B.   Statistical Methods

1. Respondent Universe and Sampling Methods

    The study sample will consist of persons who donated blood at TTIMS participating blood centers (representing nearly 60% of all US donors) and will be defined as cases and controls:

    - Cases: Blood donors who tested confirmed positive for HIV and for incident infection with HCV, or HBV based on testing nucleic acid positive, but serology negative.
    - Controls: Controls will be recruited from each participating blood center in a ratio of 2 controls per each case identified at that blood center. Donors who have confirmed false positive serology test results for HIV or HBV have been demonstrated to not carry HIV or HBV, respectively, and thus be truly negative for these infections. Research has shown that donors with false-positive results on an initial screening test are not infected.(1-3) The reason for choosing these false positive donors as controls is that operationally, blood centers (i.e., donor counselors) contact these donors anyway because of their test results. Rates of false positive serology test results for HIV and HBV are as high as 15x those of confirmed positive results in the donor population (see description of "Controls" below); thus, a sampling approach is required. Within each participating blood center, a monthly random sample of blood donors who tested false positive for HIV or HBV will be selected using the study management system. A random number generator method will be used to select a list of persons to contact for recruitment to achieve a random selection of twice the numbers of controls as cases, expected to be 1440 controls for 720 HIV cases, and 560 controls for 280 HBV/HCV cases (see below for power calculations). Selected donors will be contacted and recruited for participation in the risk factor interview.

    Table 1: Overall expected participation in risk factor interview assuming both prospective and retrospective interviews for confirmed positive and false positive donors over a 5-year period

Subject Type	HIV	HCV or HBV
Case (True positive)	720	280
Control (False positive)	1,440	560

    Power and Sample Size Calculations

    Power analyses to help guide the understanding of what levels of excess risk can be estimated are provided below. These analyses assume a case to control ratio of 1:2 and a worst case estimate of 50% case enrollment among the HIV, HCV, and HBV cases. Approximately 50-75% of controls on the randomly selected recruitment list are expected to enroll based on the REDS-II experience.

HIV case control power and sample size calculations: The detailed results from the previous Retrovirus Epidemiology Donor Study-II (REDS-II) "Transfusion-transmitted retrovirus and hepatitis virus rates and risk factors: Improving the safety of the US blood supply through hemovigilance" (OMB control number 0925-0630) provide information on the proportion of HIV, HCV, and HBV positive cases and false-positive controls who disclosed a specific risk behavior in 2011-2012. For HIV for example, being a male who had sex with another male (MSM) was the most prevalent (62% of cases) and reporting having sex with a person known to be HIV positive (27% in males and females) was the second most prevalent risk factor identified among blood donors at that time. In addition, a survey study of undisclosed risk factors in accepted male donors conducted as part of Recipient Epidemiology and Donor Evaluation Study (REDS-III), found non-compliance with the MSM deferral policy in place at that time among uninfected male blood donors to be 2.6%.(4) For HIV, we assume a maximum expected prevalence of undisclosed risk behaviors in the control group during TTIMS based on these recent data. Power to detect significant associations depends on the excess risk in cases and the baseline prevalence of each behavior in controls. With an expected sample size of 720 confirmed HIV-positive interviewed donors compared to 1,440 interviewed controls, various combinations of excess risk are shown in Table 2. The table shows the power at $\alpha=0.05$ to detect significant associations between risk factors with a prevalence of 2.6, 1.7, 0.5 and 0.25% in controls donors assuming 10, 5, 3, and 2-fold higher odds of specific risk behaviors in confirmed-positive donors.

To place these estimates in context, we will have sufficient power to detect odds ratios just above 2-fold higher for any risk behavior reported by 2.6% of controls. In addition, although the adjusted odds ratio in the REDS-II study was 3.1 for the association between HIV infection and intravenous drug use (IDU), it was not statistically significant. In that study 0.4% of false-positive controls reported IDU. With the proposed number of controls we will have sufficient power to detect an odds ratio of 5 or higher for IDU if 0.5% of controls report IDU history. Overall, the proposed ratio of cases to controls for HIV in TTIMS will be able to achieve similar power levels as achieved in the REDS-II study.

