Study Protocol

Transfusion-transmitted retrovirus and hepatitis virus rates and risk factors: Improving the safety of the US blood supply through hemovigilance

American Red Cross, Blood Systems Inc. and New York Blood Center

Background

Information on current risk factors in blood donors as assessed using analytical study designs is largely unavailable in the US. Studies of risk factor profiles among HIV-infected donors were funded by the CDC for approximately 10 years after implementation of serologic screening in the mid-1980s, whereas studies of HTLV- and HCV-seropositive (and indeterminate) donors, funded by NIH, were conducted in the early 1990s, but unfortunately, none of these studies is ongoing.[1] More recently, risk factors for HCV infection in nucleic acid positive (NAT) anti-HCV negative donors have been reported.[2]

Infection trend analyses have been conducted by the American Red Cross (ARC). [3, 4] The findings show continued HIV risk with the prevalence of HIV in first time donors hovering around 10 per 100,000 donations in each of the last 10 years and the incidence in repeat donors increasing from 1.49 per 100,000 person-years in 1999-2000 to 2.16 per 100,000 persons-years in 2007-2008.[4] While the prevalence of HCV in first time donors decreased over this time interval from 345 to 163 per 100,000 donations, the incidence in repeat donors did not decrease and evidence of incident infection in first time donors increased. Moreover specific age, gender and race/ethnicity groups were over-represented. Significantly increased incidence of both HIV and HCV were observed in 2007/2008 compared to 2005/2006. Similar analyses for HBV have shown an incidence in all donors of 3.4 per 100,000 person-years which is lower than earlier estimates, but remains higher than for HIV and HCV.[5]

Approximately 5 million US patients receive red cell transfusions each year.[6] Preventing the transmission of infections to persons requiring transfusion is of paramount importance. The greatest improvements in infectious disease blood safety come from preventing the donation of blood that is infected. To prevent such donations blood collection organizations need to be able to share and aggregate hemovigilance data. Standardized data coding, research database processes, and data collection procedures across organizations will benefit transfusion safety. Identifying and reporting risk factors for viral infections in viremic and/or confirmed positive donors is not mandated by the US FDA or required by blood organizations or standard-setting bodies. Risk factor assessment by blood organizations is currently done as part of individual donor notification and counseling by all three organizations, but not in a systematic way within or across organizations. A systematic collection of risk factor data will enhance the information already contained in the centers' databases.

A brief review of risk factors for viral infection acquisition in the general population of the US and blood donors where available are provided below. However, it is unclear whether current blood donors have the same types of risk exposures and if the proportions of attributable risk behaviors are the same as previously observed.

HIV

Among 38,000 persons in 33 states with confidential name-based HIV infection reporting in 2004, male-male sex was the most frequently reported transmission category, followed by heterosexual contact and

IDU.[7] The 1999-2002 National Health and Nutrition Examination Survey (NHANES) results show that non-Hispanic black participants (N=1283) who reported ever using cocaine/street drugs, in addition to those who tested positive for the presence of HSV-2 antibody, had a higher prevalence of HIV infection.[8] In blood donors, risk factors reported by HIV seropositive donors from over 20 years ago were different for males and females. In males, male-male sex was most common followed by IDU, sex with an IDU, but 27% did not disclose or could not identify a risk, whereas in females, sex with males at risk for HIV was most common (81% of whom were IDU) followed by IDU, but 41% did not disclose or could not identify a risk.[9, 10]

HCV

Hepatitis C is the most common blood borne infection in the United States, and risk factors are associated with percutaneous or mucosal exposures to blood or blood-derived body fluids. A national population-based survey NHANES III, 1988-1994) surveyed approximately 15,000 participants and found that a history of injection drug use was the strongest risk factor for HCV infection.[11] Other significant risk factors included 20 or more lifetime sexual partners and blood transfusion before 1992. Among blood donors, a matched case-control study of 2,300 participants showed that injection drug use was highly associated with HCV seropositivity and was the most common risk factor reported by US blood donors (about 50%).[12] Data from the Centers for Disease Control and Prevention from 1995-2000 demonstrate that most newly acquired infections are associated with injection drug use, followed by exposure to an infected sex partner or to multiple sex partners, health care work with frequent exposure to blood, and rarely, nosocomial, iatrogenic and perinatal exposures.[13] HCV NAT positive, anti-HCV negative blood donors had the following risk factors, recent injection drug use (IDU), followed by occupational exposure, sexual contact with an HCV-infected partner (who was an IDU), and perinatal exposure.[2]

HBV

The 1988-1994 NHANES data (N=40,000) demonstrate that black race, increasing number of sexual partners, and non-US place of birth were highly associated with HBV infection.[14] Centers for Disease Control and Prevention surveillance data from 1990-2004 show that the proportion of acute HBV cases reporting multiple sexual partners and male-male sex doubled over this time period, indicating that these behaviors are associated with increased risk of HBV transmission.[15]

HTLV

In a frequency-matched case-control study of former blood donors (149 HTLV-I cases, 381 HTLV-II cases, and 936 controls), blood transfusion, more than 7 lifetime sex partners, and any sex partner from an endemic area were highly associated with both HTLV-I and HTLV-II infection.[16] In addition, injection drug use or sex with an IDU were significant risk factors for HTLV-II infection.

Project Aims

This project represents a collaborative pilot research study that will include a comprehensive interview study of viral infection positive blood donors at the American Red Cross (ARC), Blood Systems Inc. (BSI) and New York Blood Center (NYBC) in order to identify the current predominant risk factors for virus positive donations and will also establish a donor biovigilance capacity that currently does not exist in the US. At this time it is not easy to integrate risk factor data and disease marker surveillance information within or across different blood collection organizations because common interview procedures and laboratory confirmation procedures are not being used and so we cannot easily tabulate and analyze behavioral risks or viral infections in US blood donors. This creates the potential for gaps in

our understanding of absolute incidence and prevalence as well as risks that could lead to transfusion-transmitted disease. Combined data are critical for appropriate national surveillance efforts. For example, this information could be used to target educational interventions to reduce donations from persons with high risk behaviors. This is particularly important in the case of behaviors associated with incident (recently acquired) infections because these donations have the greatest potential transmission risk because they could be missed during routine testing. As part of the project a comprehensive research-quality biovigilance database will be created that integrates existing operational information on blood donors, disease marker testing and blood components collected by participating organizations into a research database. The combined database will capture infectious disease and risk factor information on nearly 60% of all blood donors and donations in the country. Following successful completion of the risk factor interviews and research database development, the biovigilance network pilot can be expanded to include additional blood centers and/or re-focused on other safety threats as warranted, such as XMRV. This pilot biovigilance network will thereby establish a standardized process for integration of information across blood collection organizations.

The **Specific Aims** are to:

1) Define consensus infectious disease testing classification algorithms for HIV, HCV, HBV, and HTLV that can be used to consistently classify donation testing results across blood collection organizations in the US. This will allow for better estimates of infection disease marker prevalence and incidence in the US.

2) Determine current behavioral risk factors associated with prevalent and incident (when possible) HIV, HCV, HBV and HTLV infections in blood donors, including parenteral and sexual risks, across the participating blood collection organizations using a case-control study design.

> Hypothesis:
> The distribution of risk factors for viral infections in donated blood reported by donors in studies from more than 10 years ago will not be same as those reported today. We expect that factors such as intravenous drug use will comprise a lower proportion of identified risk in blood donors with infections, and those sexual exposures, including multiple heterosexual sex partners and male-to-male sex, will comprise a larger proportion of reported risks for infection.

