liters = 0.25; 3 liters = 0.27; 4 liters = 0.3, 5 liters = 0.35; 6 & 7 liters = 0.4; and 8-10 liters = 0.49. Example: If oxygen saturation is 90% on 4 liters of oxygen by face mask, then the ratio is 90/0.3 = 300.

Note 20: PF ratio. Calculate by dividing the pO2 (on arterial blood gases) by FiO2 (flow rate of oxygen in decimel of 1.0, e.g., 40% FiO2 = 0.4). Example: Someone with pO2 of 120 receiving 40% oxygen, the PF ratio is 120 ÷ 0.4 = 300.

Note 21: The definition of when the reaction occurred is the earliest time at which one or more of the following events were noted: pO2/FiO2 <300; SO2/FiO2 <315, and pulmonary edema on chest x-ray.

Appendix H: Interim Analysis

The statistical power for the study is based on several assumptions, including the estimated incidence of TRALI/Possible TRALI. Other assumptions include the number of units transfused per transfusion episode. Although these assumptions are based on validated processes, such as, the published literature and the pilot data generated during the design of LAPSII, there remains a small possibility that the actual data may not show the expected incidence of TRALI in the two groups under study. Therefore, an internal pilot study will be performed to re-assess sample size (Tim Friede and Meinhard Kieser. 2004. Sample size recalculation for binary data in internal pilot study designs, Pharmaceutical Statisitics, vol 3 p269-279).

The internal pilot study will result in an interim analysis. The interim analysis will be performed when the recipient record review process has been completed (including the review by the Medical Board) for 33% of subjects. At this juncture, the incidence of TRALI (including possible TRALI) will be calculated without un-blinding cases and controls. The internal pilot study data will allow improved estimates of TRALI incidence, and component distribution per transfusion episode to be computed. The sample size calculation as described in section H.4.will be re-done.

If the interim analysis suggests that an increase in the number of study recipients is needed, then two potential approaches are available. First approach will explore the possibility of completing the study with the larger sample size. The second approach will explore the possibility of reducing the power of the study, e.g., from 90% to 85% to reduce the degree of increase in sample size so that the increase in the number is not overwhelming. The use of internal pilot study data to re-assess sample size may have a modest effect on statistical power (A.L. Gould and W.J. Shih. 1998. Modifying the design of ongoing trials without unblinding, Statistics in Medicine, vol 17, p89-100). Therefore, reducing power to a nominal 85% may result in the effective power being on the order of 80%. In any event, changing the sample size will not affect type I error (i.e. 0.05 level test for analysis), as the sample size re-assessment will maintain blinding.

The first option will be preferred because it would allow maintaining the power to 90%. In this regard, a further increase in the number, e.g., by 20-40% may be achievable based on the budgetary guidelines that have been developed for the protocol which includes an additional margin of up to 40% of the record review coordinator's salary support. Also, during the design of the study, a sample size as large as 3,283 units for each group was considered feasible. This latter number represents a 2.8-fold increase (3,283 vs. 1,175). Thus, a considerable increase in the number needed might be tolerable. Nonetheless, a much larger increase in the sample size would require careful deliberations by the LAPSII working group, NHLBI, the Coordinating Center, and the Central testing laboratory to determine the feasibility of conducting a much larger study. In addition careful review of the available resources and the time frame within which the additional charts can be reviewed must be considered before embarking on a much larger sample size. These deliberations will also explore the second approach, namely, a reduction in the power of the study as a less preferred strategy.

Appendix: I

LEUKOCYTE ANTIBODY PREVALENCE STUDY – PHASE II

EXPERT REVIEW LOG FORM

	STUDY ID	TRALI (No risk factors for ALI)	TRALI (with risk factors for ALI)	ALI	TACO	TRALI/ TACO	OTHER* (Specify)	COMMENTS
1		☐	☐	☐	☐	☐		
2		☐	☐	☐	☐	☐		
3		☐	☐	☐	☐	☐		
4		☐	☐	☐	☐	☐		
5		☐	☐	☐	☐	☐		
6		☐	☐	☐	☐	☐		
7		☐	☐	☐	☐	☐		
8		☐	☐	☐	☐	☐		
9		☐	☐	☐	☐	☐		
10		☐	☐	☐	☐	☐		
11		☐	☐	☐	☐	☐		
12		☐	☐	☐	☐	☐		
13		☐	☐	☐	☐	☐		
14		☐	☐	☐	☐	☐		
15		☐	☐	☐	☐	☐		

* Other Specify E.g. Transient Obstruction, Bronchospasm, Accidental Extubation