Table 2. Power for various risk factor prevalence combinations for HIV cases compared to controls, assuming 50% case enrollment and a 1:2 case:control ratio

Infectious Marker case/control sample sizes	Prevalence of risk factor in controls – [Reference]	Odds ratio to detect in cases			
		10	5	3	2
		Power			
HIV 720/1,440	2.6%(4)	1.0	1.0	1.0	0.79
	1.7%(5)	1.0	1.0	0.97	0.62
	0.5%	1.0	0.94	0.58	0.23
	0.25%	0.99	0.71	0.33	0.14

HBV and HCV case control power and sample size calculations: The inclusion of control interviews for nucleic acid test (NAT)-only HCV and HBV cases will increase the number of cases and controls over the five-year period to a projected 1,000 and 2,000, respectively. The additional 560 control interviews will be triggered based on being HBsAg false-positive. HBsAg confirmation testing when non-neutralized along with being anti-HBc and NAT negative indicate that any HBsAg false-positive does not have an HBV infection. These controls would be informative for comparing to the up to 280 participants with NAT-only HCV and HBV infections who are planned to be interviewed as part of TTIMS. Power to detect significant associations depending on the excess risk in cases and the baseline prevalence of each behavior in controls with a sample size of 280 incident HCV and HBV confirmed positive, interviewed donors compared to 560 interviewed controls is provided in Table 3. The table shows the power at α=0.05 to detect significant associations between risk factors with a prevalence of 5.2, 2.3, 0.5 and 0.25% in controls donors assuming 10, 5, 3, and 2-fold higher odds of specific risk behaviors in confirmed-positive donors. The estimates are based on risk behaviors that were significantly associated with HCV or HBV infection in controls from the REDS-II Study; 5.2% of controls from that study reported spending 3 or more nights in jail, detention or a group home, and 2.3% of controls reported sex with an IDU.

Table 3. Power for various risk factor prevalence combinations for HCV and HBV cases compared to controls, assuming 50% case enrollment and a 1:2 case:control ratio

Infectious Marker case/control sample sizes	Prevalence of risk factor in controls – [Reference]	Odds ratio to detect in cases			
		10	5	3	2
		Power			
HBV & HCV 280/560	5.2% (5)	1.0	1.0	0.98	0.67
	2.3% (5)	1.0	1.0	0.81	0.38
	0.5%	0.96	0.60	0.27	0.12
	0.25%	0.76	0.35	0.16	0.09

For the assessment of the association between IDU and incident HCV, we will be able to estimate a significant odds ratio of less than 9 in cases compared to controls. In the REDS-II Study the association between HCV and IDU was highly significant with an odds ratio of 42.

For other uncommon risk behaviors in the uninfected population, such as those with frequency of 0.25%, if all interviewed false-positive donors are included in the analysis of infection risk factors 2060 controls can be compared to each case group. If this is done, we will have sufficient power $(1-\beta > 0.80)$ to detect odds ratios of 10 or higher in cases. Similarly, we will have sufficient power $(1-\beta = 0.80)$ to detect odds ratios of 5 or higher if the behavioral prevalence in uninfected donors is around 0.65%.

2. <u>Procedures for the Collection of Information</u>

Each organization, ARC, BSI, NYBC, and OneBlood, will interview its own donors. The TTIMS Laboratory and Risk Factor Coordinating Center (LRCC) will coordinate and train blood centers in conducting risk factor interviews by donor counselors via telephone. A tailored plan will be developed for each site based on language administration capability (i.e. English and Spanish-language capability or English-only). The risk factor interview is targeted to be conducted within 30-days of the date the index donation is confirmed to meet TTIMS eligibility definitions (for prospective interviews). Conducting these interviews as soon as possible after the detection of the infected donation is the best way to ensure the most complete and accurate disclosure of risk behaviors by the donors who are infected. Telephone interviews will be conducted for case and control interviews. HIV confirmed positive donors and a few confirmed positive HCV or HBV infected donors may be interviewed in person, at the choice of the respondent. Such in person interviews are expected to occur rarely.

Cases

HIV, HBV, and HCV cases will be contacted in accord with one of the three routes of participant contact for the study. Cases will come from the four participating blood collection organizations. Every case who is identified and eligible to be interviewed according to the study case definitions will be contacted for study recruitment. No sampling scheme will be used.

Controls

Controls for this study are intended to reflect the population of eligible blood donors. Controls will be interviewed in a ratio of 2 controls for each case. Control donor interviews will be based on a monthly random sample of all anti-HIV, or HBsAg false-positive donors from the same blood collection organization each case comes from. No other matching criteria to cases will be used so that we may assess which demographic characteristics in addition to behavioral risk factors in multivariable logistic regression analyses are predictors of infections in blood donors. For example, the change in the MSM deferral strongly supports the need for monitoring to see if increased or decreased numbers of young, male donors with HIV infection are donating blood. Controls will be interviewed contemporaneously with cases. All interviewed controls will be included in each analysis comparing risk factors in confirmed positive for each infection to the entire control group. Eligible study controls will only include anti-HIV confirmed false positive donors and HBsAg confirmed false positive donors.

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 testing the ratio of false-positive to confirmed positive donors is greater than 15:1. This ratio means that we will have far more false-positive donors than confirmed positive donors as potential participants. Similarly, HBsAg false-positive are straightforward to confirm as uninfected and will serve as an additional source of potential controls. Use of donors with HBsAg, non-neutralized, false-positive donations is of particular relevance to serve as controls for incident [serology negative, nucleic acid test positive (NAT yield)] HBV and HCV cases.