3) Determine nationally-representative infectious disease marker prevalence and incidence for HIV, HCV, HBV, and HTLV overall and by demographic characteristics of donors. This will be accomplished by forming research databases from operational data at BSI and NYBC into formats that can be combined with the ARC research database.

> Hypotheses:
> The rate of HIV, HCV, and HTLV infections in blood donors will be stable or increasing over time when data from 2010 are analyzed and compared to other years. The rate of HBV infection may be stable or even decreasing in the blood donor population over the same time period, in part due to expanded vaccination efforts or changes in the demographic characteristics of blood donors.

4) Analyze integrated risk factor and infectious marker testing data together because when taken together these may show that blood centers are not achieving the same degree of success in educational efforts to prevent donation by donors with risk behaviors across all demographic groups.

The primary contribution this study can add to a national hemovigilance system is to monitor routes and rates of infection acquisition that could lead to transfusion-transmission. Thus results from this study could also be used to assist in formulating guidelines, informing policy decisions, planning prevention programs, and targeting risk reduction interventions. Efforts to enhance hemovigilance related programs in the USA are especially important because there is currently no national hemovigilance system. In the United Kingdom, where a national hemovigilance system has been in place since 1996, the collection and analysis of data has led to specific recommendations that underpin key transfusion safety initiatives.[17]

Aim 1. Define and document consensus infectious disease testing classification algorithms for HIV, HCV, HBV, and HTLV that can be used to consistently classify donation testing results across blood collection organizations in the US, thus allowing for better estimates of infection disease marker prevalence and incidence in the US.

During task 1, one in-person meeting of testing and blood banking experts from each of the participating organizations to take place in Maryland at Westat or ARC offices will be convened to define consensus classification algorithms. This work will require discussion of the meaning of different combinations of screening and confirmatory results during this study. Table 1 below provides the current relevant tests of record for each of the organization.

Table 1. Current assays used or to be used at each organization during the study period.

Infection	ARC	BSI	NYBC
HIV-1/2			
Antibody screening	PRISM Anti-HIV1/2 Plus O EIA	Bio-Rad Anti-HIV1/2 Plus O EIA	Bio-Rad Anti-HIV1/2 Plus O EIA
NAT screening	Gen-Probe Ultrio	Gen-Probe Ultrio	Gen-Probe Ultrio
Confirmation	Sanochemia HIV-1 IFA	Sanochemia HIV-1 IFA	Sanochemia HIV-1 IFA
HCV			
Antibody screening	Ortho 3.0 ELISA	Ortho 3.0 ELISA	Ortho 3.0 ELISA
NAT screening	Gen-Probe Ultrio	Gen-Probe Ultrio	Gen-Probe Ultrio
Confirmation	Chiron RIBA 3.0	Chiron RIBA 3.0	Chiron RIBA 3.0
HBV			
HBsAg screening	Abbott PRISM	Abbott PRISM	Abbott PRISM
Anti-HBc screening	Abbott PRISM	Abbott PRISM	Abbott PRISM
NAT screening	Gen-Probe Ultrio	Gen-Probe Ultrio	Gen-Probe Ultrio
Confirmation	PRISM (HBsAg Neutralization)	PRISM (HBsAg Neutralization)	PRISM (HBsAg Neutralization)
HTLV-I/II			
Antibody screening	Abbott PRISM	Abbott PRISM	Abbott PRISM
Confirmation	California State Proprietary	Innogenetics INNO-LIA	Innogenetics INNO-LIA

The current interpretation algorithms used by BSI are included as an Appendix. Similar algorithms are used by ARC and NYBC, but a thorough vetting and discussion is necessary to define consensus interpretation of combined assay results. The interpretation algorithms will be documented and used in this and future collaborative studies.

Aim 2. Determine current behavioral risk factors associated with prevalent and incident HIV, HCV, HBV and HTLV infections in blood donors, including parenteral and sexual risks, across the participating blood collection organizations using a case-control study design.

Currently, information on risk factors for virus acquisition is not systematically collected in the USA. Donors are counseled individually and this information becomes part of individual blood donation records. However, aggregation and analysis of this information does not occur. This information would help to safeguard the nation's blood supply by providing blood centers with information to improve the pre-donation screening process. This systematic surveillance of risk factors among infected blood donors provides ongoing information about the effectiveness of donor selection and is recommended to evaluate and optimize blood policies. [18]. Risk behaviors may change over time and monitoring epidemiological trends in virus acquisition is vital for understanding which questions to ask donors on the donor history questionnaire and how to phrase those questions in order to obtain the most accurate response. This information would also help to guide donor counseling, as donor counselors would be able to provide more relevant advice to the donor to prevent spread of infection based on the risk factors the donors disclose.

> Hypothesis:
> The distribution of risk factors for viral infections in donated blood reported by donors in studies from more than 10 years ago will not be same as those reported today. We expect that factors such as intravenous drug use will comprise a lower proportion of identified risk in blood donors with infections, and those sexual exposures, including multiple heterosexual sex partners and male-to-male sex, will comprise a larger proportion of reported risks for infection.

The goal of the study is to identify self-reported risks factors for disease marker positive blood donations and to report frequencies and patterns of risk factors in the population of donors who test positive. Donors who are confirmed positive for one of the four viral infections and also donors who test repeat reactive but do not confirm positive based on supplemental/confirmatory testing for the same infections will be interviewed. The unconfirmed (false positive) donors will serve as a comparison group to the confirmed positive donors.

An estimated 9-10 million individuals donate blood every year [19, 20]. While blood donors as a whole tend to be healthier than the general population [21, 22], many infected individuals learn of their virus seropositivity only through blood donation, during which blood is operationally screened to detect viral biomarkers for HBV, HCV, HIV, and HTLV, among other pathogens. Table 2 provides the number of disease marker positive donors at our blood centers in the year 2007 and also 2009. The two different years show provide an indication of annual variability in the number of infections identified in donors, but also show that year to year results are similar.

Table 2. Confirmed positive donations collected by Blood Systems, New York Blood Center, and the American Red Cross in 2007 & 2009.

Infection	BSI	NYBC	ARC	Total
2007				
HIV-1	39	31	189	259
HCV	212	191	2132	2535

HBV	204	142	794	1140
HTLV I/II	100	78	149	327
2009				
HIV-1	31	24	194	249
HCV	315	187	1970	2472
HBV	169	134	648	951
HTLV I/II	75	69	143	287

Defining Infection Status

Confirmed Positive Donors

The combination of assays performed is used to help identify recently acquired versus long-standing (remote) viral infections. In order to truly define incident infection it is necessary to conduct detuned assays. Additional detuned testing will not be conducted as part of this study. However, sufficient information can be gained by considering the combination of tests that are positive for each infection (Table 3). For example, an HIV infection that is only identified through NAT testing is an incident infection. Whereas an infection with a relatively high NAT result coupled with low level antibody would suggest a recent seroconversion. Both of these infection types represent newly acquired infections, except in rare instances. In addition, prevalent infections in repeat donors can be reclassified as incident based on the use of a definition of a non-reactive donation less than 2 years prior to the NAT+/Ab+ donation.

Table 3. Summary of screening results for HIV, HCV and HBV used to define recent or remote infection.

Confirmatory Test Result	**Definition for the Study**
HIV	
HIV Ab-negative *and* HIV NAT-positive	Incident (recent infection)
HIV Ab-positive (detuned assay indicates recent infection) *and* HIV NAT-positive	Incident (recent infection)
HIV Ab- positive (detuned assay indicates remote infection) *and* HIV NAT-positive	Prevalent (remote infection)
HCV	
HCV Ab-negative *and* HCV NAT-positive	Incident (recent infection)
HCV Ab-positive *and* HCV NAT-positive	Prevalent (remote infection)
HBV	
HBsAg-positive *and* Anti-HBc non-reactive	Incident (recent infection)
HBsAg-positive *and* Anti-HBc reactive	Prevalent (remote infection)
HTLV	
To be defined if possible	Incident (recent infection)

To be defined if possible	Prevalent (remote infection)

During the REDS-II Molecular Surveillance study 44% of HIV infections were classified as recent, whereas only 3% of HCV infections and 14% of HBV infections met definitions for recently acquired infection. It is likely we will see similar proportions in this risk factor study.

False Positive Donors

Donations from donors that test repeat reactive that ultimately cannot be confirmed by supplemental testing are thought to be random events and therefore they represent an appropriate sample of blood donors. Studies of infected and uninfected blood donors have previously used this strategy to identify controls for case-case control studies [23, 24] and research has shown that donors with false positive results on the initial screening test are not infected [25-27].

Robust confirmation procedures are used to ensure false positive donors are indeed false positive. There is virtually no chance that a false positive donor will be a true positive donor that has been misclassified. These donors are not the same as donors who are classified as indeterminate.[28] Donors with indeterminate confirmation results will not be eligible for this study. The frequency of false positive testing results varies according to the sensitivity and specificity and other aspects of the screening tests used. For anti-HIV the ratio of false positive to confirmed positive donors is approximately 15:1, for anti-HCV 2.5:1, for HBsAg 3.3:1, and for anti-HTLV 2.6:1. The ratios mean that we will have far more false positive donors than confirmed positive donors as potential subjects for the study. Because we will be sampling HBV and HCV confirmed positive donors, for the overall study this ratio is expected to be over 10 false positive donors to 1 confirmed positive donor. Therefore we will also sample our controls (described below)

The operational processes that are used for blood test results notification for these donors represent an opportunity to sample an informative control group that has interacted with each blood center in a largely similar manner to that of cases. These donors are contacted by mail informing them of the results of testing and their false positive status. They represent appropriate controls because they have gone through confirmatory testing procedures that unambiguously establish they are not infected with one of the four viral infections of interest in this study. In addition because they have to be contacted and provided the results which include the false positive screening tests, these donors may be interested and motivated to complete the questionnaire as part of the counseling process. All controls will be serving as the comparison population for each confirmed positive infection the ratio of controls to cases will vary according to each infection.

AIM 2 METHODS & PROCEDURES

A risk factor questionnaire has been developed that focuses on the risk behaviors associated with human-to-human transmission of viral infections for which blood centers universally screen donated blood (draft included as an Appendix). The ability to ask questions about all four viruses on a single instrument is feasible since infected individuals often share risk factors for routes of virus acquisition. Although the risk of transmission varies for each virus HBV, HCV, HIV, and HTLV can all be acquired through the following routes: parenteral (examples: blood or blood product transfusion, transplantation, injection drug use, tattooing, body piercing, needlestick injury), sexual, perinatal (examples: during pregnancy, labor, delivery or breastfeeding), and household contact (examples: sharing toothbrushes or razor blades with an infected individual). These routes of acquisition and risk factors have all been

previously identified in the literature and considered to be well-established [2, 12, 18, 29-31]. Our study is designed to assess the frequency of these routes of self-reported infection acquisition. The study is not designed to assess very rare or newly hypothesized routes of infection acquisition.

This common questionnaire also represents the first step toward the establishment of a basic donor-focused nationwide hemovigilance system for viral infections, which could lead to the further reduction of the risk of virus transmission through blood transfusion. While this risk is very rare, it still exists particularly in cases where a donor has recently become infected and then donated blood. In the U.S., since the introduction of nucleic acid amplification testing (NAT) in 1998-2000 which identifies most, the residual risk estimates of HIV and HCV transmission through blood transfusion are 1 in 2 million units [32]. The residual risk of HBV, for which NAT has previously not routinely been performed, is 1 in 200,000-500,000 units using a combination of anti-hepatitis B core and hepatitis B surface antigen testing, while the HTLV residual risk is 1 in 500,000-3 million units [32]. HBV NAT testing will begin during this study at each of the participating centers.

After notification of having donated either a confirmed positive donation or false positive donation based on standard operating procedures at each organization, donors who are either confirmed or false positive for HBV, HCV, HIV, and/or HTLV will be asked to complete an interviewer-administered telephone or in-person questionnaire. We will build on current operational procedures to facilitate the ease of use of the risk factor interview instrument. As per operational procedures, except in rare circumstances, donors who are HIV positive are notified in person by blood center professional medical staff (physician or qualified donor counselor) and will be asked to complete the questionnaire at the time of notification, or based on professional judgment at a future in person meeting or by telephone call if agreement for future contact is obtained. HIV false positive donors may be interviewed in person or over the telephone depending on the donor's preference by the same donor counselors. For HBV, HCV, and HTLV the interview will be conducted over the telephone by trained donor counselors. There are strict operational procedures in place to verify that a person contacted by phone is the blood donor. If the donor's identity cannot be confirmed via standard operational procedures the interview will not be conducted.

There are three routes of study subject contact for this study (Figure 1):

1. In person interview when a donor returns to the blood center for counseling (expected for HIV) but donors positive for other viral markers could also seek in person counseling. False positive donors may also seek in-person counseling.
2. Donor initiated telephone contact following receipt of disease marker testing results and counseling materials sent to the donor via standard mail (HCV, HBV, and HTLV results and for HIV false positive results). Donors who are notified by mail whether confirmed or false positive are encouraged to call donor counselors to discuss the results and any additional questions the donors may have.
3. Donor counselor initiated phone contact in which the donor counselors contact the donor to follow-up to see if the donor received the notification letter and counseling materials. Donor counselors will attempt to contact donors by telephone call up to 3 times by telephone. If we are unable to reach a donor after 3 attempts the donor will be classified as lost to follow-up.