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 50% participation of confirmed positive cases, we will also obtain human subjects approval to conduct risk factor interviews of donors from the beginning of TTIMS (October 2015) in order to achieve the planned sample size for cases and controls. Furthermore, this will allow us to directly compare infectious marker rates and behavioral risk factors in donors just prior to the implementation with those just after implementation of the MSM donor eligibility change.

Data Analysis

The project analysts will compute descriptive statistics, such as frequencies and measures of central tendency (means and medians) in order to characterize the infected population and catalog donor-reported risk factors likely to be the route of virus acquisition in case groups. We will compare the risk factors reported by cases and controls 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.

TTIMS is a monitoring system. Primary reports will be to provide quarterly frequency results. The data required by the FDA are:

- Quarterly reports (based on achieved enrollment and completed testing at the close of the quarter)
- For each type of viral infection:
    - Number of completed interviews in the quarter by center and overall,
    - Frequency tables of age, gender, race/ethnicity, first-time or repeat status, HHS public health region, and donation type of interviewed donors,
    - Frequency tables of reported risk factors. The risk factors reported by interviewed donors will also be compared every six months to see if proportions of attributable risk behaviors change over time.
- Results of HIV recency testing
- Frequency tables of HIV recency results by the same categories listed in A.1.b.
- Frequency tables of risk factors in donors classified as having recently acquired infections (All NAT-only infections or classified as HIV recent infection based on recency testing).

Advanced Analyses

Independently for each virus, univariable and multivariable logistic regression analysis will be used to compare risk factors when the outcome variable, infection status, can be defined in a dichotomous manner. For example, to assess the association between risk behaviors and demographics when comparing recently acquired HIV to long-standing HIV infection. The multivariable analyses will be important so that we may account for potential confounding with regard to factors such as socio-economic status and education level.

The inclusion of controls will allow for more advanced statistical analyses. In addition to descriptive statistics, such as frequencies, we will be able to use multivariable logistic regression analysis to compare confirmed-positive and false-positive donors to determine the association between risk behaviors, while adjusting for demographics, Public Health Service (PHS) regions, or other factors. 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. Furthermore, advanced exploratory analyses may also be possible, such as multilevel modeling and potentially structural equation modeling. Use of these techniques could generate novel interpretations of patterns of infection in blood donors and also provide for a more direct assessment of how similar or dissimilar infections in blood donors are to the larger sets of data on infections identified through other public health surveillance, and higher risk groups surveillance. These analyses will allow for a deeper level of monitoring of the data from TTIMS and, if other public health datasets can be accessed, a direct comparison to other sources. Examples of published studies using these techniques show there is potential for new insights if these methods are applied to blood donor data.(6-8)

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

Based on our previous experience in the "Transfusion-transmitted retrovirus and hepatitis virus rates and risk factors: Improving the safety of the US blood supply through hemovigilance" (OMB control number 0925-0630), we determined telephone interviews help to improve response rates because of the sensitive nature of the questions. Participants were more willing to disclose risk behaviors over the telephone rather than face to face. In addition, we have obtained a Certificate of Confidentiality from HHS to help to protect the release of information on donor responses on the interview. The Certificate helps to ensure participants will be afforded the greatest confidentiality available to the extent law allows.

4. Test of Procedures or Methods to be Undertaken

The previously conducted "Transfusion-transmitted retrovirus and hepatitis virus rates and risk factors: Improving the safety of the US blood supply through hemovigilance" (OMB control number 0925-0630) provided a detailed test of all procedures proposed to be used in TTIMS. Over 4,000 blood donors were interviewed using nearly identical study procedures and interviews. Based on this previous project, it took on average 40 minutes to complete the questionnaire and 5 minutes to conduct verbal consent, or overall 45 minutes to complete the data collection procedures. We used this figure for our burden hour calculations in our previous information collection request. Based on our

experience with this survey over the past three years, we have adjusted our burden estimate. We decreased the average burden per response from 45 to 30 minutes.

5. <u>Individuals Consulted on Statistical Aspects and Individuals Collecting and/or Analyzing Data</u>

Individuals consulted include biostatisticians on statistical aspects of the study design; the blood centers researchers responsible for enrollment, administering questionnaires, and collection of samples; the FDA Center for Biologics Evaluation and Research, Office of Biostatistics and Epidemiology, representatives of the NIH NHLBI, and the TTIMS Steering Committee consisting of representatives from several government agencies. Data analysis will be performed by the analytic staff that includes experts in qualitative data analysis along with epidemiologists and biostatisticians, with assistance and oversight provided by the TTIMS Steering Committee.

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