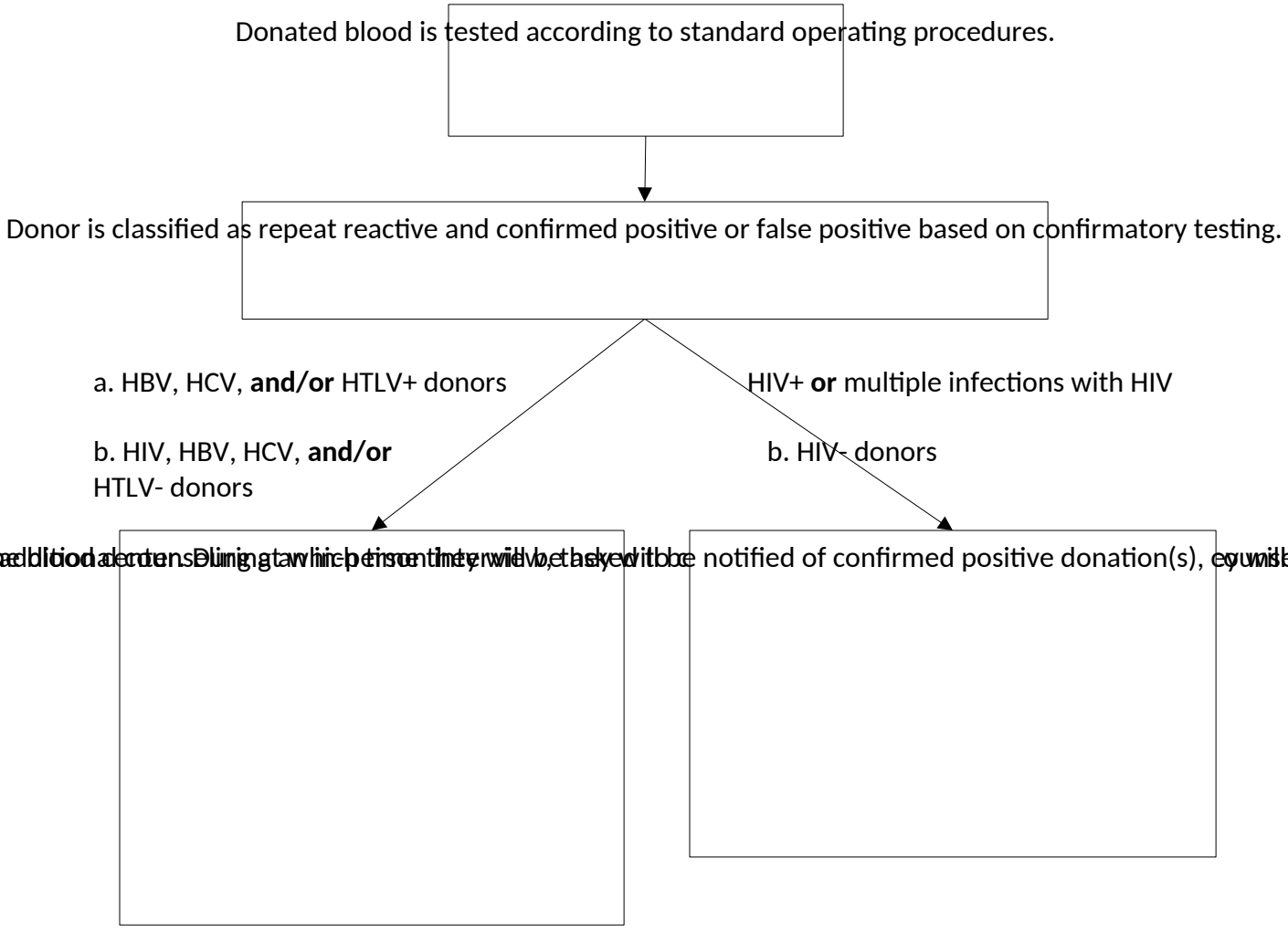
The interview form is designed to keep track of the route of study subject contact, including capturing basic information on donors whom we contact and who refuse to participate following recruitment efforts. This information will be analyzed at the end of the study to assess whether demographic differences are evident for donors who participate compared to donors who refuse.

The questionnaire will require approximately 30 minutes of the donor's time. The instrument has been developed with skip patterns so that the time of administration may vary substantially based on the risk behaviors of different donors. Donors will be interviewed by counselors who are employed by the each organization where the donor gave blood.

Completed questionnaires from all three participating organizations will be sent to Blood Systems Research Institute (BSRI) via registered mail or courier service. At BSRI the data collected on each form will be entered into a single database using optical scanning technology. BSRI researchers will not have access to the names or any other personally identifying information, except biometric identifiers.

Interviews will be conducted in English, or Spanish based on the preference stated by each donor. Consent will be obtained in the same language as the interview.

Figure 1. Study subject flow diagram for donor interviews.



<u>Subject Interviews</u>

Case recruitment procedures and study sample numbers for the interviews to be conducted will be dependent on the type of infection.

HIV and HTLV Cases

HIV and HTLV cases will be contacted in accord with one of the three routes of subject contact for the study. HIV cases will come from the three participating organizations. In order to enhance the number of study subjects we will include HIV confirmed positive donors from the Community Blood Centers of South Florida. For HTLV confirmed positive donors we will include donors from the 3 main participating organizations only (ARC, BSI, and NYBC).

HCV and HBV Cases

HCV and HBV cases will be contacted in accord with one of the three routes of subject contact for the study. Because of the number of confirmed HCV and HBV positives donors each year, we will sample cases for these infections. We will conduct 500 case interviews for each type of infection. ARC will conduct 300 HCV and 300 HBV interviews; BSI will conduct 100 HCV and 100 HBV interviews, and NYBC will conduct 100 HCV and 100 HBV interviews. We will preferentially contact all NAT-only cases and recent seroconversion cases. We expect that 75% of cases who are contacted will agree to participate in the study. These study numbers will be sufficient to provide up-to-date information on risk factors for confirmed HCV and HBV in persons who have recently donated. To achieve a sample that will be temporally representative of the year-long study interview period we will sample donors on a monthly basis. For ARC this means that 25 HCV and 25 HBV confirmed positive interviews will need to be conducted every month. For BSI and NYBC, 8 to 9 interviews for each infection will be conducted each month. HCV and HBV case interviews will be tallied starting at the beginning of every month and for each organization when the targeted number of interviews are reached HCV and HBV case interviews will end until the beginning of the next month.

Controls

Controls for this study are intended to reflect the population of eligible blood donors. Controls will not be matched to cases so that we may include demographic characteristics in addition to risk factors in our multivariable logistic regression analysis of the predictors of infections in blood donors. Controls will be interviewed contemporaneously as cases. All interviewed controls will be included in each analysis comparing risk factors in confirmed positive for each infection to the entire control group. Therefore, the ratio of controls to cases will vary depending on the infection being examined. For HIV the ratio will be approximately 7 controls per case, for HCV and HBV the ratio will be 5 controls per case, and for HTLV the ratio will be approximately 8 controls per case. Each participating organization will have to ensure that the controls in the study are similar to population of eligible donors according to age, gender, race, and first time donor status. To accomplish this each organization will monitor the demographic characteristics of control donors so that study participants resemble the eligible donor population for that blood collection organization. For example we will select controls in bins that largely resemble the eligible donor population with respect to gender, age group, race/ethnicity, and first time or repeat donor status.

<u>Risk Factor Questionnaire</u>

The risk factor interview covers content on common routes of exposure and also less common routes. In addition the questionnaire will provide each confirmed positive donor the opportunity to report in an open-ended manner how he/she believes he/she may have been infected. Three versions of the questionnaire have been developed to reflect specific administrative information for ARC, BSI and NYBC. The risk factor and related content is identical for all three centers. In addition the risk factor questionnaire includes questions on subjects' motivations for donating and a short assessment of the donor's quality of life at the time of the interview using the EuroQol Five Dimension (EQ-5D) instrument. The EQ-5D is a descriptive system of health-related quality of life consisting of five dimensions (mobility, self-care, usual activities, pain/discomfort, anxiety/depression) each of which can take one of three responses. The responses for each dimension are no problems/some or moderate problems/extreme problems, and the EQ-5D includes an open-ended overall quality of life assessment in which a standard vertical 20 cm visual analog scale (similar to a thermometer) is used for recording an individual's rating of his or her current health-related quality of life.[33] Measures of quality of life infected donors and a comparison group have not been reported in the US.

Prospective and Retrospective Interviews

The majority of risk factor interviews will be conducted soon after confirmatory testing is completed from donors who have been newly classified as true or false positive for each infection based on blood donation testing (prospective interviews). Depending on the length of study and the possibility that reduced numbers of infections possibly could be observed for unknown reasons during the planned study period and to account for the expected 75% participation of confirmed positive cases, we will also obtain human subjects approval to conduct risk factor interviews of donors from the beginning of 2010 in order to achieve the projected sample size for each infection. This issue is likely to only be relevant for HIV infections and this strategy will only be used for HIV because of the importance of achieving sufficient participation of subjects for HIV (see below).

Sample Size

The estimation of optimal sample size is difficult because of limited information available on the prevalence of risk behaviors in accepted blood donors. For this reason we have focused on a power analysis to help guide our understanding of what risk estimate results are achievable given the number of each type of true positive donors we believe we will be able to interview. A survey study of undisclosed risk factors in accepted donors conducted by the Retroviral Epidemiology Donor Study in the 1990s found a prevalence of undisclosed risks of 186 per 10,000 donors or 1.86%[34]. We assume this is the prevalence of undisclosed risks in the control group for our study. The sample size (as described below) will include 350 HIV confirmed positive donors, 500 HCV confirmed positive donors, 500 HBV confirmed positive donors, 300 HTLV confirmed positive donors. The table below shows the power (of an α=0.05 level test) to detect significant associations between risk factors with a prevalence of 1.86, 1.0 and 0.5% in controls donors assuming 2, 3, 5, and 10-fold higher prevalence of the same risk factors in confirmed positive donors, by the four infectious markers (Table 4). The higher the prevalence of the risk behaviors is in the donor population and the larger the excess risk in confirmed positive donors the higher the power will be to detect a significant difference.

Table 4. Power for various risk factor prevalence combinations in controls and cases.

Infectious Marker case/control sample	Prevalence of risk factor in controls	Odds ratio to detect in cases

sizes		10	5	3	2
HIV 350/2500	1.86%	<99.9	<99.9	91.3	51.3
	1.0%	<99.9	97.6	72.1	32.2
	0.5%	99.5	83.9	46.7	18.3
HCV and HBV 500/2500	1.86%	<99.9	<99.9	96.7	62.3
	1.0%	<99.9	99.4	82.3	39.8
	0.5%	<99.9	91.4	56.1	22.4
HTLV 300/2500	1.86%	<99.9	99.8	88.0	46.7
	1.0%	<99.9	96.1	67.3	29.2
	0.5%	99.1	80.0	42.9	16.7

For HIV risk factors, a study of 350 confirmed positive donors and 2500 false positive donors will have more than 80% power to be able to detect a 5-fold increased prevalence of risk factors in true positive cases if the risk factor prevalence is 0.5% or higher in false positive donors. If the risk factor prevalence in false positive donors is the same as reported in the previous REDS study (1.86%) we will have more than sufficient power to detect a 3-fold higher risk factor prevalence in true positive cases. For HTLV risk factors the power estimates are similar, but slightly lower at any given combination of risk factor prevalence in false positive donors and excess risk in true positive donors because of the lower number of HTLV true positive cases (300) we are planning to interview.

For HCV and HBV risk factors a study of 500 confirmed positive donors and 2500 false positive donors will have sufficient power to be able to detect a 3-fold increase in prevalence of risk factors in cases if the risk factor prevalence is 1.0% or higher in false positive donors.

These power analyses underpin our decision to seek to interview as many confirmed HIV and HTLV positive donors as possible, whereas we will sample of HCV and HBV confirmed positive donors. Assuming 75% participation of HIV and HTLV confirmed positive donors and the pre-specified maximum number of confirmed positive HCV and HBV participants, the overall number of interviews to be conducted by each organization are shown in Tables 5 and 6:

Table 5. Overall expected participation in risk factor interview assuming both prospective and retrospective interviews for HIV positive and false positive donors.

Subject Type	HIV	HCV	HBV	HTLV
Case (True positive)	350	500	500	300
Control (False positive)	2500			

Table 6. Expected confirmed positive donor participation in risk factor interview study by blood center based on 2007 data assuming both prospective and retrospective interviews for HIV positive and false positive donors.

Viral Infection	BSI	NYBC	ARC**	Total
HIV-1	40	30	280	350
HCV	100	100	300	500

HBV	100	100	300	500
HTLV I/II	80*	31	189	300
Total Cases	320	261	1069	1650
Total Controls	486	396	1618	2500

*Approximately half of HTLV infections at BSI are expected to be confirmed.
** Includes interviews of Community Blood Centers of South Florida donors.

Statistical Analyses

We will compute descriptive statistics, such as frequencies, in order to characterize the infected population and catalog donor-reported risk factors likely to be the route of virus acquisition. We will compare the risk factors reported by donors according to demographics and in different regions of the country to determine if patterns of infection acquisition vary using the Chi-square or t-test depending on the structure of the predictor variable included in the analysis. Independently for each virus, univariable and multivariable logistic regression analysis will be used to compare confirmed positive and false positive donors to determine the association between risk behaviors and demographics, and testing confirmed positive. The multivariable analysis will be important so that we may account for potential differences between cases and controls with regard to factors such as socio-economic status. In addition, analyses restricted to confirmed positive donors will also be conducted. For example, risk factors for recent and remote infections will be compared for each virus to determine if incident infections are associated with specific risk behaviors.

Participant Incentives

Incentives will be provided. Confirmed positive donors will receive $100 for completing the interview. Incentives will be provided through the same operational procedures within each organization that allow for personally identifying each donor. The participation incentive will be sent to each donor or can be picked up at the respective donor clinics within two weeks following the completion of the interview. A $50 participation incentive will be provided to false positive (control) donors. The study investigators will not be responsible for providing the incentive payments to participants, the risk factor interviewers or the study coordinators they report to, who already have access to personally identifying information, will be responsible for ensuring participation incentives are provided to participants.

Human Subjects Considerations

All human subjects and other approval requirements for this study will be met before the study can begin. A certificate of confidentiality from NHLBI has been obtained to prevent the blood centers or study investigators from being legally compelled to release information reported by donors during the interview. Confirmed positive donors will be asked to complete the questionnaire proximate to the time that they are notified of their infection status. This represents an emotionally difficult and challenging time for donors. Some donors may be completely surprised or in dismay at the results of testing. The notification process is intended to be as benign as possible. The addition of a questionnaire designed to assess risk behaviors into the notification process may be difficult for some donors to complete. Participating in the donor questionnaire is optional and is not a condition for future counseling. Donors

will be given the option of being contacted at a later time to complete the questionnaire. Donors may refuse participation and future contact.

Each infection has potentially serious consequences for the future health of the blood donor. Part of standard counseling is to encourage that donors seek full medical evaluation by their physician. However, in particular the risk of distress over HIV infection and the risk of stigma are very high. As per required operational procedures no donor identifiers that could reasonably be used to identify specific individuals will be available to the study researchers.

For false positive donors, the interview may also be useful in helping donor counselors with the counseling message and in identifying possible types of behaviors that could lead to false positive testing results. Again, as per required operational procedures no donor identifiers that could reasonably be used to identify specific individuals will be available to the study researchers.

A NHLBI Certificate of Confidentiality has been obtained to prevent the study from being compelled to release information reported by the persons who participate in this study.

Aim 3. Determine nationally-representative infectious disease marker prevalence and incidence for HIV, HCV, HBV, and HTLV overall and by demographic and/or geographic characteristics of donors. This will be accomplished by forming research databases from operational data at BSI and NYBC into formats that can be combined with the ARC research database and conducting a subsequent analysis.

An integrated database that includes laboratory testing and blood establishment computer system donor data from each organization will permit a wide-range of analyses. Although efforts to provide summary data for the participating centers have been achieved for international reporting such as for WHO, the summary nature of the data obtained from the different computers systems does not allow for more in-depth analyses.

A newly-constructed and continually maintained research database removes barriers between different database systems at the blood centers and creates a common underlying data structure. It will be patterned after the research database of ARC.

Direct benefits to blood safety and hemovigilance research include:

1. Combination of research study and operational data, while still maintaining the required integrity of the operational data warehouse databases that must comply with regulatory requirements.
2. Decreased ramp-up time for conducting time-sensitive analyses that are necessary due to regulatory initiatives or emerging issues in blood safety.
3. Greater control over denominator data making it much easier for researchers to conduct more complex analyses when different blood centers are involved.
4. Capacity to expand the number of blood centers included in future efforts based on documented procedures developed during this project.

AIM 3 METHODS & PROCEDURES

The BSI and NYBC databases will mimic that of ARC. The ARC research database is known as ARCnet. ARCnet is an extract of operational data that is migrated to a unique server. Data flow goes in one

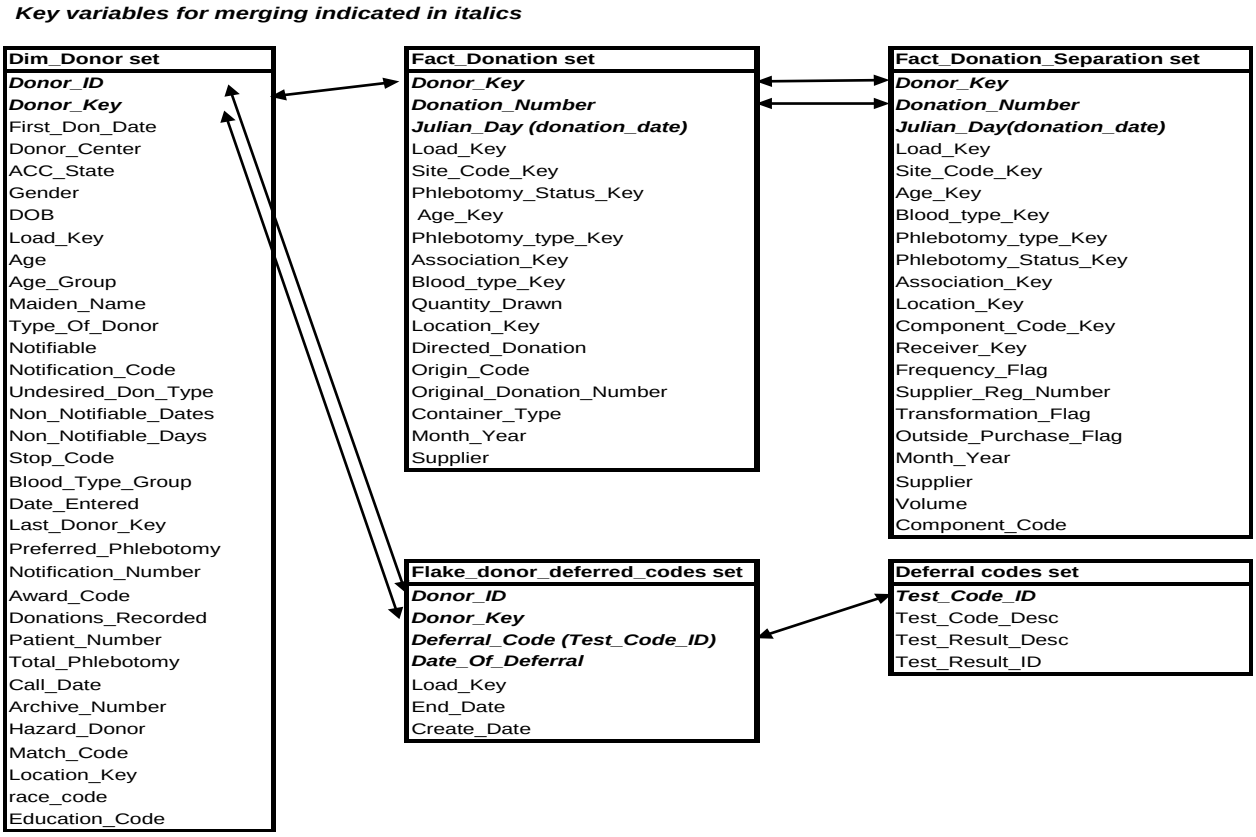
direction – the one-way process is very important for liability because it is not uncommon for ARCnet researchers to identify inconsistencies in the data that is captured from operational systems. The combined research database will be maintained at ARC. Mirroring procedures used at ARC, a plan will be developed that includes processes for formal quality control (QC) and scheduled updates at BSRI and NYBC.

Using BSI as an example, operational databases are built on a relational table structure as shown in Figure 2. To be useful for research donors and donations need to be linked by unique identifiers. In addition statistical analyses require a flat-file format as opposed to relational tables. For this reason the operational structure has to be transformed to a new format. In addition the research database does not require, nor should it include, personally identifying information. The data extracts we obtain will include only the donor identification number and blood unit identification numbers (where applicable), but NOT names, addresses or telephone numbers. Updates to the research database can be accomplished by joining new records to the database using the blood donor number. Each blood center/organization will maintain separately recorded and secure linkages to personal identifiers.

Steps in the Development of the Research Database

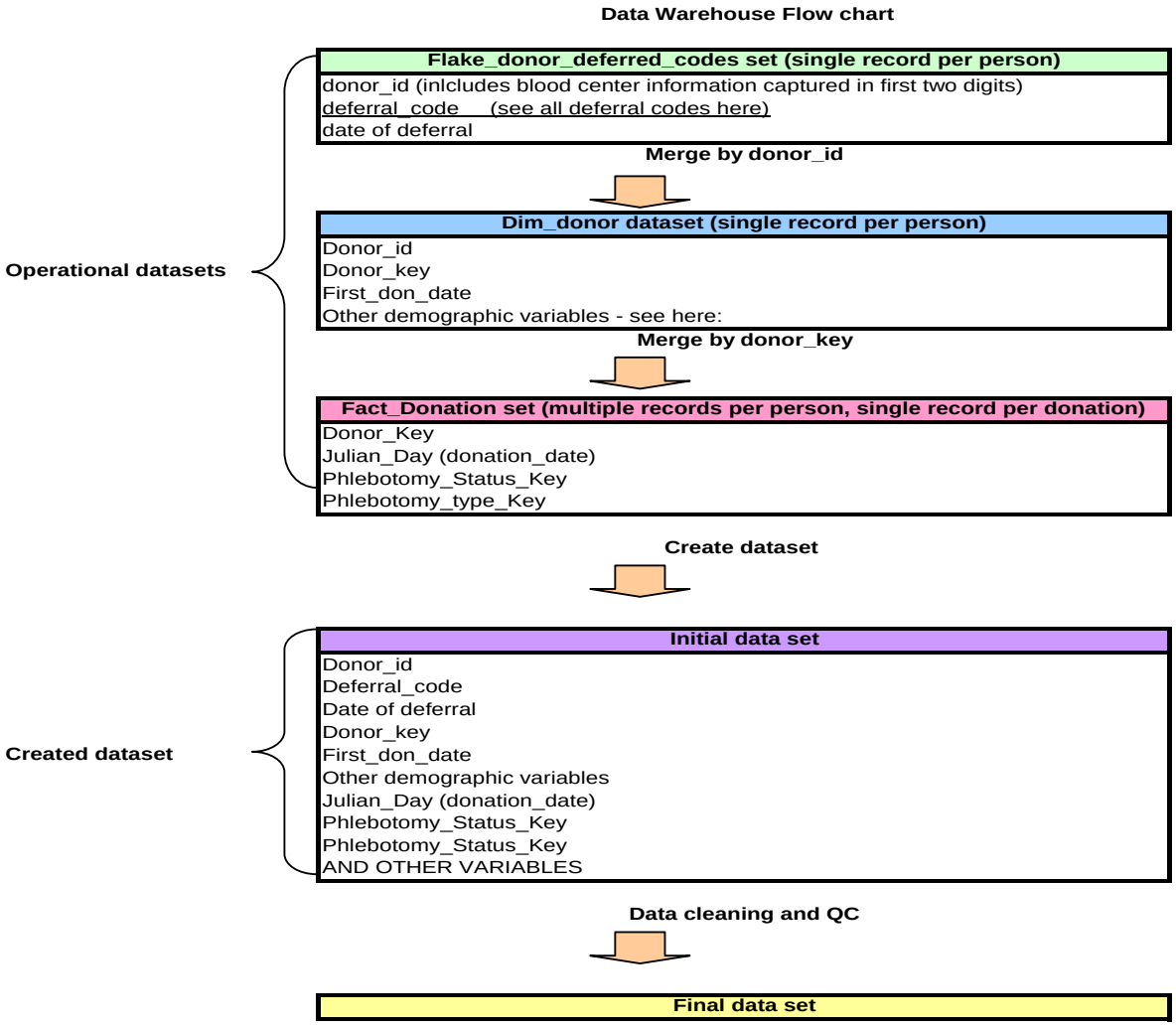
Step 1: Determine the set of variables and other data to be extracted from operational data (Figure 1). Draft common dataset contents are provide in Table 7.

Figure 2. Operational data structure and example of available data elements and key elements that link records across tables.



Step 2: The step-wise process for creating the BSI research data warehouse is depicted below (Figure 3) Once constructed it will then be prepared for export to ARC as well as for a research database to be maintained at BSRI.

Figure 3. Data acquisition and research database creation.



Step 3: Data acquisition and verification.

The process for obtaining extracts and cleaning data will be developed in detail through consultation with ARCNet database specialists. Building off of the processing approach used by ARCnet, the following procedures will largely be duplicated at BSI and NYBC.

1. Queries. Designated extraction from the regulated blood establishment production systems queries will be run on monthly intervals. Thus, for January data this extract would happen in late February.

2. QC-1. Pre-processing is done to check file integrity and that all files are present. Line counts are done and compared to adjoining months and the same month from the previous year.

3. Processing-1. The files are released to the Database Administrator (DBA) for main processing into a single month SAS dataset.

4. QC-1. Fields are checked for integrity and consistency at various steps in the processing of the monthly data. PROC FREQ and PROC PRINT are used to review the fields, and the "log" files of the programs are reviewed by the DBA. These procedures allow for the identification of potentially incorrect values such as outliers or out of range data. In addition, reports for logical inconsistency or other indicators of pre-defined unexpected changes in donations or infections are generated to assist in the QC process. Conflicts and errors are resolved if possible or flagged for further inquiry when they cannot be resolved.

5. Processing-2. The monthly data is added to the main database. Integration of any single month involves integrating donors from that month into their previous donation history. Several fields need to be recalculated each time a month is added. For example, date of previous donation, and first time or repeat presentation and donation.

6. QC-2. Once the month is integrated additional PROC FREQ/PRINT programs are run for review. Logs are also checked.

7. Supplementary/Confirmatory Test Result Addition. 6-7 weeks after the end of a month a confirmatory file from staff at the testing laboratories is provided. These data are pre-processed and given to the DBA for inclusion into the main database. Pre- and post-processing reviews are done to insure compatibility of the data to be added.

8. Once completed the database is copied from a temporary work area and overwrites the current production version of the research database. Back-up and disaster recovery copies are maintained.

The common data elements that will be available in the research database for each organization include the following donor and donation related parameters.

Table 7. Donor and donation data to be captured in the common research database.

Variable Category	**Specific Variable**
Administrative/Tracking Information	Encrypted donor number
Donor Demographics	Date of birth
	Age
	Sex (gender)
	Race
	Zip code
Donation Characteristics	Donation history (FT or RPT)
	Number of previous donations

	Type of donation (whole blood, etc.)
	Type of donor (allogeneic, etc.)
	Date of donation
Infection Testing	Anti-HIV
	HIV NAT
	Anti-HCV
	HCV-NAT
	HBsAg
	Anti-HBc
	HBV-NAT
	Anti-HTLV
	HIV confirmatory results
	HCV confirmatory results
	HBV confirmatory results
	HTLV confirmatory results
	HIV final interpretation
	HCV final interpretation
	HBV final interpretation
	HTLV final interpretation

Statistical Analysis

The infectious disease testing results will be used to stratify donors into recently acquired versus prevalent infection and reported as such. As an example, the results for BSI are provided. Recent seroconversion can be distinguished from prevalent infection based on the combination of NAT results and antibody titer for HIV and HCV (Table 8). For HBV defining infection status is more difficult, and for HTLV it may not be possible to fully classify donors into recent or prevalent infection.

Table 8. 2009 donor infection classification for BSI donors.

Infection	NAT-only yield	Recent Seroconversion	Prevalent	Total
HIV-1	0	31	0	31
HCV	6	0	309	315
HBV	Not Applicable	44	125	169
HTLV I/II	Not Applicable	Not Applicable	75	75

* Unconfirmed infections, it is assumed that approximately half of HTLV infections at BSI are expected to be confirmed.

The database will contain detailed information on dates of donation, demographic characteristics (age, sex, etc.), serology and nucleic acid testing results including confirmatory testing for each infection. The combination of results obtained from serology and nucleic acid testing allows us to identify incident infections. These data will be used to report rates of four viral infections during the initial 1-year study period (2010-2011). First-time donor and repeat donor analyses will be conducted separately. Similar to previous publications, prevalence of infection in first time donors will be calculated with associated 95%

confidence intervals.[21] Prevalence will be defined as the number of infected donations from first time donors divided by total number of first-time donations each year. Incidence is defined as the number of new infections divided by the person-years of time accrued. In repeat donors, incidence and 95% confidence intervals for each infection will be calculated using the incidence-window period model.[35, 36] Alternately incidence rates can be calculated in the classical way by dividing the number of identified infections by person-years for all repeat donors where follow-up time for a donor is the time between his/her first and last donation. For persons who become infected follow-up time is adjusted by assuming that the infection occurred halfway between the last negative and first positive donation so that the individual accrued person-time is half that of the follow-up time.

In future years if the project continues as the database matures, additional analyses can be conducted including infectious marker time trends. Trends over time can be assessed by using logistic regression with prevalence of each infection as an outcome variable and year of donation as a predictor variable. This approach will allow us to determine whether there are changes over time based on departure from a common underlying prevalence rate per year over a defined multiple-year period.

Human Subjects Considerations

The joint research database will not have personally identifying information. A unique identifying number will be given to each donor based on each center's blood donor identification number. Except for consent obtained for donation and use of information for administrative purposes, specific donor consent will not be obtained for information included in the common database. The only threat to donors is the potential for loss of confidentiality and inadvertent release of information. However because personally identifying information will not be obtained, the risk of loss of confidentially is minimal.

References

1. Busch, M.P., *Lessons and opportunities from epidemiologic and molecular investigations of infected blood donors.* Transfusion, 2006. **46**(10): p. 1663-6.
2. Orton, S.L., et al., *Risk factors for HCV infection among blood donors confirmed to be positive for the presence of HCV RNA and not reactive for the presence of anti-HCV.* Transfusion, 2004. **44**(2): p. 275-81.
3. Zou, S., et al., *Prevalence, incidence, and residual risk of major blood-borne infections among apheresis collections to the American Red Cross Blood Services, 2004 through 2008.* Transfusion.
4. Zou, S., et al., *Prevalence, incidence, and residual risk of human immunodeficiency virus and hepatitis C virus infections among United States blood donors since the introduction of nucleic acid testing.* Transfusion.
5. Zou, S., et al., *Current incidence and residual risk of hepatitis B infection among blood donors in the United States.* Transfusion, 2009.
6. Whitaker, B.I., et al., *The 2007 National Blood Collection and Utilization Survey*. 2008, The United States Department of Health and Human Services: Rockville, Maryland. p. 66.
7. Hariri, S. and M.T. McKenna, *Epidemiology of human immunodeficiency virus in the United States.* Clin Microbiol Rev, 2007. **20**(3): p. 478-88, table of contents.
8. McQuillan, G.M., et al., *Prevalence of HIV in the US household population: the National Health and Nutrition Examination Surveys, 1988 to 2002.* J Acquir Immune Defic Syndr, 2006. **41**(5): p. 651-6.
9. Doll, L.S., et al., *Human immunodeficiency virus type 1-infected blood donors: behavioral characteristics and reasons for donation. The HIV Blood Donor Study Group.* Transfusion, 1991. **31**(8): p. 704-9.
10. Petersen, L.R. and L.S. Doll, *Human immunodeficiency virus type 1-infected blood donors: epidemiologic, laboratory, and donation characteristics. The HIV Blood Donor Study Group.* Transfusion, 1991. **31**(8): p. 698-703.
11. Armstrong, G.L., et al., *The prevalence of hepatitis C virus infection in the United States, 1999 through 2002.* Ann Intern Med, 2006. **144**(10): p. 705-14.
12. Murphy, E.L., et al., *Risk factors for hepatitis C virus infection in United States blood donors. NHLBI Retrovirus Epidemiology Donor Study (REDS).* Hepatology, 2000. **31**(3): p. 756-62.
13. Alter, M.J., *Prevention of spread of hepatitis C.* Hepatology, 2002. **36**(5 Suppl 1): p. S93-8.
14. McQuillan, G.M., et al., *Prevalence of hepatitis B virus infection in the United States: the National Health and Nutrition Examination Surveys, 1976 through 1994.* Am J Public Health, 1999. **89**(1): p. 14-8.
15. Shepard, C.W., et al., *Hepatitis B virus infection: epidemiology and vaccination.* Epidemiol Rev, 2006. **28**: p. 112-25.
16. Schreiber, G.B., et al., *Risk factors for human T-cell lymphotropic virus types I and II (HTLV-I and -II) in blood donors: the Retrovirus Epidemiology Donor Study. NHLBI Retrovirus Epidemiology Donor Study.* J Acquir Immune Defic Syndr Hum Retrovirol, 1997. **14**(3): p. 263-71.

17. Stainsby, D., et al., *Serious hazards of transfusion: a decade of hemovigilance in the UK.* Transfus Med Rev, 2006. **20**(4): p. 273-82.
18. Van der Bij, A.K., R.A. Coutinho, and C.L. Van der Poel, *Surveillance of risk profiles among new and repeat blood donors with transfusion-transmissible infections from 1995 through 2003 in the Netherlands.* Transfusion, 2006. **46**(10): p. 1729-36.
19. Sullivan, M.T., et al., *Blood collection and transfusion in the United States in 2001.* Transfusion, 2007. **47**(3): p. 385-94.
20. Sullivan, M.T. and E.L. Wallace, *Blood collection and transfusion in the United States in 1999.* Transfusion, 2005. **45**(2): p. 141-8.
21. Glynn, S.A., et al., *Trends in incidence and prevalence of major transfusion-transmissible viral infections in US blood donors, 1991 to 1996. Retrovirus Epidemiology Donor Study (REDS).* Jama, 2000. **284**(2): p. 229-35.
22. Salonen, J.T., et al., *Donation of blood is associated with reduced risk of myocardial infarction. The Kuopio Ischaemic Heart Disease Risk Factor Study.* Am J Epidemiol, 1998. **148**(5): p. 445-51.
23. Custer, B., et al., *Associations between West Nile virus infection and symptoms reported by blood donors identified through nucleic acid test screening.* Transfusion, 2009. **49**(2): p. 278-88.
24. Orton, S.L., S.L. Stramer, and R.Y. Dodd, *Self-reported symptoms associated with West Nile virus infection in RNA-positive blood donors.* Transfusion, 2006. **46**(2): p. 272-7.
25. McAuley, J.D., et al., *Clinical significance of nondiscriminated reactive results with the Chiron Procleix HIV-1 and HCV assay.* Transfusion, 2004. **44**(1): p. 91-96.
26. Ownby, H.E., et al., *Loss of volunteer blood donors because of unconfirmed enzyme immunoassay screening results. Retrovirus Epidemiology Donor Study.* Transfusion, 1997. **37**(2): p. 199-205.
27. Sharma, U.K., et al., *Impact of changes in viral marker screening assays.* Transfusion, 2003. **43**(2): p. 202-14.
28. Dodd, R.Y. and S.L. Stramer, *Indeterminate results in blood donor testing: what you don't know can hurt you.* Transfus Med Rev, 2000. **14**(2): p. 151-60.
29. Cleary, P.D., et al., *Sociodemographic and behavioral characteristics of HIV antibody-positive blood donors.* Am J Public Health, 1988. **78**(8): p. 953-7.
30. Carneiro-Proietti, A.B., et al., *HTLV in the Americas: challenges and perspectives.* Rev Panam Salud Publica, 2006. **19**(1): p. 44-53.
31. Custer, B., et al., *Global epidemiology of hepatitis B virus.* J Clin Gastroenterol, 2004. **38**(10 Suppl 3): p. S158-68.
32. Dodd, R.Y., E.P. Notari, and S.L. Stramer, *Current prevalence and incidence of infectious disease markers and estimated window-period risk in the American Red Cross blood donor population.* Transfusion, 2002. **42**(8): p. 975-979.
33. EuroQolGroup. *EQ-5D a standardised instrument for use as a measure of health outcome*. 2010 [cited July 22, 2010]; Available from: http://www.euroqol.org/eq-5d/what-is-eq-5d.html.
34. Williams, A.E., et al., *Estimates of infectious disease risk factors in US blood donors. Retrovirus Epidemiology Donor Study.* JAMA, 1997. **277**(12): p. 967-972.

35. Kleinman, S., et al., *The incidence/window period model and its use to assess the risk of transfusion-transmitted human immunodeficiency virus and hepatitis C virus infection.* Transfus Med Rev, 1997. **11**(3): p. 155-72.
36. Busch, M.P., et al., *A new strategy for estimating risks of transfusion-transmitted viral infections based on rates of detection of recently infected donors.* Transfusion, 2005. **45**(2): p. 254-64.